

Dietary protein levels for juvenile matrinxã (*Brycon amazonicus*) reared in biofloc and clear water systems

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ABSTRACT - This study evaluated the potential for rearing matrinxã (*Brycon amazonicus*) with different crude protein (CP) levels and using production performance, water quality, and animal welfare as indicators. To achieve this, 720 juvenile fish (3.65 ± 0.17 g) were distributed in 24 experimental units (200 L). The study, which lasted for 60 days, followed a factorial design (2×4) with two production systems: biofloc technology (BFT) and clear water (CW) and four CP levels (24, 30, 36, and 42%) in the diet, with three repetitions for each. We found that the BFT system showed a lower total ammonia level (0.45 ± 0.15 mg/L) compared with CW (1.16 ± 0.50 mg/L). Fish produced in the BFT system exhibited better performance in terms of apparent feed conversion (1.06 ± 0.15), productivity (7.68 ± 2.38 g/m³), and survival ($76.95 \pm 9.15\%$). The type of production system affected total leukocytes and neutrophils in the blood. Juvenile matrinxã can be fed a diet containing 30% CP in both production systems. Furthermore, the results demonstrated that bioflocs do not confer a direct benefit to juvenile matrinxã in terms of their feeding; however, the BFT system is a promising alternative for increasing the survival and improving animal welfare during the rearing phase.

Keywords: feeding, fish production, ideal protein, production system

1. Introduction

Among current production technologies, the biofloc system (BFT) can be considered sustainable in terms of the use of water, physical space, and its generation of effluents (McCusker et al., 2023; Khanjani et al., 2024a). This technology guarantees an additional source of food for aquatic organisms through the conversion of nitrogenous waste into microbial flocs (Silva et al., 2018; Khanjani et al., 2023a; Dos Santos et al., 2021, 2023, 2024; McCusker et al., 2023), in addition to contributing to the improvement of water quality, which has a direct influence on the performance and reproduction of aquatic organisms (Dos Santos et al., 2023; Khanjani et al., 2024b). The water in the system can be reused in subsequent production cycles due to the presence of a microbiota that acts in the cycling of nitrogenous waste, which, when kept in suspension in the water column by continuous aeration, can be used as a nutritional source for the farmed animals (Vadhel et al., 2020; Dos Santos et al., 2023; McCusker et al., 2023).

The microbial floc present in the BFT system can serve as an additional food source, thereby reducing the feed supply and/or the amount of crude protein (CP) in the feed offered. This makes the system more economical and contributes to fish performance (Lara et al., 2017; Dos Santos et al., 2023, 2024; Khanjani et al., 2023b). The potential application of this technology has already been evaluated using juvenile tambaqui (*Colossoma macropomum*), a fish native to the Amazon and Orinoco basins, as demonstrated by Dos Santos et al. (2021, 2023, 2024). The use of the BFT system can be a valuable strategy for increasing the production of fish species of great economic interest. This method has already been successfully implemented in other countries for shrimp species, as demonstrated by Emerenciano et al. (2013b,c), for the Pacific white shrimp (*Litopenaeus vannamei*), and red tilapia (*Oreochromis niloticus*), as demonstrated by Widanarni et al. (2012).

The matrinxã, *Brycon amazonicus*, has high commercial interest due to its good acceptance in the consumer market and easy adaptation to confined systems (Nascimento et al., 2020). According to Mattos et al. (2013), it has great potential in the Amazon region and other regions of Brazil due to its omnivorous feeding habits and to its excellent production performance rates in various farming systems. However, despite being a species with great productive/economic potential (IBGE, 2021), its production technology still needs to be better structured, especially in the larviculture and rearing phase of the species, which presents intraspecific cannibalism and aggressive behavior (Carvalho et al., 2018; Ferreira et al., 2020; Izel-Silva et al., 2024).

As previously demonstrated by Izel-Silva et al. (2024), the reduction in luminosity due to the turbidity of the water in the biofloc system contributes to a reduction in aggressive behavior and an increase in the productivity of the species. A reduction in light intensity in the water leads to an increase in melatonin production, which in turn reduces the aggressive behavior of larval-stage or juvenile matrinxã (Lopes et al., 2018; Amaral et al., 2020; Cornélio et al., 2021). It may also contribute to a reduction of protein levels in the feed, as already found with tilapia (*Oreochromis* sp.) (Day et al., 2016; Luo et al., 2017; Silva et al., 2018) and carp (*Cyprinus* sp.) (Castro et al., 2016; Najdegerami et al., 2016) in the juvenile phase.

Furthermore, despite the species' market potential, matrinxã production is still in its early stages. It is therefore important to study production management strategies, such as the BFT system, which can increase productivity and reduce production costs by providing additional nutrients to the fish such as microbial protein. Dos Santos et al. (2023, 2024) discovered that it is feasible to decrease the CP levels in tambaqui feed without compromising performance or animal welfare, as well as improving the digestibility and absorption of food. Although matrinxã is not a filter-feeding species like tambaqui, it does consume zooplankton in its juvenile stage, and in the natural environment, fish between 25-50 mm standard length, for example, can have up to 80% of their diet made up of zooplankton microorganisms (mainly microcrustaceans) and insect larvae (Leite, 2004). As a result, it is not really known how much of the microorganisms present in the BFT the matrinxã could consume or how significant this intake could be in reducing the amount of feed offered or the protein in the diet. Thus, the objective of this study was to determine the optimal percentage of CP in the diet of juvenile matrinxã that would promote the best animal performance and welfare for the species, without compromising water quality in the clear water (CW) and BFT systems.

2. Material and methods

2.1. Ethical approval

The experiment was carried out at the Laboratório de Fisiologia Aplicado à Piscicultura. (LAFAP), of the Estação Experimental de Aquicultura of the Coordenação de Tecnologia e Inovação (COTEI) at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, and was approved by the Ethics Committee on the Use of Animals (CEUA) at INPA (No. 040/2020, SEI 01280.001374/2020-68).

2.2. Experimental design

Juvenile matrinxã weighing approximately 0.5 g were obtained from a commercial fish farm and acclimated for 40 days in 2,000-L fiberglass tanks with bioflocs and constant aeration. After the

acclimatization period, 720 Juvenile matrinxã (weighing 3.6 ± 0.1 g) were distributed in a factorial design (2×4) that involved two production systems (BFT and CW) and four CP levels in the diet (24, 30, 36, and 42%), with three repetitions each. Matrinxã juveniles ($n = 30$, equivalent to 150 fish/m^3) were distributed in 24 circular polyethylene tanks of 500 L (filled with 200 L of water) with intense and continuous aeration, using microperforated hoses to suspend particulate matter in the water column. Each experimental unit was maintained in isolation with no interconnectivity between them in both the BFT and CW systems. The study was conducted for 60 days.

The experimental unit in the BFT system received an inoculum of bioflocs that had been matured in the laboratory and corresponded to 50% of the volume used in each experimental unit, following the methodology of Kruppenauer et al. (2012). In the CW treatments, a 50% water exchange occurred daily throughout the experimental period.

2.3. Experimental diets

The feeds (Table 1) used in the experiment were isocaloric; the ingredients of which were ground to 1 mm, homogenized in a mixer, and processed in a commercial extruder (INBRA MX-80, Inbramaq, Ribeirão Preto, Brazil), and the 2–4 mm pellets were dried using forced ventilation (55°C). The animals were fed three times a day (8:00, 12:00, and 17:00 h) until apparent satiety, with the amount adjusted daily by observing the leftover feed 15 min after offering the feed.

Table 1 - Formulation and nutritional composition of the experimental diets with different crude protein levels for juvenile *Brycon amazonicus* in biofloc and clear water systems for 60 days

Ingredient (%)	Crude protein level			
	24%	30%	36%	42%
Soybean meal	10.45	25.35	29.72	30.58
Corn grain	44.42	30.48	16.17	3.49
Corn gluten	12.00	8.00	13.00	19.50
Corn starch	10.00	10.00	10.00	10.00
Offal meal	6.96	11.50	15.00	19.50
Fish meal	5.00	5.00	5.00	5.00
Meat and bone meal	2.00	2.00	2.00	2.00
Wheat flour	2.00	2.00	2.00	2.00
Soy oil	2.80	1.77	1.34	0.50
Dicalcium phosphate	1.80	1.05	0.60	0.00
Limestone	1.05	0.83	0.65	0.46
Inert	0.00	0.50	3.00	5.45
L-lysine	0.60	0.60	0.60	0.60
DL-methionine	0.40	0.40	0.40	0.40
Mineral supplement	0.50	0.50	0.50	0.50
BHT	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Centesimal composition (%)				
Dry matter	91.24	90.23	94.13	90.64
Crude protein	24.40	30.20	36.90	42.80
Ether extract	6.80	4.80	4.20	4.00
Crude fiber	1.30	1.50	1.90	2.00
Ash	4.80	4.58	6.84	6.82
Gross energy (kcal)	4,086.85	4,086.85	4,086.85	4,086.84

Premix: folic acid (minimum), 100.00 mg/kg; nicotinic acid (minimum), 10.00 g/kg; pantothenic acid (minimum), 5,000.00 mg/kg; BHT (minimum), 5,000.00 mg/kg; biotin (minimum), 50.00 mg/kg; cobalt (minimum), 30.00 mg/kg; copper (minimum), 1,000.00 mg/kg; choline (minimum), 80.00 g/kg; iron (minimum), 7,500.00 g/kg; iodine (minimum), 250.00 mg/kg; inositol (minimum), 4,000.00 g/kg; manganese (minimum), 2,000.00 mg/kg; magnesium (minimum), 600.00 mg/kg; potassium (minimum), 2,000.00 mg/kg; selenium (minimum), 70.00 mg/kg; vitamin A (minimum), 2,000,000.00 IU/kg; vitamin B1 (minimum), 2,000.00 mg/kg; vitamin B2 (minimum), 4,000.00 mg/kg; vitamin B12 (minimum), 10,000.00 mcg/kg; vitamin C (minimum), 80.00 g/kg; vitamin B6 (minimum), 5,000.00 mg/kg; vitamin D3 (minimum), 600,000.00 IU/kg; vitamin E (minimum), 15,000.00 IU/kg; vitamin K3 (minimum), 700.00 mg/kg; zinc (minimum), 7,500.00 mg/kg.

