Revista ARACÊ
ISSN: 2358-2472

HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF TAMBAQUI SUBMITTED TO RESTRICTION AND FEEDING FREQUENCY IN A RECIRCULATING SYSTEM FOR AQUACULTURE "RAS"

https://doi.org/10.56238/arev7n2-316

Submitted on: 01/28/2025 **Publication date:** 02/28/2025

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ABSTRACT
This study evaluated the effect of feed restriction and different feeding frequencies on the hematological and biochemical parameters of Tambaqui in a recirculating system for RAS aquaculture. Based on a randomized block experimental design, food restriction was evaluated from three groups (blocks): TR1 (Control) = daily feeding without restriction (7 times a week); TR2 = feeding six days in a row with one day of restriction (6 times a week); TR3 = feeding for five days in a row with two days of restriction (5 times a week). For food frequency, the groups (treatments) were: TF1 = 1x/day (08:00); TF2 = 2x/day (08; 00 and 17:00); TF3 = 3x/day (08:00, 12:00 and 17:00); TF4 = 4x/day (08:00, 11:00, 14:00 and 17:00). At the end of the 30-day experimental period, blood samples were collected to determine hematological and biochemical parameters. TR3 fish showed reductions in Hb,

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ISSN: 2358-2472

HT, MCV and HCM and an increase in PRT in relation to TR1 and TR2 ($p<0.05$). In the condition of food frequency evaluation, only an increase in the RBC of TF2 in relation to TF3, in the Gli of TF4 in relation to TF1 and in the PRT of TF2 in relation to TF3 ($p<0.05$) was observed. Overall, these results indicate that daily and twice-daily feeding resulted in better results in hematology and blood biochemistry, being the most suitable for tambaqui production in an intensive culture system in the RAS modality.

Keywords: Colossoma Macropomum. Blood. Plasma metabolic. Fish farming. Food Management.

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

The cost of feed is among the most expensive in fish farming, representing 60 to 80% of total costs (Kubitza, 1997; Pereira et al., 2013). The use of feeding protocols that promote the rational use of feed can significantly contribute to reducing costs and improving the profitability of fish farming. Several food management strategies have been evaluated, including food composition, feeding rate, food restriction, optimal feeding frequency; feeding times and distribution techniques (Pérez-Jiménez, 2007; Pereira-Filho et al., 2008; Santos et al., 2013; Melo et al., 2020; Caruso et al., 2012; Kondera et al., 2017; ElArabya; Amer; Kalil, 2020; Golçalvez-Júnior et al., 2021; Guilherme et al., 2022)

The feeding restriction strategy is based on the knowledge that, in their natural environments, fish can go for long periods without feeding, either as a result of fluctuation in water levels (floods/ebbs), reproductive migration and/or pre-spawning phase or the winter months (Shimeno; Kheyyali; Takeda, 1990; Navarro and Gutierrez, 1995; Cho, 2005). Feed restriction and refeeding has been widely indicated in fish management as a way to provide a reduction in feed intake and production costs without prejudice to productive performance (Abdel-Hakim et al., 2009; Santos et al., 2015; Santos et al, 2018; Peace; Pastrana; Brandão, 2018). The ability of fish to grow compensatory after a period of fasting and refeeding is already recognized (Ali; Nicieza; Wootton, 2003; Abdel-Tawwab et al., 2006; McCue, 2010). The relationship between feed restriction and compensatory growth has been described for several crop species, including tambaqui (Pereira-Filho et al., 2008; Barcellos et al., 2010; Arauco and Costa, 2012; Paz, Pastrana, and Brandão 2018; Assis et al., 2020; Garcez et al., 2023; Melo et al., 2024; Leal et al., 2024).

Another form of rational food management that has attracted attention is the implementation of optimal food frequency, which can promote better growth and lower feed conversion, reduce feed waste, and improve water quality (Gao et al., 2022). However, caution should be exercised in relation to feeding frequency, a feeding frequency below or above the ideal, for a given species, can harm the development of fish, as well as interfere with water quality and costs (Guilherme et al., 2022). Optimal feeding frequencies have been investigated in a wide range of fish species in farming systems and results vary between species (Dwyer et al., 2002; Souza et., 2014; Gomes et al., 2019; Porto et al., 2020; Cadorin et al., 2021; Guilherme et al., 2022).

