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ORIGINAL ARTICLE

Physiological responses to long fasting followed by refeeding in juveniles of pirapitinga, *Piaractus brachypomus*

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ABSTRACT

For many fish species, prolonged fasting is part of their life cycle, as there are seasonal fluctuations in the quantity and quality of food available in their natural habitat. These animals use endogenous reserves during periods of food scarcity and recover when resources become available again. We evaluated the effect of a prolonged fasting period on indicators of body reserve use, growth performance and intestinal integrity of the Amazonian serrasalmid *Piaractus brachypomus*. We distributed 66 juveniles (68.6 ± 2.2 g) in 11 tanks. The treatment consisted of 30 days fasting followed by 45 days refeeding and the control of 75 days normal feeding with 5 replicates (one tank with six fish). The six individuals in the 11th tank were used for baseline measurements. Blood parameters, muscle lipid concentration, hepatosomatic and mesenteric fat indices, somatic growth parameters and intestinal villi morphology were measured every 15 days. Glucose, triglycerides, cholesterol, total protein, the mesenteric fat and hepatosomatic indices, weight gain, specific growth rate, condition factor and total biomass decreased significantly during fasting compared to the control, but all except body condition recovered during refeeding. The length and perimeter of the intestinal villi was significantly lower during fasting compared to the control. The feeding protocol allowed *P. brachypomus* to mobilize part of their body reserves during fasting, however, in general, refeeding was sufficient to restore their body needs and growth performance compatible with that of continuously fed animals.

KEYWORDS: blood biochemistry, food deprivation, intestine histology, pirapitinga

Resposta fisiológica ao jejum prolongado, seguido por realimentação, em juvenis de *Piaractus brachypomus*

RESUMO

Muitas espécies de peixe suportam jejum prolongado como parte do seu ciclo de vida, devido a flutuações na quantidade e qualidade de alimentos disponíveis em seu habitat natural. Esses animais utilizam reservas endógenas durante períodos de escassez de alimentos e se recuperam quando voltam a estar disponíveis. Avaliamos o efeito de jejum prolongado sobre indicadores de uso de reservas corporais, desempenho e integridade intestinal no serrasalmídeo amazônico *Piaractus brachypomus*. Distribuímos 66 juvenis (68,6 ± 2,2 g) em 11 tanques. O tratamento consistiu em 30 dias de jejum seguidos de 45 dias de realimentação, e o controle de 75 dias de alimentação contínua, com 5 repetições (um tanque com seis peixes). Os seis indivíduos do 11º tanque foram usados para medidas basais. Parâmetros sanguíneos, concentração de lipídios musculares, índices de gordura hepatossomática e mesentérica, parâmetros de crescimento somático e morfologia das vilosidades intestinais foram medidos a cada 15 dias. Glicose, triglicerídeos, colesterol, proteína total, índices hepatossomático e de gordura mesentérica, ganho em peso, taxa de crescimento específico, fator de condição e biomassa total diminuíram significativamente durante o jejum em comparação com o controle, mas todos, exceto a condição corporal, recuperaram-se durante a realimentação. O comprimento e o perímetro das vilosidades intestinais foram significativamente menores durante o jejum em comparação com o controle. O protocolo de alimentação permitiu que *P. brachypomus* mobilizasse parte de suas reservas corporais durante o jejum, porém, em geral, a realimentação foi suficiente para repor suas necessidades corporais e o desempenho compatível com o de animais alimentados continuamente.

PALAVRAS-CHAVE: bioquímica sanguínea, privação alimentar, histologia intestinal, pirapitinga

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INTRODUCTION

Piaractus brachypomus (Cuvier, 1818) (Serrasalmidae), is a native South American fish naturally distributed in the Solimões-Amazonas and Orinoco rivers (Escobar *et al*. 2019; Sandoval-Vargas *et al.* 2020). The species is considered the third largest scalefish in the Amazon Basin after *Arapaima gigas* (Schinz, 1823) and *Colossoma macropomum* (Cuvier, 1818). It is of great commercial importance due to its omnivorous feeding habit, resistance to handling in captivity and easy adaptation to unfavorable limnological conditions. Its diet in nature is based on the ingestion of leaves, flowers, fruits and seeds of superior plants (Bai *et al*. 2020).

