

# Food restriction in hybrid catfish *Pseudoplatystoma reticulatum* x *Leiarius marmoratus* produced in cages: zootechnical performance, physiological metabolism and economic viability

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**ABSTRACT.** Production of the hybrid *Pseudoplatystoma reticulatum* x *Leiarius marmoratus* submitted to food restriction and re-alimentation was evaluated on commercial fish farming for zootechnical performance, physiological metabolism and economic viability. For 60 days, hybrid catfish (n=312; 562±12.00 g; 40±3.30 cm) were maintained in cages with a density of 13 fish m<sup>-3</sup>. Experimental design was totally randomized with two feeding strategies: (FR15/RE15) food restriction for 15 days, followed by re-alimentation for 15 days; (NR) no food restriction and three repetitions. Biometry was performed twice a month to determine zootechnical levels. Blood samples were collected for hematologic and biochemical analysis; and liver and intestine to determine hepatosomatic index (HSI), hepatocytes histometry and intestinal coefficient (IC). For the economic evaluation, we used the Total Operational Cost. Fish from FR15/RE15 exhibited total compensatory gain. IC and HIS were superior in FR15/RE15 at 60 days. Hematocrit, hemoglobin and erythrocyte count exhibited increased levels on FR15/RE15 at 30 and 45 days. Exposure to FR15/RE15 influenced hepatocytes histometry. Both feeding systems showed economic viability in short and long-term when fish density was 60 fish m<sup>-3</sup>. Food restriction for 15 days and re-alimentation for 15 days is indicated for hybrid catfish production in cages on market conditions.

**Keywords:** Feeding strategy; compensatory gain; economic profit.

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## Introduction

The development of technologies to improve productivity is essential to enhance the competitiveness of Brazilian aquaculture in the global market (Gallardo-Collí et al., 2020). In this context, nutrition and feeding stand out as critical factors, accounting for approximately 70% of production costs. Therefore, the creation of diets that incorporate food restriction strategies emerges as an economically relevant approach (Py et al., 2021).

A strategy is the use of compensatory growth, a phenomenon where the fish, after undergoing a period of food restriction followed by proper refeeding, experience rapid weight gain (Souza et al., 2001). This process is attributed to increased feed conversion efficiency (Savoie et al., 2017) and enhanced capacity of the gastrointestinal tract to absorb nutrients. However, for the compensatory growth to be effective, it is crucial to ensure that the fish remain in adequate health conditions throughout the production cycle (François et al., 2023).

Furthermore, compensatory growth also proves to be an effective strategy for reducing feeding costs, which represent a significant portion of expenses in fish farming systems. By optimizing feed supply, it is possible to minimize waste, improve feed conversion, and consequently increase the profitability of aquaculture operations (François et al., 2023).

The genus *Pseudoplatystoma* is among the neotropical fish with the greatest commercial importance in Brazil (Godovarthi et al., 2012). In Brazil, there are hybrids of this genus being cultivated in large scale, such as the hybrid catfish, which is the result of crossing the cachara *Pseudoplatystoma reticulatum* female,

Eigenmann and Eigenmann (1889), with the jundiá amazônico *Leiarius marmoratus* male, (Gill, 1870) (Cotrim et al., 2016). The hybrid catfish is easily adapted to diets of omnivorous fish and has an increased carcass yield compared to *P. reticulatum* and *L. marmoratus*, thus stimulating commercial interest of Brazilian fish farmers (Morshedi et al., 2011). Besides, hybrid catfish meat has a pleasant taste, with no intramuscular fishbones, firm texture and increased market value.

Fish production in cages is an intensive production system where the animals are kept in high densities in structures that allows extensive water circulation. Therefore, the country's rivers, estuaries, dams and hydroelectric reservoirs can be used for fish farming (Romanzini & Costa, 2023). Considering that there are few studies on food restriction in hybrid catfish, there is a need for research that evaluates its effects on performance, metabolic and physiological aspects to aid fish farmers with competitive strategies.

The aim of this present work is to evaluate the effect of food restriction and re-alimentation over the zootechnical performance, metabolic and physiological profile, and economic viability in hybrid catfish production (*P. reticulatum* x *L. marmoratus*) in cages in commercial aquaculture.

## Material and methods

### Ethical aspects

This study was approved by the Animal Ethics Committee (CEUA) of Mato Grosso do Sul State University-UEMS/Aquidauana, MS, Brazil, under protocol no 015/2019. The experiment was performed in partnership with a commercial fish farm at Nioaque, MS, Brazil (20°57'05"S 55°48'44"W). Six cages were used, with a volume of 4 m<sup>3</sup> each, placed in a dam of 0.5 ha of water layer.

### Experimental design and conditions

Hybrids catfish juveniles (n=312) with mean initial weight of 562±12.00 g and total length of 40±3.30 cm were randomly distributed (13 fish m<sup>-3</sup>) in two groups of two feeding strategies: (FR15/RE15) food restriction for 15 days, followed by re-alimentation for 15 days; (NR) no food restriction/continuous feeding. The treatments obtained three replications for each feeding strategy and one cage was used for each replica. After 60 days of experiment, the animals underwent fasting for further analysis.

Fish of group NR were fed manually daily at 8 am and 4:30 pm, with commercial extruded feed (particle size: 4-6mm; Guarantee levels: 32% crude protein, 6.5% ether extract, 7% crude fiber and 10% mineral matter) with a ratio of 3.0% of biomass. This same management was applied to group FR15/RE15 during re-alimentation cycles. The feed amount was adjusted biweekly, with adjustments performed after the biometries.

