Fish protein hydrolysate in diets for Nile tilapia post-larvae

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Abstract – The objective of this work was to determine the apparent digestibility coefficient (ADC) of crude protein, crude energy, fat, and dry matter of fish protein hydrolysate (FPH), made of by-products of Nile tilapia (*Oreochromis niloticus*) and whole sardines (*Cetengraulis edentulus*), and to evaluate the productive performance and muscle fiber growth of Nile tilapia post-larvae. Two trials were conducted, the first one to determine the digestibility in 120 fingerlings (70.0±2.0 g), and the second one to evaluate the productive performance of 375 post-larvae, with three days of age, which were distributed in 25 aquaria with 30 L of useful volume. Five diets were prepared based on vegetable ingredients, to which fish were included at 0, 2, 4, 6, and 8% FPH. For the evaluation of muscle growth, eight fish of each experimental unit were used. The ADC values found were: 98.29% for dry matter; 99.28% for crude protein; and 99.13% for gross energy. The best zootechnical response for the productive performance resulted from the treatment with the inclusion of fish hydrolysate at 4.75%. The diets affected the frequency of the muscle fiber diameters, mainly the growth by hyperplasia. FPH can be efficiently used, and its inclusion at 4.75% is indicated in the diets for Nile tilapia in the post-larvae stage.

Index terms: *Cetengraulis edentulus*, *Oreochromis niloticus*, digestibility, fish meal, nutrition, peptides, pisciculture.

Hidrolisado proteico de peixe em dietas para pós-larvas de tilápia-do-nilo

Resumo – O objetivo deste trabalho foi determinar o coeficiente de digestibilidade aparente (CDA) da proteína bruta, da energia bruta, da gordura e da matéria seca de um hidrolisado proteico de peixe (HPP), feito de resíduos de tilápia-do-nilo (*Oreochromis niloticus*) e sardinha inteira (*Cetengraulis edentulus*), e avaliar o desempenho produtivo e o crescimento das fibras musculares de pós-larvas de tilápia-do-nilo. Realizaram-se dois ensaios, o primeiro para a determinação da digestibilidade em 120 alevinos (70,0±2,0 g) e, o segundo, para avaliação do desempenho produtivo de 375 pós-larvas, com três dias de idade, distribuídas em 25 aquários (unidade experimental) com volume útil de 30 L. Cinco rações à base de ingredientes vegetais foram elaboradas, às quais se incluíram os peixes a 0, 2, 4, 6 e 8% de HPP. Para a avaliação do crescimento muscular, oito peixes de cada unidade experimental foram utilizados. Os valores de CDA encontrados foram: 98,29% para matéria seca; 99,28% para proteína bruta; e 99,13% para energia bruta. As melhores respostas zootécnicas quanto ao desempenho produtivo resultaram do tratamento com a inclusão do hidrolisado proteico a 4,75%. As dietas influenciaram a frequência do diâmetro das fibras musculares, principalmente o crescimento por hiperplasia. O HPP pode ser eficientemente utilizado, e sua inclusão a 4,75% é indicada em dietas de tilápia-do-nilo na fase de pós-larva.

Termos para indexação: *Cetengraulis edentulus*, *Oreochromis niloticus*, digestibilidade, farinha de peixe, nutrição, peptídeos, piscicultura.

Introduction

The production of larvae and fry in the quantity and quality to meet the growing demand of fish farming depends on efficient solutions to problems in the rearing process. Feeding outstands as the main factor among those responsible for failures in larviculture, (Phelps, 2010).

To achieve satisfactory performance indices, it is recommended that, in the initial stage, part of the dietary protein be of animal origin because of the better profile of available amino acids and minerals (Galdioli et al., 2000; NRC, 2011). Therefore, fish meal continues to be the main protein source in diets for most species of farmed fish because of the balanced amino acid profile and the composition of essential

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fatty acids, digestible energy, vitamins, and minerals (Tacon & Metian, 2008).

