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Digestibility of swine liver and meat protein hydrolysates by Nile tilapia

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Abstract – The objective of this work was to evaluate the digestibility of the protein, amino acids, and gross energy of swine liver and meat hydrolysates by Nile tilapia (*Oreochromis niloticus*). The fish were distributed into 12 tanks with conical bottoms, in a completely randomized design, and fed with the three following diets, with four replicates each: a reference diet with soybean and fish meal; and two test diets, one with swine liver hydrolysate and the other with swine meat hydrolysate. The coefficients of apparent digestibility were high for both hydrolysates, being above 83% for dry matter, 95% for crude protein, and 92% for gross energy. Regarding amino acids, the coefficients remained at 98–100% for the two hydrolysates. The digestibility percentages of the hydrolysates were higher than those of the protein ingredients, both of plant and animal origin, commonly used in the formulation of diets for this fish species. The tested hydrolysates have potential to be used in the formulation of diets for Nile tilapia.

Index terms: *Oreochromis niloticus*, animal by-products, aquaculture, digestible nutrients, protein hydrolysate.

Digestibilidade de hidrolisado proteico de fígado e carne suínos pela tilápia-do-nilo

Resumo – O objetivo deste trabalho foi avaliar a digestibilidade da proteína, dos aminoácidos e da energia bruta de hidrolisado de fígado e carne suínos pela tilápia-do-nilo (*Oreochromis niloticus*). Os peixes foram distribuídos em 12 tanques com fundo cônico, em delineamento inteiramente causalizado, tendo sido alimentados com as seguintes três dietas, com quatro repetições cada uma: dieta referência com farinha de soja e de peixe; e duas dietas teste, uma com hidrolisado de fígado suíno e outra com hidrolisado de carne suína. Os coeficientes de digestibilidade aparente foram elevados para ambos os hidrolisados, tendo sido acima de 83% para matéria seca, 95% para proteína bruta e 92% para energia. Em relação aos aminoácidos, os coeficientes permaneceram entre 98–100% para os dois hidrolisados. Os percentuais de digestibilidade dos hidrolisados foram maiores do que os dos ingredientes proteicos, tanto de origem vegetal como animal, comumente utilizados na fabricação de dietas para esta espécie de peixe. Os hidrolisados testados têm potencial para serem usados na formulação de dietas para tilápia-do-nilo.

Termos para indexação: *Oreochromis niloticus*, subprodutos animais, aquacultura, nutrientes digestíveis, hidrolisado proteico.

Introduction

Protein is the main component of organic tissues in animals and an essential nutrient for the growth and feed efficiency of fish (Hou et al., 2017). The diets offered to aquatic organisms reared in captivity are formulated with a blend of raw materials of both animal and plant origin, and protein ingredients represent the highest cost in the manufacturing process (Guimarães et al., 2008a).

Each ingredient, however, has at least one limiting characteristic in relation to its use, such as the anti-nutritional factors of plant ingredients, which reduce the bioavailability of nutrients for the organism (Dong et al., 2010). In the case of fish meal, the main input of animal origin in fish diets, such limitations include: inconsistent availability; high variability in terms of composition, which is considered an environmental liability; and high cost, which can hinder aquaculture production as a whole (Palupi et al., 2020).

Therefore, in aquaculture, diets should be formulated using inputs with nutritional quality, regional availability, and a viable cost. Animal byproducts – such as meat and bone, and feather and viscera meals – are widely used as protein ingredients, and, in Brazil, they represent important raw materials for the productive sector of the country, currently the third and fourth largest producer of poultry and swine worldwide, respectively (ABPA, 2020).

Byproducts, with a low added value, have been used as an alternative raw material to obtain high value products, such as fish feed (Gachango et al., 2017). Among these byproducts, those representing 52% of total pig live weight – such as organs, fat or lard, skin, feet, abdominal and intestinal contents, bone, and blood – stand out (Jayathilakan et al., 2012). However, the nutritional quality of these ingredients might be a limiting factor due to their varying composition, high ash content, low nutritional availability, and imbalance of amino acids (Palupi et al., 2020).

