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ANIMAL SCIENCE

Water quality, growth, and hematology in Nile tilapia cultured in biofloc systems using molasses as a carbon source

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Abstract: Biofloc technology represents a sustainable solution for aquaculture development, articularly in regions with limited water resources. We hypothesized that the addition of molasses to biofloc systems, at different carbon-to-nitrogen (C:N) ratios, could modulate hematological responses in Nile tilapia (*Oreochromis niloticus*), potentially occurring prior to detectable effects on growth performance. Therefore, this study evaluated the effects of molasses supplementation at C:N ratios of 10:1 (10M) and 20:1 (20M), compared to a control without carbon addition (0M), on water quality, growth, and hematological parameters in juvenile tilapia reared in 12,800 L tanks (stocking density: 50 fish/m^3) for 107 days in a semi-arid region of Brazil. Results showed that C:N ratios significantly altered the water's physical and chemical properties. However, final productivity (14.5 ± 1.0 kg/m^3) and survival rate (95.9 ± 1.4%) were not affected. Notably, molasses supplementation reduced albumin levels while increasing total plasma protein and globulin concentrations, indicating a positive modulation of the fish immune response. These findings suggest that carbon addition in biofloc systems can improve water quality and promote early immunophysiological responses in Nile tilapia under semi-arid conditions.

Key words: Aquaculture sustainability, Carbon-to-nitrogen ratio, Heterotrophic, Immune response, *Oreochromis niloticus*.

INTRODUCTION

The global human population is expected to approach 9.5 billion by 2050, which will significantly increase demand for high-quality protein. To meet this demand, aquaculture production must increase by an estimated 250 to 300 million metric tons (Tacon et al. 2021). The aquaculture industry is therefore crucial for future food security, requiring sustainable and environmentally responsible practices. However, achieving high yields of quality aquatic protein remains a key challenge for this rapidly growing sector (Khanjani et al. 2023).

This rising demand for food will be intensified by growing pressure on global water resources. By 2025, more than half of the world's population will live in regions where over 40% of renewable water resources are already used for human needs, further increasing pressure on availability. Many nations are already experiencing water scarcity, and others are nearing this threshold (Veth et al. 2008). The effects of climate change, reduced precipitation, and limited freshwater sources pose additional challenges, particularly in arid and semi-arid areas (Fedoroff et al. 2010), potentially restricting aquaculture expansion in these regions.

To address such limitations, new technologies that support aquaculture in water-constrained environments are essential. Biofloc technology offers a promising solution, particularly in brackish water environments, whre it can sustain livelihoods in arid regions (Barros et al. 2014). This system optimizes space usage, yields satisfactory production outcomes (Krummenauer et al. 2014), and conserves water efficiently, achieving higher productivity and lower environmental impact than traditional systems. Biofloc technology can also reduce production costs, making aquaculture more feasible in arid and semi-arid areas (Sandifer & Hopkins 1996).

Bioflocs are critical in maintaining water quality by assimilating ammonia nitrogen in culture water. With an adequade carbon source, they stimulate the growth of heterotrophic bacteria and biofloc formation, which stabilizes water conditions (Ahmad et al. 2016, Chamorro-Legarda et al. 2016, Pérez-Fuentes et al. 2016, Vilani et al. 2016). Using organic carbon to control ammonia promotes a complex microbial community comprising bacteria, algae, protozoa, zooplankton, and fungi (Crab et al. 2012, Chamorro-Legarda et al. 2016). However, carbon availability is critical for biofloc development, leading to research into alternative carbon sources, such as molasses, rice bran, and sugarcane residues (Midori et al. 2015).

