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ANIMAL PRODUCTION

Zootechnical performance and physiological responses of juvenile pirapitinga (*Piaractus brachypomus*) under different feeding rates and frequencies

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ABSTRACT: This study evaluated the effects of feeding rates and frequencies on performance and physiological responses of pirapitinga (*Piaractus brachypomus*). A total of 896 juveniles (1.32 ± 0.60 g) were stocked at a density of 1 fish/L for 40 days in a recirculating aquaculture system (RAS). Four feeding rates, 6% (T_6), 8% (T_8), 10% (T_{10}) and 12% (T_{12}) of biomass, and two feeding frequencies, F_2, at 08:30 and 16:00, and F_4, at 08:30, 11:00, 13:30 and 16:00, were tested in a 4x2 factorial arrangement with four replicates. The results for the first 30 days showed an effect of feeding rate on growth parameters, but no effect for feeding frequency in general. Between 30 and 40 days, weight gain, specific growth rate showed an effect of feeding rate, with T_6 being superior. The viscero (VSI) and hepatosomatic (HSI) indices were affected, with the lowest values for T_{12}. When fed with F_2, the lowest hemoglobin was for T_6, while there was no difference among feeding rates with F_4. Higher triglyceride levels were recorded when fed with F_2, T_6, and T_8, while the lowest was for T_{12}. Blood glucose only had an effect of feeding frequency, with higher values for F_2. Hematocrit, total protein, cholesterol, and mesenteric fat index (MFI) were not affected by feeding rate nor feeding frequency. A twice-daily feeding frequency proved sufficient for juvenile *P. brachypomus*, with an average initial weight of 1.3 g and reared in RAS, and a 12% biomass feeding rate was most efficient.

Key words: feeding strategy, RAS, Neotropical fish, *Piaractus brachypomus.*

Desempenho zootécnico e respostas fisiológicas de juvenis de pirapitinga (*Piaractus brachypomus*) sob diferentes taxas e frequências de alimentação

RESUMO: Este estudo avaliou os efeitos das taxas e frequências de alimentação no desempenho e nas respostas fisiológicas de pirapitinga (*Piaractus brachypomus*). Para isso, um total de 896 juvenis (1,32 ± 0,60 g) foram estocados a uma densidade de 1 peixe/L por 40 dias em um sistema de recirculação aquícola (RAS). Quatro taxas de alimentação, 6% (T_6), 8% (T_8), 10% (T_{10}) e 12% (T_{12}) da biomassa, e duas frequências de alimentação, F_2, às 8h30 e 16 h, e F_4, às 8h30, 11 h, 13h30 e 16 h, foram testadas em um arranjo fatorial 4x2 com quatro repetições. Os resultados dos primeiros 30 dias mostraram um efeito da taxa de alimentação nos parâmetros de crescimento, mas nenhum efeito da frequência de alimentação em geral. Após 40 dias, o ganho de peso, o ganho de peso diário e a taxa de crescimento específica diária mostraram influência da taxa de alimentação, sendo T_6 superior. Além disso, os índices víscero (IVS) e hepatossomático (IHS) foram afetados, com os menores valores para T_{12}. Quando alimentados com F_2, o menor nível de hemoglobina foi observado em T_6, enquanto não houve diferença entre as taxas de alimentação com F_4. Níveis mais elevados de triglicerídeos foram registrados quando alimentados com F_2, T_6 e T_8, enquanto o menor nível foi observado em T_{12}. A glicemia apresentou influência apenas da frequência de alimentação, com valores mais altos para F_2. Hematócrito, proteína total, colesterol e índice de gordura mesentérica (IGM) não apresentaram influência da taxa ou frequência de alimentação. Portanto, uma frequência de alimentação duas vezes ao dia mostrou-se suficiente para juvenis de *P. brachypomus*, com peso inicial médio de 1,3 g e criados em sistema de recirculação aquícola (RAS), sendo que uma taxa de alimentação de 12% da biomassa foi a mais eficiente.

Palavras-chave: estratégia de alimentação, RAS, peixes neotropicais, *Piaractus brachypomus*.

INTRODUCTION

The development of feeding strategies that promote better use of food is essential in fish production, as it directly impacts zootechnical performance and physiological responses, and influences variables related to water quality in the cultivation environment (HUANG et al., 2015, HASSAN et al., 2021; KIM et al., 2021). Among the main feeding managements adopted in aquaculture, determination of the ideal feeding rate stands out, since this variable affects growth (LIU et al., 2024; RODRIGUES et al., 2024), feed efficiency (ARYANI et al., 2017; DU et al., 2006; KIM et al., 2021; MELO et al., 2024), physiological parameters (KIM et al., 2021; MELO et al., 2024) and reproductive parameters (BHUJEL et al., 2007; XIONG et al., 2022), as well as water quality (SILVA et al., 2019).

