

Short Communication

Poultry byproducts and swine liver used in diets for Nile tilapia juveniles

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ABSTRACT. This study aimed at evaluating diets containing protein hydrolysate from poultry byproducts and swine liver (PHPPL), at different inclusion levels, for Nile tilapia (*Oreochromis niloticus*) juveniles. Seven diets were evaluated, being a negative control (free of both hydrolysate and fishmeal) and positive control (free of hydrolysate but containing fishmeal), besides five diets with 1, 2, 3, 4, or 5% inclusion of the mixed protein hydrolysate. Significant effects were observed regarding the animals' final length, with higher values with the 1% inclusion level than the negative control. The liver's quantitative evaluation revealed that inclusions above 3% might lead to severe alterations in the organ's morphology. The use of PHPPL in the diets that did not contain fishmeal has shown to be effective in maintaining the performance parameters of Nile tilapia. Thus, its use is recommended considering an inclusion level of 3%.

Keywords: *Oreochromis niloticus*; byproducts; protein hydrolysates; fish nutrition; histology; aquaculture

Byproducts derived from animal slaughtering industries are increasingly being used for animal diets, can be included in the diet after being transformed into protein hydrolysates (FAO, 2018). Increased knowledge about the biological effects of protein hydrolysates in aquatic animal's diets has been increasing in the last decades. However, many gaps remain, especially regarding species with marketing interests. According to Martínez-Alvarez *et al.* (2015), it is necessary to improve knowledge about the best levels of dietary inclusion of animal byproducts hydrolysates in micro diets and about their biological effects in reared-fish, besides gathering information on the molecular structures of peptide chains of the hydrolysates.

Studies have been carried out to analyze performance parameters in the inclusion of hydrolysates from byproducts of terrestrial animals in fish diets. In a diet offered for *Salminus brasiliensis*, Lorenz *et al.* (2018) observed higher weight gain with the inclusion of a swine liver protein hydrolysate (SLH), while Lewandowski *et al.* (2013) reported an improvement of weight gain, final length, and feed conversion with SLH inclusion in diets for *Steindachneridion melanodermatum*. In the study of Alves *et al.* (2019), the authors displayed positive results regarding the use of poultry hydrolysate protein (PHP) and SLH

as flavor enhancers for Nile tilapia (*Oreochromis niloticus*). However, there are no published results about the effects of Nile tilapia's productive performance regarding the inclusion of PHP and SLH in the diets.

The use of protein hydrolysates in diets for Nile tilapia is commercially relevant, seen that this species stands out in the productive aquaculture chain, as the fourth most produced species in the world (FAO, 2018). In this sense, this study aimed to evaluate the effects of the inclusion of blend protein hydrolysates produced with poultry and swine liver byproducts as a feed ingredient for Nile tilapia juveniles zootechnical performance, proximate body composition, and the histological aspect of the liver.

The study was carried at the Laboratory of Aquaculture of the Aquaculture Management Study Group-GEMAQ, of the University of West Paraná (UNIOESTE), in Toledo/PR, Brazil. The Ethics Committee on Animal Use approved the study, protocol number 17/19-CEUA of the Paraná Federal University.

Five isoproteic (40% crude protein) and isoenergetic (3,462 kcal of digestible energy per kg) experimental diets were used, which contained increasing levels of protein hydrolysate of poultry byproducts

Table 1. Ingredients used in the experimental diets containing different inclusion levels of PHPPL (protein hydrolysate of poultry by-products and swine liver).¹NC: negative control, ²PC: positive control, ³mineral/vitamin supplement (composition: vitamin A, vitamin D3, vitamin E, vitamin K3, vitamin B1, vitamin B2, calcium pantothenate, vitamin B6, vitamin B12, niacin, folic acid, biotin, inositol, iron sulfate, manganese sulfate, zinc sulfate, calcium iodate, sodium selenite, copper sulfate, cobalt carbonate, calcium propionate, antioxidant (BHT), mineral oil, silicon dioxide, propylgalate, citric acid).

