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Digestibility of animal and vegetable protein ingredients by pirarucu juveniles, *Arapaima gigas*

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ABSTRACT - The objective of this study was to determine the apparent digestibility coefficients of energy, protein, and amino acids in protein ingredients by pirarucu juveniles. A test was conducted with six protein ingredients: meat and bone meal, fish meal, hydrolyzed feather meal, poultry by-product meal, soybean meal, and corn gluten meal. Three repetitions were used for each tested ingredient. A reference feed was used with 430 g kg^{-1} crude protein and 19.63 kJ g^{-1} gross energy. The test feeds consisted of the replacement of 30% of the reference feeds with the test ingredients. Chromium oxide was added to the feeds at 1 g kg^{-1} as an external marker. Eighteen juveniles with an average weight of 235±36 g were used. The best apparent digestibility coefficients of protein were found for fish meal, followed by the poultry by-product meal and meat and bone meal. However, except for gluten, all the tested ingredients presented protein digestibilities above 0.70. The crude energy apparent digestibility coefficient was higher for animal ingredients, above 0.75, than for vegetable ingredients, which presented values below 0.60. Pirarucu efficiently uses the protein from the tested ingredients, regardless of origin. However, it has a preferential ability to use the energy from animal ingredients.

Key Words: amino acid, carnivore species, fish nutrition, nutritional value

Introduction

Pirarucu (*Arapaima gigas*) is a carnivorous fish and an important source of income for fishermen in the region around the Amazon basin. However, pirarucu fishing is limited and regulated by inspection and environmental agencies to keep the stock from being depleted and to ensure that ecological balance is maintained, as it is a species at the top of its food chain (Castello et al., 2011).

In recent years, this species has gained attention from the aquaculture industry because it presents both attractive characteristics for the development of its farming and a large size: it may reach more than 10 kg in a year. It has aerial respiration, a good carcass yield, and its meat is highly appreciated and valued (Imbiriba, 2001). Another important characteristic is that despite being a carnivorous fish, when it is well managed in captivity, it is not a cannibal species during its juvenile stage. After weaning, the fish accept inert food.

To formulate proper feeds for each species, it is fundamental to be aware of the digestibility of the ingredients. Digestibility information is necessary to prepare feeds that will lead to better yields, therefore optimizing feed costs, increasing productivity and profits, and reducing the environmental impact generated through excretion (Cho, 1987).

For carnivorous fish, the knowledge of animal and vegetable protein ingredient yields is crucial because large feed amounts are required to meet protein requirements. As protein-containing ingredients are the most costly ones, the possibility of using different sources may generate savings on the final cost of feeds. However, the volume of information available on captive pirarucu nutrition is limited (Cipriano et al., 2015), and it is inversely proportional to the rising number of fish farmers interested in its intensive production. Therefore, research efforts are required to collect data regarding the feed yield and nutritional needs of the species (Fracalossi and Cyrino, 2012).

This study aimed to determine the apparent digestibility of dry matter, crude protein, gross energy, and amino acids in animal and vegetable protein ingredients for pirarucu juveniles.

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Material and Methods

The experiment was conducted in Ilhéus, BA, Brazil, in accordance with ethical standards and approved by the Ethics and Biosafety Committee of Universidade Estadual de Santa Cruz (case no. 001/2015). We used 18 juvenile pirarucu with an average live weight of 235±36 g; specimens were provided by the Canta Galo Farm, Ibirataia-BA. Three individuals were housed per tank in six tanks (310 L) that were later used as feeding tanks. The tanks were arranged in a closed circulation system using a water pump (Dancor®, RJ, Brazil-75 HP) with biological filters, and constant aeration was provided by a blower (WEG of 1 HP).

Juveniles were subjected to period of adaptation to laboratory and routine management conditions for 10 days, during which they received the reference feed (Table 1) four times a day. During the adjustment period and the experimental period, daily cleaning was performed to remove feces and possible scraps of feed.

After that period, the selected fish remained in the tanks, which were used as feeding tanks. They received five meals a day: two in the morning (8.00 h and 10.00 h) and three in the afternoon (12.00 h, 14.00 h, and 16.00 h). For each meal tested, the fish went through a four-day adaptation period and then their feces were collected for a three-day period.

