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Influence of fasting and refeeding cycles on growth, health and feeding costs of the Amazonian fish *Colossoma macropomum*

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ABSTRACT

Feed restriction can be used as a management strategy to increase feed efficiency through compensatory growth (a higher growth rate after a period of restricted feeding). However, there is still a lack of information on the physiological mechanisms underlying the fish responses to feed restriction. Therefore, this study aimed at investigating the cycles of fasting and refeeding on the performance, cost feeding, health and physiological mechanisms of juvenile tambaqui *(Colossoma macropomum).* For this purpose, feeding protocols were tested for 60 days: continuous feeding (control); two days of fasting and refeeding for two days (F2R2); two days of fasting and refeeding for three days (F2R3) and two days of fasting and refeeding for four days (F2R4), on a total of 108 two-month-old tambaqui juveniles (29.53 ± 1.31 g), distributed in 300 L boxes. Weight gain (g) was lower for the F2R2 and F2R3 treatments compared to the control. Fish subjected to the F2R4 feeding strategy showed similar growth to the group of fish continuously fed. Only the restriction protocol F2R3 resulted in a reduction in blood glucose, while cholesterol was reduced in F2R2 and F2R3. There was mobilization of hepatic glycogen in the F2R3 and F2R4 treatments, while muscle lipids were not altered. The different fasting cycles did not affect leukocyte respiratory activity, hematocrit or hemoglobin. The lowest relative feed cost values were in the F2R2 and F2R4 treatments. The F2R4 protocol reduces feed cost and maintains the ability to adjust energy metabolism without causing stress in *C. macropomum*.

KEYWORDS: Compensatory growth, metabolism, tambaqui.

Influência de ciclos de jejum e realimentação no crescimento, saúde e custos de alimentação do peixe amazônico *Colossoma macropomum*

RESUMO

A restrição alimentar pode ser utilizada como estratégia de manejo para aumentar a eficiência alimentar através do ganho compensatório (i.e. taxa de crescimento acima do normal logo após um período de restrição nutricional). No entanto ainda há escassez de informações sobre os mecanismos fisiológicos subjacentes às respostas dos peixes à restrição alimentar. Portanto este estudo teve como objetivo investigar os ciclos curtos de jejum e realimentação no desempenho, custo com alimentação, saúde e mecanismos fisiológicos de juvenis de tambaqui (*Colossoma macropomum).* Para isso protocolos de alimentação foram testados durante 60 dias: alimentação contínua (controle); dois dias de jejum e realimentação por dois dias (F2R2); dois dias de jejum e realimentação por três dias (F2R3) e dois dias de jejum e realimentação por quatro dias (F2R4). Um total de 108 juvenis de tambaqui com dois meses de idade (29,53 ± 1,31 g) foram distribuídos em caixas de 300 L. O ganho de peso (g) foi menor para os tratamentos F2R2 e F2R3 em relação ao controle. Os peixes submetidos a estratégia de alimentação F2R4 apresentaram crescimento semelhante ao grupo de peixes continuamente alimentados. Apenas o protocolo de restrição F2R3 resultou em redução da glicemia, enquanto o colesterol foi reduzido em F2R2 e F2R3. Houve mobilização de glicogênio hepático nos tratamentos F2R3 e F2R4, enquanto os lipídios musculares não foram alterados. Os diferentes ciclos de jejum não afetaram a atividade respiratória de leucócitos, hematócrito ou hemoglobina. Os menores valores de custo relativo de alimentação foram no tratamento F2R2 e F2R4. O protocolo F2R4 reduz o custo da alimentação e mantém a capacidade de ajuste do metabolismo energético sem causar estresse em *C. macropomum*.

PALAVRAS-CHAVES: Ganho compensatório, metabolismo, tambaqui.

