

Metabolism and growth performance of pacu (*Piaractus mesopotamicus*) juveniles submitted to different feeding frequencies

Metabolismo e desempenho de crescimento de juvenis de pacu (*Piaractus mesopotamicus*) submetidos a diferentes frequências de alimentação

Metabolismo y rendimiento de crecimiento de pacu juveniles (*Piaractus mesopotamicus*) sometidos a diferentes frecuencias de alimentación

Received: 03/17/2021 | Reviewed: 03/26/2021 | Accept: 04/01/2021 | Published: 04/04/2021

Mariana Lins Rodrigues

ORCID: <https://orcid.org/0000-0003-4957-9626>
Universidade Estadual do Oeste do Paraná, Brazil
E-mail: lins.mariana@hotmail.com

Robson Araújo dos Santos

ORCID: <https://orcid.org/0000-0002-1628-4373>
Universidade Estadual do Oeste do Paraná, Brazil
E-mail: robson0701@gmail.com

Rebeca Maria Sousa

ORCID: <https://orcid.org/0000-0002-1195-3304>
Universidade Federal da Grande Dourados, Brazil
E-mail: rebeca_sousa31@hotmail.com

Claucia Aparecida Honorato

ORCID: <https://orcid.org/0000-0002-7161-4996>
Universidade Federal da Grande Dourados, Brazil
E-mail: clauciahonorato@ufgd.edu.br

Dacley Hertes Neu

ORCID: <https://orcid.org/0000-0001-5130-773X>
Universidade Federal da Grande Dourados, Brazil
E-mail: dacley_pesca@hotmail.com

Fábio Bittencourt

ORCID: <https://orcid.org/0000-0001-5894-7158>
Universidade Estadual do Oeste do Paraná, Brazil
E-mail: fabio.gemaq@gmail.com

Abstract

The aim of this study was to evaluate the effects of different feeding frequencies on productive performance, hepatocytes integrity, frequency of muscle fibers distribution, enzymatic activity, and proximate composition of pacu juveniles (*Piaractus mesopotamicus*). One hundred and sixty fish were distributed in 20 tanks arranged in a recirculation system. The proposed managements of feed offer were the partition of the daily amounts into one, two, three or four times during 60 days. Feed consumption were higher in fish fed four times a day, whilst the hepatosomatic index was higher in fish fed once, but similar to fish that received food two or four times a day. As for plasmatic glucose levels, fish fed twice a day displayed an increase in this parameter. The livers of fish fed two or four times a day presented larger hepatocytes and cellular nuclei. Regarding the proximate composition of carcasses, fish that received one daily feeding presented higher ether extract. It was concluded that feeding frequency is a factor that promotes changes in *P. mesopotamicus* nutritional metabolism, thus the offer of feed four times a day could be recommended for this species in this life stage.

Keywords: Feed consumption; Hepatocyte; Glucose; Muscular growth; Hepatic enzymes.

Resumo

O objetivo deste estudo foi avaliar os efeitos de diferentes frequências de alimentação no desempenho produtivo, integridade dos hepatócitos, frequência de distribuição das fibras musculares, atividade enzimática e composição centesimal de juvenis de pacu (*Piaractus mesopotamicus*). Cento e sessenta peixes foram distribuídos em 20 tanques dispostos em sistema de recirculação. As propostas de manejo da oferta de ração consistiam na partição das quantidades diárias em uma, duas, três ou quatro vezes durante 60 dias. O consumo de ração foi maior nos peixes alimentados quatro vezes ao dia, enquanto o índice hepatossomático foi maior nos peixes alimentados uma vez, mas semelhante aos peixes que receberam ração duas ou quatro vezes ao dia. Quanto aos níveis de glicose plasmática, os peixes alimentados duas vezes ao dia apresentaram aumento desse parâmetro. Os fígados de peixes alimentados duas

a quatro vezes ao dia apresentavam hepatócitos e núcleos celulares maiores. Em relação à composição centesimal das carcaças, os peixes que receberam uma alimentação diária apresentaram maior extrato etéreo. Concluiu-se que a frequência alimentar é um fator que promove alterações no metabolismo nutricional de *P. mesopotamicus*, portanto, a oferta de ração quatro vezes ao dia poderia ser recomendada para esta espécie nesta fase de vida.

Palavras-chave: Consumo de ração; Hepatócito; Glicose; Crescimento muscular; Enzimas hepáticas.

Resumen

El objetivo de este estudio fue evaluar los efectos de diferentes frecuencias de alimentación sobre el rendimiento productivo, la integridad de los hepatocitos, la frecuencia de distribución de las fibras musculares, la actividad enzimática y la composición próxima del pacú juvenil (*Piaractus mesopotamicus*). Se distribuyeron ciento sesenta peces en 20 tanques dispuestos en un sistema de recirculación. Las propuestas para gestionar el suministro de pienso consistieron en dividir las cantidades diarias en una, dos, tres o cuatro veces durante 60 días. El consumo de alimento fue mayor en los peces alimentados cuatro veces al día, mientras que el índice hepatosomático fue mayor en los peces alimentados una vez, pero similar a los peces que recibieron alimento dos o cuatro veces al día. En cuanto a los niveles de glucosa plasmática, los peces alimentados dos veces al día mostraron un aumento en este parámetro. Los hígados de los peces alimentados de dos a cuatro veces al día tenían hepatocitos y núcleos celulares más grandes. En cuanto a la composición centesimal de las canales, los peces que recibieron alimentación diaria mostraron un mayor extracto de éter. Se concluyó que la frecuencia de alimentación es un factor que promueve cambios en el metabolismo nutricional de *P. mesopotamicus*, por lo que se podría recomendar la oferta de alimento cuatro veces al día para esta especie en esta etapa de la vida.

Palabras clave: Consumo de alimento; Hepatocito; Glucosa; Desarrollo muscular; Enzimas del hígado.

1. Introduction

Providing high-quality diets that meet the nutritional demands of a species is fundamental for the productive success of fish, since it allows a better use of the available nutrients by the animal (NRC, 2011), and also the adoption of adequate feeding frequencies is essential, seen that it relates directly to the animal's metabolism, healthiness and to the deposition of nutrients in its carcass (Bittencourt et al., 2013; Santos et al., 2015). Factors such as species, age, water quality and temperature interfere in the number of daily feeding events (Hayashi et al., 2004).

The determination of feed supply frequency and quantities to be offered are important in relation to feeding management, in order to avoid excessive amounts of feed, which can act in the reduction of both waste generation and production costs, besides maintaining the water quality. On the other hand, feeding frequencies below the optimal rate are also undesirable (Cho et al., 2003). Besides, it composes an important factor within the feeding management, since it stimulates the fish to search for the feed in pre-determined moments, thus contributing for the reduction of feed conversion rates and enabling the possibility of observing the health status of the animals (Carneiro and Mikos, 2005), as well as modifying the fish's proximate composition (Burkert et al., 2008), which may exert an important influence on the quality of breeding water (Cho, 2003).

