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Intestinal histomorphometry and biometric indexes of tambaqui (*Colossoma macropomum*) grown under different feeding rates

Histomorfometria intestinal e índices biométricos de tambaqui (*Colossoma macropomum*) cultivado sob diferentes taxas de alimentação

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ABSTRACT
Optimizing the production system of tropical species in tanks requires scientific studies on proper food management, with the determination of the ideal feed rate, aiming at the best rates of zootechnical performance and carcass yield associated with the lowest production cost for the producer. The aim of this study was to evaluate the histomorphometry of the intestine and the biometric indices of tambaqui (Colossoma macropomum) grown under different feeding rates. The study was conducted at the Fish Culture Research Center of the Universidade Federal de Rondônia, located in the city of Presidente Médici in the state of Rondônia, using 225 juveniles of tambaqui, with average initial body weight and length of 713.08g±0, 95 and 10.62 cm±0.1, respectively, being submitted to different feeding rates (0.5; 0.75; 1.0; 1.25 and 1.5% of body weight) with three repetitions each, in the density of 15 juveniles per hapa making a total of 15 hapas. Commercial feed of 28% crude protein was provided, and the fish were weighed every 30 days to adjust the feed supply in relation to body weight. The animals were slaughtered with an average of 2,162 g and 43.6 cm of total length at 140 days of culture. The intestine itself presented average weight and length between the batches of feed animals with different feeding rates of 7.7 g and 73.44 cm, respectively. The results of the biometric evaluation for weight and body length, weight and length of the intestine, relative weight of the intestine, CI:CC ratio (length of the intestine: body length) and the histomorphometric evaluation of the height of the intestinal villi did not present significant differences ($P>0.05$), however, there was a linear increase in the feeding rates ($P<0.05$) of tambaquis in the fattening phase for liver weight, from 27.77 g to 34.44 g, visceral fat weight, from 83.22 g to 121.63g, hepatosomatic index and viscerosomatic fat index, maximum 1.55% and 4.42%, respectively.

Keywords: Biometric assessment, Hepatosomatic index, Production cost, Viscerosomatic index.

RESUMO
Otimizar o sistema de produção de espécies tropicais em viveiros demanda estudos quanto ao manejo alimentar adequado, com a determinação da taxa de alimentação ideal, visando os melhores índices de desempenho zootécnico e rendimento de carcaça associados ao menor custo de produção para o produtor. O objetivo deste estudo foi avaliar a histomorfometria do intestino e os índices biométricos de tambaqui (Colossoma macropomum) cultivado sob diferentes taxas de alimentação. O estudo foi desenvolvido no Centro de Piscicultura da Universidade Federal de Rondônia, localizada no município de Presidente Médici no estado de Rondônia, utilizando-se 225 juvenis de tambaqui, com peso e comprimento corporais médios iniciais de 713,08 g ± 0,95 e 10,62 cm ± 0,1, respectivamente, sendo submetidos a diferentes taxas de alimentação (0,5; 0,75; 1,0; 1,25 e 1,5% do peso corporal) com três repetições cada, na densidade de 15 juvenis por hapa perfazendo um total de 15 hapas. Foi fornecida ração comercial de 28% de proteína bruta, e os peixes foram pesados a cada 30 dias para realização do ajuste do fornecimento de ração em relação ao peso corporal. Os animais foram abatidos com média de 2.162 g e 43,6 cm de comprimento total aos 140 dias de cultivo. O intestino propriamente dito apresentou peso e comprimento médios entre os lotes de animais arraçoados

com diferentes taxas de alimentação de 7,7 g e 73,44 cm, respectivamente. Os resultados da avaliação biométrica para peso e comprimento corporal, peso e comprimento do intestino, peso relativo do intestino, relação CI:CC (comprimento do intestino: comprimento corporal) e da avaliação histomorfométrica da altura das vilosidades intestinais não apresentaram diferenças significativas (P>0,05), no entanto, houve um incremento linear as taxas de alimentação (P<0,05) dos tambaquis na fase de engorda para o peso do fígado, de 27,77 g a 34,44 g, peso da gordura visceral, de 83,22 g a 121,63 g, índice hepatossomático e índice gordura viscerossomático, máximos de 1,55% e 4,42%, respectivamente.