2.4. Water quality analyses

Temperature, pH, dissolved oxygen, and salinity were checked daily using a multiparameter probe (Akso, AK88). The total ammonia concentration (Verdouw et al., 1978) and nitrite (Boyd and Tucker, 1992) in the water were assessed three times a week. Nitrate (adapted from Papaspyrou et al., 2014) and turbidity (NTU; portable turbidimeter - Hanna, HI93703C) were measured once a week.

Total suspended solids levels were checked every week by filtering 20 mL of water through GF 50-A glass fiber filters (Strickland and Parsons, 1972). The volume of settleable solids was monitored twice a week using Imhoff cones (Avnimelech, 2007). The alkalinity (mg/L of CaCO₃) was monitored three times a week using acid-base titration and the methyl-orange indicator (APHA, 1998), with a target of maintaining a level above 50 mg/L, and, when necessary, corrections were made with sodium bicarbonate (Avnimelech, 2009). In the CW system, the alkalinity was always adjusted after changing the water in the system.

2.5. Analyses of animal performance and somatic indices

To evaluate productive performance, the weight of each fish was measured using biometric assessments at the beginning and end of the experiment. For this, the animals were anesthetized in a benzocaine solution (100 mg/L) and, from the weight obtained, the following was calculated: average final weight (FW) = final weight of all fish/number of fish; daily weight gain (DWG) = (average final weight (g) – average initial weight (g))/days; specific growth rate (SGR) = [(ln final average weight (g) – ln initial average weight (g))/days] × 100; apparent feed conversion (AFC) = feed intake (g)/weight gain (g); survival (S%) = (number of animals at the end of the experiment/number of animals at the beginning of the experiment) × 100, and productivity (g/m³) = (total biomass/tank volume)/1,000; protein efficiency ratio (PER%) = average weight gain/(CP in the diet × average feed intake).

For the analysis of the somatic indices, three fish from each experimental unit were euthanized in a benzocaine solution (300 mg/L) for dissection of the total viscera and liver, and weighted. The following calculations were used: viscerosomatic index (%) = (viscera weight / live weight) × 100 and hepatosomatic index (%) = (liver weight / live weight) × 100.

2.6. Blood collection and determination of blood parameters

To evaluate the blood parameters, three fish from each experimental unit were previously anesthetized in a benzocaine solution (100 mg/L) and had blood collected through puncture of the tail vein using a syringe with heparin anticoagulant (10 µL). The following parameters were analyzed: hematocrit (Ht), using the microhematocrit method; hemoglobin ([Hb]) concentration, using the cyanmethemoglobin method and commercial reagents; and erythrocyte count (RBC), using a Neubauer chamber and a formaldehyde-citrate solution. The total and differential leukocyte count was performed using blood samples previously stained with May-Grünwald-Giemsa-Wright (MGGW) stain according to the methodology in Tavares-Dias and Moraes (2004). The number of leukocytes and thrombocytes were quantified from the 2,000 erythrocyte count and, for the differential blood cell count, 200 leukocytes were identified and classified as lymphocytes, monocytes, or neutrophils.

From the Ht, [Hb], and RBC results, the Wintrobe indices were calculated: mean corpuscular volume (MCV) = hematocrit × 10/number of erythrocytes (fl); mean corpuscular hemoglobin (MCH) = [Hb] × 10/RBC (pg); and mean corpuscular hemoglobin concentration (MCHC) = [Hb] × 100/Ht (g/dL). The values of cholesterol, triglycerides, and glucose in the plasma were determined using the enzymatic colorimetric method using commercial kits, and total proteins were determined using the modified biuret method.

2.7. Assessments of the nutritional composition of fish and bioflocs

To determine the proximal composition of fish, three animals were collected per experimental unit, then euthanized in benzocaine (300 mg/L) and subsequently stored in a freezer at –18 °C. The samples

were dehydrated in a ventilated oven at 55 °C until constant weight and ground in a multiprocessor to obtain uniform samples. Dry matter (%) was determined in an oven at 105 °C, CP was obtained using the micro-Kjehldal method, ether extract was analyzed through continuous extraction with petroleum ether in a Soxhlet extractor, and ash was obtained via burning in a muffle furnace at 550 °C (AOAC, 2012).

For the proximal analysis of the flocs, at the end of the experiment, 80 L of suspended bioflocs were collected from each experimental unit and transferred to conical tanks with a capacity of 100 L. After decanting for 3 h, approximately 1 L of sedimented material was collected and stored at -18 °C. This material was dried in a ventilated oven at 55 °C and macerated to obtain uniform samples for the determination of CP, ether extract, and ash (AOAC, 2012).

2.8. Statistical analysis

For all water quality and performance evaluations, each cage containing 30 juvenile matrinxã was considered an experimental unit. For somatic indices and hematological variables, each fish was considered an experimental unit, with nine repetitions of each treatment. Data were first checked for normality of studentized residuals using the Shapiro-Wilk test with the InfoStat statistical program (version 2020), and homogeneity of variances using the Bartlett test with R software. Only for the proximal composition of the bioflocs, one-way analysis of variance (one-way ANOVA) was performed. All other data was subjected to two-way analysis of variance (two-way ANOVA), and the means were compared by Tukey's test. The significance level accepted for all the tests carried out was $\alpha = 0.05$.

The statistical model for one-way ANOVA used to test the effect of treatments was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

in which Y_{ij} = is the observation of experimental units ($j = 1, 2, 3$) in the factor protein level ($i = 24, 30, 36$ and 42%), μ = the overall mean, τ_i = fixed effect of factor i protein levels ($i = 24, 30, 36$ and 42%), and ε_{ij} = the random error associated with each observation Y_{ij} .

The statistical model for two-way ANOVA used to test the effect of treatments was:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk}$$

in which Y_{ijk} = value observed, μ = the overall mean, τ_i = effect of production system (Biofloc and Clear Water), γ_j = effect of protein level (24, 30, 36 and 42%), $\tau\gamma$ = effect of interaction between production system and protein level, and ε_{ijk} = random error associated with each observation Y_{ijk} .

3. Results

3.1. Water quality

No significant differences in temperature were observed between the treatments. The concentration of total ammonia in the water was only affected by the production system, with lower levels observed in the biofloc system (0.45 ± 0.15 mg/L; $P < 0.0001$). The production system had no effect on pH or nitrite concentration. Salinity (1.45 ± 0.13 ppt; $P < 0.0001$) was higher and dissolved oxygen (DO = 6.03 ± 0.10 mg/L; $P < 0.0015$) was lower in the BFT system. There were no significant differences in pH and nitrite levels at the three lowest CP levels. Dissolved oxygen was higher in the water with fish fed 24% CP and the two lowest CP levels resulted in lower salinity (Table 2).

The study found a significant interaction between the CP level and the production system in relation to nitrate, alkalinity, total suspended solids (TSS), settleable solids (SS), and turbidity ($P < 0.05$). However, only alkalinity was affected by the different CP levels in the CW system. On the other hand, reducing the CP level in the diet resulted in lower concentrations of nitrate, TSS, SS, and turbidity and an increase in the concentration of alkalinity in the BFT system (Table 3).