Providing information about productivity and physiology of fish during food restriction can generate relevant information regarding the applicability of food managements in distinct production systems. In this sense, the dietary frequency in fish rearing might influence the levels of protein, cholesterol and triglycerides in plasmatic metabolism (Dieterich et al., 2013), as well as batch uniformity (Pouey et al., 2012), growth (Santos et al., 2014), and body composition with alterations in content moisture, protein, lipid and ash (Daudpota et al., 2016). Productive performance parameters (e.g. weight gain, specific growth rate, and apparent feed conversion) may also be affected, as reported by Tsuzuki et al. (2014) and Frasca-Scorvo et al. (2017). Thus, studies on feeding dynamics could highlight the implications of the energy resources used by reared fish and provide information about the biochemical pathways involved in feeding, and such a comprehension of these biochemical processes may be useful to satisfy the energetic needs of aquatic organisms.

The pacu (*Piaractus mesopotamicus*), a migrating fish native from South America has opportunistic omnivorous food habit, being well adapted to production systems (Vaz et al., 2000). Its distribution includes the Paraná, Paraguay and Uruguay rivers basins (Calcagnotto and DeSalle, 2009). According to Urbinati and Gonçalves (2005), this species is considered one of the fish species with higher market value in Brazil, and significant volumes in native fish farming, as it is considered an easy-adaptive species to rearing conditions (IBGE, 2016; Mourad et al., 2018). This species displays high fillet yield, and its meat is appreciated for its excellent flavor.

Based on the aforementioned, the aim of this study was to verify the effects of different feeding frequencies on productive performance, plasma glucose, hepatocytes integrity, frequency of muscle fibers distribution, enzymatic activity and proximate composition in *P. mesopotamicus*.

2. Methodology

The experimental procedures adopted in this study were previously approved by the Ethics Committee on Animal Use of the State University of Western Paraná the protocol number 04/18.

Experimental design

A total of 160 pacu juveniles with mean weight of 139.16 ± 8.86 g and mean length of 19.75 ± 0.67 cm were used. In order to accommodate the animals, 20 tanks of cylindrical shape with conical bottoms were used (useful volume of 0.5 m^3), coupled with constant aeration and adapted to a recirculation system.

Water quality was kept within the tolerable limits for the species (Signor et al., 2011; Bittencourt et al., 2012; Sipaúba-Tavares and Santeiro, 2013; Rodrigues et al., 2018): pH 7.01 ± 0.02 , electrical conductivity $80.20 \pm 0.52 \mu\text{S cm}^{-1}$, dissolved oxygen $6.45 \pm 0.35 \text{ mg L}^{-1}$ and temperature 26.7 ± 1.1 °C. All parameters were measured on a weekly basis with the aid of a multiparameter probe YSI 556 (Ohio, EUA), and temperature was measured daily with a digital thermometer.

The proposed feeding management was the parceling of feed supply (Ruohonen, Kettunen and King, 2001), split into one, two, three and four daily offers, according to the scheme described below: (1) Fish fed once a day; (2) Fish fed twice a day; (3) Fish fed three times a day; (4) Fish fed four times a day. A commercial diet was used in this experiment contained 35% crude protein, 5.5% ether extract, 5.0% ash, 13% fibers, 3% calcium and 1% phosphate.

Plasmatic metabolic variable

In order to obtain blood plasma, three animals from each experimental unit was submitted to blood sampling by puncture of the caudal vein using disposable syringes containing EDTA (ethylenediaminetetraacetic acid) at 10%. For glucose determination, the methodology of Dubois et al. (1956) was performed with the aid of a spectrophotometer at 450 nm. A standard glucose solution of 1 mg mL^{-1} was used for the estimation.

Growth performance analysis

At the end of the experimental period of 60 days, all fish underwent a fasting period of 24 hours, followed by anesthesia with a benzocaine solution (100 mg L^{-1}) that was applied inside the aquariums where the fish were immersed, as recommended by Bittencourt et al. (2013). Subsequently, biometrics were performed to assess the animals' weight and length, and the following calculations were made: weight gain - WG (g) = (final weight - initial weight); specific growth rate - SGR ($\% \text{ day}^{-1}$) = $100 \times [(\ln \text{ final weight} - \ln \text{ initial weight})/\text{time}]$; daily feed intake - DFI (g) = (total feed intake/time); Feed conversion rate- FCR = (supplied feed/weight gain); protein efficiency rate - PER (%) = $100 \times (\text{weight gain}/\text{consumed crude protein})$; survival - SU (%) = (number of fish at the end of the experiment/number of fish at the beginning of the experiment) x

100, and whole eviscerated fish- WEF (g) = (weight of whole fish without viscera).

After euthanasia, samples were collected for the evaluation of somatic indexes and histological analysis. The evaluated somatic indexes were viscerosomatic fat index (VFI (%)) = $100 \times [\text{weight of visceral fat/body weight}]$ and hepatosomatic index (HI (%)) = $100 \times [\text{weight of hepatic tissue/body weight}]$.

The proximate composition of the fish carcasses was determined according to the methodology of AOAC (2005), in which all animal were eviscerated and frozen (-20 °C) before analysis. These samples were dried for 24 hours (55 °C in an air-circulating oven) for dry matter analysis and burned (550 °C) for 6 hours to access ash content. Crude protein content was analyzed by the Kjeldahl method and the ether extract by the Soxhlet method.

Enzymes assay

Tissues sub-samples of the fish's liver were homogenized with a Teflon pestle motor-driven tissue cell disruptor (IKA_ T10 basic Ultra Turrax, Staufen, Germany) into 0.02 M Tris / 0.01 M phosphate buffer pH 7.0 plus glycerol 1/1 (v/v), at 4° C under ice bath and kept a ratio 1 : 10 (tissue: buffer). Homogenates were centrifuged at 13400 x g for 3 min (Centrifuge Eppendorf 5415R), and the supernatants (crude homogenates) were preserved for analyses.

The homogenates from tissue disruption were centrifuged at 600 x g for 3 min, and the supernatants were centrifuged at 6000 x g for 8 min at 3 °C. Resultant supernatants were used as crude enzyme source. The enzyme assay was done in the following reaction medium Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were determined by an end-point type assay and read at 430 nm. The reaction medium of both enzymes was: 222 mM alanine (ALT) or 44.4 mM aspartate (AST), 11.6 mM -ketoglutarate, 0.22 mM arsenate, 0.27 mM pyridoxal phosphate and 0.1M phosphate buffer pH 7.0 (Reitman and Frankel, 1957).