The choice of carbon source in biofloc systems influences both the nutritional quality of bioflocs (Midori et al. 2015) and water quality (Silva et al. 2017). Tilapia, known for their resilience to environmental stressors, can utilize microbial flocs, making biofloc technology economically advantageous and suitable for sustainable tilapia farming (Ekasari et al. 2015). Hematological variables, widely recognized as indicators of fish well-being and health, are frequently used to assess the condition of tilapia reared in biofloc systems. These metrics can reveal health changes even before visible clinical symptoms appear, providing a reliable early assessment (Barbieri & Bondioli 2013, Schütt et al. 1996). Recent studies have reinforced the relevance of hematological analysis in aquaculture, highlighting its diagnostic potential for physiological and immunological monitoring in tilapia and other cultured species (Reshi et al. 2023, Fazio et al. 2024), as well as its applicability in detecting responses to environmental or nutritional factors before such changes are reflected in growth or survival.

Given the necessity of optimizing nutritional management strategies in aquaculture, this study aimed to evaluate the effects of molasses supplementation at different carbon-to-nitrogen (C:N) ratios on water quality, growth performance, and physiological responses of Nile tilapia (*Oreochromis niloticus*) during the grow-out phase in biofloc systems under semi-arid conditions.

MATERIALS AND METHODS

Ethics

The study followed the guidelines of the National Council for Animal Experimentation Control (CONCEA). The protocol received approval from the Ethics Committee on Studies and Research at UFRPE, Brazil (Protocol No. 23082.009150/2013 - CEUA/B19).

Biological material and experimental design

Juvenile Nile tilapia (*Oreochromis niloticus*) of the Chitralada strain, with an average weight of 73.2 ± 2.7 g, were obtained from a commercial fish farm. The experiment was conducted at the Laboratory for Experiments with Aquatic Organisms (UFRPE) in twelve fiberglass tanks with a total capacity of 1,000 L, each filled with 800 L of water. Tanks were covered with protective mesh and maintained under constant aeration and natural photoperiod. Water exchange was not performed during the trial, except for the replacement of evaporative losses using water from an on-site artesian well.

The study followed a completely randomized design with three treatments and four replicates per treatment, totaling 12 experimental units. The treatments consisted of two carbon-to-nitrogen (C:N) ratios using molasses as a carbon source—10:1 (10M) and 20:1 (20M)—and a control group (0M) without carbon addition. Each tank was stocked with 40 juvenile fish, corresponding to a stocking density of 50 fish/m³. Thus, each treatment included 160 fish. Water used in the 10M and 20M treatments originated from a biofloc-based system previously described by Silva et al. (2017). In the control group (0M), water was renewed weekly to maintain quality.

Carbon Source and Molasses Addition

During the first 10 days, molasses was added daily to treatments 10M and 20M, calculated based on the required C:N ratios, the nitrogen excreted (converted to ammonia) from feed, and the carbon content in molasses. The molasses formula (Equation 1) was adapted from Avnimelech (2009) as follows:

Molasses = [(QRation x% N Ration x% N Excretion) x (C:N)] (0.22 - 1).

Where: QRation = daily feed quantity;

%NRation = nitrogen introduced by feed (calculated as crude protein content ÷ 6.25);

%NExcretion = 50% of feed nitrogen excreted as ammonia.

The molasses used contained approximately 22 g of carbon per gram, as analyzed by the Laboratory of Fundamental Chemistry at UFRPE. After day 10, molasses was added weekly at rates of 10 and 20 g of carbon per 1 g of total ammoniacal nitrogen in the water to maintain the 10:1 and 20:1 ratios.

Feeding Protocol

Experimental fish were fed with a commercial diet, three times daily (8:00, 12:00, and 16:00 hrs). Initially, a feed with 40% crude protein (CP) was used and later reduced to 32% CP. The feeding rate ranged from 2.0% to 1.3% of biomass, adjusting from the beginning to the end of the culture period.

Water quality analysis

Water quality parameters, including temperature (°C), dissolved oxygen (mg L^{-1}), electrical conductivity (mS cm^{-1}), and pH, were monitored daily using a multiparameter probe (YSI ProPlus). Water transparency (cm) was measured with a Secchi disk, and salinity (g L^{-1}) was determined using an optical refractometer.