Palavras-chave: Avaliação biométrica, Custo de produção, Índice hepatossomático, Índice viscerossomático.

1 INTRODUCTION

Fish farming together with animal production has been experiencing substantial technological advances in the areas of genetic improvement, management, health and nutrition (ALBUQUERQUE et al., 2019), because it is an activity that every day becomes more important as a source of protein for human consumption, providing healthy food, being increasingly sought after by consumers and recommended (BOTELHO et al., 2017).

Among the species cultivated in Brazil, the tambaqui (*Colossoma macropomum*) stands out, fish originating in South America that inhabits the floodplain areas of the Orinoco and Amazon River basins (MORAIS; O'SULLIVAN, 2017). Tambaqui is the species most studied by researchers in the area in the Amazon, as it is of great commercial interest (SOUSA et al., 2016) and has conquered the local market, through its growing production in fish farming, and this has occurred due to the rusticity of the species and excellent potential for cultivation in confinement environments (MEANTE; DÓRIA, 2017).

The positive growth in the production of tambaqui is directly related to the diet used in the cultivation of the species (BEZERRA et al., 2014), observing the protein content in the feed and its offer to the animals. However, Zarpellon (2015) points out that the supply of nutrients generated in the farming environments is a worrying factor, which can cause problems for the fish and for the environment, since the high feed supply and its residues are thrown directly into the water. In this follow-up, it is necessary to take into account the sustainability of the crops, the genetics of the animals (ARARIPE et al., 2006), the composition of the rations, in addition to the rate and frequency of feeding (PORTO et al., 2020).

Since feed is one of the most expensive items in the different fish production systems, with 50% of the total cost (MARTINS et al., 2020) or more, it is necessary to adopt measures aimed at reducing this cost, for through adequate food management. Considering that the consumption of animal feed is directly related to the health of the fish (SOUZA et al., 2014), the improper adjustment can harm the feed conversion of the fish, but when done correctly, it contributes to better performance and animal efficiency.

Meer et al. (1997) state that the excess of food causes metabolic alterations, which worsens the efficiency of absorption of nutrients. On the other hand, the low supply of food directly affects the weight gain of the animals. Therefore, the ability of fish to process food is fundamental to their development and depends on the structure of the epithelium and intestinal villi, the enzymatic profile of the gastrointestinal tract and its enzymatic plasticity in relation to the environment (KUPERMAN; KUZ'MINA, 1994).

Since the basic knowledge of the digestive physiology of fish is fundamental for the development of diets that meet the nutritional requirements of cultivated species (CAVALI et al., 2020), it is observed that studies that contemplate the understanding of the morphophysiology of the digestive system are scarce in the literature , hindering the development of nutritional studies, preparation of rations, food management and minimization of the environmental impacts generated by the fish farming activity (FERNANDES et al., 2014).

Thus, the objective of this study was to evaluate the histomorphometry of the intestine and the biometric indices of tambaqui grown at different feed rates in the fattening phase.

2 MATERIAL AND METHODS

The study was conducted at the Fish Farming Research Center Carlos Eduardo Matiazze, from the Universidade Federal de Rondônia (11 ° 9 '56.08 ”S and 61 ° 53' 52.06” W), located in the municipality of Presidente Médici, state of Rondônia, in the period from August 2016 to May 2017, under approval by the Animal Ethics Committee under CEUA 018/2015.

A dug tank 20 m wide x 50 m long and depth of 1.64 m was used, totaling 1640 m^3 of water, with a flow rate of 5 liters/s, subdivided into 16 hapas with an area of 50 m^2 of galvanized mesh and coated with PVC, equipped with floating feeders of 1.5 m radius.

Were 225 juveniles of tambaqui acquired in certified fish farming were used, with initial average body weight and length of 713.08g ± 0.95 and 10.62 cm ± 0.1, respectively, grown until slaughter. The animals were distributed in a completely randomized design, submitted to five different feeding rates (0.5; 0.75; 1.0; 1.25 and 1.5% of body weight) with three repetitions or hapas, in the density of 15 juveniles / hapa making a total of 15 hapas (experimental units).