Histological analysis

The fragments of dorsal white skeletal muscle and liver were immersed in a buffered formaldehyde solution for 24 hours and then washed in alcohol 70%. After fixating the samples, fragments were dehydrated, diaphanized and included in paraffin. Then, cuts of 2-6 µm were obtained and stained with hematoxylin-eosin (HE) (skeletal muscle) or period acid of Schiff/hematoxylin (PAS-H) (liver samples). Microscopic analysis and material documentation were performed in a microscope Olympus BX41 (Tokyo, Japan).

In muscle samples, 200 muscular fibers were measured by animal, which were grouped in three diameter classes (<20, 20-50, and >50 µm), in order to evaluate the contribution of hyperplasia and hypertrophy to muscle growth (Almeida et al., 2008) in each treatment. In liver samples, we measured hepatocytes with pyknotic nuclei (%), hepatocyte area, area of the pyknotic nuclei and area of normal nuclei.

Statistical analysis

The used experimental design was entirely randomized with four treatments and five replicates, with eight animals being placed in each experimental unit. All data were submitted to a homogeneity and normality test (Levene) and to a variance analysis (ANOVA) at a 1% significance level and mean (standard deviation) were compared by the Tukey's test by software Statistic 7.1®.

3. Results

Growth performance and proximate composition of carcasses

Feeding frequency influenced both daily feed consumption and HI of fish ($p < 0.01$). The animals that received four

daily feedings consumed a larger amount of feed ($p < 0.01$) in comparison to one, two and three feeds per day. Fish that received feed on a daily basis presented higher HI ($p < 0.01$) in relation to those who consumed the diets in three distinct moments throughout the day (Table 1).

Table 1. Growth performance, somatic indexes and blood glucose of pacu juveniles (*Piaractus mesopotamicus*) submitted to different feeding frequencies.

Variables	Daily Feedings				P value
	One	Two	Three	Four	
WG (g) ¹	61.07±13.33	69.14±15.28	82.28±10.29	68.69±15.47	0.20
FCD (g) ²	98.38±1.72d	102.12±1.27c	107.78±2.13b	112.34±2.87a	<0.01
FCR ³	1.68±0.38	1.54±0.33	1.33±0.17	1.75±0.53	0.32
SGR (%.day ⁻¹) ⁴	0.91±0.15	1.02±0.19	1.14±0.16	1.01±0.27	0.36
PER (%) ⁵	1.94±0.44	2.11±0.48	2.38±0.29	1.91±0.55	0.36
SU (%) ⁶	91.43±12.78	82.85±15.64	97.14±6.39	91.42±7.82	0.29
WEF(g) ⁷	174.28±19.28	178.75±21.27	207.35±24.71	189.46±16.16	0.09
HI (%) ⁸	1.32±0.14a	1.22±0.03ab	1.09±0.13b	1.24±0.09ab	<0.01
VFI(%) ⁹	1.99±0.31	2.05±0.11	2.37±0.17	2.43±0.38	0.38

Values with different letters within the same line indicate statistical difference by the Tukey's test (1% probability). Variables: (WG)¹ - mean weight gain; (FCD)² - feed consumption daily; (FCR)³ - feed conversion rate; (SGR)⁴ - specific growth rate; (PER)⁵ - protein efficiency rate; (SU)⁶ - survival; (WEF)⁷ - eviscerated fish; (HI)⁸ - hepatosomatic index; (VFI)⁹ - viscerosomatic fat index; Mean ± standard deviation. Source: Authors (2021).

The parameters regarding the proximate composition of carcasses presented differences for ether extract of fish fed once a day ($p < 0.01$) (Table 2).

Table 2. Proximate composition of carcasses of pacu juveniles (*Piaractus mesopotamicus*) submitted to different feeding frequencies.

Variables	Daily Feedings				P value
	One	Two	Three	Four	
MS ¹	66.82±1.92	66.00±1.04	66.11 ±1.58	64.86 ±1.21	0.25
CP ²	18.17 ±0.86	19.05±1.60	18.51±0.84	18.52±1.08	0.68
EE ³	13.95±0.46a	12.40±0.85ab	11.66±0.22b	12.81±0.17ab	<0.01
MM ⁴	3.59±0.32	3.54±0.33	3.28±0.35	3.57±0.35	0.48

Values with different letters within the same line indicate statistical differences by the Tukey's test (1% probability). Variables: (MS)¹ - moisture; (CP)² - crude protein; (EE)³ - ether extract; (MM)⁴ - mineral matter. Mean ± standard deviation. Source: Authors (2021).

Fish that received two feedings a day presented higher glucose indexes in comparison to other treatments ($p < 0.01$). No significant differences were found regarding the enzymatic activity of the protein metabolism, ALT and AST in the animals' liver (Table 3).

Table 3. Plasma glucose and hepatic aminotransferases in pacu (*Piaractus mesopotamicus*) juveniles submitted to different feeding frequencies.

Variables	Daily Feedings				P value
	One	Two	Three	Four	
TGP-ALT(U/mg) ²	22.6±2.13	21.92±2.07	21.06±0.94	21.28±0.64	0.43
TGO-AST(U/mg) ³	17.14±1.62	16.22±1.63	16.12±1.75	16.17±1.34	0.70
GLU(mg dL ⁻¹) ¹	61.63±5.26b	117.18±42.36a	52.46±46.78b	65.18±8.35b	<0.01

Variables: (GLU) – Glucose plasmatic; (TGP-ALT)² – alanine aminotransferase; (TGO-AST)³ – aspartate aminotransferase. Mean ± standard deviation.

Source: Authors (2021).

Liver histopathology

Feeding frequencies promoted an increase in the size of both normal and pyknotic hepatocytes, as well as in the nuclei, and the percentage of normal and pyknotic cells ($p < 0.01$), when fish were fed twice or four times a day, as shown in Table 4. The percentage of normal cells was higher when fish were fed one or three times a day.

Table 4. Size of hepatocytes, normal and pyknotic nuclei, and percentage of normal and pyknotic cells in pacu juveniles (*Piaractus mesopotamicus*), submitted to different feeding frequencies.