To collect the feces, the fish were transferred to the digestibility tanks (200 L) 1 h after their last meal. The digestibility tanks had conical shapes, constant aeration, and biological filters and were equipped with collection devices in their bottoms, which were submersed in water and ice during the collection periods. At 7.00 h on the following day, the fish were transferred to the feeding tanks. The collection devices were detached so that the feces could be collected. The feces were packed in disposable aluminum containers to be dried in a forced-ventilation drying oven at 55 °C for 24 h.

The tanks were cleaned daily and the physical and chemical variables of the water were measured weekly. The pH, temperature, and dissolved oxygen were 6.8 to 7.0, 26.8±0.43 °C, and 7.2±1.43 mg L^{-1}, respectively.

To prepare the experimental diets, a diet was formulated as a reference (Table 1) with the use of SUPER CRAC® software. The crude protein levels were the same as those found by Ituassú et al. (2005). The ingredients were ground in a knife mill, pushed through a 0.5 mm sieve, and homogenized. The mixture was previously moistened using water at 40 °C. The feeds were processed in a meat grinder with a reversible function using a 2-mm matrix. The feed granules were dried in a kiln (55 °C) with forced ventilation for 24 h and disintegrated to present the proper size for the fish to feed on them.

Test feeds were prepared using a mixture of 70% reference feed and 30% of the ingredient to be tested. The apparent digestibility coefficients of the following six protein ingredients were tested: meat and bone meal, fish meal, hydrolyzed feather meal, poultry by-product meal, soybean meal, and corn gluten meal (Table 2). For each ingredient tested, three repetitions were used and obtained every seven days. The feces were collected in the last four days of each cycle.

The apparent digestibility coefficients of the feed and the test ingredients were determined using indirect methods with the use of chromium oxide (1 g kg^{-1}) as the external marker. The elemental contents were represented in the feeds and in the feces. The apparent digestibility coefficients of the feeds (ADCFE) were calculated according to De Silva (1989), using the following formula:

$$\text{ADCFE} = 100 - \frac{\%\text{Cr}_2\text{O}_3\text{ feed}}{\%\text{Cr}_2\text{O}_3\text{ feces}} \times \frac{\%\text{nutrients or gross energy feces}}{\%\text{nutrients or gross energy feed}}$$

The coefficients of digestibility of the ingredients (ADC_I) were calculated using the methodology employed by Bureau et al. (1999), with the following formula:

$$\text{ADCI} = \frac{\text{ADCTF} - \text{ADCRF} \times 0.7}{\text{ADCTF} \times 0.3},$$

in which ADCI is the apparent digestibility coefficient of nutrients or energy in the ingredients; ADCTF is the apparent digestibility coefficient of nutrients or energy

Table 1 - Composition of reference diet

Ingredient	Content (g kg^{-1})
Soybean meal	188.00
Wheat bran	140.00
Corn gluten meal	105.00
Corn	90.03
Fish meal	370.00
Poultry by-product meal	57.67
Corn starch	27.00
Soybean oil	8.45
Mineral and vitamin mix[1]	7.00
Sodium chloride	3.50
Cellulose	2.15
Chromic oxide III	1.00
BHT	0.20
Total	1000
Crude protein (g kg^{-1})	432.40
Gross energy (kJ g^{-1})	19.63
Ash (g kg^{-1})	143.00

BHT - butylated hydroxytoluene.

[1] Mineral and vitamin mix per kg of product: vitamin A - 6,000,000 IU; vitamin D3 - 2,250,000 IU; vitamin E - 75,000 mg; vitamin K3 - 3,000 mg; vitamin thiamine (B1) - 5,000 mg; riboflavin (B2) - 10,000 mg; pyridoxine - 8,000 mg; biotin - 2,000 mg; ascorbic acid (vitamin C) - 192,500 mg; niacin - 30,000 mg; folic acid - 3,000 mg; Fe - 100,000 mg; Cu - 600 mg; Mn - 60,000 mg; Zn - 150,000 mg; I - 4,500 mg; Cu - 15,000 mg; Co - 2,000 mg; Se - 400 mg.