The fish were fed three times a day, at 7:00 am, 11:00 am and 5:00 pm, with extruded feed with a 28% crude protein content (Table 1), according to the commercial recommendation for animals above 750g of body weight. Fish were weighed every 30 days to adjust the feed supply in relation to body weight and according to the weight gain rates observed in the literature for weekly projections.

Table 1. Guaranteed levels of the feed provided.

Item	Content (g/kg)
Crude protein (Min.. g)	280.0
Feed moisture (Max.. g)	90.0
Ether extract (Min.. g)	30.0
Crude fiber (Max.. g)	90.0
Mineral matter (Max.. g)[1]	150.0
Vitamins and aminoacids[2]	----

[1] Phosphorus (g/kg) 10.0; Calcium (Max .. g/kg) 35.0; Calcium (Min .. g/kg) 10.0; Iron (mg/kg) 68.0; Copper (mg/kg) 6.4; Manganese (mg/kg) 7.5; Zinc (mg/kg) 20.0; Cobalt (mg/kg) 0.04; Iodine (mg/kg) 0.4; Selenium (mg/kg) 0.1. [2] Vitamin A (IU/kg) 26000.0; Vitamin D3 (IU/kg) 6000.0; Vitamin E (IU/kg) 24.0; Vitamin K3 (mg/kg) 2.5; Vitamin B1 (mg/kg) 2.0; Vitamin B2 (mg/kg) 4.0; Vitamin B6 (mg/kg) 2.1; Vitamin B12 (mcg/kg) 5.0; Niacin (mg/kg) 12.0; Pantothenic acid (mg/kg) 4.0; Biotin (mg/kg) 60.0; Choline (mg/kg) 80.0; Vitamin C (mg/kg) 300.0.

After weighing, the animals were stunned by hypothermia on ice: water in a 2:1 ratio for 5 minutes (MENDES, 2013), followed by bleeding, according to the Regulation of Industrial and Sanitary Inspection of Animal Origin Products (RIISPOA) (BRASIL, 2017). Body biometric assessments measured body weight (g) and total body length (cm), given by the distance from the anterior end of the head to the end of the caudal fin.

2.1 BIOMETRIC AND HISTOMORPHOMETRIC ANALYZES OF THE INTESTINE

The biometric evaluation was performed on three specimens from each hapa, selected considering the average body weight of the batch. After stunning and bleeding, an incision was made in the abdominal cavity of the fish to remove the intestine, which was separated from the stomach, to measure weight (precision scale AS 2000 C- Mars) and length (graduated ruler), allowing the calculation of the relative weight of the intestine (PRI), according to equation (1).

$$Intestine\ weight / \ Fish\ weight\ \times 100 \quad \mathbf{(1)}$$

And the bowel length / body length (CI / CC) ratio. The liver and visceral fat of the fish were also separated for weighing, used to obtain the hepatosomatic index (IHS) and viscerosomatic fat index (IGVS), according to equations (2) and (3).

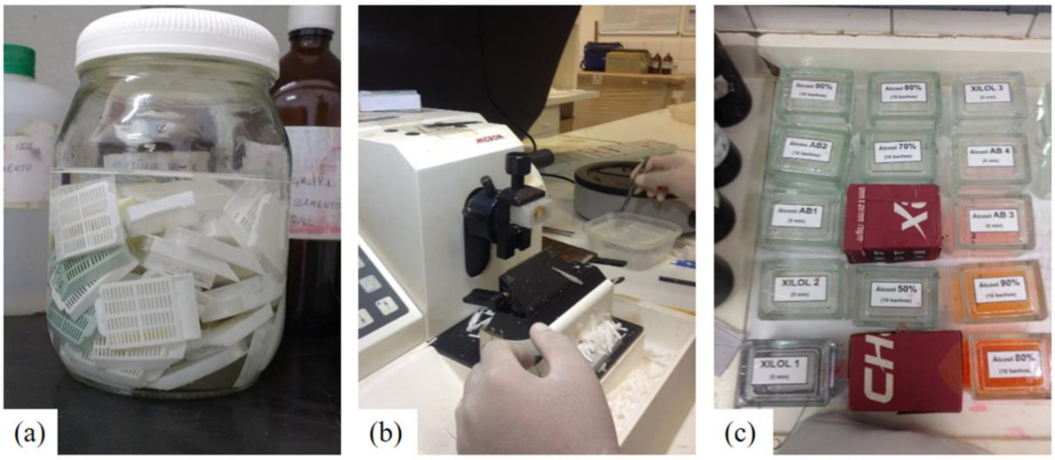
$$IHS = Liver\ weight / \ Fish\ weight\ \times 100\ (\mathbf{2})$$
$$IGVS = Visceral\ fat\ weight / \ Fish\ weight\ \times 100\ (\mathbf{3})$$

