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Tilapia by-product hydrolysate powder in diets for Nile tilapia larvae

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ABSTRACT. This study aimed to evaluate the digestibility of tilapia by-product protein hydrolysate powder (TBHP) from tilapia filleting by-product, and its inclusion in diets for Nile tilapia larvae. In order to determine the apparent digestibility coefficients, two diets were formulated, the reference diet and the test diet (20% of TBHP) and, to evaluate the inclusion, six diets were formulated, which were with 0.0, 2.0; 4.0; 6.0, and 8.0% of TBHP. The apparent digestibility coefficients of crude protein and gross energy were 89.5 and 98.3%, respectively. At levels above 4.0% of inclusion of TBHP, the weight, final length and weight gain were impaired, however, survival rate was higher ($p < 0.05$). The TBHP can be used in diets for tilapia due to the high digestibility coefficients for crude protein and gross energy. The inclusion of up to 4.0% of TBHP does not affect the reproductive performance and survival rate of Nile tilapia larvae.

Keywords: digestibility, fish nutrition, performance.

Hidrolisado proteico seco do resíduo da filetagem de tilápias em dietas para larvas de tilápia do Nilo

RESUMO. Objetivou-se avaliar a digestibilidade do hidrolisado proteico seco do resíduo da filetagem de tilápias (HPST) e sua inclusão em dietas para larvas de tilápia do Nilo. Para determinar os coeficientes de digestibilidade aparente, duas dietas foram elaboradas, referência e teste (com 20% do HPST) e para avaliação da inclusão, seis dietas foram formuladas com 0,0, 2,0; 4,0; 6,0, e 8,0% de HPST. Os coeficientes de digestibilidade aparente da proteína e energia bruta foram de 89,5 e 98,3%, respectivamente. Nos níveis acima de 4,0% de inclusão de HPST o peso e comprimento final e ganho em peso foram prejudicados, no entanto a sobrevivência foi maior ($p < 0,05$). O HPST pode ser utilizado em dietas para tilápias em função dos elevados coeficientes de digestibilidade para proteína bruta e energia bruta. A inclusão de até 4,0% de HPST não afeta o desempenho produtivo e a sobrevivência de larvas de tilápias do Nilo.

Palavras-chave: digestibilidade, nutrição de peixes, desempenho.

Introduction

The processing of residue from the Nile tilapia, including heads, viscera, skin and bones, represent a significant volume in the acquisition of the fillet, the main product sold. The fillet yield in the processing industry is 34-37% (Nguyen et al., 2010) generating more than 60% of residue (Chalamaiah, Kumar, Hemalatha, & Jyothirmayi, 2012), which display high level of high quality protein and can be used in human and animal nutrition after processing (De Paris et al., 2016).

Pursuant to other sectors such as poultry and pigs, the aquaculture sector tends to the full use of fish in order to reduce costs and add value to the product. Mechanically separated meat, the tanned skin, flour and hydrolysates are some of the products that can add value to this remainder, which can be intended for human consumption, animal feeding (flour and hydrolyzed) and clothing and accessory industry (skin). Other products such as collagen and gelatin can also be obtained from residue such as skin and scales, with use in different areas (Huang, Kuo, Wu, & Tsai, 2016; Zhang et al., 2016), such as the food industry as edible biofilms (Hosseini, Rezaei, Zandi, & Ghavi, 2013; Weng, Zheng, & Su, 2014).

The production of hydrolysates occurs under controlled temperature and pH conditions, with application of enzymes with high affinity with different substrates, like the tilapia processing residues (Robert et al., 2015; De Paris et al., 2016), in order to obtain a high quality product in comparison to the fish meal (Silva, Ribeiro, Silva, Cahú, & Bezerra, 2014). Its quality is given by the high protein content, the presence of peptides, free amino acids and flavoring components, source of essential nutrients such as amino acids and fatty acids (Dieterich et al., 2014), and bioactive components with antioxidant function (Samaranayaka & Li-Chan, 2011; Chalamaiah et al., 2012).