Table 2 - Nutritional composition based on the dry matter from ingredients assessed during the analysis of digestibility by pirarucu (*Arapaima gigas*)

	Ingredient					
	Corn gluten meal	Feather meal	Fish meal	Meat and bone meal	Poultry by-product meal	Soybean meal
Dry matter (g kg^{-1})	88.95	90.27	92.44	93.19	97.17	88.27
Crude protein (g kg^{-1})	275.25	706.78	578.34	437.47	604.47	508.71
Gross energy (kJ g^{-1})	17.43	23.03	19.22	15.57	23.81	18.26
Lipids (g kg^{-1})	95.82	170.26	229.90	168.01	200.17	107.59
Ash (g kg^{-1})	60.54	49.78	218.79	380.43	66.69	64.85
Amino acid (g kg^{-1})						
Alanine	18.8	35.9	40.5	35.2	33.5	21.0
Arginine	7.7	47.6	34.5	31.8	37.2	34.5
Aspartate	12.8	50.3	42.3	28.7	48.6	52.4
Cysteine	4.8	33.9	3.7	1.7	7.3	6.5
Glutamate	42.2	77.0	62.7	48.1	73.1	82.8
Glycine	9.8	53.5	66.0	74.3	42.2	20.9
Histidine	5.9	9.8	9.1	5.8	10.7	12.2
Isoleucine	7.9	32.4	16.5	10.1	23.4	21.9
Leucine	25.7	58.8	30.4	21.8	42.0	35.5
Lysine	5.8	23.5	32.1	21.6	32.5	29.1
Methionine	3.9	6.3	12.9	5.6	10.9	6.5
Phenylalanine	9.6	34.5	17.5	12.2	23.2	24.1
Proline	22.7	62.5	35.0	41.5	29.0	22.6
Serine	10.0	70.8	21.2	15.2	25.7	21.5
Threonine	8.3	33.6	20.0	11.9	24.0	17.4
Valine	11.5	48.6	21.6	15.8	28.9	24.0

in the test feed; and ADCRF is the apparent digestibility coefficient of nutrients or energy in the reference feed.

After drying and checking for the possible presence of scales, the samples were identified, stored in plastic containers, and kept in a freezer (−10 °C) for further analyses of dry matter (DM), mineral matter (MM), crude protein (CP), gross energy (CE), and chromium concentrations.

The analyses of crude protein, gross energy, dry matter, and mineral matter were conducted as per the methodology of AOAC (2000). The analyses of amino acids from feces and feeds were conducted by ion-exchange chromatography by Evonik Industries AG using the methodology of White et al. (1986). The chromium concentrations were analyzed with the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP OES), model Varian 710-ES, series using the methodology of Giné-Rosias (1998).

The data were subjected to one-way analysis of variance (ANOVA) and compared by the Scott-Knott test ($P<0.05$), using the statistical software R Core Team (2011).

Results

The apparent digestibility coefficients (ADC) of dry matter, crude protein, and gross energy of the evaluated ingredients for pirarucu juveniles presented significant differences ($P\leq0.05$) (Table 3).

The best apparent digestibility coefficients of dry matter (ADCDM) were found for the poultry by-product meal and fish meals, both of which were above 89.0%. The ADCDM of feather meal, soybean meal, and meat and bone meal had intermediate values, ranging from 70.8% to 79.5%. The corn gluten meal presented the lowest ADCDM.

The fish meal had the best apparent digestibility coefficients for crude protein (ADCCP), followed by the poultry by-product meal and meat and bone meal. They were followed by the soybean meal and hydrolyzed feather meal. The corn gluten meal had the lowest ADCCP.

The ingredients that presented the highest apparent digestibility coefficients of amino acids (ADCAA) were fish meal, meat and bone meal, and poultry by-product meal, with an average of 90.9%. For soybean meal, ADCAA classified in groups with higher values or intermediate values were found, with an average of 85.8%. The lowest ADCAA values were found for the hydrolyzed feather meal and the corn gluten meal, with an average of 70.8%.

For the apparent digestibility coefficients of gross energy (ADCCE), the ingredients with the best digestibilities were the poultry by-product meal and fish meal. The feather meal and meat and bone meal had intermediate ADCCE. The lowest ADCCE were found for the corn gluten meal and soybean meal.

