Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(5): 509 – 520 (2023)
www.ejabf.journals.ekb.eg

The Effect of Different FPH Methods and Levels on the Performance, Feed Utilization and Body Composition of Nile Tilapia Fry

Tasneem K. Abd El-Rady[1], Al-Azab M. Tahoun[2], Hesham F. Amin[1*]

[1]Department of Fish Processing Technology, Faculty of Fish Resources, Suez University, Suez, Egypt
[2]Department of Aquaculture, Faculty of Fish Resources, Suez University, Suez, Egypt
[*]**Corresponding author: hesham.ameen@suezuniv.edu.eg**

ARTICLE INFO

Article History:
Received: July 25, 2023
Accepted: Aug. 29, 2023
Online: Oct. 1, 2023

Keywords:
Protein hydrolysate,
Nile tilapia fry,
Growth performance,
Feed utilization;
Proximate composition,
Survival rate

ABSTRACT

The purpose of the current study was to investigate how tuna waste protein hydrolysate, which was partially substituted for fish meal (FM), affected growth performance, feed utilization, and body composition. For three months, The Nile tilapia fry (0.7 gram) samples were fed on diets including enzymatic or microbial protein hydrolysate at two levels of substitution (15 and 30%). The enzymatic fish protein hydrolysate (E-FPH) was recovered from tuna waste by employing 2% fish pepsin enzyme (0.5%), while the microbial fish protein hydrolysate (M-FPH) was created by fermentation using lactic acid bacteria. Five diets were formulated by substituting FM with 0% (control diet), 15% (E-FPH 15), 30% (E-FPH 30), 15% (M-FPH 15), and 30% (M-FPH 30). The E-FPH 30 diet demonstrated significant differences ($P< 0.05$) from other treatments in some aspects of growth performance, proximate composition, feed utilization, and protein utilization parameters. This E-FPH 30 diet treatment recorded SGR of 3.767%/ day, survival rate of 98.380%, feed intake of 9.052g, FCR of 1.475, and PER of 2.141. However, it was not superior to the control diet. Additionally, the Nile tilapia fed on E-FPH 30 diet showed the highest muscle protein content (54.54%), and lowest ether extract (18.45%), followed by E-FPH diet 15, then M-FPH 30 diet and M-FPH 15 diets. The present study concluded that partially replacing fish meal with 15% and 30% of tuna waste-derived hydrolysates, especially E-FPH has promising implications in aqua-feeds as a sustainable source of proteins and amino acids.

INTRODUCTION

Tilapia is one of the world's most promising aquaculture species. Tilapia can adapt to adequate environmental conditions in any aquaculture system. Global tilapia output is expected to increase further, reaching approximately 7.3 million tonnes by 2030 **(FAO, 2021)**. Success in growing tilapia production will depend on nutritional strategies followed to challenge mainly the shortages in feed ingredients and their costs.

More than 20 million tons fish waste is produced from fisheries industry yearly worldwide **(Caruso, 2015)**. Tuna canning industry is an example; it produced 50- 70% waste **(Guérard *et al.*, 2002; FAO, 2010)** which can be converted to sustainable valuable protein products, preventing their environmental impacts **(Abbey *et al.*, 2017)**.

Fish meal is the primary source of high quality protein in aquafeeds although it represents the main cost impeding. Many sustainable feed ingredients were proposed for use in aquafeeds to solve the economic and environmental problems **(Tacon *et al.*, 2022)**. The provision of tilapia meals closely matching optimal amino acid needs is an important strategy

Indexed in Scopus ELSEVIER DOAJ IUCAT

for resolving a number of problems, including optimising sustainable source materials, reducing feeding costs, and attenuating nitrogen loss into the environment.

Fish protein hydrolysate (FPH) consisting of relatively small bioactive peptides (2–20 amino acids) can be obtained by chemical or biological hydrolysis from fish waste **(Sarmadi & Ismail, 2010)**. FPH is considered valuable feed ingredients due to high protein content and good balanced amino acids **(Gao *et al.,* 2021)**. In general, FPH can produce safely, fast and easily by using commercial proteases enzymes such as pepsin, flavourzyme and papain at laboratory scale **(Singh *et al.,* 2014; Luna-Vital *et al.,* 2015)**. Microbial fermentation is another protein hydrolysis method that includes incubating fish waste as a source of protein with specific microorganisms that secrete hydrolytic enzymes **(Raveschot *et al.,* 2018)**. This fermentation process is safe, low cost and eco-friendly **(Lee *et al.,* 2017)**.

In previous studies, FPH was applied as feed additives to reduce costs and enhance the animals' digestion, absorption and immunity. Incorporating protein hydrolysates in feeding regime was associated with the improvement in immunity and growth performance of fish species **(Siddik *et al.,* 2021)**. This was particularly applicable for larvae and juveniles **(Xu *et al.* 2016; Siddik *et al.* 2019)**. Replacing 5% and 10% of FPH instead of fish meal improved the growth parameters of fish **(Siddik *et al.,* 2018a; Ha *et al.,* 2019)**. According to **Siddik *et al.* (2021)**, small peptides and free amino acids in FPH promote the health and growth of fish. Replacing FPH till 10% of total dietary protein in diet containing fish meal did not exhibit poor effect on the fish growth while incorporated 20% of FPH impaired feed intake and growth performance **(Xu *et al.,* 2016)**. In another study on juvenile barramundi nutrition, incorporating 50% or higher fermented or non-fermented FPH instead of fish meal reduced the digestibility of protein, fat and dry matter, which may be related to free amino acids and nucleotides **(Siddik *et al.,* 2018b)**.

