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ENERGY AND PROTEIN INGREDIENTS FOR USE IN PIAPARA (*Megaleporinus obtusidens*) DIETS: NUTRITIVE VALUE AND INTESTINAL MORPHOMETRY*

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ABSTRACT

The apparent digestibility coefficients for crude protein (ADC_{CP}), dry matter (ADC_{DM}), and gross energy (ADC_{GE}) of ingredients were determined for piapara (*Megaleporinus obtusidens*). Test diets were formulated to contain 69.5% of reference diet, 0.5% of chromium oxide, and 30% of test ingredients. The protein ingredients evaluated were tilapia processing residue meal (TPRM), feather and poultry blood meal (FPBM), poultry by-product meal (PBM), meat and bone meal (MBM), cottonseed meal (CM), corn gluten meal (CGM), and soybean meal (SM); the energy ingredients tested were corn (C), corn germ meal (CGRM), rice meal (RM), wheat bran (WB), and sorghum (S). Groups of 30 piaparas were fed twice daily during five days with test diets. Intestinal morphometry of fish were also evaluated. Digestibility coefficients of protein and energy ingredients were highest for soybean meal (ADC_{DM} = 85.8%; ADC_{CP} = 95.2%; and ADC_{GE} = 87.2%) and corn (ADC_{DM} = 94.5%; ADC_{CP} = 76.2%; and ADC_{GE} = 89.3%), respectively. Of the energy test ingredients analyzed, corn had the highest digestibility coefficients and induced beneficial changes on intestinal morphology compared to sorghum and corn germ meal. All protein ingredients showed potential for use in piapara diets, except meat and bone meal and feather and blood meal.

Keywords: nutrient; feed; digestibility.

INGREDIENTES ENERGÉTICOS E PROTEICOS PARA USO EM DIETAS DA PIAPARA (*Megaleporinus obtusidens*): VALOR NUTRICIONAL E MORFOMETRIA INTESTINAL

RESUMO

Os coeficientes de digestibilidade aparente da proteína bruta (ADC_{CP}), matéria seca (ADC_{DM}) e energia bruta (ADC_{GE}) de ingredientes foram determinados para a piapara (*Megaleporinus obtusidens*). Dietas testes foram formuladas contendo 69,5% da dieta referência, 0,5% de óxido de cromo e 30% do ingrediente teste. Os alimentos proteicos avaliados foram: farinha do resíduo de processamento da tilapia (TPRM), farinha de penas e sangue de aves (FPBM), farinha de vísceras de aves (PBM), farinha de carne e ossos (MBM), farelo de algodão (CM), glúten de milho (CGM) e farelo de soja (SM); os alimentos energéticos testados foram: milho (C), gérmen de milho (CGRM), farelo de arroz (RM), farelo de trigo (WB) e sorgo (S). Grupos de 30 piaparas foram alimentados duas vezes ao dia durante cinco dias com as dietas testes. Também foi avaliada a morfometria intestinal dos peixes. Os coeficientes de digestibilidade dos ingredientes proteicos e energéticos foram mais altos para o farelo de soja (ADC_{DM} = 85,8%; ADC_{CP} = 95,2%; e ADC_{GE} = 87,2%) e milho (ADC_{DM} = 94,5%; ADC_{CP} = 76,2%; e ADC_{GE} = 89,3%), respectivamente. Dentre os ingredientes energéticos, o milho apresentou os maiores coeficientes e induziu alterações morfométricas intestinais benéficas, quando comparado ao sorgo e gérmen de milho. Todos os ingredientes proteicos apresentaram potencial uso em dietas para a piapara com exceção da farinha de carne e ossos e farinha de penas e sangue de aves.

Palavras-chave: nutriente; alimento; digestibilidade.

INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world, contributing substantially to economic growth and food security, especially in developing countries. Reducing feed costs is a significant challenge to promoting sustainable and profitable aquaculture development, which can be achieved by replacing conventional ingredients with foods capable of promoting the growth potential of fish at a lower cost (Couto et al., 2016).