Table 3 - Apparent digestibility coefficients (ADC) for dry matter, crude protein, gross energy, and amino acid of the tested ingredients in pirarucu (*Arapaima gigas*)

ADC (%)	Ingredient						CV (%)	P-value
	Corn gluten meal	Feather meal	Fish meal	Meat and bone meal	Poultry by-product meal	Soybean meal		
Dry matter	61.2c	79.5b	89.2a	70.8b	93.5a	76.7b	7.56	0.001
Crude protein	74.2d	80.4c	97.6a	89.4b	90.3b	83.8c	2.81	0.001
Gross energy	59.8c	83.3b	89.1a	75.4b	96.2a	58.0c	6.45	0.001
Amino acid								
Alanine	75.1b	73.2b	99.3a	89.0a	96.3a	794b	7.56	0.001
Arginine	84.4b	76.4c	99.4a	93.8a	91.5a	94.6a	4.51	0.001
Aspartate	63.5b	69.6b	91.9a	80.4a	94.5a	91.1a	9.48	0.001
Cysteine	71.1b	59.3b	86.4a	70.7b	69.1b	80.7a	11.16	0.022
Glutamate	82.2b	73.3b	95.3a	87.2a	94.6a	93.7a	5.93	0.001
Glycine	67.1c	81.3b	95.8a	88.8a	97.4a	83.5b	7.89	0.001
Histidine	75.7b	72.1b	93.2a	87.8a	95.4a	93.9a	7.28	0.002
Isoleucine	62.7b	71.4b	95.0a	85.7a	92.3a	81.6a	10.11	0.003
Leucine	78.2b	69.2b	96.1a	89.2a	90.8a	81.2b	8.18	0.005
Lysine	71.3b	69.3b	98.8a	90.2a	94.7a	93.6a	7.46	0.001
Methionine	71.8b	72.5b	94.4a	86.3a	94.1a	86.2a	7.85	0.003
Phenylalanine	65.8b	68.2b	94.4a	84.2a	91.6a	76.1b	10.68	0.006
Proline	75.6b	67.4b	94.6a	87.7a	92.1a	86.6a	8.49	0.004
Serine	74.5b	72.5b	96.6a	86.8a	87.6a	87.1a	7.1	0.003
Threonine	62.9b	69.0b	96.5a	85.0a	92.2a	79.7a	9.55	0.001
Valine	65.7b	65.1b	95.4a	86.7a	88.9a	83.0a	9.99	0.002

CV - coefficient of variation.

Discussion

Carnivorous fish have high lipase and protease levels (Furné et al., 2005), which benefit the digestibility of animal protein ingredients, while production of enzymes is influenced by factors such as environmental changes (Moura et al., 2007) and dietary particle size (Polese et al., 2010). That explains why the best ADCDM were found for the poultry by-product meal and fish meal. Similar ADCDM values were found by Braga et al. (2008) for fish meal and meat and bone meals — 95.2% and 95.7% —, respectively, for dourado juveniles (33.5 g). However, Zhou et al. (2004) found similar ADCDM rates for fish meal (87.6%) and lower rates for poultry by-product meal (80.9%) for cobia juveniles (*Rachycentron canadum*) (10 g).

Among the animal ingredients, meat and bone meal had the worst dry matter digestibility, even though the protein digestibility was high. The composition of that ingredient was variable, and meals with large bone amounts tended to present lower ADCDM (Silva et al., 2013). The meat and bone meal had 38% mineral matter, which demonstrated a high amount of bone in its composition.

In studies with carnivorous fish, high protein digestibility values were observed for fish meal, as found for oscar (*Astronotus ocellatus*), 92.8% (Nascimento et al., 2012); dourado (*Salminus brasiliensis*), 94.3% (Borghesi et al., 2009); spotted sorubim (*Pseudoplatystoma corruscans*), 84.1% (Gonçalves and Carneiro, 2003); striped surubim (*Pseudoplatystoma reticulatum*), 82.8% (Silva et al., 2013); cobia (*Rachycentron canadum*), 96.3% (Zhou et al., 2004); and sea bass (*Dicentrachus labrax*), 93.5% (Davies et al., 2009).