Twice a month, before the first feeding, dissolved oxygen (7.72±0.89 mg L<sup>-1</sup>) and temperature (27.2±2.45°C) were measured with an oximeter (Alfakit AT 160) and pH (7.65±0.90 mg L<sup>-1</sup>) was measured with a portable pH meter (Quimis). Total ammonia (0 mg L<sup>-1</sup>) was measured weekly using a commercial kit (Hach, Loveland, CO, EUA). Values of the parameters for water quality were considered adequate according to Boyd and Tucker (1998).

### Biometry and zootechnical performance index

Before the experiment started and at 15, 30, 45 and 60 days, we counted and individually measured the fish with a digital pendulum scale (Ayumi) and measured their total and standard length (cm) with an ichtyometer. Feeding was suspended 24 hours prior the biometry to empty the gastrointestinal tract.

The following zootechnical indexes were determined by biometry data: weight gain (WG) (g)= mean final weight - mean initial weight; length gain (cm)= final length - initial length; feed intake (I) (kg)= feed offered daily<sup>-1</sup> (kg) x number of total experimental days; apparent feed conversion (FC)= feed provided (kg) GP<sup>-1</sup> (kg); specific growth rates (SGR) (%)= [100 x (Ln final weight- Ln initial weight) experiment days<sup>-1</sup>]; protein efficiency ratio (PER) (%)= WG / % of feed protein) x 100; Fulton's condition factor (K)= 100 x {weight (g)<sup>-1</sup> total length (cm)<sup>3</sup>}.

### Intestinal coefficient and hepatosomatic index

Six fish of each feeding strategy (two fish/repetition) were euthanized by deepening the anesthesia state (eugenol - 50 mg L<sup>-1</sup>) to collect the intestine and liver at 15, 30, 45 and 60 days. After necropsy and sampling collection, the intestine was measured and the liver weighted to determine the intestinal coefficient and hepatosomatic index, respectively, using the following equations: intestinal coefficient (IC)= intestinal length / standard body length; hepatosomatic index (HSI)= (liver weight / body weight) x 100.

### Hematologic analysis

For the hematologic analysis at 15, 30, 45 and 60 days, six fish of each feeding strategy ( $n=2$  fish/repetition) were anesthetized with eugenol ( $50 \text{ mg L}^{-1}$ ). After blood collection, the sampled fish were euthanized and removed from the experiment. These fish were subsequently used for tissue collection (liver and intestine). Blood was collected through a caudal venipuncture, using syringe and needles covered in EDTA 3%, the following were determined: hematocrit by the microhematocrit method, hemoglobin (Hb) determined by the cyanmethemoglobin method, total count of erythrocyte number (Er), with a Neubauer chamber through the Natt-Herrick method (1952). The following hematimetric indexes were calculated: medium corpuscular volume (MCV) ( $\text{Ht} \times 10/\text{Er}$ ) and medium corpuscular hemoglobin concentration (MCHC) ( $\text{Hb} \times 100 \text{ Ht}^{-1}$ ), respectively, according to Ranzani Paiva et al. (2013). For total leukocyte and thrombocyte count, we did individual blood smears, in duplicates, dried at room temperature and stained with the May Grünwald-Giemsa-Wright staining method (Tavares-Dias & Moraes, 2004).

### Energetic mechanism analysis

To evaluate the energetic mechanism at 45 and 60 days, we determined the concentration of blood glucose (glucose oxidase [GOD]), using a glucose sensor Accu Chek Active Roche. After blood centrifugation, performed for five minutes at  $9,000 \text{ g}$  at  $4^\circ\text{C}$  (BIOPLUS-S200), the plasma was obtained and concentration of triglycerides, total cholesterol and total proteins was determined by spectrophotometry.

### Analysis of liver histometry

To perform the liver histometry at 30, 45 and 60 days, a part of the liver was fixed with buffered formaldehyde 10% for 24 hours and stored with ethanol 70% until histological processing. Liver's parts were paraffin-embedded and sliced with a  $5 \mu\text{m}$  width and stained with eosin and hematoxylin. A digital system was connected to a light microscope to screen capture ten images of each slide using a 1000X magnification. Sixty hepatocytes randomly selected were measured: cytoplasm's area ( $\mu\text{m}^2$ ) and perimeter ( $\mu\text{m}$ ) and nucleus's area ( $\mu\text{m}^2$ ), perimeter ( $\mu\text{m}$ ) and diameter ( $\mu\text{m}$ ) using the software Motic 2.0 (Motic Asia, Hong Kong). The following parameters were calculated: ratio of nucleus area/cytoplasm area ( $R_{\text{anc}} = \text{nucleus area} / \text{cytoplasm area} \times 100$ ), ratio of nucleus/cytoplasm perimeter ( $R_{\text{pnc}} = \text{nucleus perimeter} / \text{cytoplasm perimeter} \times 100$ ).

### Economic analysis of food restriction strategy

For economic analysis, data on improvements, machinery and equipment were collected in 2019, and market values were adjusted for 2024. Values of investment, such as vehicles and raw material storage were divided by the area occupied by cages in relation to the fish farming total area.

