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Effects of fish protein hydrolysate on the growth performance, feed and protein utilization of Nile tilapia (*Oreochromis niloticus*)

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Abstract The effect of fish protein hydrolysate (FPH) supplementation on the growth performance, feed and protein utilization and feeding cost of Nile tilapia (*Oreochromis niloticus*) was determined. Juvenile Nile tilapia were randomly distributed into five groups and fed with five isonitrogenous (32% crude protein) and isolipidic (7% crude fat) diets. The control diet contained fish meal without FPH supplementation (basal diet). Diets 2-5 contained 10% and 30% oil layer protein hydrolysate (OLPH) and 10% and 30% of aqueous protein hydrolysate (APH), respectively. All experimental fishes were manually fed to apparent satiation in triplicate groups for 8 weeks. Fish fed with APH10 diet had significantly higher growth performance ($P < 0.05$) in terms of final fish body weight, weight gain, average daily gain (ADG) and specific growth rate (SGR). In addition, fishes fed with APH10 had significantly higher feed utilization, protein efficiency ratio (PER) and protein productive value (PPV) than the fishes fed with the other diets ($P < 0.05$). The diet containing over 10% APH caused a reduction in growth performance, feed and protein utilization, possibly resulting from the high small peptides and amino acids which were over the appropriate range for dietary protein requirements for Nile tilapia. The cost of APH10 diet exhibited 26 bahts per kg fish gain in weight, it was lower than all other test diets. Our current finding indicates that dietary APH10 could improve growth performance, feed efficiency, protein utilization, and beneficial feeding cost for Nile tilapia.

Keywords: Fish by-product, Nile tilapia, Papain, Protein hydrolysate, Growth performance

Introduction

Fish silage or fish by-products are usually discarded in large quantities. These include fish by-catch and comprise approximately 50-70% raw materials from the world fish processing industry. These fish by-products are considered as functional ingredients because they contain high nutrients which are necessary for growth in fish (Khosravi *et al*., 2015). Fish silage produced from

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fish fillets waste is a good source of protein and essential fatty acids such as polyunsaturated fatty acids (PUFAs)(Quinto *et al*., 2018); however, these by-products are traditionally used for low priced market products such as fishmeal and plant fertilizers (Guerard *et al*., 2002; Khosravi *et al*., 2015). Aquatic animal feed production mainly uses fishmeal and fish oil at approximately 20%. However, the decline in fishmeal resources has continuously caused an increase in the price of this fishmeal. Therefore, several studies have attempted to explore alternative animal protein sources that could be used for sustainable aquaculture. Although vegetable protein sources have lower costs than animal protein sources, some plant protein sources have restriction factors such as bioactive molecules deficiency and some anti-nutritional factors which may lead to poor palatability and low amount of some essential amino acids. These factors may affect the growth performance, feed and nutrient utilization (Leduc *et al*., 2018; Opstvedt *et al*., 2003; Refstie *et al*., 2004) as well as exhibit histological changes in the intestines or cause enteritis in fish (Garc á-Ortega *et al*., 2016; Miao *et al*., 2018; Smith *et al*., 2018).

Fish protein hydrolysates (FPHs) are produced by enzymatic hydrolysis to acceptable forms and easy to use for metabolism. FPHs have nutritionally high levels of indispensable amino acids. A large number of amino acids are in small fraction of peptide chains which are produced from the polypeptides during enzymatic hydrolysis. These low molecular weight bioactive peptides have been known as potential protein source with high level of essential amino acids (Cai *et al*., 2015). In addition, the small peptides in the enzymatic hydrolysate have been shown to improve digestion, absorption, growth performance, feed utilization, nutrient utilization (Khosravi *et al*., 2015; Refstie *et al*., 2004; Xu *et al*., 2016; Zheng *et al*., 2013), immuno-stimulation and disease resistance (Khosravi *et al*., 2015; Ovissipour *et al*., 2014) in several fish species such as finfish (Ha *et al*., 2019; Siddik *et al*., 2019; Siddik *et al*., 2018), shrimp (Niu *et al*., 2014; Quinto *et al*., 2018; Shao *et al*., 2018) and abalone (Goosen *et al*., 2014).