Diet	Treatment (%)						
Ingredient	NC ¹	PC ²	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Cornmeal	24.65	25.73	25.08	25.50	25.93	26.35	26.78
Soybean protein concentrate	26.09	21.33	24.82	23.55	22.28	21.01	19.73
Rice grits	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Poultry viscera meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Feather meal	6.80	6.80	6.80	6.80	6.80	6.80	6.80
Blood meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Corn bran (gluten 60%)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fishmeal 55%	0.00	5.00	0.00	0.00	0.00	0.00	0.00
PHPPL	0.00	0.00	1.00	2.00	3.00	4.00	5.00
Soybean oil	3.67	3.30	3.54	3.41	3.28	3.15	3.02
Alcohol yeast	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Wheat gluten	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.95	1.36	1.96	1.97	1.98	1.99	2.01
Lime	0.72	0.36	0.70	0.67	0.65	0.63	0.60
L-lysine HCl	0.58	0.61	0.58	0.57	0.56	0.56	0.55
L-threonine	0.55	0.56	0.55	0.55	0.56	0.56	0.56
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral/vitamin supplement ³	0.60	0.60	0.60	0.60	0.60	0.60	0.60
DL-methionine	0.40	0.38	0.40	0.39	0.39	0.38	0.38
Vitamin C	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Antifungal	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100	100	100
Nutritional levels (%)							
Starch	23.89	24.56	24.15	24.42	24.69	24.95	25.22
Digestible protein	34.45	34.13	34.41	34.36	34.32	34.27	34.23
Lipids	6.69	6.94	6.64	6.00	6.55	6.50	6.46
Total leucine	3.41	3.40	3.41	3.40	3.39	3.38	3.37
Total arginine	2.61	2.55	2.59	2.57	2.55	2.53	2.51
Total lysine	2.60	2.60	2.60	2.60	2.60	2.60	2.60
Total valine	2.23	2.23	2.23	2.23	2.22	2.22	2.22
Total threonine	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Total phenylalanine	2.05	2.00	2.04	2.02	2.01	2.00	1.98
Total isoleucine	1.61	1.59	1.61	1.60	1.59	1.58	1.58
Calcium	1.31	1.31	1.31	1.31	1.31	1.31	1.31
Crude fiber	1.29	1.18	1.26	1.24	1.21	1.18	1.15
Total histidine	1.05	1.03	1.05	1.04	1.04	1.04	1.03
Total phosphorus	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total methionine	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Available phosphorus	0.76	0.83	0.76	0.76	0.76	0.76	0.76
Total tryptophan	0.43	0.41	0.43	0.43	0.43	0.42	0.42

and swine liver (PHPPL), the product provided by the company BRF Ingredients[®] (1, 2, 3, 4 or 5%). A positive control diet (containing fishmeal) and a negative control diet (without hydrolysate or fishmeal) were used. The diets were produced according to the recommendations of Furuya (2010) and NRC (2011) (Table 1). Both the PHPPL and the produced diets were submitted to analysis of the dry matter, crude protein,

ether extract, mineral matter, and energy, according to the methodology of AOAC (2000) (Table 2).

The diet's macronutrients were milled in a hammer-type mill (Moinhos Vieira, model MS 280, Tatuí/SP, Brazil) coupled with a 0.3 mm mesh. After milling, both the micronutrients and protein hydrolysates were added, mixed for 5 min in a manual mixer. The obtained blend was extruded (Extec Máquinas, Ribeirão Preto/

Table 2. Proximate composition of the experimental diets. PHPPL: protein hydrolysate of poultry by-products and swine liver (BRF Ingredients Ltda.). ¹NC: negative control, ²PC: positive control. Analyzed composition (g kg⁻¹ natural matter): 92.20 dry matter; 72.43 crude protein; 6.98 ether extract; 4.62 mineral matter; 4.99 energy. Distribution of molecular weight in Dalton (Da) (% of peptides: <250 Da: 8.88%, 250-500 Da: 31%, 500-1000 Da: 60%.

