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Optimization of Growth Performance of Nile Tilapia (*Oreochromis niloticus*) in Biofloc Farming: Evaluating Growth Performance and System Stability Under Reduced Feeding Regimes

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ABSTRACT

This study assessed the effect of different feeding levels on the growth performance of Nile tilapia (*Oreochromis niloticus*) as well as on the stability of the biofloc system over a 9-week experimental period. Three feeding levels were tested: BFT100 (100% of a complete ration), BFT75 (75% of a complete ration), and BFT50 (50% of a complete ration). A flow-through system group (control) was feed 100% ration, while biofloc groups received 100% (BFT100), 75% (BFT75), or 50% (BFT50) of that ration. Each treatment was replicated 3 times, and the fish were reared in subsquare tanks (130 L) at a stocking density of 6.2 kg.m^{-3} per experimental unit (70 fish per tank). Growth performance was recorded at 14-day intervals. All fish were counted and weighed to determine the total biomass. A sample of 30 fish per unit was measured and weighed individually to assess variability. Growth performance was significantly affected by feeding level ($p < 0.05$), whereas survival remained high and did not differ among treatments (92.9%–98.6%). The highest specific growth rate (SGR) was observed in the flow-through control (2.72% day^{-1}), followed by BFT75 (1.92% day^{-1}) and BFT100 (1.70% day^{-1}), while BFT50 exhibited the lowest SGR (1.21% day^{-1}). A moderate level of feed reduction (BFT75) resulted in improved water quality and maintained feed efficiency, with a feed conversion rate (FCR) (0.89) similar ($p > 0.05$) to that of the control (0.75). In contrast, higher FCR values were recorded in BFT100 (1.09) and BFT50 (1.13), indicating reduced feeding efficiency under full feeding and excessive feed restriction, respectively. Notably, the BFT100 treatment was characterized by signs of organic overload under full-feeding conditions, including floc disruption, increased nitrogenous waste levels, microbial imbalance, and mortalities. Accordingly, this study concludes that the biofloc system can withstand a 25% feed reduction without adverse effects on FCR when environmental stability is maintained. Resource optimization and progressive system management are likely the most effective approaches to enhance the sustainability of biofloc technology (BFT)–based tilapia rearing.

1 | Introduction

Aquaculture is now one of the major sectors of the food industry. It is at the heart of current challenges in food security and sustainable development, including population growth, rising food demand, and environmental degradation [1, 2]. Aquatic food consumption has increased in the past decades [3, 4]. Global aquatic production is expected to increase by 10%, reaching 205 million tonnes by 2032 [5]. Capture fisheries output has plateaued; consequently, aquaculture has become an increasingly important source of food and income, thereby alleviating pressure on wild stocks [6]. As aquaculture pursues best practices to enhance efficiency and sustainability, its contribution to global food security and nutrition is likely to increase significantly in the coming years [7, 8].

However, aquaculture is facing several economic issues, primarily the high feed price, which accounts for 30%–60% of the total production cost, and the volatility of fishmeal and fish oil resulting from material shortages [1, 9]. While aquaculture is central to food security (SDG 2) and sustainable development (SDG 14), its expansion is creating environmental concerns, including high water and energy requirements, a carbon footprint, and pollution of natural ecosystems [10, 11].

Innovative aquaculture technologies, such as aquaponics and biofloc systems, are emerging to address challenges [12, 13]. Aquaponics integrates RAS aquaculture with plant culture, reducing water demand and optimizing waste disposal [14]. Similarly, biofloc technology (BFT), which mimics a natural microenvironment to filter water at low cost and provides fish with an additional food source, is very promising [15, 16]. Moreover, the "flocponic" system, which combines hydroponics and biofloc, is emerging as a new solution with various advantages, including reduced water consumption, reduced pollutant release, reduced feed intake, and reduced pathogen threat [17].

BFT is a closed system that contains suspended particles, known as flocs. These aggregates are composed of microorganisms, including bacteria, algae, protozoa, and copepods, as well as organic waste, such as uneaten food and feces [18]. The particle size of bioflocs generally ranges from 50 to 1000 µm [19]. This technology enables the production of proteins by heterotrophic bacteria and the recycling of nitrogenous waste through nitrification by autotrophic bacteria. The C/N ratio determines the bacterial activity in this system [20]. By biologically filtering nitrogenous waste and producing digestible nutrients rich in proteins and fats, these microorganisms play a significant role in maintaining water quality. The most common microorganisms prevalent are nitrifying autotrophic bacteria, phytoplankton, and heterotrophic bacteria. They fulfill primary roles in the system:

i. Improving water quality by converting toxic nitrogen compounds into nitrates and consumable proteins,
ii. Improving system efficiency by reducing the feed conversion rate (FCR) and enhancing the growth rate, and
iii. Strengthening biosecurity by outcompeting pathogens,
iv. Zero water exchange, thus permitting efficient use of limited water resources and preventing the discharge of nutrient-rich wastewater into the environment [21].

Aggregates (flocs) are kept in suspension in the water column using vigorous aeration. Aeration benefits fish and other microorganisms in the water column, particularly juvenile fish, which use 2.5 times as much oxygen as adults [22].

Floc aggregates serve as a substrate for nitrifying bacteria (*Nitrobacter* sp. and *Nitrosomonas* sp.). They oxidize ammonium to nitrite and nitrate [23]. Heterotrophic bacteria produce microbial proteins using excreted nitrogen, which fish can utilize as a source of nutrition. The flocs can contain proteins (25%–50% of the dry matter), fats (0.5%–15%), and vitamins and minerals. Thus, they are a secondary source of nutrition [24]. Maintaining a high carbon/nitrogen (C/N) ratio is essential for promoting heterotrophic bacterial activity [25]. This requires an additional source of organic carbon, such as simple sugars [26]. However, an excess of carbon may pose hazards, such as gill clogging and reduced oxygenation [27].

Additionally, the C/N ratio is used to assess water quality in BFT, evaluating inorganic nitrogen levels [28]. The biofloc's heterotrophic bacteria use carbon for metabolism and nitrogen for growth [29]. The composition of the feed (with lower protein levels and higher C/N ratios) and the effectiveness of bacteria and fish in nutrient absorption both contribute to balancing the C/N ratio [30]. A C/N ratio of 15:1 is generally sufficient to maintain optimal BFT performance at any point in time, but a C/N ratio of 10:1 is a better alternative only when bioflocs have reached maturity [31].

Daily measurements of physicochemical parameters (temperature, dissolved oxygen, pH, alkalinity, conductivity, suspended solids (SS), and floc volume) are necessary to maintain proper system operation. The main limiting factors in this system are excessive SS load and high concentrations of nitrogen compounds, which can become toxic and impair fish growth and health [32]. Additionally, a sharp drop in alkalinity may occur when autotrophic bacteria consume inorganic carbon during ammonium nitrification. This may result in pH variations that harm the biofloc system and fish [33].

BFT is better suited to microfiltering species (tilapia, carp, and shrimp), which can consume flocs and tolerate high SS concentrations. Additionally, by preventing the spread of pathogens, the bacterial colonies in biofloc systems ensure biosecurity [27].

Tilapia (*Oreochromis* sp.) is the third most farmed fish species in the world, with 6.5 million tons produced in 2022, 90% of which comes from Nile tilapia (*Oreochromis niloticus*) [34]. This fish has increased its production over the last decade, primarily due to its adaptability across various farming systems, including extensive, semi-intensive, and intensive systems, as well as its tolerance for a wide range of environmental conditions [1, 35, 36]. Nile tilapia tolerates temperatures from 20°C to 31°C [37], a pH range of 6.5 to 9 [38], and low oxygen levels (as low as 3 mg.L^{-1}) [39], which seems to be a prime factor for biofloc culture [70]. It also tolerates high levels of SS, ammonium, and nitrites while being an omnivorous and microphagous feeder, allowing it to feed on floc aggregates and digest microbial proteins directly. This unique combination makes it an ideal candidate for biofloc farming systems.