Nile tilapia, *Oreochromis niloticus*, is the second most cultured fish species after carp in the world, wherein Nile tilapia is the foremost cultured species in Thailand due to its high consumption, good adaptability and disease tolerance (Suebsong *et al*., 2019). The production of Nile tilapia in Thailand occurs primarily in semi-intensive ponds and cages floated along the rivers. Supplemental feed additives such as fertilizer and supplementary feeding are being used to increase fish production. Many feed additives have been used as supplementation for semi-intensive culture of Nile tilapia. The feed additives generally used in aquaculture are: single ingredient, simple mixtures and/or high nutritional complete feeds (Adebayo *et al*., 2004; Ai-Hafedh and Siddiqui,

1998; Fasakin *et al*., 1999). In this study, we hypothesized that the enzymatic hydrolysate of fish byproducts could be used to enhance growth performance of tilapia. This study was conducted to investigate the effect on the growth performance, feed efficiency, protein utilization and feeding cost of adding FPH in the tilapia diets.

Materials and Methods

Preparation of fish protein hydrolysate

Fish waste silage hydrolysate or by-products were collected from the local market in Phitsanulok Province, Thailand. These by-products were homogenized in a cooking blender and frozen at –20˚C until analysis. Papain was used to hydrolyze fish waste silage at a concentration of 50 ml kg^{-1} followed by 50˚C incubation in the water bath with continuous agitation for 3 hours. In the final step of hydrolysis, it was subjected to a heating process at 95˚C for 10 min to inactivate the enzyme. Solid wastes (e.g., scale, bone, fins) were separated by centrifugation at 8,000 rpm for 15 min. Four layers of fish waste silage were formed and separated as shown in Figure 1, the upper part was oil layer, followed by light fat-protein layer, then soluble protein hydrolysate layer, and the sludge or heavy lipid-protein layer. The upper oil and soluble protein layers were collected into new tubes and frozen at –20˚C until further use.

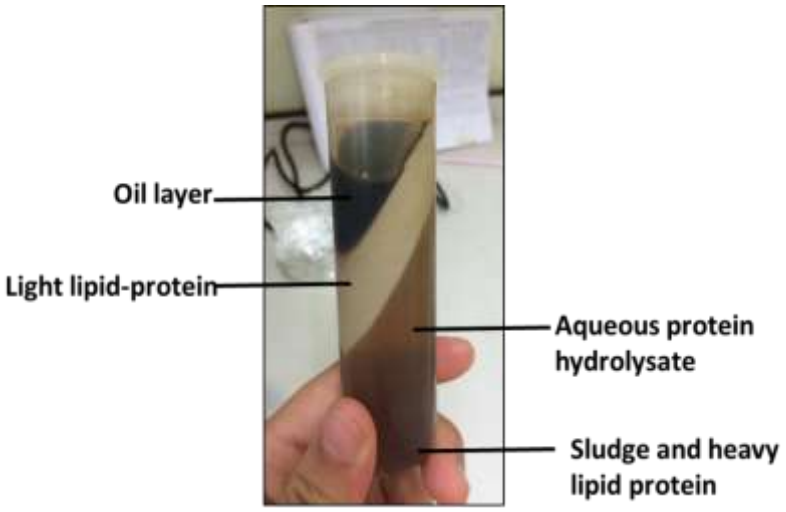

Figure 1. Four layers of fish protein hydrolysate

Experimental diets

Five experimental diets (isonitrogenous and isolipidic diets with approximately 32% crude protein and 7% crude lipid, respectively) were formulated with different levels of FPH as shown in Table 1. The basal diet had non-fish waste silage hydrolysate. The feed ingredients of diet 2 to diet 5 were set as the basal diet. Diets 2 and diet 3 were additionally sprayed with 10% and 30% oil layer protein hydrolysate (OLPH10 and OLPH30, respectively). Diet 4 and 5 were sprayed using 10% and 30% aqueous protein hydrolysate (APH10 and APH30, respectively). All feed ingredients were thoroughly blended along with the lipid sources (fish oil, oil layer and aqueous protein hydrolysate) and 350 ml kg^{-1} distilled water was added to form the feeds. It was thoroughly mixed using a kitchen mixer (Champ inter-trade, Thailand). The mixed ingredients were then pelleted at 3 mm diameter. The obtained pellets were dried in a hot air oven at 105˚C overnight and then stored at –20˚C until use.