To analyze economic viability, methodology of Operational Cost following the São Paulo's Institute of Agricultural Economics was used (Matsunaga et al., 1979). The following were calculated: The Effective Operational Cost (EOC) was composed of direct expenses (outlay); the Total Operational Cost (TOC) which adds to EOC the value of depreciation and the Total Cost (TC) that comprises the opportunity cost of invested and current capital.

Only permanent workforce was considered, with a worker responsible for the fish farming activities, with a salary of R\$ 900.00 per month, plus labor charges (42%). For the calculation, period of 30 minutes daily was considered from the worker's labor to the production of hybrid catfish in cages during the experimental period and the value was divided among the treatments with 33.3% for FR15/RE15 and 66.7% for NR.

Expenses with feed, fuel, juveniles acquisition and other materials were counted. Moreover, 2.0% of total EOC was considered for occasional expenses and 5% of the total invested for maintenance of machines, equipment and improvements (Martin et al., 1995).

Depreciation of improvements, machines and equipment was determined by a linear method with a residual value of zero, except for the vehicle value which was considered 10% of the initial value. Opportunity cost of fixed capital was calculated applying an interest rate over the mean invested capital. A 6% rate per year was used and proportionately converted for the experiment period.

A simulation was performed considering a fish density of  $60 \text{ fish m}^{-3}$  (Scorvo Filho et al., 2008), which is common on *Pseudoplatystoma* spp. production. These data were plotted in Excel and production cost and possible income were calculated.

To define rentability we used the following indicators: total profit (TP), which is a multiplication of the amount sold by their market price; gross margin (GM), which is the difference between TP and EOC; net

income (NI) which is the difference between TP and TOC; profit (P) which is the difference between TP and TC; profitability index (PI) which is the percentage ratio of P and TP and remuneration index of invested capital which is the percentage between NI and invested capital.

### Statistical analysis

Data was analyzed for normality (Shapiro-Wilk) and homogeneity (Levene), and the means were analyzed for variance (two-factor ANOVA). To compare treatments and sampling times completely random experimental design was used with a factorial arrangement 2 x 4, with two treatments (FR15/RE15 and NR) and four sampling times (15, 30, 45 and 60 days) to hematologic variables. A factorial arrangement 2 x 2 was used with two treatments (FR15/RE15 and NR) and three sampling times (45 and 60 days) for triglycerides, total cholesterol, total proteins and liver histometry. Additionally, a 2 x 3 factorial scheme was used with two treatments (FR15/RE15 and NR) and two sampling times (30, 45 and 60 days).

Means were compared with Tukey test with 5% significance. Data that did not interact with treatments and sampling time are presented as means of each treatment in all sampling times and means of all treatments at each moment. Data with interaction are shown considering the means of each treatment for each sampling time. Variables of daily weight, weight gain, length gain, specific growth rates, feed intake, condition factor and apparent feed conversion were analyzed for variance only comparing treatments.

## Results and discussion

### Zootechnical performance

There was no difference between treatments of food restriction and re-alimentation (FR15/RE15) and no restriction for the final weight and length. At the end of 60 days there was no difference ( $p > 0.05$ ) to the mean values of weight gain, specific growth rates, survival and condition factor among the feeding strategies used and tested. The fish of FR15/RE15 had increased length gain and protein efficiency ratio and decreased feed intake and best apparent feed conversion (Table 1).

**Table 1.** Zootechnical performance of hybrid catfish (*P. reticulatum* x *Leiarius marmoratus*) produced in cages and submitted to food restriction and re-alimentation or continuous feeding.

Parameters	Treatments	
	FR15/RE15	NR
Mean final weight (g)	887.24±116.29	807.85±20.73
Weight gain (g)	322.24±102.07	232.19±26.63
Final length (cm)	43.18±3.79	41.11±2.66
Length gain (cm)	6.26±1.23 <sup>a</sup>	3.47±1.11 <sup>b</sup>
Feed intake (kg)	636.96±38.65 <sup>b</sup>	1,148.18±40.79 <sup>a</sup>
Apparent feed conversion	2.12±0.72 <sup>b</sup>	5.00±0.70 <sup>a</sup>
Protein efficiency ratio (%)	1.40±0.40 <sup>a</sup>	0.56±0.08 <sup>b</sup>
Specific growth rates (cm)	6.68±0.13	6.55±0.07
Condition factor	0.80±0.14	1.74±0.01

FR15/RE15= food restriction for 15 days, followed by re-alimentation for 15 days; NR= no food restriction. Values are expressed as mean ± standard deviation. Different letters in the same line are different among themselves ( $p < 0.05$ ).

This present work indicates that restriction of 15 days followed by re-alimentation for 15 days allowed total compensation in hybrid catfish juveniles, in two restriction/re-alimentation cycles, which was presented by Ali et al. (2003), when the animals submitted to food restriction grows to be the same size as the animals of the same age which were fed continuously.

Zootechnical performance parameters did not differ between treatments, except for gain in length and protein efficiency rate, which were higher in FR15/RE15, suggesting hyperphagia, one of the main mechanisms of compensatory gain, characterized by an increase in food intake during refeeding, combined with optimized metabolic efficiency for weight recovery and growth (Souza et al., 2001). During the period of restriction, vital processes depend on energy reserves, and there is a reduction in metabolic rate. When the fish receive food again, they prioritize linear growth (length) as an adaptive strategy, favoring increased feed efficiency. The high protein efficiency and prioritization of growth in length reinforce the fish's ability to adapt to the restriction/refeeding regime without significant damage to zootechnical performance. In this work, it was observed that food consumption was higher in the NR treatment; however, apparent feed conversion showed unsatisfactory results in this treatment.