This study investigates the effect of feed management, particularly the reduction of formulated feed input, on the growth performance of tilapia and on the stability of the biofloc system. The objective of this research is to address the following question: "To what extent does feed reduction affect the growth of tilapia reared in a biofloc system and the stability of the rearing environment?" To this end, different feeding levels were tested in juvenile Nile tilapia, with growth performance and rearing environment quality evaluated.

2 | Material and Methods

2.1 | Experimental Setup

The study was conducted at the UGeRAA laboratory (Unit for the Management of Aquatic Resources and Aquaculture) at the University of Liège, in Tihange, Belgium, over a period of 9 weeks, under ethical protocol number 2759. This period included a 1-week acclimatization phase and 8 weeks of experimental trial.

At the start of the experiment, tilapia juveniles (mean initial body weight = 11.4 ± 2.1 g) were stocked into experimental tanks at a density of 6.2 kg.m^{-3}, i.e., 70 individuals in each tank, resulting in a total of 840 individuals. The experimental factor in the study was the "feeding level," evaluated in the control in flow-through vs. biofloc system under four treatments, each replicated three times. Different treatments are summarized in Table 1.

Control treatment in flow-through: 100% of the daily feed ration (tanks 1, 2, and 4); BFT100 (experimental treatment with biofloc): 100% of the daily feed ration (tanks 6, 12, and 9); BFT75 (experimental treatment with biofloc): 75% of the daily feed ration (tanks 3, 7, and 10); BFT50 (experimental treatment with biofloc): 50% of the daily feed ration (tanks 5, 8, and 11).

The experimental setup consisted of 12 fiberglass (polyester) sub-square shape tanks, each with a capacity of 130 L and covered with a mesh to prevent the fish from jumping out. Depending on the treatment, each tank was filled with either freshwater (groundwater) or biofloc.

The development of the autotrophic bacterial community was facilitated by the addition of 130 bioballs (1 cm in diameter) to the biofloc tank at the beginning of the acclimatization process. The temperature was maintained at 27°C in water and 26.5°C in the air. A photoperiod of 12 h light and 12 h dark (12 L:12D) was established using 27W LED lamps.

In the biofloc tanks, the water lost during floc decantation was replaced. Water lost through evaporation was replenished daily, representing 0.3% of the tank volume. Water renewal (adjusted by measuring the water height in the tanks) was done with fresh water stored in a 27°C holding tank. The water exchange rate in the flow-through tanks was 30% per hour. A random design was used to arrange the experimental tanks, with blocks defined by the primary disturbance, specifically the door opening. This approach helped minimize the block effect within the experimental design. The integration of these objectives and the constraints imposed by the room layout resulted in the experimental layout shown in Figure 1.

The initial feed ration corresponded to 3% of the tank biomass, was adjusted every 14 days after biomass control, and was multiplied by the food input level for the treatment. Depending on feeding behavior and physical and chemical conditions, the feeding rate was adapted.

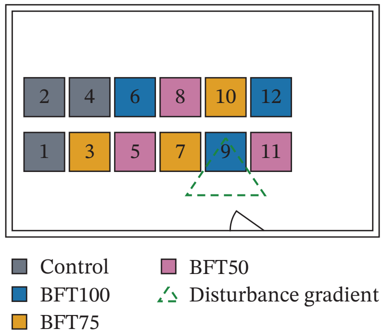


FIGURE 1 | Experimental setup.

The distributed feed ration was calculated as follows:

Initial feed ration (RA0) = B × 0.03 × FIL

B: Biomass of the tank (g)

FIL: Feed input level (%)

Feed ration on day n (RAn) (g) = $RA_{n-1} + SGR \times RA_{n-1}$

RAn−1: Feed ration on day $n-1$ (g)

SGR: Specific growth rate (%.day^{-1})

Throughout the experiment, the fish were fed a 2 mm pelleted feed (Advance, Alltech Coppens). This feed contains:

Crude protein: 50%

Lipids: 15%

Fibers: 0.7%

Ash: 6.3%

Gross energy: 21.4 MJ/kg

Digestible energy: 19 MJ/kg

The daily ration was divided into three equal portions, distributed at 9:00 a.m., 1:00 p.m., and 4:00 p.m.

2.2 | Biological Material

Juveniles originated from two spawns obtained from the UGeRAA-Chitralada strain via natural fertilization in an aquarium. The eggs, collected from the females' mouths, were incubated for 10 days in a Zug bottle at 27°C. One thousand forty-four

TABLE 1 | Summary of the experimental conditions.

Treatments	Control	BFT100[a]	BFT75[a]	BFT50[a]
Initial biomass (g)	800 ± 0.4	800 ± 0.4	800 ± 0.4	800 ± 0.4
Initial density (kg.m^{-3})	6.2	6.2	6.2	6.2
Number of individuals	70	70	70	70
Feed ration	100%	100%	75%	50%

[a]BFT, biofloc technology.

larvae were distributed into three 50-L aquariums maintained at 27°C. Since tilapia exhibit significant sexual dimorphism towards males (over 50%), and to avoid growth heterogeneity between males and females, the experiment was conducted with male juveniles. To achieve this, sex reversal was performed using 17 α-methyltestosterone (60 mg.kg^{-1} of feed) dissolved in ethyl alcohol (300 ml.kg^{-1} of feed), mixed with the fry feed (particle sizes varying over time: 0.2–0.3 mm, 0.3–0.5 mm, and 0.5–0.8 mm) and left to evaporate for 24 h before use. The feed was distributed ad libitum, with seven to eight daily feedings.

2.3 | Stabilization of Biofloc

The experimental biofloc, taken from a mother solution maintained at UGeRAA, was inoculated into nine tanks (BFT100, BFT75, and BFT50). Each tank contained 120 L of clear fresh water and 10 L of biofloc. To stabilize the system, five tilapia (100 g) were introduced to promote the proliferation of biofloc and nitrifying bacteria. For 4 weeks, aggregates of flocs (150–200 mL) and nitrifying bacteria (2 mL.tank^{-1}) were added to control nitrite levels. Feeding begins at 0.3% of the biomass, progressively increasing up to 3%, with an initial sugar addition equivalent to 100% of the feed ration, later reduced to one-third to stimulate heterotrophic bacteria [24]. The stabilization phase was performed to achieve a floc volume of 25 mL.L^{-1} and stabilize total ammonia nitrogen (TAN) and nitrite concentrations below 1 mg. L^{-1} [40]. Figure 2 illustrates the variations in nitrogen compounds during the system's stabilization phase.

2.4 | Monitoring and Management of Physicochemical Parameters

The same operator conducted water parameter measurements daily between 8:00 and 9:00 a.m., using identical instruments throughout the experiment. The target values for these parameters are defined based on the literature [41, 42].

Temperature (26–28°C): The biofloc and control tanks in the flow-through were heated, and temperature was measured daily with an oxy-thermometer (Oxymeter 330 - CellO x 325 WTW).

Dissolved oxygen and floc mixing (> 6 mg.L^{-1}): Two aerators (Superfish Hi-Oxy Disk - ø 10 cm) per tank maintain dissolved oxygen levels above 6 mg.L^{-1} and ensure floc suspension. Oxygen levels were measured daily with an oxy-thermometer (Oxymeter 330-CellO x 325 WTW).

Conductivity: Conductivity was maintained between 1000 and 3000 µS.cm^{-1} and measured daily using a conductivity meter (Multimeter HQ40D-CDC401 Hach).

Alkalinity (100–150 mg.L^{-1} of $CaCO_3$) and pH (6.8–8): Alkalinity was measured thrice weekly by titration. The target values were greater than 20 mg.L^{-1} for the flow-through and between 50 and 150 mg.L^{-1} for the biofloc. Sodium bicarbonate was added if alkalinity or pH (maintained between 6.8 and 8) falls below the thresholds.

Concentration of settleable solids: The volume of flocs was measured daily using an Imhoff cone after 30 min of settling. The target value was 20–30 mL.L^{-1}. When the value was too high, water from the tank was partially drained and left to settle for a while, allowing the flocs to settle. Clear water was returned to the tanks.

TAN < 1 mg.L^{-1}, nitrites (NO_2^-) < 1 mg.L^{-1}, and nitrates (NO_3^-) < 500 mg.L^{-1}: TAN and nitrite concentrations were measured every 2 days, and nitrate concentrations were measured weekly by spectrophotometry.