Table 1. Experimental feed formulation and chemical composition

Ingredients/ Chemical composition	**Test diets (%)**				
	T1 (basal diet)	T2 (OLPH10)	T3 (OLPH30)	T4 (APH10)	T5 (APH30)
Fishmeal	20.8	20.8	20.8	20.8	20.8
Soybean meal	30	30	30	30	30
Rice bran	27	27	27	27	27
Corn	18	18	18	18	18
Fish oil	3.2	3.2	3.2	3.2	3.2
Vitamin mix	0.5	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Fish hydrolysates were sprayed before douching the experimental feed pellets					
OLPH	0	10	30	-	-
APH	0	-	-	10	30
Chemical composition (%)					
Dry matter	2.74±0.19	2.73±0.77	2.20±0.30	2.10±0.12	2.08±0.65
Crude protein	32.72±0.31	31.38±0.70	32.31±0.83	32.09±0.72	32.52±0.69
Crude fat	6.97±0.44	7.48±0.51	7.96±0.93	7.17±0.38	7.16±0.83
Ash	7.47±0.01	7.49±0.04	7.37±0.04	7.75±0.06	7.41±0.05
Fiber	2.61±0.33	2.09±0.02	2.11±0.01	2.81±0.01	2.93±0.43

[1]Vitamin mixture (mg or IU/kg diet): A, 5,000 IU; D3, 1,000 IU; E, 5,000 mg; K, 2,000; B1, 2,500 mg; B2, 1,000 mg; B6, 1,000 mg; B12, 10 mg; inositol, 1000 mg; pantothenic acid, 3,000 mg; niacin acid, 3,000 mg; C, 10,000 mg; folic acid, 300 mg; biotin, 10 mg

[2]Mineral mixture (g/kg feed); calcium phosphate, 80; calcium lactate, 100; ferrous sulfate, 1.24; potassium chloride, 0.23; potassium iodine, 0.23; copper sulfate, 1.2; manganese oxide, 1.2; cobalt carbonate, 0.2; zinc oxide, 1.6; magnesium chloride, 2.16; sodium selenite, 0.10

Fish, facilities, and rearing system

Nile tilapia were purchased from a local private hatchery in Phitsanulok Province, Thailand and transported to the Fish Nutrition Laboratory, Department of Agriculture Science, Faculty Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand. The fishes were acclimatized in the laboratory for 14 days. During this period, the fishes were fed with a commercial diet containing 40% crude protein, 12% dry matter, fiber 4% crude fiber, and 4% crude fat (High grade9961, Samutprakarn, Thailand).

At the beginning of the experiment, 20 fishes with approximately 2.50-4.00 g/fish were placed in glass aquarium. They were randomly allocated into triplicate groups. The glass aquaria used was with 150 L capacity with a flow-through water system and aerated using airstone for 24 h. The fishes were acclimatized for 3 days prior to initiation of the experiment. The fishes were fed twice a day at 9:30 and 16:30 by hand at a rate approaching apparent satiation as determined by visualization. The glass tanks were exposed to the natural light/dark regimes. All fishes in each tank were group-weighted at two-week intervals. Water qualities were determined everyday and maintained at 30±2˚C, 4-5.5 dissolved oxygen (DO) and 7.8-8.2 pH throughout the feeding trial.

