Aquaculture Reports 35 (2024) 102000

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Feeding rate and feeding frequency during the grow-out phase of tambaqui (*Colossoma macropomum*) in earthen ponds

Ana Paula Oeda Rodrigues *, Luiz Eduardo Lima de Freitas, Patricia Oliveira Maciel-Honda, Adriana Ferreira Lima, Leandro Kanamaru Franco de Lima

Brazilian Agricultural Research Corporation, Embrapa Fisheries and Aquaculture, P.O. Box 90, Palmas, TO CEP 77008-900, Brazil

A R T I C L E I N F O

Keywords:
Amazonian fish
Semi-intensive system
Feeding management
Natural productivity
Feed conversion

A B S T R A C T

Adequate feeding protocols are extremely important for the technical and economic feasibility of fish production systems. Two feeding rates, 3% and 4% body weight per day (3%BW and 4%BW), and two feeding frequencies, twice and three times a day, were tested during 84 days at the initial grow-out phase of tambaqui (94.5 ± 6.4 g) in earthen ponds. No mortalities or difference in growth between treatments were observed during the trial. Total feed intake and feed conversion ratio were low in the fish fed at 3%BW. Fat deposition in the fish carcass and concentration of glycogen and lipid vacuoles in the hepatocytes were also low in fish fed at 3%BW. Plasma cholesterol and triglycerides were higher in the fish fed three times a day at 4%BW. Increased concentration of malonaldehyde was observed in the carcass of fish fed three times a day at 4%BW at the end of the storage period. Fish fed twice a day presented higher total feed intake per meal than fish fed three times a day, demonstrating that tambaqui was able to regulate the feed intake to benefit from a low feeding frequency. No difference was observed in pond phytoplankton and zooplankton concentration or sediment chemistry. Fish fed at 3%BW presented higher stomach content weight, demonstrating the contribution of the natural food as a complementary nutrition to tambaqui. Based on the results, tambaqui of 95–350 g reared in earthen ponds may be fed twice a day at 3%BW.

1. Introduction

Tambaqui (*Colossoma macropomum*) is an omnivorous fish species native to the Amazon and Orinoco River Basins and with high economic importance in Brazil and other countries within the Amazon region (Hilsdorf et al., 2022). Together with pacu (*Piaractus mesopotamicus*), pirapitinga (*P. brachypomus*) and their interspecific hybrids, they are the main group of native fish produced in South and Central America, with production also growing in Asian countries such as China, Indonesia, Malaysia, Myanmar, and Vietnam (Woynárovich and Van Anrooy, 2019). Tambaqui is a fast-growing fish, with high consumer acceptance, and tolerant to high levels of un-ionized ammonia (Gomes et al., 2020) and low water dissolved oxygen levels (Neves et al., 2020); all characteristics that demonstrate the importance of the species to the aquaculture industry (Hilsdorf et al., 2022).

Semi-intensive farming of tambaqui in earthen ponds is the most practiced system, usually in one or two phases, in monoculture, with initial weight of 2–5 g and final weight varying from 1 to 2.5 kg, with a yield of 5–12 tonnes ha^{-1-1} $year^{-1}$ (Valenti et al., 2021). In Brazil, tambaqui is commonly farmed in semi-intensive system in ponds or reservoirs with low water renovation and relies on rainfall as the main water supply (Lima et al., 2018). Feed represents 70–80% of the operational costs in such system. Therefore, adequate feeding management is extremely important for the technical and economic feasibility of the tambaqui production chain.

Feeding ratio and feeding frequency are interdependent parameters that affect the feed intake and feed efficiency, fish size homogeneity and, consequently, production, economic and environmental efficiency of semi-intensive and intensive aquaculture systems (Wang et al., 1998; Dwyer et al., 2002; Zhou et al., 2003; Wu et al., 2015; Muntaziana et al., 2017). Adequate feeding regime must consider the species' feeding habit, the fish life stage, and the singularities of the production system (Schnaittacher et al., 2005; Costa-Bomfim et al. 2014; Muntaziana et al., 2017). Most of the studies on feeding ratio and feeding frequency for a given fish species are conducted far from the farm conditions, in wet laboratories under strictly controlled water quality parameters, low

* Corresponding author.
E-mail address: anapaula.rodrigues@embrapa.br (A.P.O. Rodrigues).

https://doi.org/10.1016/j.aqrep.2024.102000
Received 9 November 2023; Received in revised form 19 January 2024; Accepted 24 February 2024
Available online 2 March 2024

stocking densities, and covering only initial life stages, with little or no representation of the total amount of feed fed during an entire culture cycle, and without the challenges and interactions faced in the farm environment (Anras et al., 2001; Glencross et al., 2023). In earthen pond farming systems, the natural food, mainly represented by the planktonic community in the pond, is an important dietary complement to planktivorous fish species, and should be considered in feeding management to minimize the feed costs and increase the production efficiency (Biswas et al., 2006; Kabir et al., 2019; Boyd et al., 2020).

Studies evaluating feeding strategies for the grow-out phase of tambaqui in farm conditions have only been carried out in cages (Chagas et al., 2005, 2007; Silva et al., 2007). The existing feeding protocols for the production of tambaqui in earthen ponds are mainly based on recommendations from feed industries without scientific validation. Therefore, the objective of the present study was to investigate the optimal feeding rate and feeding frequency in the grow-out phase (95–350 g) of tambaqui in earthen ponds, based on growth performance, blood parameters, liver histology, contribution of the natural food and fish meat quality.

2. Materials and methods

2.1. Experimental conditions

Two feeding rates, 3% and 4% body weight per day (3%BW and 4% BW), and two feeding frequencies, twice and three times a day, were assessed in the grow-out of tambaqui cultured in earthen ponds. The feeding rates and feeding frequencies tested were based on values empirically suggested by commercial feeding tables for omnivorous fish and by tambaqui farmers that apply some feeding control during the production cycle.

The experimental design was a completely randomized 2×2 factorial, with four replicates per treatment. A total of 16 earthen ponds of approximately 300 m^2 surface area and 70 cm depth were stocked with 300 fish per pond.

Juvenile tambaqui of average 13 g were purchased from a commercial farm (Tocantins, Brazil) and kept in earthen ponds similar to the experimental ponds for 45 days until they reached the desired weight for the trial (94.5 ± 6.4 g). During this period fish were fed three to four times a day with a commercial feed (40% crude protein, 2–3 mm pellet size). One week prior to the trial, experimental ponds were (i) drained; (ii) disinfected with $Ca(OH)_2$ (200 kg 1000 m^{-2}); (iii) limed with agricultural lime 30%CaO-18%MgO (300 kg 1000 m^{-2}); (iv) fertilized with urea (50 kg ha^{-1}), rice bran (100 kg ha^{-1}) and single superphosphate 18%P-16%Ca-8%S (67 kg ha^{-1}). Thereafter, ninety fish were individually weighed, and the mean weight was used to define the range of fish weight to be stocked in the experimental ponds. Each pond was stocked by manual selection and counting of fish. Ten bags with 30 fish each were weighed, and fish transferred from the original pond to the experimental pond.

The experiment was conducted for 84 days, from mid-March until mid-June 2019. The accumulated rainfall during such period was of 60.5, 127, 54.5 and 0 mm for March, April, May, and June, respectively. The water in the experimental ponds was replenished by taking from a reservoir and indirectly by the rain (groundwater). In the first 49 days, fish were fed with a 4–6 mm commercial feed (30.1% crude protein, 4041 kcal kg^{-1} gross energy, 4.7% fat, 16.7% ash, 20.9% neutral detergent fiber, 2.3% phosphorus, and 92.2% dry matter), and for the remaining duration of the experiment fish were fed a 6–8 mm commercial feed (27.9% crude protein, 4228 kcal kg^{-1} gross energy, 5.3% fat, 13.5% ash, 28.8% neutral detergent fiber, 1.9% phosphorus and 92.9% dry matter). Feed was manually offered at 10:00 and 16:00 (twice a day) and at 10:00, 13:00 and 16:00 (three times a day) six days a week. Each feeding would take a maximum of 30 min, and it was divided in two to avoid feed leftovers. To estimate feed intake, the amount of feed per meal per pond was weighed before each feeding and, in cases when fish stopped eating before finishing the allocated portion, the remaining feed was weighed and recorded. Every two weeks 30 fish per pond were sampled to monitor growth and adjust the feeding rate (3%BW or 4% BW).