One way to improve the nutritional quality of animal byproducts is to manufacture hydrolysates from these raw materials by breaking peptide bonds with enzymes, which results in a product containing free amino acids and small peptides with a low mineral matter content (Soares et al., 2020). Since, in the gastrointestinal tract of fish, the protein is hydrolyzed and absorbed as free amino acids or small peptides that are used in the metabolic reactions of the cells, it is suggested that there is a nutritional demand for this form of protein instead of the long-chain one (Debnath & Saikia, 2021). Studies have also shown that the inclusion of protein hydrolysates in the diets of fish improved their digestibility of protein and amino acids, growth performance, and immune system (Bui et al., 2014; Song et al., 2014; Leduc et al., 2018; Lorenz et al., 2018).

The objective of this work was to evaluate the digestibility of the protein, amino acids, and gross energy of swine liver and meat hydrolysates by Nile tilapia [*Oreochromis niloticus* (Linnaeus, 1758)].

Materials and Methods

The study was approved by the committee on animal research and ethics of Universidade Estadual do Oeste do Paraná, under Ceua Unioeste 2019/45-19, and was carried out at the Laboratory of Aquaculture of the study group on aquaculture management of the university.

The digestibility of swine liver and meat protein hydrolysates (SLH and SMH, respectively), both manufactured by BRF Ingredients (São Paulo, SP, Brazil), was evaluated. The apparent digestibility coefficients were determined for the three following experimental diets: a reference diet made of soybean and fish meal (Table 1), to determine digestible

Table 1. Composition of the reference diet used to determine the apparent digestibility coefficient for Nile tilapia (*Oreochromis niloticus*).

Ingredient	Composition (g kg^{-1})
Corn grain	298.6
Wheat meal	249.6
Soybean meal	212.2
Fish meal	178.2
Rice meal	50.0
Mineral and vitamin mix[(1)]	5.0
Salt	3.0
Choline chloride	1.0
Propionic acid	1.0
Antioxidant (BHT)	0.2
Vitamin C	1.0
Total	1,000.00

[(1)]Levels of guarantee per kilogram of product: 10,000,000 UI vitamin A, 4,000,000 UI vitamin D3, 150,000 mg vitamin E, 100,000 mg vitamin K3, 25,000 mg vitamin B1, 25,000 mg vitamin B2, 25,000 mg vitamin B6, 30,000 mcg vitamin B12, 100,000 mg niacin, 50,000 mg calcium pantothenate, 6,000 mg folic acid, 1,000 mg biotin, 200,000 mg inositol, 1,000 mg iron, 800 mg iodine, 30,000 mg manganese, 140,000 mg zinc, 800 mg selenium, 18,000 mg copper, 200 mg cobalt, 124,000 mg ethoxyquin, and 450,000 mg potassium sorbate.

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nutrients; and two test diets, consisting of 80% of the reference diet plus 20% of each tested ingredient, i.e., of SLH and SMH (Table 2). The experiment was performed in a completely randomized design, with four replicates per treatment.

The ingredients used for diet formulation were weighed, milled in a hammer mill with a 0.3 mm sieve, and homogenized according to the treatment. In addition, 0.1% chromium oxide III (Cr_2O_3) was included in the diets as an inert marker. The diets were then processed in an extruding machine and dried in a forced-circulation oven, at 55°C, for 12 hours.

For the experiment, 288 Nile tilapia, with a mean weight and length, respectively, of 185.6±27.8 g and 17.3±0.8 cm, were used, being randomly distributed into 12 tanks with conical bottoms and a useful volume of 500 L. Feeding was performed daily using 3.0% of the biomass (Tran-Ngoc et al., 2019), divided into five feeding events at 8:00 a.m., 11:00 a.m., 2:00 p.m., 4:00 p.m., and 6:00 p.m.

The fish were acclimatized to the experimental conditions for seven days, during which they were fed the experimental diets. Each conical tank (experimental unit) was assigned randomly to one of the three diets, resulting in four replicates per diet and per ingredient. The average of the water parameters in the experimental units was 24.71±0.68ºC, 6.81±0.16, and 5.20±0.15 mg L^{-1} for temperature, pH, and dissolved oxygen, respectively. The parameters were monitored using the YSI Professional Plus multi-parameter water quality meter (YSI, Yellow Springs, OH, USA). Every day, after the first and last feeding

Table 2. Nutritional composition of the ingredients and experimental diets used in the digestibility assay with Nile tilapia (*Oreochromis niloticus*)(1).