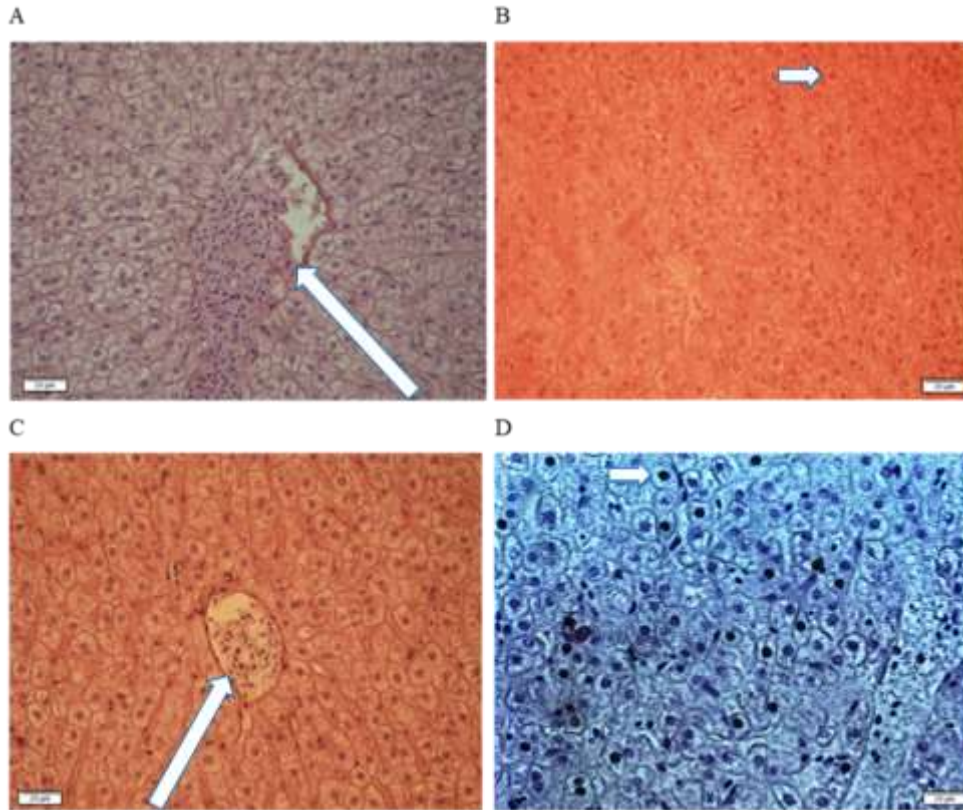
Variables (µm)	Daily Feeding				P value
	One	Two	Three	Four	
TH-N ¹	18.53±0.98c	22.31±1.73ab	19.90±0.93bc	23.77±2.03a	<0.01
TH-P ²	17.79±0.52b	22.19±1.44a	18.96±1.07b	23.88±2.38a	<0.01
TN-N ³	5.87±0.44b	7.00±0.42a	6.11±0.17b	7.23±0.30a	<0.01
TN-P ⁴	5.28±0.42b	6.68±0.50a	5.64±0.27b	6.68±0.51a	<0.01
PC-N ⁵	82.55±12.14a	42.05±16.71b	84.26±11.11a	42.02±11.76b	<0.01
PC-P ⁶	17.45±12.14b	57.95±16.71a	15.74±11.11b	57.98±11.76a	<0.01

Values with different letters within the same line indicate statistical difference by the Tukey's test at 1% probability. Variables: (TH-N)¹ - size of normal hepatocyte; (TH-P)² - size of pyknotic hepatocyte; (TN-N)³ - size of normal nuclei; (TN-P)⁴ - size of pyknotic nuclei; (PC-N)⁵ - percentage of normal cells; (PC-P)⁶ - percentage of pyknotic cells. Mean ± standard deviation.

Source: Authors (2021).

Concerning the evaluation of the animals' livers, regardless of the used feeding frequency, the tissue did not present any apparent lesion (Figure 1). The histological cuts demonstrated a cord distribution of hepatocytes, and the sinusoids were coated with endothelial cells (Figure 1A, C). Hepatocytes presented clear cytoplasm with central nuclei and round shape (Figure 1B) and in some regions, it was dislocated to the cell's periphery (Figure 1D).

Figure 1. Photomicrograph of hepatocytes of pacu juveniles submitted to different feeding frequencies. (A) fish fed once a day, demonstrating cord display of hepatocytes (line), PAS/H 40x; (B) fish fed twice a day, with centralized nuclei (white arrow) H/E 40x; (C) fish fed three times a day, with the conservation of the cordonal arrangement (line) H/E 40x; (D) fish fed four times a day, with a pyknotic nuclei (tip of white arrow), PAS/H 40x.



Source: Authors (2021).

Muscle histomorphometric

The distribution frequencies of skeletal muscle fibers of pacu did not present significant differences among feeding frequencies ($p > 0.01$), as shown in Table 5.

Table 5. Frequency of muscle fibers distribution in different diameter classes of pacu juveniles (*Piaractus mesopotamicus*), submitted to different feeding frequencies.

Distribution Frequencies	Daily Feedings				P value
	One	Two	Three	Four	
20 μm	5.83 \pm 3.41	6.51 \pm 3.98	4.43 \pm 2.44	4.52 \pm 2.00	0.53
20-50 μm	49.83 \pm 15.12	39.22 \pm 12.56	38.44 \pm 11.74	44.73 \pm 16.83	0.13
>50 μm	44.34 \pm 17.97	54.27 \pm 14.98	56.13 \pm 12.77	50.75 \pm 17.19	0.23

Values with distinct letters within the same line indicate significant differences by the Tukey's test at 1% probability. Mean \pm standard deviation.

Source: Authors (2021).

4. Discussion

Growth performance

Increasing feeding frequencies led to higher feed ingestion, although it did not interfere with the growth rate of fish. The lack of effects on growth might be related to feeding habits and metabolism, factors that may interfere the utilization of nutrients and consequently the nutritional status of fish (Sampaio et al., 2007). Souza et al. (2014) showed that distinct feeding frequencies did not affect productive performance and physiological variables of tambaqui juveniles (*Colossoma macropomum*), while Sousa et al. (2012) demonstrated similar results for Nile tilapia juveniles. However, the authors suggest that when higher fractionations of offered feeds were performed, tilapia juveniles displayed a better use of nutrients - which was reflected in its productive performance, thus highlighting that metabolic processes are related to intrinsic characteristics of a species' development.

According to Marques et al. (2008), lower feeding frequencies reduces the amounts of consumed feed, due to the limited storage capacity of the fish's digestive system, thus influencing its physiological processes of compensatory growth (Hornick et al., 2000), and in the maintenance of the body right after re-feeding, so that the animals reach its adequate growth (Cho et al., 2006). In several species as the goldfish (*Carassius auratus*), tilapia (*Oreochromis niloticus*), common snook (*Centropomus parallelus*) and pacu (*Piaractus mesopotamicus*), increased feeding frequencies leads to improved productive performance, especially due to the increase in feed consumption and use efficiency (Dieterich et al., 2013; Tsuzuki et al., 2014; Santos et al., 2014; Zhao et al., 2016).

Feed intake increased along with feeding frequency, being higher in fish fed four times a day, thus demonstrating that fish submitted to higher feed frequencies grew equally in comparison to other groups. It is noteworthy that lower feed frequencies lead to increased appetite, aggression and voracity in fishes (Qin et al., 1997), as the catabolic process that unleash the activation of hormones such as cortisol is related to stress in animals (Nelson and Cox, 2011). An optimal frequency may improve the intestinal digestive function of fish, leading to higher growth and utilization of dietetic components (Wu et al., 2018). High feed rates promoted an increase in gastric motility, hampering the digestive and absorption functions of nutrients (Volkoff et al., 2017).