Fishmeal produced from whole fish besides the high protein content is also a great source of fatty acids and minerals and has been widely used in diets due to its high palatability and balanced amino acid profile (Gatlin et al., 2007). However, the intensification of feed production has increased demand for this ingredient, resulting in less availability and elevated fish feed costs (Klinger and Naylor, 2012; Campos et al., 2018), which currently account for 70% of total production costs. Thus, combining protein and energy ingredients in a competitive and environmentally friendly manner is a major challenge for feed formulators (Mungkung et al., 2013).

Knowledge of the nutritional value of each ingredient is critical when using new raw materials in feed formulations. The determination of apparent digestibility coefficients (ADC) provides the amount of energy and nutrients contained in the fraction of food ingested that is not excreted in the feces (Choubert et al., 1979; De Silva and Anderson, 1998; NRC, 2011) but is used by the animal metabolism (Che et al., 2017). Digestibility tests make it possible to evaluate the potential of ingredients for use in the diet of aquaculture species (Gaylord and Gatlin, 1996; Che et al., 2017; Campos et al., 2018) and are essential to enable formulation of nutritionally balanced diets that maximize fish yields (Oliveira-Filho and Fracalossi, 2006; Glencross et al., 2016), but also to limit fish waste products and improve homeostasis, enhancing the resistance to disease and stress (Pezzato et al., 2009).

The nutritional value of feedstuffs varies from species to species and fish development stage (NRC, 2011) due to physiological and morphological differences in the digestive tract and can also be affected by the processing of the ingredients, which can be hydrolyzed and extruded under high heat and pressure, improving digestibility (Fontoura et al., 2014). In addition to the digestibility of food, it is important to determine the possible metabolic and physiological changes that organisms may experience following the inclusion of different ingredients in the diet. These feedstuffs may have antinutritional factors in their composition that may make it difficult to absorb certain nutrients or even cause adverse physiological effects in animals, which may result in changes to the taste and color of the meat (Schwarz et al., 2011). Conversely, these foods may be a significant source of vitamins, folates, and minerals such as calcium, potassium, magnesium, and iron and have positive effects on fish growth (Roy et al., 2010).

The piapara *Megaleporinus obtusidens* (formerly known as *Leporinus obtusidens*) (Ramirez et al., 2017) is a freshwater fish species native to South America with omnivorous eating habits (Durães et al., 2001) very appreciated in sport fishing for its behavior during angling (Moro et al., 2013) and of great commercial importance due to its good potential yield and wide acceptance in the consumer market (Tataje and Zaniboni-Filho, 2010). Studies on piapara nutrition are scarce but essential for formulating well-balanced diets. This study aimed to determine the ADCs of dry matter, crude protein, and gross energy in protein and energy ingredients and the possible intestinal morphometry changes in fish fed different diets.

MATERIAL AND METHODS

The experimental procedures of this study were approved by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on Animal Use (CEUA), São Paulo State University (UNESP), protocol n° 23/2018.

Protocol and experimental design

The experiment was conducted at the Aquaculture Laboratory of the College of Agricultural and Technological Sciences (FCAT/UNESP), Dracena Campus. Fish tanks were provided with continuous aeration and water from a flowing artesian well at a renewal rate of approximately 3.5 times per day. The system has an electric aerator coupled to silicone hoses and porous stones to provide dissolved oxygen, and water temperature was maintained constant using 150-W thermostats. Fish were maintained on a 12-h light–dark cycle using fluorescent lamps and a digital timer.

A total of 1,440 piaparas (26.9 ± 7.6 g; mean ± SD), distributed in 48 130-L polyethylene aquaria (feeding tanks) at a stocking rate of 30 fish/tank, were used in the study. Water parameters were monitored and remained within the acceptable range for the species: temperature, 25.7 ± 0.4°C; dissolved oxygen concentration, 6.8 ± 0.8 mg L^{-1}, monitored using a YSI 55 probe; and pH, 7.6 ± 0.1, monitored using a YSI Professional Plus probe (YSI Incorporated, Yellow Springs, Ohio, USA). Before the experiment was initiated, the fish were acclimated to laboratory conditions for 20 days and fed a commercial feed twice daily until apparent satiety.