Suspended solids (SS): SS were measured weekly and should range between 200 and 500 mg.L^{-1} in biofloc and < 50 mg.L^{-1} in flow-through.

C/N ratio: Liquid beet molasses (37.5% carbon content; [43]) is an organic carbon source. It was added daily at 9:00 a.m. at a rate of 33% of the total daily feed ration to maintain a C/N ratio of ~12 [44]). Molasses are readily available, inexpensive, and dissolve quickly [28].

2.5 | Monitoring of Zootechnical Parameters

The measured zootechnical parameters were survival rate (SR; %), average weight gain (g), SGR (%/day), feed conversion ratio (FCR), and yield (kg.m^{-3}.day^{-1}). All fish were counted and

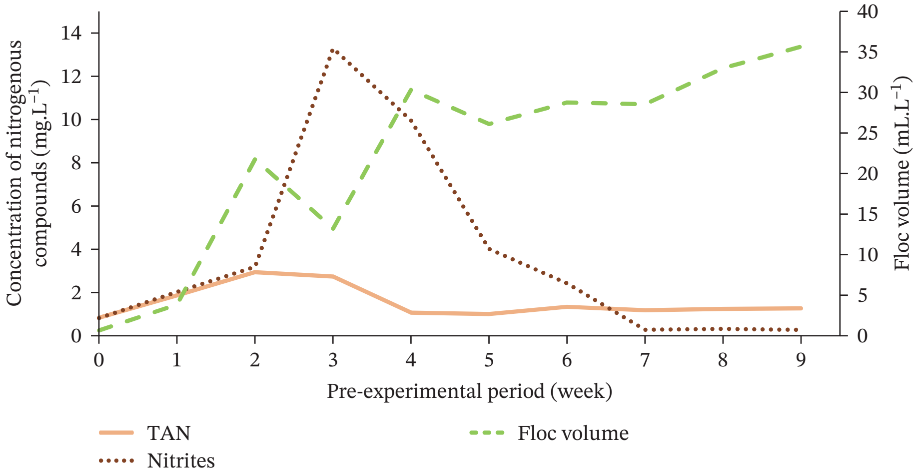


FIGURE 2 | Evolution of total ammonia nitrogen (TAN), nitrite (NO_2^-) concentrations, and floc aggregates in the rearing environment during the 9 weeks preexperimental period (mean across all treatments).

weighed to determine the total biomass. In addition, 30 individuals from each experimental unit were randomly selected and individually measured and weighed to assess their growth performance and variability. Weight measurements were taken using a PBS Kern balance ($e = 0.01$ g), and total length was recorded with a stop rule (±1 mm). These measurements were conducted every 14 days to monitor growth progression in the selected individuals. The calculation methods for these parameters are provided below.

$$SR\ (\%) = \frac{Nf}{Ni} \times 100$$

N_f: Final number of fish

N_i: Initial number of fish

$$SGR\ (\%/day) = \frac{\ln(WMf) - \ln(WMi)}{t} \times 100$$

WM_f: Final mean weight (g)

WM_i: Initial mean weight (g)

t: Duration of the experiment (days)

$$FCR = \frac{TRD}{Bf - Bi}$$

B_f: Final biomass (g)

B_i: Initial biomass (g)

TRD: Total feed ration distributed (g)

$$Yield\ (Yld)\ (kg/m^3/year) = \frac{Bf - Bi}{V \times t} \times 365$$

B_f: Final biomass (kg)

B_i: Initial biomass (kg)

V: Tank volume (m^3)

t: Duration of the experiment (days)

$$Fulton's\ condition\ factor\ (K) = \frac{W}{L^3} \times 100$$

W: Individual weight (g)

L: Length of the individual (cm)

2.6 | Statistical Analyses

The growth performance parameters of the fish, as well as the physical and chemical characteristics of the rearing environment, were analyzed using analysis of variance (ANOVA) in R version 4.4.1, provided that the assumptions of normality of residuals and homogeneity of variances were met [45]. When the normality assumption was not satisfied, a Kruskal–Wallis test was used. If significant differences were detected, pairwise comparisons between treatments were conducted using post hoc tests, specifically the Tukey test for ANOVA and the Dunn-Bonferroni test for the Kruskal–Wallis test. A significance level of $\alpha = 0.05$ was used to determine pairwise differences for the Tukey test. In contrast, the Bonferroni correction method employed a significance level of $\alpha \approx 0.0083$ for the Dunn test [46]. Spearman's correlation was used to evaluate the relationship between floc volume and growth parameters in R version 4.4.1.

3 | Results

3.1 | Water Quality and Biofloc Characteristics

The temperature stayed within the 26–28°C range. The flow-through control exhibited a significantly lower temperature (26.4°C) than the biofloc treatments: BFT100 (26.7°C), BFT75 (26.8°C), and BFT50 (26.6°C). The temperature variations were predominantly observed between 26.1°C and 27.2°C, encompassing 90% of the data (Table 2).

The dissolved oxygen concentration was significantly higher in the BFT50 treatment (7.3 mg.L^{-1}) than in the other treatments (T0, BFT100, and BFT75), which averaged 7 mg.L^{-1}.

Alkalinity was significantly higher in the control (224 mg.L^{-1} of $CaCO_3$) compared to the biofloc treatments, which averaged 52 mg.L^{-1}. No influence of feed input was noted. Despite adhering to the target range (>50 mg.L^{-1}), abrupt variations of ±40 mg.L^{-1} were observed within 24 h, with a minimum value of 17 mg.L^{-1} recorded in the BFT100 treatment.

The pH was also higher in the flow-through control (8.16) compared to the biofloc treatments, which averaged 7.45. Extreme pH values in the biofloc treatments ranged from 6.44 to 7.83, with occasional deviations from the recommended range of 6.8 to 8.

Conductivity progressively increased from 1600 µS.cm^{-1} to 2300 µS.cm^{-1} in the biofloc treatments, remaining below the upper limit of 3000 µS.cm^{-1}. It was significantly lower in the flow-through control (811 µS.cm^{-1}). Feed input had no notable effect on this parameter.

TABLE 2 | Temperature, dissolved oxygen, pH, alkalinity, and conductivity (mean ± standard deviation, $n = 3$).

	Variables				
Treatments	**Temperature (°C)**	**Dissolved oxygen (mg/L)**	**pH**	**Alkalinity (mg.L^{-1})**	**Conductivity (µs.cm^{-1})**
T0	26.4 ± 0.5^a	7.0 ± 0.5^a	8.16 ± 0.09^a	224 ± 12^a	811 ± 21^a
BFT100	26.7 ± 0.4^b	7.1 ± 0.4^a	7.48 ± 0.35^b	55 ± 28^b	1921 ± 269^b
BFT75	26.8 ± 0.3^c	7.0 ± 0.3^a	7.42 ± 0.31^{bc}	54 ± 24^b	1981 ± 256^b
BFT50	26.6 ± 0.4^d	7.3 ± 0.3^b	7.44 ± 0.22^c	47 ± 18^b	1987 ± 251^b
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: According to Dunn's test, means accompanied by different upper script letters are significantly different in each column ($p < 0.0083$). The absence of a letter indicates a nonsignificant difference.

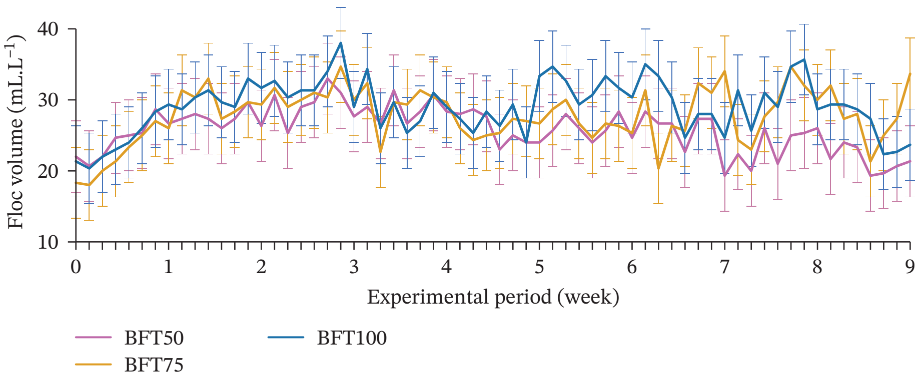


FIGURE 3 | Evolution of floc volume over time in the three experimental treatments (mean ± standard deviation, $n = 3$).