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INTRODUCTION

Fish demonstrate a remarkable ability to cope with variations in food availability throughout the year as a result of seasonal fluctuations in food supply, during migration or spawning (Marqueze et al. 2018). During food deprivation, the mobilization of body reserves is regulated by the endocrine system (Dar et al. 2018) and hepatic glycogen is the first substrate to be mobilized in order to maintain glycemic levels (Lu et al. 2019). Other reserves, such as mesenteric fat, can also be utilized, which allows for increased levels of fatty acids and glycerol in the bloodstream (Favero et al. 2018) and muscle protein, which, via proteolysis, mobilizes amino acids to the tissues (Favero et al. 2020). When submitted to different restriction and refeeding protocols, alterations in hematological parameters, such as hematocrit, total protein, erythrocytes and hemoglobin, can also occur (Assis et al. 2020).

The adoption of feed management with restriction and refeeding cycles has been widely used in fish farming as a strategy to reduce feed and labor costs (Paz et al. 2018; Roa et al. 2019; Favero et al. 2020). In addition, this practice improves water quality (Nebo et al. 2018) and growth rate (Oh and Park 2019) and allows animals to achieve compensatory growth (Ashouri et al. 2020). The compensatory gain response occurs when the animal kept in restriction followed by refeeding reaches the same weight as those fed without restriction (Favero et al. 2020). During the post-fasting refeeding period, fish may present hyperphagia, which allows them to recover body reserves (Assis et al. 2020). This process also increases food efficiency, which includes greater digestion and absorption of nutrients, as well as reducing metabolic costs (Santos et al. 2021).

Colossoma macropomum Cuvier 1816 originates from the Amazon basin and leads the production from native fish farming in Brazil, with 267,060 tons in 2022 (Peixe BR 2023). This stems from its tolerance, rapid growth (Araújo et al. 2018), acceptance of artificial feed and adaptation to breeding in confinement (Santos et al. 2018). This species is widely consumed, not only in Brazil, but in other South American countries such as Colombia, Peru and Venezuela (Woynárovich and Anrooy 2019).

Although there are studies of compensatory growth on *C. macropomum* (Ituassú et al. 2004; Santos et al. 2018; Roa et al. 2019; Assis et al. 2020; Santos et al. 2021; Melo et al. 2024), there is still a shortage of information about the physiological mechanisms underlying compensatory gain in this species, as well as the possibility that the process of food restriction can induce immunological changes and generate stress. Additionally, this approach is of great interest to the productive sector, since feed in fish farming accounts for more than 60% of the cost of production. In this scenario, studies related to feed management are important for ensuring the sustainability of the activity and for avoiding wastage of feed and reducing production costs (Santos et al. 2018).

Thus, the present study aims to investigate the effects of alternating cycles of fasting and refeeding on zootechnical performance, feed costs, hematological characteristics and metabolic parameters, as well as the impact on the health of juvenile *C. macropomum*.

MATERIALS AND METHODS

Ethical statement

All the procedures described below comply with the guidelines of the National Animal Experimentation Control Council (CONCEA – MCTIC). This proposal was evaluated and approved by the Ethics Committee for the Use of Animals (CEUA) of Nilton Lins University (Approval No. 003/2018).

Fish and experimental conditions

Juvenile tambaqui were acquired from Fazenda Santo Antônio (Presidente Figueiredo, Amazonas, Brazil) and stored at the laboratory of Physiology and Fish Metabolism at Nilton Lins University (Manaus, Amazonas, Brazil). A total of 108 juvenile tambaqui two months old were acclimated and kept under observation for 10 days. Subsequently, they were weighed (29.53 ± 1.31 g), measured (9.56 ± 0.72 cm) and randomly distributed in twelve 300 L circular polyethylene tanks with nine fish in each. All the tanks were coupled to a water recirculation system and had constant aeration. The filtration system was composed of a 1.000 L reservoir tank, with a mechanical glass fiber filter and a biological filter with 100 L of filter media.

Water quality parameters, such as temperature (28.82 ± 0.76ºC), dissolved oxygen (4.79 ± 0.60 mg L^{-1}), and pH (4.79 ± 0.60 mg L^{-1}), were randomly evaluated three times a week with a multiparameter probe (AK88, AKSO), since all treatments had a single biological filter, and total ammonia (1.5 ± 0.01 mg L^{-1}) was evaluated fortnightly using the method of Verdouw, Van Echteld, Dekkers (1978).