This work also proves that food restriction can differ for different species (Ali et al., 2003). Salomão et al. (2017), using the same restriction period in Nile tilapia, *Oreochromis niloticus*, (Linnaeus, 1758) did not observe compensatory growth after re-alimentation. The results of the present work support the findings of Cotrim et al. (2016), that evaluated 30 days of partial food restriction with different feeding rates (5; 2; 0.5 and 0% of body weight) followed by 90 days of re-alimentation (5% of body weight) in hybrid catfish, *P. reticulatum* x *Leiarius marmoratus* (being the same hybrid as in this study). The study found total compensation in the groups with partial restriction with 2 and 0.5% of body weight; however, this present study is the first investigation about total food restriction in hybrid catfish.

For the intestinal coefficient we observed an interaction ( $p < 0.001$ ) between feeding strategy and sampling times (Table 2). After 60 days, the fish that went through food restriction exhibited increased intestinal coefficient ( $p < 0.001$ ) (Table 2).

For HSI, there was no interaction among treatments and sampling times ( $p = 0.575$ ). Regardless of the feeding strategy, there is an effect of time for HSI (total value) and at the end of the experimental period the fish that went under restriction exhibited higher HSI.

**Table 2.** Intestinal coefficient and hepatosomatic index hybrid catfish (*P. reticulatum* x *Leiarius marmoratus*) produced in cages and submitted to food restriction and re-alimentation or continuous feeding.

Treatments	Time (days)				Total
	15	30	45	60	
Intestinal coefficient					
FR15/RE15	1.35±0.13 <sup>b</sup>	1.21±0.05 <sup>b</sup>	1.29±0.30 <sup>b</sup>	2.01±0.64 <sup>Aa</sup>	1.49±0.32
NR	0.76±0.59	1.10±0.11	1.22±0.19	1.28±0.17 <sup>B</sup>	1.13±0.32
Total	1.06±0.50	1.16±0.10	1.25±0.23	1.64±0.58	
Hepatosomatic index (%)					
FR15/RE15	1.07±0.45	1.09±0.02	1.61±0.55	1.78±0.39	1.44±0.40 <sup>A</sup>
NR	0.81±0.29	0.74±0.14	0.89±0.26	1.49±0.24	1.09±0.40 <sup>B</sup>
Total	0.94±0.37 <sup>b</sup>	0.92±0.21 <sup>b</sup>	1.25±0.55 <sup>ab</sup>	1.63±0.34 <sup>a</sup>	

FR15/RE15= food restriction for 15 days, followed by re-alimentation for 15 days; NR= no food restriction. Values are expressed as mean ± standard deviation. Lowercase letters differ on lines and uppercase letters differ on columns ( $p < 0.05$ ).

During the restriction, vital processes are maintained through endogenous energy storage, leading to weight loss. This occurs in several organs, especially the liver, which plays a significant role in glycogen metabolism (Souza et al., 2001). Superior HSI on FR15/RE15 fish indicates an increase in the liver's weight, due to increased workload with triglycerides and hepatic glycogen mobilization. This indicates that food restriction affects energy storage in the liver to support vital processes due to lack of food.

### Hematologic parameters

For the erythrogram, leukogram and thrombogram, we did not observe interaction between feeding strategy and sampling time. We observed increased values of hematocrit and number of circulating erythrocyte in FR15/RE15. After 30 days, regardless of the feeding strategy, we observed an increase of hematocrit and decrease of medium corpuscular hemoglobin concentration (MCHC).

In FR15/RE15, values of Er ( $p = 0.026$ ) and Ht ( $p = 0.015$ ) were higher compared to treatment without restriction, regardless of sampling time. Hb concentration, after 30 and 60 days increased ( $p = 0.009$ ), whereas Ht exhibited an increase ( $p = 0.001$ ) at 30 days.

There was no interaction between the treatments and sampling time to leukocyte and thrombocyte total count. Regardless of the treatment, leukocyte increased ( $p < 0.001$ ) at 30 and 45 days; thrombocyte increased ( $p = 0.0089$ ) at 30 days when compared with data from day 15 (Table 3).

The values found for hematocrit were superior than the indicated by the literature for hybrid surubim (*P. reticulatum* x *P. corruscans*), according to Saran Neto et al. (2009). At 30 and 45 days, these values were 94.34 and 90.41% higher, respectively, and at 60 days, the increase was 67.15% higher compared to 15 days. However, both treatments exhibited this increase, suggesting a health state for both treatments. Increase of hematocrit or the number of erythrocytes after stress might suggest hemoconcentration or hemodilution by osmoregulatory dysfunction (Tavares-Dias & Moraes, 2003). However, these values do not indicate a negative alteration in the fish health state, whereas according to Tavares-Dias et al. (2004) hemoglobin concentration for healthy fish is 10 g 100 dL<sup>-1</sup>. In the present study, at 30 and 45 days, an increase of 33.68 and 14.92%, respectively, was observed, and at 60 days, the values remained adequate.

Despite the increase in hematocrit and hemoglobin values in the group with dietary restrictions, these levels did not exceed critical limits reported in the literature. On the contrary, the results reflect a positive adaptive capacity of the fish, indicating that food restriction, followed by refeeding, did not compromise the blood homeostasis and health status of the animals. This physiological adaptation can be advantageous in production systems, as it highlights the fish's ability to support alternative feeding regimes without significant damage to their performance and well-being.