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Overfeeding can increase production costs and compromise water quality, such as by increasing ammonia concentrations (OH et al., 2019; KIM et al., 2021). Conversely, some studies have reported better performance under *ad libitum* feeding regimes, attributing this result to greater nutrient availability (GUO et al., 2020; CADORIN et al., 2022). These discrepancies highlighted the lack of a standardized feeding protocol across different species.

In addition to feeding rate, feeding frequency is also a determining factor in aquaculture production, potentially influencing apparent feed conversion (BOOTH et al., 2008), growth (CANTON et al., 2007; WU et al., 2019), lot uniformity (ABE et al., 2022), and physiological parameters (GÜROY et al., 2022). Thus, even with diets formulated according to the nutritional requirements of the cultured species, optimizing feed management is crucial for efficient nutrient utilization and reduced production costs (SILVA et al., 2019). However, factors such as economic limitations, labor availability, and the species being cultured affect the choice of the ideal feeding frequency (VILLARROEL et al., 2011; GÜROY et al., 2022; WANG et al., 2007). LI et al. (2014) observed that a feeding frequency of three to five times a day improved the growth of juvenile *Megalobrama amblycephala*. Higher frequencies, however, reduced performance and caused stress, increasing hepatic oxidation rates and decreasing immunity. In contrast, other studies demonstrated benefits of increasing feeding frequency, such as reduced feed conversion, increased daily weight gain, and increased growth rate (DWYER et al., 2002; XIE et al., 2011; HUANG et al., 2015). These inconsistencies reinforce the need for species-specific studies.

Pirapitinga, *Piaractus brachypomus* (Characiformes), is an omnivorous fish native to the Orinoco, La Plata, and Amazon river basins. It is of great economic importance in South American countries such as Brazil, Colombia, Peru, and Venezuela (JORGE et al., 2018; FERREIRA et al., 2021) and is also farmed in Asian countries such as Thailand (SUPAMATTAYA & LERSSUTTHICHAWAL, 2005) and India (KUMAR et al., 2018; DEVI et al., 2020). Although, it can reach up to 20 kg in the wild its slaughter weight in captivity varies between 1.5 and 2.0 kg. Studies have demonstrated good results for larviculture (DAVID et al., 2011; ANGELES-ESCOBAR et al., 2021; FERREIRA et al., 2023) and juvenile production in recirculating aquaculture systems (RAS) (VÁSQUEZ-TORRES et al., 2011; FERREIRA et al., 2021; FAVERO et al., 2022). However, there is a lack of information on the use of specific feeding strategies, which directly impacts the commercial production of this species. Therefore, this study evaluated the effects of different feeding rates and frequencies on the growth performance and physiological responses of juvenile pirapitinga (*Piaractus brachypomus*) raised in a recirculating aquaculture system (RAS).

MATERIALS AND METHODS

Experimental protocol

The study was carried out in the Laboratório de Aquacultura (LAQUA) of the Universidade Federal de Minas Gerais (UFMG), with approval by Research Ethics Committee (Protocol 283/2023, Belo Horizonte, Brazil).

A total of 896 juvenile *P. brachypomus* were distributed in 32 circular tanks of 28 L of useful volume (density 1 fish/L), in a RAS with mechanical (acrylic wool) and biological (crushed stones of 1 to 2 cm) filters, supplementary aeration and heaters with thermostats to control water temperature and acclimatized for two weeks. After acclimatization, all fish were individually weighed (mean initial body weight of 1.32 ± 0.60 g) with a digital scale – Marte Cientifica, model AD5002, São Paulo, Brazil) and measured for total length (2.33 ± 1.28 cm) with an ichthyometer.

The animals were fed a commercial extruded diet (Aquos Starter 45 – Total®, Bom Jesus da Penha, Minas Gerais, Brazil - 1.8 mm, 45% crude protein, 8.5% ether extract, 4% crude fiber, 15% mineral matter, 2% calcium and 1% phosphorus) during the 40 days of the experiment. The feeding strategies employed four feeding rates, based on 6, 8, 10 and 12% of biomass (T_6, T_8, T_{10} and T_{12}, respectively) and two feeding frequencies (F_2, 08:30 and 16:00, and F_4, 08:30, 11:00, 13:30 and 16:00), in a completely randomized design, under a 4 x 2 factorial arrangement with four replicates.

Feeding rates were calculated based on biomass and adjusted at each biometric. The feed remaining after 30 minutes of feeding was collected, dried in an oven (Nova Ética/Ethink Technology, Vargem Grande Paulista, São Paulo, Brazil) at 55 ºC, and then weighed to determine the feed intake.

Zootechnical performance

Biometric measurements were performed after 10, 20, 30, and 40 days of experimentation to determine fish weight and total length. The obtained data were used to determine the following zootechnical parameters:

Final weight (FW) (g);
Total length (cm)
Weight gain (WG) = final weight (g) – initial weight (g);
Specific growth rate (SGR) (% day^{-1}) = 100 x [(*ln* final weight) - (*ln* initial weight)]/interval between biometrics;
Feed conversion ratio (FCR) = feed intake (g fish^{-1})/ weight gain (WG) (g).