During 60 days, four treatments were performed with three repetitions, these being: Control, continuous feeding; F2R2, two days of fasting followed by two days of refeeding; F2R3, two days of fasting followed by three days of refeeding; F2R4, two days of fasting followed by four days of refeeding.

The diet consisted of a commercial extruded diet of 28% crude protein with 5% ether extract (Supra®, Manaus, Amazonas, Brazil), which was provided twice a day (9:00 and 17:00) until apparent satiety. Consumption was measured by the difference between the initial and final weight of the total amount of food provided, for this each experimental unit had its individual pot of food which was weighed and replenished weekly until the end of the experiment.

Growth assessment

At the end of the experimental period, the fish were fasted for a period of 24 h. Animals from all experimental units were weighed (g) on a digital scale (semi-analytical-BG, Gehaka) and the total length (cm) was measured with a measuring tape to calculate the following production performance metrics:

Weight gain: WG = final weight – initial weight

Specific growth rate: SGR = 100 × (ln final weight – ln initial weight)/days of experiment

Feed conversion rate: FCR = feed intake/weight gain

Sampling and analysis of blood, plasma and tissue

At the end of the 60 days of experiment, a single collection was carried out where three fish were chosen from each experimental unit (n=12) at random and directed to the container with anesthetic for blood, plasma and tissue sampling.

A sample of 0.5 mL of blood was collected by puncture of the caudal vessel and dispensed into two microtubes containing 10 µL of anticoagulant (heparin or fluoridated EDTA) and into another microtube without the anticoagulant to obtain the serum.

Heparinized blood was used to evaluate leukocytes and other hematological variables (hemoglobin concentration, erythrocyte count and hematocrit). Plasma was separated by centrifugation of blood (3,000 rpm for 10 minutes at 4ºC) with fluorinated EDTA and used for metabolic analysis (glucose and triglycerides). Serum was obtained after coagulation at room temperature for 2 hours and for subsequent use for analysis of cholesterol and total protein. The fish were euthanized by deepening the anesthetic plane whereby the animals remained immersed in the benzocaine solution (200 mg L^{-1} of water) for a longer period for tissue collection (liver, dorsal muscle and visceral fat). The visceral fat was weighed to calculate the viscerosomatic fat index (VSFI) using the formula:

VSFI = (tissue weight/body weight) x 100

Counting of erythrocytes (RBC) was conducted in a Neubauer chamber using blood previously fixed in formalin-citrate buffer. To determine the hematocrit (Hct), the microhematocrit tubes were centrifuged at 10,000 rpm for 5 minutes, and the reading was performed on a measuring card that provides the hematocrit values.

The hemoglobin concentration (Hgb) was determined using the cyanmethemoglobin method. Plasma was used to determine blood glucose levels and the concentration of triglycerides, while serum was used to measure cholesterol and total protein. Labtest kits (Labtest Diagnóstica S.A.) which use enzymatic assays and reading in a spectrophotometer, according to the manufacturer’s recommendations (Ranzani-Paiva et al. 2013) were used for these analyses.

The median portion of the dorsal musculature was used to analyze the concentration of muscle lipid according to the technique proposed by Bligh and Dyer (1959), based on lipid extraction with chloroform and methanol. The liver was used to determine hepatic glycogen using the methodology proposed by Moon, et al. (1989).

