FISH PROTEIN HYDROLYSATE AS AN INGREDIENT IN DIETS FOR *Arapaima gigas* JUVENILES*

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ABSTRACT

This study evaluated the dietary inclusion of a fish protein hydrolysate (FPH) derived from from tilapia trimmings, on physiological and growth parameters of juveniles of *Arapaima gigas*. A total of 180 arapaima juveniles (91.4 ± 2.7 g) were used in a complete randomized design with six treatments (n = 3). Fish were fed to apparent satiation four times a day for eight weeks, with diets containing increasing inclusion levels of FPH (0, 4, 8, 12, 16 and 20%). FPH diets did not affect growth and hemato-biochemical parameters of arapaima juveniles. The FPH from tilapia trimmings seems to be a suitable ingredient for arapaima over 90 g feeds, at least up to 20% inclusion level. No bioactive effects of the FPH could be detected.

Keywords: alternative ingredient; tilapia trimmings; carnivorous fish

HIDROLISADO PROTÉICO DE PEIXE EM DIETAS PARA JUVENIS DE *Arapaima gigas*

RESUMO

Este estudo avaliou a inclusão na dieta de um hidrolisado de proteína de peixe (FPH) derivado de aparas de tilápia sobre parâmetros fisiológicos, crescimento e composição proximal do músculo de juvenis de *Arapaima gigas*. Cento e oitenta juvenis de arapaima (91,4 ± 2,7 g) foram utilizados em delineamento inteiramente casualizado, com seis tratamentos (n = 3). Os peixes foram alimentados até saciedade aparente quatro vezes por dia durante oito semanas, com dietas contendo níveis crescentes de inclusão de FPH (0, 4, 8, 12, 16 e 20%). As dietas de FPH não afetaram o crescimento e os parâmetros hemato-bioquímicos de juvenis de arapaima. O FPH de aparas de tilápia parece ser um ingrediente adequado para rações de arapaima acima de 90 g, pelo menos até 20% de inclusão. Nenhum efeito bioativo da FPH pode ser detectado.

Palavras-chave: ingrediente alternativo; aparas de tilápia; peixe carnívoro

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INTRODUCTION

Arapaima gigas is one of the most important native species for Brazilian aquaculture. It is a carnivorous species with big growth rates, high fillet yield and high market value, which makes it very attractive for fish farming (IMBIRIBA, 2001; OLIVEIRA *et al.*, 2012; VALLADÃO *et al.*, 2016). However, there are limitations in juveniles supply mainly due to absence of techniques for artificial reproduction and high mortality rates observed in arapaima early life (NÚÑEZ *et al.*, 2011; LIMA *et al.*, 2015). Diets with an optimal cost-effectiveness and reducing susceptability to oportunistic pathogens are also lacking for this species. The use of diets with high biological value and immunostimulant action ingredients could reduce growth losses and mortality rates due to parasites.

Fish protein hydrolysates (FPH) have been described to possess bioactive peptides and amino acids which can act as antibiotics, antibacterial agents, antioxidants, and regulators of the activity of certain digestive enzymes (GILL *et al.*, 1996). FPH are considered potential ingredients for the aquafeed industry, due to its high protein content, as well as having flavoring action (CONCEIÇÃO *et al.*, 2012; HE *et al.*, 2013). The inclusion up to 10% FPH in juvenile diets for croaker, *Pseudosciaena crocea*, provided improvements in growth and immunological parameters (TANG *et al.*, 2008). However, these benefits are not present for all species. The addition of 15% and 25% FPH in diets for juvenile turbot, *Scophthalmus maximus*, reduced protein and energy digestibility due to gastrointestinal disorders (OLIVA-TELES *et al.*, 1999).

The objective was to evaluate the dietary inclusion level of protein fish hydrolyzate on growth and haematological parameters of arapaima juveniles.

MATERIAL AND METHODS

This study has been approved by the Ethical Committee of Animal Experimentation and Research of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil (Protocol Number 016/2016).

Arapaima juveniles (91.4 ± 2.7 g; 23.0 ± 0.6 cm) were simultaneously housed (10/0.15 m^3) in 150 L tanks, adding up 18 experimental units with open water system and constant aeration, under a full randomised design with six treatments and three replicates, at Fish Farming Station of Instituto Nacional de Pesquisas da Amazônia (INPA, Manaus, Brazil).

