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Apparent digestibility of juveniles of matrinxã *Brycon amazonicus* fed diets with different protein levels

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ABSTRACT. The objective of the present study was to determine the apparent digestibility of juveniles of matrinxã *Brycon amazonicus* fed diets with different levels of protein. The experimental design was completely randomized, where the treatments consisted of four levels of protein in the diets (28, 32, 36 and 40%) and five replicates (water boxes) with 10 juveniles of matrinxã each. Diets were offered four times a day, and feces were collected twice a week, along 28 days, for digestibility analysis. Data collected were subjected to polynomial regression at 5% significance. Differences ($p < 0.05$) were observed in dry matter digestibility, with higher digestibility according to increasing levels of protein in the diets. This behavior was directly expressed in the digestibility of nutrients by juveniles of matrinxã, such as % ash, % crude protein, apparent digestible energy, % digestibility of apparent digestible energy and energy: protein ratio. However, this was not observed ($p > 0.05$) in the digestibility of % ether extract. Digestibility of N, P, Ca, Mg, Cu and Zn presented linear positive ($p < 0.05$) behavior, in which the use of these minerals increased with the increase in the level of protein in the diets. However, the digestibility of K, Fe and Mn reduced ($p < 0.05$) with increasing protein levels in the diets. Thus, it can be concluded that the rise in protein level in diets for juveniles of matrinxã improves nutrient digestibility. The results of this study indicated that 40% crude protein in the diets provided better results.

Keywords: *Brycon amazonicus*; dry matter; enzymes; protein efficiency.

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Introduction

Matrinxã (*Brycon amazonicus*) is a fish species native to neotropical freshwaters of the Amazon and Tocantins-Araguaia river basins (Howes, 1982), characterized by its omnivorous feeding habit, with a tendency to carnivory, and a high commercial profitability (Gadelha & Araújo, 2013). According to Gadelha and Araújo (2013), matrinxã in housing system accept pelleted feed, grains, fruits and agricultural by-products, showing fast growth.

This species ranks as the second most cultivated in the State of Amazonas, Brazil, being found in 14% of fish farms with excavated ponds, and in 68% of fish farms that use igarapé channels (Lima et al., 2015; Estevão-Rodrigues, Lima, & Estevão-Rodrigues, 2017). Izel, Pereira-Filho, Melo, and Macêdo (2004) also affirms that the increased demand for matrinxã production in controlled environments is due to its fast adaptation to housing system, great acceptance of commercial diets and high commercial value. However, the food cost for this species can considerably raise its market price, accounting for up to 90% production final cost and increasing up to 50% market value (Lima et al., 2015; Barbosa & Lima, 2016).

These oscillations are mainly due to the high protein requirement of this species regarding its feeding habit. The knowledge of the digestibility of crude protein in the composition of the diets is very important, because this nutrient is directly related to the energy and protein metabolism, besides the own performance (Abimorad & Castellani, 2011; Boscolo, Signor, Freitas, Bittencourt, & Feiden, 2011).

In addition, the correct requirement of crude protein in the diets allows to provide an ideal metabolic balance to the fishes (Souza et al., 2013). Considering the above, the present study aimed to determine the apparent digestibility of diets with different levels of protein to juveniles of matrinxã.

Material and methods

The experiment was carried out in the Laboratory of Aquatic Organisms Nutrition (Nilton Lins University), Manaus, State of Amazonas, Brazil, from January to March 2017, in a completely randomized design. Animal care procedures followed guidelines established by the Ethics Committee on Animal Research of the Nilton Lins University (Protocol n. 009/2017, Ceua/UNL).

The experimental design was completely randomized, where the treatments consisted of four levels of protein in the diets (28, 32, 36 and 40%) and five replicates (water boxes) with 10 juveniles of matrinxã each, totaling 200 experimental units (fishes). Fish were obtained in a commercial hatchery with 51.4 ± 1.1 g initial weight and 22.1 ± 0.8 cm initial length.

These were transported to Nilton Lins University in 80 L plastic bags supplied with water and oxygen, being acclimatized in polyethylene circular water boxes with 300 L for one week, feeding a commercial diet with 28% crude protein. Then, fish were moved to polyethylene circular water boxes with 200 L according to experimental treatments, for feces collection. Before the onset of the experimental period, animals were subjected to an adaptation period to diets and facilities of one week.

Diets were produced based on formulations specifically developed for matrinxã (Izel et al., 2004). The feeds contained similar nitrogen and energy levels, but each one contained different levels of crude protein (28, 32, 36 and 40%; Table 1). To produce the feed, the mixture was homogenized, being added 10% of water, and pelletized using an industrial grinder. Pellets of 3 to 4 mm were dried in a forced circulation oven (45°C for 24 hour). Feed was stored at -20°C in hermetic containers and portions used in daily feeding were weighed immediately before use and kept in coolers. All diets were added with 0.5% chromic oxide (Cr_2O_3).

