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Less feed does not mean lower growth: The impact of feeding frequency on fishes reared in BFT enriched with sodium chloride

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ABSTRACT - The objective of this work was to analyze the impact of different feeding frequencies on the growth and hematological parameters of freshwater fish species (tambaqui, *Colossoma macropomum*; matrinxã, *Brycon amazonicus*) subjected to biofloc technology enriched with sodium chloride. We tested two feeding frequencies: T1 = feeding all experimental days for tambaqui, except on Sundays; T2 = feeding all experimental days for tambaqui, except on Sundays and Wednesdays; M1 = feeding all experimental days for matrinxã, except on Sundays; and M2 = feeding all experimental days for matrinxã, except on Sundays and Wednesdays. The growth performance of tambaqui and matrinxã were not affected by the treatments. The MCHC for matrinxã was the only hematological parameter significantly different. The physiological status (Kn = condition factor) of the M2 treatment was the only parameter that presented a significant difference in relation to the central value (Kn = 1.00). Water quality parameters remained within the range indicated for the rearing of both species. The feeding frequencies tested do not negatively affect tambaqui or matrinxã.

Keywords: aquaculture, biofloc, *Brycon amazonicus*, *Colossoma macropomum*, fish farming, salt

1. Introduction

Modern aquaculture differs largely from traditional aquaculture due to the search for increased productivity per area or volume through the control of several water, physiological, health, and feeding parameters (Kumar et al., 2018). Among the features linked to feeding parameters, one of the most fundamental in relation to a reduction in feed intake is feeding frequency (Gilannejad et al., 2019). The optimal feeding frequency varies according to the species being studied and its respective feeding habits, water quality, animal size, feed composition, and rearing system (Baloi et al., 2016).

The management of processes related to the supply of feed is one of the most important factors in commercial aquaculture enterprises, as feed is the most important input within the variable cost of producing aquatic organisms (Baloi et al., 2016). Depending on the feed frequency used, there may be a stimulus to the intake of inert feed in the water column. Thus, biofloc technology (BFT) is indicated for promoting supplementary microfood based on protein macroaggregates (biofloc) (Ahmad et al., 2017).

For the filtration of the microbial protein present in biofloc, two Amazonian native freshwater species appear to be the main candidates: tambaqui (*Colossoma macropomum*) and matrinxã (*Brycon amazonicus*). Tambaqui is the most important native fish species reared in Brazil and has a diet based on the intake of seeds, fruits, and zooplankton (Wood et al., 2017). Matrinxã is also a characid species that reaches up to 4.0 kg, and in nature, it feeds on plant remains, fruits, seeds, insects, etc. (Marinho-Pereira et al., 2014). Both species have an established consumer market, digestibility of vegetable protein, and many satisfactory results in aquaculture with alternative feeding frequencies (Rocha et al., 2018; Santos et al., 2018; Barros et al., 2019).

However, in BFT system, the nutrient loading is processed by microbes, but sometimes the nutrient loading can be too much for the microbes to handle. In such case, there can be an issue of ammonia and nitrite spikes. These nitrite spikes occur due to the greater sensitivity of nitrite-oxidizing bacteria (NOB) to aquaculture conditions in water (temperature, pH, dissolved oxygen, dissolved solids, alkalinity), which makes stabilizing and proliferating this group of bacteria (Ruiz et al., 2020). If nitrite levels remain high, this nitrite will be absorbed by the fish and will oxidize hemoglobin into methemoglobin, making it impossible for the red cells to transport oxygen and killing the animal through anoxia (Kir and Sunar, 2018).

To avoid nitrite mortality, when nitrite levels are high, some aquaculture producers carry out water renewal, something that is not sustainable in the long term or impossible in places and regions where water resources are scarce. Therefore, to prevent water exchange, one of the best-known techniques used by aquaculture farmers to reduce the nitrite absorption capacity of fish is the use of sodium chloride (NaCl). A decrease in nitrite toxicity occurs because Cl^- ions are also absorbed by chloride cells via the same physiological pathway (Jia et al., 2015).

In natural environments, salinity levels characterize different aquatic environments, segregating freshwater from estuarine and marine environments. Within this differentiation, it is necessary to understand that the ions Na^+ and Cl^- are determinant during the analysis of the degree of osmolarity of the aquatic fluid and its respective concentrations, which is fundamental in decision-making in relation to the preference for aquatic environments with low levels of salinity (close to 4 ppt) and tends to favor the osmoregulatory processes of fish species that have a natural habitat in environments lacking salinity and low osmolarity (Griffith, 2017).