**Table 3.** Erythrogram of hybrid catfish and absolute values of leukocyte and thrombocyte (*P. reticulatum* x *Leiarius marmoratus*) produced in cages and submitted to food restriction and re-alimentation or continuous feeding.

Treatments	Time (days)				
	15	30	45	60	
	Erythrocyte ( $\times 10^6 \mu\text{L}^{-1}$ )				Total
FR15/RE15	2.24 $\pm$ 0.92	4.35 $\pm$ 1.62	4.62 $\pm$ 1.82	2.75 $\pm$ 1.25	3.12 $\pm$ 1.53 <sup>A</sup>
NR	2.17 $\pm$ 0.99	3.40 $\pm$ 0.80	3.68 $\pm$ 0.32	2.25 $\pm$ 0.98	2.62 $\pm$ 1.26 <sup>B</sup>
Total	2.20 $\pm$ 0.91 <sup>b</sup>	3.87 $\pm$ 1.32 <sup>a</sup>	4.14 $\pm$ 1.35 <sup>a</sup>	2.54 $\pm$ 1.16 <sup>b</sup>	
	Hemoglobin (g dL <sup>-1</sup> )				Total
FR15/RE15	10.66 $\pm$ 9.16	14.25 $\pm$ 3.78	12.57 $\pm$ 2.93	8.98 $\pm$ 2.80	10.55 $\pm$ 4.47
NR	9.57 $\pm$ 6.98	12.11 $\pm$ 2.59	10.04 $\pm$ 3.27	9.42 $\pm$ 1.33	9.95 $\pm$ 3.25
Total	10.11 $\pm$ 7.78 <sup>ab</sup>	13.17 $\pm$ 3.28 <sup>a</sup>	11.30 $\pm$ 3.24 <sup>ab</sup>	9.16 $\pm$ 2.30 <sup>b</sup>	
	Hematocrit (%)				Total
FR15/RE15	29.50 $\pm$ 15.93	57.33 $\pm$ 14.79	56.17 $\pm$ 12.25	49.31 $\pm$ 15.50	49.55 $\pm$ 16.27 <sup>A</sup>
NR	28.80 $\pm$ 14.27	51.67 $\pm$ 10.80	46.67 $\pm$ 10.25	39.20 $\pm$ 15.93	41.03 $\pm$ 15.30 <sup>B</sup>
Total	29.11 $\pm$ 14.04 <sup>b</sup>	54.50 $\pm$ 12.70 <sup>a</sup>	51.42 $\pm$ 11.86 <sup>a</sup>	44.91 $\pm$ 16.32 <sup>a</sup>	
	MCV (fL)				Total
FR15/RE15	161.41 $\pm$ 39.90	140.02 $\pm$ 45.61	121.58 $\pm$ 7.44	202.66 $\pm$ 83.81	155.96 $\pm$ 41.44
NR	146.52 $\pm$ 35.44	153.34 $\pm$ 30.46	127.42 $\pm$ 12.92	199.56 $\pm$ 59.23	156.70 $\pm$ 54.72
Total	152.47 $\pm$ 23.63	146.67 $\pm$ 35.44	124.50 $\pm$ 9.96	201.10 $\pm$ 56.41	
	MCHC (g dL <sup>-1</sup> )				Total
FR15/RE15	49.37 $\pm$ 10.78	24.83 $\pm$ 2.91	23.83 $\pm$ 1.90	18.19 $\pm$ 2.69	27.20 $\pm$ 12.22
NR	29.28 $\pm$ 3.15	21.34 $\pm$ 2.68	21.51 $\pm$ 2.42	22.93 $\pm$ 6.95	23.76 $\pm$ 3.74
Total	37.31 $\pm$ 16.99 <sup>a</sup>	23.08 $\pm$ 3.15 <sup>b</sup>	22.67 $\pm$ 2.32 <sup>b</sup>	20.55 $\pm$ 5.46 <sup>b</sup>	
	Total leukocyte (n° of cells $\times 10^3$ )				
FR15/RE15	205.69 $\pm$ 104.92	512.39 $\pm$ 120.25	494.66 $\pm$ 267.59	251.62 $\pm$ 155.73	308.86 $\pm$ 196.05
NR	194.15 $\pm$ 131.44	340.87 $\pm$ 143.75	371.18 $\pm$ 124.05	226.56 $\pm$ 119.12	260.54 $\pm$ 136.35
Total	199.92 $\pm$ 113.55 <sup>a</sup>	426.63 $\pm$ 146.96 <sup>a</sup>	432.92 $\pm$ 209.05 <sup>a</sup>	241.02 $\pm$ 140.67 <sup>b</sup>	
	Thrombocyte count (n° of cells $\times 10^3$ )				
FR15/RE15	34.67 $\pm$ 16.88	95.04 $\pm$ 40.56	79.47 $\pm$ 37.90	52.51 $\pm$ 45.32	58.97 $\pm$ 44.13 <sup>A</sup>
NR	33.96 $\pm$ 25.59	55.79 $\pm$ 30.04	55.70 $\pm$ 13.41	38.12 $\pm$ 22.64	42.78 $\pm$ 41.67 <sup>B</sup>
Total	34.31 $\pm$ 20.67 <sup>b</sup>	75.42 $\pm$ 38.30 <sup>a</sup>	67.59 $\pm$ 29.81 <sup>ab</sup>	46.42 $\pm$ 37.32 <sup>ab</sup>	

FR15/RE15= food restriction for 15 days, followed by re-alimentation for 15 days; NR= no food restriction. Values are expressed as mean  $\pm$  standard deviation. Lowercase letters differ on lines and uppercase letters differ on columns ( $p < 0.05$ ).