However, the application of hydrolysates in industrial processes, its storage and transportation are obstacles, because they are in liquid form, as highlighted by De Paris et al. (2016). The same authors, using spray drying technique in the protein hydrolysates produced from the residue of the tilapia filleting (head, carcass and skin), obtained positive results with the recovery of the product in dry form, which has a high nutritional quality and microbiological safety. Thus, this process tends to facilitate the handling and stability of the product. According to Robert et al. (2015), the characteristics attributed in *in vitro* studies of the hydrolyzed residue of tilapia filleting, such as high nutritional potential, balanced aminoacitic profile, antimicrobial activity and others, should also be evaluated in *in vivo* assays.

In this context, this study aims to evaluate the digestibility and inclusion in the diet for Nile tilapia larvae of the tilapia filleting by-product powder (TBHP), obtained by enzymatic process using the carcass, head and skin and subjected to spray drying.

Material and methods

Digestibility evaluation

The study was conducted in the laboratory of Aquaculture and Fish Nutrition, of the Grupo de Estudos de Manejo na Aquicultura (GEMAq), from the Universidade Estadual do Oeste do Paraná (Unioeste), campus of Toledo, State Paraná, Brazil, approved by the Ethics Committee in Animal and Practical classes experimentation - CEEAAP/ Unioeste under animal testing protocol number 149996.

Two diets were elaborated in order to evaluate digestibility, the reference and test diets, both with 0.01% chromic oxide added as inert marker. The test diet contained 20% of TBHP and 80% of the reference diet (Table 1). The diets were extruded in an Extruder Ex Micro® and dried in forced ventilation oven for 48 hours at 55°C.

It was employed 180 tilapias with average weight of 55.1±3.52 g, randomly distributed in eight cylindrical tanks of conical bottom, with feces collector. The fish passed through a period of seven days of adaptation period, followed by the collection period. The feeding was performed five times a day (08, 11; 13:30; 15:30 and 17:30 hours) until apparent satiety.

Table 1. Reference diet.

Ingredients	Quantity (%)
Soybean	28.60
Rice	25.00
Corn grain	16.88
Fish meal	15.00
Wheat	8.00
Poultry meal by-products	5.00
Premix-APP	1.00
Salt	0.30
Propionic acid	0.20
Antioxidant (BHT)	0.02
Total	100.00

After the collection period, the feces were dried in forced ventilation oven at 55°C for 72h and it was carried out physical and chemical analysis (crude protein, ether extract, dry matter, mineral matter and energy) as well as the feed and the test ingredient, according to Association of Official Analytical Chemists (AOAC, 2000). It was also held chromium oxide analysis of feces and diets, according to the methodology described by Bremer Neto, Graner, Pezzato, Padovani, and Cantelmo (2003).

The obtained results were used to calculate the diet digestibility coefficients, according to the following Equation 1:

$$CDA\ (\%) = 100 - \{100 \times [(\%Indicator_D / \%Indicator_F) \times (N_F/N_D)]\} \quad (1)$$

where:

$CDA(\%)$ = Apparent digestibility coefficient in the diet;
$\%\ Indicator_{Diet}$ = Percentual of the indicator present in the diet;
$\%\ Indicator_{Feces}$ = Percentual of the indicator present in the feces;
N_{Feces} = Quantity of nutrients in the feces;
N_{Diet} = Quantity of nutrients in the diet.

Then, it was calculated the TBHP digestibility coefficient Equation 2:

$$CDA_{ing} = CDA(\%)\ Dt + (CDA(\%)\ Dt - CDA(\%)\ Ref)*[b*N_{ref})/ a*N_{ing})] \quad (2)$$

where:
CDA_{ing} = Coefficient of digestibility of the ingredient;
$CDA(\%)_{Dt}$ = Coefficient of digestibility of the test diet;
$CDA(\%)_{Ref}$ = Coefficient of digestibility of the reference diet;

N_{Ref} = Quantity of nutrients in the reference diet.