Table 2 - Mean \pm standard deviation of water quality variables of the biofloc (BFT) and clear water (CW) systems verified over 60 days during the production of juvenile matrinxã (*Brycon amazonicus*) fed diet with different crude protein levels

Variable	Temperature (°C)	DO (mg/L)	pH	Salinity (ppt)	Ammonia (mg/L)	Nitrite (mg/L)
BFT	27.04 \pm 0.13	6.03 \pm 0.10B	7.19 \pm 0.28	1.45 \pm 0.13A	0.45 \pm 0.15B	0.49 \pm 0.22
CW	27.04 \pm 0.12	6.16 \pm 0.11A	7.27 \pm 0.18	0.06 \pm 0.03B	1.16 \pm 0.50A	0.38 \pm 0.30
Protein level (%)						
24	26.98 \pm 0.14	6.26 \pm 0.10b	7.43 \pm 0.06b	0.69 \pm 0.68a	0.53 \pm 0.25	0.22 \pm 0.09a
30	27.08 \pm 0.08	6.08 \pm 0.11a	7.26 \pm 0.08ab	0.75 \pm 0.76ab	0.80 \pm 0.35	0.39 \pm 0.17ab
36	27.06 \pm 0.12	6.07 \pm 0.12a	7.20 \pm 0.14ab	0.83 \pm 0.82b	0.83 \pm 0.50	0.46 \pm 0.27ab
42	27.03 \pm 0.17	5.98 \pm 0.08a	7.03 \pm 0.24a	0.79 \pm 0.79ab	1.07 \pm 0.77	0.68 \pm 0.28b
BFT						
Protein level (%)						
24	26.96 \pm 0.13	6.23 \pm 0.04	7.44 \pm 0.09	1.31 \pm 0.09	0.31 \pm 0.06	0.24 \pm 0.04
30	24.08 \pm 0.07	5.99 \pm 0.08	7.24 \pm 0.09	1.44 \pm 0.04	0.51 \pm 0.21	0.40 \pm 0.08
36	27.08 \pm 0.17	5.98 \pm 0.04	7.17 \pm 0.12	1.57 \pm 0.15	0.47 \pm 0.14	0.61 \pm 0.19
42	27.03 \pm 0.25	5.93 \pm 0.07	6.98 \pm 0.16	1.50 \pm 0.11	0.53 \pm 0.09	0.72 \pm 0.72
CW						
Protein level (%)						
24	27.01 \pm 0.17	6.28 \pm 0.15	7.42 \pm 0.01	0.07 \pm 0.03	0.75 \pm 0.10	0.20 \pm 0.13
30	27.09 \pm 0.10	6.17 \pm 0.04	7.28 \pm 0.07	0.05 \pm 0.01	1.09 \pm 0.10	0.38 \pm 0.25
36	27.04 \pm 0.08	6.16 \pm 0.11	7.21 \pm 0.19	0.09 \pm 0.05	1.19 \pm 0.47	0.31 \pm 0.27
42	27.04 \pm 0.11	6.02 \pm 0.05	7.17 \pm 0.26	0.07 \pm 0.01	1.61 \pm 0.76	0.64 \pm 0.42
Two-way ANOVA						
System	NS	<0.0015	NS	<0.0001	<0.0001	NS
Protein	NS	<0.0002	<0.0016	<0.0391	NS	<0.0196
Interaction	NS	NS	NS	NS	NS	NS

DO - dissolved oxygen; NS - not significant.

Means followed by different letters in the same column differ according to ANOVA and Tukey's test ($P < 0.05$).

3.2. Animal performance and somatic indices

No significant differences were found in FW, SGR, DWG, PER, and feed intake between the production systems (Table 4). However, fish produced in the biofloc system showed higher productivity (7.68 \pm 2.38 g/m³; $P < 0.0001$) and survival rates 76.95 \pm 9.15%, and lower apparent feed conversion (AFC = 1.06 \pm 0.15; $P < 0.0424$) (Table 4).

The 24% CP level had generally the worst performance, while there was no significant difference among the 30, 36, and 42% CP levels. Additionally, the CP levels did not affect animal survival. In BFT, the hepatosomatic (HSI = 2.45 \pm 0.48%; $P < 0.0006$) and viscerosomatic (VSI = 11.27 \pm 2.94%; $P < 0.0042$) indices were higher. The CP levels did not have a significant effect on the HSI. However, juveniles that were fed with 42% CP had a lower VSI (7.73 \pm 1.22%; $P < 0.0181$) compared with those fed 24% CP (12.30 \pm 3.82%; Table 4).

3.3. Blood parameters

Blood parameters (Tables 5 and 6) of the fish (in BFT and CW) showed no significant differences between the systems. The blood parameters of the juveniles fed different CP levels in the diet also showed no significant differences, except for Hb concentration ($P < 0.0262$). The 42% CP level in the diet showed a higher (9.28 \pm 0.78 g/dL) hemoglobin concentration than those fed the 24% CP diet (8.26 \pm 3.88 g/dL), while there was no difference among the three highest CP levels.

Table 3 - Mean \pm standard deviation of water quality variables of the biofloc (BFT) and clear water (CW) systems verified over 60 days during the production of juvenile matrinxã (*Brycon amazonicus*) fed diet with different crude protein levels

Variable	Nitrate (mg/L)	Alkalinity CaCO ₃ (mg/L)	TSS (mg/L)	SSV (mg/L)	Turbidity (UNF)
BFT	129.98 \pm 36.83A	50.59 \pm 11.16A	192.54 \pm 44.07A	12.15 \pm 8.25A	76.47 \pm 19.11A
CW	9.61 \pm 4.09B	45.34 \pm 3.55B	25.33 \pm 6.80B	0.19 \pm 0.08B	4.73 \pm 1.37B
Protein level (%)					
24	52.13 \pm 49.09c	55.24 \pm 8.71c	80.33 \pm 69.00c	1.70 \pm 1.87a	26.93 \pm 26.25c
30	58.60 \pm 51.92bc	52.20 \pm 4.35c	101.25 \pm 85.02b	5.55 \pm 6.47b	38.94 \pm 37.28b
36	80.70 \pm 79.50ab	45.04 \pm 3.61b	117.33 \pm 99.04ab	6.57 \pm 7.08ab	45.44 \pm 45.57ab
42	87.78 \pm 89.05a	38.68 \pm 3.97a	136.83 \pm 99.0a	10.87 \pm 13.22a	51.08 \pm 49.41a
BFT					
Protein level (%)					
24	96.49 \pm 10.76Ab	63.79 \pm 1.78Aa	141.00 \pm 27.37Ad	3.30 \pm 1.04Ac	50.32 \pm 8.88Ad
30	104.35 \pm 19.68Ab	56.07 \pm 1.28Ab	177.50 \pm 24.07Ac	10.90 \pm 4.35Ab	72.74 \pm 6.78Ac
36	152.29 \pm 20.00Aa	46.05 \pm 4.95Ac	207.00 \pm 19.83Ab	12.90 \pm 2.19Ab	86.73 \pm 8.79Ab
42	166.82 \pm 32.80Aa	36.48 \pm 4.64Ad	244.67 \pm 15.73Aa	21.50 \pm 9.87Aa	96.08 \pm 5.09Aa
CW					
Protein level (%)					
24	7.76 \pm 0.58Ba	48.11 \pm 1.64Ba	19.67 \pm 10.67Ba	0.10 \pm 0.10Aa	3.53 \pm 1.41Ba
30	12.84 \pm 8.01Ba	48.32 \pm 0.80Ba	25.00 \pm 7.05Ba	0.20 \pm 0.001Ba	4.16 \pm 1.08Ba
36	9.11 \pm 0.53Ba	44.04 \pm 2.24Aab	27.67 \pm 2.93Ba	0.23 \pm 0.06Ba	5.14 \pm 1.09Ba
42	8.74 \pm 2.17Ba	40.87 \pm 1.86Ab	29.00 \pm 2.63Ba	0.23 \pm 0.06Ba	6.07 \pm 0.64Ba
Two-way ANOVA					
System	<0.0001	<0.0003	<0.0001	<0.0001	<0.0001
Protein	<0.0037	<0.0001	<0.0002	<0.0082	<0.0001
Interaction	<0.0027	<0.0001	<0.0010	<0.0092	<0.0001

TSS - total suspended solids; SSV - settleable solids volume; NS - not significant.

Means followed by different letters, uppercase for comparison of production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test ($P < 0.05$).

We found an interaction ($P < 0.0223$) between production systems and CP level for the variable Ht. Juveniles produced in the BFT system had a lower average Ht value ($30.06 \pm 2.05\%$) than those produced in the CW system and feeding the 24% CP diet ($33.22 \pm 4.32\%$). Hematocrit was not affected by CP levels in juveniles in the CW system, and there was no significant difference in Ht in the higher CP levels in the BFT (Table 5). The profile of defense cells (Table 6) showed differences only between the BFT and CW systems in terms of total leucocytes and neutrophils ($P < 0.0001$), with higher averages in the juvenile matrinxã reared in BFT (total leucocytes = $36.31 \pm 12.54 \times 10^3/\mu\text{L}$ and neutrophils = $0.81 \pm 1.11 \times 10^3/\mu\text{L}$) compared with the CW system (total leucocytes = $29.74 \pm 9.74 \times 10^3/\mu\text{L}$ and neutrophils = $0.09 \pm 0.22 \times 10^3/\mu\text{L}$).