The study complied with official Brazilian guidelines for the care and use of animals for scientific and educational purposes (CONCEA, protocol # 14/2018 CEUA/CNPASA), and with the National Management System of Genetic Heritage and Associated Traditional Knowledge (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – AB3C898).

2.2. Fish growth performance

At the end of the experiment, fish were fasted for 24 h and harvested to calculate survival rate and final biomass (fish were weighed in groups of ten per bag). Thirty-four fish per pond, representative of the mean size, were sampled for final body weight and final total length.

The following growth performance parameters were calculated:

- Total weight gain (kg) = final biomass – initial biomass;
- Specific growth rate (% day^{-1}) = 100 × [(ln final weight - ln initial weight)/number of days];
- Daily feed intake (% body weight day^{-1}) = [total feed intake (as fed basis)/(final biomass + initial biomass/2)]/number of days × 100;
- Survival = 100 × (final fish count/initial fish count);
- Feed conversion = total feed intake (as fed basis)/total weight gain.

2.3. Basic nutrient composition

At the beginning of the experiment, four groups of 20 fish each were sampled for initial carcass composition analysis. At the end of the experiment, eight fish per pond were sampled for final carcass composition analysis. Fish carcass samples and feed samples were analysed according to methods described in AOAC (1990) for dry matter, ash, crude protein, and fat. Gross energy was measured by calorimetry. Nutrient carcass deposition for protein and energy was calculated using the formula: [(final biomass × final carcass protein/energy) – (initial biomass × initial carcass protein/energy)]/total intake of protein/energy × 100.

2.4. Hematological analysis

Blood samples were collected from four fish per pond on days 28, 56 and 84. Fish were previously fasted for 24 h. Blood was collected from the caudal vein using 10% EDTA as anti-coagulant. Plasma was collected by centrifuging the total blood at 1500 × *g* for 10 min at 4 °C. Total blood on day 84 was used for total hemogram, including: (a) hematocrit; (b) total red blood cell (RBC); (c) hemoglobin concentration; (d) mean corpuscular volume (MCV); (e) mean corpuscular hemoglobin concentration (MCHC), as described by Maciel and Affonso (2021). Plasma glucose, triglycerides, total protein, and cholesterol were evaluated on days 28, 56 and 84 using commercial kits (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil) and spectrophotometric readings.

2.5. Liver histology

At the end of the trial, fish sampled for blood were euthanized with an overdose of eugenol for liver histomorphological analysis. Liver samples were fixed in 4% buffered formalin, included in paraffin, and stained with hematoxylin and eosin (Suvarna et al., 2013). The area of hepatocytes and the area of hepatocytes nuclei were randomly measured in 50 cells per fish (Rodrigues et al., 2017) under light microscope using a digital camera and image analysis system (Leica DM 2500, Heidelberg, Germany).

2.6. Lipid oxidation rate

At the end of the trial, four fish per pond were sampled for lipid oxidation rate assay. Fish were stored at −18 °C and analysed on days 0, 30, 60 and 90 of storage. Approximately 5.0 g of epaxial and hypaxial muscle samples without any bones were collected for the assay. Triplicate samples were analysed for malonaldehyde concentration using a spectrophotometer following the thiobarbituric acid in trichloroacetic acid extract method (Vyncke, 1970).

2.7. Primary productivity and fish stomach content

Pond plankton availability was analysed on days 0, 28, 56 and 84 of the experiment. Phytoplankton nets (20 μm mesh; 30 cm diameter) and zooplankton nets (68 μm mesh; 37 cm diameter) were dragged for 10 m along each pond, approximately 30 cm below the surface, resulting in a total water volume filtered of 0.71 and 1.75 m^3, respectively. Samples were stored in 4% formalin until analysed. Abundance and composition of the phyto- and zooplankton community (taxonomic groups) were analysed in 1-mL subsamples per pond using Neubauer and Sedwick-Rafter chambers, respectively.

Two fish per pond were sampled for stomach content analysis on days 28, 56 and 84. Fish were not fed commercial feed 24 h prior to sampling. Fish were euthanized with overdose of eugenol and stomach sampled and fixed in 4% formalin. Stomach content was qualitatively and quantitatively analysed, and total food weight in the stomach recorded. Food items were grouped in the following categories: (a) insects – including parts of adults, larvae and nymphs; (b) plants – seeds and plant tissue; (c) phytoplankton; (d) sediment – sand grains; (e) copepods; (f) cladocerans; (g) rotifers; (h) feed pellets; (i) partially digested matter – when the food item could not be identified. Based on the results, frequency of occurrence (FO) of each food item was calculated by dividing the number of stomachs in which the item occurred by the number of stomachs with food (Hyslop, 1980). The relative abundance (RA) of each food item was estimated considering the total amount of food in the stomach as 100% (Silva et al., 2008).

2.8. Water quality and pond sediment

Pond water dissolved oxygen concentration, temperature, and transparency were measured at 08:00, and pH at 16:30, six days a week, using a multiparameter probe and Secchi disc. Water alkalinity, hardness, total ammonia nitrogen, and nitrite were measured weekly, and carbon dioxide every two weeks, using commercial colorimetric kits (Alfakit).

Ponds presenting dissolved oxygen concentration below 2 mg L^{-1} and total transparency were fertilized during the experiment at two weeks intervals. Fertilization was done with half of the initial urea and single superphosphate concentrations.

Pond bottom sediment was sampled before and after the experiment. Sediment samples were collected from each pond at three locations (near the water inlet, in the middle of the pond, and near the water outlet), at 20 cm depth with a van Veen grab sampler. Excess water was discarded after 24 h of sample settling. The three sediment samples per pond were pooled, and dried in ventilated oven at 45 °C for later analysis of organic carbon, organic matter, phosphorus in resin and total nitrogen (van Raij et al., 2001).

2.9. Statistical analysis

Data on fish growth performance, carcass nutritional composition, histology, hemogram, number of fertilization episodes, water quality and bottom sediment chemistry were subjected to bi-factorial analysis of variance (PROC GLM, SAS 9.0, SAS, Cary, NC, USA), followed by Tukey's test. Blood biochemical data, stomach content weight, phyto- and zooplankton concentration, and malonaldehyde concentration in fish meat were analysed according to the evaluation interval and using mixed model (PROC MIXED, SAS 9.0, SAS, Cary, NC, USA) to analyse repeated measures in time – where the best covariance matrix structure was evaluated according to the Akaike information criterion. Each pond was considered as an experimental unit and treated as random effect. Feeding rate, feeding frequency, evaluation intervals and their interactions were treated as fixed effects. Student t-test was used to compare the means. Normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test) of the residues were tested and confirmed. Relative abundance and frequency of occurrence of the stomach contents data were analysed by the nonparametric Kruskal-Wallis test considering the treatments resulting from the interaction between feeding rate and feeding frequency as independent samples. Chemical parameters from the initial and final sediment samples were compared by Wilcoxon's test. The level of statistical significance adopted for all analysis was 0.05. Data is expressed as mean ± standard deviation.

3. Results

3.1. Fish growth performance

No fish mortality was observed during the trial. Initial and final biomass, weight gain, final body weight and final total length did not differ between treatments ($p > 0.05$) (Table 1). Equally, growth pattern of fish from the different treatments was relatively similar throughout the study (Fig. 1). Total feed intake, total feed intake per feeding, feed conversion ratio and daily feed intake were higher in the fish group fed at 4%BW than in group fed at 3%BW ($p < 0.05$) (Table 1). Total feed intake per feeding was higher in fish fed twice a day than those fed three times a day ($p < 0.05$) (Table 1).

3.2. Carcass composition and liver histology

No significant difference was observed in the fish carcass deposition of protein and energy among treatments ($p > 0.05$) (data not shown); except higher fat content observed in the carcass of fish fed at 4%BW ($p < 0.05$) (Table 2). The area of the hepatocytes was also larger in fish fed at 4%BW ($p < 0.05$), but without significant difference in the area of the hepatocyte nuclei (Table 2). Overall, most of the fish presented slightly swollen hepatocytes by the presence of vacuoles of glycogen and fat, with central nuclei and granular and eosinophilic cytoplasm (Fig. 2). Swollen polyhedron-shape hepatocytes due to more abundant vacuoles that dislocate the cytoplasm to the periphery of the cell were more frequent in fish fed at 4%BW (Fig. 1).

3.3. Hematological analysis

RBC, hemoglobin and MCV in fish after 84 days of trial were not different between treatments ($p > 0.05$) (Table 2), except hematocrit and MCHC that varied according to feeding frequency. Hematocrit was higher and MCHC were lower in fish fed twice a day ($p < 0.05$) (Table 2).