Egypt is considered the third country for the global tilapia production (**FAO, 2020**). To maintain the growing production requires many factors, especially the cost of fish feed formulation which increased dramatically by increasing fish meal price. Applied FPH prepared simply from fish wastes would reduce the costs of aquafeeds and improve the environmental impacts.

Therefore, this study focused on the partial replacement (15% and 30%) of biological FPH as bio-economy and sustainable protein source instead of fish meal in diets formulation of juvenile tilapia and investigated their growing performance.

MATERIALS AND METHODS

1. Materials and reagents

Bigeye tuna waste composed of backbone, head and fins collected from the line of tuna canned product belong to fish processing unit of the Faculty of Fish Resources, Suez University, Egypt. The collected waste which accounted for 29.7% of the total weight of tuna fish was frozen until processed.

Analytical grade chemicals (HCl, NaOH and pepsin enzyme, etc.) were purchased from Piochem Chemicals, Egypt.

Microbiological media and bacterial strain (MRS) Agar and broth media and nutrient broth media was obtained from Oxoid Ltd, England. The broth of *Lactobacillus plantarum ss. plantarum* (oral-Jensen 1919, DSM 20174) was bought from Cairo Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

2. Methods

2.1. Enzymatic and microbial methods for FPH preparation

After thawing the frozen Bigeye tuna waste (20 Kg) at 5 ± 2°C overnight, waste was grinded using the electric grinder. FPHs were prepared based on enzymatic hydrolysis or microbial fermentation as follows:

Enzymatic hydrolysis (E-FPH) procedures were performed as described by **Jamil *et al.* (2016)** and **Bhingarde *et al.* (2018)**. A grinded tuna waste was mixed with distilled water (1:1 w/v) and pH was adjusted to 2.5 using 2N HCl then subjected to heat treatment by steaming machine (85˚C for 10min) to inactivate endogenous enzymes. 2% (v/w) of 0.5% pepsin enzyme was added to the mixture and incubated at 37°C for 5h. Then, enzymatic hydrolysis was inactivated by heating at 85ºC for 15min using steaming machine. After centrifugation at 6000 rpm for 20min, the enzymatic hydrolysate was collected, and pH was adjusted to 6 using 2N NaOH, and then hydrolysate was dried at 60˚C for 24h (E-FPH).

Microbial hydrolysis (M-FPH) steps were carried according to **Samaddar and Kaviraj (2014)** and **Khiari and Mason (2018)**. At first, starter culture of *Lactobacillus plantarum* was cultured into 5ml nutrient broth and incubated at 37°C for 48h. Then, 2ml of culture was transferred into 100ml of sterile Lactobacilli MRS (DeMan, Rogosa and Sharpe) broth and incubated at 37°C for 48h to get the adequate microbial inoculum (LAB) (10^8 CFU/ml) for fermentation process. A grinded tuna waste (10kg) was mixed with 3.2L of glucose solution (175g/ L), and pH was adjusted to 5. Then, 800ml of LAB inoculum was added, and the mixture was incubated at 37°C for 9 days. After centrifugation at 6000 rpm for 20min, the hydrolysate was collected and pH was adjusted to 6 with 2N NaOH. Then, the hydrolysate was dried at 60˚C for 24h (M-FPH).

2.2. Preparation of experimental diet treatments

Five experimental diets (35 percent protein) were formulated using five different FPH partial replacements from fish meal (control diet, E-FPH at 15% and 30%, and M-FPH at 15% and 30%). Tables (1, 2) listed the composition and proximate analysis of each experimental diet. Each ingredient in the experimental diet was finely powdered, homogenised for 45 minutes and dried for 8 hours at 60˚C. Dried diets were packed into airtight jars and stored in a cool and dry place until the feeding trial commenced.

2.3. Experimental fish and system

Specimens of the Nile tilapia fry (*Oreochromis niloticus*) of two- day of old were obtained from a commercial tilapia hatchery located in El-Sharkia Government. The fries were transferred in oxygenated plastic bags to the Aquaculture laboratory. Before starting the experimental feeding trial, fish were acclimatized to new environment for 15 days in two 60-liter plastic tanks in order to adapt to the experimental conditions. After the adaptation period, fish were assigned to the experimental tanks in duplicate with initial mean weight (0.72±0.02g) and stocking density (30 fry/ tank) into ten (90L) conical plastic tanks, suitable for the collection of feces, Experimental aeration was performed using a 0.5hp- air blower (Vortex MODEL; XGB-370), which compresses air in the experimental tank through air stones along the day. A dechlorinated tap water was the source of continuous fresh water.