The survival rate was also calculated at each biometric by directly counting the animals and using the following formula:
Survival Rate (%) = 100 x (final number of fish/initial number of fish).

Blood analysis

At the end of the experimental period (40 days) and after a 24-h fast, three fish from each experimental unit (n = 12 fish/treatment) were anesthetized with 50 mg L^{-1} eugenol (FERREIRA et al., 2021) for blood collection by caudal venipuncture. Blood was collected using heparinized syringes (32G or 4 mm gauge needle) and transferred to microtubes containing 10% sodium heparin. Hemoglobin concentration was determined from whole blood using a commercial colorimetric kit (Quibasa-Bioclin <www.bioclin.com>), while hematocrit was measured using the method established by GOLDENFARB et al. (1971). Total plasma protein was determined using a RHB0-90 Brix (0 to 90%) analog refractometer.

The remaining blood was centrifuged at 4000 rpm for 10 min to separate the plasma; and subsequently, determine the concentrations of glucose, triglycerides and cholesterol by an enzymatic-colorimetric method using commercial kits (Quibasa-Bioclin <www.bioclin.com>) and reading on a Bioclin 100® semi-automatic analyzer (Quibasa-Bioclin <www.bioclin.com>).

Somatic indices

After blood collection, the same fish were euthanized by immersion in an overdose of eugenol (285 mg/L) (MATTIOLI et al., 2017) for removal of the viscera (stomach, liver, gallbladder, spleen, and intestine) and mesenteric fat, which were weighed to calculate the following indices:
VSI (Viscerosomatic Index) = (viscera weight/body weight) x 100;
HSI (Hepatosomatic Index) = (liver weight/body weight) x 100
MFI (Mesenteric Fat Index) = (mesenteric fat weight/ body weight) x 100.

Statistical analysis

The obtained data were checked for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. The data were subsequently subjected to two-way ANOVA to compare the effect of feeding rate (T_6, T_8, T_{10}, and T_{12}), the effect of feeding frequency (F_2 and F4), and that of their interaction. Tukey's post hoc test was applied to compare means at the 5% significance level. All analyses were performed with SAS statistical software, version 9.04 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Water quality parameters

Table 1 shows the results of the water quality parameters during the 40 experimental days. No significant differences were observed for the feeding rate and feeding frequency factors, nor was there any interaction between the factors for any of the parameters analyzed.

Zootechnical performance

Table 2 and table 3 shows the results for the growth parameters and survival during the 40 days of the experiment. Biometric differences between feeding rates were observed between days 1 and 10 of growth. Feeding rates T_{10} and T_{12} had higher final weight (FW), total length (TL), weight gain (WG), specific growth rate (SGR) and feed intake (FI). However, there were no significant differences in feed conversion ratio (FCR) and survival. Differences were also observed between feeding frequencies for FI, where the lowest frequency (F_2) showed higher FI compared to F_4. There was no interaction between the factors for all growth performance parameters evaluated, nor for survival.

Between days 11 and 20, the lowest feeding rate (T_6) had a higher SGR when compared to the highest feeding rate (T_{12}). Lower FCR was also found for T_6, compared to T_{10} and T_{12}. However, FI increased in line with the increase in feeding rates. For feeding frequency, the lower frequency (F_2) had a higher TL. No interaction between factors was observed during this period.

Between days 21 and 30 days, significant differences were observed for feeding rate for all growth performance parameters, except TL and survival. The highest feeding rate (T_{12}) had higher FW, WG, SGR, and FI when compared to the lowest (T_6). However, the highest feeding rates (T_{10} and T_{12}) showed the highest FCR. No significant differences

Table 1 - Water quality parameters in the culture of juvenile pirapitinga (*Piaractus brachypomus*) during 40 days and subjected to different feeding rates and frequencies.

	Temperature (ºC)	Dissolved oxygen (mg L^{-1})	Salinity (g L^{-1})	Electrical conductivity (mS cm^{-1})	pH	Total ammonia (mg L^{-1})
Feeding rate						
T_6	27.86 ± 0.52	3.98 ± 1.23	0.28 ± 0.06	0.48 ± 0.13	6.80 ± 0.09	1.92 ± 0.56
T_8	27.67 ± 0.53	3.04 ± 0.81	0.31 ± 0.03	0.57 ± 0.05	6.77 ± 0.08	1.70 ± 0.48
T_{10}	28.08 ± 0.45	3.49 ± 1.16	0.31 ± 0.04	0.52 ± 0.10	6.78 ± 0.11	1.86 ± 0.57
T_{12}	28.01 ± 0.43	2.93 ± 0.58	0.30 ± 0.06	0.57 ± 0.10	6.78 ± 0.09	1.97 ± 0.60
P-value	0.3047	0.1763	0.7048	0.2667	0.9694	0.8112
Feeding frequency						
F_2	27.94 ± 0.55	3.26 ± 1.08	0.31 ± 0.04	0.57 ± 0.07	6.78 ± 0.10	1.92 ± 0.59
F_4	27.87 ± 0.44	3.47 ± 0.98	0.29 ± 0.05	0.51 ± 0.12	6.79 ± 0.08	1.80 ± 0.49
P-value	0.7131	0.5652	0.4476	0.0961	0.7294	0.5903
P-value Interaction	0.0914	0.5816	0.8608	0.7005	0.4767	0.9151