Food deprivation appears as part of the natural life cycle of many fish species in the wild (Navarro and Gutiérrez 1995). In the Brazilian Amazon, seasonal fluctuations in the water level of rivers and lakes generate qualitative and quantitative differences in food availability for fish (Izel *et al*. 2004). During deprivation, animals can use different endogenous energy sources and show a capacity for growth recovery after diet normalization (Navarro and Gutiérrez 1995; Pérez-Jiménez *et al*. 2012). In captivity, mobilization through the degradation of liver glycogen into glucose (Barcellos *et al*. 2010; Li *et al*. 2018; Nebo *et al*. 2018), and the utilization of lipids in liver (Marqueze *et al*. 2018), muscle (Favero *et al*. 2018) and adipose tissue (Nebo *et al*. 2018), have been observed, whereas many species use muscle protein as the main source of energy during periods of food shortage (Furné *et al*. 2012).

Due to their great biological diversity, fish show a great variety of structures and abilities compared to other animal groups. Omnivorous and herbivorous fish can change the structure and absorptive properties of their digestive system in response to changes in diet (Abelha *et al*. 2001). For example, nutrient absorption can be improved by increasing intestinal surface without increasing intestine length and, in situations of food deprivation, the intestine can be increased in size as a strategy to extend the amount of time that food remains in the digestive tract, thereby improving nutrient absorption efficiency (Silveira *et al*. 2009). The length of microvilli can also be altered according to fish nutritional status and may decrease in situations of prolonged fasting (Rotta 2003).

Metabolic and/or productive responses under different feeding regimes and involving fasting and refeeding periods have been studied for several species of fish, such as *Labeo rohita* (Hamilton, 1822) (Dar *et al*. 2018), *Oreochromis niloticus* (Linnaeus, 1758) (Palma *et al*. 2010), *Oncorhynchus mykiss* (Walbaum) (Nikki *et al*. 2004) as well as native Brazilian species such as *Piaractus mesopotamicus* (Holmberg, 1887) (Ortiz *et al*. 2008), *C. macropomum* (Ituassú *et al*. 2004; Santos *et al*. 2010; Assis *et al*. 2020) and *Brycon amazonicus* (Spix and Agassiz, 1829) (Urbinati *et al*. 2014). For *P. brachypomus* juveniles, there are three published studies using short (Favero *et al*. 2022) and/or alternating (Rodríguez and Landines 2011; Rodríguez and Landines-Parra 2018) fasting and refeeding protocols, aiming at compensatory growth. The present study, therefore, aimed at evaluating the effect of a prolonged fasting period followed by refeeding on captive *P. brachypomus* juveniles by analyzing the response of indicators of the use of body reserves, growth performance and histological alterations in the foregut.

MATERIAL AND METHODS

Fish and experimental conditions

The experiment was performed at the Aquaculture Laboratory (LAQUA) at Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil, and followed a protocol approved by the ethics committee on the use of animals at UFMG (protocol 11/2022 CEUA/UFMG).

Overall we used 66 *P. brachypomus* juveniles (mean initial body weight 68.6 ± 2.2 g, mean total length 13.8 ± 0.4 cm), acquired from the fish farm Biofish Aquicultura, in Porto Velho (Rondônia state, Brazil). Sixty fish were distributed in 10 tanks of 100 L useful volume (six fish per tank) in a water recirculation system for the experimental treatments, and six were kept in a separate tank for baseline measurements. The fish were acclimatized for 15 days, receiving a commercial extruded diet (Laguna Onívoros Alevinos, 2.6 mm, Socil: 36% crude protein, 7% ether extract, 5% crude fiber, 9% mineral matter, 1–1.8% calcium and 1% phosphorus), twice a day (10h00min and 16h00min). The averages and standard deviations of water quality parameters measured during the acclimatization period and weekly during the experimental period were: temperature of 28.43 ± 1.03 °C and dissolved oxygen of 4.47 ± 0.45 mg L^{-1} (measured using a YSI multiparameter probe, EcoSense® DO200A, Yellow Springs Instrument Co. Inc., Yellow Springs, OH, USA); pH of 6.36 ± 0.78 (measured using a K39-0014PA pH meter, KASVI, São José dos Pinhais, PR, Brazil); and total ammonia of 0.20 ± 0.11 mg L^{-1} (measured using a Alfakit colorimetric test commercial kit; www.alfakit.com.br).