3.4. Nutritional composition of the fish and the bioflocs

For the mean composition values of the juvenile matrinxã at the end of the study (Table 7), there was no significant difference in CP content in the carcass. However, the ether extract content was higher ($P < 0.0058$) in juveniles reared in the BFT system ($22.04 \pm 2.36\%$) compared with those in the CW system ($20.35 \pm 1.69\%$). Additionally, a significant interaction was observed with dry matter ($P < 0.0365$) and ash ($P < 0.0327$). No difference was found in the dry matter content in the nutritional composition of matrinxã reared in the BFT system and fed 30 to 42% CP levels. The nutritional composition of the biofloc did not show any significant differences in CP or ether extract at different levels of CP. However, the mineral matter content was lower ($24.53 \pm 2.16\%$; $P < 0.0003$) when the percentage of CP in the diet was higher (Table 8).

Table 4 - Mean \pm standard deviation of productive parameters and somatic indices of juvenile matrixã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days

Variable	FW (g)	SGR (%/day)	DWG (g/day)	AFC	Productivity (g/m ³)	Survival (%)	Feed intake (g)	VSI (%)	HSI (%)	PER (%)
BFT	66.16 \pm 18.33	4.78 \pm 0.53	1.04 \pm 0.30	1.06 \pm 0.15A	7.68 \pm 2.38B	76.95 \pm 9.15A	1458.72 \pm 374.34A	11.27 \pm 2.94A	2.45 \pm 0.48A	3.00 \pm 0.48
CW	64.34 \pm 24.83	4.62 \pm 0.75	1.02 \pm 0.41	1.16 \pm 0.13B	4.57 \pm 1.39A	50.00 \pm 12.31B	929.48A \pm 309.71B	8.32 \pm 2.25B	1.59 \pm 0.44B	2.90 \pm 0.69
Protein level (%)										
24	41.23 \pm 19.19b	3.94 \pm 0.67b	0.62 \pm 0.32b	1.25 \pm 0.07a	3.60 \pm 1.41b	60.00 \pm 14.46	758.20 \pm 328.86b	12.30 \pm 3.82a	2.13 \pm 0.94	3.61 \pm 0.27a
30	71.81 \pm 15.436a	4.95 \pm 0.36a	1.14 \pm 0.26a	1.09 \pm 0.13ab	6.69 \pm 2.05a	62.78 \pm 15.83	1303.68 \pm 285.43a	9.56 \pm 2.28ab	2.04 \pm 0.48	3.19 \pm 0.40a
36	66.88 \pm 17.10ab	4.78 \pm 0.49a	1.06 \pm 0.28ab	1.06 \pm 0.10b	6.74 \pm 2.27a	68.33 \pm 18.23	1295.29 \pm 452.90a	9.59 \pm 2.55ab	2.03 \pm 0.64	2.69 \pm 0.32b
42	81.07 \pm 12.38a	5.13 \pm 0.72a	1.29 \pm 0.21a	1.04 \pm 0.17b	7.46 \pm 2.46a	62.78 \pm 23.52	1419.23 \pm 381.00a	7.73 \pm 1.22b	1.89 \pm 0.51	2.31 \pm 0.30b
BFT										
Protein level (%)										
24	38.32 \pm 6.63	3.93 \pm 0.16	0.57 \pm 0.12	1.27 \pm 0.08	4.17 \pm 0.95	72.22 \pm 3.85	945.69 \pm 254.38	14.62 \pm 3.99	2.83 \pm 0.73	3.38 \pm 0.18Aa
30	73.41 \pm 10.46	5.01 \pm 0.17	1.17 \pm 0.17	0.98 \pm 0.09	8.31 \pm 1.07	75.56 \pm 1.93	1516.74 \pm 102.91	11.52 \pm 0.68	2.38 \pm 0.30	3.46 \pm 0.32Aa
36	72.28 \pm 2.87	5.00 \pm 0.11	1.15 \pm 0.05	1.05 \pm 0.05	8.56 \pm 1.39	78.89 \pm 11.70	1665.41 \pm 214.82	10.37 \pm 0.95	2.37 \pm 0.29	2.64 \pm 0.10Ab
42	80.62 \pm 8.65	5.19 \pm 0.14	1.29 \pm 0.14	0.95 \pm 0.10	9.68 \pm 0.94	81.11 \pm 15.40	1729.84 \pm 237.76	8.60 \pm 1.05	2.23 \pm 0.49	2.51 \pm 0.26Ab
CW										
Protein level (%)										
24	44.14 \pm 29.18	3.96 \pm 1.04	0.68 \pm 0.48	1.23 \pm 0.08	3.03 \pm 1.74	47.78 \pm 7.70	593.50 \pm 376.33	9.98 \pm 2.09	1.42 \pm 0.46	3.38 \pm 0.02Aa
30	70.21 \pm 21.86	4.88 \pm 0.55	1.11 \pm 0.37	1.19 \pm 0.07	5.08 \pm 1.23	50.00 \pm 11.55	1090.60 \pm 238.57	7.60 \pm 1.05	1.70 \pm 0.37	2.91 \pm 0.28Bb
36	61.48 \pm 25.20	4.56 \pm 0.66	0.98 \pm 0.42	1.02 \pm 0.16	4.91 \pm 0.95	57.75 \pm 18.95	925.17 \pm 235.96	8.80 \pm 3.68	1.70 \pm 0.78	2.74 \pm 0.10Aabc
42	81.53 \pm 17.55	5.07 \pm 0.45	1.30 \pm 0.29	1.18 \pm 0.15	5.25 \pm 0.65	44.44 \pm 11.71	1108.60 \pm 130.17	6.88 \pm 0.58	1.54 \pm 0.23	2.11 \pm 0.20Ac
Two-way ANOVA										
System	NS	NS	NS	<0.0424	<0.0001	<0.0001	<0.0001	<0.0042	<0.0006	NS
Protein	<0.0082	<0.0052	<0.0083	<0.0092	<0.0001	NS	<0.0007	<0.0181	NS	<0.0001
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.0197

FW - final weight; SGR - specific growth rate; DWG - apparent feed gain; AFC - apparent feed conversion; VSI - viscerosomatic index; HSI - hepatosomatic index; PER - protein efficiency ratio. Means followed by different letters, uppercase for comparison of production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test (P<0.05).

Table 5 - Mean \pm standard deviation of blood parameters of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days

Variable	Ht (%)	Hb (g/dL)	RBC ($\times 10^6/\mu\text{L}$)	MCV (fl)	MCH (pg)	MCHC (g/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)	Total protein (g/dL)
BFT	34.79 \pm 4.68	8.80 \pm 2.42	1.71 \pm 0.22	202.23 \pm 35.57	54.75 \pm 16.37	26.74 \pm 8.18	201.71 \pm 33.62	613.75 \pm 166.58	106.11 \pm 9.06	3.11 \pm 0.42
CW	36.03 \pm 4.13	8.91 \pm 1.01	1.72 \pm 0.27	215.23 \pm 44.87	53.10 \pm 10.41	24.90 \pm 3.03	204.53 \pm 47.07	585.40 \pm 188.19	107.62 \pm 11.7	3.31 \pm 0.09
Protein level (%)										
24	33.22 \pm 4.3b	8.26 \pm 3.88a	1.71 \pm 0.20	197.00 \pm 34.56	53.50 \pm 20.16	27.96 \pm 11.24	222.74 \pm 43.59	689.39 \pm 145.04	102.71 \pm 4.14	3.08 \pm 0.44
30	36.44 \pm 4.70a	8.25 \pm 0.93ab	1.78 \pm 0.21	205.75 \pm 26.82	50.08 \pm 6.39	24.51 \pm 2.82	197.78 \pm 46.39	599.84 \pm 195.99	106.38 \pm 2.35	3.17 \pm 0.30
36	34.50 \pm 4.11b	9.02 \pm 1.11ab	1.65 \pm 0.29	216.93 \pm 56.70	53.77 \pm 14.54	26.30 \pm 3.16	193.82 \pm 35.05	566.03 \pm 160.22	110.15 \pm 16.29	3.09 \pm 0.16
42	37.47 \pm 3.55a	9.28 \pm 0.78b	1.71 \pm 0.26	223.23 \pm 37.65	55.33 \pm 9.63	24.98 \pm 3.19	198.13 \pm 32.58	543.03 \pm 180.70	110.20 \pm 3.19	3.51 \pm 0.33
BFT										
Protein level (%)										
24	30.06 \pm 2.05Bb	7.68 \pm 1.02	1.65 \pm 0.17	158.40 \pm 22.46	47.86 \pm 9.04	25.62 \pm 3.05	216.58 \pm 38.59	710.14 \pm 175.28	104.49 \pm 34.04	3.59 \pm 0.96
30	36.56 \pm 5.54Aa	8.99 \pm 0.94	1.78 \pm 0.15	205.88 \pm 26.50	50.75 \pm 4.98	24.90 \pm 3.10	203.07 \pm 20.76	606.94 \pm 129.52	102.32 \pm 28.70	3.39 \pm 1.05
36	35.17 \pm 4.64Aa	9.17 \pm 1.20	1.67 \pm 0.27	216.05 \pm 48.59	56.84 \pm 14.53	26.30 \pm 3.68	202.77 \pm 36.00	611.24 \pm 210.65	118.78 \pm 27.98	3.21 \pm 1.09
42	37.39 \pm 1.75Aa	9.35 \pm 0.54	1.76 \pm 0.27	217.85 \pm 37.17	54.79 \pm 12.26	25.07 \pm 2.01	184.40 \pm 33.76	526.67 \pm 210.65	101.90 \pm 29.45	3.04 \pm 0.01
CW										
Protein level (%)										
24	36.39 \pm 3.58Aa	8.83 \pm 1.13	1.78 \pm 0.22	208.59 \pm 41.55	50.40 \pm 9.08	24.29 \pm 2.14	228.90 \pm 49.63	668.65 \pm 114.07	100.93 \pm 34.12	2.88 \pm 0.96
30	36.33 \pm 4.04Aa	8.71 \pm 0.96	1.79 \pm 0.27	205.63 \pm 28.74	49.42 \pm 7.81	24.11 \pm 2.64	192.50 \pm 63.87	592.73 \pm 254.44	110.42 \pm 28.14	2.97 \pm 1.10
36	33.83 \pm 3.66Aa	8.86 \pm 1.06	1.63 \pm 0.32	217.81 \pm 66.85	56.70 \pm 15.44	26.30 \pm 2.78	184.87 \pm 33.68	520.83 \pm 198.67	101.52 \pm 35.54	2.98 \pm 0.82
42	37.56 \pm 4.88Aa	9.21 \pm 0.99	1.67 \pm 0.26	288.87 \pm 39.49	55.87 \pm 6.79	24.89 \pm 4.18	211.85 \pm 26.29	559.39 \pm 156.25	118.51 \pm 28.07	3.74 \pm 0.90
Two-way ANOVA										
System	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein	<0.0092	<0.0262	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	<0.0223	NS	NS	NS	NS	NS	NS	NS	NS	NS