Composition ($g\ kg^{-1}$)	Ingredient		Diet		
	SLH	SMH	Reference	SLH	SMH
Dry matter	909.5	948.8	942.8	962.1	957.3
Crude protein	688.9	813.9	293.1	370.9	400.7
Gross energy ($kcal\ kg^{-1}$)	5,297.50	5,009.00	4,193.00	4,645.00	4,452.50
Essential amino acids					
Arginine	34.7	47.9	19.2	23.3	25.7
Histidine	18.9	45.8	7.2	10.0	15.4
Isoleucine	28.4	30.1	10.1	14.2	14.6
Leucine	58.7	54.6	20.3	28.9	27.7
Lysine	53.5	68.5	17.0	25.1	28.3
Methionine	17.6	22.2	5.2	7.8	8.8
Phenylalanine	32.6	26.5	11.9	16.4	15.1
Threonine	31.1	32.4	11.2	15.9	15.9
Tryptophan	3.2	5.2	2.5	3.3	3.5
Valine	38.8	35.3	11.9	18.0	17.1
Total essential amino acids	317.5	368.5	116.5	162.9	172.1
Semi-essential amino acids					
Cysteine	11.5	9.6	2.7	4.9	4.6
Tyrosine	22.6	20.3	7.9	11.3	10.6
Total semi-essential amino acids	34.1	29.9	10.6	16.2	15.2
Non-essential amino acids					
Alanine	40.1	51.8	17.5	22.8	24.9
Asparagine	73.0	85.7	28.1	39.5	39.6
Glutamine	85.2	121.3	47.4	57.1	63.4
Glycine	37.3	60.3	21.9	26.0	30.2
Proline	33.3	42.6	19.8	23.4	25.0
Serine	31.0	30.9	13.4	17.3	17.0
Total non-essential amino acids	299.9	392.6	148.1	186.1	200.1
Amino sulfonic acid					
Taurine	1.2	2.9	1.1	1.1	1.5

(1)SLH, swine liver hydrolysate; and SMH, swine meat hydrolysate.

event, the system was cleaned and 50% of the water inside the tanks was exchanged to remove metabolites. During ten consecutive days, feces were collected at 7:00 a.m. using a detachable 250 mL recipient placed at the bottom of each conical tank, transferred to aluminum trays, and then stored at -20ºC.

The feed ingredients, test diets, and collected feces were subjected to physicochemical analyses and to amino acid profile determination (Table 2). The percentages of dry matter and crude protein were obtained according to the methodology of Association of Official Analytical Chemists (AOAC) (Horwitz, 2000). The gross energy has been verified via an adiabatic oxygen bomb calorimeter (IKA Werke, Staufen, Baden-Württemberg, Germany). To calculate the apparent digestibility of the diets, the chromium oxide of feces and diets were quantified according to Bremer Neto et al. (2003).

The analysis of amino acids was carried out in a UV-VIS auto-analyzer, at 570 nm, using high-pressure liquid chromatography in cation exchange resin columns and post-column derivation with ninhydrin, producing Ruhemann's purple (Friedman, 2004). Before the analysis, the samples were hydrolyzed with HCl 6.0 mol L^{-1} for 22 hours, at 110°C, according to Crestfield et al. (1963). Tryptophan was determined after enzymatic hydrolysis with Pronase, at 40°C, for 24 hours, followed by a colorimetric reaction with 4-(Dimethylamino)benzaldehyde in 10.6 mol L^{-1} sulfuric acid using an automatic UV-VIS analyzer, at 590 nm, as described in Delhaye & Landry (1992). The analyzes were performed at the commercial laboratory CBO Análise Laboratoriais Ltda., located in the municipality of Valinhos, in the state of São Paulo, Brazil.

The coefficients of apparent digestibility for dry matter, protein, amino acids, and gross energy of the evaluated diets (CAD_{Diet}) and ingredients (CAD_{Ing}) were calculated using the following equations:

CAD_{Diet} = 100 - [100×((% marker of diet / % marker of feces)×(% nutrient or gross energy in feces / % nutrient or gross energy in diet))]

$CAD_{Ing} = CAD_{TD} + [(CAD_{TD} - CAD_{RD}) \times ((0.8 \times N_{Ref}) / (0.2 \times NI))]$,

where CAD_{TD} is the coefficient of apparent digestibility of the test diet, CAD_{RD} is the coefficient of apparent digestibility of the reference diet, N_{Ref} is the nutrient (%) or gross energy of the reference diet, and NI is the nutrient (%) or gross energy of the ingredient; the first equation was used to calculate both CAD_{TD} and CAD_{RD}.