Weekly water samples were collected to analyze total ammonia nitrogen (TAN) (mg N - [NH_4 + NH_3] L^{-1}), nitrite (mg N-NO_2 L^{-1}), nitrate (mg N-NO_3 L^{-1}), inorganic phosphate (mg P-PO_4 L^{-1}), alkalinity (mg $CaCO_3$ L^{-1}), turbidity (NTU), settleable solids (SS) (mL L^{-1}), and total suspended solids (TSS) (g L^{-1}). TAN, nitrite, nitrate, inorganic phosphate, and alkalinity were measured using a photometer (YSI 9500). Settleable solids and turbidity were assessed with Imhoff sedimentation cones and a turbidimeter, respectively.

Growth performance

Biometric assessments were conducted biweekly on a random sample representing 20% of the population. The following growth performance metrics were evaluated: survival rate, feed conversion ratio (FCR), daily weight gain (DWG), specific growth rate (SGR), and final biomass.

Hematological analysis

At the end of the culture period, six fish per experimental unit were randomly sampled for hematological analysis. Fish were anesthetized using clove oil (5% solution in P.A. alcohol, applied at 1 mL L^{-1} of water). Once fully sedated, blood samples were collected by puncturing the caudal vein with a 1 mL syringe pre-rinsed with a 3% EDTA anticoagulant solution.

Erythrocyte and leukocyte counts were conducted using the hemocytometer method in a Neubauer chamber with 0.01% Merck® Toluidine Blue diluted in 0.9% saline solution. To minimize variability and ensure consistency, all cell counts were performed by the same trained technician following standardized procedures. Hemoglobin concentration was determined using the cyanmethemoglobin method with a Hemoglobin LabTest® kit, while hematocrit levels were assessed via the microhematocrit method. Plasma protein levels were measured with a handheld refractometer, and hematimetric indices, including mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), were also calculated.

Additional blood samples were drawn from the same fish without anticoagulant. These samples were centrifuged to separate plasma for albumin determination, conducted using the LabTest® Albumin kit. Globulin values were calculated by subtracting albumin from total plasma protein.

Statistical analysis

Pearson's normality test and Cochran's homoscedasticity test were applied at a 5% significance level to assess sample normality and variance homogeneity, respectively. A one-way analysis of variance (ANOVA) was conducted to identify differences among treatments. When significant differences were detected, Tukey's test was applied as a post hoc analysis, also at a 5% significance level. The analyses followed the standards outlined by Zar (1996) and were performed using SyEAPRO software (v.2).

RESULTS

Water quality parameters

The mean values of evaluated water quality parameters during the 107-day culture period of Nile tilapia in the biofloc systems are presented in Table I. Temperature and dissolved oxygen showed no significant differences ($P > 0.05$) among treatments, and dissolved oxygen maintained around 5 mg L^{-1} across all groups, indicating consistent aeration efficiency.

Significant differences ($P < 0.05$) were observed in pH, electrical conductivity, transparency, and salinity among treatments. The pH in the control (0M) treatment averaged 7.1, significantly higher than the 10M treatment (6.5) and slightly higher than 20M (6.8). Electrical conductivity was highest in the 10M and 20M treatments, with values of 3.6 and 3.5 mS cm^{-1}, respectively, compared to 2.8 mS cm^{-1} in the control.

Water transparency varied markedly between treatments, with the 0M group averaging 22.7 cm, significantly greater than the 10M and 20M treatments, which averaged 6.0 and 5.5 cm, respectively. Salinity followed a similar trend, with the 0M treatment showing a lower mean (1.4 g L^{-1}) compared to the 10M and 20M treatments (2.7 and 2.4 g L^{-1}, respectively), reflecting the influence of molasses addition on these parameters.