Ht - hematocrit; Hb - hemoglobin; RBC - erythrocyte count; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration; NS - not significant. Means followed by different letters, uppercase for production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test ($P < 0.05$).

Table 6 - Mean \pm standard deviation of the total and differential leukocytes counts of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days

Variable	Thrombocytes ($\times 10^3/\mu\text{L}$)	Total leukocytes ($\times 10^3/\mu\text{L}$)	Lymphocytes ($\times 10^3/\mu\text{L}$)	Monocytes ($\times 10^3/\mu\text{L}$)	Neutrophils ($\times 10^3/\mu\text{L}$)
BFT	1.21 \pm 1.45	36.31 \pm 12.54A	29.35 \pm 9.38	6.16 \pm 3.47	0.81 \pm 1.11A
CW	1.11 \pm 0.89	29.74 \pm 9.74B	24.83 \pm 8.03	4.82 \pm 2.48	0.09 \pm 0.22B
Protein level (%)					
24	1.12 \pm 1.46	31.28 \pm 11.38	26.15 \pm 7.51	4.62 \pm 3.57	0.51 \pm 1.27
30	1.15 \pm 0.97	29.17 \pm 13.04	23.70 \pm 9.62	4.99 \pm 3.49	0.48 \pm 0.82
36	0.74 \pm 0.78	34.63 \pm 10.70	28.18 \pm 8.25	6.26 \pm 2.99	0.19 \pm 0.35
42	1.60 \pm 1.42	37.42 \pm 10.86	30.60 \pm 9.78	6.20 \pm 2.01	0.63 \pm 0.83
BFT					
Protein level (%)					
24	1.37 \pm 1.86	32.73 \pm 15.78	26.76 \pm 9.49	5.16 \pm 4.82	0.81 \pm 1.79
30	0.74 \pm 0.70	36.02 \pm 11.50	28.47 \pm 7.41	6.72 \pm 4.07	0.83 \pm 1.08
36	0.72 \pm 0.79	32.23 \pm 12.98	26.05 \pm 10.2	5.85 \pm 3.14	0.34 \pm 0.43
42	1.99 \pm 1.95	44.27 \pm 7.74	36.11 \pm 8.79	6.91 \pm 1.82	1.25 \pm 0.76
CW					
Protein level (%)					
24	0.87 \pm 1.02	29.83 \pm 5.36	25.54 \pm 5.77	4.07 \pm 2.03	0.22 \pm 0.39
30	1.56 \pm 1.07	22.33 \pm 11.38	18.93 \pm 9.71	3.26 \pm 1.75	0.14 \pm 0.17
36	0.76 \pm 0.87	37.50 \pm 7.53	30.73 \pm 4.81	6.77 \pm 3.06	0.00 \pm 0.00
42	1.20 \pm 0.50	30.59 \pm 9.33	25.09 \pm 7.77	5.50 \pm 2.09	0.00 \pm 0.00
Two-way ANOVA					
System	NS	<0.0001	NS	NS	<0.0001
Protein	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS

NS - not significant.

Means followed by different letters in the same column differ according to ANOVA and Tukey's test (P<0.05).

4. Discussion

4.1. Water quality

The benefits of BFT technology include improved performance, welfare, water quality maintenance, and a source of protein (Ogello et al., 2021; Dos Santos et al., 2023, 2024). This system allows for a reduction in CP levels in feed and an increase in productivity (Dos Santos et al., 2023).

The treatments with higher CP levels (30, 36, and 42%) resulted in a lower DO concentration in the water because of the CP in the diet and the accumulation of nitrogenous excreta. The bioflocs contain heterotrophic bacterial biomass, which, along with the oxidation processes of organic matter, consumes DO and reduces its concentration (Manduca et al., 2021). However, the DO levels remained within the acceptable range for fish farming (Boyd, 1982).

The disparity in the pH values between the protein levels can be attributed to the consumption of alkalinity by the chemoautotrophic bacteria and the alkalinity adjustments made with sodium bicarbonate to maintain optimal bacterial activity within the system. Furthermore, the metabolic processes of the animals and microorganisms within the BFT system result in elevated CO₂ levels, which subsequently lead to a reduction in pH (Vinatea et al., 2010; Widanarni et al., 2012). However, these pH values remained within the ideal range for production systems, maintaining homeostasis, animal performance, and the nitrification process (Ebeling et al., 2006; Emerenciano et al., 2017; Figueroa-Espinoza et al., 2022).

Table 7 - Mean \pm standard deviation of the proximal composition of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days (values in % dry basis)

Variable	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
BFT	90.44 \pm 1.85	54.31 \pm 1.52	22.04 \pm 2.36A	8.37 \pm 0.67B
CW	89.99 \pm 2.18	55.03 \pm 1.70	20.35 \pm 1.69B	8.76 \pm 0.71A
Protein level (%)				
24	89.34 \pm 1.72a	53.94 \pm 1.07	21.18 \pm 2.88	8.64 \pm 0.68
30	91.25 \pm 1.94a	54.35 \pm 1.90	21.27 \pm 1.53	8.39 \pm 0.81
36	89.85 \pm 1.95b	55.50 \pm 1.34	20.90 \pm 2.49	8.78 \pm 0.71
42	90.68 \pm 2.03ab	55.09 \pm 1.77	21.50 \pm 1.95	8.46 \pm 0.66
BFT				
Protein level (%)				
24	88.44 \pm 0.96Ab	54.10 \pm 0.82	22.08 \pm 3.90	8.37 \pm 0.47Aa
30	91.27 \pm 2.09Aa	54.28 \pm 2.53	21.33 \pm 2.13	7.84 \pm 0.39Bb
36	90.07 \pm 1.24Aab	55.18 \pm 1.19	22.85 \pm 1.28	8.76 \pm 0.80Aa
42	91.97 \pm 0.50Aa	54.08 \pm 1.81	22.03 \pm 1.61	8.07 \pm 0.43Aab
CW				
Protein level (%)				
24	90.24 \pm 1.92Aa	53.78 \pm 1.33	20.28 \pm 1.04	8.45 \pm 0.85Aa
30	91.23 \pm 1.97Aa	54.42 \pm 1.25	21.20 \pm 0.79	8.94 \pm 0.74Aa
36	89.09 \pm 2.50Aa	55.82 \pm 1.51	18.95 \pm 1.71	8.80 \pm 0.70Aa
42	89.40 \pm 2.20Ba	56.10 \pm 1.81	20.97 \pm 2.21	8.84 \pm 0.65Ba
Two-way ANOVA				
System	NS	NS	<0.0058	<0.0473
Protein	<0.0389	NS	NS	NS
Interaction	<0.0365	NS	NS	<0.0327

NS - not significant.

Means followed by different letters, uppercase for comparison of production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test (P<0.05).

Table 8 - Mean \pm standard deviation of the proximate composition of the bioflocs collected from the biofloc system used for the production of juvenile matrinxã (*Brycon amazonicus*) fed diet with different crude protein levels for 60 days (values in % dry basis)

Protein level (%)	Crude protein (%)	Ether extract (%)	Ash (%)
24	31.07 \pm 0.76	4.30 \pm 0.52	28.87 \pm 1.33a
30	32.07 \pm 1.78	5.30 \pm 0.46	29.37 \pm 1.05a
36	32.30 \pm 2.17	4.50 \pm 0.56	29.67 \pm 3.70a
42	33.57 \pm 0.38	4.70 \pm 0.62	24.53 \pm 2.16b
One-way ANOVA	NS	NS	<0.0003

NS - not significant.