Table 1. Composition of the test diet of the Nile tilapia fry (*Oreochromis niloticus*)

Diet treatments / Ingredient	[1]Con. diet	[2]E-FPH		[3]M-FPH	
		15%	30%	15%	30%
Fish meal	15	12.9	10.76	12.7	10.2
FPH	0	2.12	4.24	2.29	4.58
Cellulose	3	2.1	1.23	2.3	1.7
Soybean meal	40	40	40	40	40
Corn gluten (60%)	8	8	8	8	8
Yellow corn grain	7.05	7.05	7.05	7.05	7.05
Rice bran	7	7.65	8.3	7.45	7.9
Wheat bran	5	5	5	5	5
Sunflower meal "unhulled"	4.5	4.5	4.5	4.5	4.5
Distillers dry grain soluble (DDGS)	4.5	4.5	4.5	4.5	4.5
Fish oil	2	2.23	2.47	2.25	2.53
Soybean oil	3	3	3	3	3
Salt	0.4	0.4	0.4	0.4	0.4
Supplement (vitamins and minerals)	0.3	0.3	0.3	0.3	0.3
Choline chloride (60%)	0.25	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100	100
Fish meal	15	12.9	10.76	12.7	10.2
FPH	0	2.12	4.24	2.29	4.58

[1]Con. diet: Control diet; [2]E-FPH: Enzymatic fish protein hydrolysate diet; [3]M-FPH: Microbial fish protein hydrolysate diet.

Table 2. Proximate analysis of tuna fish waste, enzymatic and microbial fish protein hydrolysates and experimental diets of the Nile tilapia fry (*Oreochromis niloticus*)

Proximate analysis (%)	TFW	Fish protein hydrolysate		Diet treatments (%)				
		(E-FPH)	(M-FPH)	[1]Con. diet	[2]EFPH		[3]MFPH	
					15%	30%	15%	30%
Moisture	59.4	2.05	3.24	10	10	10	10	10
On dry weight basis								
Protein	47.2	76.37	70.7	35.5	35.5	35.4	35.1	35.05
Fat	14.3	10.82	8.4	8.9	9	8.8	9	9.1
Ash	35.2	12.15	16.2	9	8.9	8.8	9	9
Carbohydrate	5.3	0.59	4.7	43.1	43.05	43.2	43.4	43.35
Fiber	0	0.07	0	3.5	3.55	3.8	3.5	3.5
Total energy (Kcal/100g)	338.7	403	376.4	416	416.05	415.85	416.08	416.21

TFW: Tuna fish waste; E-FPH: Enzymatic fish protein hydrolysate; M-FPH: Microbial fish protein hydrolysate; [1]Con. diet: Control diet; [2]E-FPH: Enzymatic fish protein hydrolysate diet; [3]M-FPH: Microbial fish protein hydrolysate diet.

The two extraction methods for FPH (enzymatically or microbial) at two different incorporation levels (15 and 30%) for the partial replacement of fish meal protein were addressed as follows:

1- Control diet (0% FPH) (diet without substitution of FPH).

2- E-FPH diet 15% (diet with 15% enzymatic FPH substituted from fish meal content in the diet)

3- E-FPH diet 30 % (diet with 30% enzymatic FPH substituted from fish meal content in the diet)

4- M-FPH diet 15% (diet with 15% microbial FPH substituted from fish meal content in the diet)

5- M-FPH diet 30% (diet with 30% microbial FPH substituted from fish meal content in the diet)

Fish were fed twice daily at 09:00 & 13:00h with 4g of diets for 90 days, and 50% of the water volume was exchanged to avoid contamination of the feces by feed debris.

2.4. Water quality parameters

A digital thermometer was used to test the water's temperature and dissolved oxygen content (DO), and a Milwaukee-PH600 pH meter was used to assess the water's pH once a week. Ammonia nitrogen (NH_3-N), nitrite (NO_2) values were detected on a biweekly basis using water analysis using photometer and test kits), and nitrate (NO_3) was weekly determined. While, alkalinity (expressed as Ca CO_3) was biweekly monitored by titration with sulfuric acid till pH point reached 4.5 **(APHA, 1998)**.

2.5. Experiment location and duration

The experiment was carried out in Aquaculture laboratories building, Faculty of Fish Resources, Suez University, Egypt during autumn and winter from November 2022 to January 2023.

2.6. Experimental scheme and data collection

Fish from each tank were randomly selected and weighed to determine parameters of growth performance and feed utilization efficiency and adjust daily feed quantity.