Chemical analyses (%)	Treatment (%)						
	NC ¹	PC ²	1(%)	2(%)	3(%)	4(%)	5(%)
Dry matter	93.84	93.48	90.85	92.76	94.06	93.60	92.85
Crude protein	39.34	39.03	38.51	39.20	39.49	38.49	38.81
Ether extract	3.45	1.73	2.80	4.43	4.43	2.86	2.71
Mineral matter	7.56	8.44	7.55	7.49	7.77	7.62	7.53
Energy	4.37	4.29	4.41	4.37	4.43	4.40	4.42

SP, Brazil) using a 1 mm matrix at the feed factory of the GEMAq, dried in a forced-ventilation oven (55°C) for 24 h, packed, identified, and stored in a freezer (-18°C).

A total of 336 Nile tilapia juveniles [initial mean of 1.42 ± 0.03 g standard deviation, SD] were used in this study. Fish were randomly distributed in 28 tanks (70 L), composing seven treatments with four replicates (12 fish per tank), in a water recirculation system, coupled with constant aeration and heating, with a 12:12 (L:D) photoperiod. The animals were fed six times a day (08:00-18:00 with 2 h intervals) until apparent satiety. Tanks and filters were cleaned daily throughout the experiment, which lasted for 28 days. Water quality parameters were weekly monitored, and the obtained values (mean \pm SD) were temperature $30.24 \pm 0.82^\circ\text{C}$, pH 6.98 ± 0.17 , dissolved oxygen 4.33 ± 1.22 mg L⁻¹, and electrical conductivity 135.0 ± 9.37 $\mu\text{S cm}^{-1}$, with the aid of a portable probe YSI 556[®]. At the end of the experimental period, fish were fasted for 24 h and euthanized with a eugenol solution (300 mg L⁻¹). Biometric parameters were acquired (weight and total length), followed by the acquisition of viscera weight.

The productive performance was evaluated by: MIW = mean initial weight (g); WG = weight gain (g), (mean final weight - mean initial weight); FL = final length (cm); SGR = specific growth rate (% d⁻¹), [(ln final weight - ln initial weight) / experimental period]; PER = protein efficiency rate (%), (weight gain / consumed crude protein) \times 100; AFC = apparent feed conversion (feed consumption / weight gain); SV = survival [(number of fish/12) \times 100]; and HSI (%) = hepatosomatic index [liver's weight (g) \times 100/final weight (g)]

Samples of the liver were processed for histological analysis. These were fixed, dehydrated in increasing alcoholic series, stained using a series of xylol, embedded in paraffin, sectioned (7 μm) with the aid of a microtome (Microm, International GmbH 69190, Walldorf, Germany) and stained with hematoxylin-

eosin. Histological slides were evaluated with the aid of an optical microscope P1 BX 50 (Olympus-Manila, Philippines), coupled with a camera (Olympus PMC 35-B, Berlin, Germany). Qualitative analyzes of the liver were performed in six samples per treatment. The qualitative evaluation of hepatic vacuolization was performed according to the criteria established by Caballero *et al.* (2004). A vacuolization score was attributed, where 0: not observed, 1: few vacuoles, 2: mean vacuolization, and 3: severe vacuolization (Fig. 1).

The obtained data were submitted to a homogeneity test (Levene), followed by one-way variance analysis (ANOVA) and Tukey's multiple comparison test at a 5% significance level. All statistical analyses were carried out in the statistical software Statistica 7.1 (2005).

The FL was higher for fish fed the diet containing 1% inclusion of PHPPL compared to both the negative control (NC) and the positive control (PC). No significant differences were found for the other parameters, such as WG, SGR, PER, AFC, SV, and HIS ($P > 0.05$) (Table 3). Severe hepatic vacuolization was observed in the negative control and the 4% inclusion diet. The analysis of the hepatic tissue demonstrated cell alterations, with fat depositions and vacuolization being observed. All evaluated diets resulted in alterations; however, the diets NC and the ones with inclusions above 3% demonstrated to be prejudicial for the liver, leading to severe vacuolization (Table 4, Fig. 1).