Diets and laboratory analysis

Experimental diets

Thirteen experimental diets were formulated, including a reference diet with conventional ingredients formulated to contain 27% of crude protein and 18.3 kJ g^{-1} of gross energy (Table 1). Another 12 diets were formulated to contain 69.5% of the reference diet and 30% of the tested ingredients, with seven protein and five energy ingredients. In the 13 experimental diets, 0.5% of the chromium oxide (Cr_2O_3) was included as the inert digestibility marker. The protein ingredients used were tilapia processing residue meal (TPRM), feather and poultry blood meal (FPBM), poultry by-product meal (PBM), meat and bone meal (MBM), cottonseed meal (CM), corn gluten meal (CGM), and soybean meal (SM). The energy ingredients tested were corn (C), corn germ meal (CGRM), rice meal (RM), wheat bran (WB), and sorghum (S).

After the ingredients and diets were ground, dry matter was determined in an air circulation oven at 105°C for 12 hr (method 930.15), mineral matter by incineration in a muffle at 550°C (method 942.05), crude protein by the Kjeldahl method (method 988.05), and ether extract through extraction with petroleum ether in a Soxhlet extractor (method 920.39), all according to the Association of Official Analytical Chemists methodology (AOAC, 2005). Crude energy analyses were performed using the calorimetric pump method (Model C2000 Control, IKA 2000, Guangzhou, China). The formulation and chemical composition of the ingredients and experimental diets are summarized in Tables 2 and 3.

Table 1. Formulation of the reference diet.

Ingredient	%
Tilapia processing residue meal[a]	11.00
Broken rice[b]	13.00
Soybean meal[c]	25.30
Corn[d]	26.10
Wheat bran[e]	20.60
Soybean oil[c]	0.50
Vitamin and mineral supplement[f]	1.50
Phosphate bicalcium[g]	1.98
Ethoxyquin[h]	0.02

[a] Indústria Brasileira do Peixe Ltda (Buritama, Brazil); [b] Arroz Marconato & Irmãos Ltda (Jaboticabal, Brazil); [c] Granol Industria, Comercio e Exportação S.A. (Bebedouro, Brazil); [d] Edinaldo Aure Mathias, Fazenda Ybyeté Porã (Rancharia, Brazil); [e] Moinho Globo Alimentos S.A. (Sertanópolis, Brazil); [f] Premix AcquaMeal Peixes - MCassab Nutrição Animal (Valinhos, Brazil), composition/kg of product: choline, 100 g; vitamin A, 1,750,000 IU; vitamin D3, 375,000 IU; vitamin E, 20,000 IU; vitamin K3, 500 mg; vitamin B1, 2,000 mg; vitamin B2, 2,500 mg; vitamin B6, 2,500 mg; vitamin B12, 5.0 mg; niacin, 8,750 mg; pantothenic acid, 7,500 mg; folic acid, 625 mg; biotin, 50 mg; vitamin C, 37.5 g; inositol, 12.5 g; iron, 15.0 g; copper, 1,250 mg; manganese, 3,750 mg; zinc, 17.5 g; cobalt, 50 mg; iodine, 100 mg; selenium, 75 mg; [g] Fosfoconannan 18, Connan Nutrição Animal (Boituva, Brazil); [h] Ethoxyquin 66,6, MCassab Nutrição Animal (São Paulo, Brazil).

Digestibility test

For fecal collection, digestibility tanks (80-L fiberglass aquaria) with a conical bottom were used. Feces were collected with 50-mL Falcon tubes kept on ice to prevent leaching of nutrients. The fish were fed two times per day (9:00 a.m. and 5:00 p.m.) with the test diets for seven days and placed in the digestibility tanks from 5:00 p.m. to 9:00 a.m. the next day, and then returned to their respective rearing tanks. The feces collected were stored at -20°C.