Table 1. Formulation and calculated composition (%) of diets for juveniles of matrinxã with different levels of protein.

Ingredients	Protein levels (%)			
	28	32	36	40
Corn starch	30.1787	21.1372	12.0958	3.0543
Soybean meal (45%)	29.1454	39.0063	48.8673	58.7283
Wheat meal (16%)	10.0000	10.0000	10.0000	10.0000
Fish meal (55%)	16.1759	15.3564	14.5369	13.7174
Meat and bone meal (45%)	10.0000	10.0000	10.0000	10.0000
Soybean oil	2.0000	2.0000	2.0000	2.0000
Dicalcium phosphate	2.0000	2.0000	2.0000	2.0000
Vit. min. supplement ¹	0.2500	0.2500	0.2500	0.2500
Min. supplement ²	0.2500	0.2500	0.2500	0.2500
Total	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels			
	28	32	36	40
D.E., kcal kg ⁻¹	2,716.85	2,740.87	2,764.88	2,788.89
Crude protein, %	28.000	32.0000	36.0000	40.0000
Lysine, %	1.7032	1.9550	2.2069	2.4588
Tryptophan, %	0.3064	0.3689	0.4314	0.4938
Threonine, %	1.0732	1.2289	1.3846	1.5403
Fat, %	5.1400	5.1800	5.2500	5.3200
Ash, %	10.8327	11.2573	11.6820	12.1066
Crude Fiber, %	3.0287	3.6639	4.2991	4.9344
Sodium, %	0.2109	0.2143	0.2177	0.2211

¹Vitamin mix (Nutreco) per kg product: vitamin A, 5,000,000 UI; vitamin D3, 1,600,000 UI; vitamin E, 60,000 UI; vitamin K3, 6,000 mg; thiamine (B1), 10,000 mg; riboflavin (B2), 10,000 mg; niacin, 60,000 mg; pyridoxine, 10,000 mg; pantothenic acid, 20,000 mg; biotin, 300 mg; folic acid, 3,000 mg; cobalamin, 12,000 µg; inositol, 40,000 mg. Ascorbic acid (vitamin C), 350,000 mg.

²Mineral mix (Nutreco) per kg product: Mg, 25,000 mg; Fe, 35,000 mg; Cu, 20,000 mg; Zn, 80,000 mg; I, 1,000 mg; Mn, 50,000 mg; Se, 200 mg; and Co, 480 mg.

The diets were offered four times a day (9:00 a.m., 12:00 a.m., 3:00 p.m and 6:00 p.m.); feces were collected twice a week, along 28 days. The collected samples (diets and feces) were stored in Petri dishes at -20°C during the experimental period, thawed at room temperature at the end of the experimental period, centrifuged at 2.296 g x 2 min., weighed and dried in a forced air circulation oven at 55°C for 24 hours. After drying, samples were analyzed in the Central Laboratory of Nilton Lins University, according to Association of Official Analytical Chemists (AOAC, 1999), to determine % Dry Matter (DM), % Crude Protein (CP), % Ether Extract (EE), % Ash (AS), % nitrogen (N) and mineral profile (% phosphorus [P], % potassium [K], calcium [Ca], magnesium [Mg], cooper [Cu], iron [Fe], manganese [Mn], and zinc [Zn]). The coefficients of apparent digestibility were calculated by the equation of Cho, Cowey, and Watanabe (1985):

$$\text{CDA (\%)} = 100 - \left[100 \times \left(\frac{\% \text{Id}}{\% \text{If}} \times \frac{\% \text{Nf}}{\% \text{Nd}} \right) \right],$$

where: CDA = coefficient of apparent digestibility; Id = chromium concentration in the diet; If = chromium concentration in the feces; Nd = nutrient in the diet; Nf = nutrient in the feces.

Gross energy was estimated based on the calculated energy values for protein = 5.64, ether extract = 9.44 and non-nitrogen extract = 4.11 kcal kg⁻¹, considering the breakdown for digestible energy (National Research Council [NRC], 2011) to calculate the apparent digestible energy values, the digestibility of apparent digestible energy (percentage of utilization), and energy: protein ratio. Statistical analysis was performed using the software Statistical Analysis System (SAS, 2008), and estimates of treatments were subjected to polynomial regression at 5% significance.