Alterations in HI are expected to occur due to changes in the offer of feed to fish. Santos et al. (2015) reported that the dynamic of endogenous utilization of energy reflects in the use of lipids and products formed through cellular stock, in both absorption and use in metabolic demand. The possible alterations related to increased feeding frequencies suggest that fish use food as a priority in order to meet the energetic demands of body maintenance and reposition of catabolized tissues, which may still be directed to growth (Pouey et al., 2012; Ribeiro et al., 2012; Zhao et al., 2016). In this sense, the somatic index suggests the use of energetic reserves. Fish fed only once a day presented similar growth in comparison to other treatments, although the liver apparently had to act more in the maintenance and supply of tissues, as shown by the HI.

The deposition of body fat in fish was directly influenced by feeding strategies. With the provision of feed once a day, metabolic processes promoted a greater fat deposition, which in addition to being available for the maintenance and functional development may benefit meat composition by improving the quality of deposited polyunsaturated fatty acids, according to the offered diet (Li et al., 2015, Zhao et al., 2016). Throughout fasting periods, the reserves of protein, glycogen and lipids tend to be reduced (Silveira, Logato and Pontes, 2009; Calvo et al., 2017).

Glycemic activity and hepatic aminotransferases

The maintenance of glucose levels was observed when fish were fed once, three or four times a day, which reinforces increased glycaemia. Throughout fasting periods, the maintenance of glycaemia is directly related to the capacity of mobilization of hepatic glycogen (glycogenolysis) - at least during the early fasting stages, and also depends of the later

activation of hepatic gluconeogenesis (Souza et al., 2014) and of the reduction in the utilization rate of glucose (Moon and Foster, 1995). Although the hepatic levels of glycogen were not assessed in this study, the use of glycogen reserves for the maintenance of glycaemia probably did not occur, seen that the maintenance of the hepatosomatic index was observed.

The plasma glucose of reared fish may suffer alterations according to the adopted dietary management, due to the dynamics of the animal's energetic reserves (Souza et al., 2014). Fish have a limited capacity to metabolize glucose, i.e. remove this molecule from the plasmatic compartment, thus resulting in possible hyperglycemia that may remain for hours (Ribeiro et al., 2012) due to the increase in hepatic glycogenesis and lipogenesis (Seixas-Filho, 2004).

Several studies demonstrated that teleost fish are capable of tolerating periods of unfavorable feeding conditions by activating behavioral changes and biochemical, physiological and structural adaptive responses (Ashouri et al., 2019; Mozanzadeh et al., 2015). Fish have the ability of reducing their energy costs in fasting periods and reusing proteins (Salem et al., 2007). Plasmatic glucose levels is a routine index to determine nutritional status (Pottinger et al., 2003) and nutritional stress (Furné et al., 2012; Pérez-Jiménez et al., 2012). It is noteworthy that the maintenance of this metabolite during fasting has been reported for other fish species (Ashouri et al., 2019; Bermejo-Poza et al., 2019; Mozanzadeh et al., 2015; Yarmohammadi et al., 2012), which reveals the capacity and plasticity of some fish species in the face of short-term fasting. In general, serum glucose are maintained by glycogenolysis (the break of glycogen in the liver by gluconeogenesis), generating glucose from lactate, amino acids and glycerol (Favero et al., 2019; Ashouri et al., 2019).

In this study, no differences were found in the liver enzyme content of the animals (AST and ALT), which did not indicate damage in hepatocytes, seen that these enzymes are produced in these cells and its increased concentration is associated with acute hepatic lesions. The enzyme AST is found mainly in mitochondria, while ALT is a hepatic cytosolic enzyme (Nelson and Cox, 2011). As transamination enzymes did not display significant differences, it could be inferred that no protein catabolism occurred. Studies of severe restrictions reported increased activity of these enzymes, suggesting higher gluconeogenesis from substrates such as amino acids and lactate during fasting periods (Favero et al., 2019; Ashouri et al., 2019), with prominent nutritional stress states (Furné et al., 2012; Pérez-Jiménez et al., 2012). However, when fish were fed once again, serum levels returned to normal. In this study, fasting was not severe in order to mobilize other molecules to supply the energy demand, with circulating glucose coming from feed.

Similar levels of AST and ALT activity indicate metabolic stability in the liver tissue, regardless of the longer period of catabolism in fish that were fed less meals a day (Thrall et al., 2015; Menga et al., 2018). Based on the findings of this study, splitting feeding portions from up to four feedings per day does not alter the protein metabolism.

Liver and muscle morphology

Due to the central role in fish metabolism, the liver is responsible for metabolic dynamics. Initial hepatic alterations during the digestion process mainly affect the structural and functional characteristics of hepatocytes (Carter and Houlihan, 2001; Fujimoto et al., 2016).

The observed morphological characteristics in the liver of pacu are in accordance with the performed descriptions for other species of Neotropical fish (Fujimoto, Cruz and Moraes, 2008). No alterations were observed regarding organ overload, which may occur due to excessive protein levels in the diet and adaptations to nutrients and additives (Honorato et al., 2013).

When fish were fed twice or four times a day, an increase in the number of hepatocytes with pyknotic nuclei was observed. Variations in the size of hepatocytes and its nucleus in response to changes in feed supply was evidenced by Ostaszewska et al. (2005) in *P. mesopotamicus* submitted to diets containing different sources of protein. According to Segner and Braunbeck (1988), the size of the hepatocyte's nucleus is directly related to the metabolic activity of the liver cells. In addition, protein synthesis rates may be shifted, leading to increases or reductions of its activity, possibly due to variations in

diet composition and quantity of provided nutrients in feed (Carter and Houlihan, 2001; Fujimoto et al., 2016).

The growth of skeletal muscle fibers is influenced by several external factors, such as nutrition (Koumans and Akster, 1995). The fact that there were no differences between the distributions in growth classes indicates that the feed contained available nutrients (e.g. amino acids), thus the fish maintained their anabolic rates and were able to synthesize proteins and form muscle cells (Brown and Cameron, 1991), a fact that can also be reinforced by the protein efficiency rate, which did not present significant differences among feeding frequencies.

The frequency of muscle fiber distribution indicates that the fish had hypertrophic growth, as a great share of the fibers were 20-50 or above 50 μm , which was also verified by Almeida et al. (2010) for the same species. A small frequency of fibers lower than 20 μm was noted in all sampled animals, showing once again that the animals continued to recruit new fibers for muscle growth, which is very important for this species, which has a great economic importance. Almeida et al. (2008) suggested that there is a strong association between MyoD and myogenin (factors that regulates muscle growth) and the growth stages of pacu and related them to hyperplastic and hypertrophic growth.