Temperature (29.09 ± 0.64 ^oC), dissolved oxygen (6.49 ± 0.51 mg L^{-1}) (YSI Pro20 Dissolved Oxygen Meter) and pH (5.84 ± 0.43) (YSI Pro10 pH) of water were daily monitored. Every week water total ammonia (0.00 mg L^{-1}) and nitrite (0.00 mg L^{-1}) concentrations were measured according to VERDOUW *et al.* (1978) and BOYD and TUCKER (1992) respectively. Water parameters remained within the comfort range for arapaima (CAVERO *et al.*, 2003; NÚÑEZ *et al.*, 2011; OLIVEIRA *et al.*, 2012).

Six isonitrogenous (46% CP) and isocaloric (4200 kcal kg^{-1}) diets were formulated with increasing levels (0, 4, 8, 12, 16 and 20%) of fish protein hydrolysate from tilapia trimmings (FPH) (Table 1). Fish protein hydrolyzed, ingredients and feedstuff were analyzed to centesimal composition (AOAC, 2005) at INPA's Fish Nutrition Laboratory.

The ingredients were milled, homogenized (0.9 mm), hydrated (20% water: volume), and pelleted (4-5 mm). The diets were dried in forced air ovens (50 ° C, 24 h) and stored (-4 °C) until use.

Fish were fed experimental diet four times a day (8:00, 11:00, 14:00 and 17:00 h) during eight weeks. At the end of growth assay, fish were fasted during 24 h.

The following performance parameters were evaluated according to the formulas (NRC, 2011):

- Survival Rate (SR) = (final number of fish x 100)/initial number of fish;
- Individual Weight Gain (IWG) = final weight (g) – initial weight (g);
- Feed Intake (FI) = (initial feed weight (g) - final feed weight (g))/number of fish;
- Feed Conversion Ratio (FCR) = feed intake (g)/weight gain (g);
- Daily Weight Growth (DWG) = WG (g)/days of experiment (days);
- Relative Growth Rate (RGR) = (e^g-1) x 100; where: e = nepper number; g = (ln(final weight) – ln(inicial weight))/(length of the assay period);
- Hepatosomatic Index (HSI) = liver weight (g)/body weight (g) x 100.

Table 1. Percentage and chemical composition of experimental diets for juvenile *Arapaima gigas* fed diets containing increasing levels of fish protein hydrolysate.

Ingredient (%)	FPH levels (%)					
	0	**4**	**8**	**12**	**16**	**20**
FPH Falbom [a, b]	0.0	4.0	8.0	12.0	16.0	20.0
Fish meal	30.0	30.0	30.0	30.0	30.0	30.0
Poultry viscera meal	19.0	15.4	12.5	8.4	5.4	3.0
Corn meal	15.0	15.0	15.0	14.0	14.0	13.5
Wheat meal	12.0	10.7	9.1	9.0	7.7	6.5
Soybean meal	10.0	10.0	10.0	10.0	10.0	9.3
Blood meal	4.0	4.0	4.0	4.0	4.0	4.0
Gelatine	4.0	4.4	4.5	5.0	5.0	5.5
Corn gluten	3.0	3.0	3.0	3.0	3.0	3.0
Soybean oil	1.0	1.5	1.9	2.5	2.9	3.2
Vitamin/mineral supplement [c]	2.0	2.0	2.0	2.0	2.0	2.0
Chemical composition and Gross energy						
Dry matter (%)	95.8	95.8	95.9	95.9	95.6	95.9
Ash (%)	9.4	9.2	9.2	9.3	9.5	10.2
Crude lipids (%)	7.4	7.3	7.5	7.4	7.5	7.5
Crude protein (%)	47.6	47.6	46.4	46.6	45.9	45.2
Gross energy (kcal kg $^{-1}$)	4235.2	4222.4	4194.4	4195.1	4179.3	4166.7