Indicators of stress and immune responses

At the end of the 60 days of experiment, a single collection was carried out where three fish were chosen from each experimental unit. The respiratory activity of leukocytes was evaluated by measuring the production of reactive oxygen species (ROS) according to the protocol of Sahoo et al. (2005), with the modifications proposed by Biller-Takahashi et al. (2013). The method consists of determining the ROS produced during the oxidative burst process by means of a colorimetric assay that is based on the reduction of the dye nitroblue tetrazolium (NBT), which results in the formation of precipitates of insoluble material with a dark blue color inside the phagocytic cells, known as formazan granules (Klein 1990). To form the precipitate, 0.1 µL of heparinized whole blood was added to 0.1 µL of NBT (Sigma, St Louis, MO, USA). The resulting solution was homogenized and incubated for 30 minutes at 25°C. After incubation, 50 µL of the homogenized suspension was transferred to a glass tube with 1 mL^{-1} of N, N-dimethylformamide (DMF) (Sigma, St Louis, MO, USA) and centrifuged at 3,000 rpm for 5 minutes. DMF is a solvent of salts and high molecular weight compounds, and thus smooth the cell wall of leukocytes and formazan granules, releasing the reduced NBT dye into the solution. The optical density of the solution was then determined using a spectrophotometer, with a reading made at a wavelength of 540 nm.

The total leukocyte count was performed using smears made from blood samples from each animal. These smears were prepared on slides, air-dried and subjected to May Grunwald-Giemsa-Wright (MGGW) staining. Counting was performed under a light optical microscope at 100 x magnification, using immersion oil, for 2,000 erythrocytes. In addition, using indirect methods, the total number of thrombocytes and leukocytes was also checked (Ranzani-Paiva et al. 2013).

Relative cost of feed

The relative cost of feed was evaluated using the model proposed by Bellaver et al. (1985), by which the average cost of feed per unit of weight gain is determined via the equation: cost (US$) = diet consumed (g) x price of diet consumed/ weight gain. The cost of the feed was US$ 1.06 per kg when purchased in January of 2024.

Statistical analysis

The data was checked for normality and variance homogeneity (Cramer von-Mises and Brown Forsythe tests), then submitted to an analysis of variance (ANOVA), and the means were compared using the Tukey test (P<0.05). Performance variables are presented as the mean ± standard error and population data are presented as the mean ± standard deviation.

RESULTS

Growth performance

The weight gain (Figure 1a) and specific growth rate (Figure 1b) of the animals in F2R4 did not differ when compared to the control group. However, both were higher than those obtained in F2R2 and F2R3, whose means did not differ from each other. The fish submitted to fasting cycles had a lower feed intake ($p < 0.05$) when compared to the control group (Figure 1c). The feed conversion rate in F2R4 was worse when compared to that of F2R3, with the other treatments presenting intermediate values (Figure 1d).

Blood, plasma and tissue

The mean hemoglobin concentration and RBC levels (Table 1) remained unchanged during fasting and refeeding cycles. The same occurred with hematocrit levels (Table 1), which did not present a statistical difference ($p > 0.05$) between treatments.

Regarding plasma glucose levels, only the restriction protocol F2R3 resulted in a reduction in blood glucose (Table 1). The triglyceride values found in the fish of the F2R2 treatment were higher when compared to fish of the other treatments and these, in turn, did not differ from each other (Table 1). The mean cholesterol levels of the fish of the F2R4 treatment showed no differences compared to those of the control treatment; though they were higher when compared to the fish of the F2R3 treatment (Table 1). Regarding serum protein levels in the fish submitted to fasting cycles, no differences were observed between them, but these levels were lower than those observed in the fish of the control treatment (Table 1). No differences between treatments were observed for muscle lipid concentrations (Figure 2a). In the fish of the F2R3 and F2R4 treatments, hepatic glycogen did not differ, but the levels were lower than in the fish of the control group; while the fish of the F2R2 treatment presented an intermediate value and did not differ from those of any other of the evaluated treatments (Figure 2b). The VSFI of the fish submitted to F2R2 was lower ($p < 0.05$) when compared to those of the other treatments, and these did not differ among them (Figure 2c).

Indicators of stress and immune responses

The respiratory activity of fish leukocytes was similar between treatments ($p > 0.05$) (Figure 3a). The same pattern was observed in the leukocyte count (Figure 3b).