Performance of Nile tilapia larvae

To evaluate the inclusion of dry protein hydrolysate from tilapia filleting in diets for Nile tilapia larvae, five diets were formulated based on vegetable ingredients, being one control diet without adding TBHP, and four with the inclusion of 2, 0; 4.0; 6.0 and 8.0% of the test ingredient based on test results obtained by digestibility coefficients (Table 2).

Table 2. Percentage composition and provision of the diets with the inclusion of dry protein hydrolysate from tilapia filleting by-product (TBHP) used in the experiment.

Ingredient	TBHP inclusion (%)				
	0.0	2.0	4.0	6.0	8.0
Soybean meal	39.59	37.95	36.31	34.67	33.03
Corn gluten (60%)	23.39	23.15	22.92	22.69	22.46
Broken rice	10.36	10.68	11.00	11.33	11.65
Corn grain	10.00	10.00	10.00	10.00	10.00
Soybean protein hydrolysate	5.00	5.00	5.00	5.00	5.00
Soybean oil	3.50	3.27	3.04	2.81	2.58
Dicalcium phosphate	3.15	2.72	2.28	1.85	1.41
Wheat gluten	2.00	2.00	2.00	2.00	2.00
Premix-APP[1]	1.00	1.00	1.00	1.00	1.00
L-lysine HCL	0.62	0.58	0.55	0.52	0.49
Salt	0.30	0.30	0.30	0.30	0.30
Limestone	0.26	0.52	0.77	1.03	1.28
L-threonine	0.26	0.25	0.24	0.24	0.23
Antifungal[2]	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.10	0.10	0.10	0.10	0.10
Propionic acid	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.07	0.07	0.07	0.07	0.06
L-tryptophan	0.03	0.04	0.04	0.04	0.04
Antioxidant (BHT)[3]	0.02	0.02	0.02	0.02	0.02
TBHP	0.00	2.00	4.00	6.00	8.00
Provision					
Lysine (%)	2.20	2.20	2.20	2.20	2.20
Methionine (%)	0.75	0.75	0.75	0.75	0.75
Threonine (%)	1.70	1.70	1.70	1.70	1.70
Tryptophan (%)	0.43	0.43	0.43	0.43	0.43
Total lipids (%)	5.24	4.99	4.73	4.476	4.22
Crude protein (%)	40.50	40.55	40.61	40.66	40.72
Digestible energy (kcal kg^{-1})	3500.0	3500.0	3500.0	3500.0	3500.0
Digestible protein (%)	38,60	38,60	38,60	38,60	38,60

[1]Security levels by kilogram of product: vit. A - 500.000 UI; vit. D3 - 200.000 UI; vit. E - 5.000 mg; vit. K3 - 1.000 mg; vit. B1 - 1.500 mg; vit. B2 - 1.500 mg; vit. B6 - 1.500 mg; vit. B12 - 4.000 mg; folic acid - 500 mg; calcium pantothenate - 4.000 mg; vit. C - 15.000 mg; biotin - 50 mg; inositol - 10.000; nicotinamide - 7.000; choline - 40.000 mg; cobalt - 10 mg; cooper - 500 mg; iron - 5.000 mg; iodine - 50 mg; manganese - 1.500 mg; selenium - 10 mg; zinc - 5.000 mg; [2]Algomix Agrobusiness Ltda; [3]BHT = butyl hydroxy toluene.

It was used 450 Nile larvae tilapia distributed in 30 aquariums with working volume of 25 L. Each aquarium was filled with 15 larvae with an average weight of 0.065±0.008 g and total length of 1.57±0.12 cm. The larvae were fed with their respective diets four times a day (08, 11, 14 and 17 hour), and the tanks were cleaned in the first and after the last feeding, with renovation of 20% of the water of the aquariums.