2.7. Calculations of fish growth performance

Parameters of growth performance (final weight, average daily weight gain weight gain, specific growth rate, survival rate) were calculated as follows:

Growth performance parameter	Equation
Weight gain (WG), g/ fish	**Final weight (g) - Initial weight (g)**
Daily weight gain (DWG), g/ fish/ day	**Weight gain (g)/ time (days)**
Specific growth rate (SGR), %/ day	**(Ln Final weight – Ln Initial weight)/ duration (days)× 100**
Survival rate (SR), %	**(Final number of fish / Initial number of fish) × 100**

Ln : Natural logarithm

2.8. Calculations of feed utilization

Feed utilization efficiency (feed conversion ratio, protein productive value, protein efficiency ratio and energy utilization) was calculated as follows:

Feed utilization parameter	Equation
Feed conversion ratio (FCR)	**Total feed intake (g)/ wet weight gain (g)**
Protein efficiency ratio (PER)	**(Wet weight gain (g) / Total protein intake (g))**
Protein productive value (PPV)	**(Retained protein (g)/ /protein intake(g)) × 100**
Energy utilization (EU)	**(Retained energy (Kcal)/ energy intake (Kcal)) × 100**

2.9. Analytical methods

Diets and fish proximate analyses were performed on two replicates using standard methods **(AOAC, 2012)**. The Kjeldahl technique (N6.25) was used to assess the crude

protein content. The ash content was evaluated by the overnight incineration of a pre-weighed sample in a silica crucible in a muffle furnace at 550ºC. A drying oven at 105ºC for 3 hours was used to specify the moisture content. The Soxhlet method is used to extract crude fats using petroleum ether **(AOAC, 2006)**. **AOAC (2007)** assessed crude fiber in diet treatments using the filter bag technique. Caloric values of diet treatments were calculated from the following equation of **Falch *et al.* (2010)**:

Caloric value (kcal/100g) = (lipid × 9) + (carbohydrate × 4) + (protein × 4)

3. Statistical analysis

Data were analyzed using statistics of package for social science **(SPSS) version 22. (2014)**. Mean ± Standard error (SE) and one-way ANOVA in SPSS were used to analyze the data of growth performance and feed utilization. Duncan's test was used to determine the significances of differences among treatments. The significance level was tested at 0.05 **(Duncan, 1955)**.

RESULTS AND DISCUSSION

1. Water quality parameters

Table (3) illustrates the water quality readings taken throughout the experiment in the experimental tanks. Compared to this study, **Leal *et al.* (2010)** reported identical values for water temperature (28.7°C), pH (8.1) and ammonia (0.14mg/ l), but different values for the dissolved oxygen and nitrite (3.5 mg/l and 0.08 mg/l, respectively) were recorded. All water quality parameters (temperature, pH, ammonia, nitrite and nitrate) were within the acceptable range for tilapia culture though some results are different from those of **Da Silva *et al.* (2017)**, including water temperature, dissolved oxygen and pH since water temperature was 26.26°C 0.50°C, dissolved oxygen was 5.93 mgL-1, and the pH was 6.23. The results of **Sandoval-Gallardo *et al.* (2022)** with respect to the Nile tilapia fed with herring hydrolysates supplemented feeds are similar in terms of temperature (28°C) and dissolved oxygen (5.0mg L-1) for the water quality parameters in this investigation.

Table 3. Water quality parameters for the experimental tanks

Treatments / Parameter	[1]Con. diet	[2]E-FPH		[3]M-FPH	
		15%	30%	15%	30%
pH	7.98	7.83	7.7	7.75	7.80
Temperature (Cº)	27.00	27.00	27.00	27.00	27.00
Dissolved oxygen (Do) (mg/l)	5.1	5.0	5.0	5.0	5.0
Total ammonia nitrogen (mg/l)	0.21	0.20	0.19	0.19	0.19
Nitrite (mg/l)	0.29	0.28	0.29	0.27	0.28
Nitrate (mg/l)	30.0	30.0	30.1	30.1	30.0
Alkalinity (mg/l)	161	155	165	156	161

[1]Con. diet: Control diet; [2]E-FPH: Enzymatic fish protein hydrolysate diet; [3]M-FPH: Microbial fish protein hydrolysate diet.

2. Growth performance parameters

The impact of various FPH levels and procedures on growth performance parameters and survival rate is shown in Table (4). The experimental treatments varied significantly from one another ($P \leq 0.05$). The highest final weight (FW), weight gain (WG) and average daily

weight gain (ADWG) were in the fish meal basal diet (control diet) (7.25g, 6.53g and 0.10g, respectively), followed by E-FPH 30% (6.85g, 6.13g and 0.10g, respectively), E-FPH 15% (6.35g, 5.62g and 0.09g, respectively), M-FPH 30% , (5.82g, 5.15g and 0.08g, respectively), and M-FPH 15% (5.45g, 4.81g and 0.08g, respectively). These findings are supported by those of **Siddik *et al.* (2018a)** who found that, the juvenile barramundi's specific growth rate (SGR) decreased as the amounts of tuna hydrolysate (TH) and fermented tuna hydrolysate (FTH) in their diets increased, and added that the control treatment showed the best growth performance parameters, including final body weight (FBW), weight gain (WG) and SGR. In addition, **Khieokhajonkhet and Surapon (2020)** reported that the juvenile Nile tilapia's final body weight, weight gain and average daily weight gain were 418.71g, 14.78% and 0.18g, respectively. While, **Sandoval-Gallardo *et al.* (2022)** showed that final weight and weight gain of the Nile tilapia with initial weight of 0.49g and fed on diet containing herring hydrolysates 30% were 3.63 and 3.14g, respectively.