Several studies have been carried out to understand the physiological behavior of native species (with surubim, *Pseudoplatystoma corruscans*; pacamã, *Lophiosilurus alexandri*; and pacu, *Piaractus mesopotamicus*) in these low-salinity environments. Therefore, in addition to tambaqui and matrinxã, all of these previously mentioned species may be considered stenohaline due to their tolerance of low saline concentrations in water (Santos and Luz, 2009; Jomori et al., 2012).

Therefore, the objective of this research was to analyze the impact of different feeding frequencies on the growth and hematological parameters of freshwater fish species subjected to biofloc technology enriched with sodium chloride.

2. Material and methods

2.1. Ethics

This experiment was conducted according to the Brazilian laws and regulations for scientific ethics (Law No. 11.794 of October 8th, 2008; Resolution CONCEA/MCTI No. 49 of May 7th, 2021) and was approved by the Ethics Committee on the Use of Animals (CEUA), case no. 0420200094/2020.

2.2. Location and fish used in the experiment

This experiment was carried out in Manaus, Amazonas, Brazil (03°06'02.81" S 59°58'37.06" W; WGS84). Before the beginning of the experiment, tambaqui and matrinxã juveniles (males and females) were harvested in an aquaculture farm and stocked in a round 3,000-L tank for a quarantine period of seven days. After quarantine, 84 fish (males and females) were initially weighed (tambaqui = 42.2 ± 0.7 g; matrinxã = 40.0 ± 1.2 g), measured (tambaqui = 10.8 ± 0.2 cm; matrinxã = 12.2 ± 0.1 cm) and distributed homogeneously in each experimental unit (tambaqui = eight animals per tank, 1.16 kg m^{-3} of initial density; matrinxã = six animals per tank, 0.83 kg m^{-3} of initial density). All fish were subjected to osmotic acclimatization by the addition of 1.0 g L^{-1} sodium chloride, avoiding a sudden osmotic change in the experimental units until it reached 4 ppt salinity level.

2.3. Experimental design and experimental units

During the 40 experimental days, the experiment was conducted in accordance with a completely randomized design (triplicate) with two different feeding frequencies in the BFT system under low salinity for each species: T1 = feeding all experimental days for tambaqui, except on Sundays; T2 = feeding all experimental days for tambaqui, except on Sundays and Wednesdays; M1 = feeding all experimental days for matrinxã, except on Sundays; and M2 = feeding all experimental days for matrinxã, except on Sundays and Wednesdays.

The 12 experimental units (three experimental units for species) consisted of a round 310-L polyethylene tank (290 L of useful volume) equipped with a 0.5 CV blower aeration system with porous stones for air diffusion in water. Constant and uninterrupted aeration was necessary to increase the amount of dissolved oxygen that was constantly consumed by the oxidative reactions inherent to the nitrification processes. The water initially used to fill the experimental units came from a semi-artesian well located at the same experimental location.

The feed used in this experiment was commercial, with 42% crude protein and 2.5 mm of feed pellet (Supra®, a product of Alisul Alimentos S.A.; São Leopoldo, Rio Grande do Sul, Brazil), offered twice a day (08:00 and 17:00 h) to apparent satiation and according to the feeding frequencies of each treatment (same feed for each species). All Sundays were reserved for cleaning and maintenance of the integrated experimental system for rearing of the two species in the BFT system.

2.4. Measurement of water quality and bioreactor management

The water quality of the experimental units was monitored once a day (09:00 h) by evaluating the following parameters with water multiparameter (Akso AK88v2®; Akso Produtos Eletrônicos Ltda, São Leopoldo, RS, Brazil): pH, temperature, dissolved oxygen, and total dissolved solids (TDS). Water concentrations of total ammoniacal nitrogen (TAN) and nitrite were determined by commercial colorimetric kit (Alfakit®, Florianópolis, SC, Brazil). To maintain a minimum carbon:nitrogen ratio (CN ratio) of 6:1, we added white crystal sugar (40% of the total carbon from sucrose; $C_{12}H_{22}O_{11}$) when the TAN concentration exceeded 1.0 mg L^{-1}.

Thus, after carbon addition, we added 50 mL of inoculum from a specific bioreactor for heterotrophic bacteria (BIO-H) cultivated under the following conditions and with 20 L of water: absence of light, continuous aeration, presence of TAN, white crystal sugar to provide a CN ratio of 15:1, total alkalinity above 120 mg L^{-1} of $CaCO_3$, and 20 g of probiotic product enriched with nonpathogenic heterotrophic bacteria from the genera *Bacillus* sp. and *Lactobacillus* sp.