T_6, T_8, T_{10} and T_{12}: 6, 8, 10 and 12% of biomass, respectively. F_2 and F_4: feeding 2 or 4 times a day, respectively.

were observed in feeding frequency for all growth performance parameters, except for feed intake, where increased feeding frequency led to lower FI. No interaction between the factors was observed during this period.

Between 31 and 40 days, the lowest feeding rate (T6) showed the greatest weight gain (WG) and specific growth rate (SGR), and the lowest FI, when compared to the other feeding rates. No significant differences were found in feeding frequency, except for FI, where lower frequency led to increased FI. Furthermore, there was no interaction between the factors for all evaluated growth performance parameters and for survival.

Blood parameters

Table 4 shows the blood variables for the juvenile *P. brachypomus* subjected to different feeding managements after 40 experimental days. Significant differences for feeding rate were only observed for triglycerides, with the highest and lowest triglyceride concentrations being for T_6 and T_{12}, respectively.

The F_2 feeding frequency had higher concentrations of hemoglobin, glucose, and plasma triglycerides compared to F_4. An interaction between feeding rate and feeding frequency was found for hemoglobin and triglyceride concentrations (Table 5). A lower hemoglobin concentration was observed for T_6 at the same feeding frequency (F_2). However, a higher hemoglobin concentration was found for T_{12} and F_2 compared to T_{12} and F_4. Higher triglyceride concentrations were observed for T_6 and T_8 compared to the other feeding rates at the same feeding frequency (F_2). Furthermore, triglyceride concentrations were higher for T_6 and F_2 compared to T_6 and F_4.

Somatic indices

Differences in somatic indices (Table 6) were observed only for the viscerosomatic (VSI) and hepatosomatic (HSI) indices, with both having lower values at the highest feeding rate (T_{12}). No significant differences were found for feeding frequency nor for the interaction between factors.

DISCUSSION

Knowledge of feeding strategies, such as feeding rates and frequencies, is essential for successful fish production, as these practices directly influence animal performance, survival, and physiology. In the present study, *Piaractus brachypomus* showed good adaptation to experimental conditions in the RAS systems, as evidenced by survival rates above 85% over the 40 days of the experiment, regardless of feeding rate or feeding frequency. Similar results were observed by SILVA et al. (2007), who reported good adaptation of juvenile tambaqui, *Colossoma macropomum*, in cages subjected to different feeding rates and frequencies for 45 days. More recent studies reinforce this adaptive robustness in recirculating systems. PETILLO et al. (2025) observed that juvenile *C. macropomum*, kept under different densities in RAS, did not present changes

Table 2 - Zootechnical performance and survival of juvenile pirapitinga (*Piaractus brachypomus*) subjected to different feeding rates and feeding frequencies (1 - 10 days and 11 - 20 days).