The feeding strategy adopted for a given species can interfere with the well-being and health of fish (McCue, 2010; Gonçalves-Junior et al., 2021). The analysis of hematological and biochemical parameters are important practices in the evaluation of fish welfare, as they reflect physiological conditions such as stress and health (Tavares-Dias and Moraes, 2004; Fazio et al., 2019; Petillo et al., 2025) and help to understand the effects of different feeding management on fish.

The tambaqui, *Colossoma macropomum*, is a freshwater fish, native to the Amazon and Orinoco river basins (Araújo-Lima; Goulding, 1998). Characteristics such as robustness, omnivorous feeding habits, acceptance of artificial feeds, mastery of captive breeding techniques, good zootechnical characteristics and adaptability to different cultivation systems (Val; Oliveira, 2021) makes this native species one of the most attractive for production in national fish farming, placing it as the second most produced in Brazil (Anuário da Piscicultura, 2023). In 2022, the production of this species reached about 109.7 thousand tons (IBGE, 2023). As a result of its great economic importance in national fish farming, there are many efforts to improve the management and production systems of tambaqui with a view to increasing the production and profitability of the activity (Silva; Gomes; Brandão, 2007; Souza et al., 2014; Porto et al., 2020; Guilherme et al., 2022; Garcez et al., 2023; Melo et al., 2024; Leal et al., 2024).

Therefore, the aim of this study was to evaluate the effect of feed restriction and different feeding frequencies on the hematological and biochemical parameters of tambaqui in a recirculating system for RAS aquaculture.

METHODOLOGY

STATEMENT OF ETHICS

The experiment was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Amazonas (UFAM) registered under No. 23105.006372/2024-52.

EXPERIMENT

The experiment was carried out at the Experimental Aquaculture Laboratory (LAqEx) of the Faculty of Agrarian Sciences FCA/UFAM. The experiment lasted thirty (30) days and was conducted in a randomized block design. Each of them (3) was used to evaluate the effect of feed restriction on zootechnical performance and hematological and biochemical parameters of tambaqui juveniles. Within each block, food frequencies of 1, 2, 3 and 4 times a day were tested.

Dietary restriction was evaluated from three treatments (RT). TR1 = Control daily feeding without restriction (7x/week); TR2 = feeding six days in a row with one day of restriction (6x/week); TR3 = feeding for five days in a row with two days of restriction (5x/week). Regarding feeding frequency, the treatments (TF) were: TF1 = 1x/day (08:00); TF2 = 2x/day (08:00 and 17:00); TF3 = 3x/day (08:00, 12:00 and 17:00); TF4 = 4x/day (08:00, 11:00, 14:00 and 17:00).

The experimental units consisted of 12 PVC tanks with a volumetric capacity of 310L, of which 250L of useful volume were used. The water output to the filtering structures occurred through the piping in the center of the tanks, while the treated water input was carried out by the taps located on the sides of the tanks.

The experimental and recirculation system of each block consisted of a complex of 4 production boxes, each with a capacity of 250L, and a box of filtering structures with a capacity of 500L, totaling 1500L. The water came from a semi-artesian well.

In each experimental unit, 35 fish with an average weight of 3.5 ± 0.14 g were stocked, from the Santo Antônio Farm, located in Rio Preto da Eva, AM. Fish feeding on all days and times was carried out until apparent satiety with a commercial diet of the Multi Peixe 32% type (Multifós Nutrição Animal, Vilhena, RO, Brazil), containing 32% of crude protein and pellets of 4 to 6 mm in diameter.

The biometrics were performed in two moments, at the beginning (day 0) and at the end (day 30) of the experiment. Always before each biometric procedure, the animals were anesthetized according to Inoue et al. (2011).