Means followed by different letters in the same column differ according to ANOVA and Tukey's test (P<0.05).

The use of sodium bicarbonate to stabilize water alkalinity and pH in BFT systems (Emerenciano et al., 2017) resulted in an increase in salinity. The alkalinity concentrations in both the BFT and CW systems were influenced by the CP level in the feed. The decrease in alkalinity values in the CW system may be attributed to the daily water renewal, which causes ion losses, unlike the BFT system (Dos Santos et al., 2023). The observed variation in alkalinity values among the studied systems is likely attributed to their distinct characteristics, as observed by Dos Santos et al. (2023). In their study, which tested different levels of CP in the feed in two production systems (BFT and CW) using tambaqui, the authors found higher alkalinity values in BFT.

Frequent monitoring of the concentration of total ammonia is necessary as it tends to increase with the amount of feed provided, with the accumulation of biomass in the tanks and according to the level of protein in the diet (Leira et al., 2017). Total ammonia results from the decomposition of organic matter, feed, and excreta. The BFT system showed statistically lower levels of ammonia, thus indicating the efficiency of ammonia assimilation by nitrifying and heterotrophic bacteria in the system (Ebeling et al., 2006). Similar results were reported by Dos Santos et al. (2021, 2023).

There were no significant differences in nitrite concentrations between the BFT and CW production systems. This may be attributed to the changes of water and stabilization of nitrifying bacteria. However, higher average values were observed in the BFT system and in diets with higher protein levels, which was likely due to the increased nitrogen intake from the feed. The interaction between the factors reflected these effects, with the BFT system providing a greater amount of nitrate than the CW system. This was due to the presence of chemoautotrophic bacteria, which promoted a greater conversion of nitrogen compounds. The effect was intensified by the greater amount of nitrogen in the feed. The nitrate concentrations in the factors studied (Table 3) support the findings of Mansour and Esteban (2017) and Dos Santos et al. (2023), who reported higher nitrate concentrations in the BFT system compared with the CW system. This was observed in tilapia raised using different carbon sources and protein levels, as well as in tambaqui in two production systems (BFT and CW) and with three CP levels, respectively.

In a BFT system, it is crucial to maintain TSS levels below 500 mg/L and SS volume below 50 mL/L to ensure proper functioning of the system (Hargreaves, 2013). The mean values of the experiment remained within the range used for other species, such as Nile tilapia raised in a biofloc system (Avnimelech, 2009) and juvenile tambaqui (Dos Santos et al., 2023). These species did not experience any mortality or clinical-behavioral alterations. The BFT system used in this study showed that higher protein diets led to an increase in TSS and SS, resulting in greater nitrogen excretion and microbial floc formation (Silva et al., 2018). The reduction of light entering the BFT system had the effect of increasing the turbidity of the water. This may suggest better animal welfare, as lower light intensity has been shown to reduce aggressive behavior in the species (Lopes et al., 2018; Amaral et al., 2020; Cornélio et al., 2021).

4.2. Animal performance, somatic indices, and blood parameters

Several studies have shown that the BFT system can enhance the productive performance of fish. This is because the microorganisms present in the biofloc system become bioavailable as a complementary food source (Dos Santos et al., 2021; Pires et al., 2022; Dos Santos et al., 2023, 2024). The study found that the performance of juvenile matrinxã in the two production systems evaluated was mainly related to the formation of microbial flocs and water quality in the BFT system. This suggests that the fish may be utilizing the flocs and experiencing better welfare, resulting in better performance compared with those reared in the CW system (Dos Santos et al., 2023).

The apparent feed conversion of the juveniles in the BFT system was found to be lower. This may have been due to the animals exhibiting a more optimal use of the food provided, given that the high turbidity of the system reduces aggressive behavior and stress among the animals, which also contributes to a higher survival rate of the species (Amaral et al., 2020; Cornélio et al., 2021; Izel-Silva et al., 2024). This supports the findings of Luo et al. (2014), who observed a decrease in the feed conversion ratio of Nile tilapia from 1.4 in a recirculating aquaculture system to 1.2 in a biofloc system, resulting in a 22% increase in the final weight of the fish. Nevertheless, the outcomes of this study indicate that matrinxã, despite not being filter feeders like the tilapia, may ingest particles from the BFT system. This is evidenced by the higher productivity and survival of the fish in the BFT system than in the CW system. The quantities of bioflocs consumed by the matrinxã are insignificant for their growth or for the reduction of protein in the diet; however, there are potential benefits for the health and welfare of the animals, as indicated by the survival rate and some of the physiological parameters discussed below.

Based on the results for performance, it is evident that a diet containing 24% CP does not meet the nutritional requirements of juvenile matrinxã in either of the systems. This leads to lower feed intake and productivity, as well as higher feed conversion. Mattos et al. (2018) evaluated the effect of different CP levels (30, 35, 40, and 45%) on juvenile matrinxã (approximately 3 g) in a continuous flow system and found no significant difference in the performance of the animals fed the three highest CP levels in the diet. The difference observed in this study may be related to the production system and the composition of the nutrients in the diet. Sgnaulin et al. (2021) evaluated the impact of reducing dietary protein on the production of *Piaractus mesopotamicus* and observed that the fish reared in the BFT system had a lower requirement for CP in their feed due to the utilization of nutrients from the bioflocs. Despite this, the present study found no interaction between the production system and the level of protein in the feed. This suggests that the juvenile matrinxã did not consume enough microbial flocs to increase or compensate for their growth in relation to the diets. Nevertheless, there was a noticeable improvement in feed conversion.

In addition to performance parameters, body indices have been used to assess factors affecting nutrient assimilation and nutritional status in fish (Silva et al., 2018). This study found that the BFT and CW systems had a statistically significant effect on the viscerosomatic and hepatosomatic indices of the juvenile matrinxã. However, the effect of CP levels did not affect the differences in the hepatosomatic index.

The blood parameters of the juvenile matrinxã in the BFT and CW systems did not indicate any effect on the homeostasis of fish. This suggests that the water quality and microbiological action did not induce stress in the animals. These results support the findings for Nile tilapia (*O. niloticus*) and tambaqui (*C. macropomum*) in BFT systems compared with CW systems (Dos Santos et al., 2021, 2024; Figueroa-Espinoza et al., 2022). The reduction in protein levels in the diet did not harm the physiological homeostasis of the fish, which supports the findings of Dos Santos et al. (2024). They also observed no significant effects on the metabolism and/or welfare indicators of juvenile tambaqui when reducing protein levels from 32 to 24% in BFT and CW systems.

Nonetheless, the fish showed a significant reduction in hemoglobin levels when they received the feed with the lowest protein content. This suggests a nutritional insufficiency that may have decreased oxygen demand and, as a result, reduced weight gain. Furthermore, the hematocrit level exhibited an interaction between the production systems and protein content, and the BFT system intensified the effects of protein restriction in the diet, resulting in a lower hematocrit level in fish feed with the lowest protein level.

Total leucocytes and neutrophils showed statistical differences between the juveniles in the BFT and CW systems, with higher averages in the matrinxã reared in the BFT system. As observed by Dos Santos et al. (2024), this difference can be attributed to prolonged contact with the microorganisms present in the BFT system. These authors found not only a higher number of monocytes in tambaqui (*C. macropomum*) raised in BFT, but also an increase in their respiratory activity.

4.3. Nutritional composition of the fish and the bioflocs

The nutritional composition of the feed of aquatic organisms can be a determinant of nutrient assimilation (Silva et al., 2018). The biofloc system can achieve a balance between protein, lipids, and ash in feed (Marinho-Pereira et al., 2020). The production of microorganisms in the system is affected by the type of feed and the CP level of the diets (Pires et al., 2022). This study shows that the nutritional composition of the animals was not affected by the production system. The levels of composition were similar between the BFT and CW systems in terms of crude protein. The results suggest that the CP in the feed can be reduced without compromising the efficiency of bioflocs as a feed supplement in BFT systems (Dos Santos et al., 2023). The results for CP are comparable to those reported by Eid et al. (2021), but lower than the values reported by Figueroa-Espinoza et al. (2022), who evaluated juvenile Nile tilapia in a biofloc system with experimental diets.

The bioavailability of protein, lipids, mineral matter, and microorganisms in microbial flocs within the BFT system can vary depending on the feed, stage of development of the cultivated organisms, and production management in the environment (Pires et al., 2022; Figueroa-Espinoza et al., 2022). The protein levels in the flocs, ranging from 31.0 to 33.5%, fall within the recommended range of 12 to 49% in the literature (Emerenciano et al., 2013a; Dos Santos et al., 2023). Therefore, the protein content in bioflocs can vary depending on the farming system (age and C:N ratio) and the formulation of the added feed (Figueroa-Espinoza et al., 2022).