The completely randomized experimental design consisted of two groups, each one with five replicates (five tanks with six fish each): (1) the control group – fish continuously fed with commercial extruded diet (composition as described above) twice a day (10h00min and 16h00min) until apparent satiety for 75 days; and (2) fasting group – fish submitted to fasting for 30 days and then re-fed for 45 days with extruded commercial diet (composition as described above) twice a day (10h00min and 16h00min), until apparent satiety.

Blood parameters, tissue and somatic indices

The analysis of haematological parameters was performed for the six fish in the baseline tank at day 0 of the experimental period, and six fish (one replicate) from each experimental

group at days 15, 30, 45, 60 and 75 of the experimental period (corresponding to day15 and 30 of the fasting period and day 15, 30 and 45 of the refeeding period in the fasting group). The fish were carefully removed from the tanks with a net and contained in a damp cloth while blood was removed by caudal venipuncture, using heparinized syringes. An aliquot of whole blood was separated to determine total hemoglobin concentration, measured by the cyanomethemoglobin reaction method using a commercial kit (Ref. No. K023-1 QUIBASA Química Basic Ltda. Bioclin®)

The remaining aliquot of blood was centrifuged for 10 minutes at 4000 rpm for plasma separation and determination of glucose, triglycerides and cholesterol concentrations, performed by colorimetric method using commercial kits (Bioclin® - Belo Horizonte, Brazil - www.bioclin.com) and with spectrophotometer reading (Biochrom Libra S22 UV/Vis). Total plasma protein concentration was determined using a refractometer (RHC 200-ATC, Huake Instrument Co., Ltd).

After blood collection, the fish were euthanized with an overdose of eugenol (285 mg L^{-1}) for later removal of the liver, and portions of white muscle and mesenteric adipose tissue for the analysis of tissue and somatic indices. Portions of white muscle were used to determine total lipid concentration, following the methodology of Bligh and Dyer (1959). In addition, liver and mesenteric adipose tissue were weighed to calculate the hepatosomatic index and mesenteric fat index, respectively, using the following formulas:

- Hepatosomatic index (%) = (liver weight/body weight) x 100
- Mesenteric fat index (%) = (mesenteric fat weight/body weight) x 100.

A sample of the foregut was also retrieved for analysis of the intestinal villi. The samples were fixed in Bouin's solution for 24 h and then washed in 70% alcohol for dehydration in an increasing alcoholic series, followed by diaphanization in a series of xylols, inclusion in histological paraffin and sectioning at a thickness of 2–3 µm (Santos *et al.* 2021). Three slides were made for each sample. The slides were stained using the hematoxylin-eosin technique (Santos *et al.* 2021). The material was analyzed and photo documented under a microscope (Nikon-E200 Microscope). The length and perimeter of 10 intestinal villi were measured per slide using ImageJ® software (version 1.53h 2021).

Growth performance

Biometric sampling for analysis of growth performance was done for the same individuals used for blood and tissue analysis. After removal from the tanks and euthanization (and prior to tissue removal) the fish were weighed on a digital scale (model Marte – AD5002) and measured for total length using a 30 cm polyethylene ruler. Final weight was determined, and weight gain, total biomass (sum of mean weights of fish in each tank) specific growth rate and Fulton´s condition factor was calculated according to the following formulas:

- Weight gain (g) = final weight – initial weight
- Total biomass (g) = ∑ mean weights of fish in each tank
- Specific growth rate (%) = 100 x [(*ln* final weight) - (*ln* initial weight) / days between samples]
- Fulton´s condition factor (K) = 100 x [final weight/(total length)^3]

Feed intake per fish was determined by weighing the recipients of feed offered and the leftovers taken from each tank every 15 days.

Statistical analysis

All response variables were tested and confirmed for data normality and homoscedasticity of variance (Shapiro-Wilk and Levene tests, respectively), and were compared between the two experimental groups and the five time-points (day 15, 30, 45, 60 and 75) using two-way ANOVA. A pairwise comparison of means was carried out for significant ANOVAs using Tukey's test. The data of the baseline group was compared with each experimental group at each time point using a *t* test. All tests were performed at a significance level of 5%. All analyses were made using the statistical program SigmaPlot® Software, version 12.0.