Samples were thawed, centrifuged (10 min, 435 *g*, 4°C), and oven-dried with forced-air ventilation at 45°C until constant weight. The chemical composition and energy content of fecal samples, including dry matter (DM), mineral matter (MM), crude protein (CP), and gross energy (GE), were determined according to AOAC methodologies (AOAC, 2005) as previously described. The concentration of chromium oxide was determined by the colorimetric method of Furukawa and Tsukahara (1966) using a spectrophotometer. The calculation of the apparent digestibility coefficient of nutrients was performed according to NRC (2011). The apparent digestibility coefficients of dry matter, protein, and gross energy of the test ingredients were calculated according to the methodology described by Bureau et al. (1999), based on the proportion of the reference diet and test ingredients.

Table 2. Nutrient composition of tested ingredients.

Ingredient	Dry matter (%)	Mineral matter (%)	Crude protein (%)	Ether extract (%)	Gross energy (kJ g^{-1})
Protein ingredients					
TPRM[a]	93.6	24.4	57.6	10.6	18.9
FPBM[b]	91.5	2.6	87.0	4.3	22.6
PBM[b]	95.6	14.4	72.0	6.6	20.2
MBM[c]	93.6	46.3	34.9	8.7	12.5
CM[d]	91.5	9.7	36.3	0.5	18.9
CGM[e]	91.9	1.7	70.5	2.0	24.5
SM[f]	90.0	6.5	52.0	1.8	19.5
Energy ingredients					
C[g]	88.0	1.3	11.2	4.1	18.9
CGRM[e]	94.6	1.9	24.1	6.1	17.1
RM[h]	89.0	9.7	15.5	13.2	19.9
WB[i]	89.9	4.4	19.3	3.1	18.9
S[j]	87.3	1.3	21.6	2.4	18.4

TPRM, tilapia processing residue meal; FPBM, feather and poultry blood meal; PBM, poultry by-products meal; MBM, meat and bone meal; CM, cottonseed meal; CGM, corn gluten meal; SM, soybean meal; C, corn; CGRM, corn germ meal; RM, rice meal; WB, wheat bran; S, sorghum. [a] Indústria Brasileira do Peixe Ltda (Buritama, Brazil); [b] Alimenta Agroindústria e Comércio de Alimentos (Flórida Paulista, Brazil); [c] Frigorífico Better Beef (Rancharia, Brazil); [d] Óleos Menu Indústria e Comércio (Guararapes, Brazil); [e] Ingredion Brasil Ing. Indústria Ltda (São Paulo, Brazil); [f] Granol Indústria. Comércio e Exportação (Bebedouro, Brazil); [g] Edinaldo Aure Mathias e Outra, Fazenda Ibiete Porã (Rancharia, Brazil); [h] Arroz Marconato & Irmãos (Jaboticabal, Brazil); [i] Moinho Globo Alimentos S.A. (Sertanópolis, Brazil); [j] Gran Vale Alimentos Eireli EPP (São José dos Campos, Brazil).

Table 3. Composition of test diets formulation (%).

Ingredient												
Reference diet (%)	69.5	69.5	69.5	69.5	69.5	69.5	69.5	69.5	69.5	69.5	69.5	69.5
TPRM (%)	30.0											
FPBM (%)		30.0										
PBM (%)			30.0									
MBM (%)				30.0								
CM (%)					30.0							
CGM (%)						30.0						
SM (%)							30.0					
C (%)								30.0				
CGRM (%)									30.0			
RM (%)										30.0		
WB (%)											30.0	
S (%)												30.0
Chrome oxide (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrition composition												
Dry matter (%)	93.0	92.1	91.6	93.8	91.3	91.3	91.2	4.1	3.4	4.7	4.4	4.0
Mineral matter (%)	13.2	6.8	9.8	21.1	8.6	6.4	8.0	6.4	6.4	9.0	7.4	6.4
Crude protein (%)	36.7	44.7	41.5	34.9	36.3	40.3	34.5	22.5	26.1	24.2	27.5	21.6
Ether extract (%)	5.0	3.3	3.1	5.4	2.0	3.8	2.2	3.7	3.6	6.4	1.4	2.1
Crude fiber (%)	0.6	0.6	0.6	0.6	0.5	0.6	0.6	0.5	0.6	0.6	0.6	0.5
Gross energy (kJ g^{-1})	18.4	19.8	19.0	16.3	18.6	20.2	18.8	17.6	18.9	22.5	18.8	16.3