To evaluate the zootechnical performance it was obtained weight values (g), total length (cm), weight gain (final weight - initial weight), final biomass (g) and survival rate (%). The data of productive performance of the larvae were submitted to analysis of variance ($p < 0.05$) between inclusion levels TBHP and when significant, Tukey test was used to compare the averages. Analyses were performed in the Statistica 7.0 program.

Results and discussion

Digestibility evaluation

The chemical composition values, apparent digestibility coefficient, digestible protein and digestible energy of TBHP are shown in Table 3. The chemical composition of the obtained hydrolysates of the residue from fish processing or from the whole fish varies widely, mainly attributed by the origin and composition of the raw material or process applied to obtain the final product (Abdul-Hamid, Bakar, & Bee, 2002; Dieterich et al., 2014; De Paris et al., 2016). Abdul-Hamid, Bakar, and Bee (2002) reported percentages of crude protein of 37.7 to 49.6% total fat 2.6 to 2.8% and moisture of 1.6 to 4.0% in dry hydrolysates by spray drying tilapia meat. De Paris et al. (2016), evaluating the process of drying by atomization of protein hydrolysates of filleting residue (carcass, head and skin), found values of crude protein, total fat, humidity and ash of 44.80, 0.32, 2.14 and 21.06%, respectively, a similar chemical composition to the one of TBHP.

Table 3. Chemical composition, apparent digestibility coefficient, digestible protein and energy from dry tilapia filleting by-product (TBHP).

Chemical composition		Apparent digestibility coefficient	
Crude protein (%)	47.25	Crude protein (%)	89.61
Gross energy (kcal kg^{-1})	3676.50	Gross energy (%)	98.31
Dry matter (%)	94.89	Dry matter (%)	79.52
Ash (%)	19.58		
Total lipids (%)	2.27		
Calcium (%)	0.73	Digestible protein (%)	42.34
Phosphorus (%)	4.51	Digestible energy (kcal kg^{-1})	3614.37

The composition of the hydrolysates is reflex of the substrate used as a raw material (Klompong, Benjakul, Kantachote, & Shahidi, 2009; Dieterich et al., 2014), as well as the centrifugation steps and filtration when applied for the removal of solids and when it is necessary the lipid fraction. For industry, it is important the implementation of measures that aim at standardization of raw materials, as the manufacturing stages, in order to obtain a product with quality and constant chemical composition required by market.

The TBHP digestibility coefficients were high for protein (89.61%) and gross energy (98.31%), in order to facilitate their inclusion in diets for tilapia. The results of this study corroborate with Abdul-Hamid et al. (2002), that, when evaluating dry hydrolysates of tilapia meat *in vitro,* reported values ranging from 88.4 to 92.0% of protein digestibility. Bui, Khosravi, Fournier, Herault, and Lee (2014) evaluated different protein hydrolysates for *Pagrus major* and observed higher digestibility values of crude protein (90.0%) for animals fed with diets with protein hydrolysate of tilapia.

The benefits of the use of protein hydrolysates related to the digestibility are associated with the product composition that shows low molecular weight peptides (Aksnes, Hope, Jönsson, Björnsson, & Albrektsen, 2006; Chalamaiah et al., 2012; Dieterich et al., 2014, Silva et al., 2014; Robert et al., 2015) which contributes to its increased absorption, resulting in high digestibility (Abdul-Hamid et al., 2002; Aksnes et al., 2006; Dekkers, Raghavan, Kristinsson, & Marshall, 2011; Bui, Khosravi, Fournier, Herault, & Lee, 2014).

Performance of the larvae

The inclusion of up to 4.0% of TBHP in diets for tilapia larvae did not affect the productive performance of the animals. Larvae fed with levels of 6,0 and 8,0% of TBHP presented final weight, final length and inferior weight gain. However, survival was significantly greater in the treatment with 8,0% of TBHP when compared to the control, demonstrating positive effect on the survival of larvae, fundamental in production efficiency at this stage (Table 4).

Table 4. Productive performance of Nile tilapia larvae fed with diets containing levels of dry tilapia filleting by-product (TBHP).