The evaluation of water quality was carried out systematically throughout the experiment. Parameters such as pH, dissolved oxygen, conductivity, total dissolved solids (TDS) and temperature were monitored daily using an Akso AK88v2® multiparameter meter (Akso Produtos Eletrônicos Ltda, São Leopoldo, RS, Brazil). For the analysis of nitrogenous compounds (ammonia, nitrite and nitrate), alkalinity, hardness and total solids, the methods described by Macedo (2005) were followed. In addition, turbidity was checked weekly using an Alfakit AT10P® benchtop turbidimeter (Alfakit Ltda, Florianópolis, SC, Brazil).

The zootechnical performance indicators were determined based on the measurements taken at the beginning and end of the experiment for the following indices: Weight gain: (GP) = final weight – initial weight; Biomass gain: (GB) = ; Final Density: (DF) = ; Specific growth rate: (TCE) = 100*(Ln Average Final Weight – Ln Average Initial

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ISSN: 2358-2472

Weight) /time (days); Apparent feed conversion (CAA) = CMFi / (Final average weight – Initial average weight); Apparent feed efficiency (EAA) = ; Survival: (S) = 100*(no. of fish at the end of the experiment/no. of fish at the beginning of the experiment).

Blood collection (puncture of the caudal vessel) was performed using syringes with anticoagulant EDTA (ethylenediaminetetraacetic acid). Hematocrit (Ht, %) was determined using the microhematocrit technique. The hemoglobin concentration (Hb, g.dL-1) was determined according to the cyanomethemoglobin method. The red cell count (RBC, 106.mm-3) was performed in a Neubauer chamber using an optical microscope. After the initial analyses, the following indices were determined: mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg) and mean corpuscular hemoglobin concentration (MCHC, g.dL-1) (Wintrobe, 1934).

Blood plasma values of glucose, total cholesterol, triglycerides and total proteins were determined with the aid of commercial Labtest® kits (Labtest Diagnóstica S/A, Lagoa Santa, MG, Brazil)

The data collected related to the growth of the animals, water quality and physiologistic and biochemical parameters were submitted to the Homogeneity Normality Tests to enable the use of Analysis of Variance (ANOVA). Statistically significant differences (P < 0.05) were assessed using Tukey's test. Both tests used α = 5% (Bhujel, 2011).

RESULTS

The water quality parameters remained within the recommended values for tambaqui farming in intensive systems (Lima et al., 2019; Costa et al., 2022). Despite the significant variations observed for pH, DO, temperature, conductivity, turbidity, alkalinity, ammonia and nitrate in the feed restriction (RT) treatments (Table 1) and for turbidity and total solids in the feed frequency (TF) treatments (Table 2), all values were within the tolerable limits for the species (Zaniboni Filho; Pedron; Ribolli, 2018).

Table 1. Water quality parameters of experimental cultivation of juvenile tambaqui (*Colossoma macropomum*) submitted to feed restriction in a recirculating system (RAS).

Acronym	Unit	TR1	TR2	TR3
ph	[H+]	5.44 ± 0.71a	5.27 ± 0.63b	5.59 ± 0.52 A
Cond.	µS cm-1	660.91 ± 94.15b	701.97 ± 75.90b	547.23 ± 69.89 A
Temp.	°C	28.07 ± 0.72	27.98 ± 0.70	28.07 ± 0.74
OD	L-1 mg	7.19 ± 0.91b	7.63 ± 0.64a	7.72 ± 0.71 A
TDS	L-1 mg	280.29 ± 40.79b	356.49 ± 37.9b	338.52 ± 40.10 to
Salt.	Ppt	0.35 ± 0.05b	0.44 ± 0.03b	0.43 ± 0.03 to

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ISSN: 2358-2472

Lac.	(mg CaCO $^{L-1}$)	25.25 ± 19.75c	10.23 ± 3.93a	17.21 ± 8.66 ^{to}
Turb.	NTU	2.31 ± 1.27a	3.12 ± 1.68a	1.79 ± 1.16 ^{b}
NH3	L-1 mg	7.71 ± 3.23	4.90 ± 1.59	3.42 ± 1.60
NO2	L-1 mg	0.58 ± 0.55	1.12 ± 1.30	0.63 ± 0.63
NO3	L-1 mg	29.67 ± 14.72 ^{b}	36.32 ± 7.39a	20.29 ± 10.09 ^{c}
ST	L-1 mg	428.33 ± 147.16	538.75 ± 11.82	507.50 ± 145.32