TABLE 3 | Total ammonia nitrogen (TAN), nitrites, nitrates, total suspended solids, and floc volume (mean ± standard deviation, $n = 3$).

	Variables				
Treatments	**TAN (mg.L^{-1})**	**NO_2^- (mg.L^{-1})**	**NO_3^- (mg.L^{-1})**	**TSS (mg.L^{-1})**	**Vf (mL.L^{-1})**
T0	0.65 ± 0.19^{a}	0.24 ± 0.38^{a}	35 ± 9^{a}	16 ± 11^{a}	—
BFT100	2.21 ± 0.78^{bc}	3.05 ± 3.43^{b}	437 ± 133^{b}	473 ± 120^{b}	29 ± 6^{a}
BFT75	2.25 ± 0.63^{b}	3.42 ± 2.01^{c}	459 ± 152^{b}	443 ± 66^{bc}	28 ± 5^{b}
BFT50	1.89 ± 0.43^{c}	2.34 ± 1.18^{b}	469 ± 155^{b}	409 ± 67^{c}	26 ± 4^{c}
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: In each column, means followed by different upper script letters are significantly different from each other according to Dunn's test ($p < 0.0083$). The absence of a letter indicates no significant difference.
Abbreviations: NO_2^-, nitrites; NO_3^-, nitrates; TAN, total ammonia nitrogen; TSS, total suspended solids; Vf, floc volume.

The FIL significantly affects the floc volume, which increases with higher feed input (Figure 3).

The floc volume varied during the experimental period, typically ranging from 20 to 34 mL.L^{-1} (90% of the data), with extreme values ranging from 10 mL.L^{-1} to 42 mL.L^{-1} in specific experimental units. The level of food input did not significantly affect the nitrate concentration in the biofloc treatments, which continued to increase linearly throughout the experiment (Table 3). The mean total suspended solid (TSS) concentration was significantly higher in the BFT100 treatment (473 mg.L^{-1}) compared to BFT50 (409 mg.L^{-1}). The BFT75 treatment (443 mg.L^{-1}) did not differ significantly from the other biofloc treatments. TSS concentration in the flow-through system was substantially lower (16 mg.L^{-1}) than in the biofloc treatments. TSS levels were relatively stable in the BFT75, BFT50, and control treatments, whereas the BFT100 treatment exhibited greater variability, with a mean of 288 mg.L^{-1} and a maximum of 688 mg.L^{-1}.

In the biofloc treatments, TAN concentrations remained relatively stable around the mean, although peaks of up to 4.68 mg.L^{-1} were observed in the BFT100 treatment without feeding restriction.

On the other hand, nitrite (NO_2^-) concentrations varied over time, with numerous peaks, more pronounced in the BFT100 and BFT75 treatments, where they reached 8.55 mg.L^{-1} and 8.34 mg.L^{-1}, respectively. Conversely, the flow-through control exhibited stable nitrogen compound concentrations throughout the experiment.

TAN, nitrite, and nitrate concentrations were significantly lower in the flow-through control (0.65 mg.L^{-1}, 0.24 mg.L^{-1}, and 35 mg.L^{-1}, respectively) than in the biofloc treatments. The TAN concentration in BFT100 did not differ significantly from that in other treatments, but it was significantly lower in BFT50 than in BFT75. Regarding nitrites, the average concentrations did not differ between BFT50 and BFT100, while BFT75 exhibited significantly higher concentrations.

3.2 | Growth Performance

Figure 4 illustrates the evolution of the cumulative SR over time across the three experimental treatments and the flow-through control.

The average SR across all treatments was 96.5% (Table 4). This rate ranges between 98.1% and 92.9% across treatments, with no significant differences observed. However, 68% of the mortality occurred in experimental units 6 (BFT100), 9 (BFT100), and 8 (BFT50), leading to lower SR (92.9%, 87.1%, and 92.3%, respectively). Additionally, a SR of 92.9% was recorded in the BFT100 treatment, with mortality primarily occurring during the fifth week of the experiment, seemingly due to changes in floc quality and physicochemical water parameters.

This deterioration in floc quality was observed across all experimental units in the BFT100 treatment, with several indicators suggesting potential impacts on fish welfare: the fish stopped feeding, surfaced in search of air, and mortalities

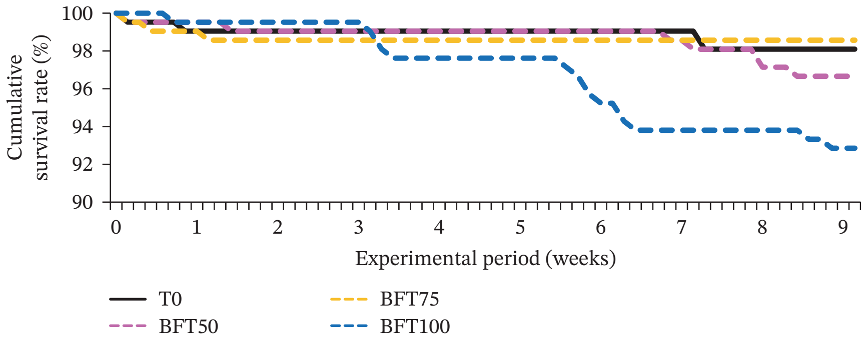


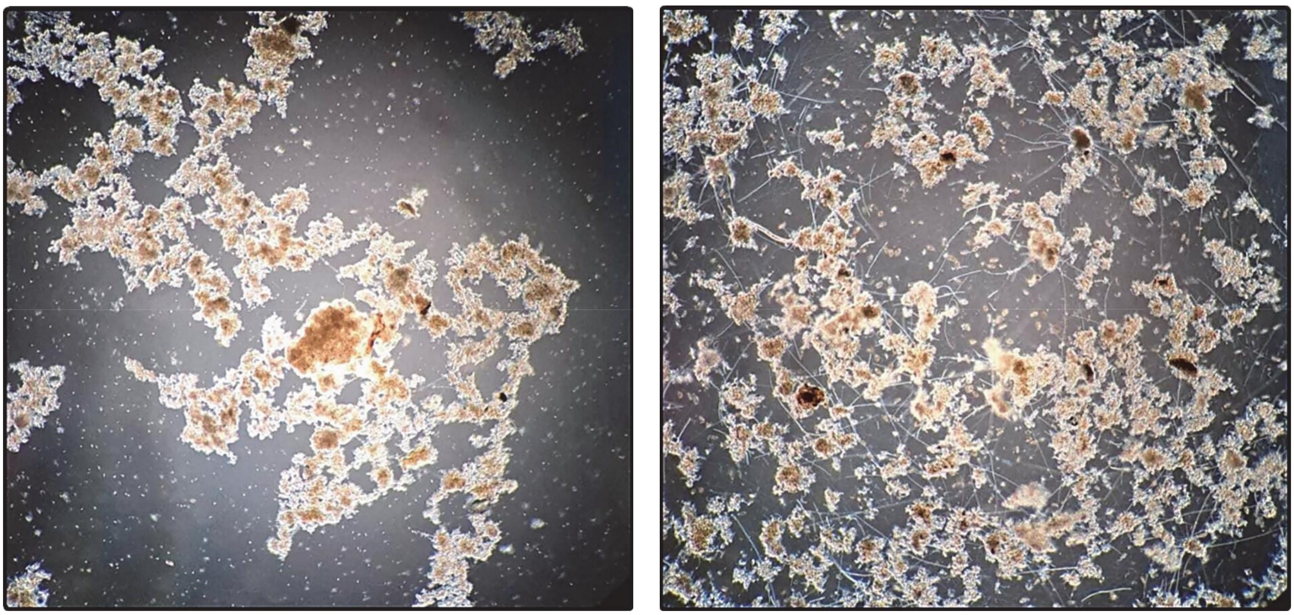
FIGURE 4 | The cumulative survival rate over time in the three experimental treatments and the control.