TPRM, tilapia processing residue meal; FPBM, feather and poultry blood meal; PBM, poultry by-products meal; MBM, meat and bone meal; CM, cottonseed meal; CGM, corn gluten meal; SM, soybean meal; C, corn; CGRM, corn germ meal; RM, rice meal; WB, wheat bran; S, sorghum.

Intestinal villus morphometry

Three fish from each tank were euthanized and laparotomy was performed to remove a portion of the intestine for histological analysis. The samples were cut longitudinally and washed in a phosphate buffered saline solution (PBS, pH 7.5), attached to a rectangular cardboard to keep the villi apparent, and fixed in a formaldehyde solution (4% in PBS). After fixation, samples were washed in 70% ethanol and again in an increasing ethanol series (70, 90, and 100%), diaphanized in a xylol series, and finally embedded in Histosec® medium (Merck, Darmstadt, Germany). The slides were cut at 5-µm thickness, stained with hematoxylin-eosin (HE), and photo-documented using a BX43 photomicroscope (Olympus Life Science, Tokyo, Japan) coupled to a digital video camera. The visual comparison of the intestinal villi of the fish was performed using image analysis software (cellSens Standard®, Olympus Life Science, Tokyo, Japan). The parameters evaluated were villus height, epithelium thickness, and total villus width at three points. Ten random readings of each intestine sample were performed.

Statistical analysis

The experiment was arranged in a completely randomized design. Protein ingredients were analyzed separately from energy ingredients. The data were tested for normality (Cramer-von Mises test) and homogeneity of variances (Brown–Forsythe test). The data were analyzed using analysis of variance (ANOVA) and the treatment means were compared using the Tukey's test at $p < 0.05$. All analyses were performed using SAS v.9 software package (SAS Institute, 2009).

RESULTS

Digestibility test

The results for the apparent digestibility coefficients of dry matter (ADC_{DM}), crude protein (ADC_{CP}), and gross energy (ADC_{GE}) of protein and energy ingredients are shown in Table 4. Significant differences were observed in ADC_{DM} and ADC_{CP} in both feed groups. In the case of ADC_{GE}, significant differences were observed only between the energy ingredients.

Feather and poultry blood meal (89.0%) and corn gluten meal (87.5%) had the highest ADC_{DM} values of all protein ingredients tested, whereas tilapia processing residue meal (69.5%) and meat and bone meal (56.5%) had the lowest ADC_{DM} values for protein ingredients. The energy ingredient with the highest ADC_{DM} was sorghum (97.2%) and the lowest ADC_{DM} values were found for rice meal (71.8%) and corn germ meal (48.0%).

The protein ingredients with the highest ADC_{CP} values were soybean meal (95.2%), followed by cottonseed meal (91.3%), poultry by-product meal (90.8%), and corn gluten meal (89.2%); sorghum (82.1%), wheat bran (81.4%), and corn (76.2%) had the highest ADC_{CP} values for energy ingredients, whereas corn germ meal (56.9%) had the lowest ADC_{CP}. Among energy ingredients, corn (89.3%), rice meal (84.5%), and wheat bran (68.8%) had the highest ADC_{GE}, whereas sorghum (52.0%) and corn germ meal (39.5%) had the lowest ADC_{GE} values.