Parameters	Inclusion of TBHP (%)				
	0.0	2.0	4.0	6.0	8.0
Initial weight (g)	0.06	0.06	0.06	0.06	0.06
Initial length (cm)	1.57	1.57	1.57	1.57	1.57
Final weight (g)	1.52[a]	1.30[ab]	1.27[ab]	1.10[bc]	0.89[c]
Final length (cm)	4.33[a]	4.08[abc]	4.08[ab]	3.85[bc]	3.70[c]
Weight gain (g)	1.49[a]	1.22[ab]	1.21[ab]	1.04[bc]	0.82[c]
Biomass (g)	9.76	8.05	12.27	10.36	10.34
Survival (%)	42.70[b]	41.30[b]	64.00[ab]	62.70[ab]	77.30[a]

Distinct lower case in the same line indicate significant difference by Tukey test ($p < 0,05$) for inclusion levels of TBHP.

According to Baldisserotto (2013), the inclusion of hydrolyzed protein diets for fish larvae can be beneficial until a certain percentage, because they are more readily absorbed by the enterocytes, due to stimulate the activity of intracellular peptidases and thus facilitating the assimilation of the amino acids. However, the absorption of nutrients and the productive performance of the animals can be affected by the inclusion of high percentages of hydrolysates in the diet, fact that was found by Espe, Sveier, Høgøy, and Lied (1999) and Hevroy et al. (2005). They evaluated different levels of fish hydrolysates for the Atlantic salmon (*Salmo salar*) and found a decrease in productive performance of animals fed with high levels of the test ingredient.

These results can be explained by the fact that high levels of hydrolyzed reflect the decrease of secretion of digestive enzymes by the large amount of small peptides and free amino acids in the gastrointestinal tract (Ovissipour, Kenari, Nazari, Motamedzadegan, & Rasco, 2014). Moreover, the high amount of these nutrients in the intestine increases absorption, blood plasma concentration and consequently, its catabolism. Both by increasing the degradation of newly synthesized proteins as well as by the oxidation of amino acids, since they cannot be stored in the tissues, making them unavailable for the synthesis of new proteins (Espe, Sveier, Høgøy, & Lied, 1999; Hevroy et al., 2005).

Bui et al. (2014) evaluated the replacement of fishmeal by different types of hydrolysates, being present the dry protein hydrolysate of tilapia processing residue in *Pagrus major* diets, and they reported positive effect on animal performance in relation to the final weight, specific growth and efficiency protein rate. However, it is noteworthy that it was used the amount of 4.0% of the different types of hydrolysates in diets tests, confirming the results observed in this experiment to the same level. These authors also observed a higher ultimate survival in animals subjected to challenge by intraperitoneal injection of the pathogen *Edwardsiella tarda*, when compared to treatment without hydrolysate protein in the diet.

The increased survival observed due to the increase of hydrolysate levels in the diet may be related to the stimulation of the immune system of animals. According to Zheng, Liang, Yao, Wang, and Chang (2013), this is due to the fact that hydrolysates show low molecular weight peptides with bioactive properties, immunostimulant and antibacterial function. Studies have shown that the activity of lysozyme, a protein related to the control of pathogens in fish (Puangkaew et al., 2004), was higher in animals fed with the hydrolysate protein of fish (Liang, Wang, Chang, & Mai, 2006; Tang, Wu, Zhao, & Pan, 2008; Hermannsdottir et al., 2009).

The immunostimulatory effects of fish hydrolysate protein may contribute to the control of diseases that devastate fish farming (Murray et al. 2003; Martínez-Alvarez, Chamorro, & Brenes, 2015) in a practical way by the inclusion in diets (Bui et al., 2014), reducing the use of chemicals that are difficult to implement and bear high environmental impact.

Conclusion

The TBHP may be used in diets for tilapia due to the high digestibility coefficients for crude protein and gross energy. The inclusion of up to 4.0% TBHP does not affect the productive performance and survival of Nile tilapia larvae.

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