Growth performance and biofloc composition

The zootechnical performance results of Nile tilapia after 107 days of cultivation in biofloc

Table I. **Mean values ± standard deviation of the physicochemical variables of the water quality monitored in the culture of Nile tilapia, in the fattening phase, with biofloc technology for 107 days (range - minimum and maximum, in parentheses; n = 4).**

Variables	Treatments			
	0M	10M	20M	P - value
Temperature (°C)	25.5 ± 1.4[a] (21.7 – 30.5)	24.9 ± 1.1[a] (21.2 – 26.9)	24.9 ± 1.1[a] (21.2 – 27.2)	0.0585
Dissolved oxygen (mg L^{-1})	4.9 ± 0.5[a] (3.5 – 6.5)	5.0 ± 0.5[a] (3.7 - 6.7)	5.0 ± 0.6[a] (3.5 - 6.6)	0.9310
pH	7.1 ± 0.3[a] (6.2 - 8.0)	6.5 ± 0.3[b] (6.0 - 7.3)	6.8 ± 0.3[ab] (6.2 - 7.4)	<0.0001
Electric conductivity (mS cm^{-1})	2.8 ± 0.2[a] (2.4 - 3.7)	3.6 ± 0.2[b] (3.2 - 4.3)	3.5 ± 0.2[b] (3.1 - 4.1)	0.0009
Transparency (cm)	22.7 ± 7.4[a] (11.0 - 37.2)	6.0 ± 1.3[b] (4.0 - 7.7)	5.5 ± 1.4[b] (4.2 - 8.7)	<0.0001
Salinity (g L^{-1})	1.4 ± 0.6[a] (0.7 - 2.2)	2.7 ± 0.9[b] (2.0 - 5.2)	2.4 ± 0.8[b] (1.3 - 4.3)	0.0164

Different letters within the same row indicate significant differences between treatments (*P* < 0.05) based on Tukey's test. Treatments 10M and 20M correspond to C ratios of 10:1 and 20:1 with molasses addition, respectively; 0M represents the control treatment without molasses.

technology, along with the bromatological analyses of the added molasses and the microbial flocs produced, are shown in Table II and Table III, respectively.

The use of molasses did not significantly affect the zootechnical performance of Nile tilapia across the different C:N ratios. Survival rate, Feed Conversion Ratio (FCR), Daily Weight Gain (DWG), Specific Growth Rate (SGR), and biomass showed no significant differences between treatments ($P > 0.05$) (Table II).

Hematological analysis

The hematological variables of tilapia cultured with molasses in a biofloc system are presented in Table IV and Figure 2. No significant differences were found in the mean values of erythrocytes, hematocrit, hemoglobin, mean corpuscular volume (MCV), or mean corpuscular hemoglobin concentration (MCHC) across the different C:N ratio treatments with molasses addition ($P > 0.05$).

However, the 20M treatment led to a significant increase in leukocyte count ($P < 0.05$). Additionally, the use of molasses across different C:N ratios was associated with a decrease in albumin concentration and a significant increase in plasma protein and globulin levels ($P < 0.05$), as shown in Figure 2.

Table II. **Zootechnical performance (mean ± standard deviation; n = 4) of tilapia during the 107-day fattening phase using biofloc technology. Treatments: 10M and 20M with molasses added at C: N ratios of 10:1 and 20:1, respectively; 0M as the control without molasses.**

Variables	Treatments			
	0M	10M	20M	P - value
Initial weight (g)	76.2 ± 4.6	71.2 ± 1.3	72.1 ± 2.9	0.1029
Final weight (g)	317.1 ± 16.8	314.8 ± 23.9	276.3 ± 35.7	0.0675
Survival (%)	94.4 ± 4.3	96.9 ± 4.7	96.7 ± 3.8	0.6461
FCR[1]	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.2	0.2421
DWG[2] (g day^{-1})	2.2 ± 0.1	2.3 ± 0.2	1.9 ± 0.3	0.0616
SGR[3] (% day^{-1})	1.3 ± 1.0	1.4 ± 0.1	1.2 ± 0.1	0.4114
Biomass (Kg m^{-3})	14.9 ± 0.8	15.3 ± 1.6	13.4 ± 2.2	0.2191

The absence of letters indicates no significant differences between treatment (P > 0.05). [1]Feed Conversion Ratio (FCR); [2]Daily Weight Gain (DWG); [3]Specific Growth Rate (SGR).