No significant differences were recorded for the highest SGR (%/day) between the control diet and the E-FPH 30% diet, as they were 3.86 and 3.76%/ day, respectively. Although there were significant differences among the E-FPH extracted enzymatically and M-FPH extracted microbially, no significant differences were detected between the two selected incorporation levels (15 and 30%) in the same extraction method. The results of SGR are higher than the findings of **Khieokhajonkhet and Surapon (2020)** on the juvenile Nile tilapia fed the aqueous protein hydrolysate (APH) with 10% diet (2.93%/ day), while the current values are similar to the results of **Siddik *et al.* (2018b)** and **Chaklader *et al.* (2020)** carried on juvenile barramundi fed on tuna hydrolysate (TH) at an inclusion level of 15% (3.39%/day) and on 90% poultry by-product meal (PBM) supplemented with 10% tuna hydrolysate (3.43 %/ day).

Whereas, the survival rate was not significantly affected via the experimental replacement of fish meal for FPH. They ranged between 96.67% in 30% replacement with microbial FPH diet and 98.38% in 30% replacement with enzymatic FPH diet. It was confirmed that the fish survival rate during the feeding trial of all tested diets was not affected by the inclusion of FPH in the diets **(Siddik *et al.*, 2018a**; **Khieokhajonkhet & Surapon, 2020)**.

Table 4. Effect of FPH methods and levels on growth performance parameters and survival rates of the Nile tilapia

Treatments / Parameter	[1]Con. diet	[2]E-FPH		[3]M-FPH	
		15%	30%	15%	30%
Initial weight (g)	0.715±0.015[a]	0.725±0.025 [a]	0.715±0.015 [a]	0.740±0.010 [a]	0.725±0.025 [a]
Final weight (g)	7.250±0.050 [a]	6.350±0.050 [c]	6.850±0.100 [b]	5.450±0.050 [e]	5.825±0.075 [d]
Weight gain (g)	6.535±0.065 [a]	5.625±0.025 [c]	6.135±0.085 [b]	4.810±0.160 [e]	5.150±0.050 [d]
Dialy weight gain (g)	0.109±0.001 [a]	0.094±0.001 [c]	0.103±0.002 [b]	0.081±0.003 [e]	0.086±0.001[d]
Specific growth rate (%/day)	3.862±0.047 [a]	3.618±0.046 [b,c]	3.767±0.011[a,b]	3.328±0.038 [d]	3.474±0.079 [c,d]
Survival rate (%)	98.335±1.665[a]	96.715±0.045[a]	98.380±1.620 [a]	98.335±1.665 [a]	96.670±0.000 [a]

Different letters in the same row denote significant difference ($P< 0.5$); [1]Con. diet: Control diet; [2]E-FPH: Enzymatic fish protein hydrolysate diet; [3]M-FPH: Microbial fish protein hydrolysate diet.

3. Feed utilization parameters

Regarding parameters presented in Table (5), the E-FPH 30% diet recorded the highest feed intake (9.052g) followed by control diet (8.988g), E-FPH 15% diet (8.86g), M-FPH 30% diet (8.651g) and M-FPH 15% diet (7.813g). These results vary with those of **Siddik *et al.* (2018a)** who elucidated that the highest feed intake was observed in the control fish group fed on the fish meal-based diet. All the experimental treatments in this study had significant effects on feed conversion ratio (FCR); this value was 1.375g/ g for the control diet, followed by E-FPH 30% diet, E-FPH 15% diet, M-FPH 15% diet and M-FPH 30% diet (1.475, 1.575, 1.625 and 1.680 g/g; respectively). Additionally, **Siddik *et al.* (2018a)** recorded that, FCR was significantly higher in the barramundi fish fed on non-fermented tuna hydrolysate 75% diet (4.36 g/g), while **Siddik *et al.* (2018b)** reported that FCR was 1.19g/ g after feeding on tuna hydrolysate of 15%. FCR results of all treatments in this study were higher than those observed in the study of **Sandoval-Gallardo *et al.* (2022)** since the FCR of the Nile tilapia (*O. niloticus*) fed on supplemented feeds containing herring hydrolysates of 30% was 1.22g/ g.

The results of protein efficiency ratio (PER) revealed that the best PER was observed for the control diet (2.27g/ g), followed by E-FPH 30% diet (2.14 g/g) and the other treatments. Protein productive value (PPV) as affected by the extraction method and the level of fish protein hydrolysate inclusion in the diets revealed that, no significant differences between fish meal basal diet and E-FPH 30 % diet recorded the highest PPV (37.314% and 35.164%, respectively). The diet included enzymatic protein hydrolysate (E-FPH) recorded the higher PPV values as compared to the diet containing microbial protein hydrolysate (M-FPH). According to this study, protein utilization (PER and PPV) is below the values recorded in the work of **Khieokhajonkhet and Surapon (2020)** during feeding the juvenile Nile tilapia with aqueous protein hydrolysate at 10% (5.51g/ g and 42.74 %, respectively). On the other hand, the PER in this research is similar to that found by **Kim *et al.* (2022)** where PER of olive flounder fed on the tuna by-product meal of 40% and 50% diets were 2.00 and 1.96g/ g, respectively while PER of E-FPH 30% diet had the same PER recorded for the Nile tilapia fed on the substitution of fish meal by 20% shrimp protein hydrolysate (2.14g/ g) **(Leal *et al.*, 2010)**.