Blood biochemical parameters varied along the trial ($p < 0.05$) (Table 3). Glucose, total protein, and cholesterol presented similar values between days 56 and 84, and higher than on day 28. Triglycerides was increased only at the end of the trial on day 84. Glucose level was higher in fish fed twice a day than three times a day ($p < 0.05$) (Table 3). Significant interaction between feeding rate and feeding frequency was observed for triglycerides and cholesterol levels, in which fish fed three times a day at 4%BW presented higher concentrations of those parameters ($p < 0.05$) (Table 3).

3.4. Lipid oxidation rate

After 90 days of storage at −18 °C, lipid oxidation rate in tambaqui meat was increased, as seen by the increase in the concentration of malonaldehyde ($p < 0.05$) (Fig. 3). Significant interaction between

Table 1
Growth performance of tambaqui fed over 84 days at two feeding rates (3% and 4% body weight) and two feeding frequencies (twice or three times a day) in a 2×2 factorial design.

Variables	Treatments				*p*-values		
	3%2x	3%3x	4%2x	4%3x	Rate	Frequency	Rate * Frequency
Initial biomass (kg)	27.7 ± 1.6	27.9 ± 2.8	27.9 ± 2.6	29.5 ± 0.6	0.3902	0.4094	0.4917
Final biomass (kg)	96.8 ± 6.9	95.2 ± 6.8	99.6 ± 8.8	106.2 ± 5.9	0.0788	0.5040	0.2775
Weight gain (kg)	69.1 ± 5.7	67.3 ± 5.7	71.7 ± 7.6	76.7 ± 5.4	0.0758	0.6152	0.2974
Final individual weight (g)	340.53 ± 22.59	341.23 ± 24.96	353.82 ± 36.84	365.53 ± 32.06	0.2292	0.6830	0.7169
Final total length (cm)	25.41 ± 0.31	25.67 ± 0.78	25.52 ± 0.74	25.91 ± 0.51	0.5808	0.3040	0.8385
Total feed intake (kg)	104.4 ± 11.6^{B}	97.8 ± 7.3^{B}	127.2 ± 15.0^{A}	132.5 ± 10.8^{A}	0.0003	0.9084	0.3165
Total feed intake/meal (kg)	52.2 ± 5.8^{Ba}	32.6 ± 2.4^{Bb}	63.6 ± 7.5^{Aa}	44.2 ± 3.6^{Ab}	0.0009	<0.0001	0.9700
Feed conversion ratio	1.5 ± 0.0^{B}	1.5 ± 0.1^{B}	1.8 ± 0.1^{A}	1.7 ± 0.1^{A}	<0.0001	0.2850	0.9328
Daily feed intake (% day^{-1})	2.0 ± 0.1^{B}	1.9 ± 0.1^{B}	2.4 ± 0.1^{A}	2.3 ± 0.1^{A}	<0.0001	0.2397	0.8088

[A,B] Different superscript letters indicate significant difference between feeding rates.
[a,b] Different superscript letters indicate significant difference between feeding frequencies.

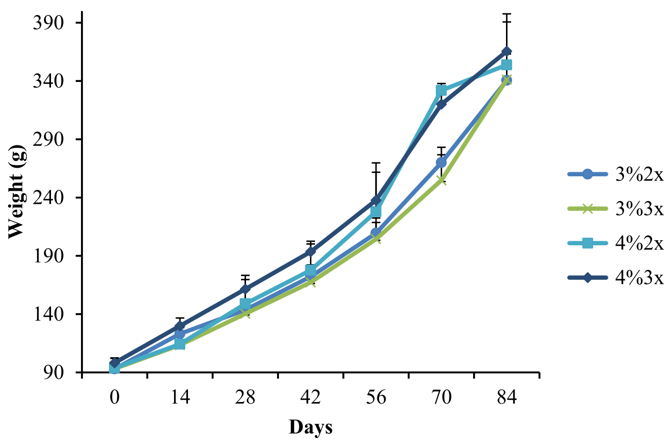

Fig. 1. Growth curve of tambaqui fed over 84 days at two feeding rates (3% and 4% body weight) and two feeding frequencies (twice or three times a day) in a 2×2 factorial design.

feeding rate and feeding frequency was detected by the higher concentration of malonaldehyde in the fish fed three times a day at 4%BW after 90 days of storage (data not shown).

3.5. Primary productivity and fish stomach content

A reduction in phytoplankton abundance was observed during the trial in all treatment ponds ($p < 0.05$), but it did not differ between treatments ($p > 0.05$) (Table 4). Significant interaction between feeding rate and time was observed, with higher abundance of zooplankton being recorded in the ponds fed at 3%BW for 84 days ($p < 0.05$) (Table 4). The number of fertilization episodes required per pond did not differ between treatments ($p > 0.05$) (Table 4).

Fish fed at 3%BW presented heavier stomach content weight ($p < 0.05$) (Table 4). Time did not affect the weight of the stomach content ($p > 0.05$) (Table 4). Cladocerans, copepods, plants, insects, and sediment were observed in the stomach of the tambaqui reared in earthen ponds. However, phytoplankton and rotifers were not observed in the stomach content. No significant difference was observed for the food items, except for sediment. High abundance of sediment was found in fish fed three times a day at 3%BW. The most abundant and frequent food items were insects and cladocerans in all treatments ($p < 0.05$). Plants were frequent but not abundant in all treatments.

3.6. Water quality and pond sediment

Except for water pH, other water quality parameters did not differ between treatments ($p > 0.05$) (data not shown). Mean dissolved oxygen, temperature and transparency in the ponds were 2.7 ± 0.72 mg L^{-1}, 29.5 ± 0.2 °C and 53.3 ± 8.1 cm, respectively. Mean values of un-ionized ammonia, nitrite, alkalinity, hardness, carbon dioxide were 0 mg L^{-1}, 0.1 ± 0.0 mg L^{-1} N-NO_2, 70.5 ± 14.1 mg L^{-1} $CaCO_3$, 71.6 ± 15.9 mg L^{-1} $CaCO_3$ and 5.6 ± 0.6 mg L^{-1}, respectively. Interaction between feeding rate and feeding frequency was observed for water pH, as lower values (7.4 ± 0.2) ($p < 0.05$) were observed in ponds where fish were fed twice a day at 3%BW (Fig. 4).

Chemical parameters of the sediment did not vary between treatments after 84 days of the feeding trial ($p > 0.05$) (Fig. 3). Carbon dioxide, organic matter, pH, and nitrogen in the sediment did not differ between beginning and end of the trial. Nevertheless, significant increase in the concentration of phosphorus in the sediment was observed from 10.8 mg dm^{-3} at the beginning to 56.8 mg dm^{-3} at the end of the trial ($p < 0.05$) (Fig. 3).

Table 2
Fat carcass composition (as is), hepatocyte areas and blood parameters of tambaqui fed over 84 days at two feeding rates (3% and 4% body weight) and two feeding frequencies (twice or three times a day) in a 2×2 factorial design.

Variables (%)	Treatments				*p*-values		
	3%2x	3%3x	4%2x	4%3x	Rate	Frequency	Rate * Frequency
Crude fat	7.18 ± 0.51^{B}	6.61 ± 0.70^{B}	8.19 ± 0.72^{A}	7.47 ± 0.71^{A}	0.0474	0.1191	0.8927
Area of hepatocyte (μm^2)	179.49 ± 6.58^{B}	173.18 ± 15.34^{B}	206.08 ± 28.44^{A}	218.70 ± 28.07^{A}	0.0060	0.7755	0.3991
Area of hepatocyte nucleus (μm^2)	16.59 ± 1.89	15.27 ± 1.22	15.18 ± 0.74	16.35 ± 1.55	0.8127	0.9187	0.1047
Hematocrit (%)	36.2 ± 1.82^{a}	35.1 ± 1.89^{b}	37.4 ± 2.15^{a}	35.3 ± 1.87^{b}	0.2018	0.0310	0.4089
RBC (10^6 μL^{-1})	1.35 ± 0.22	1.53 ± 0.35	1.47 ± 0.31	1.37 ± 0.34	0.8247	0.5947	0.0934
Hemoglobin (g dL^{-1})	10.2 ±0.69	10.4 ±0.41	10.5 ±0.66	10.5 ±0.68	0.3173	0.3572	0.4003
MCV (fL)	270.2 ± 35.6	244.9 ± 69.1	269.2 ± 94.1	286.1 ± 142.0	0.6143	0.2649	0.1099
MCHC (g dL^{-1})	28.5 ± 2.35^{b}	29.8 ± 1.59^{a}	28.4 ± 2.0^{b}	29.6 ± 1.71^{a}	0.7685	0.0087	0.9184

[A,B] Different superscript letters indicate significant difference between feeding rates.
[a, b] Different superscript letters indicate significant difference between the feeding frequencies.
RBC - red blood cells; MCV - mean corpuscular volume; MCHC - mean corpuscular hemoglobin concentration.