However, worldwide fish meal production has staganated by approximately 6–7 million tons in recent years, and with the increasing demand, its value has increased, raising production costs in aquaculture systems (Tacon & Metian, 2008; Merino et al., 2010). Therefore, several studies have been conducted on the total or partial replacement of fish meal in the preparation of diets for aquatic organisms, in order to maintain the nutritional standards of the feeds (Teixeira et al., 2006).

An alternative with great potential is the use of by-products of the fish-processing industry, in the form of protein hydrolysate and fish of low-commercial value. The hydrolysis process is defined as the protein breakdown into peptides of various sizes. This breakdown can be achieved chemically (with acids or bases), or biologically (with enzymes). The enzymatic process is more promising, as it generates a product with high functionality and nutritional value (Naylor et al., 2009; Pasupuleti & Braun, 2010).

The composition of a protein hydrolysate, produced from fish residue, may contain on average 78.75% crude protein, 3.42% fat, and 12.51% ash (Nilsang et al., 2005). The partial replacement of the fish meal with this product, even at low percentages, shows a beneficial effect on the productive performance and immunological activity of several fish species, mainly in the early stages of life (Refstie et al., 2004; Hevroy et al., 2005).

Tang et al. (2008) have shown that including up to 10% fish protein hydrolysate (FPH) in the diet of yellow croaker (*Pseudosciaena crocea*) improved the growth and immunological parameters of this fish. Likewise, Lian et al. (2005) and Chotikachinda et al. (2013) found positive effects with FPH supplementation in the diet of the studied species. The results found in these studies may be related to the high palatability of FPH, which contains biologically active, immune-stimulating peptides with antibacterial properties that can be produced during the hydrolysis process (Kotzamanis et al., 2007).

The objective of this work was to determine the apparent digestibility coefficient (ADC) of crude protein, crude energy, fat, and dry matter of FPH, made of by-products of Nile tilapia (*Oreochromis niloticus*) and whole sardines (*Cetengraulis edentulus*), and to evaluate the productive performance and muscle fiber growth of Nile tilapia post-larvae.

Materials and Methods

Digestibility and zootechnical performance trials were carried out at the aquaculture and fish nutrition laboratory of Grupo de Estudos de Manejo na Aquicultura (Gemaq), of Universidade Estadual do Oeste do Paraná (Unioeste), Campus Toledo, in the municipality of Toledo, PR, Brazil.

The production of FPH consisted of 80% residues from Nile tilapia filleting (heads, viscera, scales, fins, vertebral column, and adhered tissues) and 20% whole sardine which were used to enhance the aroma and taste of FPH, in order to improve the final product attractiveness (Broggi, 2014). This raw material was crushed in a CAF food processor , model Boca 5, 250-watt power and 30 kg h^{-1} capacity; then, a water volume equivalent to 20% of the mass value was added and homogenized. The mixture was placed for approximately 5 min in a RW 20 mechanical stirrer (Ika Brasil Equipamentos Laboratoriais, Analíticos e Processos Ltda., Campinas, SP, Brazil). Subsequently, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were added at 0.01%. The temperature was adjusted to 60°C in a water bath to promote the enzymatic activity. Alcalase enzyme was added at 0.5% and stirred continuously for 60 min. After this time, the enzymatic activity was stopped by raising the temperature to 85°C for 15 min, and a food preservative (citric acid) was added. At the end of the process, the liquid hydrolysate was filtered through a 0.5 mm steel sieve to remove small bones.

To determine digestibility, the diets were elaborated with an extruded practical feed (used as reference), and an extruded test feed composed of 80% of the reference feed and 20% of the ingredient to be tested, adding 0.1% chromium oxide, used as inert marker (NRC, 2011) (Table 1).