To avoid mortality generated by sudden nitrite spikes, we supplied 15 L of water from a specific bioreactor for the cultivation of NOB bacteria (BIO-NOB) every time the nitrite concentration reached 1.5 mg L^{-1} in the experimental units. In the BIO-NOB, we used the following inputs and steps to start the specific nitration process: a biological filtration activator (Stability®, a product of Seachem Co.;

Madison, Georgia, USA); continuous aeration; addition of broken brick as a support medium for bacterial fixation; and water quality with high levels of nitrite, total alkalinity above 120 mg L^{-1}, and negligible levels of TAN.

Sodium bicarbonate was added whenever necessary to maintain the pH (close to 7.8) and total alkalinity (above 120 mg L^{-1}) at levels that allow the growth and maintenance of heterotrophic and chemoautotrophic bacteria in the BIO-NOB (Marinho-Pereira et al., 2020). After the water input from the BIO-NOB into the experimental units to control the levels of nitrite occurred, the salinity was measured with the aid of multiparameter water quality equipment (Akso®, model AK88; São Leopoldo, Rio Grande do Sul, Brazil), and we constantly adjusted the water salinity level with sodium chloride to 4 ppt.

2.5. Growth performance and physiological status (Kn factor)

To perform the biometric procedures (initial, partial, and final) and blood collection, all the fish were removed from the tanks with the aid of a net and anesthetized with eugenol (Oliveira et al., 2021). The growth performance was determined using the following parameters: survival rate (%), weight gain (WG), biomass gain (BG), specific growth rate (SGR), daily feed intake (DFI), and feed conversion ratio (FCR).

With the biometric data obtained, it was possible to assess the body condition and evaluate the nutritional and physiological status of individuals (Gubiani et al., 2020). The Kn factor (Kn) was obtained using the ratio between the final observed weight (Wo) and the expected weight (We) to the observed length (Lt). Additionally, a logarithmic weight-length relationship was established, and the constants “a” and “b” were later used to form another equation fundamental for estimating We as a function of Lt (Tavares-Dias et al., 2008b).

2.6. Blood sampling and hematological analysis

At the end of the experimental period, fresh blood samples were collected through caudal venipuncture (Castro et al., 2021) for determination of hematocrit (Ht), hemoglobin (Hb), and erythrocyte (RBC) count. Hematocrit (%) was evaluated by the microhematocrit centrifugation technique. Hemoglobin concentration (g dL^{-1}) was determined using the cyan-methemoglobin technique. The erythrocyte count (millions mm^{-3}) was determined with a Neubauer hemocytometer using an light microscope. At the end of the blood analysis, it was possible to determine the following indices: mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g L^{-1}) (Witeska et al., 2022).

2.7. Statistical analysis

Growth performance, Kn factor, water quality, and hematological data were analyzed for normality and homogeneity by Shapiro–Wilk’s and Levene’s tests, respectively. The data are reported as the mean ± standard deviation of the mean and were evaluated by Student’s t test (for intraspecific comparisons). The following mathematical model was adopted:

$$t_{ij} = \frac{\mu_i - \mu_j}{\sqrt{\frac{\sigma_i^2}{n_i} + \frac{\sigma_j^2}{n_j}}}$$

in which t_{ij} is the t-statistic, μ_i is the mean of treatment *i*, σ_i is the standard deviation of treatment *i*, and n_i is the number of the observations of treatment *i*. Before carrying out the statistical analyses, all the data were transformed (arc-sin) and analyzed at the 0.05 level of confidence (P<0.05) (Bhujel, 2008).

2.8. Theory/calculation

$$\text{Weight gain} = \text{final weight} - \text{initial weight}$$

$$\text{Biomass gain} = \text{final biomass} - \text{initial biomass}$$

$$\text{Survival rate (\%)} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{feed intake}}{\text{biomass gain}}$$

$$\text{Daily feed intake} = \text{feed intake} \times \text{day}^{-1} \times \text{biomass}^{-1}$$

$$\text{Specific growth rate} = \frac{\ln \text{final body weight} - \ln \text{initial body weight}}{\text{experimental days}} \times 100$$

$$\text{Kn factor} = \frac{Wo}{We}$$

$$\text{Weight expected} = aLt^{b}$$

$$\text{Mean corpuscular volume} = \frac{Ht \times 10}{RBC}$$

$$\text{Mean corpuscular hemoglobin} = \frac{Hb}{RBC}$$

$$\text{Mean corpuscular hemoglobin concentration} = \frac{Hb \times 100}{Ht}$$

3. Results

There was also no statistically significant difference between the treatments tested for any of the water quality and growth performance parameters (Tables 1 and 2; $P>0.05$). No mortality was observed for tambaqui (Table 3), and matrinxã exhibited a degree of mortality when stocked in the experimental units (Table 4).

There were also no statistically significant differences in relation to the hematological parameters, except for the MCHC (Tables 5 and 6; $P<0.05$). The welfare of the animals and the Kn factor did not significantly differ between the treatments tested (Figure 1).