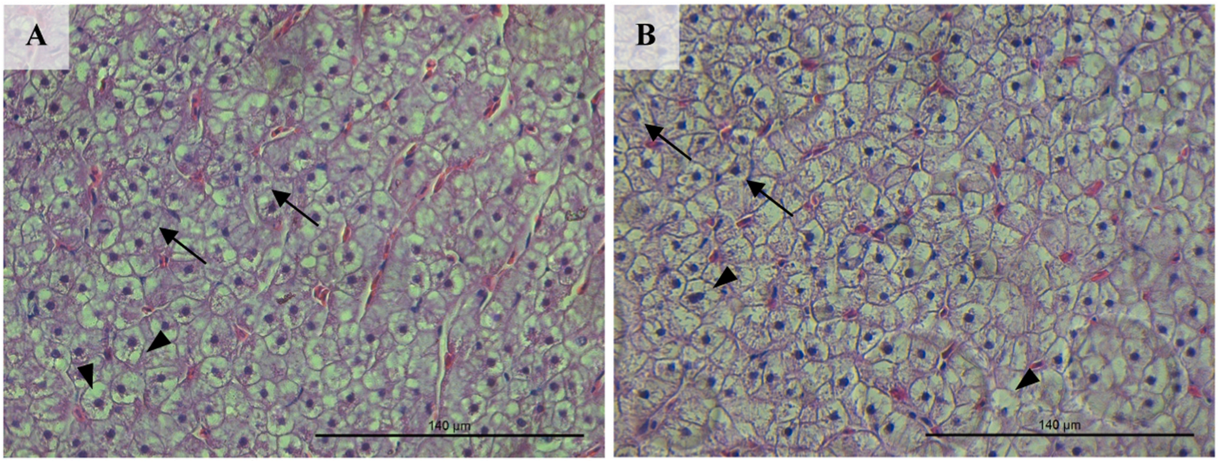

Fig. 2. (A) Liver tissue sample of tambaqui fed twice a day at 3% body weight. Slightly swollen hepatocytes by glycogen (arrow) and fat (arrowhead) vacuoles. (B) Liver tissue sample of tambaqui fed three times a day at 4% body weight. Swollen hepatocytes of polyhedron shape due to higher occurrence of glycogen (arrow) and fat (arrowhead) vacuoles. Hematoxylin and eosin staining; 400× magnification.

Table 3
Plasma glucose (Glu), total proteins (TP), triglycerides (Tri) and cholesterol (Chol) of tambaqui fed over 84 days at two feeding rates (3% and 4% body weight) and two feeding frequencies (twice and three times a day) in a 2×2 factorial design.

Variables	Time	Treatments				*p*-values[1]			
		3%2x	3%3x	4%2x	4%3x	Time	Rate	Frequency	Rate * Frequency
Glu	28d	88.2 ± 7.7^{Yx}	81.9 ± 15.8^{Yy}	87.5 ± 14.6^{Yx}	76.5 ± 6.9^{Yy}	<0.0001	0.9829	0.0182	0.8576
(mg dL^{-1})	56d	110.8 ± 5.5^{Xx}	98.9 ± 10.9^{Xy}	104.2 ± 17.8^{Xx}	110.0 ± 13.1^{Xy}				
	84d	120.7 ± 26.3^{Xx}	104.9 ± 18.7^{Xy}	125.3 ± 16.2^{Xx}	101.2 ± 12.7^{Xy}				
TP	28d	3.1 ± 0.3^{Y}	2.9 ± 0.3^{Y}	2.8 ± 0.1^{Y}	3.0 ± 0.3^{Y}	<0.0001	0.5446	0.7157	0.3976
(g dL^{-1})	56d	3.3 ± 0.2^{X}	3.2 ± 0.3^{X}	3.2 ± 0.2^{X}	3.3 ± 0.2^{X}				
	84d	3.2 ± 0.2^{X}	3.4 ± 0.1^{X}	3.3 ± 0.2^{X}	3.2 ± 0.3^{X}				
Tri	28d	261.1 ± 13.3^{YA}	234.1 ± 36.0^{YB}	265.7 ± 40.3^{YA}	292.2 ± 63.5^{YA}	0.0001	0.2596	0.9078	0.0354
(mg dL^{-1})	56d	265.0 ± 35.7^{YA}	248.5 ± 27.2^{YB}	279.5 ± 54.9^{YA}	317.6 ± 34.8^{YA}				
	84d	390.4 ± 91.8^{XA}	339.5 ± 53.7^{XB}	284.2 ± 60.0^{XA}	356.7 ± 62.0^{XA}				
Chol	28d	105.9 ± 16.6^{YA}	100.6 ± 13.4^{YB}	99.9 ± 17.8^{YA}	107.5 ± 7.6^{YA}	<0.0001	0.1915	0.8396	0.0310
(mg dL^{-1})	56d	125.1 ± 9.6^{XA}	115.0 ± 5.7^{XB}	120.7 ± 18.1^{XA}	144.3 ± 9.3^{XA}				
	84d	135.2 ± 24.4^{XA}	117.5 ± 21.2^{XB}	130.6 ± 26.0^{XA}	139.2 ± 33.9^{XA}				

[X, Y, Z] Different superscript letters indicate significant difference between sampling dates (time).
[x, y] Different superscript letters indicate significant difference between feeding frequencies.
[A, B] Different superscript letters within the same feeding frequency indicate significant difference between feeding rates.
[1] *p*-values for the interaction rate*time, frequency*time and rate*frequency*time were $p > 0.05$.

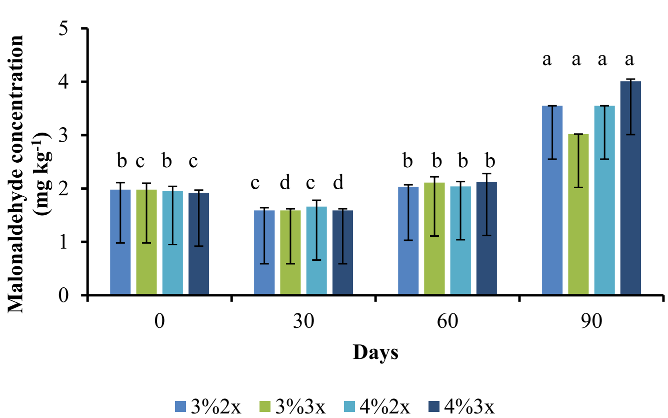

Fig. 3. Malonaldehyde concentration in tambaqui stored at −18 °C for 90 days. Fish were fed over 84 days at two feeding rates (3% and 4% body weight) and two feeding frequencies (twice and three times a day) in a 2×2 factorial design. [a,b,c,d] Different superscript letters indicate significant difference in the malonaldehyde concentration over the days of storage within the same treatment ($p < 0.05$).

4. Discussion

4.1. Fish growth performance and natural food intake

Growth of tambaqui reared in earthen ponds did not differ between the different feeding rates and feeding frequencies tested in the present study. Field studies assessing feeding protocols for tambaqui were conducted in cages. For tambaqui reared in cages in floodplain lakes, the protocol for better growth and feed conversion ratio (2.9) for fish size 55–205 g was 5%BW fed twice a day (Chagas et al., 2005). Setting aside the differences that may be related to feed, water quality, stocking density and fish genetic origin, our results of lower feeding rate with higher feed efficiency can also be attributed to a lower dependence on artificial feed, highlighting the importance of the natural food as complementary nutrition to tambaqui in earthen ponds. Similar observation has been reported by Arbeláez-Rojas et al. (2002), with higher growth and better feed conversion ratio in tambaqui reared in earthen ponds than in an intensive raceway system.

Intake of planktonic organisms, mainly zooplankton, has been described for larval stages (Sipaúba-Tavares and Braga, 2007) and grow-out (Gomes and Silva, 2009) of tambaqui in captivity, as well as in the wild throughout the growth cycle (Honda, 1974; Goulding and Carvalho, 1982; Silva et al., 2000). Accordingly, high frequency of

Table 4
Abundance of phytoplankton (Phyto) and zooplankton (Zoo), number of fertilization episodes (Fert) and weight of the stomach content (GW) over 84 days of tambaqui cultured in earthen ponds at two feeding rates (3% and 4% body weight) and two feeding frequencies e (twice and three times a day) in a 2×2 factorial design.