For histomorphometric analyzes, two samples (5 mm) of the proximal intestine of each fish were taken, followed by evisceration, fixed in 10% formalin for 24 hours and conserved in 70% alcohol to assess the height of the intestinal villi. The histological processing of the samples took place in part at the Animal Anatomy Laboratory of the Universidade Federal de Rondônia and in the Veterinary Pathology Laboratory of the Universidade Federal de Jataí.

In their processing (Figure 1), the samples underwent dehydration procedures in an increasing series of ethyl alcohol (70, 80, 90, 96 and 100%) and clearing in two xylol PA batteries (C_8H_{10}), remaining for 30 minutes in each step. Subsequently, paraffin infiltration was carried out in two batteries (I and II), with the paraffin temperature between 56 and 58 °C, for 30 minutes each. After that, it was included in paraffin, where the samples are packed with molten paraffin between 56 and 58 °C and kept at room temperature, or under light refrigeration, for 12 hours until the time of cutting. With the blocks ready, a microtomy with 5 micrometer cuts (µm) was followed.

The material is placed in a histological bath with heated water and captured with a glass slide and taken to the oven at 37 °C for at least 12 hours for drying. Then, staining was carried out, following the methodology of Luna (1968) for staining in Hematoxylin and Eosin (H&E).

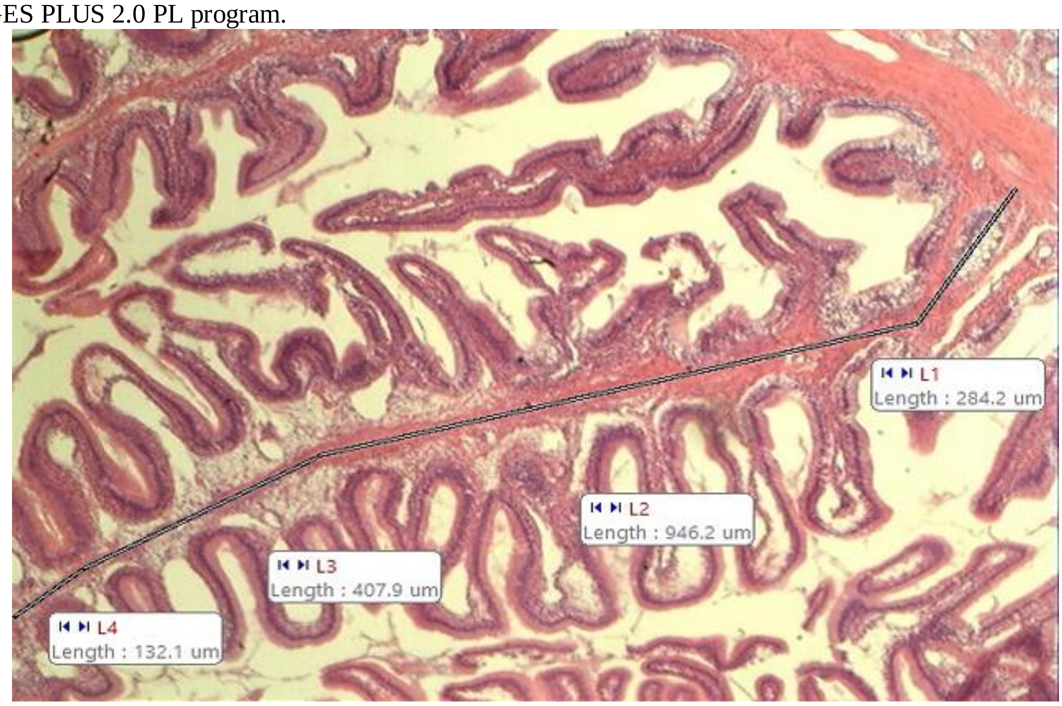
Figure 1. Sample processing. (a) Dehydration, (b) Diaphanization and (c) microtomy and staining.