Sample collection and analysis

At the 8th week of the feeding experiment, survived fishes were bulk weighed and counted from each replicate to calculate the growth performance and survival rate as follows:

Weight gain (WG, %):

Weight gain = [final weight (g) – initial weight (g)]/initial weight (g) x 100

Average daily gain, (ADG, g/day):

ADG = final weight (g) – initial weight (g)/days

Specific growth rate, (%):

Specific growth rate =ln [final weight (g) – initial weight (g)]/days x100

Survival rate, (%):

Survival rate = (number of final fish)/initial number of fish x 100

To determine the experimental feed utilization, the weight of consumed adiet was recorded and the nutrient utilization of each experimental diet calculated as follows:

Feed conversion ratio (FCR):

FCR = total feed intake (g)/weight gain (g)

Rate of feed intake (%/fish):

$$\text{Rate of feed intake} = \frac{F \times 100}{\frac{W_0+W_t}{2} \times \frac{N_0+N_t}{2} \times t}$$

Where F = dry weight of consumed diet (g)

N_0 = number of initial fish

W_0 = average weight of initial fish (g)

N_t = number of final fish

W_t = average weight of final fish (g)

Feed efficiency (FE, %):

$FE = (1/FCR) \times 100$

Feed efficiency ratio (FER):

FER = weight gain (g)/dry feed intake (g)

To determine protein utilization, the whole body of fishes at the initial and final stages were incubated at 105˚C until constant dry. Samples were then finely ground using pestle and mortar. Whole-body fish protein was determined according to the standard protocol (AOAC, 1997) using four replications for all analyses. Whole-body protein content was used to calculate protein utilization parameters as follows:

Protein efficiency ratio (PER):

PER = wet weight gain (g)/ total protein intake (g)

Protein productive value (PPV):

PPV = protein gain of fish (g)/ total protein intake (g)

Proximate composition

All samples including feedstuff, test diets and whole-body fish were used in four replications for proximate analysis following the standard protocol (AOAC, 1997). All samples were minced and ground using mortar and pestle. Moisture was carried out using the Memmert model (UL50, Germany) at 105˚C until the weight became constant. Crude protein was analyzed using the Kjeldatherm® block heating system and distillation units (semi-automatic Kjeldahl, Gerhardt Vapodest, 45s, Germany) following the Kjeldahl method (N × 6.25). Crude lipid was conventionally extracted by petroleum ether using a classic soxhlet apparatus (Gerhardt, Germany). The crude fiber was digested with acid and basic digestion. Ash was incinerated by the combustion method at 550˚C for 6 h using a muffle furnace (Carbolite ELF 11/14, England).

Economic analysis

All the costs of feed ingredients and feed consumed were used to determine the economic efficiency of the test diet. Feeding cost was calculated

based on the feed formulation (Table 1), the price of each ingredient per kg and feed consumed showed as follows:

Feeding cost (baht/kg)

Feeding cost = price of feed ingredient (baht/kg)/amount of feeding ingredient used (kg)

Feeding cost (bath/kg fish gain)

Feeding cost = (price of diet/feed consumed)/weight gain × 100

Statistical analysis

All experimental data were analysed using one-way analysis of variance (ANOVA) using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test (DMRT) was carried out to determine the difference among mean values. All data are represented as the mean value ± standard error of the mean (S.E.M.). $P < 0.05$ value was used to determine statistical difference.

Results

Growth performance

All experimental fishes promptly accepted the different diets during the 8 weeks of the culture period. Dietary FPH inclusion in Nile tilapia diets has a significant effect on the growth performance, feed and protein utilization as shown in Table 2 to Table 4. Fishes fed with the APH10 diet had the highest growth performance in terms of FBW, weight gain, ADG, and SGR compared to the control group ($P < 0.05$) while the fish fed with the control diet showed no significant difference in fish fed with OLPH10 and OLPH30 diets (Table 2). The fish survival rate during the feeding trial of all test diets further confirmed that all experimental diets did not affect fish welfare. The survival rate was high across all test diets with no significant effect with regards to FPHs supplementation ($P > 0.05$).

Feed utilization

The use of 10APH showed the highest value in terms of total feed intake, rate of feed intake, feed efficiency and feed efficiency ratio (Table 3). A significantly higher FCR was found in the control group. However, fish fed with APH30 diets had the lowest rate of feed intake, feed efficiency and feed efficiency ratio.