TABLE 4 | Survival rate, specific growth rate, feed conversion rate, yield, and Fulton's condition factor (mean ± standard deviation, $n = 3$).

	Variables					
Treatments	**SR (%)**	**SGR (%.j^{-1})**	**FCR (%)**	**Yld (kg.m^{-3}.an^{-1})**	**K**	**Feed quantity (g)**
T0	98.1 ± 2.2	2.72 ± 0.02^{a}	0.75 ± 0.02^{a}	151.0 ± 7.70^{a}	1.74 ± 0.03	7267.4 ± 33.8
BFT100	92.9 ± 5.8	1.70 ± 0.13^{b}	1.097± 0.07^{bc}	59.2 ± 13.20^{b}	1.76 ± 0.03	4126.6 ± 243.1
BFT75	98.6 ± 0.0	1.92 ± 0.02^{b}	0.89 ± 0.02^{ac}	79.5 ± 1.34^{b}	1.74 ± 0.03	4555.4 ± 19.0
BFT50	96.5 ± 3.9	1.21 ± 0.12^{c}	1.13 ± 0.17^{b}	37.3 ± 6.35^{c}	1.70 ± 0.03	2670.9 ± 28.2
p-Value	ns	<0.0001	0.0029	<0.0001	ns	—

Note: In each column, means followed by different upper script letters are significantly different according to the Tukey test ($p < 0.05$). The absence of a letter indicates no significant difference. *K*, Fulton's condition factor.
Abbreviations: FCR, feed conversion rate; SGR, specific growth rate; SR, survival rate; Yld, yield.

occurred sequentially. The flocs also failed to settle; their color appeared more "milky" than in other tanks. A microscopic examination of the floc aggregates (Figure 5) enables us to understand the phenomenon, which is likely due to the development of filamentous organisms. Figure 5 illustrates the differences in bioflocs observed in two tanks: Tank 3 (BFT 75) (Figure 5a) contains functional biofloc with no sedimentation issues or adverse effects on fish appetite, whereas Tank 6 (BFT100) (Figure 5b) shows nonsettling floc aggregates and fish that are not feeding. These aggregates are connected by abundant filaments identified as unicellular fungi.



(a) (b)

FIGURE 5 | Floc aggregates observed using an Olympus BX40 optical microscope (×400): experimental BFT 75: tank 3 (a) and BFT 100: tank 6 (b).

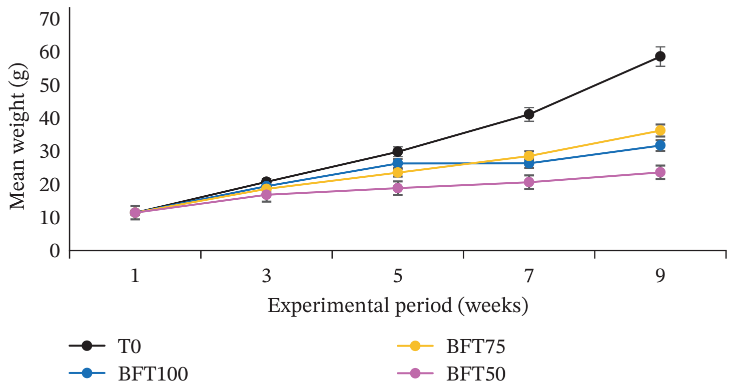


FIGURE 6 | Evolution of the mean body weight over time across the four experimental treatments.

The growth curves presented in Figure 6 effectively reflect the results of this study regarding the SGR, particularly highlighting the deterioration of the biofloc aquaculture environment in the BFT100 treatment during the fifth week of the experiment, as well as the fluctuations observed throughout the experimental period.

The control group's SGR over 9 weeks was 2.72%.day^{-1}. This value is significantly higher than the SGR values for the BFT100 (1.70%.day^{-1}), BFT75 (1.92%.day^{-1}), and BFT50 (1.21%.day^{-1}) treatments. The SGR for the most restrictive feed treatment (BFT50) is significantly lower than the other two biofloc treatments (BFT100 and BFT75), for which SGR values are not significantly different.

The FCR for the flow-through control group (0.75) was significantly lower than for the BFT100 and BFT50 treatments but not significantly different from the FCR of the BFT75 treatment (0.89). The latter is not different from the FCR of BFT100 (1.09). The least-fed treatment, BFT50, showed a significantly higher FCR than the BFT75 treatment (1.13 vs. 0.89) but did not differ from the control group with no feeding restriction.

The flow-through control group's yield was significantly higher (151 kg.m^{-3}.year^{-1}) than that of the biofloc treatments. The reduction in feed supply to 50% in the biofloc system had an impact on the yield, as the BFT50 treatment resulted in a yield of 37.3 kg.m^{-3}.year^{-1}, which is significantly lower than that of the BFT100 and BFT75 treatments (59.2 kg.m^{-3}.year^{-1} and 79.5 kg.m^{-3}.year $^{-1}$, respectively).

Fulton's condition factor (K) indicates the fish condition, reflecting whether the fish are sufficiently nourished. In our study, the condition factor did not differ significantly across the four treatments, ranging from 1.70 to 1.76.

The control (T0) received the highest feed input (7267.4 ± 33.8 g), followed by BFT75 (4555.4 ± 19.0 g) and BFT100 (4126.6 ± 243.1 g). The lowest feed allocation was observed in the BFT50 treatment (2670.9 ± 28.2 g).

3.3 | Correlation Between Floc Volume and Zootechnical Performance

Among the variables considered, only the SR showed a significant correlation with floc volume ($\rho = -0.6439$; $p = 0.0238$). No significant correlation was observed between floc volume and the SGR (SGR: $\rho = -0.2177$; $p = 0.4967$), FCR (FCR: $\rho = -0.0177$; $p = 0.9565$), yield (Yld: $\rho = -0.1340$; $p = 0.6779$), or Fulton's condition factor (K: $\rho = 0.0288$; $p = 0.9293$).

4 | Discussion

This study aimed to optimize Nile tilapia growth performance in biofloc systems by balancing formulated feed input. Physicochemical parameters, such as temperature and dissolved oxygen, remained within standards across all treatments [41]. However, biofloc groups exhibited distinct conductivity, pH, and alkalinity values compared to the flow-through control. These differences were likely due to higher water renewal in the control group (30% h^{-1}), which maintained TAN, nitrite (NO_2^-), and nitrate (NO_3^-) concentrations below critical thresholds [47].

To maintain stable biofloc conditions, daily water renewal (2.5%–4.1%) and sodium bicarbonate additions were applied to buffer pH and alkalinity fluctuations caused by CO_2 production and nitrification ([48]). Nitrite levels fluctuated [40], reaching up to 8.55 mg.L^{-1} in BFT100, especially in treatments with high feed input. Nitrate accumulation (NO_3^-) peaked at 680 mg.L^{-1}, consistent with other findings [49].

Conductivity in the biofloc system (BFT) remains below the recommended thresholds [50] and is not significantly affected by feeding rate, in accordance with [51], likely due to water renewal promoting ionic leaching.

In biofloc systems, a progressive accumulation of SS is frequently reported during feed restriction trials, with concentrations reaching up to 1000 mg.L^{-1} without significant effects on fish survival or growth [52, 53]. This accumulation tends to be more pronounced in treatments subjected to lower levels of feed restriction, which is consistent with our observations [48, 54, 55].

However, solid control is essential in biofloc systems to limit physiological effects, particularly at the gill level [56]. In the present study, mean TSS concentrations were maintained below 500 mg.L^{-1} in the BFT75 and BFT50 treatments. In contrast, in BFT100, despite a higher water renewal rate (4.08%.day^{-1}), this threshold occasionally exceeded the critical levels reported in the literature [44]. Floc volume increased with feed input but remained below the critical threshold of 50 mL.l^{-1} due to daily settling operations.