Source: Author's file.

In light microscopy using a 4x magnifying glass, eight intact villi of each fish (in µm) were measured, making up 24 villi per hapa. Height was measured from the apex to the beginning of the muscle layer (Figure 4) using the MOTIC IMAGES PLUS 2.0 ML software.

Figure 2. Measurements of a villus of the anterior portion of the tambaqui intestine made with the MOTIC IMAGES PLUS 2.0 PL program.



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2.2 STATISTICAL ANALYSIS

The treatment averages were analyzed using the SAS statistical program, by analysis of variance and regression, using linear, quadratic and cubic orthogonal contrasts, being adopted at 95% reliability (α=0.05).

3 RESULTADS

The five different feeding rates evaluated in the research did not have a significant effect ($P>0.05$) on the animal and intestine biometric measurements, given by body weight and length, intestine weight and length, relative intestine weight and the relationship bowel length: body length (CI:CC). They also did not change ($P>0.05$) the height of the intestinal villi in the anterior fraction of the tambaqui intestine in the fattening phase (Table 2). The villus height of the tambaqui anterior intestine (Figure 3) in the present study did not differ significantly between feeding rates ($P>0.05$), with an average of 1608 µm.

The fish were slaughtered with an average body weight of 2.162 g at 140 days of cultivation and an average of 43.6 cm in total length. The intestine itself presented average weight and length between the batches of feed animals with different feeding rates of 7.7 g and 73.44 cm, respectively.

Figure 3. Villus of the anterior portion of tambaqui intestine in H&E staining and 4x magnification microscopy. There was no difference in height in the villi between the images analyzed from (a) to (d).

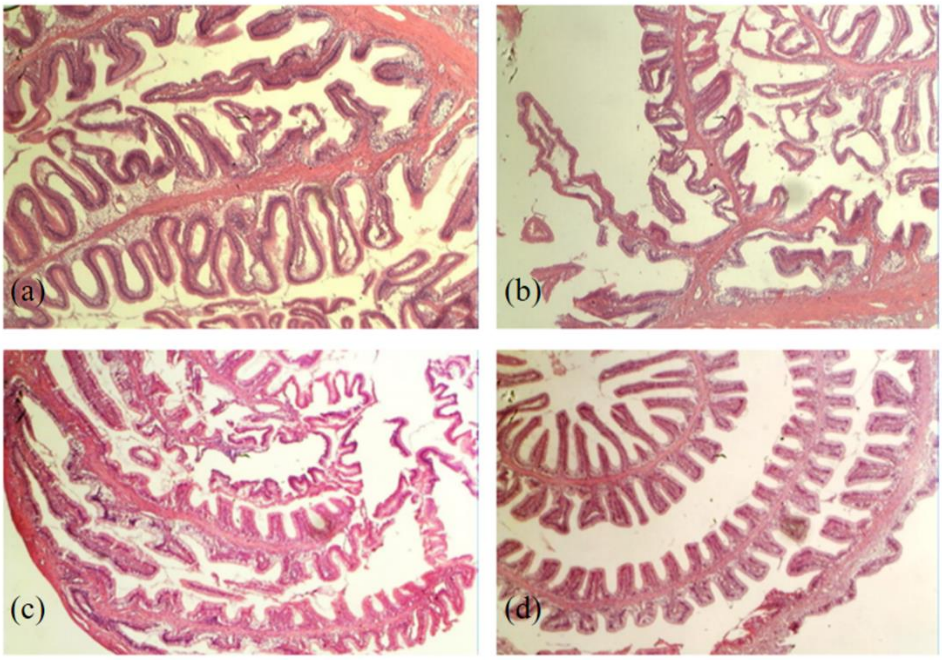

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Table 2. Averages of the biometric and morphometric variables of the tambaqui intestine grown at different feeding rates.