	FW (g)	TL (cm)	WG (g)	SGR (% day^{-1})	FI (g fish^{-1})	FCR	Survival (%)
---1 - 10 days---							
Feeding rate							
T_6	4.04 ± 0.38^a	5.30 ± 0.20^c	1.31 ± 0.25^c	6.90 ± 0.95^c	0.07 ± 0.01^d	0.62 ± 0.07	98.80 ± 1.84
T_8	2.63 ± 0.25^c	5.58 ± 0.12^{bc}	1.84 ± 0.33^b	8.70 ± 1.12^b	0.10 ± 0.01^c	0.57 ± 0.03	98.36 ± 2.80
T_{10}	3.15 ± 0.33^b	5.77 ± 0.26^{ab}	2.38 ± 0.43^a	10.26 ± 1.23^a	0.12 ± 0.01^b	0.61 ± 0.04	98.06 ± 1.90
T_{12}	3.69 ± 0.43^a	6.01 ± 0.15^a	2.73 ± 0.38^a	11.19 ± 0.96^a	0.14 ± 0.01^a	0.61 ± 0.09	98.06 ± 2.80
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001	0.4139	0.9410
Feeding frequency							
F_2	3.38 ± 0.66	5.72 ± 0.31	2.07 ± 0.66	9.28 ± 1.94	0.11 ± 0.02^a	0.60 ± 0.05	97.84 ± 2.74
F_4	3.37 ± 0.64	5.61 ± 0.33	2.06 ± 0.64	9.25 ± 2.01	0.10 ± 0.02^b	0.61 ± 0.07	98.80 ± 1.77
P-value	0.9406	0.5946	0.9406	0.9264	0.0395	0.6364	0.3218
P-value Interaction	0.3548	0.5946	0.3548	0.2248	0.1700	0.4285	0.5125
---11 - 20 days---							
	FW (g)	TL (cm)	WG (g)	SGR (% day^{-1})	FI (g fish^{-1})	FCR	Survival (%)
Feeding rate							
T_6	7.15 ± 1.68	7.23 ± 0.48	4.52 ± 1.78	9.77 ± 2.97^a	0.15 ± 0.01^d	0.69 ± 0.07^a	98.21 ± 2.98
T_8	6.88 ± 0.97	7.12 ± 0.24	3.73 ± 1.13	7.77 ± 2.08^{ab}	0.25 ± 0.01^c	0.84 ± 0.06^{ab}	96.27 ± 4.61
T_{10}	6.98 ± 1.93	7.07 ± 0.43	3.29 ± 2.29	6.12 ± 3.65^{ab}	0.33 ± 0.02^b	0.93 ± 0.18^b	94.19 ± 8.08
T_{12}	6.34 ± 0.98	7.38 ± 0.56	2.29 ± 0.95	4.43 ± 1.63^b	0.42 ± 0.02^a	0.98 ± 0.16^b	94.19 ± 9.42
P-value	0.7060	0.3940	0.0766	0.0046	<0.0001	0.0006	0.6870
Feeding frequency							
F_2	7.18 ± 1.57	7.37 ± 0.49^a	3.79 ± 1.95	7.48 ± 3.45	0.29 ± 0.10	0.84 ± 0.09	97.09 ± 3.89
F_4	6.50 ± 1.20	7.03 ± 0.31^b	3.12 ± 1.51	6.57 ± 3.10	0.29 ± 0.11	0.89 ± 0.22	94.34 ± 8.33
P-value	0.1938	0.0170	0.2539	0.3541	0.7853	0.2818	0.3221
P-value Interaction	0.4515	0.0733	0.4589	0.4489	0.9773	0.1383	0.4835

FW = final weight; TL = total length; WG = weight gain; SGR = specific growth rate; FI = feed intake; FCR = feed conversion ratio. Means followed by different letters in the same column indicate differences by Tukey's test ($P < 0.05$). T6, T8, T10 and T12: 6, 8, 10 and 12% of biomass, respectively. F2 and F4: feeding 2 or 4 times a day, respectively. Values are expressed by mean ± standard deviation (SD).

in zootechnical parameters, blood indicators or water quality parameters, thus demonstrating that this species tolerates these conditions well without compromising its development or well-being.

Results for zootechnical performance showed that the highest feeding rates (T_{10} and T_{12}) promoted the best responses for FW, TL, WG, SGR, and FI between 1 and 10 days of growth. According to JOBLING (2012), during the early stages of fish farming, there is a greater need for nutrients and energy to cover high metabolic rates and accelerated growth, resulting in a greater amount of feed consumed per biomass compared to the later growth phase.

Between days 11 and 20, the lowest feeding rate (T6) promoted the highest specific growth rate (SGR), the lowest feed intake (FI), and the best feed conversion ratio (FCR) when compared to T_{12}. For the period between 21 and 30 days, better growth responses (FW, WG, and SGR) were again observed for the highest feeding rates (T_{10} and T_{12}), as was the case for the period between 1 and 10 days as well; however, the lowest rates (T_6 and T_8) promoted better results for FCR. These results agreed with EIRAS et al. (2025), who observed that juvenile *C. macropomum* achieved better zootechnical performance with feeding rates between 11.87 and 12.5% of biomass per day, in RAS, considering an evaluation period of up to 28 days. The similarity between the findings reinforces that, even near the end of the experimental period, higher feeding rates continue to favor growth, while lower rates can optimize FCR. SALARO et al. (2008) also found better FCR with lower feeding rates for *Hoplias lacerdae*. According to the authors, high feeding rates can increase production costs due

Table 3 - Zootechnical performance and survival of juvenile pirapitinga (*Piaractus brachypomus*) subjected to different feeding rates and feeding frequencies (21 - 30 days and 31 - 40 days).