The digestible nutrients and gross energy of the ingredients were quantified by the following equation: $DN = (NI / CAD_N) \times 100$, where DN is the digestible nutrient (%) or gross energy (kcal kg^{-1}), NI is the nutrient (%) or gross energy (kcal kg^{-1}) of the ingredient, and CAD_N is the apparent digestibility coefficient of the nutrient (%) or gross energy (kcal kg^{-1}) of the ingredient.

Data were subjected to Shapiro-Wilk's normality test and Levene's homogeneity test. A t-test was applied to check the differences between apparent digestibility coefficients, digestible nutrients, and gross energy of the ingredients of the two test diets. Statistical analyses were performed considering a significance level of 5%, using the Statistica, version 7.0, software (TIBCO Software Inc., Palo Alto, CA, USA).

Results and Discussion

The coefficients of apparent digestibility were high for both hydrolysates, with percentages above 83% for dry matter, 95% for crude protein, and 92% for gross energy (Table 3). SMH showed a higher digestibility of these three parameters, but only stood out statistically regarding crude protein, although it presented higher digestible values for dry matter and crude protein when compared with SLH.

The values obtained for the apparent digestibility coefficients of SLH and SMH show that the hydrolysis process is efficient in releasing nutrients and energy from these raw materials. There were, however, statistical differences for percentage of protein digestibility and digestible protein between SMH and SLH, which is related to the composition of the raw materials used as a substrate for the manufacturing of the hydrolysates, whose chemical composition and nutritional quality are not altered by the hydrolysis process (Dieterich et al., 2014). The amount of crude protein was 81.39 and 68.89% in SMH and SLH, respectively (Table 1), a result similar to that observed when comparing the apparent digestibility percentages of dry matter and gross energy.

The digestibility coefficients of the hydrolysates evaluated in the present study were higher than those of the protein ingredients – such as poultry viscera meal, meat and bone meal, and blood meal – that are commonly used in the formulation of diets for Nile tilapia (Guimarães et al., 2008b; Dong et al., 2010). Furthermore, the coefficients of digestibility of crude protein for SLH and SMH were higher than those reported by Tran-Ngoc et al. (2019) for vegetable meals, such as those of rice, rapeseed, soybean, sunflower, and distiller's dried grain with solubles, showing values of 84.0, 87.8, 92.2, 90.2, and 89.2%, respectively.

The apparent digestibility coefficients of SLH and SMH were higher than those of the hydrolysates from feather meal, feather protein, and hydrolyzed swine liver (Tran-Ngoc et al., 2019; Cardoso et al., 2021). However, only SLH showed high coefficients compared with those found for hydrolyzed swine mucus protein, which were of 100, 97.12, and 96.62% for dry matter digestibility, crude protein, and gross energy, respectively (Cardoso et al., 2021).

The amino acid coefficients of digestibility of both SLH and SMH were high and remained at 98–100% (Table 4). Significant differences were observed for essential amino acids – such as histidine, leucine, lysine, and methionine –, whose percentages were higher for SMH (Table 4). However, the values for

Table 3. Apparent digestibility, digestible nutrients, and gross energy of swine liver and meat hydrolysates (SLH and SLM, respectively) offered to Nile tilapia (*Oreochromis niloticus*)[(1)].

Ingredient	Coefficient of apparent digestibility			Digestible nutrient		
	Dry matter (%)	Crude protein (%)	Gross energy (%)	Dry matter (g kg^{-1})	Crude protein (g kg^{-1})	Gross energy (kcal kg^{-1})
SLH	83.69±5.2	95.74±0.8b	92.37±3.6	761.2±47.1b	659.6±5.4b	4,893.42±190.28
SMH	89.59±2.9	98.27±0.8a	96.76±0.6	850.2±79.8a	799.9±6.6a	4,846.94±29.740

[(1)]Means followed by different letters, in the same column, differ significantly by the t-test, at 5% probability. Data expressed as the mean±standard deviation.

Table 4. Apparent digestibility and digestible amino acids of swine liver and meat hydrolysates (SLH and SLM, respectively) offered to Nile tilapia (*Oreochromis niloticus*)[(1)].