For the feed production, the ingredients were initially milled in a hammer-type grinder with a 0.7 mm sieve, and, then, weighed and mixed manually. The crushed feed was moistened with 20% water, and extruded in an ExMicro apparatus (Exteec Máquinas, Ribeirão Preto, SP, Brazil), with 10 kg h^{-1} production capacity. After this processing, the feeds were oven dried at 55°C for 12 hours.

Nile tilapia juveniles (120) with a mean mass of 70±2 g were used. Fish were randomly distributed in six 90 L conical tanks suitable for the collection of feces. The juveniles remained in the tanks for seven days in the tanks, in order to adapt to the diets and to the experimental conditions. The treatments, randomly assigned at the beginning of the adaptive period, consisted of three tanks with reference feed, and three tanks with test feed. Fish were fed at 09:00, 12:00, 15:00, and 18:00 h. Two hours after the last feeding, the tanks were cleaned, and 70% of the water volume was exchanged, to avoid contamination of the feces by feed debris. Finally, the collecting cups were attached to the bottom of the tanks and, at 07:00 h of the following day, feces were collected and immediately stored in a freezer at -15ºC until the start of the analyses.

The coefficients of apparent digestibility and gross energy of the nutrients in the diets and FPH were calculated according to the NRC equations (2011), adapted for 20% of the feed inclusion, as follows: $CDA(n) = \{100 - [100 \times (\%Cr_2O_3d \times \%Cr_2O_3f) \times (\%Nf / \%Nd)]\}$, in which: CDA (n) is the nutrient digestibility coefficient; $\%Cr_2O_3d$ is the percentage of chromium oxide in the diet; $\%Cr_2O_3f$ is the percentage of chromium oxide in feces; %Nf is the percentage of nutrient in feces; %Nd is the percentage of nutrient in the diet. And $CDA (i) = \{CDAdt + [(CDAdt - CDAdr) \times (0.8 \times Ddr / 0.2 \times Ding)]\}$, in which: CDA (i) is the apparent digestibility coefficient of the ingredient; 0.8 is the percentage of the reference diet; CDAdt is the apparent digestibility coefficient of the test diet; 0.2 is the percentage of the ingredient; and CDAdr is the apparent digestibility coefficient of the reference diet.

Table 1. Composition of the reference diet used for determination of the apparent digestibility coefficient (ADC) of fish protein hydrolysate (FPH).

Ingredient	Percentage (%)
Soybean meal	27.51
Rice meal	25.00
Corn grain	17.87
Fish meal	15.00
Wheat gluten	8.00
Poultry meal	5.00
Supplement (mineral + vitamin)[(1)]	1.00
Salt	0.30
Chromium oxide	0.10
Antifungal	0.20
Antioxidant	0.02
Total	100.00
Calculated values	
Starch (%)	32.00
Calcium (%)	1.26
DE tilapia (kcal kg^{-1})	3,118
Fat (%)	3.12
Methionine (%)	0.57
Digestible protein (%)	29.73
Threonine (%)	1.19

[(1)]Guarantee levels by kilogram of product: 500,000 IU vitamin A; 200,000 IU vitamin D3; 5.000 mg vitamin E; 1,000 mg vitamin K3; 1,500 mg vitamin B1; 1,500 mg vitamin B2; 1,500 mg vitamin B6; 4,000 mg vitamin B12; 15,000 mg vitamin C; 50 mg biotin; 7,000 mg nicotinamide; 500 mg folic acid; 4,000 mg calcium pantothenate; 10,000 IU inositol; 40,000 mg choline; 10 mg Co; 500 mg Cu; 5,.000 mg Fe; 50 mg I; 1,500 mgMn; 10 mgSe; and 5,000 mg Zn.

The reference feed and the test feed were analyzed for dry matter, ash, crude protein, fat, energy, and chromium oxide. The following components of the feces were analyzed: crude protein, gross energy, fat, and chromium oxide. The analyses of the diets and feces followed the methodology described by Horwitz (2005), except for the chromium analysis that followed the protocol described by Bremer Neto et al. (2003).