The maintenance of the zootechnical performance attributes a positive aspect for the preference of PHPPL inclusion, upon the use of fishmeal. Lewandowski *et al.* (2014) evaluated swine liver hydrolysates in diets for jundiá (*Rhamdia voulezi*) and mandi (*Pimelodus britski*) larvae and did not observe prejudices in performance parameters. In this study, the observed values of SV were positive, seen that de diets containing PHPPL did not affect this parameter, which

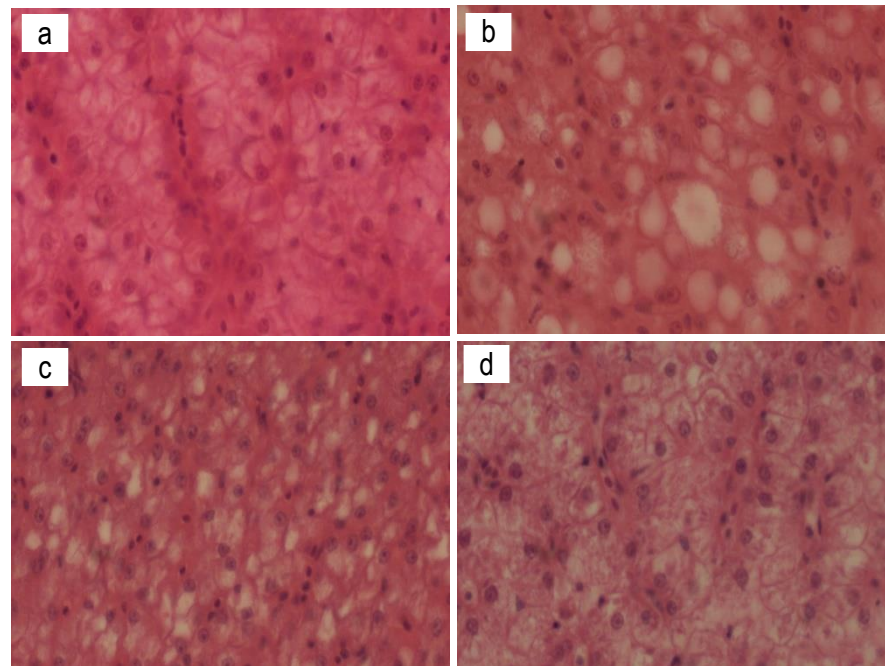


Figure 1. Photomicrography of liver of Nile tilapia (*Oreochromis niloticus*) with hepatocytes vacuolization in different degrees of severity (HE 40x). a) Defined hepatocyte cell limit and well-marked nucleus (score = 0), b) presence of small intracellular vacuoles, without shape alteration, and positioning of hepatocyte nucleus (score = 1), c) hepatocytes with apparent and large vacuoles (score = 2), d) severe vacuolization of the cytoplasm, loss of cellular shape and presence of periphery nucleus (score = 3).

Table 3. Mean values of performance and proximate composition of Nile tilapia (*Oreochromis niloticus*) fed diets containing different inclusion levels of PHPP (protein hydrolysate of poultry by-products and swine liver). According to Tukey's test, means followed by distinct letters within the same line represent a significant difference ($P < 0.05$). ¹NC: negative control (without PHPP and fishmeal), ²PC: positive control (without PHPP, with fishmeal). MIW: mean initial weight, WG: weight gain, FL: final length, SGR: specific growth rate, PER: protein efficiency rate, AFC: apparent feed conversion, SV: survival, HIS: hepatosomatic index.