The data presented are composed of means ± standard deviation obtained from the experimental units (01 water tank = 01 replicate). Letters superscript only on the lines where statistical differences occurred (P < 0.05). Legend: Conductivity (Cond.); Temperature (Temp.); Dissolved Oxygen (DO); Total Dissolved Solids (TDS); Salinity (Salt.); Alkalinity (Alc.); Turbidity (Turb.); Ammonia (NH3); Nitrite (NO2); Nitrate (NO3); Total Solids (ST).

Table 2. Water quality parameters of the experimental cultivation of juvenile tambaqui (*Colossoma macropomum*) fed with various feeding frequencies in a recirculating system (RAS).

Acronym	Unit	TF1	TF2	TF3	TF4
ph	[H+]	5.43 ± 0.18	5.47 ± 0.11	5.40 ± 0.19	5.42 ± 0.18
Cond.	μS $^{cm-1}$	638.37 ± 81.25	638.63 ± 81.11	638.68 ± 80.79	638.33 ± 80.97
Temp.	°C	28.05 ± 0.05	28.05 ± 0.04	28.02 ± 0.05	28.04 ± 0.08
OD	L-1 mg	7.63 ± 0.27	7.56 ± 0.22	7.50 ± 0.19	7.53 ± 0.21
TDS	L-1 mg	327.39 ± 35.18	324.06 ± 41.08	323.94 ± 40.67	325.04 ± 42.46
Salt.	Ppt	0.41 ± 0.05	0.41 ± 0.05	0.40 ± 0.04	0.41 ± 0.05
Lac.	(mg CaCO $^{L-1}$)	21.22 ± 10.84	19.28 ± 12.51	13.25 ± 3.74	20.50 ± 10.48
Turb.	NTU	1.26 ± 0.53a	1.70 ± 0.82b	3.76 ± 0.67d	2.83 ± 0.84c
NH3	L-1 mg	4.15 ± 4.33	5.90 ± 3.60	5.61 ± 0.48	7.46 ± 4.28
NO2	L-1 mg	0.68 ± 0.80	0.83 ± 0.73	0.81 ± 0.93	1.25 ± 0.70
NO3	L-1 mg	28.20 ± 9.32	28.06 ± 14.79	32.24 ± 10.39	27.05 ± 5.78
ST	L-1 mg	371.67 ± 154.14a	338.33 ± 50.08A	608.33 ± 64.49b	636.11 ± 125.94b

The data presented are composed of means ± standard deviation obtained from the experimental units (01 water tank = 01 replicate). Letters superscript only on the lines where statistical differences occurred (P < 0.05). Legend: Conductivity (Cond.); Temperature (Temp.); Dissolved Oxygen (DO); Total Dissolved Solids (TDS); Salinity (Salt.); Alkalinity (Alc.); Turbidity (Turb.); Ammonia (NH3); Nitrite (NO2); Nitrate (NO3); Total Solids (ST).

ZOOTECHNICAL PERFORMANCE

The survival rate did not differ significantly between treatments ($p > 0.05$). However, the parameters final mean weight (MPW), weight gain (WG), final biomass (BF), biomass gain (GB), final density (DF) and specific growth rate (TCE) showed statistically significant differences between the restriction and feeding frequency treatments ($p < 0.05$) (Tables 3 and 4).

Table 3. Zootechnical indices of tambaqui (*Colossoma macropomum*) juveniles submitted to daily feeding (TR1) and feeding restriction of one day per week and refeeding (TR2) and two consecutive days per week and refeeding (TR3) for 30 days in a recirculating system (RAS).