RESULTS

Blood parameters, tissue and somatic indices

No significant differences were observed between fasting and control groups for hemoglobin concentration (Figure 1a). However, significantly lower hemoglobin concentrations were observed in the fasting group at day 30 ($p = 0.012$), day 45 ($p = 0.035$) and day 75 ($p < 0.001$), compared to the baseline group. Plasma glucose concentration for the fasting group was significantly lower than that of the control at day 30 ($p = 0.015$), but was significantly higher than that of the control and similar to baseline at day 75 (Figure 1b).

Plasma triglycerides were significantly lower in the fasting group then the control at day15 ($p < 0.001$) and day 30 ($p < 0.001$), but recovered with refeeding, when they did not differ significantly from the control, both groups with higher values than the baseline (Figure 1c). Plasma cholesterol for the fasting group was significantly higher at day 15 ($p < 0.001$) when compared to the control and baseline (Figure 1d).

Plasma total protein concentration for the fasting group was significantly lower at day 15 ($p = 0.029$) and day 30 ($p < 0.001$) and day 60 ($p = 0.047$) compared to the control group and baseline (Figure 1e).

No significant differences were found between the groups for total muscle lipid concentration (Figure 1f). The hepatosomatic index was significantly lower ($p < 0.001$) in

the fasting group at day 30 compared to the control, but was significantly higher than that of the control and baseline at day 45, and remained similar to the control group for remainder of the refeeding period (Figure 1g). The mesenteric fat index in the fasting group was significantly lower than that of the control from day 30 to day 60 (Figure 1h).

Growth performance

No mortality was recorded during the entire experimental period. Final weight did not differ between fasting and control groups during the whole experimental period (Figure 2a). The fasting group had lower weight gain than the control at 30 days of fasting ($p < 0.05$), but subsequently became similar