Variables	Time	Treatments				p-values[1]			
		3%2x	3%3x	4%2x	4%3x	Time	Rate	Frequency	Rate*Time
Phyto (ind 10^3 L^{-1})	0d	37.87 ± 9.92^X	32.86 ± 22.66^X	35.84 ± 12.46^X	24.85 ± 9.79^X	<0.0001	0.0670	0.8868	0.7820
	28d	19.27 ± 4.45^Y	26.20 ± 5.70^Y	17.33 ± 10.82^Y	26.53 ± 18.16^Y				
	56d	17.62 ± 8.96^{YZ}	20.07 ± 9.61^{YZ}	13.32 ± 1.52^{YZ}	19.84 ± 11.15^{YZ}				
	84d	17.84 ± 15.70^{ZW}	7.75 ± 5.35^{ZW}	4.21 ± 2.70^{ZW}	5.19 ± 3.66^{ZW}				
Zoo (ind L^{-1})	0d	405.56 ± 366.69^a	262.88 ± 342.20^a	244.26 ± 182.58^a	24.14 ± 19.32^a	<0.0001	0.4141	0.4749	0.0264
	28d	188.37 ± 40.86^a	404.19 ± 183.69^a	469.88 ± 354.44^a	599.30 ± 434.85^a				
	56d	385.93 ± 374.56^a	390.12 ± 304.72^a	527.09 ± 467.66^a	432.21 ± 75.16^a				
	84d	1090.70 ± 947.26^a	246.51 ± 154.55^a	175.23 ± 70.90^b	111.28 ± 111.15^b				
Fert		3.00 ± 0.82	2.50 ± 1.00	3.25±1.71	2.75±0.50	NA	0.6573	0.3808	NA
GW (g)	28d	0.25 ± 0.19 ^{A}	0.55 ± 0.77^A	0.30 ± 0.32^B	0.19 ± 0.09^B	0.9989	0.0340	0.8522	0.4539
	56d	0.33 ± 0.17^A	0.40± 0.55^A	0.22 ± 0.13^B	0.32 ± 0.14^B				
	84d	0.66 ±0.50^A	0.38 ± 0.27^A	0.10 ± 0.08^B	0.14 ± 0.07^B				

[X,Y,Z,W] Different superscript letters indicate significant difference between sampling dates (time).
[a,b] Different superscript letters within the same sampling date indicate significant difference between feeding rates.
[A,B] Different superscript letter indicate significant difference between feeding rates.
[1] *p*-values for frequency*time, rate*frequency, and rate*frequency*time interactions were $p > 0.05$.
ind - individuals; NA – not applicable.

occurrence of cladocerans and insects in the stomachs of tambaqui in this study support such findings, demonstrating that tambaqui benefit from the plankton available in the pond.

The heavier stomach content of fish fed at 3%BW points to the greater intake of natural food items available in the pond. Higher intake of zooplankton and insects, which are rich in protein (Silva et al., 2000), may have contributed to a growth rate similar to those fish fed at 4%BW.

The time of sampling did not affect the weight of the stomach content, showing that when fish are deprived from artificial feed, they do not increase natural food intake as they grew. The type of natural food items ingested by tambaqui changes according to the growth stage, with planktonic crustaceans becoming less important as fruits and seeds intake increases in the natural environment (Honda, 1974; Abelha et al., 2001). This may explain the constant intake of zooplankton during the present study.

4.2. Feeding efficiency, carcass composition and physiological responses

Feeding frequency did not affect feed efficiency or carcass composition, whereas they were affected by feeding rate. Fish fed at 4%BW consumed higher amount of feed, resulting in excess energy, increased feed conversion ratio and carcass fat deposition. In addition, fish fed at 4%BW three times a day presented higher blood triglycerides and cholesterol levels. High blood cholesterol may be due to high dietary intake of cholesterol or its precursor fatty acids (Caula et al., 2008), whereas high blood triglycerides may result from increased fat mobilization rate (McCue, 2010). Increased feed intake and feed conversion ratio as a function of increased feeding rate was also observed in Brazilian sardine (*Sardinella brasiliensis*), associated with higher fat carcass deposition and higher blood cholesterol and triglycerides levels (Baloi et al., 2017). Such reduction in the feed utilization efficiency was followed by a reduction in some digestive enzymes activity in the sardine, demonstrating that lower feeding rates may stimulate physiological adaptations that compensate the low food intake and increase utilization efficiency.

Increased size of hepatocytes in fish fed at 4%BW, caused by larger amount of glycogen and lipids vacuoles, may be a sign of cellular degeneration and, together with the increased carcass fat deposition, suggests lipogenesis due to higher feed intake (Jiang et al., 2020). Signs of cellular degeneration, although to a low extent, caused by vacuoles in the cytoplasm, were observed in the majority of fish liver, which can be attributed to high feed intake in semi-intensive farming systems (Fernandes Junior et al., 2016). Increased values of plasma glucose, total protein, triglycerides, and cholesterol observed during tambaqui grow-out may also be due to high feed intake in the specific farming system.

Higher feed intake per meal observed in the fish fed twice a day than in those fed three times a day demonstrated that tambaqui in grow-out was able to regulate the feed intake to benefit from a low feeding frequency. Such adaptation is commonly observed in response to lower feeding frequency, and it is linked to the physical capacity of the fish stomach (Dwyer et al., 2002; Baloi et al., 2016). The latter, together with fish growth performance, is determinant to stablish the ideal feeding frequency. Additionally, in commercial farms, another important factor is the cost of labor to feed fish; for fish farms that use manual feeding, the greater the frequency, the higher the cost of feeding (NRC, 2011). The higher plasma glucose levels found in fish fed twice a day can also be due to this adaptation. In juvenile Lebranche mullet (*Mugil liza*), the increase in plasma glucose levels observed at higher feeding frequencies was related to an increase in total feed intake and, consequently, in the availability of dietary carbohydrates (Calixto da Silva et al., 2020). In our study, as the feeding rate was fixed, total feed intake did not differ between the tested feeding frequencies. However, the higher total feed consumption per meal in fish fed twice a day can have provided a larger amount of carbohydrates per meal for tambaqui, increasing the levels of plasma glucose.

Fish fed twice a day also presented higher values of hematocrit, which may result from stress events, hemoconcentration, or response to anemia (Campbell, 2004). Nevertheless, no changes in total proteins or RBC were observed that could lead to either hemoconcentration or anemia. The reduced MCHC, but stable hemoglobin concentration, was expected and does not interfere in fish homeostasis. Despite the overall variations in the hematological parameters of fish fed twice a day, the hemogram values were within the normal range reported for a healthy tambaqui (Costa et al., 2019; Izel-Silva et al., 2020; Maciel and Affonso, 2021), indicating that both feeding frequencies were suitable for the species.

4.3. Lipid oxidation rate

Feed composition and the amount of feed fed to fish in intensive systems may affect lipid oxidation rate, for example, by pro- and antioxidant chemicals and their excess intake (Fogaça and Sant'ana, 2009). The longer the exposure and storage of fish, higher the rancidification, a trend that was observed in the fish meat of all treatments of the present study. Meat of fish fed three times a day at 4%BW and stored for 90 days presented high concentration of malonaldehyde, concurring with the high carcass fat deposition. Nevertheless, it is important to note that the