Table 2. Growth performance of Nile tilapia fed all test diets for 8 weeks

Experimental diets	Growth performance[1]					
	IBW[2]	FBW[3]	Weight gain[4]	Average daily gain[5]	Specific growth rate[6]	Survival rate[7]
Control	2.84±0.10	10.02±0.78^{bc}	251.46±27.20^{bc}	0.13±0.01^{bc}	2.24±0.14^{bc}	100
OLPH10	2.83±0.40	11.32±1.33^{b}	297.08±46.49^{b}	0.15±0.02^{b}	2.45±0.21^{b}	100
OLPH30	2.88±0.78	11.65±0.58^{b}	309.77±20.23^{b}	0.16±0.01^{b}	2.51±0.09^{b}	100
APH10	2.81±0.50	14.78±1.48^{a}	418.71±52.08^{a}	0.18±0.21^{a}	2.93±0.18^{a}	100
APH30	2.87±0.65	9.23±0.25^{c}	223.98±8.83^{c}	0.11±0.00^{c}	2.10±0.05^{c}	100

[1]Values represented as means (n=3), the standard error of means (SEM) were calculated with pooled samples. The values within the same column with different letters are shown the significant differences ($p<0.05$); [2]IBW = Initial body weight [3]FBW = Final body weight; [4]Weight gain (%) = (final weight-initial weight)/initial weight x 100; [5]ADG, Average daily gain (g/day) = final weight – initial weight/days; [6]SGR, Specific growth rate (%) = (lnW2-lnW1/T) x 100, W1 = initial weight, W2 = final weight, T = cultured period; [7]Survival rate (%) = number of final fish/number of initial fish x 100

Table 3. Feed efficiency of Nile tilapia fed fish protein hydrolysate for 8 weeks

Experimental diets	Feed efficiency of tilapia fed test diets[1]				
	Feed intake[2]	Feed conversion ratio[3]	Rate of feed intake[4]	Feed efficiency[5]	Feed efficiency ratio[6]
Control	8.88±0.46^{b}	1.24±0.07^{a}	0.12±0.00^{a}	80.52±4.44^{bc}	0.50±0.06^{b}
OLPH10	9.28±0.61^{b}	1.11±0.11^{b}	0.12±0.00^{a}	90.85±8.49^{ab}	0.62±0.11^{a}
OLPH30	9.53±0.23^{b}	1.09±0.05^{b}	0.12±0.00^{a}	92.27±4.61^{ab}	0.64±0.05^{a}
APH10	12.82±2.34^{a}	1.07±0.07^{b}	0.13±0.01^{a}	93.88±6.65^{a}	0.73±0.03^{a}
APH30	9.12±0.53^{b}	1.07±0.05^{b}	0.10±0.01^{b}	70.29±6.99^{c}	0.40±0.05^{b}

[1]Values represented as means (n=3), the standard error of means (SEM) were calculated with pooled samples. The values within the same column with different letters are shown the significant differences ($p<0.05$); [2]Feed intake (g) = total feed consumed; [3]FCR, Feed conversion ratio = Feed intake/Weight gain; [4]Rate of feed intake=dry feed consumed/ (weight gain/2)×(number of final fish/2)×days; [5]Feed efficiency (%) = (1/FCR) x 100; [6]Feed efficiency ratio = weight gain/fed diet

Protein utilization

Protein utilization was significantly influenced by both oil layer and aqueous protein hydrolysate supplementation (Table 4). Protein utilization (PER and PPV) was higher in fishes fed with APH10 than the other diets. Fishes fed with the diet containing APH30 has significantly lower protein utilization than all other treatments (Table 4).