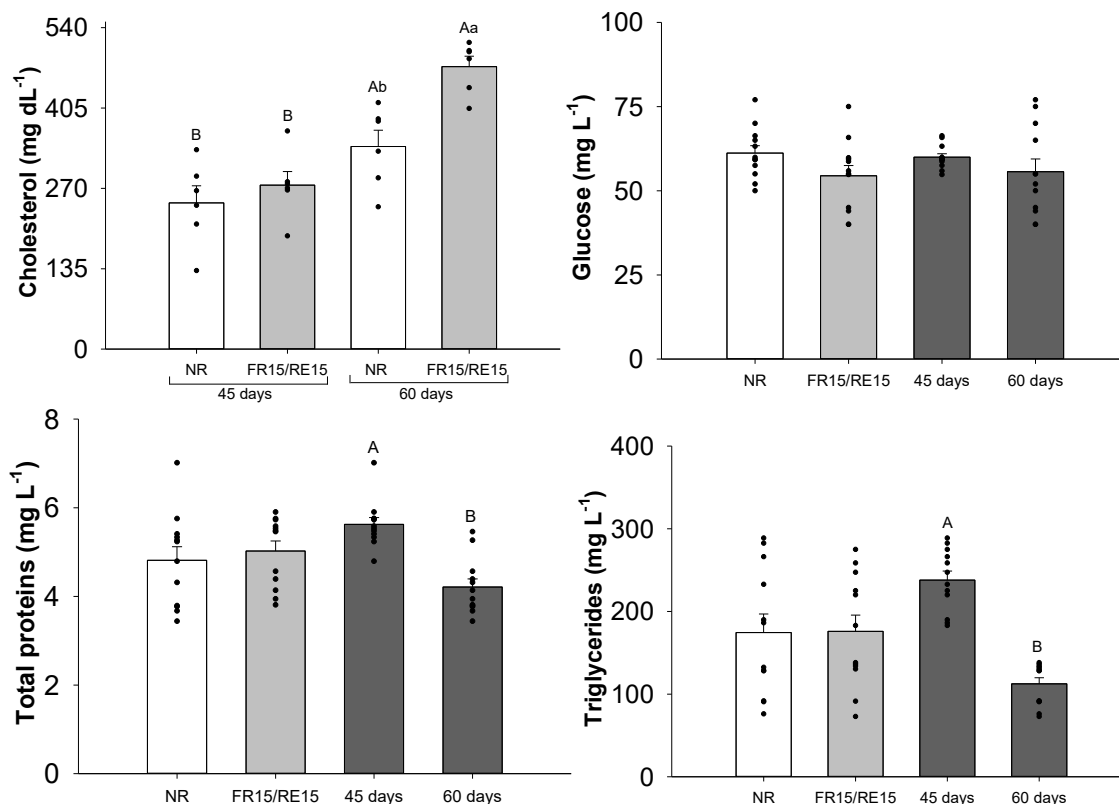
### Biochemical parameters

There was no interaction between the feeding strategy and the times and treatments evaluated for glucose. In the parameters of total proteins and triglycerides, at 45 days, both treatments presented higher values compared to 60 days, but there was no difference between treatments ( $p > 0.05$ ). For cholesterol there was an interaction between time and evaluated treatment, at 60 days the FR15/RE15 showed an increase (Figure 1).

Biochemical parameters are also capable of indicating the physiological state of fish considering that the physiology might not be altered when the animal is submitted to cyclic periods of food deprivation and re-alimentation, especially because blood analysis can indicate these alterations (Morshedi et al., 2011; Tavares-Dias & Moraes, 2003). In this study, food restriction followed by re-alimentation maintained glucose, total protein, and triglyceride concentrations similar to animals fed continuously; however, FR15/RE15 cholesterol showed increase at 60 days. According to Godavarthy et al. (2012) restriction leads to a drop in insulin, which consequently carries out the synthesis of cholesterol, a precursor for the synthesis of stress hormones (glucocorticoids), and this increase is related to the production of gluconeogenesis and adrenocorticoids, in addition to compounds such as ketones, acetate, acetyl-CoA, glucose, which help combat stress by providing energy. This demonstrates that after the fish were re-fed, the production of gluconeogenesis was reestablished, due to the increase in cholesterol concentration.

The maintenance of adequate levels of glucose, total proteins and triglycerides, combined with the absence of hematological indicators of anemia, strengthens the hypothesis that the fish maintained their metabolic efficiency even during periods of food restriction. This approach can be useful for jointly evaluating the health and productivity of animals under different feeding practices, providing important information for optimizing nutritional strategies in production systems.

Moreover, we observed that the energetic metabolism of hybrid catfish exhibits plasticity for adaptation on cycles of food restriction and that the strategy of 15 days of restriction followed by 15 days of re-alimentation presents satisfactory results of energetic and physiologic mechanisms.



**Figure 1.** Seric biochemical profile (mean  $\pm$  standard deviation) of hybrid catfish (*P. reticulatum*  $\times$  *L. marmoratus*) produced in cages, submitted to food restriction and re-alimentation. FR15/RE15= food restriction for 15 days, followed by re-alimentation for 15 days; NR= no food restriction. Values are expressed as mean. Lowercase letters differ on lines and uppercase letters differ on columns ( $p < 0.05$ ).