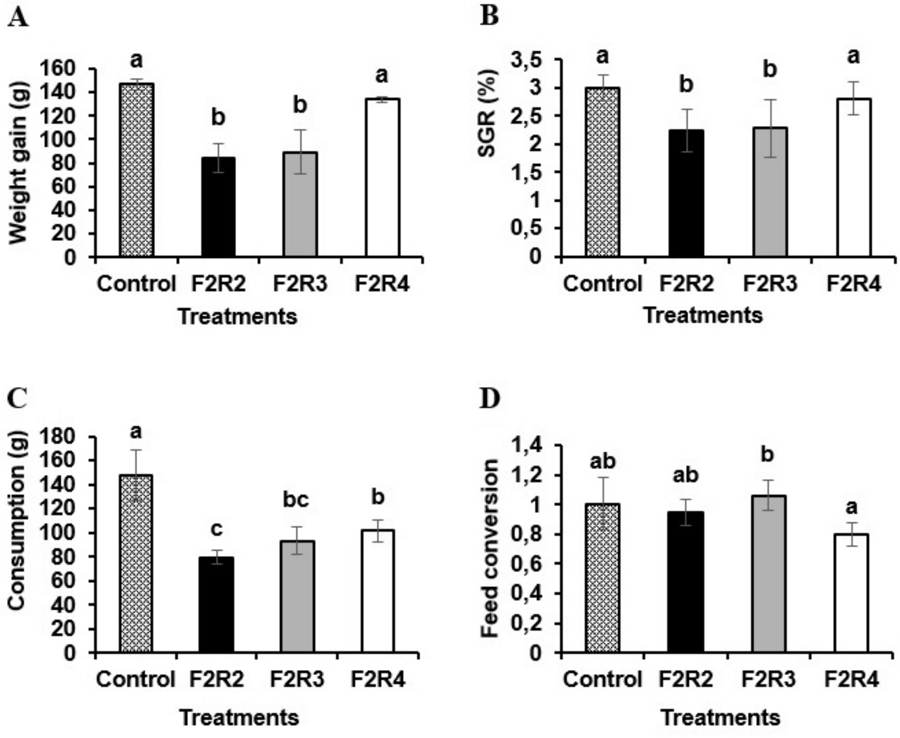

Figure 1. Mean and standard error of weight gain (**A**), specific growth rate SGR (**B**), consumption (**C**) and feed conversion (**D**) of juvenile *C. macropomum* submitted to control: continuous feeding; F2R2: two days of fasting followed by two days of refeeding; F2R3: two days of fasting followed by three days of refeeding; F2R4: two days of fasting followed by four days of refeeding, after the 60 days of the experiment. Different letters indicate statistical difference according to the Tukey test ($p < 0.05$).

Table 1. Mean and standard deviation of hemoglobin levels, red blood cell (RBC), hematocrit, plasma glucose, triglycerides, cholesterol and serum protein in juvenile tambaqui (*C. macropomum*) subjected to short cycles of two days of fasting and two (F2R2), three (F2R3) and four (F2R4) days of refeeding, after 60 experimental days.

Variables	Control	F2R2	F2R3	F2R4
Hemoglobin (g/dL^{-1})	8.55 ±1.15	8.48±1.84	7.68 ±2.32	7.31 ±1.97
RBC (x10 6 mL^{-1})	1.59±2.13	1.61±1.46	1.83±1.11	1.63±1.09
Hematocrit %	46.16±10.6	43.72±5.74	39.0 ±10.0	43.4 ±7.35
Plasma glucose (mg dL^{-1})	46.05±2.50a	40.49±1.64ab	35.50±2.71b	42.93±3.01a
Triglycerides (mg dL^{-1})	284.55±5.91b	429.48±9.82a	322.27±9.08b	358.21±11.04b
Cholesterol (mg dL^{-1})	111.65±8.95b	117.90±12.16ab	126.89±14.44a	109.94±9.00b
Serum protein (mg dL^{-1})	3.67±0.35a	3.20±0.35b	3.12±0.20b	3.26±0.26b

Different letters indicate statistical difference using the Tukey test ($p < 0.05$).