5. Conclusions

The study found that juvenile matrinxã can be fed a diet containing 30% CP in both the BFT and CW systems. The results demonstrate that microbial flocs do not confer a direct benefit to juvenile matrinxã in terms of their feeding. However, the presence of the bioflocs and the increased turbidity in the water lead to an increase in the survival rate of the juveniles and improves animal welfare. Consequently, it is crucial to monitor the system to ensure that it is functioning optimally and prevents future losses, thereby promoting greater availability of juveniles in the BFT system for production.

Data availability

The entire dataset supporting the results of this study is available upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Affonso, E. G.; Suita, S. M. and Freitas, T. M. **Data curation:** Cunha, R. B. and Silva, M. A. **Formal analysis:** Cunha, R. B. and Silva, M. A. **Funding acquisition:** Affonso, E. G. **Investigation:** Cunha, R. B.; Silva, M. A. and Abe, H. A. **Methodology:** Cunha, R. B.; Affonso, E. G. and Silva, M. A. **Project administration:** Affonso, E. G.; Silva, M. A. and Freitas, T. M. **Resources:** Affonso, E. G. **Supervision:** Affonso, E. G.; Suita, S. M.; Silva, M. A. and Freitas, T. M. **Visualization:** Cunha, R. B.; Silva, M. A. and Abe, H. A. **Writing – original draft:** Cunha, R. B. **Writing – review & editing:** Affonso, E. G.; Suita, S. M.; Silva, M. A.; Abe, H. A. and Freitas, T. M.

Conflict of interest

The authors declare no conflict of interest.

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References

Amaral, A. P.; Silva, E. C. C. and Carvalho, T. B. 2020. Melatonin reduces aggressiveness and improves oxidative status of matrinxã (*Brycon amazonicus*) subjected to social challenge. *Fish Physiology and Biochemistry* 46:1019-1024. <https://doi.org/10.1007/s10695-020-00768-x>

- AOAC - Association of Official Analytical Chemists. 2012. Official methods of analysis of AOAC International. 19th ed. AOAC International, Washington, DC.
- APHA - American Public Health Association. 1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, American Water Works Association, Water Environment Federation. Washington, DC.
- Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture* 264:140-147. <https://doi.org/10.1016/j.aquaculture.2006.11.025>
- Avnimelech, Y. 2009. *Biofloc technology: a practical guide book*. 2nd ed. The World Aquaculture Society, Baton Rouge, Louisiana, USA.
- Boyd, C. E. 1982. *Water quality management for pond fish culture*. Elsevier Science, Amsterdam.
- Boyd, C. E. and Tucker, C. S. 1992. *Water quality and pond soil analyses for Aquaculture*. Alabama Agricultural Experiment Station, Auburn University, Auburn, AL.
- Carvalho, T. B.; Souza, E. C. M.; Pinheiro-da-Silva, J. and Villacorta-Correa, M. A. 2018. Effect of body size heterogeneity on the aggressive behavior of larvae of matrinxã, *Brycon amazonicus* (Characiformes, Bryconidae). *Acta Amazonica* 48:304-310. <https://doi.org/10.1590/1809-4392201800541>
- Castro, M. G.; Castro, M. J.; De Lara, A. R.; Monroy, D. M. C.; Ocampo, C. J. A. and Davila, F. S. 2016. Length, weight, and condition factor comparison of *Carassius auratus* (Linnaeus, 1758) juveniles cultured in biofloc system. *International Journal of Fisheries and Aquatic Studies* 4:345-350.
- Cornélio, J. P. S.; Braga, L. G. T. and Villacorta-Correa, M. A. 2021. Can turbid water and refuges with aquatic macrophytes increase the survival and growth of *Brycon amazonicus* larvae? *Research, Society and Development* 10:e227101522737. <https://doi.org/10.33448/rsd-v10i15.22737>
- Day, S. B.; Salie, K. and Stander, H. B. 2016. A growth comparison among three commercial tilapia species in a biofloc system. *Aquaculture International* 24:1309-1322. <https://doi.org/10.1007/s10499-016-9986-z>
- Dos Santos, R. B.; Izel-Silva, J.; Fugimura, M. M. S.; Suita, S. M.; Ono, E. A. and Affonso, E. G. 2021. Growth performance and health of juvenile tambaqui, *Colossoma macropomum*, in a biofloc system at different stocking densities. *Aquaculture Research* 52:3549-3559. <https://doi.org/10.1111/are.15196>
- Dos Santos, R. B.; Izel-Silva, J.; de Medeiros, P. A.; Fugimura, M. M. S.; de Freitas, T. M.; Ono, E. A.; Claudiano, G. S. and Affonso, E. G. 2023. Dietary protein requirement for tambaqui cultivated in biofloc and clear water systems. *Aquaculture International* 31:1685-1704. <https://doi.org/10.1007/s10499-023-01047-1>
- Dos Santos, R. B.; Izel-Silva, J.; de Medeiros, P. A.; Fugimura, M. M. S.; de Freitas, T. M.; Gallani, S. U.; Claudiano, G. S.; Ono, E. A. and Affonso, E. G. 2024. Immunophysiology of tambaqui fed with different levels of dietary protein in biofloc and clear water system. *Aquaculture International* 32:1067-1079. <https://doi.org/10.1007/s10499-023-01205-5>
- Ebeling, J. M.; Timmons, M. B. and Bisogni, J. J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture* 257:346-358. <https://doi.org/10.1016/j.aquaculture.2006.03.019>
- Eid, A. E.; Showhdy, K. G.; Ali, M. A. M. and Elfeky, A. 2021. Effect of reuse biofloc water on growth performance, feed utilization of Nile tilapia fingerlings (*Oreochromis niloticus*). *Egyptian Journal of Nutrition and Feeds* 24:315-326. <https://doi.org/10.21608/ejnf.2021.193029>
- Emerenciano, M.; Gaxiola, G. and Cuzon, G. 2013a. Biofloc technology (BFT): a review for aquaculture application and animal food industry. p.301-327. In: *Biomass now cultivation and utilization*. Matovic, M. D., ed. IntechOpen. <https://doi.org/10.5772/53902>
- Emerenciano, M.; Cuzon, G.; Paredes, A. and Gaxiola, G. 2013b. Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc. *Aquaculture International* 21:1381-1394. <https://doi.org/10.1007/s10499-013-9640-y>
- Emerenciano, M.; Cuzon, G.; Goguenheim, J.; Gaxiola, G. and AQUACOP. 2013c. Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. *Aquaculture Research* 44:75-85. <https://doi.org/10.1111/j.1365-2109.2011.03012.x>
- Emerenciano, M. G. C.; Martínez-Córdova, L. R.; Martínez-Porchas, M. and Miranda-Baeza, A. 2017. Biofloc technology (BFT): a tool for water quality management in aquaculture. p.91-109. In: *Water quality*. Tutu, H., ed. In Tech. <https://doi.org/10.5772/66416>
- Ferreira, J. N.; Barros, I. B. A. and Carvalho, T. B. 2020. Effect of water temperature on aggressiveness and physiological parameters of juvenile matrinxã, *Brycon amazonicus* (Spix and Agassiz, 1829). *Applied Animal Behaviour Science* 231:105091. <https://doi.org/10.1016/j.applanim.2020.105091>
- Figueroa-Espinoza, J.; Rivas-Vega, M. E.; Mariscal-López, M. A.; Emerenciano, M. G. C.; Martínez-Porchas, M. and Miranda-Baeza, A. 2022. Reusing water in a biofloc culture system favors the productive performance of the Nile tilapia (*Oreochromis niloticus*) without affecting the health status. *Aquaculture* 558:738363. <https://doi.org/10.1016/j.aquaculture.2022.738363>