### Hepatocyte histometry

Feeding strategy, sampling time and their interaction had an impact over hepatocyte histometry. At 30 and 45 days, FR15/RE15 exhibited hepatocytes with nucleus and cytoplasm with increased area and perimeter compared to NR. At 45 days there was an interaction of treatments and sampling time on histometric parameters of hepatocytes, such as nucleus and cytoplasm area, ratio of nucleus and cytoplasm area, nucleus and cytoplasm perimeter (Table 4).

Nucleus and cytoplasm area on FR15/RE15 exhibited increased values at 30 and 45 days. However, the ratio of nucleus and cytoplasm area at 30 days was smaller under restriction and similar in other times. At 30 and 45 days, the nucleus and cytoplasm perimeter were higher in FR15/RE15 compared to NR; however, at 60 days they were similar.

Hepatic histometric parameters are biomarkers for nutrition and metabolic condition and the health state of fish (Rodrigues et al., 2017). Food restriction management for 15 days followed by re-alimentation for 15 days presented increased values compared to NR at 30 and 45 days; however, at 60 days of re-alimentation, similar results.

Increase of hepatosomatic index might be attributed to regeneration of energy storage at the liver, such as lipid and glycogen (Rodrigues et al., 2017). Lipid mobilization as an energy source in food restriction is confirmed by increase of free plasmatic fatty acids in extended periods of fasting, whereas the fatty acid levels are restored at re-alimentation. Lipid mobilization involves the breakdown of triglycerides to free fatty acids

and glycerol into the bloodstream. This transformation usually occurs in target tissues such as the liver, which justifies the increase of morphometric parameters of hepatocytes (Silva et al., 2014). Thus, suggests that food restriction did not alter the cellular activity of the liver, considering there was no drastic alteration in its structure.

**Table 4.** Histological and morphometric characteristics of hepatocytes of hybrid catfish (*P. reticulatum* x *Leiarius marmoratus*) produced in cages and submitted to food restriction and re-alimentation or continuous feeding.

Treatments	Time (days)			Total
	30	45	60	
Nucleus's area (μm <sup>2</sup> )				Total
FR15/RE15	10.54±2.58 <sup>cA</sup>	15.00±3.18 <sup>Aa</sup>	12.82±2.40 <sup>b</sup>	13.07±3.26
NR	9.52±2.27 <sup>cB</sup>	11.85±2.11 <sup>Bb</sup>	12.65±2.40 <sup>a</sup>	11.56±2.58
Total	10.03±2.48	13.42±3.12	12.73±2.40	
Cytoplasm's area (μm <sup>2</sup> )				Total
FR15/RE15	111.16±29.19 <sup>cA</sup>	148.01±40.41 <sup>Aa</sup>	125.20±32.67 <sup>b</sup>	130.24±37.95 <sup>A</sup>
NR	86.18±22.15 <sup>aB</sup>	116.29±30.10 <sup>Bb</sup>	135.87±78.21 <sup>c</sup>	116.11±56.45 <sup>B</sup>
Total	98.67±28.73	132.15±38.96	130.54±60.85	
Ratio of nucleus area/cytoplasm area				Total
FR15/RE15	9.65±3.26 <sup>A</sup>	10.29±3.15	10.82±3.06	10.33±3.17
NR	11.51±3.16 <sup>B</sup>	11.03±6.62	10.44±6.64	10.93±6.01
Total	10.58±3.34	10.66±5.19	10.63±5.24	
Perimeter's nucleus (μm)				Total
FR15/RE15	11.40±1.42 <sup>cA</sup>	13.66±1.41 <sup>Aa</sup>	12.63±1.20 <sup>b</sup>	12.71±1.59
NR	10.85±1.30 <sup>cB</sup>	12.15±1.07 <sup>Bb</sup>	12.55±1.17 <sup>a</sup>	11.97±1.34
Total	11.13±1.38	12.90±1.46	12.59±1.18	
Perimeter's cytoplasm (μm)				Total
FR15/RE15	40.28±5.45 <sup>cA</sup>	47.13±6.62 <sup>Aa</sup>	41.95±5.51 <sup>b</sup>	43.48±6.60
NR	35.45±4.78 <sup>cB</sup>	41.02±4.93 <sup>Bb</sup>	42.88±4.61 <sup>a</sup>	40.33±5.58
Total	37.87±5.66	44.08±6.58	42.42±5.08	

FR15/RE15= food restriction for 15 days, followed by re-alimentation for 15 days; NR= no food restriction. Values are expressed as mean  $\pm$  standard deviation. Lowercase letters differ on lines and uppercase letters differ on columns ( $p < 0.05$ ).

Therefore, this work demonstrates that fasting did not harm the physiological capacity of hybrid catfish to grow, as indicated by the compensatory growth. We suggest that 15 days of food restriction followed by 15 days of re-alimentation to be added to the food management of this fish, aiming increased compensatory growth and consequently increased production.

### Economic analysis

The necessary investment to hybrid catfish production (*P. reticulatum* x *L. marmoratus*) in six cages was estimated at R\$13,982.74. The most expensive item were the cages, with 63.21% of total investment, followed by the vehicle used by the worker to travel to the fish farm, with 16.55%.