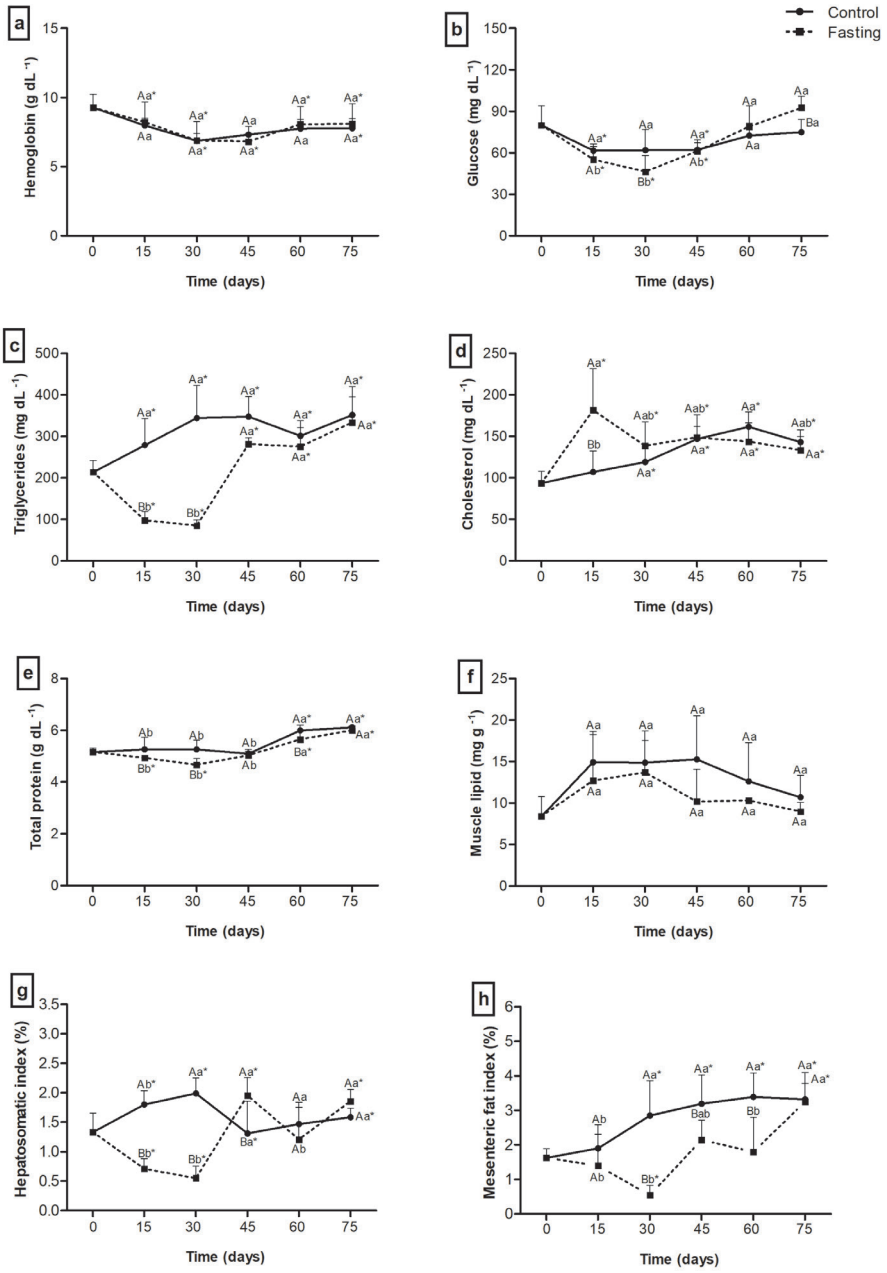

Figure 1. Hemoglobin (A); glucose (B); triglycerides (C); cholesterol (D); total protein (E); muscle lipid (F); hepatosomatic index (G); and mesenteric fat index (H) in *Piaractus brachypomus* juveniles submitted to 30-day fasting followed by 45-day refeeding and their control group submitted to 75 days of normal feeding. Both groups share the data of the baseline values at day 0. Different capital letters between the control and fasting group within the same sampling time, and different lowercase letters within groups indicate statistically significant pairwise differences according to a post-hoc Tukey test. Asterisks indicate significant differences between the control or fasting group at each time-point and the baseline group according to a t-test. Data points represent the mean and bars the standard deviation of the measurements of six fish. All comparisons were statistically significant at $p < 0.05$.