Variables	Feeding rate (% of body weight)					Averages	Contrasts[2]				CV (%)
	0.50	0.75	1.00	1.25	1.50		L	Q	CB	QT	
Total weight (g)	2.115	2.225	1.996	2.203	2.270	2.162	NS	NS	NS	NS	11.82
Body length (CC. cm)	43.50	44.33	42.55	44.28	43.67	43.65	NS	NS	NS	NS	3.61
Intestine weight (g)	7.85	8.25	8.87	7.02	6.73	7.74	NS	NS	NS	NS	17.12
Bowel length (CI. cm)	71.44	77.56	70.11	73.89	74.11	73.44	NS	NS	NS	NS	10.59
Liver weight (g)[3]	27.77	30.46	34.16	39.36	34.44	33.24	0.0105	NS	NS	NS	11.68
Visceral fat weight (g)[3]	83.22	85.38	86.72	100.27	121.63	95.45	0.0074	NS	NS	NS	15.73
Relative weight of intestine (PRI. %)	0.37	0.37	0.45	0.32	0.30	0.36	NS	NS	NS	NS	19.11
Ratio CI:CC	1.76	1.78	1.58	1.65	1.75	1.70	NS	NS	NS	NS	9.44
Hepatosomatic index (%)[3]	1.32	1.38	1.72	1.79	1.68	1.55	0.0006	NS	NS	NS	10.46
Viscerosomatic fat index (%)[3]	3.94	3.99	4.34	4.50	5.36	4.42	0.0001	NS	NS	NS	5.74
Villus (µm)	1693	1753	1476	1634	1482	1608	NS	NS	NS	NS	16.33

[1] P = 5% was considered. [2] Contrast: L = linear. Q = quadratic. CB = cubic. QT = quartic. NS = not significant at 5% and CV = Coefficient of variation. [3] Weight of the Liver (g) = 20.8186 + 7.2819 * Rate (R^2 = 64.00); Visceral fat weight (g) = 59.0596 + 19.4396 * Rate (R^2 = 81.20); Hepatosomatic index = 1.0263 + 0.3136 * Rate (R^2 = 68.46); Viscerosomatic fat index = 3.0052 + 0.7765 * Rate (R^2 = 85.50).

4 DISCUSSION

Tambaqui's natural diet includes zooplankton, phytoplankton, fruits and seeds, being classified as an omnivorous-opportunistic species that is easily accepted by artificial diets and adapted to captive breeding (HONDA, 1974; LOPERA-BARRERO et al., 2011). The generalist food characteristic of tambaqui makes the response to feeding rates directly related to the cycle phase and the characteristic of the cultivation environment given especially by the presence of primary production in the nursery (PORTO et al, 2017), with lower consumption oxygen at higher temperatures and lower feed efficiency of the species with increasing maturity (SOUSA et al., 2016).

Rodrigues (2014) carried out a review on the tambaqui food management and observed differences in the crude protein content and feeding rates offered in the cultivated in excavated nurseries and net tanks, cited feeding rates of 2% and 1.5% of the weight to animals above 500g and 1000g, respectively. They also highlighted the lack of information for fish in the fattening phase due to the higher cost of research with larger animals.

The CI:CC ratio varies according to the species' eating habits, ranging from 0.2 to 2.5 in carnivorous fish, between 0.6 and 8.0 in omnivores and from 0.8 to 15.0 and herbivores (BALDISSEROTTO, 2010). In this study, the average CI:CC ratio obtained was 1.70, which is in accordance with the literature for omnivorous fish. However, less relation to the length of the intestine can be observed for animals fed with less fibrous diets or of better quality. In this study, the carry over effect resulting from the residue of the higher feeding rates impacting the water quality of the nursery, may have contributed to the abundance in primary production and availability of food in the fattening phase, which culminated in non-significant responses ($P>0.05$) regarding body and intestinal biometric measurements.

Changes in the length of the intestine can be compensated for by variations in the area of the intestinal mucosa and by the variability of cell types found in the epithelium of this mucosa (BORGES et al., 2010). Schwars et al. (2011) described a linear increase in the intestine length of Nile tilapia larvae in relation to the increase in the levels of mannan-oligosaccharide (MOS) prebiotics in diets. On the other hand, Lima et al. (2017) did not observe significant differences in the weight and length of the tambaqui intestine when using probiotics in the feed or dissolved in water during transport.