Table 1 - Effect of alternative feeding frequencies on water parameters of tambaqui reared in biofloc technology enriched with sodium chloride

Parameter	Treatment with tambaqui[1]	
	T1	T2
Dissolved oxygen (mg L^{-1})	6.2 ± 0.1	6.2 ± 0.2
Temperature (°C)	26.8 ± 1.0	26.5 ± 0.3
pH	7.9 ± 0.1	7.8 ± 0.2
Total ammonia nitrogen ($NH_3 + NH_4^+$)	0.4 ± 0.1	0.2 ± 0.0
Nitrite (NO_2^-)	1.0 ± 0.2	0.6 ± 0.3
Sedimentable solids (mL L^{-1})	8.7 ± 2.5	5.3 ± 2.3

[1] T1 = feeding all experimental days for tambaqui, except on Sundays; T2 = feeding all experimental days for tambaqui, except on Sundays and Wednesdays.
All the data are presented as the means ± standard deviations obtained from experimental units (1 tank = 1 replicate).

Table 2 - Effect of alternative feeding frequencies on water parameters of matrinxã reared in biofloc technology enriched with sodium chloride

Parameter	Treatment with matrinxãi[1]	
	M1	M2
Dissolved oxygen (mg L^{-1})	6.1 ± 0.1	6.3 ± 0.4
Temperature (°C)	26.1 ± 0.2	26.3 ± 0.8
pH	7.9 ± 0.06	7.9 ± 0.1
Total ammonia nitrogen (NH_3 + NH_4^+)	0.5 ± 0.1	0.4 ± 0.2
Nitrite (NO_2^-)	1.47 ± 0.5	1.3 ± 1.0
Sedimentable solids (mL L^{-1})	8.2 ± 3.0	7.3 ± 4.5

[1] M1 = feeding all experimental days for matrinxã, except on Sundays; M2 = feeding all experimental days for matrinxã, except on Sundays and Wednesdays.
All the data are presented as the means ± standard deviations obtained from experimental units (1 tank = 1 replicate).

Table 3 - Effect of alternative feeding frequencies on the growth performance of tambaqui reared in biofloc technology enriched with sodium chloride

Parameter	Treatment with tambaqui[1]	
	T1	T2
Initial weight (g)	42.1 ± 1.0	42.3 ± 0.3
Initial length (cm)	10.8 ± 0.3	10.7 ± 0.1
Final weight (g)	71.0 ± 4.9	69.4 ± 1.8
Final length (cm)	12.8 ± 0.3	12.9 ± 0.1
Survival rate (%)	100.0	100.0
Weight gain (g)	28.9 ± 4.2	27.1 ± 2.1
Biomass gain (g)	230.7 ± 33.1	216.7 ± 16.5
SGR (% day^{-1})	1.3 ± 0.0	1.2 ± 0.0
DFI (g feed $fish^{-1}$ day^{-1})	1.05 ± 0.1	1.08 ± 0.0
FCR	0.95 ± 0.1	1.00 ± 0.08

SGR - specific growth rate; DFI - daily feed intake; FCR - feed conversion ratio.
[1] T1 = feeding all experimental days for tambaqui, except on Sundays; T2 = feeding all experimental days for tambaqui, except on Sundays and Wednesdays.
All the data are presented as the means ± standard deviations obtained from experimental units (1 tank = 1 replicate).

Table 4 - Effect of alternative feeding frequencies on the growth performance of matrinxã reared in biofloc technology enriched with sodium chloride

Parameter	Treatment with matrinxã[1]	
	M1	M2
Initial weight (g)	39.7 ± 0.2	40.2 ± 1.8
Initial length (cm)	12.2 ± 0.2	12.2 ± 0.2
Final weight (g)	49.0 ± 1.9	51.5 ± 8.4
Final length (cm)	13.7 ± 0.5	13.3 ± 1.0
Survival rate (%)	77.8 ± 25.5	83.3 ± 28.9
Weight gain (g)	9.3 ± 1.8	11.3 ± 5.4
Biomass gain (g)	58.0 ± 14.1	98.0 ± 32.5
SGR (% day^{-1})	0.5 ± 0.1	0.6 ± 0.5
DFI (g feed $fish^{-1}$ day^{-1})	0.73 ± 0.1	0.77 ± 0.1
FCR	3.85 ± 0.9	2.35 ± 0.8

SGR - specific growth rate; DFI - daily feed intake; FCR - feed conversion ratio.
[1] M1 = feeding all experimental days for matrinxã, except on Sundays; M2 = feeding all experimental days for matrinxã, except on Sundays and Wednesdays.
All the data are presented as the means ± standard deviations obtained from experimental units (1 tank = 1 replicate).