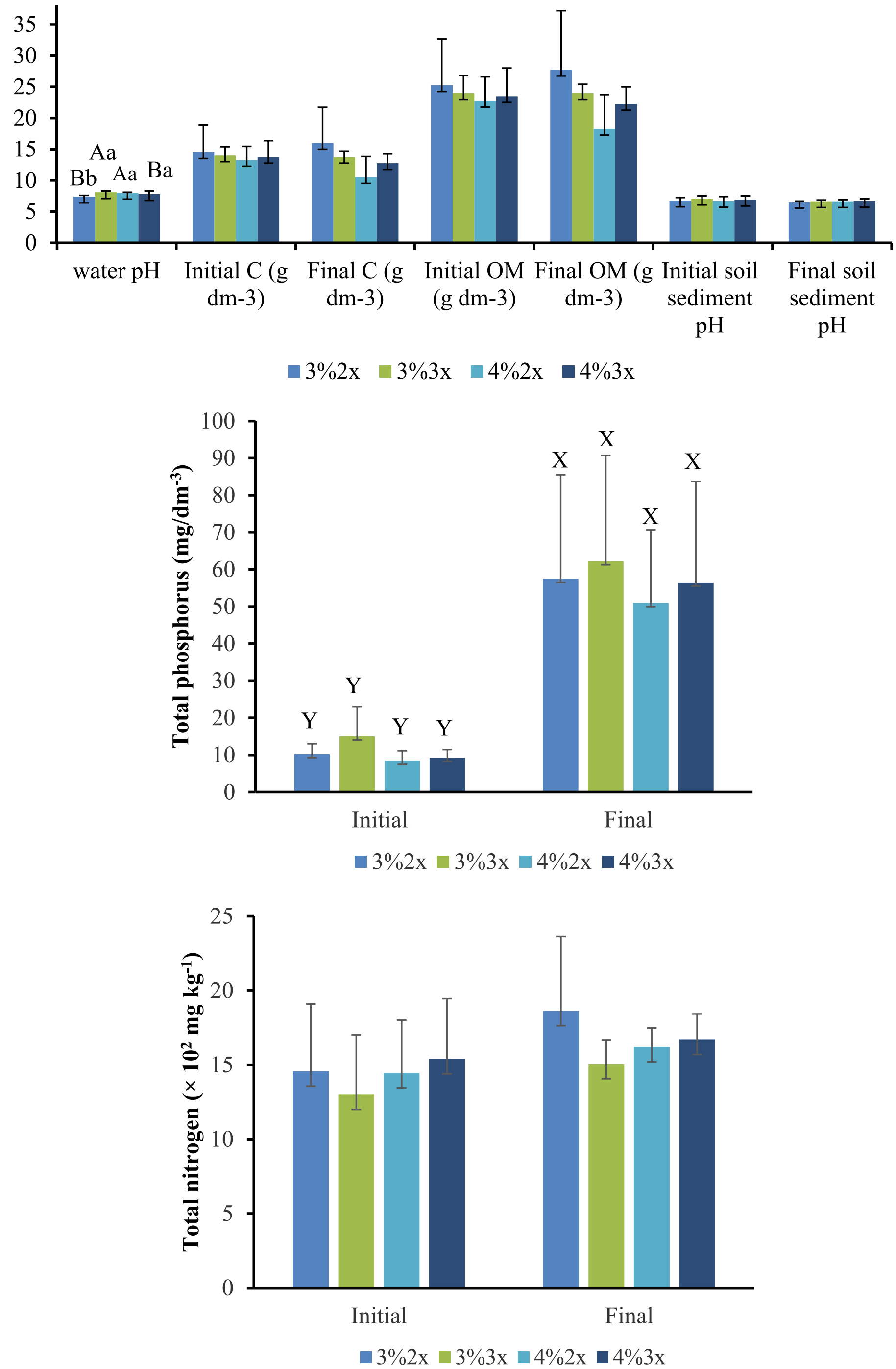

Fig. 4. Mean water pH, and pond bottom sediment organic carbon (C), organic matter (OM), pH, phosphorus (P), and total nitrogen (N) at beginning and end of 84 days of tambaqui feeding at two feeding rates (3% and 4% body weight) and two feeding frequencies (twice and three times a day) in a 2×2 factorial design. [A, B] Different superscript letters indicate significant difference between feeding rates within the same feeding frequency. [a, b] Different superscript letters indicate significant difference between feeding frequencies within the same feeding rate. [X,Y] Different superscript letters indicate significant difference between sampling dates (time).

malonaldehyde concentrations during the storage period tested were below the sensory rejection levels of 5.0–8.0 mg kg^{-1} fish (Bogdanović et al., 2012). The fish tested in the present trial were from the initial grow-out stage, and studies are warranted in the final grow-out stage (1–2.5 kg fish) to assess the oxidation curve of those products meant for immediate consumption.

4.4. Primary productivity, water quality, and sediment

The phytoplankton community is strongly influenced by weather and anthropogenic factors (Sipaúba-Tavares et al., 2019). During wet season, low luminosity has direct effect upon primary productivity (Kopp et al., 2016) and, consequently, on water dissolved oxygen concentrations. During most part of the study local rainfall was high, which may explain the reduction in primary productivity, even with increased fish biomass and organic load.

Water quality parameters remained within the recommended range for neotropical fish species (Baldisseroto and Gomes, 2020), except for dissolved oxygen, which was below 4 mg L^{-1}. Low levels of dissolved oxygen were also observed in the morning in other studies in ponds with tambaqui in the Amazon region (Gomes and Silva, 2009; Izel-Silva et al., 2020) and in cages in floodplain lakes (Chagas et al., 2005, 2007), as well as with tambatinga (♀*Colossoma macropomum* × ♂*Piaractus brachypomus*) reared in seasonal ponds during the wet season (Lima et al., 2018). Low oxygen levels (< 1 mg L^{-1}) are common in the natural habitat of tambaqui, and the species developed some physiological adaptations that allow survival and growth in such conditions (Neves et al., 2020).

Phosphorus, nitrogen, organic matter, and organic carbon did not vary with the feeding rate or feeding frequency. Organic matter and organic carbon levels were within acceptable values for aquaculture (Boyd et al., 2002) and similar to previously reported values in a study that characterized fertilized ponds for tambaqui farming (Souza et al., 2021). Nitrogen levels were also within range reported for fish ponds sediments, which are nitrogen-rich (Dróżdż et al., 2020). On the other hand, initial and final values for phosphorus in the sediment were lower than commonly reported values in ponds (Dróżdż et al., 2020). Knowing that the sediment can absorb high amounts of phosphorus (Boyd et al., 2002), the values reported in this study may reflect the originally low concentration of phosphorus in the soil. Considering the low phosphorus availability in the soil, it is suggested that low phosphorus was available in the water, which may have limited the phytoplankton growth, together with the low accumulation of other compounds in the sediment. Additionally, soil pH values were near neutral possibly due to adequate levels of organic matter in the sediment and the carbon:nitrogen ratio of 10:1 in the ponds during the study period. Such conditions are favorable to the microbial growth and organic matter decomposition (Boyd et al., 2002; Jiménez-Montealegre et al., 2005).

No significant difference in primary productivity and the same number of fertilization episodes needed in the ponds during the study may indicate that the differences in nutrient input in the water due to the feed was not sufficient to affect primary productivity or sediment chemistry of the ponds. Increase in the zooplankton community was observed after 28 days as a response to the initial abundance of phytoplankton, as reported by Khan and Bari (2019), who studied the plankton community in both fertilized and artificial formulated feed treated fish ponds, and by Li et al. (2019) in the natural environment. Nevertheless, the abundance of the zooplankton reduced in most of the treatments by day 84, possibly due to low availability of food source, i.e., phytoplankton, and the initial intense grazing, as described by Li et al. (2019). Significant increase in zooplankton was observed in the ponds where fish were fed at 3%BW twice a day, which may justify the increase in the fish stomach content during the same period in those ponds.

5. Conclusion

Tambaqui fed at 3%BW presented the same growth and better feed utilization efficiency than fish fed at 4%BW. The species was able to regulate feed intake to benefit from a low feeding frequency, which implies in lower cost of labor to feed fish manually. Based on the results found in this study for the initial grow-out phase (95–350 g) of tambaqui cultured in earthen ponds, the recommended feeding rate and frequency is 3% of body weight twice a day.

Funding

This study is part of the grant "BRSAqua - Ações estruturantes e inovação para o fortalecimento das cadeias produtivas da Aquicultura no Brasil" (Structuring actions and innovation to strengthen Aquaculture productive chains in Brazil Component project - Nutrition and Feeding) funded by the Brazilian Development Bank (BNDES), Brazilian Agricultural Research Corporation (Embrapa) and Ministry of Agriculture, Livestock and Supplies (MAPA), through the National Council for the Scientific and Technological Development (CNPq grant no. 421502/2017-7).

CRediT authorship contribution statement

Ana Paula Oeda Rodrigues: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **Patricia Oliveira Maciel-Honda:** Formal analysis, Investigation, Methodology, Writing – original draft, Data curation. **Luiz Eduardo Lima de Freitas:** Investigation, Methodology, Resources, Writing – review & editing. **Leandro Kanamaru Franco de Lima:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Adriana Ferreira Lima:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors are thankful to the undergraduate students who assisted during the feeding trial and to the staff at the Animal Nutrition Laboratory, Southeast Livestock Embrapa for the compositional analysis assays.

References

Abelha, M.C.F., Agostinho, A.A., Goulart, E., 2001. Plasticidade trófica em peixes de água doce. Acta Sci. Biol. Sci. 23, 425–434. https://doi.org/10.4025/actascibiolsci.v23i0.2696.