Intestinal villus morphometry

The results for morphometric analysis of intestinal villi of piaparas fed different protein and energy ingredients are shown in Table 5. No significant differences were observed in epithelium width and height of piapara juveniles fed protein and energy ingredients, but epithelium thickness was significantly different in piaparas fed protein ingredients. Intestinal epithelium thickness was significantly increased in fish fed tilapia processing residue meal (55.1 µm) and feather and poultry blood meal (57.1 µm) than in fish fed soybean meal (39.9 µm), meat and bone meal (41.8 µm), and corn gluten meal (42.1 µm).

There were significant differences in intestinal villus height and width between piaparas fed different energy-based diets. Villus height was increased in fish fed corn (455.2 µm) compared to fish fed sorghum (346.9 µm) and corn germ meal (334.2 µm). Piaparas fed corn also had wider villi (114.6 µm) than those fed sorghum (92.2 µm).

Table 4. Apparent digestibility coefficients of dry matter, crude protein and gross energy of different ingredients in piapara diets (%).

Ingredient	Dry matter (%)	Crude protein (%)	Gross energy (%)
Protein ingredients			
TPRM	69.5±3.8[bc]	85.5±1.2[b]	89.4±3.8
FPBM	89.0±10.2[a]	84.9±3.6[b]	90.8±10.0
PBM	83.7±7.4[ab]	90.8±1.7[ab]	94.1±4.0
MBM	56.5±2.6[c]	52.2±1.9[c]	92.0±4.7
CM	84.3±11.2[ab]	91.3±4.8[ab]	84.5±4.6
CGM	87.5±10.4[a]	89.2±6.4[ab]	88.4±7.2
SM	85.8±3.3[ab]	95.2±0.4[a]	87.2±2.3
Energy ingredients			
C	94.5±12.3[ab]	76.2±11.5[a]	89.3±10.6[a]
CGRM	48.0±5.7[d]	56.9±2.0[b]	39.5±6.8[d]
RM	71.8±6.5[c]	67.8±7.6[ab]	84.5±5.1[ab]
WB	74.9±10.5[bc]	81.4±2.4[a]	68.8±10.2[bc]
S	97.2±3.5[a]	82.1±6.9[a]	52.0±6.9[cd]

Note: Values are presented as mean ± SE (n = 4). Different letters in the same column denote significant differences (p <0.05) by Tukey´s test. Abbreviations: TPRM, tilapia processing residue meal; FPBM, feather and poultry blood meal; PBM, poultry by-products meal; MBM, meat and bone meal; CM, cottonseed meal; CGM, corn gluten meal; SM, soybean meal; C, corn; CGRM, corn germ meal; RM, rice meal; WB, wheat bran; S, sorghum.

Table 5. Intestinal villi morphometry in piapara fed different diets (µm).

Ingredient	Intestinal villi		
	Height (µm)	Width (µm)	Thickness (µm)
Protein ingredients			
TPRM	377.1±62.1	104.6±13.1	55.1±7.2[a]
FPBM	375.8±59.7	109.0±12.4	57.1±7.4[a]
PBM	423.1±51.7	105.5±7.8	50.3±4.4[ab]
MBM	342.7±42.3	88.8±9.4	41.8±6.6[bc]
CM	369.8±86.5	108.3±15.9	49.5±6.5[abc]
CGM	400.8±44.7	97.6±4.3	42.1±3.7[bc]
SM	382.6±134.5	108.0±36.1	39.3±1.5[c]
Energy ingredients			
C	455.2±54.0[a]	114.6±13.9[a]	47.3±7.9
CGRM	334.2±56.2[b]	96.4±13.7[ab]	45.4±8.9
RM	390.3±73.1[ab]	99.3±10.7[ab]	44.9±6.2
WB	366.6±83.0[ab]	97.9±11.2[ab]	44.3±4.0
S	346.9±21.1[b]	92.2±4.3[b]	42.1±2.7

Note: Values are presented as mean ± SE (n = 4). Different letters in the same column denote significant differences (p <0.05) by Tukey´s test. Abbreviations: TPRM, tilapia processing residue meal; FPBM, feather and poultry blood meal; PBM, poultry by-products meal; MBM, meat and bone meal; CM, cottonseed meal; CGM, corn gluten meal; SM, soybean meal; C, corn; CGRM, corn germ meal; RM, rice meal; WB, wheat bran; S, sorghum.