Results and discussion

Results of apparent digestibility of macronutrients are listed in Table 2. Differences ($p < 0.05$) were detected in dry matter digestibility ($y = 4.273x + 56.62$ $R^2 = 0.98$), with higher values of digestibility with increasing levels of protein in the diets. This increased digestibility of dry matter is directly expressed in the digestibility of nutrients by juveniles of matrinxã, such as % ash ($y = 3.874x + 41.93$ $R^2 = 0.99$) and % crude protein ($y = 5.597x + 50.855$ $R^2 = 0.75$). However, this behavior was not observed ($p > 0.05$) in the ether extract digestibility. From these, fish fed diets containing higher level of crude protein (40%) showed better digestibility of dry matter, mineral matter and crude protein.

Table 2. Apparent digestibility of macronutrients for diets containing different protein levels to juveniles of matrinxã.

Coefficients (%)	Protein levels (%)				p-value	Effect	CV (%)
	28	32	36	40			
Dry matter	61.61	64.05	69.52	74.03	0.03	LP	11.32
Mineral matter	45.88	49.51	53.66	57.41	0.02	LP	13.13
Ether extract	50.60	50.00	52.01	50.29	0.06	ns	2.87
Crude protein	58.68	61.43	62.20	77.08	0.03	LP	2.63

CV - Coefficient of variation. p-value - Coefficient of probability. LP - Linear positive. ns - non-significant.

The digestibility parameters of macronutrients showed that the increase in protein level in the diets raised the digestibility and use of nutrients by the metabolism of matrinxã juveniles. These results indicate that increased digestibility of dry matter by the organism of juveniles of matrinxã directly influence the digestibility of other nutrients. Thus, considering the cost-benefit relation, a better use of nutrients by the fish organism provide a better development in controlled environment (Izel et al., 2004; Arbeláez-Rojas, Inoue, & Moraes, 2011).

Physiologically, juveniles of matrinxã tend to exhibit high compensatory growth (Urbinati, Sarmiento, & Takahashi, 2014), leading to the conclusion that the ideal nutrient balance in the diets, mainly protein (Izel et al., 2004), can improve the use of all nutrients by the fish metabolism, expressing this in body development. As considered to other fish species, the determination of the diet digestibility coefficient for matrinxã is important to determine the ability of digestion and absorption of nutrients from the ingredients ingested by fish, besides providing information about the use of the main nutrients (Magalhães Júnior et al., 2016).

In this study, the protein levels in the diets did not affect the digestibility of fats (ether extract). Lima, Silveira, and Tuesta (2015a) affirm that protein is the most important, metabolic and economically, nutrient in diets for fishes, independent of species, being responsible for both energy metabolism and body development. Thus, when protein levels meet the nutritional requirements, fats are exclusively destined for deposition as a reserve of energy, which explains because the protein levels did not affect the fats digestibility.

The same physiological behavior is observed in other species with carnivorous or omnivorous habits, such as tambaqui (Oishi, Nwanna, & Pereira Filho, 2010), pirarucu (Watson, Stewart, & Teece, 2013; Lima, Rodrigues, Varela, Torati, & Maciel, 2015b), and pacu (Signor et al., 2010; Ribeiro et al., 2017). Boscolo et al. (2011) also affirm that this increase in the use of nutrients by the organism is clearly verified from the increase in apparent digestible energy and percentage of energy that is being used, in addition to a gradual reduction in the energy: protein ratio, as observed in this study. It is important to point out that carnivorous and omnivorous fish have high protease levels, which naturally affect the digestibility of food protein according to the protein availability provided to fish, especially the relationship between enzyme production and substrate available (Polese et al., 2010; Cipriano et al., 2016).

Results of apparent digestibility of minerals are listed in Table 3. Digestibility of nitrogen ($y = 9.016x + 3.43$ $R^2 = 0.89$), phosphorus ($y = 14.986x + 31.73$ $R^2 = 0.97$), calcium ($y = 7.545x + 61.73$ $R^2 = 0.94$), magnesium ($y = 13.392x + 14.565$ $R^2 = 0.89$), copper ($y = 9.726x + 42.19$ $R^2 = 0.92$) and zinc ($y = 4.248x + 65.83$ $R^2 = 0.99$) showed linear positive ($p < 0.05$) behavior, with increased use of these minerals with increasing levels of protein in the diets. In turn, digestibility of potassium ($y = -1.7261x + 96.035$ $R^2 = 0.72$), iron ($y = -13.973x + 96.605$ $R^2 = 0.89$) and manganese ($y = -14.67x + 77.575$ $R^2 = 0.93$) reduced ($p < 0.05$) with increasing protein level in the diets.

As for the digestibility of minerals, although most of them can be absorbed by fish from the aquatic environment (Bakke, Glover, & Kroghdahl, 2010), the juveniles of *matrinxã* showed the same behavior observed for digestibility of other nutrients, both for mineral matter in general (ash) and macro- and micro-minerals, except potassium, iron and manganese. The results of the individual analysis of mineral digestibility showed a behavior similar to that observed in digestibility of total mineral matter, where the higher level of crude protein (40%) provided results with great increase or decrease in the digestibility. Furthermore, the reduced digestibility of these specific minerals may indicate that the exacerbated increase in protein levels, and consequently nitrogen, in diets for juveniles of *matrinxã* may cause metabolic imbalances, and subsequently, diminish the absorption and use of other nutrients, as commented by Lima et al. (2015a).