The significant effects were obseved on the energy utilization (EU %) as affected by different FPH extraction methods and levels in the diets. The highest EU was found for the control diet (20.39%), followed by E-FPH 30% (18.77%), while the lowest values were showed by two levels of M-FPH (15.679 and 15.931%).

Table 5. Effect of FPH methods and levels on feed intake and feed utilization parameters of the Nile tilapia

Treatments / Parameter	[1]Con. diet	[2]E-FPH		[3]M-FPH	
		15%	30%	15%	30%
Feed intake	8.988± 0.253 [a]	8.860±0.180 [a]	9.052±0.279 [a]	7.813±0.148 [b]	8.651±0.070 [a]
FCR	1.375±0.025 [d]	1.575±0.025 [b]	1.475±0.025 [c]	1.625±0.025 [a,b]	1.680±0.030 [a]
PER	2.277 ± 0.041[a]	1.994 ± 0.031 [c]	2.141 ± 0.036 [b]	1.949 ± 0.029 [c]	1.887 ± 0.033 [c]
PPV	37.314 ± 1.682[a]	31.996 ± 1.074 [b,c]	35.164 ± 2.046 [a,b]	29.828 ± 0.370 [c]	29.553 ± 0.728 [c]
EU	20.396± 0.840[a]	17.321± 0.646[b,c]	18.774 ± 1.410[a,b]	15.679 ± 0.017[c]	15.931±0.399 [b,c]

Different letters in the same row denote significant difference (P<0.5); [1]Con. diet: Control diet; [2]E-FPH: Enzymatic fish protein hydrolysate diet; [3]M-FPH: Microbial fish protein hydrolysate diet; FCR: Feed conversion ratio; PER: Protein efficiency ratio; PPV: Protein productive value; EU: Energy utilization.

4. Final body composition analysis

As demonstrated in Table (6), no significant difference was recorded between the tilapia chemical composition of all treatments in terms of moisture (74.9- 75.7%), ether extract (18.45- 20.35%) and ash (16.81- 17.86%) contents. On the other hand, there was significant difference ($P \leq 0.05$) in crude protein contents between fish fed on M-FPH diet and both control diet and E-FPH diets, where the protein contents ranged from 54.54 to 50.84%. In the same trend of this study, **Siddik *et al.* (2018a)** reported that protein, lipid and ash contents of barramundi (*Lates calcarifer*) fish fed on fermented and non-fermented tuna hydrolysate as fishmeal protein replacement ingredients recorded values ranging from 13.22 - 14.67%, 3.50 - 4.08% and 3.69 - 3.89%, respectively, for wet weight. The moisture content in this study coincides with that repoted for juvenile barramundi fed on 10% tuna hydrolysate (TH) (73.22%) **(Siddik *et al.*, 2019)**. In contrast, **Suma *et al.* (2023)** postulated that, the protein contents of the Nile tilapia muscle fed with diets containing 0%, 0.5%, 1% and 2% FPH showed an increasing trend with the increase in FPH in the dietary diets (64.06%, 64.56, 66.31% and 65.73%, respectively).

Table 6. Effect of FPH methods and levels on body composition of the Nile tilapia fry fed the test diets

Treatments / Parameter	[1]Con. diet	[2]E-FPH		[3]M-FPH	
		15%	30%	15%	30%
Moisture	77	75.1 ± 0.011[a]	74.9 ± 0.057 [a]	75.7 ± 0.202 [a]	75 ± 0.127 [a]
On dry weight basis					
Crude protein	58	54.49±0.395 [a]	54.29±0.525 [a]	54.54±1.140 [a]	51.10±1.400[a,b]
Ether extract	25	19.25±0.250 [a]	19.65±0.650 [a]	18.45±0.550 [a]	19.35±0.650 [a]
Ash	15.5	17.05±1.655 [a]	17.18±0.400 [a]	17.86±1.240 [a]	17.15± 0.950 [a]

Different letters in the same row denote significant difference ($P< 0.5$); [1]Con. diet: Control diet; [2]E-FPH: Enzymatic fish protein hydrolysate diet; [3]M-FPH: Microbial fish protein hydrolysate diet.

CONCLUSION

The main objective of this study was the enzymatic and microbiological hydrolysis of FPH from tuna waste and its partial substitution by 15% or 30% of fish meal in experimental diets for the Nile tilapia fry (*Oreochromis niloticus*). The experimental results of the Nile tilapia juvenile's proximate composition, feed utilization, growth performance parameters and water quality suggest that partially replacing fish meal by 15% and 30% tuna waste-derived hydrolysates, especially enzymatic protein hydrolysate (E-FPH), has promising implications in aquafeeds as a sustainable source of proteins and amino acids.