Amino acid	Coefficient of apparent digestibility (%)		Digestible nutrient (g kg^{-1})	
	SLH	SMH	SLH	SMH
Essential amino acids				
Arginine	98.64±0.4	99.28±0.7	34.2±0.1b	47.5±0.3a
Histidine	98.18±0.9b	99.45±0.2a	18.6±0.1b	45.5±0.1a
Isoleucine	97.85±2.0	99.05±0.8	27.8±0.5b	29.8±0.2a
Leucine	97.55±0.9b	98.92±0.5a	57.3±0.5a	54.0±0.2b
Lysine	98.92±0.2b	99.61±0.4a	52.9±0.1b	68.2±0.3a
Methionine	97.71±0.7b	99.02±0.2a	17.2±0.1b	21.9±0.1a
Phenylalanine	97.63±1.4	98.33±0.9	31.8±0.4a	26.0±0.2b
Threonine	95.55±0.8	96.90±0.8	29.7±0.3	31.3±0.2
Tryptophan	98.35±2.8	99.19±0.9	3.1±0.1b	5.1±0.1a
Valine	97.87±2.1	98.76±0.8	37.9±0.8a	34.8±0.3b
Semi-essential amino acids				
Cysteine	97.51±2.2	98.11±1.5	11.2±0.2a	9.4±0.1b
Tyrosine	98.15±1.8	98.82±0.9	22.1±0.4a	20.0±0.1b
Non-essential amino acids				
Alanine	96.70±1.8	98.64±1.0	38.7±0.7b	51.0±0.5a
Asparagine	99.24±0.4	99.33±0.3	72.4±0.3b	85.1±0.3a
Glutamine	98.94±0.4	99.35±0.3	84.2±0.3b	120.5±0.3a
Glycine	96.15±2.0	98.58±0.8	35.8±0.7b	59.4±0.5a
Proline	96.70±1.4	98.25±0.5	32.2±0.4b	41.8±0.2a
Serine	96.99±1.4	97.32±1.8	30.0±0.4	30.0±0.5
Amino sulfonic acid				
Taurine	100.00±0.00	100.00±0.00	1.2±0.0b	2.9±0.0a

[(1)]Means followed by different letters, in the same row, differ significantly by the t-test, at 5% probability. Data expressed as mean±standard deviation.

digestibility only differed for threonine and serine. Regarding the other 17 amino acids, SLH presented a higher digestible composition only for leucine, phenylalanine, valine, cysteine, and tyrosine.

The percentage of amino acids and coefficients of digestibility and, consequently, the value of digestible amino acids of the hydrolysates evaluated in the present study were higher than those found for the ingredients commonly used in the formulation of diets for Nile tilapia, including fish meal, canola meal, meat and bone meal, soybean meal, and protein concentrates and hydrolysates (Xavier et al., 2014; Vidal et al., 2017; Bibi et al., 2021; Cardoso et al., 2021). This result is attributed to the breakage of peptide bonds from proteins by enzymatic hydrolysis, which generates free amino acids or small peptides that improve nutrient bioavailability and use by the organism (Dieterich et al., 2014; Soares et al., 2020). Despite the significant differences observed for the coefficients of digestibility of histidine, leucine, lysine, and methionine, SMH stood out regarding the values for amino acid digestibility due to its higher protein percentage.

In both SLH and SMH, the concentration of tryptophan was lower than that of the other studied amino acids. However, due to its elevated percentage of digestibility, the value of digestible tryptophan was higher than the nutritional demand of this amino acid by Nile tilapia, which is of 2.9 g kg^{-1} (Zaminhan et al., 2017). In comparison, considering that tryptophan is the least present amino acid in raw materials of animal origin, fish meal and meat and bone meal do not provide enough digestible tryptophan to meet the nutritional demands of this fish species (Guimarães et al., 2008a, 2008b).

Considering that lysine and methionine are usually the first limiting amino acids of the ingredients used in the formulation of diets for Nile tilapia, as well as the obtained results, SLH and SMH meet the nutritional demands of this species, which has a higher nutritional requirement for lysine, arginine, and threonine (Furuya, 2010).

Conclusions

1. Swine liver and meat hydrolysates (SLH and SMH, respectively) allow of a high digestibility of dry matter, crude protein, and gross energy, as well as a high use of amino acids, by Nile tilapia (*Oreochromis niloticus*).

2. SLH and SMH are potential ingredients for the formulation of diets for Nile tilapia.

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