

Short Communication

Compelling palatability of protein hydrolysates for Nile tilapia juveniles

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ABSTRACT. This study aimed at determining the compelling palatability of different protein hydrolysates for Nile tilapia juveniles (*Oreochromis niloticus*). Four isoproteic (30% crude protein) and isoenergetic (3,200 kcal kg⁻¹) experimental diets were formulated, with a 5% inclusion level of poultry hydrolysate protein (PHF), or swine liver hydrolysate protein (PHS), or feather hydrolysate protein (PHP) and a control treatment containing 5% of fishmeal (FPE). Four juveniles (2.9 ± 0.01 g) were distributed in 10 L tanks and fed four times a day, and before each feeding event, diets were drawn. The same amount of pellets from each diet was offered, and feeding behaviors were recorded during three minutes for each feeding event, referred to the time of capture of the first pellet, number of pellet rejections, and number of approximations without capturing pellets and quantity of consumed pellets. A significant effect ($P < 0.05$) was observed regarding a higher consumption of pellets and palatability index for PHF, followed by PHS, FPE, and PHP. Therefore, it was concluded that PHF provided the highest compelling palatability for Nile tilapia juveniles, by displaying a 10.82% increase of the palatability index, 17% of final feed consumption, and presented a rejection number 6.89 times lower than FPE.

Keywords: *Oreochromis niloticus*; feed attractant; palatability agent; nutrition; behavior; aquaculture

Nile tilapia *Oreochromis niloticus* is a well-adapted species that inhabits several regions of the world, especially in places with tropical and subtropical climatic conditions, which are considered favorable for its adaptability, rearing and good performance (Higuchi *et al.*, 2013; Brito *et al.*, 2017).

In intensive fish farms, feed usually represents high operational costs, reaching up to 70% of the total expenses (Crivellini *et al.*, 2006; Furuya, 2010), with protein is the most expensive ingredient of feeds, whilst is the principal nutrient related to a fish's growth (Boscolo *et al.*, 2005; Tacon & Metian, 2008; Furuya, 2010; NRC, 2011; Zho & Yue, 2012). One way of reducing costs with commercial feeds is reducing the use or completely replacing fishmeal, but a significant obstacle concerning this alternative is the acceptability of different ingredients by the animals, seen that diets rich in fishmeal has a well-known acceptance due to its high palatability (Kotzamanis *et al.*, 2007).

The hydrolysis process is a chemical reaction performed with the addition of specific enzymes that modify the functional properties of raw materials when added, thus promoting protein breakdown in amino acids and peptides of multiple sizes, with high protein levels and digestibility (Ronnestad *et al.*, 2007; Fries *et al.*, 2011). This process provides the cleavage of proteins, resulting in nitrogen compounds of low molecular weight, which may act as attractive feeding substances since they are detected by the fish's gustatory system (Halver & Hardy, 2002; Broggi *et al.*, 2017).

Research shows that diets containing fish-based hydrolysate proteins blended with other animal's processing wastes, even in low levels, have been presenting positive effects on productive performance, immune system activities and digestibility in the first developmental stages of several species (Chotikachinda *et al.*, 2013; Ovissipour *et al.*, 2014; Srichanun *et al.*,

2014; Silva *et al.*, 2017). Therefore, the present study was designed to determine the compelling palatability of poultry hydrolysate protein (PHF), swine liver hydrolysate protein (PHS), and feather hydrolysate protein (PHP) for Nile tilapia.

The essay was performed in the Laboratory of Aquaculture of the Aquaculture Management Study Group - GEMAg, of the University of West Paraná (UNIOESTE), Campus Toledo, PR, Brazil, from September 2016 to January 2017. The procedures used in the present study were approved by the Ethics Committee on Animal Use (CEUA) of the State University of West Paraná, according to Experimental Certificate of Animal Use N°45/17.