Table 4. Protein utilization in tilapia fed fish protein hydrolysate for 8 weeks

Experimental diets	**Protein utilization**[1]	
	Protein efficiency ratio[2]	Protein productive value[3]
Control	3.82 ± 0.08^{c}	23.75 ± 2.05^{c}
OLPH10	4.44 ± 0.30^{b}	32.25 ± 2.43^{b}
OLPH30	4.51 ± 0.30^{b}	36.24 ± 1.63^{b}
APH10	5.51 ± 0.43^{a}	42.74 ± 1.63^{a}
APH30	2.82 ± 0.13^{d}	14.57 ± 0.80^{d}

[1]Values represented as means (n=3), the standard error of means (SEM) were calculated with pooled samples. The values within the same column with different letters are shown the significant differences ($p<0.05$); [2]Protein efficiency ratio (PER) = wet weight gain (g)/ total protein intake (g); [3]Protein productive value (PPV) = protein gain of fish (g)/ total protein intake (g)

Economic analysis

Data on the economic efficiency of Nile tilapia fed with diet containing FPHs is presented in Table 5. The total cost of all FPHs inclusion in diets was higher than the control. An increase of 1 kg fish mass which resulted from the diet with 10% APH had the lowest cost (Table 5).

Table 5. Economic analysis of Nile tilapia fed fish protein hydrolysate

Experimental diets	**Feeding cost**	
	Total cost (Baht/kg diet)[1]	Feeding cost (Baht/kg fish gain)[2]
Control	21.44	28.57
OLPH10	24.48	26.80
OLPH30	24.44	26.47
APH10	24.14	26.00
APH30	24.94	35.80

[1]Cost of feed ingredients (Bath/kg) ; [2]Feeding cost (Baht/kg gain) = (feeding cost/kg x total eaten feed)/weight gain

Discussion

The results of the present study showed positive effects in fishes fed with both OLPHs and APHs supplementation in diets indicating that FPHs inclusion in diets did not affect Nile tilapia growth performance throughout the 8 weeks of culture period. In addition, the survival rate of all treatments clearly confirmed that experimental diets had no adverse effects. Although the growth performance of fish fed with APH30 was slightly diminished, it was not significantly different with the control group ($P > 0.05$). These results were in accordance with several studies which showed that the minimum FPH supplementation in diets could support growth and feed efficiency compared with the basal diet as observed in several fish species such as Japanese flounder, *Paralichthys olivaceus* (Zheng *et al*., 2012), Atlantic salmon, *salmo salar* (Espe *et al*., 2012; Hevroy *et al*., 2005; Refstie *et al*., 2004) and cobia, *Rachycentron canadum* (Mach and Nortvedt, 2011). Fishes fed with 10-30% OLPH had higher growth performance than the control group, however, it was noticed that the oil layer was mainly composed of phospholipids which are active on the surface between phosphates and peptides. This promoted the formation of oil in the water surface after centrifugation. In the previous study further reported that the upper layer contained approximately 80% fat which may contribute to the decreased growth of fish due to high lipid content (Batista *et al*., 2010).

The minimum level of APH10 supplementation gave the highest final body weight. This could be due to the presence of low molecular weight peptide chains (Aksnes *et al*., 2006; Zheng *et al*., 2012) resulting to higher feed intake (HevrØY *et al*., 2005; Refstie *et al*., 2004), protein digestibility (HevrØY *et al*., 2005; Khosravi *et al*., 2015), and supplying a balance of indispensable amino acids in diet (Mach and Nortvedt, 2011). Growth performance of fish fed diet containing APH30 exhibited growth suppression, however, it was not significantly different compared to the control group. FPH has long been manufactured by autolysis and acidic hydrolysis using fish silage and/or fish by-products. These are the processes that might reduce indispensable amino acids especially tryptophan (Jackson *et al*., 1984; Shahidi *et al*., 1995). Fish silage or fish by-product autolysis always occurs upon storage resulting to bitter flavoring agents and lipid auto-oxidation (HevrØY *et al*., 2005; Refstie *et al*., 2004). Thus, high dietary FPH inclusion might negatively affect essential amino acid deficiency that results in reduced feed efficiency and growth performance in Nile tilapia.

It is concluded that fish silage or fish by-product has a potential use as an alternative feed supplement for Nile tilapia. The use of 10% APH gave the highest growth performance, feed and protein utilization. In addition, feed cost analysis showed that 10% APH had the lowest inputs in terms of feeding cost to gain 1 kg of fish body weight. However, APH supplementation higher than 10% may reduce growth performance in Nile tilapia.

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