Despite these conditions, survival exceeded 92.8% across treatments. BFT100 exhibited the lowest SR (92.9%), with a peak mortality in the fifth week, which may be associated with deterioration in floc quality and variations in water quality parameters. Filamentous fungi and floc disintegration were observed in the BFT100 treatment. Although such phenomena may be favored under unfavorable conditions, such as C/N imbalance [57], high organic loading, or insufficient mixing, the available data do not allow a direct causal relationship to be established between these observations and the recorded mortalities but rather suggest a temporal association.

Similarly, although dissolved oxygen concentrations remained above 6 $mg.L^{-1}$, elevated nitrite concentrations were recorded during this period. Based on previously described physiological mechanisms, nitrite exposure has been shown to impair oxygen transport and induce stress responses in aquatic organisms [58]; however, its involvement in the stress observed in the present study remains hypothetical and cannot be confirmed without additional physiological measurements. The corrective measures implemented (reduction of feed input, adjustment of solids management, increased water renewal, and cessation of molasses supplementation) led to progressive stabilization of the system and a subsequent reduction in mortality.

FCR ranged from 0.89 (BFT75) to 1.13 (BFT50), with BFT75 showing the best performance. The low FCR in BFT50 indicates underfeeding, as the applied ration was below the optimal level for Nile tilapia [59]. In BFT100, the FCR was 1.09, higher than the control (0.75), which may be due to late mortalities and inaccuracies in estimating feed intake under biofloc conditions.

Interestingly, the FCRs of BFT75 and BFT100 did not differ significantly, showing improved FCR with feed reduction. This may also be due to higher feed input in the BFT75 than in the BFT100 during system disturbances, combined with limited evidence of aggregate consumption [60]. The similar FCR between BFT75 and the control suggests that biofloc farming can enable a 25% reduction in feed without compromising feed efficiency [61, 62]. The control system's low FCR (0.75) is attributed to optimized feeding, moderate stocking density, high-protein diet, and precise rationing [60]. Overall, the FCR values observed (BFT100, BFT75, and BFT50) align with industry norms for biofloc systems (0.9–1.25) [54, 63, 64].

However, as a limitation for comparison, the flow-through system group (control) does not provide microbial protein, and the hydrodynamic conditions, as well as the associated energy expenditure of the fish, differ from those in the biofloc system. Moreover, feed rationing is generally more precise in clear-water systems than in biofloc systems.

SGR was lower in biofloc treatments (1.70%, 1.92%, and 1.21%.day^{-1}) compared to the control (2.72%.day^{-1}). Particularly in BFT50, the lower SGR reflects the inability of fish to compensate for a 50% feed reduction through floc consumption, consistent with prior findings that beyond a 20%–30% reduction, flocs alone are insufficient [21, 65, 66]. Future studies will explore more moderate reductions (e.g., 90% and 80% rations) and use nitrogen isotope tracers to confirm aggregate ingestion [67].

Contrary to expectations, the unrestricted feeding in BFT100 yielded 2.5 times less biomass than the control system, due to impaired environmental quality and partial feed intake [56]. Nonetheless, the absolute yields in BFT100, BFT75, and BFT50 remained above baseline standards [64], possibly due to the lower stocking density and higher dietary protein content.

No significant effect of treatment on the condition factor (K) was observed, although the values were slightly lower than those reported in similar studies (1.80–1.82) [68]. This likely results from reductions in ration during unstable physicochemical periods (3% to 2% biomass). However, variations in fish age, sex, and diet across studies limit direct comparisons.

Spearman correlation analysis showed that increased floc volume was associated with reduced fish survival. At the same time, growth performance, feed efficiency, yield, and condition factor were not significantly affected under the experimental conditions. High floc volumes can disrupt the environmental balance by increasing SS and reducing dissolved oxygen availability, potentially affecting growth and survival [69].

To improve robustness under intensive conditions, future experiments should increase stocking densities, reduce dietary protein levels, and integrate automated sedimentation systems to stabilize floc volume. The observed floc instability is consistent with the accumulation of SS, as reported in other studies [70]. Moreover, the unmeasured C/N ratio in this experiment, inferred only from feed and molasses inputs, may have contributed to microbial imbalance and physicochemical instability. Since the C/N ratio governs microbial dynamics and nitrogen cycling, it is a critical control point requiring further monitoring and refinement in biofloc systems.

Furthermore, to validate these findings and assess long-term sustainability, future research should include longer trial durations, measured C/N ratio, and economic analysis of feed cost savings versus operational adjustments in biofloc management. The application of molecular tools to track microbial shifts under varying C/N ratios would also enhance understanding of flocs.

5 | Conclusions

This study demonstrates that biofloc systems can reduce the need for an external nutrient source in Nile tilapia aquaculture without negatively affecting feed efficiency. Moderate ration levels as low as 25% below the complete ration do not harm feed conversion. However, the more significant decrease is observed in the 50% complete ration, which sharply decreases performance, highlighting the nutritional constraints of biofloc per se. On the other hand, high feed inputs in biofloc systems showing signs of instability may be associated with microbial imbalances, accumulation of nitrogenous compounds, and degraded rearing conditions, potentially affecting fish performance and survival; however, a direct causal link with mortality cannot be established based on the present data. The effectiveness of BFT depends on maintaining system stability, controlling the carbon-to-nitrogen ratio, and aligning floc production with fish feeding capacity. Future research should focus on refining feeding strategies and biofloc management using large-scale trials and advanced analytical tools.

6 | Key Contribution

Effects of three feeding levels (100%, 75%, and 50% of the maximal ration) on the growth performance of Nile tilapia

(*Oreochromis niloticus*) and the stability of a biofloc system over a 9-week experimental period.

Author Contributions

Lydie Bambi Langa and Amaël Bossan contributed to the conceptualization, methodology, data analysis, and drafting of the manuscript. Vincent Gennotte and Carole Rougeot contributed to the drafting and critical revision of the manuscript.

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Disclosure

All authors have read and accepted the submitted manuscript version.

Ethics Statement

This study was approved by the Animal Ethics Committee of the University of Liège (protocol code 2759 and date of approval: 31 January 2025).

Consent

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All datasets produced and/or examined during this study can be obtained from the corresponding author upon reasonable request.

References

1. L. Bambi Langa, S. Wamuini Lunkayilakio, P. Mafwila Kinkela, V. Gennotte, and C. Rougeot, "Fish Farming Activities in Mbanza-Ngungu: Farmer Socio-Professional Profiles, Production Practices, and Improvement Opportunities for Sustainable Aquaculture," *Sustainability* 16, no. 17 (2024): 7481.

2. M. S. Bank, M. Metian, and P. W. Swarzenski, "Defining Seafood Safety in the Anthropocene," *Environmental Science & Technology* 54, no. 14 (2020): 8506–8508.

3. FAO, "The State of World Fisheries and Aquaculture," 2020 (2020), FAO.

4. G. Kennedy, M. B. Ahern, L. L. Iannotti, S. Vie, L. Sherburne, and S. H. Thilsted, "Considering the Food Environment Can Help to Promote the Consumption of Aquatic Foods for Healthy Diets," *Frontiers in Sustainable Food Systems* 7 (2023).

5. FAO, "The State of World Fisheries and Aquaculture," 2024 (2024), FAO.

6. C. E. Boyd, A. A. McNevin, and R. P. Davis, "The Contribution of Fisheries and Aquaculture to the Global Protein Supply," *Food Security* 14, no. 3 (2022): 805–827.

7. T. Garlock, F. Asche, J. Anderson, et al., "Aquaculture: The Missing Contributor in the Food Security Agenda," *Global Food Security* 32 (2022): 100620.

8. G. C. Mair, M. Halwart, Y. Derun, and B. A. Costa-Pierce, "A Decadal Outlook for Global Aquaculture," *Journal of the World Aquaculture Society* 54, no. 2 (2023): 196–205.

9. OCDE/FAO, "OECD–FAO Agricultural Outlook *2023–2032*," OECD (2023).

10. B. Belton, D. C. Little, W. Zhang, P. Edwards, M. Skladany, and S. H. Thilsted, "Farming Fish in the Sea Will Not Nourish the World," *Nature Communications* 11, no. 1 (2020): 5804.

11. Q. Jiang, N. Bhattarai, M. Pahlow, and Z. Xu, "Environmental Sustainability and Footprints of Global Aquaculture," *Resources, Conservation and Recycling* 180 (2022): 106183.