Variable	Inclusion levels of the protein hydrolysate of poultry byproducts and swine liver (%)						
	NC ¹	PC ²	1%	2%	3%	4%	5%
Performance							
MIW (g)	1.42 ± 0.01	1.42 ± 0.04	1.44 ± 0.01	43 ± 0.03	1.42 ± 0.04	1.41 ± 0.04	1.39 ± 0.02
WG (g)	15.19 ± 1.20	15.30 ± 0.35	17.03 ± 1.49	15.83 ± 1.25	16.20 ± 1.04	15.29 ± 1.42	16.05 ± 1.02
FL (cm)	8.94 ± 0.22b	9.04 ± 0.11b	9.59 ± 0.30a	9.34 ± 0.12ab	9.16 ± 0.27ab	9.37 ± 0.12ab	9.21 ± 0.12ab
SGR (%)	8.77 ± 0.24	8.79 ± 0.04	9.08 ± 0.26	8.88 ± 0.22	8.98 ± 0.16	8.80 ± 0.36	9.00 ± 0.25
PER (%)	2.66 ± 0.38	2.90 ± 0.12	2.85 ± 0.23	2.88 ± 0.25	2.58 ± 0.31	2.70 ± 0.21	2.67 ± 0.25
AFC	1.03 ± 0.14	0.94 ± 0.04	0.95 ± 0.04	0.93 ± 0.08	1.05 ± 0.14	1.00 ± 0.07	1.04 ± 0.10
SV (%)	87.50 ± 10.75	88.88 ± 9.62	100 ± 0.00	97.91 ± 4.16	87.50 ± 15.95	97.22 ± 4.81	91.66 ± 11.78
HIS (%)	2.60 ± 0.18	2.26 ± 0.19	2.28 ± 0.72	2.40 ± 0.27	2.58 ± 0.31	2.32 ± 0.12	2.47 ± 0.40

is an important parameter to be evaluated in experiments considering new feed ingredients (Santos *et al.*, 2019).

Another relevant aspect regards the composition of the peptide fragments, as the PHPP used in this study was composed of 8.88% peptides of <250 Da, 31% between 250-500 Da, and 60% between 500-1000 Da. Aksnes *et al.* (2006) demonstrated that low concen-

trations of peptides <100 Da have influenced in the productive performance of *Gadus morhua* due to reduced feed efficiency, while higher concentrations of small-size peptides are essential for fish's growth and nutrients use. Therefore, it is possible that the size and composition of PHPP fragments did not contribute to the improvement of growth parameters, and there is a need for a balance of peptide size and sufficient amounts in order to influence growth performance.

Table 4. Vacuolization score: 0: unobserved, 1: low vacuolization, 2: mean vacuolization, 3: severe vacuolization.

Inclusion levels of protein hydrolysate of poultry byproducts and swine liver (PHPPL) (%)							
Variables	NC ¹	PC ²	1%	2%	3%	4%	5%
V. score	1-3	0-1	0-2	0-2	0-2	0-3	0-2

Nutrition is closely related to the liver, which may change its function and morphology. The appearance of hepatic steatosis is closely related to nutritional disturbances, especially regarding fatty acids imbalance (Tacon, 1996; Caballero *et al.*, 2004). As demonstrated by Bombardelli *et al.* (2009) and Tessaro *et al.* (2014), there is a close relationship between increased energy levels and hepatic steatosis. Liland *et al.* (2015) obtained similar results with inclusions of byproducts (poultry, poultry grease, and swine blood meal) for Atlantic salmon (*Salmo salar* L.), showing that the diets containing such ingredients led to small and moderate lesions, while diets without hydrolysates similar to a commercial feed (with high plant-based ingredients), presented moderate to severe lesions. In this sense, it can be considered that the diets with up to 3% inclusion of PHPPL displayed an adequate balance of fatty acids (similar to the diet containing fishmeal), as it was possible to observe less vacuolization in the liver.

The PHPPL did not cause issues regarding its use as a feed ingredient for Nile tilapia juveniles in terms of zootechnical performance, and the inclusion level of 1% contributed to the final length of fish, while a 3% inclusion did not lead to liver alterations. Therefore, this inclusion level can be efficiently used in diets for Nile tilapia juveniles to replace fishmeal.

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