[a] *FPH amino acid composition (g 100 g^{-1}): arginine (2.8); histidine (0.65); isoleucine (1.54); leucine (2.87); lysine (3.17); methionine (1.01); phenylalanine (1.66); threonine (1.64); tryptophan (0.31); valine (1.99); cysteine (0.3); tyrosine (1.12); glutamic acid (5.42); glycine (4.35); serine (1.53); proline (2.66); alanine (3.02); aspartate (3.61); taurine (0.32); hidroxiprolina (1.28);* [b] *Chemical composition: dry matter (92%); ash (8.6%); lipids (1%); protein (41.25%);* [c] *Premix Nutron®. Brazil: Mn 26 mg; Zn 140 mg; Fe 100 mg; Cu 14 mg; Co 0.2 mg; I 0.6 mg; Se 0.6 mg. A 10000 UI; D3 4000 UI; E 100 mg; K 5 mg; B1 25 mg; B2 25 mg; B6 25 mg; B12 30 mg; niacin 100 mg; folic acid 5 mg; panthotenic acid 50 mg; biotin 0,8 mg; colin 2000 mg; inositol 50 mg; C 350 mg.*

Blood samples (1.5 mL) were taken by caudal venipuncture (6 fish tank^{-1}), with ethylenediamine tetraacetic acid (EDTA-10%) coated syringes for hematological and biochemical parameters evaluation. From these a subsample (3 fish tank^{-1}) were euthanized with anesthetic overdose (0.4 mL eugenol L^{-1} water) to collect the liver and calculate the HSI. From these subsamples, fish muscle were collected and frozen to posterior chemical composition analysis.

The blood samples were used for hematocrit (GOLDENFARB *et al.*, 1971) and hemoglobin [Hb] (BLAXHALL and DAISLEY, 1973) percentage analysis. Erythrocyte number (RBC) was counted in a Neubauer chamber in diluted blood (1:200) (NATT and HERRICK, 1952). Hematimetric parameters such as mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were obtained according to WINTROBE (1934). Determination of glucose, total protein, cholesterol, triglycerides and albumin from plasma were performed after whole blood centrifugation (4º C, 12000 rpm/180 s), using commercial kits and spectrophotometric readings.

Data with parametric distribution (FI, FCR, DWG, RGR, SR, Hb, Ht, MCHC, glucose, protein, cholesterol, triglycerides and albumin) were analysed by one-way ANOVA (p<0.05). Data with nonparametric distribution (HSI, RBC, MCH, MCV) were analyzed by Kruskal-Wallis test (p<0.05). Data under the significance level were submitted to regression analysis to find the best model fit (CurveExpert Professional v 2.6.2). Since no model tested showed a suitables fit, Tukey test (p<0.05) were used to narrow down the dose interval in order to obtain de closest value (ZAR, 2013).

RESULTS

All diets were equally accepted by fish, and no mortalities were observed during the trial. Growth parameters and HSI were not affected by inclusion of FPH in the diets (Table 2). Arapaima juveniles show a RGR of 1.5% day^{-1} (coefficient of variation (CV) = 4.3%); growing 2.2 g day^{-1} (CV = 7.8%) and 70 g month^{-1} (CV = 7.1%) among treatments.

All haematological and biochemical parameters, exception for haematocrit (Ht), showed no diferences among treatments (p>0,05). The 12% FPH diet decreased Ht compared to the control (Table 3).

Table 1. Performance parameters (mean ± standard deviation) of *Arapaima gigas* juveniles fed diets with increasing levels of fish protein hydrolysate (FPH) for 60 days. FI: feed intake; IWG: individual weight gain; FCR: feed conversion rate; DWG – daily weight gain; RGR: specific growth rate; SR: survival rate.

Parameter	FPH Levels (%)‡						*p-value*
	0	**4**	**8**	**12**	**16**	**20**	
FI (g)	281.86 ± 12.6	260.00 ± 7.5	259.85 ± 14.9	259.09 ± 5.0	262.93 ± 18.5	275.78 ± 7.1	0.15^{ns}
IWG (g)	158.59 ± 6.5	132.67 ± 2.5	148.54 ± 1.4	135.05 ± 3.5	131.29 ± 3.0	136.36 ± 2.1	0.92^{ns}
FCR	1.78 ± 0.7	1.96 ± 0.3	1.75 ± 0.1	1.92 ± 0.4	2.00 ± 0.4	2.02 ± 0.2	0.95^{ns}
DWG (g day^{-1})	2.52 ± 1.0	2.10 ± 0.4	2.36 ± 0.2	2.14 ± 0.5	2.08 ± 0.5	2.17 ± 0.3	0.91^{ns}
RGR (% day^{-1})	1.56 ± 0.4	1.41 ± 0.1	1.52 ± 0.1	1.45 ± 0.2	1.40 ± 0.2	1.44 ± 0.1	0.92^{ns}
HSI (%)	1.72 ± 0.4	1.61 ± 0.2	1.63 ± 0.4	1.59 ± 0.3	1.61 ± 0.6	1.47 ± 0.4	0.90^{ns}
SR (%)	100	100	100	100	100	100	-

ns: no significance (p>0.05).