Fish meals differ with regard to their quality. They may present excess minerals in their composition, generally due to the high presence of scales and bones, which results in reduced protein contents and nutritional values (Sampaio et al., 2001).

Silva et al. (2013), in a study with *P. reticulatum*, observed a similar ADCCP for meat and bone meal (87.4%), a higher ADCCP for poultry by-product meal (99.4%), and a lower ADCCP for soybean meal. Lee (2002) observed similar ADCCP values for meat and bone meal (90.0%) and soybean meal (84.0%) for *Sebastes schlegeli*. Similar values were observed by Borghesi et al. (2009) for poultry by-product meal for dourado (*Salminus brasiliensis*) (91.3%).

The ADCCP of feather meal and corn gluten meal in pirarucu were lower than those found for dourado (91.3% and 93.5%, respectively) (Borghesi et al., 2009) and for corn gluten meal (94.4%) for cobia (Zhou et al., 2004). However, the ADCCP were higher than those found by Gonçalves and Carneiro (2003) for feather meal (39.5%) for spotted sorubim (*P. corruscans*).

The protein quality of an ingredient depends on the profile of the amino acids and their availability (Rollin et al., 2003). Although the ADCCP is directly related to the digestibility of amino acids, there may be differences in ADCCP for some of them (Zhang et al., 2015). The formulation of diets based on the amount of available amino acids may result in considerably improved performances (Rawles et al., 2006).

Lysine is considered the first limiting amino acid to fish growth (Abboudi et al., 2006). The lysine requirements of commonly farmed fish species fall between 32 and 62 g kg^{-1} of the total consumed protein (Wilson, 2002). Zhou et al. (2004) found a lysine ADC for cobias close to those found in this study for fish meal (97.5%), meat and bone meal (84.5%), and poultry by-product meal (91.8%). However, they found a higher value for corn gluten meal (96.9%). Similarly, methionine is an indispensable amino acid for normal growth for fish (Zhou et al., 2006). The methionine ADC obtained were close to those found for cobias by Zhou et al. (2004) for fish meal (95.9%) and poultry by-product meal (92.5%).

Vegetable ingredients presented an ADCCE below 60.0%, whereas the animal ones presented values above 75.0%. These results demonstrated that pirarucu had a lower capacity to digest energy in vegetable ingredients. Higher ADCCE for animal ingredients were also observed for the carnivorous fish *R. canadum* (Zhou et al., 2004), *Sebastes schlegeli* (Lee, 2002), and *P. corruscans* (Gonçalves and Carneiro, 2003).

Studies with trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*), also carnivorous species, demonstrated that the production of alpha-amylase was reduced and hindered energy digestion as carbohydrates. In turn, lipase activity may be higher than that found for omnivorous species (Hidalgo et al., 1999; Furné et al., 2005).

Nonetheless, fish are able to modify their enzyme production throughout time, according to the food they receive. This adaptation varies according to the species. Lundstedt et al. (2004) assessed the response of the enzyme profile of *P. corruscans* given different feeds and found increased amylase production with high levels of carbohydrates.

Braga et al. (2008) assessed the digestibility of ingredients in dourado juveniles (35.51 g) and observed close ADCCE rates for poultry by-product meal (95.3%) and fish meal (93.9%) and better digestibilities for meat and bone meal (75.4%) and corn gluten meal (95.7%). The ADCCE for soybean meal was similar to the 61.7% found by Gonçalves and Carneiro (2003) for *P. corruscans* (9.8 g).

The feather meal ADCCE for pirarucu was similar to those found by Bureau et al. (1999) for trout juveniles (81.0%, 24 g) and by Lee (2002) for *Sebastes schlegeli* juveniles (85.0%, 30 g). However, Silva et al. (2013) found worse digestibility for surubim (53.8%). In addition to the differences among the studied fish species, the lack of standardization for certain ingredients, such as the amount of ash, contributed to different values.

Conclusions

Pirarucu adapts to the use of feeds with animal and vegetable ingredients. The protein in the tested ingredients is well digested regardless of its origin. However, the energy digestibility from vegetable ingredients is smaller than that from animal ingredients.

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