12. K. R. Salin and N. T. Vinh, "Biofloc Technology in Aquaculture," in *Frontiers in Aquaculture Biotechnology*, (Elsevier, 2023): 69–88.

13. A. K. Verma, M. H. Chandrakant, V. C. John, R. M. Peter, and I. E. John, "Aquaponics as an Integrated Agri-Aquaculture System (IAAS): Emerging Trends and Future Prospects," *Technological Forecasting and Social Change* 194 (2023): 122709.

14. L. A. Ibrahim, H. Shaghaleh, G. M. El-Kassar, M. Abu-Hashim, E. A. Elsadek, and Y. Alhaj Hamoud, "Aquaponics: A Sustainable Path to Food Sovereignty and Enhanced Water Use Efficiency," *Water* 15, no. 24 (2023): 4310.

15. G. Liu, M. Verdegem, Z. Ye, et al., "Advancing Aquaculture Sustainability: A Comprehensive Review of Biofloc Technology Trends, Innovative Research Approaches, and Future Prospects," *Reviews in Aquaculture* 17, no. 1 (2025).

16. M. G. C. Emerenciano, M. H. Khanjani, M. Sharifinia, and A. Miranda-Baeza, "Could Biofloc Technology (BFT) Pave the Way Toward a More Sustainable Aquaculture in Line With the Circular Economy?" *Aquaculture Research* 2025, no. 1 (2025).

17. S. M. Pinho, J. P. de Lima, L. H. David, et al., "FLOCponics: The Integration of Biofloc Technology With Plant Production," *Reviews in Aquaculture* 14, no. 2 (2022): 647–675.

18. M. T. Jamal, M. Broom, B. A. Al-Mur, et al., "Biofloc Technology: Emerging Microbial Biotechnology for the Improvement of Aquaculture Productivity," *Polish Journal of Microbiology* 69, no. 4 (2020): 401–409.

19. X. Lyu, Y. Guo, W. Liu, et al., "Evaluation of Long-Term Operational and Production Performance of Biofloc Technology (BFT) System Within Various Particle Size," *Aquaculture* 578 (2024): 740090.

20. M. H. Hussien, M. M. Zayd, A. E. Elshafey, et al., "Comparing the Effect of Different Carbon Sources on Growth, Blood Parameters, Immunity, and Water Quality in Nile Tilapia (*Oreochromis niloticus*) Cultured in a Biofloc System," *Aquaculture Reports* 42 (2025): 102755.

21. M. Mugwanya, M. A. O. Dawood, F. Kimera, and H. Sewilam, "Biofloc Systems for Sustainable Production of Economically Important Aquatic Species: A Review," *Sustainability* 13, no. 13 (2021): 7255.

22. M. A. Zafar and M. M. Rana, "Biofloc Technology: An Eco-Friendly "Green Approach" to Boost up Aquaculture Production," *Aquaculture International* 30, no. 1 (2022): 51–72.

23. J. Wang, L. Yang, Y. Zhang, H. Zhang, and J. Liu, "Partial Nitrification Characteristics of an Immobilized Carrier in Municipal Wastewater Under Low-Temperature Shock: The Role of the Nitrifying Bacterial Community Structure," *Water* 15, no. 9 (2023): 1714.

24. J. A. Hargreaves, "Biofloc Production Systems for Aquaculture Southern Regional Aquaculture Center," (2013).

25. U. Nisar, D. Peng, Y. Mu, and Y. Sun, "A Solution for Sustainable Utilization of Aquaculture Waste: A Comprehensive Review of Biofloc Technology and Aquamimicry," *Frontiers in Nutrition* 8 (2022).

26. B. T. Iber, D. Torsabo, A. S. Kamaruzzan, et al., "Exploring Carbon Sources in Biofloc Technology for Enhanced Aquaculture and Environmental Sustainability," *Discover Food* 5, no. 1 (2025): 207.

27. Y.-B. Yu, J.-H. Lee, J.-H. Choi, et al., "The Application and Future of Biofloc Technology (BFT) in Aquaculture Industry: A Review," *Journal of Environmental Management* 342 (2023): 118237.

28. C. Li, X. Zhang, Y. Chen, et al., "Optimized Utilization of Organic Carbon in Aquaculture Biofloc Systems: A Review," *Fishes* 8, no. 9 (2023): 465.

29. Y. Avnimelech, "Carbon/Nitrogen Ratio as a Control Element in Aquaculture Systems," *Aquaculture* 176, no. 3-4 (1999): 227–235.

30. K. Tayyab, S. Aslam, M. A. Ghauri, A. S. Abbas, and A. Hussain, "Consequential Study on Different Levels of C/N Ratios Used in Biofloc-Based Aquaculture System," *Brazilian Journal of Biology* 83 (2023).

31. Y.-B. Yu, J.-H. Choi, J.-H. Lee, A.-H. Jo, K. M. Lee, and J.-H. Kim, "Biofloc Technology in Fish Aquaculture: A Review," *Antioxidants* 12, no. 2 (2023): 398.

32. I. G. Lopes, L. B. Braos, M. C. P. Cruz, and R. M. Vidotti, "Valorization of Animal Waste From Aquaculture Through Composting: Nutrient Recovery and Nitrogen Mineralization," *Aquaculture* 531 (2021): 735859.

33. J. M. Ebeling, M. B. Timmons, and J. J. Bisogni, "Engineering Analysis of the Stoichiometry of Photoautotrophic, Autotrophic, and Heterotrophic Removal of Ammonia–Nitrogen in Aquaculture Systems," *Aquaculture* 257, no. 1–4 (2006): 346–358.

34. F. FAO and A. D, "The State of World Fisheries and Aquaculture," FAO (2022).

35. G. Ashouri, S. H. Hoseinifar, E. El-Haroun, R. Imperatore, and M. Paolucci, "Tilapia Fish for Future Sustainable Aquaculture," *In Novel Approaches Toward Sustainable Tilapia Aquaculture* (2023): 1–47.

36. A. M. El-Sayed and K. Fitzsimmons, "From Africa to the World—the Journey of Nile Tilapia," *Reviews in Aquaculture* 15, no. S1 (2023): 6–21.

37. J. N. Leonard and P. V. Skov, "Capacity for Thermal Adaptation in Nile Tilapia (*Oreochromis niloticus*): Effects on Oxygen Uptake and Ventilation," *Journal of Thermal Biology* 105 (2022): 103206.

38. O. Omweno, J. Getabu, A. Omondi, R. and, and P. S. Orina, "Water Quality Effects on Growth and Survival of *Oreochromis jipe* and, *Oreochromis niloticus*, Species in Aquaculture," in *Water Quality - New Perspectives. IntechOpen*, (2024).

39. W. Emam, H. Lambert, and C. Brown, "The Welfare of Farmed Nile Tilapia: A Review," *Frontiers in Veterinary Science* 12 (2025).

40. G. Abakari, G. Luo, and E. O. Kombat, "Dynamics of Nitrogenous Compounds and Their Control in Biofloc Technology (BFT) Systems: A Review," *Aquaculture and Fisheries* 6, no. 5 (2021): 441–447.

41. M. H. Khanjani, A. Mohammadi, and M. G. C. Emerenciano, "Water Quality in Biofloc Technology (BFT): An Applied Review for an Evolving Aquaculture," *Aquaculture International* 32, no. 7 (2024): 9321–9374.

42. S. McCusker, M. B. Warberg, S. J. Davies, et al., "Biofloc Technology as Part of a Sustainable Aquaculture System: A Review on the Status and Innovations for Its Expansion," *Aquaculture, Fish and Fisheries* 3, no. 4 (2023): 331–352.

43. F. P. Serra, C. A. P. Gaona, P. S. Furtado, L. H. Poersch, and W. Wasielesky, "Use of Different Carbon Sources for the Biofloc System Adopted During the Nursery and Grow-Out Culture of *Litopenaeus vannamei*," *Aquaculture International* 23, no. 6 (2015): 1325–1339.