Acronym	Unit	TR1	TR2	TR3
PMI	g	5.86 ± 1.46	6.49 ± 1.57	4.62 ± 0.97
PMF	g	39.72 ± 1.03 ^{to}	32.94 ± 5.48b	26.05 ± 6.11c
GP	g	33.86 ± 2.32 ^{to}	26.45 ± 5.19b	21.43 ± 5.67c
BI	Kg	0.21 ± 0.05	0.23 ± 0.05	0.16 ± 0.03
BF	Kg	1.39 ± 0.04 ^{to}	1.15 ± 0.19b	0.91 ± 0.21c
GB	Kg	1.19 ± 0.08 ^{to}	0.93 ± 0.18b	0.75 ± 0.20c
DI	kg $^{m-3}$	0.792 ± 0.21	0.908 ± 0.22	0.647 ± 0.14

DF	kg $^{m-3}$	5.56 ± 0.14 ^{A}	4.61 ± 0.77b	3.65 ± 0.14c
TEC	%	6.45 ± 0.82 ^{A}	5.45 ± 0.84b	5.73 ± 0.82b
CAA	Reason	1.08 ± 0.16	1.00 ± 0.13	1.00 ± 0.09
EAA	%	94 ± 0.15	101 ± 0.13	101 ± 0.10
S	%	99.0 ± 0.2	87.0 ± 0.24	100 ± 0.0

The data presented are composed of means ± standard deviation obtained from the experimental units (01 water tank = 01 replicate). Legend: initial average weight (PMI); final average weight (MPW); weight gain (WG); initial biomass (BI); final biomass (BF); biomass gain (GB); initial density (DI); final density (DF); specific growth rate (TCE); Apparent feed conversion (ACR); Apparent feed efficiency (EAA); Survival(s).

Table 4. Zootechnical indices of experimental cultivation of juvenile tambaqui (*Colossoma macropomum*) fed one (TF1), two (TF2), three (TF3) and four (TF4) times/day for 30 days in a recirculating system (RAS)

Acronym	Unit	TF1	TF2	TF3	TF4
PMI	g	6.03 ± 1.90	6.09 ± 2.29	5.31 ± 0.64	5.19 ± 1.29
PMF	g	26.87 ± 10.74b	35.08 ± 5.96a	35.61 ± 4.54a	34.05 ± 6.58a
GP	g	20.84 ± 8.85b	29.00 ± 5.60a	30.29 ± 5.09a	28.86 ± 5.80a
BI	Kg	0.21 ± 0.07	0.21 ± 0.02	0.19 ± 0.02	0.18 ± 0.05
BF	Kg	0.94 ± 0.38b	1.23 ± 0.21a	1.25 ± 0.16a	1.19 ± 0.23a
GB	Kg	0.73 ± 0.3b	1.01 ± 0.20A	1.06 ± 0.18a	1.01 ± 0.20A
DI	kg $^{m-3}$	0.84 ± 0.27	0.85 ± 0.32	0.74 ± 0.09	0.73 ± 0.18
DF	kg $^{m-3}$	3.76 ± 1.50b	4.91 ± 0.83a	4.98 ± 0.64a	4.77 ± 0.92a
TEC	%	4.91 ± 0.28	5.96 ± 1.07	6.34 ± 0.79	6.29 ± 0.56
CAA	Reason	0.89 ± 0.03a	0.98 ± 0.10a	1.12 ± 0.11b	1.11 ± 0.06b
EAA	%	113 ± 0.04a	103 ± 0.11a	90 ± 0.9b	90 ± 0.5b
S	%	100 ± 0.0A	84 ± 0.28a	99 ± 0.64a	98 ± 0.02a

The data presented are composed of means ± standard deviation obtained from the experimental units (01 water tank = 01 replicate). Legend: initial average weight (PMI); final average weight (MPW); weight gain (WG); initial biomass (BI); final biomass (BF); biomass gain (GB); initial density (DI); final density (DF); specific growth rate (TCE); Apparent feed conversion (ACR); Apparent feed efficiency (EAA); Survival(s).

Dietary restriction significantly influenced the PMF, GW, BF, GB and FD indices, with the highest values observed in TR1, followed by TR2 and TR3 ($p < 0.05$). Fish submitted to TR1 showed the best results of specific growth rate (TCE) when compared to TR2 and TR3, which did not differ from each other.

In the analysis of the feeding frequency (TF) treatments, the fish of TF1 showed the lowest values of PMF, GP, BF, GB and DF compared to the other treatments. The apparent feed conversion (AAC) was lower in TF1 and TF2, while the survival rate was higher in TF2 ($p < 0.05$) (Table 4).