Economic evaluation of experimental conditions (13 fish  $\text{m}^{-3}$ ) demonstrates impracticability for a brief period (GM < 0), extended period (NI < 0) and not being economically attractive ( $p < 0$ ) (Table 5). As a simulation, with an alteration of fish density (60 fish  $\text{m}^{-3}$ ), both treatments are able for a brief period (GM > 0), extended period (NI > 0) and is economically attractive ( $p > 0$ ) when compared to the opportunity cost of 6% per year.

FR15/RE15 had a smaller total cost and higher profit than NR, considering that feed cost was 55% smaller and expenses with workforce was 50% smaller at FR15/RE15. Economic performance of FR15/RE15 was superior to NR.

The results of installation cost of cages to produce hybrid catfish (*P. reticulatum* x *L. marmoratus*) are in accordance with the expectation. In a work by Sabaini et al. (2015), cost of project implementation to produce spotted surubim (*Pseudoplatystoma* spp.) in cages was approximately R\$ 182,000.000. The most representative items of this investment were the installation of the system (cages, floats, anchors and rope) with 67.7%, which was similar to this present work. The presence of cages in investments were also verified by other authors (Ayroza et al., 2011).

Feed and workforce were the main expenses of operational cost. Kubitza (1999) indicates that feed expenses in a fish farming may vary from 40 to 70% of total production cost. Similar values were found by (Ayroza et al., 2011) when cages were used. Kubitza (1999) states that feed and juveniles investments comprise 86% of total production cost for surubim, the same inclination was reported by Sabaini et al. (2015).



**Table 5.** Economic evaluation of *P. reticulatum* x *Leiarius marmoratus* production submitted to food restriction and re-alimentation and continuous alimentation in a fish density of 13 fish m<sup>-3</sup> and simulation of fish density of 60 fish m<sup>-3</sup> for a two-month cycle.

	Experiment (13 fish m <sup>-3</sup> )		Simulation (60 fish m <sup>-3</sup> )	
	FR15/RE15	NR	FR15/RE15	NR
<i>Costs</i>				
Labor	308.64	617.28	1,234.57	2,469.14
Fuel	182.88	186.81	206.50	269.50
Juvenile	99.00	99.00	432.00	432.00
Medicines	89.10	89.10	388.80	388.80
Portion (32% PB)	636.96	1,148.18	1,116.93	1,844.89
Incidental expenses	2.79	2.79	45.71	45.71
Maintenance	58.26	58.26	58.26	58.26
Total EOC	1,377.63	2,201.42	3,482.77	5,508.30
Depreciation	178.11	178.11	178.11	178.11
Total TOC	1,555.74	2,379.53	3,660.88	5,686.41
Opportunity cost	8.39	8.39	8.39	8.39
Total TC	1,564.13	2,387.92	3,669.27	5,694.80
<i>Revenues</i>				
Production in 2 months (kg)	72.24	61.22	597.00	548.00
Sale price (R\$ kg <sup>-1</sup> )	16.00	16.00	16.00	16.00
Total revenue	1,155.84	979.52	9,552.00	8,768.00
<i>Indicators</i>				
Gross margin (R\$)	-221.79	-1,221.90	6,069.23	3,259.70
Net margin (R\$)	-399.90	-1,400.01	5,891.12	3,070.20
Profit (R\$)	-408.29	-1,408.40	5,882.73	3,073.20
Profitability index (% per cycle)	-35.32	-143.78	61.58	35.05
Return on invested capital (% per cycle)	-2.14	-5.61	21.87	7.11

Values in R\$ for the productive cycle for March, 2024 (US\$ 1 = R\$ 5.05).

The strategy used in this study presented an economy of 55% in feed, which was expected considering that the amount of feed intake was smaller. However, even though the fish went through fasting for a period, or even partial reduction (Cotrim et al., 2016), the animals reached the same weight as the fish that did not fast. Furthermore, the reduction in feed consumption contributes to a decreased dependence on feed inputs, promoting a more efficient use of natural resources. This approach strengthens sustainable management by balancing economic gains with environmental benefits.

The item with the most presence in this work was the workforce. However, food restriction also reduced the expenses in this item. Olasunkanmi and Yusuf (2014) also observed this tendency, which emphasizes the use of this system, especially in small scale production, since they verified that the second most expensive item of variable cost was workforce, with 31.96%.

Food restriction can facilitate team and supply management considering the less intensive use of workforce and equipment/material in production, which is beneficial to the farmer with a production cost reduction due to workforce excessive cost, as observed in the present work.

The simulations demonstrated that fish density can alter the production costs creating scale gains, whereas only with a fish density simulation of 60 fish m<sup>-3</sup> the production limit is viable, and the farmer reaches satisfactory results of economic viability. FR15/RE15 treatment is even more satisfactory, because with decreased feed intake and workload, it is possible to obtain improved profit. Thus, the combination of reduced operational costs, lower environmental impact, and increased production efficiency makes the presented strategy a sustainable solution for aquaculture management.

## Conclusion

Food restriction of 15 days followed by re-alimentation for 15 days can be used in hybrid catfish production in cages in commercial conditions for 60 days. The fish can be adapted to this management and restriction causes total compensatory gain with no damage to their health. Besides that, this strategy is economically feasible and attractive when a density of 60 fish m<sup>-3</sup> is used.

## Data availability

The data supporting the findings of this study are included in the text and tables. Raw data for all figures are available from the corresponding authors upon reasonable request.

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