The zootechnical performance of Nile tilapia post-larvae was evaluated in 375 three-day-old Nile tilapia post-larvae, which were distributed in 25 aquaria of 30 L useful volume each, in a completely randomized design, with five treatments and five replicates, in which the experimental unit consisted of an aquarium with 15 animals. Each aquarium had its own water aeration and heating system. The aquaria were siphoned twice a day: once in the morning and again in the late afternoon. Fish were fed at 08:00, 10:00, 12:00, 14:00, and 18:00 h. The experiment lasted 40 days.

Five feeds were prepared based on ingredients of plant origin, with five different percentages of FPH: 0, 2, 4, 6, and 8%. The experimental diets were formulated according to the NRC recommendation (2011) (Table 2). The feeds were extruded according to the procedure described for the digestibility trial; however, shortly after drying, the feeds were ground again to obtain a 0.7 mm feed.

At the end of the experimental period, fish were fasted for 24 hours, then the individual measurements of mass (g) and total length (cm) were recorded. The evaluated zootechnical performance variables were: final mass, mass gain, specific growth rate, protein efficiency rate, survival rate, and batch uniformity.

In both experiments, the water temperature, dissolved oxygen, and pH were measured daily using a

HI 9828 multi-parameter (Hanna Instruments Portugal Ltda., Póvoa de Varzim, Portugal) and remained within the recommended amounts for the species cultivation (Ridha & Cruz, 2001). The average values obtained in the digestibility trial were: temperature, 25.12°C ± 1.15°C; dissolved oxygen, 4.55±0.64 mg L^{-1}; and pH, 6.68±0.26. The average values obtained in the zootechnical performance test were: water temperature, 26.26°C±0.50°C; dissolved oxygen, 5.93±0.34 mg L^{-1}; and pH, 6.23±0.40.

In the morphometric analyses of muscle fiber, eight fish from each experimental unit were used to evaluate muscle growth. These samples were fixed in 10% buffered formalin for 24 hours, and processed for inclusion in paraffin. Cross sections (5 μm) were obtained in a microtome and stained with hematoxylin-eosin. Using an image analysis system, morphometry was conducted to determine the smallest diameter of 200 muscle fibers per animal. The data on diameter (D) were separated, as described in Assis et al. (2004), into the following classes: c10, $D \leq 10$ μm; c20, $10 < D \leq 20$ μm; c30, $20 < D \leq 30$ μm; and c40, $30 < D \leq 40$ μm. On the basis of the results of diameter classification, the degree of hypertrophic and hyperplastic growth of muscle fibers was evaluated according to Alami-Durante et al. (2010).

Data on zootechnical performance and histology were subjected to the analysis of variance, and the means were compared by the Tukey's test, at 5% probability. The best level of FPH inclusion was determined by a

Table 2. Composition of experimental diets based on plant ingredients with fish protein hydrolysate (FPH) inclusion for post-larvae of Nile tilapia (*Oreochromis niloticus*).

Ingredient	FPH (%)				
	0	2	4	6	8
Soybean meal	39.79	39.26	39.29	39.19	39.21
Corn gluten	25.10	24.31	23.07	21.12	21.10
Corn grain	12.57	12.89	13.10	13.51	13.13
FPH	0.00	2.00	4.00	6.00	8.00
Rice meal	5.00	5.00	5.00	5.00	5.00
Wheat gluten	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	3.85	3.84	3.82	3.81	3.80
Sobean protein isolate	3.00	3.00	3.00	3.00	3.00
Supplement (mineral + vitamin)[(1)]	1.00	1.00	1.00	1.00	1.00
L-threonine	0.23	0.22	0.22	0.21	0.21
Antifungal	0.20	0.20	0.20	0.20	0.20
Soybean oil	4.07	3.09	2.11	1.13	0.15
Vitamin C	0.10	0.10	0.10	0.10	0.10
L-tryptophan	0.06	0.06	0.05	0.05	0.05
DL-methionine	0.01	0.01	0.02	0.02	0.03
Antioxidant	0.02	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00	100.00
Calculated values					
Linoleic acid (%)	03.12	2.70	2.28	1.86	1.44
Starch (%)	21.40	21.41	21.41	21.41	21.41
Calcium (%)	1.10	1.10	1.10	1.10	1.10
DE tilapia (kcal kg^{-1})	3,500	3,500	3,500	3,500	3,500
Fat (%)	6.21	6.20	6.19	6.19	6.18
Methionine (%)	0.75	0.75	0.75	0.75	0.75
Digestible protein (%)	41.30	41.30	41.30	41.30	41.30
Threonine (%)	1.70	1.70	1.70	1.70	1.70
Tryptophan	0.43	0.43	0.43	0.43	0.43