Table 5 - Effect of alternative feeding frequencies on the hematological parameters and body condition (Kn factor) of tambaqui reared in biofloc technology enriched with sodium chloride

Parameter	Treatment with tambaqui[1]	
	T1	T2
Hemoglobin (g dL^{-1})	6.35 ± 0.85	6.27 ± 0.15
Hematocrit (%)	25.88 ± 3.05	24.42 ± 1.59
RBC (millions mm^{-3})	1.25 ± 0.17	1.16 ± 0.28
MCV (fL)	208.35 ± 16.94	219.45 ± 53.63
MCH (pg)	51.11 ± 5.26	56.86 ± 16.75
MCHC (g dL^{-1})	24.51 ± 0.56	25.73 ± 1.57
Condition factor (Kn)	1.01 ± 0.02	0.98 ± 0.02

RBC - erythrocyte count; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.
[1] T1 = feeding all experimental days for tambaqui, except on Sundays; T2 = feeding all experimental days for tambaqui, except on Sundays and Wednesdays.
All the data are presented as the means ± standard deviations obtained from experimental units (1 tank = 1 replicate).

Table 6 - Effect of alternative feeding frequencies on the hematological parameters and body condition (Kn factor) of matrinxã reared in biofloc technology enriched with sodium chloride

Parameter	Treatment with matrinxã[1]	
	M1	M2
Hemoglobin (g dL^{-1})	6.97 ± 0.48	7.64 ± 0.32
Hematocrit (%)	28.56 ± 1.15	28.94 ± 1.68
RBC (millions mm^{-3})	1.49 ± 0.28	1.42 ± 0.19
MCV (fL)	195.06 ± 28.95	204.79 ± 15.20
MCH (pg)	47.09 ± 5.61	54.11 ± 4.87
MCHC (g dL^{-1})	24.19 ± 0.72b	26.40 ± 0.42a
Condition factor (Kn)	0.94 ± 0.10	1.05 ± 0.03

RBC - erythrocyte count; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.
[1] M1 = feeding all experimental days for matrinxã, except on Sundays; M2 = feeding all experimental days for matrinxã, except on Sundays and Wednesdays.
All the data are presented as the means ± standard deviations obtained from experimental units (1 tank = 1 replicate). In each line, means that are significantly equal have the same letter (P>0.05). Statistical comparisons were only intraspecific.

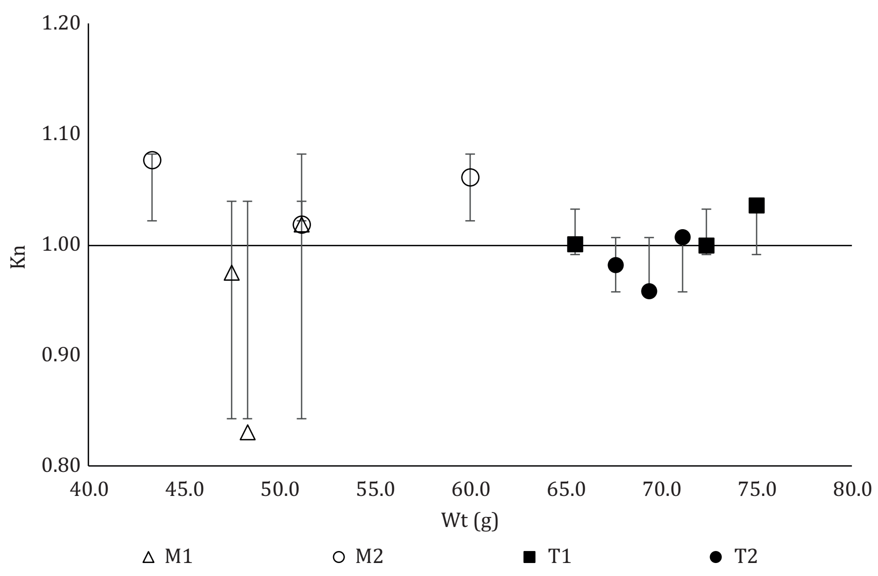

Figure 1 - Relationship between the condition factor (Kn) and total weight (Wt) of tambaqui and matrinxã reared at different feeding frequencies in biofloc technology enriched with sodium chloride.

4. Discussion

In general, fish metabolism is directly affected by daily oscillation of temperature (Pinho et al., 2017). In the BFT system, the most suitable temperature range for nonpathogenic heterotrophic bacteria and AOB and NOB groups is 28 to 30 °C (Ruiz et al., 2020). However, the mean temperature observed during our experiment (26.4 ± 0.30 °C) was sufficient for TAN and nitrite oxidation and control (Table 1).