REFERENCES

Abbey, L.; Glover-Amengor, M.; Atikpo, M. O.; Atter, A. and Toppe, J. (2017). Nutrient content of fish powder from low value fish and fish byproducts. Food Science and Nutrition, 5(3): 374-379.

AOAC. (2006). Method 991.36. Fat (Crude) in meat and meat products. Official Methods of Analysis of AOAC International. 18th Edition. Arlington, TX (USA).

AOAC. (2007). Official Method of Analysis 18th Ed., AOAC International, Gaithersburg, MD, Method 962.09

AOAC. (2012). AOAC Official Method 942.05. Ash of animal feed. In: G. Latimer (Editor). Official Methods of analysis of AOAC International, 19th Edition. Gaithersburg, MD (USA).

APHA. (1998). Standard methods for the examination of water and wastewater, 20.

Bhingarde, O. R.; Koli, J. M.; Patange, S. B.; Sonavane, A. E.; Shingare, P. E.; Relekar, P. P. and **Mulye, V. B.** (2018). Effect of different concentration of Pepsin Enzyme on extraction of Fish Protein Hydrolysate from Malabar Sole Fish (*Cynoglossus macrostomus*). Bulletin of Environment, Pharmacology and Life Sciences, 7 (9): 94-103.

Caruso, G. (2015). Fishery wastes and by-products: a resource to be valorized. Journal of Fisheries Sciences.com, 9(4): 80-83.

Chaklader, M. R.; Fotedar, R.; Howieson, J.; Siddik, M. A. and **Foysal, M. J.** (2020). The ameliorative effects of various fish protein hydrolysates in poultry by-product meal based diets on muscle quality, serum biochemistry and immunity in juvenile barramundi, *Lates calcarifer*. Fish & Shellfish Immunology, 104: 567-578.

Da Silva, T. C.; Rocha, J. D. A. M.; Moreira, P.; Signor, A. and **Boscolo, W. R.** (2017). Hidrolisado proteico de peixe em dietas para pós-larvas de tilápia-do-nilo. Pesquisa Agropecuária Brasileira, 52(7): 485-492.

Duncan, D. B. (1955). New multiple range test. Biometrics, 11(1): 1-42.

Falch, E.; Overrien, I.; Solberg, C. and **Slizyte, R. (2010).** Composition and calories. In Seafood and Seafood Product Analysis, Part III, Boca Raton, FL: CRC Press, Taylor and Francies Group, L.M.L. Nollet, & F. Toldrά (Eds), pp. 257-288.

FAO. (2010). Fisheries topics: resources. Tuna resources. Text byJacek Majkowski. In: FAO Fisheries and Aquaculture Department [online]. Rome. http://www.fao.org/fishery/topic/12251/en.Accessed May 10, 2010.

FAO. (2020). The state of World Fisheries and Aquaculture 2020. Sustainability in action. Organization FaA, editor. Rome: Food and Agriculture Organization of the United Nations.

FAO. (2021). Food and Agriculture Organization of the United Nations (FAO) Yearbook — Fishery and Aquaculture Statistics (p. 110). FAO.

Gao, R.; Yu, Q.; Shen, Y.; Chu,Q.; Chen,G.; Fen, S.; Yang, M.; Yuan, L.; McClements, D. J. and **Sun, Q.** (2021). Production, bioactive properties, and potential applications of fish protein hydrolysates: Developments and challenges. Trends in Food Science and Technology, 110: 687–699.

Guérard, F.; Guimas, L. and **Binet, A. J.** (2002). Production of tuna waste hydrolysates by a commercial neutral protease preparation. Journal of molecular catalysis B: Enzymatic, 19: 489-498.

Ha, N.; Jesus, G. F. A.; Gonçalves, A. F. N.; de Oliveira, N. S.; Sugai, J. K.; Pessatti, M. L.; Mouriño, J. L. P.; El Hadi, P. and **Fabregat, T.** (2019). Sardine (*Sardinella spp.*) protein hydrolysate as growth promoter in South American catfish (*Rhamdia quelen*) feeding: Productive performance, digestive enzymes activity, morphometry and intestinal microbiology. Aquaculture, 500: 99-106.

Jamil, N. H.; Halim, N. R. A. and **Sarbon, N. M.** (2016). Optimization of enzymatic hydrolysis condition and functional properties of eel (*Monopterus sp.*) protein using response surface methodology (RSM). International Food Research Journal, 23(1): 1–9.

Khiari, Z. and **Mason, B.** (2018). Comparative dynamics of fish by-catch hydrolysis through chemical and microbial methods. LWT - Food Science and Technology, 97: 135-143.

Khieokhajonkhet, A. and **Surapon, K.** (2020). Effects of fish protein hydrolysate on the growth performance, feed and protein utilization of Nile tilapia (*Oreochromis niloticus*). International Journal of Agricultural Technology, 16(3): 641-654.