Table III. **Centesimal composition of bioflocs produced after 107 days and molasses used in cultivation of tilapia fertilized with molasses in C:N 10 and 20:1 ratios.**

Centesimal composition (% MS)*	C:N Ratios		Molasses
	10:1	20:1	
Crude protein (CP)	29.9	30.3	4.5
Ethereal extract (EE)	5.0	16.9	5.1
Crude fiber (CF)	23.3	17.6	1.2
Mineral matter (MM)	35.0	36.5	2.3

***Determined. A difference in the values of EE and CF could be observed, between the flocs formed in the C:N ratios.**

DISCUSSION

Tilapia are well-suited to biofloc technology (BFT), particularly within the optimal temperature range of 26 to 30°C, where growth, feed conversion, reproduction, health, and handling tolerance are enhanced (Avnimelech 2009). Although some temperature values in this study were slightly below this range, no adverse effects on fish performance were observed.

Following (Avnimelech 2009), pH levels for tilapia in BFT should ideally be between 6.0 and 8.5. In the 10M treatment, however, pH was significantly lower (*P* < 0.05) compared to the 0M control. This decrease may result from higher microbial respiration in the 10M treatment, as bacteria consume carbonate and bicarbonate ions during their development, thereby lowering pH when using a 10:1 C:N ratio (Ebeling et al. 2006).

Dolomitic limestone was applied in all tanks to maintain alkalinity above 150 mg L^{-1} $CaCO_3$. In the 10M and 20M treatments, where water addition was limited to compensate for evaporation losses, the high evaporation rates of the semi-arid region led to increased dissolved ion concentration, elevating electrical

Table IV. **Mean ± standard deviation (n =4) of hematological parameters in tilapia cultured with biofloc technology under different C:N ratio management. 0M: control without molasses; 10M and 20M: molasses added at C:N ratios of 10:1 and 20:1, respectively.**

Variables	Treatments			
	0M	10M	20M	P - value
Erythrocyte (10^6 cél µL^{-1})	2.1 ± 0.5	2.1 ± 0.4	2.0 ± 0.2	0.8404
Hematocrit (%)	25.0 ± 3.3	25.0 ± 2.3	26.5 ± 3.9	0.5121
Hemoglobin (g dL^{-1})	6.7 ± 0.5	6.5 ± 0.4	7.0 ± 0.9	0.4534
MVC (fl)	121.7 ± 37.3	122.1 ± 30.1	133.7 ± 23.9	0.7518
MCHC (%)	27.4 ± 2.7	26.9 ± 2.9	26.8 ± 3.9	0.9259

The absence of letters indicates no significant differences between treatment (*P* > 0.05). MCV = (Hematocrit / Erythrocyte count) × 10. MCHC = (Hemoglobin / Hematocrit) × 100.

conductivity and salinity in tanks with molasses addition.

Ammonia from fish excretion and microbial decomposition in water can negatively impact fish performance, increasing disease risk and even mortality (Avnimelech 2009). Keeping nitrite levels low in biofloc systems aligns with findings by Ebeling et al. (2006) and Chamorro-Legarda et al. (2016), who noted that in heterotrophic systems with mature microbial communities, inorganic nitrogen compounds are typically kept low due to their conversion into microbial biomass.

The assimilation of ammoniacal nitrogen by heterotrophic bacteria for microbial floc formation was somewhat limited in controlling total ammonia nitrogen (TAN) concentrations. In the 0M treatment, ammonia levels were regulated by frequent water exchanges, which may have also contributed to lower nitrite and nitrate levels. High nitrite concentrations can be toxic to fish, and it is recommended to keep values below 1.0 mg L^{-1} (Avnimelech 2009). In the 10M and 20M treatments, nitrite values were consistently lower than in 0M, reflecting the action of nitrifying bacteria.

Various carbon sources, including dextrose, rice bran, and molasses, have proven effective in biofloc formation, reducing ammonia and improving water quality (Serra et al. 2015, Silva et al. 2017). Organic carbon sources such as molasses dissolve more rapidly than slower-degrading sources like rice bran (Serra et al. 2015) and cassava starch (Silva et al. 2017). Molasses at C:N ratios of 10:1 and 20:1 was effective in inducing microbial floc formation, resulting in higher levels of suspended and settleable solids. Additionally, the low water exchange in these treatments led to increased inorganic phosphate, settleable solids, and total suspended solids.