For pH, we obtained an overall value of 7.89 ± 0.11. Aride et al. (2007) determined that a pH close to neutral (between 6.0 and 7.0) favors the homeostasis of tambaqui, and for matrinxã, Tavares-Dias and Sandrim (1998), Marques et al. (2004), and Arbeláez-Rojas and Moraes (2009) reported mean pH values of 7.2 to 7.6 in individuals subjected to aquaculture conditions.

Dissolved oxygen levels ranged between 6.1 and 6.3 mg L^{-1}. In an aquaculture case, if there is a sudden reduction in dissolved oxygen levels, tambaqui has more adaptative features than matrinxã, including an adaptation that promotes an increase in RBC in plasma (Ferreira et al., 2010; Wood et al., 2017; Abdel-Tawwab et al., 2019).

The ammonia and nitrite concentrations obtained were within the standard limits for the rearing of matrinxã and tambaqui (Brandão et al., 2005; López and Anzoátegui, 2013). Among all the inorganic compounds present in aquaculture units, ammonia and nitrite are the most limiting agents for intensive aquaculture (Souza et al., 2019). In the nitrogen cycle, while AOB-type bacteria transform ammonia into nitrite (one of the most lethal nitrogenous compounds in commercial breeding environments), NOB-type bacteria are the only ones capable of continuing the nitrogen cycle, oxidizing nitrite to nitrate, a less toxic form of nitrogen (Bossier and Ekasari, 2017). In other words, for the efficient removal of these two chemical compounds, regular applications of nonpathogenic heterotrophic bacteria are vital because AOB-type bacteria take advantage of the nitrogen present in ammonia for their own cell growth and replication (Abrar et al., 2019).

Some productive factors were crucial for these ammonia and nitrite levels to not reach lethal levels: dissolved oxygen levels were maintained above 4.0 mg L^{-1}; total alkalinity was above 120 mg L^{-1}; there was a continuous supply of heterotrophic bacteria and nitrating agents through the use of BIO-H and BIO-NOB bioreactors; and the levels of the sedimentable solids (mL L^{-1}) were periodically measured and controlled (Ruiz et al., 2020).

These sedimentable solids are essential for the conservation of ammonia and nitrite within the recommended range for the rearing of aquatic animals, as the number of colonizing bacteria varies between 1×10^6 and 1×10^9 mL^{-1} of the total biofloc biomass (Ahmad et al., 2017). Bakar et al. (2015) determined that higher C:N ratios promote an increase in sedimentable solids (biofloc), resulting in the mitigation of possible accumulations of ammonia and nitrite in the system.

Accurate analysis of growth performance is fundamental for understanding the development of both species subjected to experimental conditions. Initially, we needed to observe the mortality of matrinxã, which should not be attributed to salinity; rather, it was due to the low density of individuals per cubic meter of water, resulting in an increase in the aggressiveness of the animals stocked in the experimental unit (Arbeláez-Rojas and Moraes, 2009). Unfortunately, the mortality measured had a negative influence in some growth parameters, mainly biomass gain and FCR.

Nevertheless, tropical freshwater fish may experience mortality in saline environments. Brol et al. (2017) evaluated the effect of salinity (8 ppt) and stocking density (400 and 800 fishes m^{-3}) on the growth performance of Nilotic tilapia (*Oreochromis niloticus* – lineage GIFT) and red tilapia (*Oreochromis* sp.). The respective average survival ranged from 72.9 ± 7.3 to 90.6 ± 4.7%.

Tambaqui obtained a mean SGR of 1.27 ± 0.1%, and matrinxã had a mean SGR of 0.6 ± 0.3%, which are lower than those obtained by Fiúza et al. (2015) and Mattos et al. (2018), respectively. Fiúza et al. (2015) obtained a mean SGR of 1.85 ± 0.8% when evaluating the growth performance of tambaqui juveniles (initial weight = 57. 4 ± 5.5 g) reared in clear water and water from a recirculating system for

84 days under four different salinity conditions (0, 5, 10, and 15 ppt). Mattos et al. (2018) reported a mean SGR of 4.72 ± 0.2% for matrinxã juveniles (initial weight = 3.2 ± 0.2 g) subjected to four different levels of crude protein in the feeding diet (30, 35, 40, and 45%) for 60 days.

We observed an overall mean WG of 28.0 ± 3.1 g for tambaqui, value lower than those reported by Silva et al. (2013) (WG = 34.9 ± 1.8 g, after 45 days) and Fiúza et al. (2015) (WG = 338.8 ± 19.6 g, after 84 days). For matrinxã we obtained a mean WG of 10.3 ± 3.6 g, value below that observed by Brandão et al. (2005) (WG = 65.8 ± 5.0 g, after 60 days) and Mattos et al. (2018) (WG = 51.1 ± 5.8 g, after 60 days).