[(1)]Guarantee levels per kilogram of product: 500,000 IU vitamin A; 200,000 IU vitamin D3; 5,000 mg vitamin E; 1,000 mg vitamin K3; 1,500 mg vitamin B1; 1,500 mg vitamin B2; 1,500 mg vitamin B6; 4,000 mg vitamin B12; 15,000 mg vitamin C; 50 mg biotin; 7,000 mg nicotinamide; 500 mg folic acid; 4,000 mg calcium pantothenate; 10,000 IU inositol; 40,000 mg choline; 10 mg Co; 500 mg Cu; 5,000 mg Fe; 50 mg I; 1,500 mg Mn; 10 mg Se; and 5,000 mg Zn.

quadratic equation, associated with the linear response plateau (LRP) model. Data were calculated by the statistical software SAEG (Universidade Federal de Viçosa, Viçosa, MG, Brazil).

Results and Discussion

The centesimal composition showed that FPH had 40.74% crude protein, 54.06% lipids, 3.23% ash, and 6.429 kcal kg^{-1} of gross energy (values based on dry matter). These values are similar to other hydrolysates mentioned in the literature, produced with fish residues, such as: shrimp, with 46.7% crude protein (Bueno-Solano et al., 2009); tilapia, with 37.7% crude protein (Abdul-Hamid et al., 2002); and croaker, with 47.09% crude protein (Martins et al., 2009). The nature and quality of the raw material are two of the factors that can affect the results obtained from the composition, and that can affect characteristics as the quality and functionality of the final product (Choi et al., 2009).

The values for ADCs of crude protein, gross energy, fat, and dry matter were, respectively, 99.28, 99.13, 97.64, and 98.29%. The high-protein digestibility may be associated with the increased solubility, and a structural unfolding of the protein molecule into smaller peptide units occurred during hydrolysis (Van Der Plancken et al., 2003). These smaller molecules, generated by the hydrolysis process, show considerably higher-intestinal absorption kinetics for solutions that contain only dipeptides and tripeptides, or partially hydrolyzed proteins, than those composed only of free amino acids (Fairclough et al., 1980; Zhanghi & Mattews, 2010).

The speed of absorption of free amino acids and small peptides is attributed to factors such as competition between amino acids and the transport of amino acids. Free amino acids are rapidly absorbed only in the proximal small intestine, whereas dipeptides and tripeptides are absorbed in both the proximal and distal portions of the small intestine; dipeptides and tripeptides have a faster rate of absorption (Frenhani & Burini, 1999; Ronnestad & Morais, 2008).

In vivo digestibility of FPH is still rarely evaluated. However, in vitro digestibility shows slightly lower results than those found in the present study. Hevroy et al. (2005) found 95% dry matter, 98% gross energy, and 91% lipids; and Foh et al. (2011) mentioned values of 93.2% protein digestibility.

The values of productive performance – final mass, mass gain, specific growth rate, and batch uniformity – showed a quadratic effect ($p<0.05$) with the FPH inclusion. However, survival was not affected ($p>0.05$) by the experimental diets (Table 3). The association of the quadratic effect with the LRP model was adjusted only for the final mass, mass gain (indicating the same value), and specific growth rate, where the maximum inclusion determined were 4.75 and 4.77%, respectively (Figure 1).