BLOOD AND BIOCHEMICAL PARAMETERS

Fish submitted to TR3 treatment had the lowest values of hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), in addition to the highest levels of total proteins (PRT) when compared to the other treatments ($p < 0.05$) (Table 5). The other parameters RBC, CHCM, GLI, TRG and CLT did not differ significantly between the treatments ($p > 0.05$).

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ISSN: 2358-2472

Table 5. Blood parameters of tambaqui (*Colossoma macropomum*) juveniles submitted to daily feeding (TR1) and feeding restriction of one day per week and refeeding (TR2) and two consecutive days per week and refeeding (TR3) for 30 days in a recirculating system (RAS).

Parameter	Unit	TR1					TR2				TR3	
Hb	g.dL-1	4,9	±	0.9a		4,7	±	0.9a		3,5	±	1.1b
RBC	106.mm-3	1,4	±	0,3		1,5	±	0,2		1,6	±	0,4
Ht	%	27,9	±	2.5a		29,3	±	4.8a		24,6	±	3.5b
VCM	Fl	200,5	±	35.4a		192,9	±	31.7a		158,3	±	38.2b
HCM	pg	35,3	±	9.9a		30,9	±	7.4ab		24,4	±	10.4b
CHCM	g.dL-1	17,5	±	2.9a		16,2	±	3.8ab		15,1	±	4.7b
GLI	mg.dL-1	97,0	±	13,7		94,6	±	9,8		90,8	±	12,1
CLT	mg.dL-1	442,4	±	181,4		369,0	±	135,5		463,0	±	131,4
TRG	mg.dL-1	697,5	±	321,3		556,5	±	241,0		401,3	±	170,2
PRT	g.dL-1	1,18	±	0.7 to		1,25	±	0.8a		2,10	±	1.4 b

The data presented are composed of means ± standard deviation obtained from the sampled fish (n=15 per treatment). Legend: hemoglobin (Hb); number of red blood cells (RBC); hematocrit (Ht); Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); Plasma Glucose (GLI); Total Cholesterol (CLT); Triglycerides (TRG); Total Proteins (PRT). Different letters indicate a significant difference between the treatments (p<0.05).

In the food frequency experiments, there was a slight significant increase in the RBC of TF2 in relation to TF3 ($p < 0.05$), indicating a better physiological condition in this group. No significant differences were observed for Hb, Ht, MCV, HCM, and MCHC ($p > 0.05$) (Table 6).

Table 6. Blood parameters of tambaqui (*Colossoma macropomum*) juveniles fed one (TF1), two (TF2), three (TF3) and four (TF4) times/day for 30 days in a recirculating system (RAS)

Parameter	Unit	TF1	TF2	TF3	TF4
Hb	g.dL-1	4.14 ± 1.37	4.16 ± 1.39	4.26 ± 0.9	4.37 ± 0.7
RBC	106.mm-3	1.53 ± 0.2 abs	1.75 ± 0.4 A	1.41 ± 0.3 b	1.42 ± 0.3 b
Ht	%	26.9 ± 4.7	28.9 ± 3.9	26.2 ± 3.6	27.1 ± 4.3
VCM	Fl	177.6 ± 36.0	172.9 ± 47.8	188.9 ± 26.9	195.9 ± 42.6
HCM	pg	25.5 ± 7.8	26.9 ± 11.1	35.4 ± 7.9	32.7 ± 7.1
CHCM	g.dL-1	15.7 ± 5.1	14.3 ± 4.4	17.7 ± 3.7	16.3 ± 2.6
GLI	mg.dL-1	87.0 ± 13.9 b	93.9 ± 7.8 abs	96.7 ± 10.9 abs	99.0 ± 12.3 A
CLT	mg.dL-1	486.2 ± 176.2	462.2 ± 129.4	368.5 ± 129.4	376.7 ± 148.2
TRG	mg.dL-1	473.1 ± 233.9	555.5 ± 273.8	377.2 ± 164.7	626.9 ± 314.8
PRT	g.dL-1	1.26 ± 0.7 AB	2.20 ± 1.4 A	1.03 ± 0.8 b	1.88 ± 2.0 abs

The data presented are composed of means ± standard deviation obtained from the sampled fish (n=15 per treatment). Legend: hemoglobin (Hb); number of red blood cells (RBC); hematocrit (Ht); Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); Plasma Glucose (GLI); Total Cholesterol (CLT); Triglycerides (TRG); Total Proteins (PRT). Different letters indicate a significant difference between the treatments (p<0.05).