Anras, M.L.B., Beauchaud, M., Juell, J.E., Covès, D., Lagardère, J.P., 2001. Environmental factors and feed intake: rearing systems. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell Science, Massachusetts, pp. 157–188.

AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Arbeláez-Rojas, G.A., Fracalossi, D.M., Fim, J.D.I., 2002. Body composition of tambaqui, *Colossoma macropomum*, and matrinxã, *Brycon cephalus*, when raised in intensive (igarapé channel) and semi-intensive (pond) culture systems. Rev. Bras. Zootec. 31, 1059–1069. https://doi.org/10.1590/s1516-35982002000500001.

Baldisseroto, B., Gomes, L.C., 2020. Espécies nativas para piscicultura no Brasil, third ed. Editora UFSM, Santa Maria.

Baloi, M., de Carvalho, C.V.A., Sterzelecki, F.C., Passini, G., Cerqueira, V.R., 2016. Effects of feeding frequency on growth, feed efficiency and body composition of juvenile Brazilian sardine, *Sardinella brasiliensis* (Steindachner 1879). Aquac. Res. 47, 554–560. https://doi.org/10.1111/are.12514.

Baloi, M.F., Sterzelecki, F.C., Sugai, J.K., Passini, G., Carvalho, C.V.A., Cerqueira, V.R., 2017. Growth performance, body composition and metabolic response to feeding rates in juvenile Brazilian sardine *Sardinella brasiliensis*. Aquac. Nutr. 23, 1458–1466. https://doi.org/10.1111/anu.12521.

Biswas, G., Jena, J.K., Singh, S.K., Muduli, H.K., 2006. Effect of feeding frequency on growth, survival and feed utilization in fingerlings of *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) in outdoor rearing systems. Aquac. Res. 37, 510–514. https://doi.org/10.1111/j.1365-2109.2006.01457.x.

Bogdanović, T., Šimat, V., Frka-Roić, A., Marković, K., 2012. Development and application of Quality Index Method Scheme in a shelf-life study of wild and fish farm affected bogue (*Boops boops*, L.). J. Food Sci. 77, 99–106. https://doi.org/10.1111/j.1750-3841.2011.02545.x.

Boyd, C., Wood, C., Thunjai, T., 2002. Aquaculture Pond Bottom Soil Quality Management. Corvallis: Pond Dynamics/Aquaculture Collaborative Research Support Program. Oregon University, p. 41.

Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G.J., Teletchea, F., Tomasso Jr, J.R., Tucker, C.S., Valenti, W.C., 2020. Achieving sustainable aquaculture: historical and current