5. Conclusion

Different feeding frequencies did not compromise the growth of pacu (*P. mesopotamicus*). Thus, feed supply into four feeding events is recommended for the development of this species at this life stage.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

- Almeida, F. L. A., Carvalho, R. F., Pinhal, D., Padovani, C. R., Martins, C., & Dal Pai-Silva, E. M. (2008). Differential expression of myogenic regulatory factor MyoD in pacu skeletal muscle (*Piaractus mesopotamicus* Holmberg 1887: Serrasalminae, Characidae, Teleostei) during juvenile and adult growth phases. *Micron*, 39, 1306-1311. <https://dx.doi.org/10.1016/j.micron.2008.02.011>
- Almeida, F. L. A., Pessotti, N. S., Pinhal, D., Padovani, C. R., Leitão, N. J., Carvalho, R. F., Martins, C., Portella, M. C., & Pai Silva, M. D. (2010). Quantitative expression of myogenic regulatory factors MyoD and myogenin in pacu (*Piaractus mesopotamicus*) skeletal muscle during growth. *Micron*, 41, 997-1004.
- Ashouri, G., Mahboobi-Soofiani, N., Hoseinifar, S. H., Torfi-Mozanzadeh, M., Mani, A., & Khosravi, A., Carnevali, O. (2019). Compensatory growth, plasma hormones and metabolites in juvenile *Siberian sturgeon* (*Acipenser baerii*, Brandt 1869) subjected to fasting and re-feeding. *Aquaculture Nutrition*, 00, 1–10. <https://dx.doi.org/10.1111/anu.13002>
- AOAC (2005). Official methods of Analysis of Official Analytical Chemists international (18a ed.). Arlington, VA: Association of Official Analytical Chemists
- Bermejo-Poza, R., Villarroel, M., Pérez, C., Chavarri, E. G., Díaz, M. T., Torrent, F., & Fuente, J. (2019). Fasting combined with long catch duration modifies the physio-metabolic response and flesh quality of rainbow trout. *Aquaculture Research*, 51, 1244-1255. <https://dx.doi.org/10.1111/are.14475>
- Bittencourt, F., Souza, B. E., Neu, D. H., Rorato, R. R., Boscolo, W. R., & Feiden, A. (2013). Eugenol e benzocaína como anestésico para juvenis de *Cyprinus carpio* Linnaeus, 1758 (Carpa comum). *Revista Brasileira de Ciências Agrárias*, 8, 163-167. <https://dx.doi.org/10.5039/agraria.v8i1a2225>
- Bittencourt, F., Souza, B. E., Lui, T., Borella, M. I., Boscolo, W. R., Feiden, A., & Romagosa, E. (2012). Protein diets promote the maturation of oocytes and spawning of *Piaractus mesopotamicus* kept in cages. *Journal of Applied Ichthyology*, 28, 886-893.
- Brown, C. R., & Cameron, J. N. (1991). The relationship between specific dynamic action (SDA) and protein synthesis rates in the Channel catfish. *Physiological and Biochemical Zoology*, 64, 298–309.
- Burkert, D., Andrade, D. R., Sirol, R. N., Salaro, A.L., Rasguido, J. E. A., & Quirino, C. R. (2008). Rendimento do processamento e composição química de filés de surubim cultivado em tanques-rede. *Revista Brasileira de Zootecnia*, 37, 1137-1143. <http://dx.doi.org/10.1590/S1516-35982008000700001>
- Calcagnotto, D., & DeSalle, R. (2009). Population genetic structuring in pacu (*Piaractus mesopotamicus*) across the Paraná-Paraguay basin: evidence from microsatellites. *Neotropical Ichthyology*, 7: 607-616. <https://dx.doi.org/10.1590/S1679-62252009000400008>