	FW (g)	TL (cm)	WG (g)	SGR (% day^{-1})	FI (g fish^{-1})	FCR	Survival (%)
---21 - 30 days---							
Feeding rate							
T_6	9.80 ± 0.50^{c}	8.42 ± 0.51	2.65 ± 1.54^{b}	3.40 ± 2.35^{b}	0.29 ± 0.01^{d}	0.74 ± 0.10^{a}	95.53 ± 5.64
T_8	11.90 ± 0.87^{b}	9.01 ± 0.58	5.01 ± 1.31^{ab}	5.53 ± 1.56^{ab}	0.48 ± 0.02^{c}	0.94 ± 0.12^{a}	93.75 ± 6.54
T_{10}	12.83 ± 1.14^{ab}	9.04 ± 0.71	5.84 ± 1.79^{a}	6.35 ± 2.32^{ab}	0.67 ± 0.06^{b}	1.36 ± 0.19^{b}	91.51 ± 7.85
T_{12}	14.24 ± 2.27^{a}	9.34 ± 0.90	7.89 ± 3.02^{a}	8.08 ± 2.90^{a}	0.84 ± 0.04^{a}	1.52 ± 0.39^{b}	92.85 ± 6.93
P-value	< 0.0001	0.0826	0.0007	0.0081	< 0.0001	< 0.0001	0.7119
Feeding frequency							
F_2	12.48 ± 2.30	9.11 ± 0.82	5.29 ± 2.91	5.58 ± 2.85	0.59 ± 0.22^{a}	1.10 ± 0.32	93.97 ± 6.07
F_4	11.90 ± 1.88	8.79 ± 0.64	5.40 ± 2.58	6.09 ± 2.82	0.55 ± 0.20^{b}	1.18 ± 0.42	92.85 ± 7.34
P-value	0.2560	0.2008	0.8903	0.5621	0.0312	0.3016	0.6735
P-value Interaction	0.7261	0.4734	0.9188	0.8356		0.1353	0.5328
---31 - 40 days---							
	FW (g)	TL (cm)	WG (g)	SGR (% day^{-1})	FI (g fish^{-1})	FCR	Survival (%)
Feeding rate							
T_6	17.43 ± 2.08	10.45 ± 0.38	7.62 ± 1.82^{a}	5.69 ± 1.02^{a}	0.69 ± 0.12^{b}	1.41 ± 0.60	88.39 ± 8.37
T_8	15.20 ± 1.61	10.04 ± 0.28	3.30 ± 1.53^{b}	2.42 ± 1.04^{b}	0.84 ± 0.25^{ab}	2.50 ± 1.41	85.26 ± 6.52
T_{10}	16.69 ± 1.83	10.39 ± 0.51	3.86 ± 2.34^{b}	2.61 ± 1.53^{b}	0.87 ± 0.25^{ab}	1.96 ± 1.35	87.94 ± 8.73
T_{12}	15.71 ± 0.94	10.22 ± 0.22	2.85 ± 1.65^{b}	2.02 ± 1.18^{b}	0.91 ± 0.30^{a}	2.51 ± 0.94	85.26 ± 11.20
P-value	0.0711	0.1688	0.0004	< 0.0001	0.0153	0.1667	0.8847
Feeding frequency							
F_2	15.91 ± 1.95	10.28 ± 0.46	4.11 ± 2.52	2.98 ± 1.78	1.03 ± 0.18^{a}	2.49 ± 1.22	85.49 ± 8.43
F_4	16.60 ± 1.65	10.27 ± 0.31	4.70 ± 2.82	3.39 ± 2.06	0.63 ± 0.10^{b}	1.70 ± 0.97	87.94 ± 9.04
P-value	0.2661	0.9857	0.4349	0.3889	<0.0001	0.0530	0.5065
P-value Interaction	0.8120	0.7443	0.7784	0.6801	0.1643	0.3817	0.5537

FW = final weight; TL = total length; WG = weight gain; SGR = specific growth rate; FI = feed intake; FCR = feed conversion ratio. Means followed by different letters in the same column indicate differences by Tukey's test ($P < 0.05$). T6, T8, T10 and T12: 6, 8, 10 and 12% of biomass, respectively. F2 and F4: feeding 2 or 4 times a day, respectively. Values are expressed by mean ± standard deviation (SD).

to feed waste and worsening water quality. At the end of the experimental period (31 to 40 days), the lowest feeding rate (T6) resulted in greater weight gain (WG) and specific growth rate (SGR) than the other rates, lower feed intake (FI), and no significant differences for final weight (FW), total length (TL), or feed conversion ratio (FCR). The optimal feeding rate, defined as the lowest amount of feed per meal capable of promoting maximum growth with the best FCR, can vary according to the weight range of the animals and the type of production system (EIRAS et al., 2025). During the experimental period of 11 to 40 days, the fish of the present study presented average weights ranging from 7 g (11-20 days) to 18 g (31-40 days), highlighting the importance of standardizing feeding strategies to achieve the best zootechnical performance.

Indeed, feeding rates can be influenced by several factors, such as species, age, and production system (EL-ARABY et al., 2020; CATARINO et al., 2019; MANLEY et al., 2015). Previous studies with *C. macropomum* indicated that optimal production performance depends on both juvenile weight and the rearing system. For example, juveniles weighing between 95 and 350 g raised in earthen ponds performed best when fed 3% of biomass (RODRIGUES et al., 2024), while juveniles weighing between 43 and 400 g kept in biofloc systems responded best to rates of 4% of biomass (NAKAYAMA et al., 2022).