Weight gain and SGR could vary from an experiment to another because of small differences in experimental conditions: water quality, area and volume of tanks (space area for swimming and animal welfare), intrinsic genetic factors, feed (% of crude protein, types of animal protein, amino acids), and other reasons.

The mean DFI obtained was 1.07 ± 01 g feed fish^{-1} day^{-1}, an inferior value against that presented by Fiúza et al. (2015), which ranged between 1.0 ± 0.1 to 5.7 ± 0.4 feed fish^{-1} day^{-1}. In addition, for matrinxã, we observed a mean DFI of 0.76 ± 0.1 g feed fish^{-1} day^{-1}, which was higher than the mean DFI of 0.03 g feed fish^{-1} day^{-1} obtained by Marques et al. (2004) throughout the analysis of four different stocking densities (24, 48, 72, and 96 fish m^{-3}) in matrinxã growth performance (initial weight = 2.0 ± 0.82 g) over 20 days. This high difference in DFI is explained by the difference in initial weight between the studies.

Finally, for FCR, we obtained means of 0.97 ± 0.1 (tambaqui) and 3.08 ± 1.1 (matrinxã). The values for matrinxã are consistent with the mortality observed in the treatments, which resulted in a negative influence on the final biomass and, consequently, on FCR. In comparison with the findings of other works, our matrinxã data were superior to the average FCR of 1.29 ± 0.2, 1.33 ± 0.0, and 1.49 ± 0.3 obtained by Marques et al. (2004), Brandão et al. (2005), and Mattos et al. (2018), respectively. The tambaqui FCR data were less than the FCR values determined by Silva et al. (2013) and Fiúza et al. (2015) of 0.99 ± 0.06 and 1.3 ± 0.01, respectively (considered only animals raised at a salinity of 5 ppt).

In hematological analysis, it is important to emphasize that Hb, Ht, and RBC may present relevant changes that serve as a basis for identifying physiological stresses caused by osmoregulatory adaptations linked to fish farming, which were originally from fresh water, in water environments with low salinity.

The matrinxã data for Hb, Ht, and RBC agree with the parameters established by Tavares-Dias et al. (2008a) for captive breeding of matrinxã (Hb range = 5.5-8.1 g dL^{-1}; Ht range = 23.0-35.0%; RBC range = 1.13-1.56 10^{6} mm^{-3}). The average Hb concentration, average Ht, and average RBC density analyzed for tambaqui were less than those determined by Tavares-Dias and Sandrim (1998) (Hb range = 9.0-13.8 g dL^{-1}; Ht range = 30.0-56.0%), except for RBC (2.0-4.0 10^{6} mm^{-3}), and by Gomes et al. (2006) (mean Hb = 13.05 ± 1.09 g dL^{-1}; mean Ht = 30.71 ± 3.79 %; mean RBC = 2.45 ± 0.40 10^{6} mm^{-3}).

Hematocrit, Hb, and RBC values outside the standard range for raising situations may indicate anemia (Burgos-Aceves et al., 2019). Nevertheless, Ranzani-Paiva et al. (1999) reported that oscillations in hematological values may also be a result of specific conditions at the breeding site. Aride et al. (2007) reported physiological deficiencies in tambaqui subjected to pH values that exceeded 6.0, and for matrinxã, Arbeláez-Rojas et al. (2002) described a better productive performance in environments with a profile characteristic of the numerous small watercourses existing in the Amazon Rainforest, popularly known by the indigenous term “igarapé”: average temperature of 25.8 °C; pH oscillating between 4.6 and 5.6; average dissolved oxygen concentration of 5 mg L^{-1}; full transparency; and continuous flow of water, with sustained swimming being an important factor that aids in the growth process of matrinxã (Arbeláez-Rojas and Moraes, 2009).

Mean corpuscular volume, MCH, and MCHC values are similar to those determined by Gomes et al. (2006) (MCV = 134.01 ± 32.51 fL; MCH = 57.17 ± 12.73 pg; MCHC = 44.33 ± 6.59 %) and Tavares-Dias et al. (2008a) (MCV = 191.7-253.8 fL; MCHC = 17.0-30.7 g dL^{-1}) for tambaqui and matrinxã, respectively. Mean corpuscular volume, MCH, and MCHC are fundamental for fully analyzing the capacity of plasma to transport oxygen and carbon dioxide (Kumar and Banerjee, 2016) and to determine a diagnosis of anemia (Witeska et al., 2022). The only hematological parameter that was significantly different among the treatments ($P<0.05$) was MCHC for matrinxã. Nevertheless, all the derived erythrocyte parameters remained within the stipulated baseline for both species (Gomes et al., 2006; Tavares-Dias et al., 2008a).