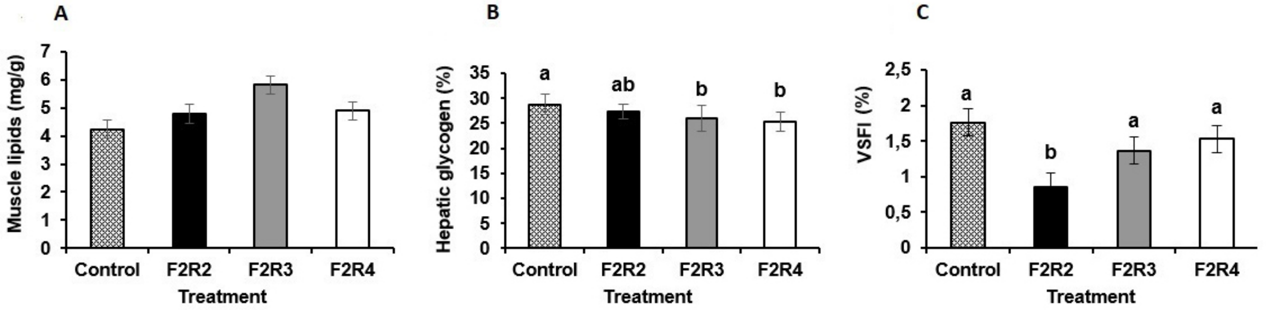

Figure 2. Muscle lipid (**A**), hepatic glycogen (**B**) and viscerosomatic fat index VSFI (**C**) levels of juvenile *C. macropomum* submitted to control: continuous feeding; F2R2: two days of fasting followed by two days of refeeding; F2R3: two days of fasting followed by three days of refeeding; F2R4: two days of fasting followed by four days of refeeding, after the 60 days of the experiment. Different letters indicate statistical difference according to the Tukey test ($p < 0.05$).

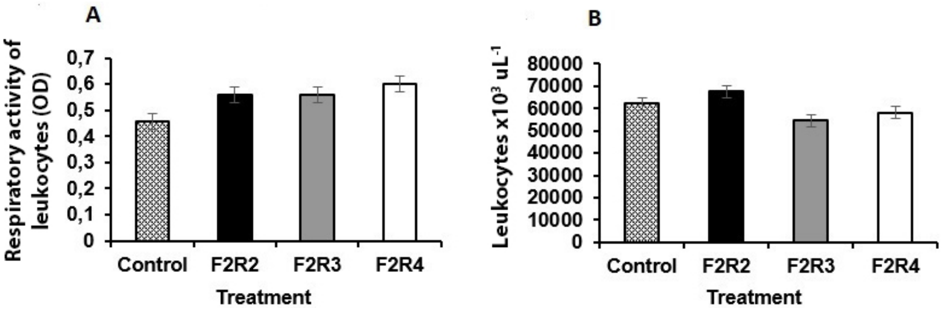

Figure 3. Means and standard error for respiratory activity of leukocytes (**A**) and number of leukocytes (**B**) of juvenile *C. macropomum* submitted to control: continuous feeding; F2R2: two days of fasting followed by two days of refeeding; F2R3: two days of fasting followed by three days of refeeding; F2R4: two days of fasting followed by four days of refeeding, after the 60 days of the experiment. Different letters indicate statistical difference according to the Tukey test ($p < 0.05$).

Relative cost of feeding

The relative cost of feeding per kg of weight gain was US$ 0.98 for the control treatment, and the relative costs of the F2R2 and F2R3 treatments were US$ 0.91 and US$ 1.04, respectively. On the other hand, the F2R4 treatment showed the lowest relative cost - US$ 0.85.

DISCUSSION

In the study we aimed at determining the effects of alternating cycles of fasting and refeeding on zootechnical performance and health of juvenile *C. macropomum*, as well as the feeding costs. We have shown that two days of fasting and refeeding for four days caused total growth compensation, no negative effects on hematological variables, no stress or change in the fish's innate immunity indicators, in addition to presenting a low relative cost of food. In contrast, the F2R2 and F2R3 protocols were more severe, negatively affecting the evaluated physiological responses.