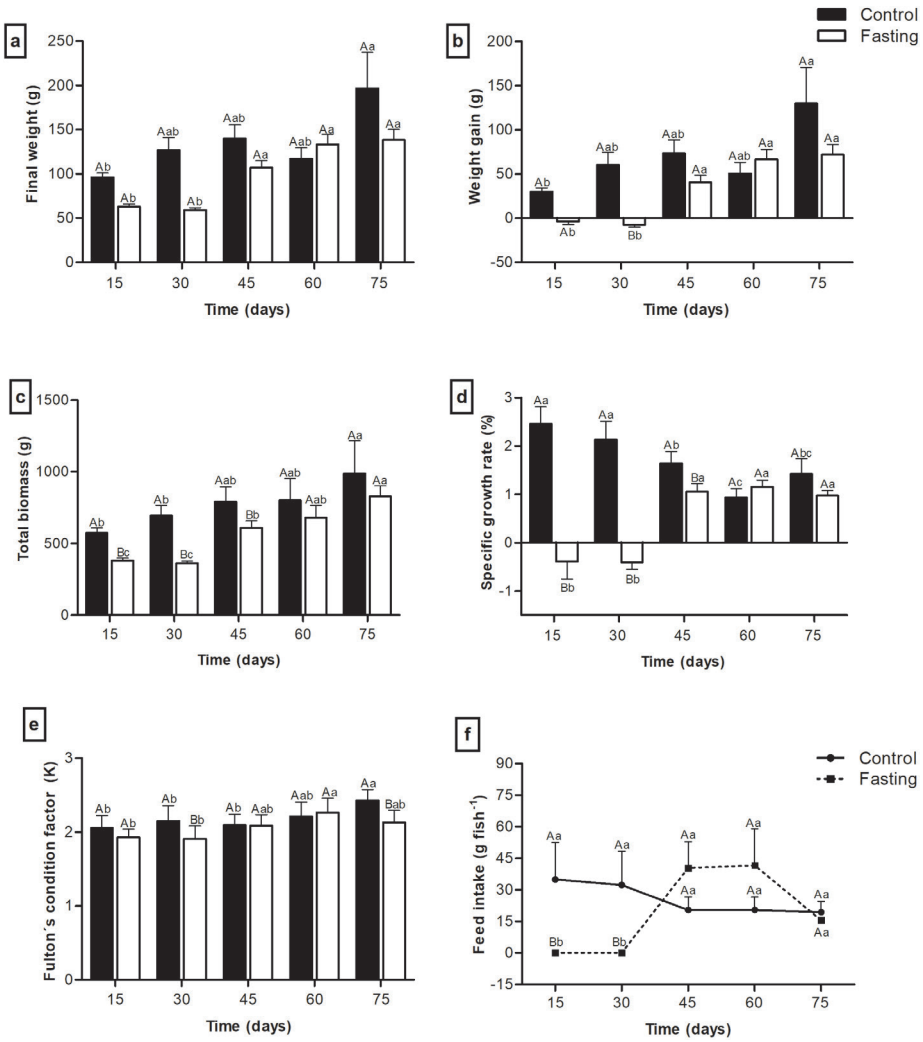

Figure 2. Final weight (A); weight gain (B); total biomass (C); specific growth rate (D); Fulton´s condition factor K (E); and feed intake (F) in *Piaractus brachypomus* juveniles submitted to 30-day fasting followed by 45-day refeeding and their control group submitted to 75 days of normal feeding. Different capital letters indicate a significant difference between the control and fasting group at each sampling time (15 and 30 days of fasting and 15, 30 and 45 days of refeeding). Different lowercase letters indicate significant within-group differences along the experimental period according to a Tukey test. Columns are the mean and bars the standard deviation of the measurement of six fish. All comparisons were statistically different at $p < 0.05$.

to the control and remained so in the refeeding periods until the end of the experiment (Figure 2b).

Total biomass was significantly lower in the fasting group compared to the control at days 15, 30 and 45 days (15 days after refeeding). The difference between the groups became non significant at day 60 (at 30 days of refeeding) (Figure 2c).

The specific growth rate was significantly lower in the fasting group compared to the control groups throughout the fasting period and for the first 15 days of refeeding, and did not differ significantly from the control for the rest of the refeeding period (Figure 2d). Fulton´s condition factor was significantly lower in the fasting group than in the control at days 30 and 75 (45 days of refeeding) (Figure 2e). Feed intake did not differ significantly between the fasting and control group during the refeeding period (Figure 2f).

Intestinal villi

The length of intestinal villi length was significantly lower in the fasting group at days 15 and 30, but significantly higher at day 60 (30 days of refeeding) compared to the control (Table 1). Within the fasting group, villi length also varied significantly with time, being significantly longer in the refeeding period than in the fasting period ($p < 0.001$).

The fasting group had a significantly smaller villi perimeter than the control group at day 30 (30 days of fasting) ($p = 0.018$) (Table 1), but the perimeter of villi enlarged during the refeeding period, when the difference to the control group became non significant. Villi perimeter differed significantly between the baseline and fasting groups at day 30, and between the baseline and control groups at days 30, 45 and 60.

Table 1. Length and perimeter of the intestinal villi of *Piaractus brachypomus* juveniles submitted to long fasting followed by refeeding. The baseline measurements at day 0 are common to both groups. Days 15 and 30 of the experimental period correspond to the fasting period in the treatment. Values are the mean ± standard deviation for six fish.

Experimental period	Fasting	Control
Villi length (µm)		
day 0	11255.0 ± 5611.0	11255.0 ± 5611.0
day 15	7782.2 ± 4228.4 Bb	14263.4 ± 4145.2 Ab
day 30	5845.2 ± 3034.0 Bb	18971.4 ± 5879.2 Aa*
day 45	10099.4 ± 959.6 Aa	8010.2 ± 1004.6 Ac
day 60	13942.6 ± 2448.0 Aa	7547.3 ± 2991.6 Bc
day 75	10288.2 ± 3057.9 Aa	9248.2 ± 2110.6 Ac
Villi perimeter (µm)		
day 0	9561.0 ± 3151.0	9561.0 ± 3151.0
day 15	7729.0 ± 3830.6 Aa	10703.3 ± 2751.2 Aa
day 30	4837.3 ± 1569.5 Bb*	15365.0 ± 5969.5 Aa*
day 45	7372.7 ± 557.4 Aa	6405.0 ± 557.4 Aa*
day 60	10081.3 ± 2170.0 Aa	6720.0 ± 1251.1 Aa*
day 75	17282.4 ± 9524.4 Aa	7508.5 ± 1576.1 Aa

Means followed by different capital letters in a line indicate significant differences between the control and fasting groups, and means followed by different lowercase letters in a column indicate significant differences within group according to a post-hoc Tukey test. Asterisks indicate differences between the control or fasting group and the baseline group at each time-point according to a *t* test.