Four isoproteic (30% crude protein, CP) and isoenergetic (3,200 kcal kg⁻¹) experimental diets were formulated, according to the nutritional recommendations proposed by Furuya (2010). The three hydrolysates diets were produced and supplied by the company BRF Ingredients. The hydrolysates were made through a process of enzymatic hydrolysis in one of the factories of the company in Concórdia-SC, Brazil. The evaluated diets were: FPE (control) = diet with a 5% inclusion level of fishmeal; PHF = diet with 5% inclusion level of poultry hydrolysate protein; PHS = diet with 5% inclusion level of swine liver hydrolysate protein; and PHP = diet with 5% inclusion level of feather hydrolysate protein (Table 1).

Ingredients were grounded and sieved (0.3 mm) in a hammer type mill previously to diets' formulation. For the extrusion of diets, blends were moistened with 20% of water and homogenized, then placed in a mechanical mixer type "Y" for 15 min. The diets were extruded (1.0 mm) in an equipment model Ex-Micro, which holds a capacity of 10 kg h⁻¹. After extrusion, all diets were dried in a forced ventilation oven for 24 h (55°C).

Four juveniles with a mean weight of 2.9 ± 0.01 g were placed in four aquariums (10 L) individually coated with a waterproofing latex material and equipped with aeration systems and temperature control by using 15 W thermostats. Each aquarium had a transparent lid with a centralized hole for pellets' addition.

Throughout the experiment, water temperature, pH and dissolved oxygen were, respectively: 25.12 ± 1.15°C, 6.68 ± 0.26 and 4.55 ± 0.64 mg L⁻¹, being following the recommended by Ridha & Cruz (2011) for the species' development. A polystyrene barrier was placed around the aquariums, isolating them from the laboratory routine, thus minimizing possible effects on the animals' behavior.

All four fish underwent an adaptation and training 15 days, so they would adapt to human presence during feeding events, where behaviors were registered, and the number of pellets was counted, until apparent satiety. At this time, fish were fed a commercial diet containing 40% CP, and its pellets were 1 mm. After the adaptation period, the essay was initiated.

Fish were fed four times a day at 8, 11, 14 and 17 h. Daily, all aquariums were siphoned to remove feces and food scraps. In feeding events, each fish received 20 pellets of the diet. The experimental design was completely randomized, with four replicates per day. A draw for each diet was daily performed for each fish, and a 3 min filming period was set with a camera (model BE Unique 4K Ultra HD) when the pellets were introduced in the aquariums. The essay lasted for eight days, and 128 footages were obtained (4 fish × 4 feedings, 16 essays per day), and for each tested feed, 32 essays were performed.

The essays were performed based on the methodology described by several authors (Kasumyan & Morsi, 1996; Kasumyan & Doving, 2003; Kasumyan & Sidorov, 2012). These authors proposed the palatability index as a quantitative estimate of taste preference (in percentage), using the following calculation: $IP = ((R-C)/(R+C)) \times 100$, in which IP is the palatability index, R refers to pellet consumption of the tested feed and C is the pellet consumption of the control diet.

After data collection, all footages were examined, and the following feeding behaviors were analyzed: a) capture the first pellet (seconds), b) rejections of the pellet after capture, c) approaches without capturing pellets, and d) the number of consumed pellets. The palatability index was calculated for each treatment. The data were submitted to parametric variance analysis (ANOVA), and in case of significant effect ($P < 0.05$), the Tukey's multiple comparison test was performed at a 5% significance level. Before all analyses, the errors normality (Shapiro-Wilk) and homogeneity of variances (Levene's test) were verified. All statistical evaluations were performed with the aid of the software Statistic 7.1 (2005).

Significant differences were observed ($P < 0.05$) in pellets consumption and number of rejections after capture (Table 2). The PHF presented higher compelling palatability, with its palatability index being 10.82%, higher than PHS (8.73%) and PHP (-6.66%), in comparison to the diet containing FPE. The mean value of pellet consumption referred to the PHF treatment was higher ($P < 0.05$) in comparison to PHP, but equal to the others ($P > 0.05$). An increase of 17% on pellets consumption was observed in PHF in relation to FPE. PHP provided the lowest consumption.