During the 60-day experimental period, no mortality was recorded. However, due to the lower weight gain and the lower growth rate observed in fish submitted to treatments F2R2 and F2R3, only partial compensatory growth was observed. On the other hand, fish under the F2R4 treatment, which obtained responses of weight gain and SGR that were similar to the fish of the control treatment, demonstrated a total compensatory growth. Similar to the present study, growth compensation for *C. macropomum* was also complete when the fish were subjected to cycles of two days of restriction and five days of refeeding (Santos et al. 2018), or with one day of restriction and six days of refeeding (Assis et al. 2020). Additionally, the findings of our study suggest that shorter cycles than those already reported in the literature can be used, what may positively impact feed efficiency.

The growth response may vary among the various fasting protocols, from total compensation, overcompensation or no compensation possible (Roa et al. 2019; Santos et al. 2021; Melo et al. 2024). Several factors can affect this response, such as species, water temperature, nutritional status prior to fasting, duration of deprivation and refeeding period (Costa et al. 2019; Porto et al. 2024). One of the physiological mechanisms that enables compensatory gain is hyperphagia (Ali, et al. 2003). In this study, hyperphagia has been observed during refeeding, however, none of the treatments exceeded the feed intake of the control group during the experimental period. Hyperphagia was identified by increased swimming activity and food intake at the time of food offering. Additionally, the food conversion observed in the fish of the F2R4 treatment indicates that the fish were able to take advantage of the food more efficiently. These results corroborate those presented by Morshedi et al. (2017) in a study using Siberian sturgeon (*Acipenser baerii* Brandt 1869) with cycles of two and four days of fasting, followed by 26 and 32 days of refeeding, respectively, in which the fish did not demonstrate higher final feed consumption, but presented a satisfactory feed conversion response.

In the present study, the absence of negative an effect on hemoglobin, RBC and hematocrit results allows us to confirm that the fish maintained their state of health even when submitted to different fasting and refeeding cycles. The hematological variables are used as tools for diagnosing health conditions (Santos and Tavares-Dias 2010). The ability of fasting to alter normal physiological conditions is variable, and may be related to age, species, or even the diet offered in the period preceding fasting (Serra et al. 2016). Food deprivation induced *Nibea albiflora* Richardson 1846 to oxidative stress by increasing glutathione levels (Jiao et al. 2020), but it can also increase stress resistance in *Danio rerio* Hamilton 1822 through lipid catabolism (Lu et al. 2019), thus showing variable responses.

To supply their demand for energy, the fish in the F2R4 treatment showed the ability to mobilize mainly the hepatic glycogen reserves in order to maintain glycemic homeostasis. This finding is in line with the results of Roa et al. (2019), who found no significant differences in glycemic concentrations in short cycles of restriction and refeeding of *C. macropomum*. Typically, when fish go through periods of food deprivation, their glucose concentrations are maintained at a stable basal level, which is due to increased breakdown of glycogen, a process known as glycogenolysis (Navarro and Gutiérrez 1995).

The same pattern was observed in the levels of triglycerides, with the exception of those of the fish in the F2R2 treatment, which presented high levels in relation to the fish of the other treatments. This can be attributed to the fact that this treatment subjected the fish the greatest number of days on food restriction (30 days, alternating every 2 days), which probably mobilized a greater amount of triglycerides in the circulation, though the other treatments maintained values that were close to the average. It is pertinent to note that, according to Weatherley and Gill (1981), triglyceride and total protein levels may present variable responses, and contradictory findings may result from differences in fish species and their physiology.

For cholesterol, only the fish of the F2R4 treatment achieved levels similar to those of the control. Under normal feeding conditions, endogenous cholesterol is directly regulated by the dietary levels offered (Caruso et al. 2012; Zhu et al. 2014). Fasting, in turn, causes an increase in circulating cholesterol levels due to the stimulation of endogenous synthesis (Zhu et al. 2014), which may explain this variation. Regarding serum protein levels, there were no differences among treatments, and they remained close to the values of the fish in the control. This indicates that the short cycles of fasting and refeeding were not enough for the glycogen and lipid reserves to be completely consumed. Favero et al. (2018) observed that longer fasting cycles (18 days) of Pacu (*Piaractus mesopotamicus*) resulted in a sharp drop in serum protein levels, which shows the effectiveness of the short fasting and refeeding cycles used in our study.