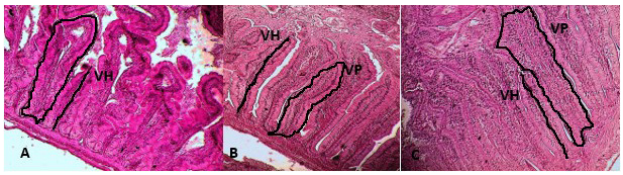

Figure 3. Portion of the foregut of *P. brachypomus* (100x magnification) indicating examples of measurement of villi length (VL) and villi perimeter (VP). Staining with hematoxylin-eosin. A – Baseline group; B – Control group; C – Fasting group. This figure is in color in the electronic version.

DISCUSSION

The present study evaluated the feeding strategy of 30 days of fasting and 45 days of refeeding for aquacultured juveniles of pirapitinga (*Piaractus brachypomus*) and monitored the use of body reserves, growth performance and foregut histology. The fasting period had no significant effect on hemoglobin concentration, similarly to the results of Assis *et al.* (2020) for *C. macropomum* juveniles submitted to short periods of one or two days of food restriction. Changes in hemoglobin concentration may be related to the body's need to improve oxygen maintenance (Nikinmaa *et al.* 1984) and even a month-long fasting apparently did not elicit this response in *P. brachypomus*, at least not after one cycle of fasting/refeeding.

The glucose level of the fasting group decreased significantly after 30 days of fasting, but recovered after 15 days of refeeding. Food restriction can affect glucose levels in fish (Gomes *et al.* 2001). The need to obtain glucose is met by the breakdown of glycogen in the liver (glycogenolysis), or via gluconeogenesis from lactate, amino acids and glycerol (Polakof and Panserat 2016). Studies on fasting with other species show that glucose can remain unchanged (Barcellos *et al.* 2010; Costas *et al.* 2011; Nebo *et al.* 2018, Silva *et al.* 2019; Assis *et al.* 2020), while others increase (Caruso *et al.* 2010) or even decrease (Favero *et al.* 2018; Favero *et al.* 2020), as was observed in the present study. The behavior of glucose depends on fasting duration and the species-specific metabolism (Caruso *et al.* 2010). The physiological effects of fasting depend on the species of fish being studied (Furné *et al.* 2012), fish size and water temperature (Glencross and Bermudes 2011), photoperiod (Biswas *et al.* 2002), diet (Hilton 1982) and the duration of fasting and refeeding periods (Reigh *et al.* 2006).

The plasma triglyceride concentration decreased significantly in the fasting group. Fasting can inhibit lipogenesis and induce the mobilization of lipid stores through lipolysis and β-oxidation of fatty acids (Li *et al.* 2018). In our study, we observed that *P. brachypomus* mobilized lipid reserves from adipose tissue, as evidenced by the decrease in the mesenteric fat index at 30 days of fasting while the level of lipids in muscle did not change. However, the mobilization of this reserve was insufficient to maintain the triglyceride level during the fasting period. However, 15 days of refeeding were sufficient for recovery of the blood triglyceride level to approximately that of the control group.

The plasma cholesterol level increased significantly in the fasting group after 15 days of fasting but decreased to similar levels to that of the control group at 30 days of fasting. Cholesterol can be obtained from the diet (exogenous route) or can be synthesized (endogenous route) by the liver and intestine (Maita *et al.* 2006) and transported by lipoproteins (Zhu *et al.* 2014). Our results show that cholesterol was not used as an energy source by *P. brachypomus*. Cholesterol response was variable among species submitted to different fasting and refeeding strategies (Favero *et al.* 2020). It increased in *Acipenser baerii* (Brandt, 1869) (Shirvan *et al.* 2020), *Piaractus mesopotamicus* (Favero *et al.* 2020) and *Lophiosilurus alexandri* (Steindachner, 1876) (Silva *et al.* 2019), decreased in *Acipenser naccarii* (Bonaparte, 1836), *Oncorhynchus mykiss* (Furné *et al.* 2012), and *Dentex dentex* (Linnaeus, 1758) (Pérez-Jiménez *et al.* 2012) and remained unchanged in *Acipenser baerii* (Jafari *et al.* 2019) and *Oreochromis niloticus* (Nebo *et al.* 2018).