Overall, the different feeding frequencies used did not affect the zootechnical performance parameters of the animals. Similar results were reported by COSTA-BOMFIM et al. (2014), who also found no differences in the growth performance of

Table 4 - Blood variables of juvenile pirapitinga (*Piaractus brachypomus*) subjected to different feeding rates and feeding frequencies during 40 days of cultivation.

	Hemoglobin (g dL^{-1})	Hematocrit (%)	Total protein (g dL^{-1})	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})
Feeding rate						
T_6	6.93 ± 0.98	24.89 ± 3.01	4.44 ± 0.33	88.76 ± 25.01	76.74 ± 21.33	200.53 ± 91.60^{a}
T_8	7.02 ± 1.14	25.04 ± 3.54	4.33 ± 0.35	89.20 ± 24.85	80.60 ± 20.58	184.84 ± 66.25^{ab}
T_{10}	7.41 ± 0.68	25.93 ± 4.16	4.38 ± 0.33	84.08 ± 16.55	85.69 ± 16.11	173.97 ± 50.89^{ab}
T_{12}	7.15 ± 1.01	27.53 ± 2.96	4.28 ± 0.32	81.44 ± 11.58	80.12 ± 23.63	149.35 ± 49.58^{b}
P-value	0.2998	0.0652	0.4649	0.4842	0.5269	0.0417
Feeding frequency						
F_2	7.32 ± 0.86^{a}	26.48 ± 3.87	4.39 ± 0.31	90.34 ± 21.29^{a}	81.63 ± 19.37	191.69 ± 80.91^{a}
F_4	6.94 ± 1.04^{b}	25.21 ± 3.18	4.32 ± 0.35	81.40 ± 18.15^{b}	79.95 ± 21.76	162.65 ± 48.96^{b}
P-value	0.0463	0.0953	0.3151	0.0326	0.6969	0.0245
P-value Interaction	0.0186	0.2391	0.4831	0.3416	0.3552	0.0065

Means followed by different letters in the same column indicate differences by Tukey's test ($P < 0.05$). T_6, T_8, T_{10} and T_{12}: 6, 8, 10 and 12% of biomass, respectively. F_2 and F_4: feeding 2 and 4 times a day, respectively. Values are expressed by mean ± standard deviation (SD).

Rachycentron canadum fed at frequencies of 1, 2, 3, 4, or 6 times a day. The authors recommended the lowest frequency (once a day), highlighting economic benefits such as reduced labor costs and greater profitability. Similarly, ASSIS et al. (2025) investigated the impact of feeding frequency (two or three times a day), combined with a weekly restriction cycle, on juvenile *C. macropomum*. Although, they did not observe significant differences in zootechnical performance, fish fed twice a day showed greater development of intestinal villi, suggesting possible physiological advantages in digestion and nutrient absorption.

Conversely, higher feed intake was observed in fish subjected to the lowest feeding frequency throughout the 40 experimental days, with the exception only of the period between 11 and 20 days, in which there was no significant difference. In the study by RODRIGUES et al. (2024), juvenile tambaqui cultivated in earthen ponds showed similar responses, and the authors highlighted the species' ability to regulate feed intake to benefit from a low feeding frequency.

Lower feeding frequencies may be accompanied by greater physiological stress, as indicated by the higher plasma glucose and triglyceride concentrations, as well as higher hemoglobin concentrations, observed in the present study. Therefore, adopting lower feeding frequencies

Table 5 - Interaction (mean ± standard deviation) for hemoglobin and triglycerides of juvenile pirapitinga (*Piaractus brachypomus*) subjected to different feeding rates and feeding frequencies after 40 days of cultivation.

Feeding rate	----------Feeding frequency----------	---------Hemoglobin (g dL^{-1})----------	--------Triglycerides (mg dL^{-1})-------
T_6	F_2	6.63 ± 1.07^{Ba}	249.06 ± 100.29^{Aa}
T_8		7.39 ± 0.67^{Aa}	207.23 ± 57.63^{ABa}
T_{10}		7.57 ± 0.55^{Aa}	162.98 ± 68.14^{BCa}
T_{12}		7.68 ± 0.76^{Aa}	147.51 ± 60.76^{Ca}
T_6	F_4	7.22 ± 0.83^{Aa}	152.01 ± 52.18^{Ab}
T_8		6.65 ± 1.44^{Aa}	162.44 ± 69.01^{Aa}
T_{10}		7.25 ± 0.78^{Aa}	184.96 ± 22.56^{Aa}
T_{12}		6.62 ± 0.97^{Ab}	151.19 ± 37.96^{Aa}

Capital letters indicate difference by Tukey's test ($P < 0.05$) between different feeding rates within the same feeding frequency. Lower case letters indicate difference by Tukey's test ($P < 0.05$) between feeding rates across feeding frequencies. T_6, T_8, T_{10} and T_{12}: 6, 8, 10 and 12% of biomass, respectively. F_2 and F_4: feeding 2 and 4 times a day, respectively. Values are expressed by mean ± standard deviation (SD).