Table 1. Composition of experimental diets used in the evaluation of compelling palatability with Nile tilapia (*Oreochromis niloticus*). ¹Crude protein content. ²Guarantee levels per kilogram of the 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 mg; nicotinamide - 7,000 mg; choline - 40,000 mg; cobalt - 10 mg; copper - 500 mg; iron - 5,000 mg; iodine - 50 mg; manganese - 1,500 mg; selenium - 10 mg; zinc - 5,000 mg. ³Butil-hidroxi-toluene.

Ingredients	Treatments			
	Fishmeal (Control)	Swine liver hydrolysate protein	Feather hydrolysate protein	Poultry hydrolysate protein
Soy bran (45%) ¹	52.20	50.74	49.82	50.47
Maize (8.5%) ¹	21.88	22.62	22.20	22.85
Rice grits (8.5%) ¹	10.00	10.00	10.00	10.00
Wheat bran (15.6%) ¹	5.00	5.00	5.00	5.00
Fishmeal (55%) ¹	5.00	0.00	0.00	0.00
Swine liver hydrolysate protein (70%) ¹	0.00	5.00	0.00	0.00
Feather hydrolysate protein (79%) ¹	0.00	0.00	5.00	0.00
Poultry hydrolysate protein (72%) ¹	0.00	0.00	0.00	5.00
Soybean oil	3.08	2.66	3.97	2.72
Dicalcium phosphate	1.34	1.99	2.02	1.91
Mineral-vitamin supplement	0.50	0.50	0.50	0.50
Calcitic lime	0.35	0.85	0.83	0.90
Salt	0.30	0.30	0.30	0.30
Choline chloride (60%)	0.10	0.10	0.10	0.10
Antifungal (Calcium propionate)	0.10	0.10	0.10	0.10
Vitamin C (35%)	0.10	0.10	0.10	0.10
Antioxidant (BHT) ³	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Calculated Values				
Starch (%)	29.19	29.47	29.09	29.58
Calcium (%)	1.00	1.00	1.00	1.00
Digestible energy (kcal kg ⁻¹)	3,200	3,200	3,200	3,200
Total phosphorus (%)	0.80	0.80	0.80	0.80
Fat (%)	5.52	5.12	6.08	5.02
Crude protein (%)	30.00	30.00	30.00	30.00

Significant differences were observed in the feeding behavior referred to the number of rejections after capture ($P < 0.05$). The lowest mean value of this variable was observed in PHF, with 0.29 pellets, followed by PHS with 1.15 pellets, while the highest rejection rate was observed in PHP, with 3.12 pellets. However, no differences were verified between FPE, PHF and PHS ($P > 0.05$), but the data allows the observation that PHF presented several pellet rejections 6.89 times higher about FPE. Approximations without capturing pellets were 0.43 for PHF, 0.62 for PHS, 1.00 for FPE and 1.37 for PHP.

Although this study did not focus on the chemical composition of the ingredients, it should be stressed that free amino acids were already studied and evaluated as feed stimulants (Table 3). The feed's amino acid composition was assessed by the method MA-009 (White *et al.*, 1986; Hagen *et al.*, 1989) at a commercial laboratory (CBO Laboratory Analysis Ltda., Valinhos-SP).

The differences found for the palatability index, pellets consumption and number of rejections after capture for PHF might be related to the content of amino acids present in protein hydrolysates coming from by-products of the poultry industry (Oliveira *et al.*, 2014), seen that PHF presented a higher amino acid content in comparison to PHS and PHP (Table 3). Amino acids are essential components for a diet's palatability since they send chemical signs to the animal's gustatory system. The presence of free amino acids and peptides of low molecular weight is important for a diet's palatability (Naylor *et al.*, 2009; Kasumyan & Doving, 2003; NRC, 2011).