The nutritional composition of biofloc is highly dynamic and can be influenced by factors such as environment, carbon source, salinity, light intensity, and microbial communities (Emerenciano et al. 2013). Our results differ from those of Azim & Little (2008), who, using wheat flour in BFT for tilapia, reported values of 38% crude protein (CP), 3% ether extract (EE), and 12.5% ash content (AC). (Widanarni et al. 2012) found similar values (40–50% CP, 10–25% EE, 25–30% AC) using molasses. Future studies should focus on optimizing biofloc concentrations and managing microbial communities to enhance biofloc nutritional composition for aquaculture.

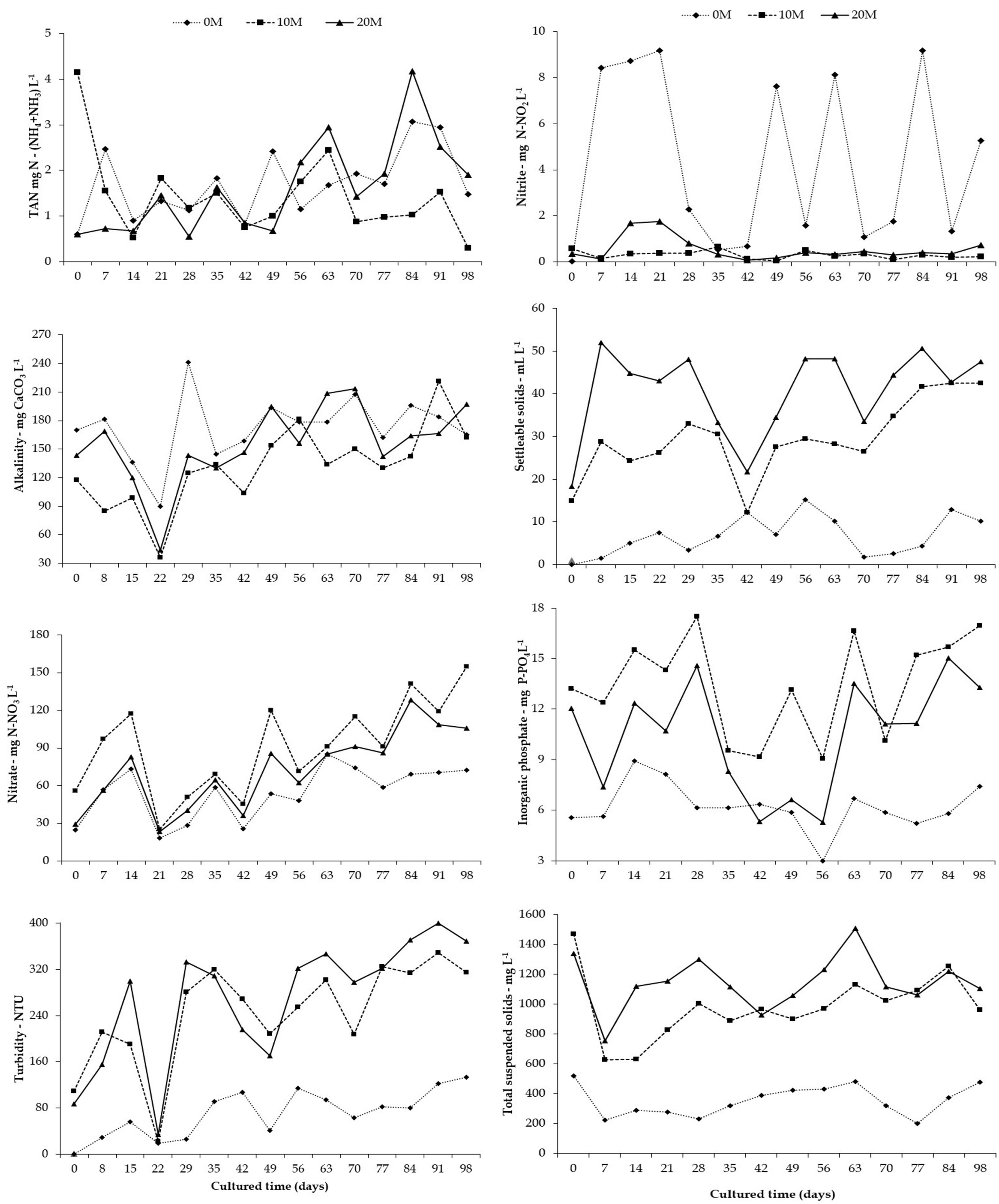

Figure 1. Weekly mean values of water quality parameters monitored over 107 days of Nile tilapia culture in a biofloc system. Treatments 10M and 20M correspond to molasses additions at carbon-to-nitrogen (C:N) ratios of 10:1 and 20:1, respectively, while 0M represents the control group without molasses supplementation.

BFT has shown positive effects on tilapia performance (Figueiredo et al. 2022). The zootechnical results in this study align with (Lima et al. 2015), who observed that a tilapia density of 45 fish m^{-3} in BFT produced 17 kg m^{-3}, with a survival rate of 91% and average fish weight of 400 g after 128 days. In BFT, water use efficiency was high (133 L kg^{-1} fish) compared to intensive green water systems, where Milstein et al. (2001) reported water consumption of 800 L kg^{-1}. In the 0M treatment, frequent water exchange increased water consumption to 871 L kg^{-1}, highlighting the advantages of BFT in water-scarce regions like the semi-arid Pajeú area.

Hematological studies on fish provide insight into physiological responses to pathogens, helping improve productivity while maintaining homeostasis (Barros et al. 2015). The 20M treatment showed significantly higher leukocyte counts, indicating an increase in immune cells. The number of leukocytes can vary with physiological state, and BFT may balance stress and productivity, stimulating immune responses (Crab et al. 2012). Although (Azim & Little 2008) found no differences in leukocyte counts between treatments, they observed that BFT enhanced fish immune responses. In our study, significant increases ($P < 0.05$) in plasma protein and globulin in the 10M and 20M treatments further suggest an immunostimulatory effect of BFT. However, such increases in leukocytes and globulins could also