Table 6 - Somatic indices of juvenile pirapitinga (*Piaractus brachypomus*) subjected to different feeding rates and feeding frequencies during 40 days of cultivation.

	--------------VSI (%)--------------	--------------HSI (%)--------------	--------------MFI (%)--------------
Feeding rate			
T_6	8.14 ± 1.04^{a}	1.58 ± 0.38^{ab}	1.25 ± 0.42
T_8	7.48 ± 0.92^{ab}	1.52 ± 0.45^{ab}	1.50 ± 0.61
T_{10}	7.45 ± 1.12^{ab}	1.79 ± 0.47^{a}	1.44 ± 0.54
T_{12}	7.18 ± 0.76^{b}	1.40 ± 0.35^{b}	1.62 ± 0.50
P-value	0.0082	0.0138	0.1269
Feeding frequency			
F_2	7.70 ± 0.92	1.64 ± 0.42	1.48 ± 0.53
F_4	7.42 ± 1.10	1.51 ± 0.44	1.42 ± 0.54
P-value	0.1589	0.1197	0.5797
P-value interaction	0.6002	0.2964	0.4086

VSI: viscero-somatic index; HSI: hepatosomatic index; MFI: mesenteric fat index. Means followed by different letters in the same column indicate differences by Tukey's test ($P < 0.05$). T_6, T_8, T_{10} and T_{12}: 6, 8, 10 and 12% of biomass, respectively. F_2 and F_4: feeding 2 and 4 times a day, respectively. Values are expressed by mean ± standard deviation (SD).

may be advantageous from a production perspective, if it does not compromise fish welfare. Therefore, a joint assessment of physiological parameters is essential to prevent decisions based solely on zootechnical performance from resulting in harm to animal health.

The analysis of the interaction between feeding rate and feeding frequency revealed significant effects on the blood parameters evaluated. The lowest hemoglobin concentration was observed in fish fed twice daily at a feeding rate of 6% of biomass and in fish fed four-times-daily feeding at a feeding rate of 12% of biomass. For triglycerides, the lowest levels were observed in fish fed twice daily at a feeding rate of 12% of biomass, indicating that both the quantity and frequency of feed supply jointly influence blood biochemical parameters. Specific combinations of feeding rate and frequency can minimize stress indicators, suggesting that optimized feeding strategies not only promote growth but also improve welfare. In this context, the higher performance observed for T_{12} indicated that; although, higher feeding rates may increase certain stress parameters in some regimes (GAO et al., 2022), the combination used with T_{12} allowed maximizing growth and feed efficiency, without significantly compromising fish welfare.

The analysis of somatic indices found the highest HSI with T_{10}, while T_6, T_8, and T_{12} showed no significant differences. The highest value for VSI was recorded with T_6, which differed only from T_{12}, indicating that the lowest VSI rate occurred at the highest feeding rate. The indices HSI and VSI are recognized as good indicators of nutrient utilization and storage, especially lipids, in the liver and abdominal cavity (SAMAD et al., 2025). Although, increases in HSI and VSI are common with higher feeding rates (MIZANUR et al., 2014; HASSAN et al., 2021), the absence of an increase in visceral energy deposition at the highest feeding rate of present study suggested that the applied rate was adequate, reflecting good nutrient utilization efficiency and feed utilization for growth, without excess accumulation in the tissues.

The reduction in VSI observed with T_{12} contrasts with studies such as those by MIHELAKAKIS et al. (2002), which reported increased VSI and HSI with increasing feeding rate for *Pagrus major*, attributing this effect to hepatic fat accumulation and excess circulating nutrients. On the other hand, CHO et al. (2007) observed no significant differences in the HSI of juvenile *P. orbignyanus* subjected to different feeding rates, demonstrating that factors such as species, diet type, and rearing conditions can modulate these indices. Similarly, KIM et al. (2021) found that the HSI and VSI of juvenile *Siniperca scherzeri* were not affected by different feeding rates, suggesting that the relationship between feeding rates and these indices may be more complex than generally assumed. It is worth noting that interpreting HSI or VSI in isolation can also be limiting, as these indices alone do not fully reflect an animal's nutritional or physiological status. Therefore, it is crucial to analyze these parameters in conjunction with zootechnical performance and biochemical indicators, to reach more accurate inferences about nutrient utilization efficiency and animal welfare.

CONCLUSION

A twice-daily feeding frequency proved sufficient for juvenile *P. brachypomus*, with an average initial weight of 1.3 g and reared in RAS, and a 12% biomass feeding rate was most efficient. This regime resulted in better growth and nutrient utilization, accompanied by improved morphometric indices and biochemical parameters, demonstrating its suitability for this rearing phase.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All protocols were approved by the Ethics Committee on the Use of Animals (CEUA / UFMG - nº 283/2023) of the Universidade Federal de Minas Gerais (UFMG). Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

DATA AVAILABILITY STATEMENT

Data may be available on request.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

We declare that this work did not use any artificial intelligence resources for the conception and writing of the manuscript.

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