Table 3. Haematological and biochemical parameters (mean ± standard deviation) of *Arapaima gigas* juveniles fed diets with increasing levels of fish protein hydrolysate (FPH) for 60 days. Hb: hemoglobin; Ht: hematocrit; Et: erythrocyte; MCHC: mean corpuscular hemoglobin concentration; MHC: mean corpuscular hemoglobin; MVC: mean corpuscular volume; Glc: glucose; Prt: total protein; Cls: total cholesterol; Trg: triglyceride; Alb: albumine.

Parameter	FPH Levels (%)‡						*p-value*
	0	**4**	**8**	**12**	**16**	**20**	
Hb (g dl^{-1})	10.47 ± 1.4	10.28 ± 1.1	10.47 ± 1.2	9.85 ± 1.6	10.20 ± 1.3	9.84 ± 1.4	0.54^{ns}
Ht (%)	32.33 ± 2.6^{b}	29.86 ± 2.3^{ab}	31.06 ± 2.0^{ab}	28.61 ± 4.2^{a}	30.69 ± 2.6^{ab}	30.06 ± 2.3^{ab}	0.01*
Et (x10^{6})	1.44 ± 0.3	1.47 ± 0.1	1.48 ± 0.1	1.39 ± 0.2	1.51 ± 0.2	1.50 ± 0.2	0.30^{ns}
CHCM (g*dL*$^{-1}$)	32.43 ± 3.7	34.48 ± 3.4	33.70 ± 2.6	34.39 ± 2.5	33.18 ± 3.0	32.71 ± 3.6	0.26^{ns}
HCM (*pg*)	79.60 ± 32.9	70.10 ± 8.7	70.81 ± 7.6	71.04 ± 6.7	68.42 ± 10.4	66.32 ± 11.9	0.56^{ns}
VCM (*fL*)	243.04 ± 85.5	203.19 ± 14.2	203.26 ± 14.2	206.61 ± 12.4	206.11 ± 27.2	203.02 ± 30.3	0.43^{ns}
Glc (mg dL^{-1})	22.34 ± 9.6	19.09 ± 8.6	24.67 ± 10.2	24.66 ± 14.3	18.68 ± 8.3	25.56 ± 12.6	0.24^{ns}
Prt (g dL^{-1})	3.44 ± 0.5	3.43 ± 0.6	2.91 ± 0.9	3.22 ± 0.8	3.23 ± 0.8	3.29 ± 0.8	0.33^{ns}
Cls (mg L^{-1})	88.59 ± 28.0	82.93 ± 28.0	76.92 ± 20.5	81.41 ± 30.3	76.40 ± 24.1	87.31 ± 24.1	0.61^{ns}
Trg (mg L^{-1})	46.72 ± 13.1	43.91 ± 12.6	55.08 ± 18.5	48.20 ± 18.0	45.97 ± 15.7	48.88 ± 17.7	0.41^{ns}
Alb (g dL^{-1})	0.84 ± 0.1	0.80 ± 0.1	0.76 ± 0.1	0.78 ± 0.2	0.79 ± 0.1	0.81 ± 0.1	0.48^{ns}

ns: no significance. Means with different superscripts in the same line are significantly different (p<0.05).

DISCUSSION

Fish protein hydrolysate inclusion did not influence feed palatability provided by its free amino acids content, which can act as feeding attractant (CHOTIKACHINDA *et al.*, 2013). It has also been reported that prolonged or uncontrolled hydrolysis of fish protein may result in bitterness (KRISTINSSON and RASCO, 2000), decreasing fish intake. Good acceptance of all experimental feeds and the similar feed intake suggest that diets showed no bitter taste.