These good hematological and physiological data resulted in an adequate Kn factor (Figure 1). A better growth performance meant closer proximity to the centralizing Kn value = 1.0 (as observed in Figure 1), regardless of the species studied. Freshwater aquatic animals are highly dependent on osmoregulation, a fundamental physiological process for the maintenance of salts during water absorption. Therefore, in environments where the concentration of salts (mainly Na^+ and Cl^-) is similar to or greater than that in blood plasma, as was the case at a salinity level of 4 ppt, the tendency is for a reduction in energy expenditure related to osmoregulation itself, allowing an increase in energy supply for physiological factors related to body growth and other biological functions (breathing, swimming, digestion, etc.; Griffith, 2017).

Marques et al. (2004) reported a mean Kn of 1.17 ± 0.0, which was greater than the mean Kn of 1.00 ± 0.1 observed for matrinxã in our study. Tavares-Dias et al. (2008b) described a Kn of 1.00 for tambaqui and matrinxã juveniles that were nearly 90 days old and randomly collected in semi-excavated ponds.

Santos et al. (2015) evaluated the growth performance of tambaqui reared in semi-excavated ponds, with a stocking density of 1.0 fish m^{-2} and fed commercial fish feed or cassava mass, verified that the Kn factor could range within the same productive cycle between values above and below the centralizing value of Kn = 1.0, depending on issues related to climatic seasonality (season of the year) and the direct relationship between the metabolic rate and the age of the animal. By correlating the hematological and condition factor Kn data, it is possible to affirm that the relationship between the observed weight (Wo) and the expected weight (We) is an efficient tool for evaluating aquaculture conditions in closed or intensive farming systems.

The growth and hematological results observed for both species shows that feeding only five days a week can provide the nutritional needs for tambaqui and matrinxã reared in biofloc technology. These growth performance data and feeding results are important due to the reduced or compatible FCR with agroeconomic activity that may generate profits for producers and investors.

Therefore, several extremely important factors must be considered to improve the direct relationship between produced biomass and feed intake: efficient management of water quality; stocking density compatible with the animal size and the respective period inside the production cycle (larviculture, second growth, and grow out phases); a feeding diet that offers adequate levels of crude protein in relation to the animal size/age; and an increase in the supply of inert feed available for consumption (phytoplankton and zooplankton) for filter feeder species (Crab et al., 2012).

The effects of fasting and refeeding cycles have already been studied for both species. Despite their reduced spawning capacity, matrinxã did not exhibit any damage in terms of growth, centesimal composition, gonadal development, fertilization, hatching, or survival (Carvalho and Urbinati, 2005; Camargo and Urbinati, 2008). Santos et al. (2018) studied the behavior of tambaqui under conditions of feed restriction and refeeding in an intensive cage rearing environment and reported that a feeding frequency of five days of feeding and two days of fasting is the best feeding management for reducing costs without negative effects on growth, a result that coincides with those achieved in our study.

A comparison of the two species tested in our study revealed that, compared with tambaqui, matrinxã is considered to have the lowest capacity to consume inert feed in the aquatic environment because it has thicker gill rakers that are fewer and more separated from each other (Arbeláez-Rojas et al., 2002). The use of biofloc by fish is fundamental for increasing feed diversity in aquaculture environments, allowing the recycling of unused feed and protein by the species reared (Avnimelech, 2007).

5. Conclusions

The treatments tested showed comparatively similar results for growth performance and hematological conditions for both species reared in biofloc technology enriched with sodium chloride during the early growth phase (above 40 g). Therefore, upon analyzing these results and comparing our findings with those of other related studies, we found that the feeding frequency tested does not negatively affect tambaqui or matrinxã rearing. Consequently, we recommend to the fish farmers of tambaqui and matrinxã the feed frequency of five days per week.

Author contributions

Conceptualization: Marinho-Pereira, T. and Aride, P. H. R. **Data curation:** Oliveira, A. T. **Formal analysis:** Marinho-Pereira, T.; Aride, P. H. R. and Oliveira, A. T. **Funding acquisition:** Cavero, B. A. S. **Investigation:** Marinho-Pereira, T. and Oliveira, C. P. F. **Methodology**: Cavero, B. A. S.; Oliveira, C. P. F. and Aride, P. H. R. **Project administration:** Cavero, B. A. S. **Resources:** Cavero, B. A. S. **Supervision:** Oliveira, A. T. **Validation:** Oliveira, C. P. F. and Oliveira, A. T. **Visualization:** Oliveira, C. P. F. and Oliveira, A. T. **Writing – original draft**: Marinho-Pereira, T. **Writing – review & editing**: Aride, P. H. R. and Oliveira, A. T.

Conflict of interest

The authors declare no conflict of interest.

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