- Calvo, N. S., Stumpf, L., Cortés-Jacinto, E., Castillo Díaz, F., & López Greco, L. S. (2017). Mobilization of energetic reserves during starvation in juveniles of different size of the redclaw crayfish *Cherax quadricarinatus*. *Aquaculture Nutrition*, 24, 952–960. <https://dx.doi.org/10.1111/anu.12631>
- Carneiro, P. C. F., & Mikos, J. D. (2005). Frequência alimentar e crescimento de alevinos de jundiá, *Rhamdia quelen*. *Ciência Rural*, 35, 187-191. <http://dx.doi.org/10.1590/S0103-84782005000100030>
- Carter, C. G., Houlihan, D. F. (2001). Protein synthesis. In: Wright, PA, Anderson, PM (Ed.). Nitrogen excretion. New York: Academic, Fish physiology, 20.
- Cho, S. H., Lim, Y. S., Lee, J. H., Lee, J. K., & Park, S. (2003). Effects of feeding rate and feeding frequency on survival, growth, and body composition of ayu post-larvae *Plecoglossus altivelis*. *Journal of the World Aquaculture Society*, 34, 85-91. <https://dx.doi.org/10.1111/j.1749-7345.2003.tb00042.x>
- Cho, S. H., Lee, S., Park, B. H., Ji, S., Lee, J., Bae, J., & Oh, S. Y. (2006). Compensatory growth of juvenile olive flounder, *Paralichthys olivaceus* L., and changes in proximate composition and body condition indexes during fasting and after refeeding in summer season. *Journal of the World Aquaculture Society*, 37, 169- 174. <https://dx.doi.org/10.1111/j.1749-7345.2006.00023.x>
- Daudpota, A. M., Abbas, G., Kalhor, I. B., Shah, S. S. A., Kalhor, H., Hafeez-ur-Rehman, M., & Ghaffar, A. (2016). Effect of feeding frequency on growth performance, feed utilization and body composition of juvenile Nile tilapia, *Oreochromis niloticus* (L.) reared in low salinity water. *Pakistan Journal of Zoology*, 48, 171-177.
- Dieterich, T. G., Potrich, F. R., Lorenz, E. K., Signor, A. A., Feiden, A., & Boscolo, W. R. (2013). Performance parameters of juvenile pacu fed at different feeding frequencies in net cages. *Pesquisa Agropecuária Brasileira*, 48, 1043–1048. <http://dx.doi.org/10.1590/S0100-204X2013000800033>
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956.) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350-356. <https://dx.doi.org/10.1021/ac60111a017>
- Favero, G. C., Boaventura, T. P., Ferreira, A. L., Silva, A. C. F., Porto, L. A., & Luz, R. K. (2019). Fasting/re-feeding and water temperature promote the mobilization of body reserves in juvenile freshwater carnivorous catfish *Lophiosilurus alexandri*. *Aquaculture*, 734223, 1-6. <https://dx.doi.org/10.1016/j.aquaculture.2019.734223>
- Frasca-Scorvo, C. M. D., Losekann, E., Queiroz, J. F., Scorvo-Filho, J. D., & Turco, P. H. N. (2017). Avaliação da frequência alimentar no desempenho de tilápia em uma represa rural. -- Jaguariúna: Embrapa Meio Ambiente, Boletim de Pesquisa e Desenvolvimento / Embrapa Meio Ambiente, ISSN 1516-4675, 75.
- Furné, M., Morales, A. E., Trenzado, C. E., García-Gallego, M., Hidalgo, M. C., Domezain, A., & Rus, A. S. (2012). The metabolic effects of prolonged starvation and re-feeding in sturgeon and rainbow trout. *Journal of Comparative Physiology B*, 182, 63–76. <https://dx.doi.org/10.1007/s00360-011-0596-9>
- Fujimoto, R. Y., Cruz C., & Moraes, F. R. (2008). Análise de efluente e histologia da pele, fígado e rim de pacus (*Piaractus mesopotamicus*) suplementados com cromo trivalente. *Boletim do Instituto de Pesca*, 34, 117-124.
- Fujimoto, R., Santos, F. B., Dias, H. M., Ramos, F. M., Silva, D. J. F., & Honorato, C. A. (2016). Feeding frequency on the production viability of production and quantitative descriptors of parasitism in angelfish. *Ciência Rural*, 46, 304-309. <https://dx.doi.org/10.1590/0103-8478cr20141704>
- Hayashi, C., Meurer, F., Boscolo, W. R., Lacerda, C. H. F., & Kavata, L. C. B. (2004). Frequência de arraçoamento para alevinos de lambari do rabo-amarelo (*Astyanax bimaculatus*). *Revista Brasileira de Zootecnia*, 33, 21–26. <https://dx.doi.org/10.1590/s1516-35982004000100004>
- Hornick, J. L., Van Eenaeme, C., Gérard, O., Dufrasne, I., & Istasse, L. (2000). Mechanisms of reduced and compensatory growth. *Domestic Animal Endocrinology*, 19, 121-32. [https://dx.doi.org/10.1016/S0739-7240\(00\)00072-2](https://dx.doi.org/10.1016/S0739-7240(00)00072-2)
- Honorato, C. A., Assano, M., Cruz, C., Carneiro, D. J., & Machado, M. R. F. (2013). Histologia do intestino de tilápia do Nilo alimentados com dietas contendo diferentes fontes de proteína. *Nucleus Animalium*, 5, 85-92. <https://dx.doi.org/10.3738/1982.2278.771>
- IBGE- Instituto brasileiro de geografia e estatística (2016). Pesquisa pecuária municipal.
- Koumans, J. T. M., & Akster, H. A. (1995). Myogenic cells in development and growth of fish. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 110, 3-20.
- Li, P. Y., Wang, J. Y., Song, Z. D., Zhang, L. M., Zhang, H., Li, X. X., & Pan, Q. (2015). Evaluation of soy protein concentrate as a substitute for fishmeal in diets for juvenile starry flounder (*Platichthys stellatus*). *Aquaculture*, 448, 578–585. <https://dx.doi.org/10.1016/j.aquaculture.2015.05.049>
- Marques, N. R., Hayashi, C., Galdioli, E. M., Soares, T., & Fernandes, C. E. B. (2008). Frequência de alimentação diária para alevinos de carpa-capim (*Ctenopharyngodon idella*, V.). *Boletim do Instituto de Pesca*, 34, 311 – 317.
- Menga, X. L., Shuai, L., Chao-Bin, Q., Zhen-Xiang, Z., Wen-Pan, H., Li-Ping, Y., Rong-Hua, L., Wen-Jun, L., & Guo-Xing, N. (2018). Intestinal microbiota and lipid metabolism responses in the common carp (*Cyprinus carpio* L.) following copper exposure. *Ecotoxicology and Environmental Safety*, 160, 257–264. <https://dx.doi.org/10.1016/j.ecoenv.2018.05.050>
- Moon, T. W., & Foster, G. D. (1995). Tissue carbohydrate metabolism, gluconeogenesis and hormonal and environmental influences. In: Hochachka, P.W., Mommsen, P. (eds). *Metabolic Biochemistry. Biochemistry and Molecular Biology of Fishes*, 4, 65-100. [https://dx.doi.org/10.1016/S1873-0140\(06\)80007-X](https://dx.doi.org/10.1016/S1873-0140(06)80007-X)
- Mourad, N. M. N., Costa, A. C., Freitas, R. T. F., Serafini, M. A., Reis Neto, R. V., & Felizardo, V. O. (2018). Weight and morphometric growth of Pacu (*Piaractus mesopotamicus*), Tambaqui (*Colossoma macropomum*) and their hybrids from spring to winter. *Pesquisa Veterinária Brasileira*, 38, 544-550. <http://dx.doi.org/10.1590/1678-5150-pvb-4808>
- Mozanzadeh, M. T., Yaghoubi, M., Yavari, V., Agh, N., Marammazi, G. J., & Popovic, N. T. (2015). Somatic and physiological responses to cyclic fasting and re-feeding periods in sobaity sea bream (*Sparidentex hasta*, Valenciennes 1830). *Aquaculture Nutrition*, 23, 181 - 191. <https://dx.doi.org/10.1111/anu.12379>