- Hargreaves, J. A. 2013. Biofloc production systems for Aquaculture. Southern Regional Aquaculture Center 4503:1-12.
- IBGE - Instituto Brasileiro de Geografia e Estatística. 2021. PPM - Produção Pecuíária municipal. Available at: <<https://sidra.ibge.gov.br/tabela/3940#resultado>>. Accessed on: Aug. 28, 2022.
- Izel-Silva, J.; dos Santos, R. B.; Medeiros, P. A.; Suita, S. M.; Wasielesky Jr, W.; Fugimura, M. M. S. and Affonso, E. G. 2024. *Brycon amazonicus* larviculture cannibalism is reduced in biofloc systems. *Aquaculture* 579:740180. <https://doi.org/10.1016/j.aquaculture.2023.740180>
- Khanjani, M. H.; Mozanzadeh, M. T.; Sharifinia, M. and Emerenciano, M. G. C. 2023a. Biofloc: A sustainable dietary supplement, nutritional value and functional properties. *Aquaculture* 562:738757. <https://doi.org/10.1016/j.aquaculture.2022.738757>
- Khanjani, M. H.; Sharifinia, M. and Hajirezaee, S. 2023b. Biofloc: a sustainable alternative for improving the production of farmed cyprinid species. *Aquaculture Reports* 33:101748. <https://doi.org/10.1016/j.aqrep.2023.101748>
- Khanjani, M. H.; Sharifinia, M. and Emerenciano, M. G. C. 2024a. Biofloc Technology (BFT) in Aquaculture: What goes right, what goes wrong? A scientific-based snapshot. *Aquaculture Nutrition* 2024:496572. <https://doi.org/10.1155/2024/7496572>
- Khanjani, M. H.; Mozanzadeh, M. T.; Sharifinia, M. and Emerenciano, M. G. C. 2024b. Broodstock and seed production in biofloc technology (BFT): an updated review focused on fish and penaeid shrimp. *Aquaculture* 579:740278. <https://doi.org/10.1016/j.aquaculture.2023.740278>
- Krummenauer, D.; Seifert-Júnior, C. A.; Poersch, L. H.; Foes, G. K.; Lara, G. R. and Wasielesky Junior, W. 2012. Cultivo de camarões marinhos em sistema de bioflocos: análise da reutilização da água. *Atlântica* 34:103-111.
- Lara, G.; Hostins, B.; Bezerra, A.; Poersch, L. and Wasielesky Jr, W. 2017. The effects of different feeding rates and re-feeding of *Litopenaeus vannamei* in a biofloc culture system. *Aquacultural Engineering* 77:20-26. <https://doi.org/10.1016/j.aquaeng.2017.02.003>
- Leira, M. H.; Cunha, L. T.; Braz, M. S.; Melo, C. C. V.; Botelho, H. A. and Reghim, L. S. 2017. Qualidade da água e seu uso em pisciculturas. *PUBVET* 11:11-17. <https://doi.org/10.22256/pubvet.v11n1.11-17>
- Leite, R. G. 2004. A alimentação de juvenis de matrinxã, *Brycon amazonicum* (Pisces, Characidae), em áreas inundadas da Ilha de Marchantaria, Amazonas, Brasil. *Acta Amazonica* 34:661-664. <https://doi.org/10.1590/S0044-59672004000400018>
- Lopes, A. C. C.; Villacorta-Correa, M. A. and Carvalho, T. B. 2018. Lower light intensity reduces larval aggression in matrinxã, *Brycon amazonicus*. *Behavioural Processes* 151:62-66. <https://doi.org/10.1016/j.beproc.2018.01.017>
- Luo, G.; Gao, Q.; Wang, C.; Liu, W.; Sun, D.; Li, L. and Tan, H. 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture* 422-423:1-7. <https://doi.org/10.1016/j.aquaculture.2013.11.023>
- Luo, G.; Zhang, N.; Cai, S.; Tan, H. and Liu, Z. 2017. Nitrogen dynamics, bacterial community composition and biofloc quality in biofloc-based systems cultured *Oreochromis niloticus* with poly- β -hydroxybutyric and polycaprolactone as external carbohydrates. *Aquaculture* 479:732-741. <https://doi.org/10.1016/j.aquaculture.2017.07.017>
- Manduca, L. G.; Silva, M. A.; Alvarenga, E. R.; Alves, G. F. O.; Ferreira, N. H.; Teixeira, E. A.; Fernandes, A. F. A.; Silva, M. A. and Turra, E. M. 2021. Effects of different stocking densities on Nile tilapia performance and profitability of a biofloc system with a minimum water exchange. *Aquaculture* 530:735814. <https://doi.org/10.1016/j.aquaculture.2020.735814>
- Mansour, A. T. and Esteban, M. A. 2017. Effects of carbon sources and plant protein levels in a biofloc system on growth performance, and the immune and antioxidant status of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology* 64:202-209. <https://doi.org/10.1016/j.fsi.2017.03.025>
- Marinho-Pereira, T.; Faria Junior, C. H.; Rincón, L. M. G.; Britto, E. N.; Cavero, B. A. S.; Aride, P. H. R. and Oliveira, A. T. 2020. Tecnología biofloc: datos, estudios y experiencias para el desarrollo de la acuicultura latinoamericana. *Brazilian Journal of Development* 6:7847-7862. <https://doi.org/10.34117/bjdv6n2-187>
- Mattos, B. O.; Bueno, G. W.; Honczaryk, A.; Pereira-Filho, M. and Roubach, R. 2018. Níveis de proteína bruta na dieta de juvenis de matrinxã (*Brycon amazonicus*). *Revista de Ciências Agrárias* 61:1-6. <https://doi.org/10.22491/rca.2018.2649>
- Mattos, B. O.; Costa, A. C.; Leal, R. S.; Freitas, R. T. F.; Pimenta, M. E. S. G. and Freato, T. A. 2013. Desempenho produtivo de *Brycon orthotaenia* em tanques-rede sob diferentes densidades de estocagem. *Ciancia Rural* 43:1057-1062. <https://doi.org/10.1590/S0103-84782013005000058>
- McCusker, S.; Warberg, M. B.; Davies, S. J.; Valente, C. S.; Johnson, M. P.; Cooney, R. and Wan, A. H. L. 2023. Biofloc technology as part of a sustainable aquaculture system: A review on the status and innovations for its expansion. *Aquaculture Fish and Fisheries* 3:331-352. <https://doi.org/10.1002/aff.2.108>
- Najdegerami, E. H.; Bakhshi, F. and Lakani, F. B. 2016. Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. *Fish Physiology and Biochemistry* 42:457-465. <https://doi.org/10.1007/s10695-015-0151-9>
- Nascimento, G. B.; Amaral, L. V.; Ramos, N. C.; Litaiff, N. R.; Ribeiro, M. W. S.; Ladislau, D. S. and Oliveira, A. T. 2020. Parâmetros hematológicos do Matrinxã *Brycon amazonicus* (Characidae: Bryconinae) criados em cativeiro na região Amazônica. *Brazilian Journal of Development* 6:3303-3315. <https://doi.org/10.34117/bjdv6n1-238>

- Ogello, E. O.; Outa, N. O.; Obiero, K. O.; Kyule, D. N. and Munguti, J. M. 2021. The prospects of biofloc technology (BFT) for sustainable aquaculture development. *Scientific African* 14:e01053. <https://doi.org/10.1016/j.sciaf.2021.e01053>
- Papaspyrou, S.; Smith, C. J.; Dong, L. F.; Whitby, C.; Dumbrell, A. J. and Nedwell, D. B. 2014. Nitrate reduction functional genes and nitrate reduction potentials persist in deeper estuarine sediments. Why? *Plos One* 9:e94111. <https://doi.org/10.1371/journal.pone.0094111>
- Pires, D. C.; Bezerra, G. A.; Watanabe, A. L.; Buglione Neto, C. C.; Bicudo, A. J. A. and Hisano, H. 2022. Optimal dietary protein level for pacu *Piaractus mesopotamicus* juveniles reared in biofloc system. *Aquaculture* 556:738274. <https://doi.org/10.1016/j.aquaculture.2022.738274>
- Sgnaulin, T.; Pinho, S. M.; Durigon, E. G.; Thomas, M. C.; Mello, G. L. and Emerenciano, M. G. C. 2021. Culture of pacu *Piaractus mesopotamicus* in biofloc technology (BFT): insights on dietary protein sparing and stomach content. *Aquaculture International* 29:2319-2335. <https://doi.org/10.1007/s10499-021-00748-9>
- Silva, M. A.; Alvarenga, E. R.; Alves, G. F. O.; Manduca, L. G.; Turra, E. M.; Brito, T. S.; Sales, S. C. M.; Silva Junior, A. F.; Borges, W. J. M. and Teixeira, E. A. 2018. Crude protein levels in diets for two growth stages of Nile tilapia (*Oreochromis niloticus*) in a biofloc system. *Aquaculture Research* 49:2693-2703. <https://doi.org/10.1111/are.13730>
- Strickland, J. D. H. and Parsons, T. R. 1972. A practical handbook of seawater analysis. 2nd ed. Fisheries Research Board of Canada, Ottawa. <https://doi.org/10.25607/OBP-1791>
- Tavares-Dias, M. and Moraes, F. R. 2004. Hematologia de peixes teleósteos. Villimpress, Ribeirão Preto.
- Vadhel, N.; Pathan, J.; Shrivastava, V.; Akolkar, N.; Kumar, S.; Misra, C. K. and Mahapatra, K. D. 2020. Comparative study on the performance of genetically improved rohu "Jayanti" and native rohu, *Labeo rohita* fingerlings reared in biofloc system. *Aquaculture* 523:735201. <https://doi.org/10.1016/j.aquaculture.2020.735201>
- Verdouw, H.; Van Eched, C. J. A. and Dekkers, E. M. J. 1978. Ammonia determination based on indophenol formation with sodium siliclylate. *Water Research* 12:397-402. [https://doi.org/10.1016/0043-1354\(78\)90107-0](https://doi.org/10.1016/0043-1354(78)90107-0)
- Vinatea, L.; Galvez, A. O.; Browdy, C. L.; Stokes, A.; Venero, J.; Haveman, J.; Lewis, B. L.; Lawson, A.; Shuler, A. and Leffler, J. W. 2010. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceways culture with zero water exchange: interaction of water quality variables. *Aquacultural Engineering* 42:17-24. <https://doi.org/10.1016/j.aquaeng.2009.09.001>
- Widanarni; Ekasari, J. and Maryam, S. 2012. Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp, cultured at different stocking densities. *Hayati Journal of Biosciences* 19:73-80. <https://doi.org/10.4308/hjb.19.2.73>