Concerning the feeding behavior referred to the time of capture of the first pellet, no differences were observed ($P < 0.05$) (Table 2). However, it was verified that for all diets, fish rapidly captured pellets, showing no rejection. The rapid pursuit for feed is one of the stages of the feeding behavior that may be stimulated by using ingredients in the diet with good tractability. When stimulating the extraoral taste response of the

Table 2. Mean values of the compelling palatability test of different protein hydrolysates for Nile tilapia (*Oreochromis niloticus*), compared to fishmeal (control). Means followed by distinct letters within columns indicate significant differences by the Tukey's test ($P < 0.05$).

Treatments	Palatability index (%)	Pellets consumption (%)	Number of rejection of pellets after capture	Number of approximations without capturing pellets	Time for the capture of the first pellet (seconds)
Control	0	83.64 ± 22.03 ^{ab}	2.00 ± 2.09 ^{ab}	1.00 ± 1.34	0.87 ± 0.45
Poultry hydrolysate protein	10.82	97.86 ± 5.67 ^a	0.29 ± 0.75 ^b	0.43 ± 0.78	0.85 ± 0.39
Swine liver hydrolysate protein	8.73	89.37 ± 18.21 ^{ab}	1.15 ± 2.10 ^{ab}	0.62 ± 1.06	0.68 ± 0.42
Feather hydrolysate protein	-6.66	74.37 ± 26.38 ^b	3.12 ± 2.99 ^a	1.37 ± 1.84	0.92 ± 0.45

Table 3. Free amino acids of the protein hydrolysates used in the evaluation of compelling palatability for Nile tilapia (*Oreochromis niloticus*).

Chemical Composition	Poultry hydrolysate protein (%)	Swine liver hydrolysate protein (%)	Feather hydrolysate protein (%)
Aspartic acid	0.81	0.45	0.04
Glutamic acid	1.11	1.05	0.10
Serine	0.56	0.40	0.02
Glycine	0.36	0.18	Undetected
Histidine	0.23	0.42	0.17
Taurine	0.36	0.08	0.05
Arginine	1.42	0.24	0.03
Threonine	0.66	0.56	0.10
Alanine	1.08	1.08	0.05
Proline	0.49	0.46	0.06
Tyrosine	1.01	0.49	0.10
Valine	1.17	0.89	0.11
Methionine	0.79	0.27	0.10
Cystine	0.16	0.15	Undetected
Isoleucine	0.91	0.60	0.14
Leucine	1.91	1.54	0.13
Phenylalanine	0.97	0.67	0.08
Lysine	1.36	0.86	0.03
Asparagine	Undetected	0.40	0.02
Glutamine	Undetected	0.18	Undetected
Total	15.36	10.97	1.33

animal, one triggers the behavior of searching for food (Kasumyan & Doving, 2003). The rapid capture is desirable in aquaculture, seen that it may reflect in lower losses of nutrients to the water, resulting in complete ingestion of the balanced diet, thus causing less environmental impact (Cyrino *et al.*, 2010).

The feeding stimulants that presented higher consumption percentages were PHF and PHS. When studying poultry viscera meal as attractants for Nile tilapia juveniles, Boscolo *et al.* (2001) also observed satisfactory results. Swine liver, tilapia carcass, and sardine hydrolysates were evaluated in the diet of Iguaçú surubim *Steindachneridion melanodermatum* and *Rhamdia quelen* by Lewandowski *et al.* (2013) and Decarli *et al.* (2016). These authors highlighted that, in

comparison to their control treatments, the swine liver hydrolysate provided the greatest results of weight gain, the final length of fish and feed conversion.

The process of enzyme hydrolysis produces free amino acids and peptides that besides improving diet's acceptance by fish, may improve its performance and health because during the enzymatic hydrolysis many bioactive peptides are formed (Kotzamanis *et al.*, 2007). Therefore, PHF and PHS have great potential in aquaculture. However, it is understood that more studies are necessary in order to evaluate the potential of hydrolysates in the improvement of performance and health status.

The PHF flavoring increased palatability index and final feed consumption and provided the lower rejec-

tion of pellets, as well as a lower number of approximations without capturing the pellets in relation to the control diet containing fishmeal. Therefore, it can be efficiently used to stimulate the consumption of feed for Nile tilapia juveniles with fishmeal are replaced.

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