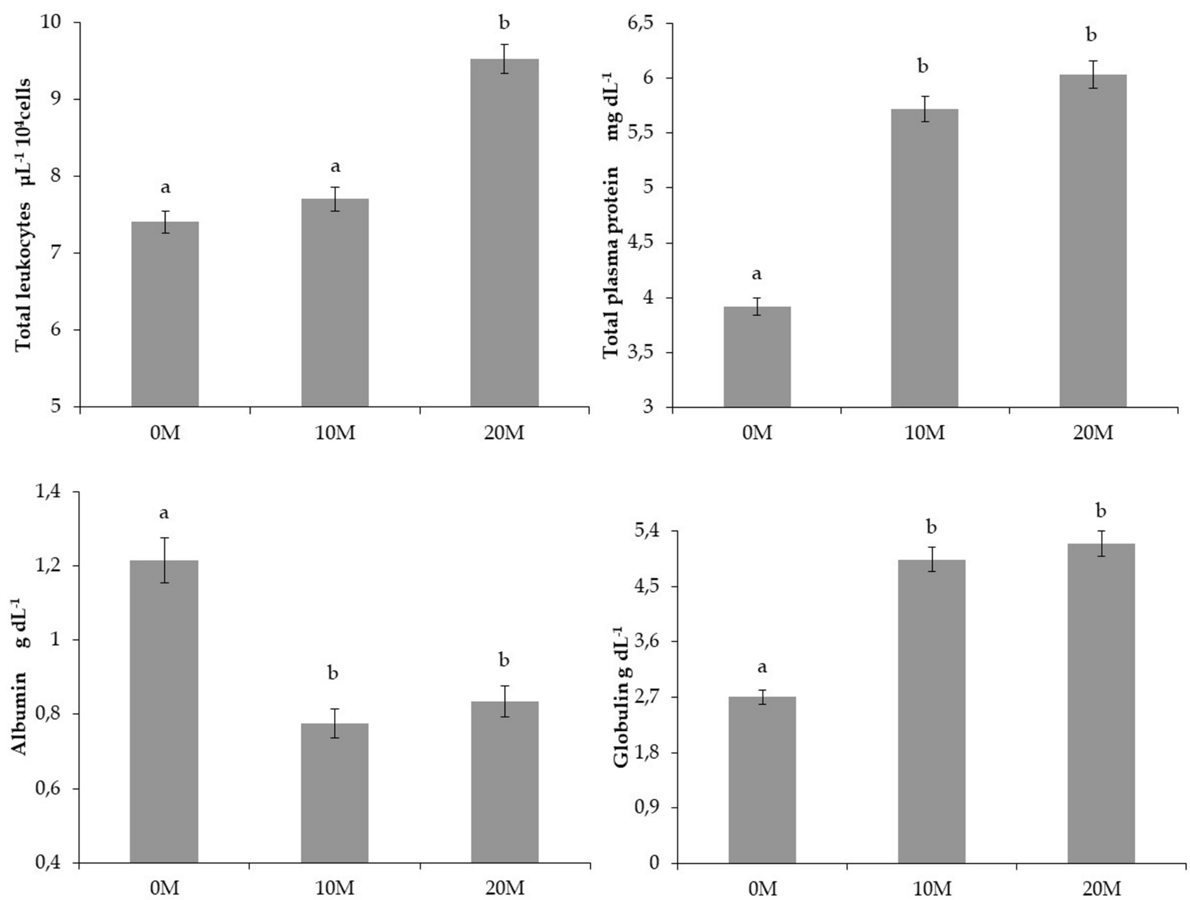

Figure 2. Mean values of total leukocytes (cells µL^{-1}; $P = 0.0179$), total plasma protein (g dL^{-1}; $P < 0.0001$), albumin (g dL^{-1}; $P < 0.0001$), and plasma globulin (g dL^{-1}; $P < 0.0001$) in tilapia cultured with biofloc technology using C:N ratios of 10:1 (10M) and 20:1 (20M) with molasses addition; 0M represents the control without molasses. Columns with different letters indicate significant differences according to Tukey's test ($P < 0.05$).

indicate chronic inflammation or latent stress, which warrants further investigation.

A limitation of the present work is its reliance on manual hematological techniques, a factor that can reduce repeatability because of inherent variability and the absence of standardized procedures. Future studies should consider adopting automated cell counting techniques, which offer greater precision and reproducibility (Fazio et al. 2024). In addition, while total plasma protein, albumin, and globulins (calculated by difference) were assessed, no fractionation analysis was performed. Advanced analytical approaches, including agarose gel or capillary electrophoresis, enable the separation of α-, β-, and γ-globulin fractions (Campos-Sánchez et al. 2024), offering more detailed immunophysiological information.

Further research is needed to explore BFT's impacts on hygiene, animal welfare, and physiological responses under adverse conditions. Such studies will deepen our understanding of BFT's effects on growth, water quality, hematology, and immune response, supporting discussions on health and nutrition in BFT. This study highlights the semi-arid Pajeú region's potential for expanding this sustainable aquaculture technology, with implications for meeting market demand efficiently.

In conclusion, the different C:N ratios applied in biofloc systems did not result in significant differences in zootechnical performance of Nile tilapia. However, the 10:1 ratio showed a favorable balance between water quality stability and productive indicators, suggesting its potential applicability in water limited regions. The observed hematological and biochemical responses, particularly the increase in leukocytes and globulins, indicate possible immunostimulation under molasses supplementation, though potential signs of physiological stress cannot be ruled out. Further studies using standardized analytical tools and deeper immunological profiling are recommended to clarify these responses and optimize carbon management strategies in BFT for tropical aquaculture systems.

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Data availability

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