Kim, J.; Baek, S. I.; Cho, S. H. and **Kim, T.** (2022). Evaluating the efficacy of partially substituting fish meal with unfermented tuna by-product meal in diets on the growth, feed utilization, chemical composition and non-specific immune responses of olive flounder (*Paralichthys olivaceus*). Aquaculture Reports, 24: 101150.

Leal, A. L. G.; de Castro, P. F.; de Lima, J. P. V.; de Souza Correia, E. and **de Souza Bezerra, R.** (2010). Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus, L.*) feeds. Aquaculture international, 18: 635-646.

Lee, E. J.; Hur, J.; Ham, S. A.; Jo, Y.; Lee, S.; Choi, M. J. and **Seo, H. G.** (2017). Fish collagen peptide inhibits the adipogenic differentiation of preadipocytes and ameliorates obesity in high fat diet-fed mice. International Journal of Biological Macromolecules, 104: 281-286.

Luna-Vital, D. A.; Mojica, L.; Mejia, E. G. D.; Mendoza, S. and **Loarca-Pi˜na, G.** (2015). Biological potential of protein hydrolysates and peptides from common bean (*Phaseolus vulgaris L.*): A review. Food Research International, 76: 39–50.

Raveschot, C.; Cudennec, B.; Coutte, F.; Flahaut, C.; Fremont, M.; Drider, D. and **Dhulster, P.** (2018). Production of bioactive peptides by *Lactobacillus* species: from gene to application. Frontiers in Microbiology, 9: 2354.

Samaddar, A. and **Kaviraj, A**. (2014). Processing of fish offal waste through fermentation utilizing whey as inoculum. International Journal of Recycling of Organic Waste in Agriculture, 3: 1-8.

Sandoval-Gallardo, J. M.; Osuna-Ruiz, I.; Martínez-Montaño, E.; Hernández, C.; Hurtado-Oliva, M. Á.; Bañuelos-Vargas, M. I.; Rios-Herrera, G. D.; Heredia. J. B.; Ramírez-Pérez, J. S. and **Salazar-Leyva, J. A.** (2022). Use of Pacific thread herring (*Opisthonema libertate*) protein hydrolysates in Nile tilapia (*Oreochromis niloticus*) feeds: Productive performance and antioxidant enzymes on organisms exposed to a heat-induced stress. Aquaculture Research, 53(17): 6135-6147.

Sarmadi, B. H. and **Ismail, A.** (2010). Antioxidative peptides from food proteins: A review. Peptides, 31(10): 1949–1956.

Siddik, M. A. B.; Howieson, J.; Fotedar, R. and **Partridge, G. J.** (2021). Enzymatic fish protein hydrolysates in finfish aquaculture: Areview. Reviews in Aquaculture, 13(1): 406–430. https://doi.org/10.1111/raq.12481

Siddik, M. A. B.; Howieson, J.; Ilham, I. and **Fotedar, R.** (2018b). Growth, biochemical response and liver health of juvenile barramundi (*Lates calcarifer*) fed fermented and non-fermented tuna hydrolysate as fishmeal protein replacement ingredients. PeerJ, 6: e4870. https://doi.org/10.7717/peerj.4870

Siddik, M. A. B.; Howieson, J.; Partridge, G. J.; Fotedar, R. and **Gholipourkanani, H.** (2018a). Dietary tuna hydrolysate modulates growth performance, immune response, intestinal morphology and resistance to Streptococcus iniae in juvenile barramundi, *Lates calcarifer*. Scientific Reports, 8(1): 15942.

Siddik, M.A.B.; Howieson, J. and **Fotedar, R.** (2019). Beneficial effects of tuna hydrolysate in poultry by-product meal diets on growth, immune response, intestinal health and disease resistance to *Vibrio harveyi* in juvenile barramundi, *Lates calcarifer*. Fish and Shellfish Immunology, 89: 61–70.

Singh, B. P.; Vij, S. and **Hati, S.** (2014). Functional significance of bioactive peptides derived from soybean. Peptides, 54: 171–179.

SPSS "Statistical Package for the Social Sciences version 22." (2014). IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.

Suma, A. Y.; Nandi, S. K.; Abdul Kari, Z.; Goh, K. W.; Wei, L. S.; Tahiluddin, A. B.; Seguin, P.; Herault, M.; Mamun, A.A.; Isaías, G.T. and **Anamul Kabir, M.** (2023). Beneficial effects of graded levels of fish protein hydrolysate (FPH) on the growth performance, blood biochemistry, liver and intestinal health, economics efficiency, and disease resistance to *Aeromonas hydrophila* of pabda (*Ompok pabda*) fingerling. Fishes, 8(3): 147.

Tacon, A.G.J.; Metian, M. and **McNevin, A.A.** (2022). Future Feeds: Suggested Guidelines for Sustainable Development. Reviews in Fisheries Science and Aquaculture., 30: 271–279.

Xu, H.; Mu, Y.; Zhang, Y.; Li, J.; Liang, M.; Zheng, K. and **Wei, Y.** (2016). Graded levels of fish protein hydrolysate in high plant diets for turbot (*Scophthalmus maximus*): effects on growth performance and lipid accumulation. Aquaculture, 454: 140-147.