DISCUSSION

Studies on ADCs for several species have demonstrated the benefits of including fishmeal in diet formulations to maximize the growth of fish due to its balanced amino acid profile. However, the increasing price of this ingredient due to its high demand has prompted the search for alternative protein sources (Mohanta et al., 2009). In the current study, ADCs for protein ingredients of animal and vegetable origin were evaluated with satisfactory results for all ingredients, except meat and bone meal (ADC_{DM} = 56.5%; ADC_{CP} = 52.2% and ADC_{GE} = 92.0%).

The digestibility of an ingredient depends mainly on its chemical composition and the digestive abilities of the species. The digestibility of fishmeal varies according to the quality of the raw material (Fernandes et al., 2004). The digestibility coefficient of dry matter provides an estimate of the overall digestibility of the ingredient. Low DM digestibility coefficient values may indicate the ingredient has a large amount of non-digestible material (Li et al., 2013) or an elevated imbalance between nutrients, which may have influenced the results for meat and bone meal and tilapia processing residue meal. A reduction in dry matter digestibility caused by high levels of mineral matter in the fishmeal has been reported in the Nile tilapia *Oreochromis niloticus* (Kitagima and Fracalossi, 2011) and the channel catfish *Ictalurus punctatus* (Xavier et al., 2014).

Piapara is an omnivorous fish with a diversified diet and high adaptability to different types of feedstuffs. Crude protein digestibility coefficients exceeding 80% were observed for most protein ingredient, except for meat and bone meal (52.2%). The ability to efficiently use nutrients from plant-based ingredients as a result of morphological and physiological adaptations, as demonstrated in Nile tilapia (Gonçalves et al., 2009), may explain the higher coefficients for crude protein observed in piaparas fed corn gluten meal (89.2%), cottonseed meal (91.3%), and soybean meal (95.2%) in our study. Similarly, other studies also reported that vegetable protein ingredients had higher digestibility coefficients than some protein ingredients of animal origin (Pezzato et al., 2002; Sallum et al., 2002; Abimorad and Carneiro, 2004; Oliveira-Filho and Fracalossi, 2006).

Piaparas fed feather and blood meal showed relatively higher digestibility coefficients (ADC_{DM} = 89.0%, ADC_{CP} = 84.9% and ADC_{GE} = 90.8%) compared to Nile tilapia, snakehead *Channa aurantimaculata*, and cobia *Rachycentron canadum* (Pezzato et al., 2002; Yu et al., 2013; Chi and Wang, 2016) fed similar diets. The high ADCs obtained for piapara in our study may be related to the use of a raw material of higher quality, the type of processing of the ingredient, or its lower mineral content.

Among the protein test ingredients, poultry by-product meal showed good results for use in piapara feeds (ADC_{DM} = 83.7%, ADC_{CP} = 90.8% and ADC_{GE} = 94.1%), which are in line with most studies with omnivorous and carnivorous species (Pezzato et al., 2002; Gaylord and Barrows, 2008; Hernandéz et al., 2010; Signor et al., 2012; Yu et al., 2013). Poultry by-product meal is recognized for its high protein and lipid content in addition to well-balanced amino acid profile, which often results in higher digestibility coefficients for crude protein than those observed for fishmeal (Pezzato et al., 2002; Kitagima and Fracalossi, 2011), and thus represents a potential substitute for fishmeal.

In general, piaparas showed good digestibility of the protein test ingredients, except meat and bone meal. Feather and poultry blood meal showed great potential for use in piapara diets, but further research is needed.

The energy ingredients evaluated showed satisfactory digestibility coefficients, except for piaparas fed diets containing corn germ meal, which yielded digestibility coefficients below 60%. Defatted corn germ meal (CGM) is a by-product of starch separation with a high content of crude fiber that contains phytic acid in its composition. In Nile tilapia, increased dietary crude fiber in the diet resulted in lower ADCs (Lanna et al., 2004). In general, fish do not have enzymes capable of digesting the fiber fraction, and the high fiber content in CGM increases the speed of passage of food through the digestive tract (Lanna et al., 2004). Thus, the shorter retention time of CGM may have reduced the efficiency of digestion and absorption of nutrients (Soares et al., 2017).