44. M. Emerenciano, L. R. Martínez-Córdova, M. Martínez-Porchas, and A. Miranda-Baeza, "Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture," in *Water Quality*, (2017): 91–109.

45. R. R. Sokal and F. J. Rohlf, "Biometry: the Principles and Practice of Statistics in Biological Research," 2013, https://www.researchgate.net/publication/44554870.

46. R. C. Campbell, R. R. Sokal, and F. J. Rohlf, "Biometry: The Principles and Practice of Statistics in Biological Research," *Journal of the Royal Statistical Society. Series A (General)* 133, no. 1 (1970): 102.

47. M. E. Abd El-Hack, M. T. El-Saadony, M. M. Nader, et al., "Effect of Environmental Factors on Growth Performance of Nile Tilapia (*Oreochromis niloticus*)," *International Journal of Biometeorology* 66, no. 11 (2022): 2183–2194.

48. M. A. Silva, É.R. Alvarenga, F. F. B. D. Costa, et al., "Feeding Management Strategies to Optimize the use of Suspended Feed for Nile Tilapia (*Oreochromis niloticus*) Cultivated in Bioflocs," *Aquaculture Research* 51, no. 2 (2020): 605–615.

49. G. Luo, J. Xu, and H. Meng, "Nitrate Accumulation in Biofloc Aquaculture Systems," *Aquaculture* 520 (2020): 734675.

50. S. M. Pinho, L. H. C. David, S. Goddek, M. G. C. Emerenciano, and M. C. Portella, "Integrated Production of Nile Tilapia Juveniles and Lettuce Using Biofloc Technology," *Aquaculture International* 29, no. 1 (2021): 37–56.

51. V. T. Rebouças, N. N. Caldini, D. D. H. Cavalcante, F. J. R. D. Silva, and M. V. D. C.E. Sá, "Interaction Between Feeding Rate and Area for Periphyton in Culture of Nile Tilapia Juveniles," *Acta Scientiarum. Animal Sciences* 34, no. 2 (2012).

52. M. E. Azim and D. C. Little, "The Biofloc Technology (BFT) in Indoor Tanks: Water Quality, Biofloc Composition, and Growth and Welfare of Nile Tilapia (*Oreochromis niloticus*)," *Aquaculture* 283, no. 1–4 (2008): 29–35.

53. L. Long, J. Yang, Y. Li, C. Guan, and F. Wu, "Effect of Biofloc Technology on Growth, Digestive Enzyme Activity, Hematology, and Immune Response of Genetically Improved Farmed Tilapia (*Oreochromis niloticus*)," *Aquaculture* 448 (2015): 135–141.

54. D. D. H. Cavalcante, F. R. D. S. Lima, V. T. Rebouças, and M. V. Carmo-e-Sá, "Nile Tilapia Culture Under Feeding Restriction in Bioflocs and Bioflocs Plus Periphyton Tanks," *Acta Scientiarum Animal Sciences* 39, no. 3 (2017): 223–228.

55. J. A. Pérez-Fuentes, C. I. Pérez-Rostro, M. P. Hernández-Vergara, and M.D. C. Monroy-Dosta, "Variation of the Bacterial Composition of Biofloc and the Intestine of Nile Tilapia *Oreochromis niloticus*, Cultivated Using Biofloc Technology, Supplied Different Feed Rations," *Aquaculture Research* 49, no. 11 (2018): 3658–3668.

56. L. K. Oliveira, L. Pilz, P. S. Furtado, E. L. C. Ballester, and Á. J. D. A. Bicudo, "Growth, Nutritional Efficiency, and Profitability of Juvenile GIFT Strain of Nile Tilapia (*Oreochromis niloticus*) Reared in Biofloc System on Graded Feeding Rates," *Aquaculture* 541 (2021): 736830.

57. M. H. Azhar, S. Suciyono, D. S. Budi, M. F. Ulkhaq, M. Anugrahwati, and J. Ekasari, "Biofloc-Based Co-Culture Systems of Nile Tilapia (*Oreochromis niloticus*) and Redclaw Crayfish (*Cherax quadricarinatus*) With Different Carbon–Nitrogen Ratios," *Aquaculture International* 28, no. 3 (2020): 1293–1304.

58. W. J. Wasielesky, L. H. Poersch, T. G. Martins, and K. C. Miranda-Filho, "Chronic Effects of Nitrogenous Compounds on Survival and Growth of Juvenile Pink Shrimp," *Brazilian Journal of Biology* 77, no. 3 (2017): 558–565.

59. D. Deswati, E. Yani, S. Safni, O. Norita Tetra, and H. Pardi, "Development Methods in Aquaponics Systems Using Biofloc to Improve Water Quality (Ammonia, Nitrite, Nitrate) and Growth of Tilapia and Samhong Mustard," *International Journal of Environmental Analytical Chemistry* 102, no. 19 (2022): 7824–7834.

60. S. B. Mengistu, H. A. Mulder, J. A. H. Benzie, and H. Komen, "A Systematic Literature Review of the Major Factors Causing Yield Gap by Affecting Growth, Feed Conversion Ratio and Survival in Nile Tilapia (*Oreochromis niloticus*)," *Reviews in Aquaculture* 12, no. 2 (2020): 524–541.

61. A. Kusmiatun, D. A. S. Utami, T. Firnaeni, et al., "Effects of Feeding Rate Reduction on the Growth Performance and Feed Utilization of Pacific White Shrimp Reared Using Biofloc System," *Jurnal Riset Akuakultur* 19, no. 4 (2025): 331–343.

62. B. C. D. Silva, K. V. Rosa, H. Massago, R. D. L. Serafini, and F. D. N. Vieira, "Nile Tilapia Nursery Feeding Management in a Biofloc System," *Ciência e Agrotecnologia* 46 (2022).

63. M. H. Rahman, M. M. Haque, M. N. Islam, and M. Arifuzzaman, "Growth and Production Performance of Tilapia (*Oreochromis niloticus*) in Intensive and Semi-Intensive Tank Based Aquaculture System Using Floating Feed," *International Journal of Fisheries and Aquatic Studies* 9, no. 4 (2021): 290–296.

64. S. Zimmermann, A. Kiessling, and J. Zhang, "The Future of Intensive Tilapia Production and the Circular Bioeconomy Without Effluents: Biofloc Technology, Recirculation Aquaculture Systems, Bio-RAS, Partitioned Aquaculture Systems and Integrated Multitrophic Aquaculture," *Reviews in Aquaculture* 15, no. S1 (2023): 22–31.

65. M. H. Khanjani, M. Sharifinia, and M. G. C. Emerenciano, "Biofloc Technology (BFT) in Aquaculture: What Goes Right, What Goes Wrong? A Scientific-Based Snapshot," *Aquaculture Nutrition* 2024, no. 1 (2024).

66. L. Stockhausen, G. B. Ferreira, B. E. Koch, B. C. Silva, and A. Jatobá, "Dietary Cost of Nile Tilapia Subject to Food Restriction in a Biofloc System," *Arquivo Brasileiro De Medicina Veterinária e Zootecnia* 76, no. 5 (2024).

67. D. Krummenauer, P. C. Abreu, L. Poersch, et al., "The Relationship Between Shrimp (*Litopenaeus vannamei*) Size and Biofloc Consumption Determined by the Stable Isotope Technique," *Aquaculture* 529 (2020): 735635.

68. A. ., "Growth Pattern, Condition Factor and First Capture-Maturity Size of Nile Tilapia (*Oreochromis Niloticus*) in a Fish Pond," *International Journal of Innovative Studies in Aquatic Biology and Fisheries* 9, no. 1 (2024): 14–18.

69. Md E. Hosain, S. M. N. Amin, M. Karim, et al., "Effect of Biofloc Volume on Growth, Survival, Economics and Proximate Composition of *Macrobrachium rosenbergii* Postlarvae Cultured in Brackish Water Biofloc System," *Blue Biotechnology* 2, no. 1 (2025): 4.

70. L. G. Manduca, M. A. D. Silva, É. R. D. Alvarenga, et al., "Effects of Different Stocking Densities on Nile Tilapia Performance and Profitability of a Biofloc System With a Minimum Water Exchange," *Aquaculture* 530 (2021): 735814.