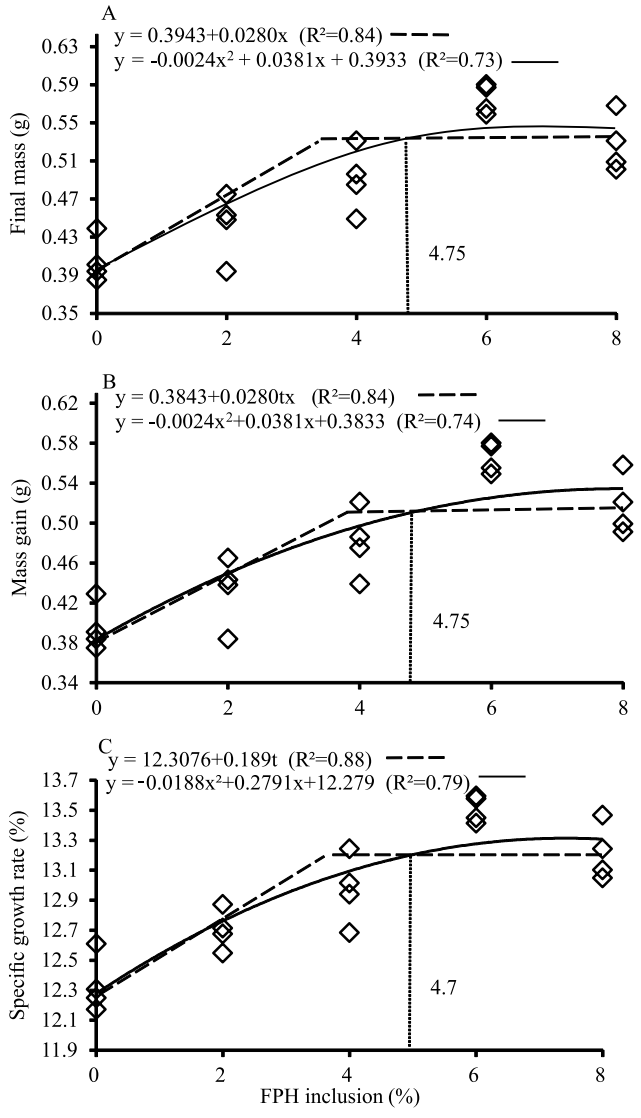

Figure 1. Best fish protein hydrolysate (FPH) inclusion level for final mass (A), mass gain (B), and specific growth rate (C), by means of the first intercept of the quadratic equation with LRP plateau.

Earlier studies have shown that diets supplemented with FPH can improve the productive performance of several fish species in the early stages of life (Kotzamanis et al., 2007; Tang et al., 2008; Chotikachinda et al., 2013). These findings are corroborated by the data obtained in the present study.

The main causes of the positive effect of FPH are associated with improved palatability and high digestibility of the protein, which stimulates digestive enzymes and channels nutrients for biomass production (Hevroy et al., 2005; Zheng et al., 2013). The positive effects on the growth rate and the final mass of salmon juveniles, fed on a diet containing 5 to 8% FPH, are mentioned by Berge & Storebakken (1996). Likewise, Zheng et al. (2013) observed that flounder fed 3.7% FPH showed a better growth and feed efficiency. The inclusion of FPH above 50% may lead to a decrease of feed intake, which is caused by a change in the palatability of the hydrolysate due to bitter-tasting peptides or rancid lipid compounds, which result in decreased feed consumption and animal growth (Hevroy et al., 2005). The inclusion of FPH affected the frequency distribution of muscle fibers ($p<0.05$), with a higher number of fibers with a diameter below 10 µm; and the treatment with 2% of FPH resulted in 74.06% fibers in this size class (Table 4). This indicates growth by hyperplasia, which is characterized by fiber diameters lower than 20 µm (Rowlerson & Veggetti, 2001; Johnston & Hall, 2004).