Corn yielded satisfactory ADCs for piapara (ADC_{DM} = 94.5%, ADC_{CP} = 76.2%, and ADC_{GE} = 89.3%). Corn is an important energy ingredient used in feed that is rich in starch, which is easily digested by animals. Moreover, the high-quality protein in corn combined with the grinding process leave it exposed and readily available, stimulating rapid digestion (Tonini et al., 2012), which explains the high ADC_{CP} found for this ingredient.

Sorghum has a nutritional value similar to that of corn and could be a potential substitute for corn as feed ingredient. However, although crude protein digestibility was high (82.1%), the energy digestibility of sorghum (52.0%) was lower than the coefficients obtained with speckled catfish *Pseudoplatystoma coruscans* (Gonçalves and Carneiro, 2003), red tilapia *Oreochromis* sp. (Campeche et al., 2011), and pacu *Piaractus mesopotamicus* (Sanchez et al., 2016). Sorghum has a large amount of fiber in the form of lignin, pentosans, and cellulose, in addition to phenolic compounds such as tannin (Sanchez et al., 2016) and this large amount of non-starch polysaccharides and indigestible fibers may have contributed to its lower digestibility compared to corn.

Rice meal yielded satisfactory ADCs for protein (67.8%) and gross energy (84.5%) and is another energy ingredient with potential for use in piapara feeds, although it generally has a high concentration of phytic acid. In juvenile grass carp *Ctenopharyngodon idella*, chemical treatment of broken rice to reduce phytic acid content improved productivity and increased deposition of phosphorus in the bones (Costenaro-Ferreira et al., 2013), showing the potential for use of this ingredient through processing.

Intestinal villi are specialized for absorption (Junqueira and Carneiro, 2012) and can undergo changes in their morphology and histology in response to the ingredients in the diet (Schwarz et al., 2011). Histological analysis revealed significant differences in the villus epithelium thickness of piaparas fed diets with different protein sources. The smaller thickness observed in piaparas fed soybean meal (39.3 μm) may be related to the antinutritional factors in soybeans causing the villi to taper (Van Den Ingh et al., 1991), which may trigger inflammation in the intestinal mucosa

(Sohrabnezhad et al., 2017) depending on the tolerance of each species and the type of processing (Escaffre et al., 2007).

Intestinal changes associated with the inclusion of plant sources in the diets may be related to the large amount of structural carbohydrates and antinutritional factors such as enzyme inhibitors or components capable of generating an inflammatory response in the villi, which can damage the gastrointestinal tract and reduce the ADC of nutrients and adversely affect the growth of fish (Martínez-Llorens et al., 2012). The more intact the mucosa is, the greater the villus height and the absorptive capacity of nutrients in the villi (Junqueira and Carneiro, 2013).

Villus height was significantly increased in piaparas fed corn (455.2 µm) than in fish fed diets with sorghum (346.9 µm) and defatted corn germ meal (334.2 µm). All the energy ingredients tested are of vegetable origin, and thus rich in carbohydrates and fiber that can induce fermentation in the fish gut, in addition to antinutritional factors as observed in pacu (Fabregat et al., 2011) and jundiá *Rhamdia quelen*) (Pretto et al., 2017). The small villus width in piaparas fed sorghum (92.2 µm) suggests that the presence of tannin in its composition caused a reduction in intestinal absorptive capacity and decreased the digestibility of energy.

CONCLUSION

Overall, digestibility coefficients were high for all protein ingredients with the exception of meat and bone meal, indicating that both animal and vegetable based ingredients have potential for use in piapara diets. Among the protein ingredients, protein digestibility was highest for soybean meal. Digestibility coefficient values were satisfactory for most energy ingredients, except for corn germ meal.

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