Bakke et al. (2010) affirm that these minerals, together with sodium and chlorine, act directly on the acid-base balance of the organism. These are directly related to metabolic changes from feed, especially oscillations in protein metabolism besides a fundamental role in intracellular processes, such as cellular signaling and energy metabolism (Le Boucher et al., 2012).

Results of energy metabolism are presented in Table 4. The behavior in dry matter digestibility also affected energy metabolism, with gradual increase ($p < 0.05$) in apparent digestible energy ($y = 96.12x + 4460.00$ $R^2 = 0.94$) and digestibility of apparent digestible energy ($y = 0.924x + 86.04$ $R^2 = 0.88$). However, the energy: protein ratio showed a gradual reduction ($p < 0.05$) with increasing levels of protein in the diets ($y = -1.401x + 15.87$ $R^2 = 0.94$).

The same pattern observed for protein digestibility was found for the energy metabolism of juveniles of *matrinxã*. Higher levels of crude protein resulted in better use of the energy by fish. Mattos, Bueno, Honczaryk, Pereira-Filho, and Roubach (2018) reported that protein levels from 40 to 45% may provide a satisfactory growth to juveniles of *matrinxã* due to the positive influence on energy metabolism. The same authors also commented that, at this stage, *matrinxã* present a high demand for protein to meet its requirements of protein and energy.

Table 3. Apparent digestibility of minerals for diets containing different protein levels to juveniles of *matrinxã*.

Coefficient (%)	Protein levels (%)				p-value	Effect	CV (%)
	28	32	36	40			
Nitrogen	14.95	20.27	25.35	43.31	0.01	LP	18.01
Phosphorus	46.80	59.63	80.58	89.77	0.02	LP	13.85
Potassium	93.40	93.10	92.53	87.83	0.03	LN	6.52
Calcium	69.33	78.40	81.04	93.60	0.01	LP	15.81
Magnesium	33.51	34.29	52.20	72.18	0.01	LP	9.84
Copper	53.50	61.74	66.42	84.36	0.02	LP	14.52
Iron	82.57	64.34	63.50	36.27	0.01	LN	9.85
Manganese	66.83	41.02	36.22	19.53	0.02	LN	10.36
Zinc	70.33	74.13	78.21	83.13	0.01	LP	9.36

CV - Coefficient of variation. p-value - Coefficient of Probability. LP - Linear positive. LN - Linear negative.

Table 4. Apparent Digestible Energy (ADE), Coefficient of Apparent Digestibility of Digestible Energy (Cdade) and Energy/Protein ratio (E:CP) of diets containing different protein levels to juveniles of *matrinxã*.

Variables	Protein levels (%)				p-value	Effect	CV (%)
	28	32	36	40			
ADE, kcal kg ⁻¹	4,531.89	4,674.43	4,777.27	4,818.04	0.04	LP	2.49
CDADE, %	86.38	88.57	89.20	89.25	0.03	LP	13.13
E:CP	14.88	12.55	11.47	10.57	0.01	LN	2.88

CV - Coefficient of variation. p-value - Coefficient of Probability. LP - Linear positive. LN - Linear negative.

Furthermore, Ferreira, Aride, Silva, and Val (2013) reported that higher levels of protein to *matrinxã* (around 40%), especially at the juvenile stage, meet the ideal energy demands for body mass development. It

is important to mention that the main energy reserves in fish are stored in the liver, muscles and, especially, around the viscera, in the form of glycogen and fat. Changes in these endogenous reserves are directly related to protein levels in the diets, indicating the energy metabolism of the fish according to the level of protein used in the diets (Arbeláez-Rojas et al., 2011).

Diets with insufficient protein levels tend to reduce growth and lower feed efficiency due to the mobilization of protein from some tissues to maintain vital fish functions. On the other hand, on a high protein diet, some will be used for muscle formation and growth, the rest will be converted to energy, which should be avoided as much as possible, since protein comprises the most expensive fraction of the diet (Signor et al., 2010; Vieira, 2017). In this sense, diets for fish, especially at initial stages, should contain a balanced protein level to each stage aiming the maintenance of a good balance in the energy: protein ratio, as observed in this study where the protein level of 40% provided this result to matrinxã at the juvenile stage.

Conclusion

It can be concluded that the raise in protein level in diets for juveniles of matrinxã improves nutrient digestibility. The results of this study indicated that 40% crude protein in the diets provided better results.

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