Plasma glucose (GLI) was significantly higher in TF4 compared to TF1, while PRT levels were higher in TF2 than in TF3 ($p < 0.05$). CLT and TRG were not affected by food frequency ($p > 0.05$).

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ISSN: 2358-2472

DISCUSSION

The results indicate that water quality remained within the recommended levels for tambaqui farming in intensive systems (Lima et al., 2019; Costa et al., 2022), corroborating other studies that evaluated different feeding strategies in recirculation systems (Zaniboni Filho; Pedron; Ribolli, 2018).

The influence of feed restriction on productive performance observed in the present study reinforces the findings of Garcez et al. (2023), who reported better zootechnical indices for juvenile tambaqui when subjected to daily feeding in excavated nurseries. Similar results were found in Nile tilapia (*Oreochromis niloticus*) fed daily, which showed greater weight and biomass gain compared to groups under dietary restriction (Arauco; Costa, 2012).

However, some research points to benefits of partial dietary restriction, as reported by Assis et al. (2020), who recommended a 1-day/week restriction for tambaqui in recirculation systems. Similar strategies were applied to pirapitinga (*Piaractus brachypomus*) (Favero et al., 2021) and to the tambacu hybrid (*Colossoma macropomum* x *Piaractus mesopotamicus*) (Paz; Pastrana; Brandão, 2018), resulting in lower feed consumption without significant impairment to productive performance. However, the reduction of 2 days/week negatively impacted feed conversion and growth in the present study, reinforcing that daily feeding is still the most effective strategy for tambaqui juveniles in RAS systems.

Regarding feeding frequency, the present study suggests that feeding fish twice a day is the best strategy to optimize zootechnical performance. This conclusion is in line with the findings of Souza et al. (2014); Porto et al. (2020) and Leal et al. (2024), who observed better growth rates and feed utilization for tambaqui under this diet.

From a physiological point of view, the feeding restriction of 2 days/week negatively impacted the blood parameters of the fish, significantly reducing Hb, Ht, MCV and HCM. This response suggests a possible suppression in the hematopoietic system or an adaptation to nutritional stress, as indicated by Fazio (2019). Previous research has reported similar reductions in these parameters for tambaqui in prolonged periods of restriction (Assis et al., 2020; Roa et al., 2019). However, no significant differences were found in hematocrit and hemoglobin concentrations for *P. brachypomus* (Favero et al., 2021) and *Hoplosternum littorale* (Rossi et al., 2015) after using both cycles of feed restriction and refeeding.

According to Ahmed, Reshi and Fazio (2020) physiological responses vary with species, age, nutritional status and exogenous factors such as water temperature, salinity among other factors. Therefore, the different results in hematological variables are probably due to differences between species or production system.

The increase in plasma PRT levels of TR3 fish in relation to the other treatments suggests that fish deprived of food for 2 days/week used proteins as an energy source, maintaining their levels of GLI, CLT and TRG without changes in relation to TR1 and TR2. As reviewed by McCue (2010), in some animals glucose levels do not change in fasting due to glycogen stores being replenished through gluconeogenesis at the expense of proteins. This result contrasts with those obtained by Hernández, Hurtado-Oliva and Peña (2019) and Assis et al. (2020) who did not find changes in PRT levels in fish subjected to feed restriction.

The metabolic strategy that fish use as a source of energy under fasting varies between species and also depends on environmental conditions, the physiological state of the fish, the use of nutrients, including carbohydrates, lipids and proteins from different organs of the body. Therefore, while some fish species use lipids after the liver's glycogen stores are depleted as their primary energy source, others use muscle protein as their primary energy source (McCue 2010; Hernández; Hurtado-Oliva; Peña, 2019).