Although plasma total protein decreased significantly in the fasting group during fasting, refeeding promoted its general recovery. Protein mobilization is also a way to maintain vital processes in fish when subjected to periods of fasting (Sheridan and Mommsen 1991). In this way, muscle proteolysis can occur when remaining available reserves are widely depleated (Navarro and Gutierrez 1995). Catabolism of proteins and amino acids can occur to meet the oxidative needs of tissues, as well as the mobilization of glycogen

to release glucose as energy fuel. Thus, plasma protein concentration may decrease through hemodilution, induced fasting, stress situations, or failure of hepatocytes due to lipid accumulation (Sala-Rabanal *et al*. 2003).

The length and perimeter of intestinal villi of *P. brachypomus* decreased during fasting and increased at 30 days of refeeding relative the control group. These measurements of intestinal villi of fish are parameters of the integrity of the intestinal mucosa and are indicators of the digestive and food absorption capacity of the fish intestine (Ferreira *et al*. 2014). The morphology of villi varies with the age, feeding habits and the environment where fish live (Baldisserotto *et al*. 2014) and respond to available diet and the physiological state of the animals (Dawood 2021; Dawood *et al*. 2023).

In *O. niloticus*, the length of the intestinal villi varied according to food availability (Honorato *et al*. 2013). In our study, the reduction in length and perimeter of villi was directly related to fasting, as the fish of the fasting group may not have developed their intestinal villi as much as the control group. Fasting causes changes that can compromise digestive activity (Ostaszewska *et al*. 2006), causing a reduction in villi height and length and reducing the area of epithelium, which, as a consequence, decreases absorption capacity (Shaibani *et al*. 2013). The reduction in the length and perimeter of the villi indicates a lack of food in the digestive tract (Green and McCormick 1999), while the increase in these measures can be understood as an improvement in the integrity of the mucosa (Carvalho *et al*. 2011), since the balance between renewal and loss of cells, which normally occurs in the intestine, maintain villi size and digestive and intestinal absorption capacities (Ferreira *et al*. 2014).

It is important for the integrity of the intestinal mucosa that the density of villi be compatible with their length, determining the area of the intestinal lumen, a fundamental space for food passage and thus relevant in animal production (Schwarz *et al*. 2010). Therefore, more in-depth studies should be carried out to determine the effects that fasting can have on the development of these animals in relation to the absorption of nutrients in the intestine when fasting, mainly considering growth responses, and the capacity of adaptation to imposed conditions.

The 30 days of fasting did not affect final weight, but promoted a decrease in weight gain, Fulton´s condition factor, specific growth rate and total biomass of *P. brachypomus* juveniles, but all parameters except the condition factor recovered to the levels of the control by the end of the refeeding period. Similar results were observed by Ituassú *et al*. (2004) for juvenile *C. macropomum* submitted to four periods of fasting (0, 14, 21 and 28 days) and by Soares *et al*. (2007) for peacock bass fingerlings (*Cichla monoculus*, Spix and Agassiz, 1831) submitted to one day of feed restriction and six days of refeeding.

Fulton´s condition factor is an important index that compares body weight with length and is related to the physiological state of fish in the culture environment, assessing whether these animals are using food efficiently (Palma *et al*. 2010). Although the condition factor of the fasted animals was significantly lower than that of the control at 45 days of refeeding, it did not influence the growth of the fish. Our results for growth performance parameters suggest compensatory responses in the fasting group that allowed the recovery of growth performance. The fasting and refeeding protocol used in our study was not intended for use in commercial production of *P. brachypomus*, as we aimed at better understanding the physiological limits of this species when subjected to a prolonged period of fasting. However, it is important to emphasize that the use of adequate fasting and refeeding protocols in commercial fish production can be economically advantageous as it allows the reduction in feeding costs due to that less food is supplied to the animals (Correa *et al.* 2020; Jafari *et al.* 2019), in labor costs (Oh and Park 2019), and in the contamination of the aquatic environment (Jafari *et al.* 2019).

CONCLUSIONS

The feeding protocol used in the present study allowed pirapitinga juveniles to survive and mobilize part of their body reserves, except for muscle lipid mobilization, during a prolonged period of fasting (30 days). However, in general, the refeeding period of 45 days was sufficient for these animals to restore their bodily needs, intestinal integrity and growth performance. It should be further investigated if body condition and growth performance are affected in the long term using more extreme fasting cycles.

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DATA AVAILABILITY

The data that support the findings of this study are available, upon reasonable request, from the corresponding author.