Amino acids are considered at feed stimulants for most fish species (VELEZ *et al.*, 2007; BARATA *et al.*, 2009). However, turbot juveniles, *Scophthalmus maximus*, fed diets up to 35% FPH in substitution of fish meal showed no feed intake increase, as inosins rather than free amino acids are probably associated with feeding stimulation in this species (MACKIE and ADRON, 1978). It should be tested in the future whether free amino acids are in fact phagostimulants for arapaima.

Some studies show better growth performance with high inclusion levels of FPH, as observed in Sea bass, *Dicentrarchus labrax,* fed diets up to 25% FPH (KOTZAMANIS *et al.*, 2007) and in salmon fed with up to 24% FPH inclusion (HEVRØY *et al.*, 2005), and juvenile croaker, *Pseudosciaena crocea,* with FPH inclusion up to 10% (TANG *et al.*, 2008). In the present study, the zootechnical parameters were similar to that observed for arapaima reared in experimental conditions according to ITUASSÚ *et al.* (2005) that reported the individual weight gain of 110.9 ± 56.0 g and 1.5 ± 0.5% of specific growth rate in arapaima juveniles (initial weight of 120.7 ± 3.5 g) fed a diet with 43.4% of crude protein for 45 days. However, our results for arapaima as well as the results for *Steubacheridion melanodermatum* fed diets containing 4% and 6% of tilapia and sardine protein hydrolysate, respectively (LEWANDOWSKI *et al.*, 2013), and for juvenile turbot, *Scophthalmus maximus*, with the addition of 15% and 25% FPH (OLIVA-TELES *et al.*, 1999), show no benefits of FPH on growth performance. In the later study FPH even lead to reduced protein and energy digestibility due to gastrointestinal disorders. So there may be species specificity for tolerance to higher levels of FPH, eventually with faster growing species (e.g., croaker and arapaima) tolerating higher levels compared to slower growing fish (e.g., turbot). The explanation for these conflicting results can also be related with FPH quality which is influenced by raw material and the manufacturing process conditions (FURLAN and OETTERER, 2002).

Moreover, it would be of interest to test high FPH hydrolysates in larvae and post-larvae stages of arapaima, as positive effects of FPH inclusion levels up to 10% feed basis, on growth performance and intestinal maturation, have been demonstrated in early stages of marine fish (CAHU and ZAMBONINO INFANTE, 2001; CONCEIÇÃO *et al.*, 2011). FPH diets showed no indication of negative or positive effects on arapaima welfare. This is in line with observations for turbot (*S. maximus*) fed up to 20% FPH diets (XU *et al.*, 2016) and red snapper (*P. major*) fed with protein hydrolyzate from krill (*Euphausia superba*), shrimp (*Litopenaeus vannamei*) and tilapia (BUI *et al.*, 2014). Furthermore, hematocrit values do not indicate any change in metabolism as observed for juvenile coho salmon (*Oncorhynchus kisutch*) (MURRAY *et al.*, 2003).

Fish protein hydrolysates could enhance several health aspects of fish due to putative bioactive compounds positively affecting fish immune system (MURRAY *et al.*, 2003; KHOSRAVI *et al.*, 2015). Haemato-biochemical parameters are important tool for evaluating the stressing agents effects which may compromise animal health (BLAXHALL, 1972; WENDELAAR BONGA, 1997). From our results, it is possible to suggest that diets formulated with FPH did not impair the physiological homeostasis of fish, as observed in *Pagrus major* fed with marine protein hydrolysates (KHOSRAVI, 2015). In addition, the inclusion of these bioactive compounds did not compromise the lipid and protein metabolism of fish, which is desirable for the maintenance of their performance; this same situation was observed in *Pagrus major* (BUI *et al.*, 2014; KHOSRAVI *et al.*, 2015) and *Cyprinius carp* L. (LATIF *et al.*, 2016) fed diet with marine and vegetable proteins hydrolysate, respectively. However, the haemato-biochemical parameters analized in the present study do not provide evidence for the presence of such bioactive peptides in the tested FPH based on tilapia trimmings. Still, further studies would be needed to confirm this, including monitoring of

innate immune parameters and performance of challenge trials with pathogens.

CONCLUSIONS

FPH from tilapia trimmings can be safely used as an ingredient for feeds of arapaima over 90 g, with an inclusion level up to 20%. No bioactive effects of the FPH could be detected.

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