These results, together with the evaluation of zootechnical performance, indicate that the ideal is daily feeding. Although the blood parameters did not reveal significant differences between TR1 and TR2 ($p>0.05$), it was observed that TR1 showed better zootechnical performance indices than TR2.

When evaluating the results of the feed frequency (TF) experiments, the fish in TF1 showed the lowest values of PF, GP, BF, GB and DF compared to the other RTs. In relation to AAC and AAS, TF1 and TF2 showed the lowest AAC and while survival was higher in TF2 ($p<0.05$). The lowest AAC occurred because the fish were fed only once a day. However, in relation to growth and weight gain, they presented the worst results.

As in the condition of feed restriction, feeding frequency can influence the hematological and metabolic parameters of fish (Navarro; Gutiérrez, 1995; Silva et al., 2020; Gonçalves-Junior et al., 2021; Guilherme et al., 2022). Despite the relevance of physiological studies in the evaluation of the effects of the feeding strategies adopted for fish, most studies disregard these analyses and focus their efforts on the evaluation of the effects on zootechnical and economic performance.

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In the present study, a slight but significant increase in the RBC of TF2 in relation to TF3 ($p<0.05$) was observed for 30 days in RAS, indicating a better physiological condition of this group in relation to the others, since a greater number of circulating red blood cells can contribute to better oxygen transport. However, there were no significant changes ($p>0.005$) for Hb, Ht, MCV, HCM and CHCM. In previous studies, submission to different feeding frequencies did not influence the hematological parameters Hb, HT, and RBC of juvenile sturgeon, *Acipenser stellatus* (Dicu et al., 2013), Nile tilapia, *Oreochromis niloticus* (Hisano et al., 2020), tiger pufferfish, *Takifugu rubripes* (Gao et al., 2022), and Oscar, *Astronotus ocellatus* (Lopera-Barreto et al., 2024).

Regarding biochemical parameters, significant increases ($p<0.05$) were found in the Gli of TF4 in relation to TF1 and in the PRT of TF2 in relation to TF3 ($p<0.05$). CLT and TRG were not affected by food frequency ($p>0.05$). The higher glucose values in the fish fed 4x/day compared to those fed only 1x/day is expected due to the greater availability of food throughout the day prior to the blood collection. In relation to PRT, the fish fed 2x/day showed better protein utilization than those fed 3x/day.

The effects of feed restriction on the biochemical parameters studied here vary according to the species and system. Souza et al. (2014) did not observe differences in C *macropomum* blood glucose levels fed 2, 4, 6 and 8x/day. Silva et al. (2020) reported for mullet (*Mugul liza*) that increased feeding frequency promoted increases in blood content of total proteins, glucose, triglycerides, and cholesterol, possibly due to increased nutrient intake and absorption.

Dicu et al. (2013) obtained higher glucose values in *Acipenser stellatus* specimens fed 2x/day than those fed 4x/day, while the other parameters remained the same. Plasma glucose, triglycerides, and cholesterol values did not alter *Astronotus acellatus* under different feeding frequencies (Lopera-Barreto et al., 2024). However, although they did not evaluate the levels of total proteins in plasma, these last authors observed significant increases in serum albumin levels of fish fed 2 and 4x/day.

In view of the results obtained for the blood parameters of tambaqui, it can be suggested that the ideal feeding frequency is twice a day. This suggestion corroborates those already obtained for tambaqui, which indicate that when fed 2x/day, this species has better productive performance (Souza et. al., 2014; Porto et al., 2020; Leal et al., 2024).

CONCLUSION

Based on the results, we concluded that daily feeding and twice a day resulted in better results in hematology and blood biochemistry, being the most suitable for tambaqui production in an intensive culture system in the RAS modality.

ACKNOWLEDGMENTS

To the teams of the Experimental Aquaculture Laboratory (LAQEX/UFAM), the Effluent Monitoring and Treatment Laboratory (LMTE/UFAM) and the Laboratory of Physiology and Biotechnology of Aquatic Organisms (LAFBOA/UFAM)

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