Regarding the villus height, a result similar to the present study was found by Zarpellon (2015) in the anterior fraction of the intestine, evaluating feeding rates of 0.7; 1.4; 2.1; 2.8 and 4.5% of body weight for pirapitinga, with an average weight of 440 g. However, a quadratic effect was observed in the middle portion of the intestine, with a feeding rate of 2.1% showing greater villus height.

The intestinal mucosa is fundamental in the digestive, absorptive and metabolic processes in teleost fish (CASPARY, 1992) and, for digestion and absorption of nutrients from the diet, an efficient area of the intestinal lumen is necessary, with high villi and mature enterocytes (ZAPELLON, 2015; CAVALI et al., 2020). Thus, the intestinal mucosa maximizes the digestive process and represents an extensive area of digestion and absorption of nutrients (MACARI; MAIORKA, 2000), the height of the villi being directly proportional to the absorption area. The integrity of the intestinal mucosa can be assessed from measurements of villus height, according to Ferreira et al. (2014), since the balance between cell renewal and cell loss that normally occurs in the intestine determines a constant cell renewal, maintaining the size of the villi and consequently the digestive capacity and intestinal absorption.

Honorato et al. (2011), describe that the height of the intestinal villi of tilapia was influenced mainly by the diet containing fish silage as a source of protein of animal origin. Research has also been carried out with probiotics for aquatic animals, evaluating the positive effects on the intestinal morphology of animals, such as benefits on the microvilli structure and absorptive surface (CARVALHO et al. 2011; MELLO et al. 2013; FERREIRA et al., 2014).

Basic knowledge of the digestive physiology of fish is essential for the development of diets that meet the nutritional requirements of cultivated species and adequate food management (RODRIGUES, 2014; PORTO et al., 2020). However, there is little information available in the literature involving the morphophysiology of the digestive system of fish, especially in native species of tropical climate (FERREIRA et al, 2014).

The dynamics of endogenous energy use can be estimated by monitoring the hepatosomatic (IHS) and viscerosomatic fat (IGVS) indices, and the changes in these indices reflect the use of lipid, protein and glycogen (COLLINS; ANDERSON, 1995). With low feeding rates, visceral lipid deposits are used as an energy reserve after depleting the liver reserves, which, after food intake, stores carbohydrates and lipids, being considered an initial source of endogenous energy for fish (SOUZA, 2002). In low-power situations, this reserve is used to supply energy through glycogenolysis and gluconeogenesis (SAITA, 2011).

The weight of visceral fat increased ($P<0.10$) by 19.4 g and the weight of the liver by 7 g for each 1% increase in the feeding rate, as well as the IHS and IGVS, which proportionally increased the amount of feed offered. Paula (2009) in a study evaluating the performance of tambaqui, pirapitinga and the tambatinga hybrid (*C. macropomum* x *P. brachypomum*) cultivated in nurseries fertilized in the fattening phase, obtained IGVS with an average of 2.75% in tambaqui with final average weight of 337.13 g in 270 days of cultivation. Fernandes et al. (2014) found values of 5.4, 6.2 and 6.4% visceral fat index in 107, 137 and 167 days of culture, respectively, when evaluating the carcass characteristics and performance parameters of tambaqui at different times cultivation fed commercial feed.

The increase observed in the IHS and IGVS indexes (Table 2) shows that fish fed with lower feeding rates used the glycogen reserves available in the liver, as well as also obtained energy through visceral fat deposits, which according to Gomes (2018), it is considered a large energy storage site in teleosts. The recovery of mesenteric energy stores is not as fast as that of the liver, indicating, again, that the liver is used as an initial source of endogenous energy

(COLLINS; ANDERSON, 1995). Despite this, the use of visceral fat as the first available reserve was reported by Weatherley and Gill (1981) and Zamal and Ollevier (1995).

5 CONCLUSION

The results of the biometric evaluation for body weight and length, weight and length of the intestine, relative weight of the intestine, CI:CC ratio and the histomorphometric evaluation of the height of the intestinal villi did not present significant differences. However, the rate of 0.5% of body weight shows less deposition of visceral and hepatic fat, without compromising the total weight of the animal.

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