The process of hyperplasia is predominant in the initial life stages of animals (Almeida et al., 2010); nevertheless, as indicated in the present study, factors such as nutrition may affect the dynamics of muscle fiber growth. With the increased FPH inclusion corresponding to the post-larvae mass gain, there was a frequency decrease in the c10 class muscle fiber and a consequent increase in the c20 class, that is, there was a negative correlation between mass gain and class <10 (−0.64), and a positive correlation for class c20 (0.62). In a study conducted with 35-day-old pacu post-larvae, Leitão et al. (2011) also show the effect of diet on the proportion of muscle fibers in hypertrophy and hyperplasia.

Table 3. Perfomance of post-larvae of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of fish protein hydrolysate (FPH) inclusion.

Variable	Fish protein hydrolysate inclusion (%)					Coeficient of variation (%)	p-value
	0	2	4	6	8		
Final mass (g)[(1)]	0.40±0.02	0.44±0.03	0.50±0.03	0.57±0.01	0.52±0.03	18.84	<0.0001**
Mass gain (g)[(2)]	0.39±0.02	0.42±0.03	0.48±0.03	0.56±0.01	0.52±0.03	13.61	<0.0001**
Specific growth rate (%)[(3)]	12.33±0.19	12.63±0.13	12.97±0.23	13.51±0.09	13.21±0.19	3.34	0.0180*
Batch uniformity (%)[(4)]	65.62±3.15	72.54±5.52	77.12±4.34	74.48±2.17	66.62±4.33	8.63	0.0003**
Survival (%)[(5)]	88.33±6.38	83.33±6.66	90.00±11.57	90.00±0.67	86.67±10.88	9.08	0.8385[ns]

[(1)]Final mass (quadratic effect, $FM = -0.024t^2 + 0.381t + 0.3933$, $R^2=73$). [(2)]Mass gain (quadratic effect, $MG = -0.0024t^2 + 0.0381t + 0,3833$, $R^2=0,74$). [(3)]Specific growth rate (quadratic effect, $SGR = -0.0188t^2 + 0.2791t + 12.279$, $R^2=0,79$). [(4)]Batch uniformity (quadratic effect, $BU = -0.6537t^2 + 5.1505t + 63.892$, $R^2=52$). [(5)]Survival (no effect). [ns]Nonsignificant. * and **Significant at 5 and 1% probability, respectively.

Table 4. Occurrence frequency of white muscle fibers in diameter classes – c10 (<10), c20 (10–20), c30 (20–30), and c40 (>30) –, in post-larvae of Nile tilapia (*Oreochromis niloticus*) diets with different levels of fish protein hydrolysate (FPH) inclusion.

Diameter class (µm)	Fish protein hydrolysate inclusion (%)					Coeficient of variation (%)	p-value
	0	2	4	6	8		
c10	58.25ab	74.06b	68.38ab	60.87ab	51.83a	20.76	0.0225*
c20	41.75	25.94	31.56	38.36	44.41	34.99	0.0580[ns]
c30	0.00a	0.00a	0.06a	0.71a	3.34b	76.90	<0.0001**
c40	0.00a	0.00a	0.00a	0.06a	0.42a	11.73	0.0002**

[ns]Nonsignificant. * and **Significant at 5 and 1% probability, respectively.

Conclusions

1. The apparent digestibility of crude protein (99.28%), fat (99.13%), dry matter (97.64%), and energy utilization (98.29%) show that the fish protein hydrolysate can be used efficiently by Nile tilapia (*Oreochromis niloticus*).

2. The growth of muscle fibers is characterized by hyperplasia.

3. The inclusion of the fish protein hydrolysate at 4.75% is indicated in diets for Nile tilapia, in the post-larval phase.

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