- Nelson, D. L., & Cox, M. M. (2011). *Princípios de bioquímica de Lehninger*. (6ª ed.), Editora Artmed, Porto Alegre, RS, NRC. National Academies Press. Washington D.C., USA.
- NRC (2011). *Nutrient requirements of fish and shrimp*. National Academies Press.
- Ostaszewska, T., Dabrowski, K., Czumińska, K., Olech, W., & Olejniczak, M. (2005). Rearing of pike-perch larvae using formulated diets-first success with starter feeds. *Aquaculture Research*, 36, 1167-1176. <https://dx.doi.org/10.1111/j.1365-2109.2005.01332.x>
- Pérez-Jiménez, A., Cardenete, G., Hidalgo, M. C., García-Alcázar, A., Abellán, E., & Morales, A. E. (2012). Metabolic adjustments of *Dentex dentex* to prolonged starvation and refeeding. *Fish Physiology and Biochemistry*, 38, 1145-1157. <https://dx.doi.org/10.1007/s10695-011-9600-2>
- Pottinger, T., Rand-Weaver, M., & Sumpter, J. (2003). Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 136, 403-417. [https://dx.doi.org/10.1016/s1096-4959\(03\)00212-4](https://dx.doi.org/10.1016/s1096-4959(03)00212-4)
- Pouey, J. L. O. F., Rocha, C. B., Tavares, R. A., Portelina, M. K., & Piedras, S. R. N. (2012). Frequência alimentar no crescimento de alevinos de peixe-rei *Odontesthes humensis*. *Semina*, 33, 2423-2428. <https://dx.doi.org/10.5433/1679-0359.2012v33n6p2423>
- Qin, J., Fast, A. W., DeAnda, D., & Weidenbach, R. P. (1997). Growth and survival of larval snakehead (*Channa striatus*) fed different diets. *Aquaculture*, 148, 105-113. [https://dx.doi.org/10.1016/S0044-8486\(96\)01378-6](https://dx.doi.org/10.1016/S0044-8486(96)01378-6)
- Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28, 56-63. <https://dx.doi.org/10.1093/ajcp/28.1.56>
- Ribeiro, F. A. Z., Vasquez, L. A., Fernandes, J. B. K., & Sakomura, N. K. (2012). Feeding level and frequency for freshwater angelfish. *Revista Brasileira de Zootecnia*, 41, 1550-1554. <https://dx.doi.org/10.1590/S1516-35982012000600033>
- Rodrigues, A. T., Bergamin, G. T., & Mansano, C. F. M. (2018). Desempenho de juvenis de tilápia-do-Nilo (*Oreochromis niloticus*) e pacu (*Piaractus mesopotamicus*) em sistema de recirculação de água. XIV Fórum Ambiental Alta Paulista, 857- 871. ISBN: 978-85-68242-76-6.
- Ruohonen, K., Kettunen, J., & King, J. (2001). Experimental design in feeding experiments. In: *Food Intake in Fish* (Houlihan, D, Boujard, T, Jobling, M eds), 88-107. Blackwell Science, Oxford.
- Salem, M., Silverstein, J., Rexroad, C.E., & Yao, J. (2007). Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). *BMC Genomics*, 8, 328-343.
- Sampaio L. A., Oliveira M., & Tesser M. B. (2007). Produção de larvas e juvenis do Peixe-rei marinho *Odontesthes argentinensis* submetidos à diferentes frequências alimentares. *Revista Brasileira de Agroecologia*, 13, 271-274.
- Santos, E. L., Santos, I. V. V. S., Lira, R. C., Silva, C. F., Moura, S. C. S., Ferreira, A. J. S., & Silva, R. M. (2014). Frequência de arraçamento para alevinos de Tilápia do Nilo (*Oreochromis niloticus*). *Revista Agropecuária técnica*, 35, 171-177.
- Santos, M. M., Calumby, J. A., Coelho Filho, P. A., Soares, E. C., & Gentelini, A. L. (2015). Nível de arraçamento e frequência alimentar no desempenho de alevinos de tilápia-do-Nilo. *Boletim do Instituto de Pesca*, 41, 387-395.
- Segner, H., & Braunbeck, T. (1988). Hepatocellular adaption to extreme nutritional conditions in ide, *Leuciscus idus melanotus* L. (Cyprinidae). A morphofunctional analysis. *Fish Physiology Biochemistry*, 5, 79-97. <https://dx.doi.org/10.1007/BF01875645>
- Seixas Filho, J.T. (2004). Uma revisão sobre o papel do carboidrato e da proteína no metabolismo de peixes com hábitos alimentar carnívoro e onívoro. *Augustus* 09, 32-51.
- Silveira, U. S., Logato, P. V. R., & Pontes, E. C. (2009). Utilização e metabolismo dos carboidratos em peixes. *Revista Eletrônica Nutritime*, 6, 817-836.
- Sipauba-Tavares, L. H., & Santeiro, R. M. (2013). Fish farm and water quality management. *Acta Scientiarum. Biological Sciences*, 35, 21-27. <https://dx.doi.org/10.4025/actasciobiolsoci.v35i1.10086>
- Signor, A. A., Feiden, A., Bittencourt, F., Potrich, F. R., Deparis, A., & Boscolo, W. R. (2011). Fósforo na alimentação de pacu (*Piaractus mesopotamicus*). *Revista Brasileira Zootecnia*, 40, 2646-2650. <https://dx.doi.org/10.1590/S1516-35982011001200005>
- Sousa, R. M. R., Agostinho, C. A., Oliveira, F. A., Argentim, D., Novelli, P. K., & Agostinho, S. M. M. (2012). Productive performance of Nile tilapia (*Oreochromis niloticus*) fed at different frequencies and periods with automatic dispenser. *Arquivos Brasileiro de Medicina Veterinária e Zootecnia*, 64: 192-197. <http://dx.doi.org/10.1590/S0102-09352012000100027>
- Souza, R. C., Campeche, D. F. B., Campos, R. M. L., Figueiredo, R. A. C. R., & Melo, J. F. B. (2014). Frequência de alimentação para juvenis de tambaqui. *Arquivos Brasileiro de Medicina Veterinária e Zootecnia*, 66: 927-932. <http://dx.doi.org/10.1590/1678-41625557>
- Statsoft, INC. (2005). *Statistica (data analysis software system)*, version 7.1.
- Tsuzuki, M. Y., Guarizi, J. D., Annuniação, W. F., & Sorandra, C. (2014). Frequência alimentar e densidade de estocagem para juvenis de robalo-peva em tanques-rede. *Boletim do Instituto de Pesca*, 40: 629 - 637.
- Thrall, M. A., Weiser, G., Allison, R. W., & Campbell, T. W. (2015). *Hematologia e bioquímica clínica veterinária* (2ª ed.), Editora Guanabara Koogan
- Urbinati, E. C., & Gonçalves, F. D. (2005). Pacu (*Piaractus mesopotamicus*), In: *Baldisseroto, B, Gomes, LC Espécies nativas para a piscicultura no Brasil*, editora UFSM, Santa Maria, Rio Grande do Sul, Brasil.

Vaz, M. M., Torquato, V. C., & Barbosa, N. D. C. (2000). Guia ilustrado de Peixes da Bacia do Rio Grande.

Volkoff, H., Sabioni, R. E., Coutinho, L. L., & Cyrino, J. E. P. (2017). Appetite regulating factors in pacu (*Piaractus mesopotamicus*): Tissue distribution and effects of food quantity and quality on gene expression. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 203: 241–254. <https://dx.doi.org/10.1016/j.cbpa.2016.09.022>

Wu, Y., Ren, X., Zhou, J., Lin, H., Wang, X., & Ma, H. (2018). Feed intake, growth, feed utilization, body composition and waste output of juvenile hybrid bream at different feeding frequencies. *Aquaculture Nutrition*, 1–6. <https://dx.doi.org/10.1111/anu.12852>

Yarmohammadi, M., Shabani, A., Pourkazemi, M., Soltanloo, H., & Imanpour, M. (2012). Effect of starvation and re-feeding on growth performance and content of plasma lipids, glucose and insulin in cultured juvenile Persian sturgeon (*Acipenser persicus* Borodin, 1897). *Journal of Applied Ichthyology*, 28: 692–696. <https://dx.doi.org/10.1111/j.1439-0426.2012.01969.x>

Zhao, S., Han, D., Zhu, X., Jin, J., Yang, Y., & Xie, S. (2016). Effects of feed-ing frequency and dietary protein levels on juvenile allogynogenetic gibel carp (*Carassius auratus gibelio*) var. CAS III: Growth, feed utilization and serum free essential amino acids dynamics. *Aquaculture Research*, 47: 290–303. <https://dx.doi.org/10.1111/are.12491>