Stored lipids are the main sources of energy and are located in adipose tissue (muscle and liver) (Sheridan 1994; Weil et al. 2013). However, in this experiment, it was found that muscle lipids were not mobilized as an energy source during brief fasting cycles, since no treatment differed significantly from the control group. These results contrast with the findings of Roa et al. (2019), who observed a reduction in muscle lipid concentration in *C. macropomum* subjected to short cycles of five days of feeding followed by two days of deprivation (5F/2D) and two days of feeding followed by four days of deprivation (2F/4D). This species demonstrated the effective mobilization of muscle lipids, corroborating previous findings in other Amazonian fish species, such as matrinxã and pacu (Takahashi et al. 2011; Urbinati et al. 2014).

The observed variation can be attributed to the longer period of fasting adopted in this study (4 days), compared to the shorter cycles mentioned in the previous research. However, our results indicate that the treatments did not severely affect the energy reserves in the liver. In order to maintain homeostasis, in the early stages of fasting, hepatic glycogen is usually the first substrate to be mobilized as an energy source (Babaei et al. 2016). This study reflects this dynamic, evidencing the mobilization of energy reserves in the liver, and showed little variation between the fish in the treatments.

Fat deposition in the abdominal cavity can be used by fish under conditions of food restriction (Souza et al. 2002), as was observed for the F2R2 treatment with the reduction of VSFI, which, due to its shorter cycle, was subjected to a greater number of fasting days throughout the experiment. Salgado-Ismodes et al. (2020) showed that, in rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) under fasting cycles of three days a week with alternating weeks, somatic visceral fat showed a decreasing linear trend, with fasting and refeeding cycles showing the use of reserves. Takahashi et al. (2011) performed 36 days of fasting and refeeding cycles in pacu (*P. mesopotamicus* Holmberg 1887) and also observed reduction of visceral lipids to maintain the energy demand of fish without subsequent recovery of their stock.

There was also no change in the indicators of innate immunity that were evaluated. These are the body's first line of defense and part of these responses include leukocytes, which are able to recognize pathogens, phagocytes and degrade them (Bols et al. 2001). When phagocytosis is initiated, there is an increased consumption of molecular oxygen (oxidative burst). Oxygen is reduced, via NADPH oxidase, to superoxide anion which, in turn, is a precursor to various reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH-) and oxygen anions (O_2). ROS are highly oxidizing and actively contribute to the destruction of microorganisms (Verlhac and Gabaudan 1997). In our study, fasting cycles did not negatively affect the innate immunity of *C. macropomum* since both the respiratory activity of leukocytes and the leukocyte count did not differ from the fish of the control treatment. A period of 30 days of fasting was not able to negatively affect the immune response capacity of juvenile pacu, even after performing a bacterial challenge (Gimbo 2015).

The relative feed cost of the F2R4 treatment was the lowest observed, although there is no statistical analysis for this variable, fish production is an economy of scale, and small savings in the production of a unit of live weight can result in considerable profit to the producer (Jena et al. 2017). Thus, the F2R4 cycle could be an interesting strategy to reduce feed consumption since it incurred a lower cost, without affecting fish weight gain and reducing possible negative impacts on water quality.

CONCLUSION

We concluded that juveniles of *C. macropomum* can attain partial compensatory growth after short cycles of fasting of two days followed by refeeding of two and three days (J2R2, J2R3) and total compensation with two days of fasting followed by four days of refeeding (J2R4). In addition to reducing food costs, restriction for short periods maintained the ability to adjust energy metabolism without exhausting energy reserves and without causing stress in animals subjected to up to two days of fasting followed by refeeding for four days.

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