perspectives and future needs and challenges. J. World Aquac. Soc. 51, 578–633. https://doi.org/10.1111/jwas.12714.
Calixto da Silva, E., Sterzelecki, F.C., Alves Musialak, L., Sugai, J.K., Castro, J. de J.P., Pedrotti, F.S., Magnotti, C., et al., 2020. Effect of feeding frequency on growth performance, blood metabolites, proximate composition and digestive enzymes of Lebranche mullet (Mugil liza) juveniles. Aquac. Res. 51, 1162–1169. https://doi.org/10.1111/are.14466.
Campbell, T.W., 2004. Hematology of fish. In: Thrall, M.A. (Ed.), Veterinary Hematology and Clinical Chemistry. Lippincott Willianms & Willkins, pp. 277–289.
Caula, F.C.B., de Oliveira, M.P., Maia, E.L., 2008. Teor de colesterol e composição centesimal de algumas espécies de peixes do estado do Ceará. Ciênc. Tecnol. Aliment. 28, 959–963. https://doi.org/10.1590/S0101-20612008000400031.
Chagas, E.C., Gomes, L.D.C., Martins, H., Roubach, R., 2007. Tambaqui productivity reared in cages with different feeding rations. Cienc. Rural 37, 1109–1115. https://doi.org/10.1590/s0103-84782007000400031.
Chagas, E.C., Gomes, L.D.C., Martins, H., Roubach, R., De, Paula, Lourenço, J.N., 2005. Tambaqui growth reared in cages in a floodplain lake under different feeding rate. Pesqui. Agropecu. Bras. 40, 833–835. https://doi.org/10.1590/s0100-204×2005000800015.
Costa, O.T.F., Dias, L.C., Malmanna, C.S.Y., Ferreira, C.A.L., Carmo, I.B., Wischneski, A. G., Sousa, R.L., Cavero, B.A.S., Lameiras, J.L.V., Dos-Santos, M.C., 2019. The effects of stocking density on the hematology, plasma protein profile and immunoglobulin production of juvenile tambaqui (*Colossoma macropomum*) farmed in Brazil. Aquaculture 499, 260–268. https://doi.org/10.1016/j.aquaculture.2018.09.040.
Costa-Bomfim, C.N., Pessoa, W.V.N., Oliveira, R.L.M., Farias, J.L., Domingues, E.C., Hamilton, S., Cavalli, R.O., 2014. The effect of feeding frequency on growth performance of juvenile cobia, *Rachycentron canadum* (Linnaeus, 1766). J. Appl. Ichthyol. 30, 135–139. https://doi.org/10.1111/jai.12339.
Dróżdż, D., Malińska, K., Mazurkiewicz, J., Kacprzak, M., Mrowiec, M., Szczypiór, A., Postawa, P., Stachowiak, T., 2020. Fish pond sediment from aquaculture production - current practices and the potential for nutrient recovery: a review. Int. Agrophys. 34, 33–41. https://doi.org/10.31545/intagr/116394.
Dwyer, K.S., Brown, J.A., Parrish, C., Lall, S.P., 2002. Feeding frequency affects food consumption, feeding pattern and growth of juvenile yellowtail flounder (*Limanda ferruginea*). Aquaculture 213, 279–292. https://doi.org/10.1016/S0044-8486(02)00224-7.
Fernandes Junior, A.C., de Carvalho, P.L.P.F., Pezzato, L.E., Koch, J.F.A., Teixeira, C.P., Cintra, F.T., Damasceno, F.M., Amorin, R.L., Padovani, C.R., Barros, M.M., 2016. The effect of digestible protein to digestible energy ratio and choline supplementation on growth, hematological parameters, liver steatosis and size-sorting stress response in Nile tilapia under field condition. Aquaculture 456, 83–93. https://doi.org/10.1016/j.aquaculture.2016.02.001.
Fogaça, F.H.S., Sant'ana, L.S., 2009. Lipid oxidation in fishes: action mechanism and prevention. Arch. Vet. Sci. 14, 117–127. https://doi.org/10.5380/avs.v14i2.13995.
Glencross, B., Fracalossi, D.M., Hua, K., Izquierdo, M., Mai, K., Øverland, M., Robb, D., Roubach, R., Schrama, J., Small, B., Tacon, A., Valente, L.M.P., Viana, M.T., Xie, S., Yakupityage, A., 2023. Harvesting the benefits of nutritional research to address global challenges in the 21st century. J. World Aquac. Soc. 54, 343–363. https://doi.org/10.1111/jwas.12948.
Gomes, L.C., Silva, C.R., 2009. Impact of pond management on tambaqui, *Colossoma macropomum* (Cuvier), production during growth-out phase. Aquac. Res. 40, 825–832. https://doi.org/10.1111/j.1365-2109.2009.02170.x.
Gomes, L.C., Simões, L.N., Araújo-Lima, C.A.R.M., 2020. Tambaqui (*Colossoma macropomum*). In: Baldisseroto, B., Gomes, L.C. (Eds.), Espécies nativas para piscicultura no Brasil, third ed. Editora UFSM, Santa Maria, pp. 17–56.
Goulding, M., Carvalho, M.L., 1982. Life history and management of the tambaqui (*Colossoma macropomum*, Characidae): an important Amazonian food fish. Rev. Bras. Zool. 1, 107–133. https://doi.org/10.1590/S0101-81751982000200001.
Hilsdorf, A.W.S., Hallerman, E., Valladão, G.M.R., Zaminhan-Hassemer, M., Hashimoto, D.T., Dairiki, J.K., Takahashi, L.S., Corrêa Albergaria, F.C., Gomes, M.E. S., Venturieri, R.L.L., Moreira, R.G., Cyrino, J.E.P., 2022. The farming and husbandry of *Colossoma macropomum*: from Amazonian waters to sustainable production. Rev. Aquac. 14, 993–1027. https://doi.org/10.1111/raq.12638.
Honda, E.M.S., 1974. Contribuição ao conhecimento da biologia de peixes do Amazonas. II-Alimentação de tambaqui. *Colossoma bidens* (Spix). Acta Amaz. 4, 47–53. https://doi.org/10.1590/1809-43921974042047.
Hyslop, E.J., 1980. Stomach contents analysis—a review of methods and their application. J. Fish Biol. 17, 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x.
Izel-Silva, J., Ono, E.A., Queiroz, M.N., Santos, R.B., Affonso, E.G., 2020. Aeration strategy in the intensive culture of tambaqui, *Colossoma macropomum*, in the tropics. Aquaculture 529, 735644. https://doi.org/10.1016/j.aquaculture.2020.735644.
Jiang, M., Zhao, H., Zhai, S.W., Newton, R.J., Shepherd, B., Tian, J., Lofald, A.G., Teh, S., Binkowski, F.P., Deng, D.F., 2020. Nutritional quality of different starches in feed fed to juvenile yellow perch, *Perca flavescens*. Aquac. Nutr. 26, 671–682. https://doi.org/10.1111/anu.13026.
Jiménez-Montealegre, R., Verdegem, M.C.J., van Dam, A.A., Verreth, J.A., 2005. Effect of organic nitrogen and carbon mineralization on sediment organic matter accumulation in fish ponds. Aquac. Res. 36, 983–995. https://doi.org/10.1111/j.1365-2109.2005.01307.x.
Kabir, K.A., Schrama, J.W., Verreth, J.A.J., Phillips, M.J., Verdegem, M.C.J., 2019. Effect of dietary protein to energy ratio on performance of Nile tilapia and food web enhancement in semi-intensive pond aquaculture. Aquaculture 499, 235–242. https://doi.org/10.1016/j.aquaculture.2018.09.038.
Khan, N.S., Bari, J.B.A., 2019. The effects of physico-chemical parameters on plankton distribution in poultry manure and artificial formulated feed treated fish ponds, Noakhali, Bangladesh. Int. J. Fish. Aquat. Stud. 7, 1–7.
Kopp, R., Řezníčková, P., Hadašová, L., Petrek, R., Brabec, T., 2016. Water quality and phytoplankton communities in newly created fish ponds. Acta Univ. Agric. Silvic. Mendel. Brun. 64, 71–80. https://doi.org/10.11118/actaun201664010071.
Li, C., Feng, W., Chen, H., Li, X., Song, F., Guo, W., Giesy, J.P., Sun, F., 2019. Temporal variation in zooplankton and phytoplankton community species composition and the affecting factors in Lake Taihu – a large freshwater lake in China. Environ. Pollut. 245, 1050–1057. https://doi.org/10.1016/j.envpol.2018.11.007.
Lima, A.F., Rodrigues, A.P.O., Maciel, P.O., Prysthon, A., Flores, R.M.V., Bezerra, T.A., 2018. Small-scale fish farming in seasonal ponds in Brazil: technical and economic characterization. Lat. Am. J. Aquat. Res. 46, 314–329. https://doi.org/10.3856/vol46-issue2-fulltext-7.
Maciel, P.O., Affonso, E.G., 2021. Praziquantel against monogeneans of tambaqui (*Colossoma macropomum*). Aquac. Int. 29, 2369–2386. https://doi.org/10.1007/s10499-021-00753-y.
McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 156, 1–18. https://doi.org/10.1016/j.cbpa.2010.01.002.
Muntaziana, A.M.P., Amin, S.M.N., Kamarudin, M.S., Rahim, A., Romano, N., 2017. Feeding frequency influences the survival, growth and body lipid content of striped snakehead, *Channa striatus* (Bloch) fry. Aquac. Res. 48, 2602–2606. https://doi.org/10.1111/are.13001.
Neves, L.C., Favero, G.C., Beier, S.L., Ferreira, N.S., Palheta, G.D.A., Melo, N.F.A.C., Luz, R.K., 2020. Physiological and metabolic responses in juvenile *Colossoma macropomum* exposed to hypoxia. Fish Physiol. Biochem. 46, 2157–2167. https://doi.org/10.1007/s10695-020-00868-8.
NRC (National Research Council), 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.
Rodrigues, R.A., Saturnino, K.C., Fernandes, C.E., 2017. Liver histology and histomorphometry in hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*) reared on intensive fish farming. Aquac. Res. 48, 5083–5093. https://doi.org/10.1111/are.13325.
Schnaittacher, G., King, V.W., Berlinsky, D.L., 2005. The effects of feeding frequency on growth of juvenile Atlantic halibut, *Hippoglossus hippoglossus* L. Aquac. Res. 36, 370–377. https://doi.org/10.1111/j.1365-2109.2005.01218.x.
Silva, C.C., Ferreira, E.J.G., Deus, C.P., 2008. Dieta de cinco espécies de Hemiodontidae (Teleostei, Characiformes) na área de influência do reservatório de Balbina, rio Uatumã, Amazonas, Brasil. Iheringia Ser. Zool. 98, 465–468. https://doi.org/10.1590/S0073-47212008000400008.
Silva, C.R., Goes, L.C., Brandão, F.R., 2007. Effect of feeding rate and frequency on tambaqui (*Colossoma macropomum*) growth, production and feeding costs during the first growth phase in cages. Aquaculture 264, 135–139. https://doi.org/10.1016/j.aquaculture.2006.12.007.
Silva, J.A.M., Pereira-Filho, M., Oliveira-Pereira, M.I., 2000. Seasonal variation of nutrients and energy in tambaqui's (*Colossoma macropomum* Cuvier, 1818) natural food. Rev. Bras. Biol. 60, 599–605. https://doi.org/10.1590/s0034-71082000000400009.
Sipaúba-Tavares, L.H., Braga, F.D.S., 2007. The feeding activity of *Colossoma macropomum* larvae (tambaqui) in fishponds with water hyacinth (*Eichhornia crassipes*) fertilizer. Braz. J. Biol. 67, 459–466. https://doi.org/10.1590/S1519-69842007000300010.
Sipaúba-Tavares, L.H., Millan, R.N., Capitano, É.C.O., Scardoelli-Truzzi, B., 2019. Abiotic parameters and planktonic community of an earthen fish pond with continuous water flow. Acta Limnol. Bras. 31, e13 https://doi.org/10.1590/S2179-975×3018.
Souza, R.A.L., Takata, R., Souza, A. da S.L., Silva Júnior, M.L., Silva, F.N.L., 2021. Caracterização de sedimentos em viveiros de piscicultura na Amazônia Oriental, Brasil. Res. Soc. Dev. 10 (1), 10. https://doi.org/10.33448/rsd-v10i1.11815.
Suvarna, S.K., Layton, C., Brancroft, J.D., 2013. Bancroft's Theory and Practice of Histological Techniques, seventh ed. Churchill Livingstone Elsevier, London.
Valenti, W.C., Barros, H.P., Moraes-Valenti, P., Bueno, G.W., Cavalli, R.O., 2021. Aquaculture in Brazil: past, present and future. Aquac. Rep. 19, 100611 https://doi.org/10.1016/j.aqrep.2021.100611.
van Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais. Instituto Agronômico, Campinas.
Vyncke, W., 1970. Direct determination of the thiobarbituric acid value in thichloroacetic extracts of fish as a measure of oxidative rancidity. Feete-Scifen Anstrichm. 72, 1084–1087. https://doi.org/10.1002/lipi.19700721218.
Wang, N., Hayward, R.S., Noltie, D.B., 1998. Effect of feeding frequency on food consumption, growth, size variation, and feeding pattern of age-0 hybrid sunfish. Aquaculture 165, 261–267. https://doi.org/10.1016/S0044-8486(98)00266-X.
Woynárovich, A., Van Anrooy, R., 2019. Field guide to the culture of tambaqui *Colossoma macropomum*, Cuvier, 1816, FAO Fisheries and Aquaculture Technical Paper.
Wu, Y., Han, H., Qin, J., Wang, Y., 2015. Effect of feeding frequency on growth, feed utilization, body composition and waste output of juvenile golden pompano (*Trachinotus ovatus*) reared in net pens. Aquac. Res. 46, 1436–1443. https://doi.org/10.1111/are.12297.
Zhou, Z., Cui, Y., Xie, S., Zhu, X., Lei, W., Xue, M., Yang, Y., 2003. Effect of feeding frequency on growth, feed utilization, and size variation of juvenile gibel carp (*Carassius auratus gibelio*). J. Appl. Ichthyol. 19, 244–249. https://doi.org/10.1046/j.1439-0426.2003.00453.x.