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The effect of partial replacement of fish meal protein by dietary hydrolyzed fish protein concentrate on the growth performance of orange-spotted grouper epinephelus coioides

Abstract

This study investigated the effect of partial fish meal protein replaced by hydrolyzed fish protein concentrate [HFPC] on the growth performance of orange-spotted group erl. *Epinephelus coioides* (1.77 g initial weight) were fed five isonitrogenous [45%] and isolipidic [11%] diets containing different levels of HFPC to replace 0, 5, 10, 15 and 20% [0FS, 5FS, 10FS, 15FS and 20FS] of fish meal protein, respectively. Each diet was fed to triplicate groups of fish three times a day until satiation for six weeks. The weight gain percentages of fish fed diets containing HFPC to replace 5, 10, and 20% fish meal protein were significantly [$p<0.05$]higher than those of fish fed the control diet. The crude protein, crude lipid and ash contents of muscle of fish fed diets containing HFPC were lower than those of fish fed the control diet. The results indicated that diets included HFPC can improve growth performance of fish, and up to 20% fish meal protein can be replaced by HFPC in the diets for orange-spotted grouper *Epinephelus coioides*.

Keywords: Fish meal, Hydrolyzed fish protein concentrate, Replacement, Orange-spotted grouper

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Abbrevations: HFPC, Hydrolyzed Fish Protein Concentrate; NFE, Nitrogen Free Extract; ANOVA, A One-Way Analysis of Variance; FCR, Feed Conversion Ratios; ADC, Apparent Digestibility Coefficient

Introduction

In the recent decades, the interest in grouper aquaculture has been reported to increase rapidly in several Asian countries.[1,2] As a marine carnivorous fish, grouper require high level protein in the diet,[3,4] and most of practical feed contain high amount of fish meal as a main protein source.[5] However, the increasing price of fish meal is the most important concern related to economics and availability of this ingredient in the future,[6,7] and fish feed industry challenges to reduce dietary fish meal level. Therefore, the replacement of fish meal with alternative protein sources in fish diet is ongoing research priority to fish nutrition.[8]

Many studies have investigated on the use of terrestrial plant and animal by-products as protein sources to partial or total replacement of fish meal in marine carnivorous diets.[9-16] Deficient amino acids and the presence of anti-nutritional factors are the limiting factors in using of plant protein sources in fish diet,[17-20] and also the problem associated with animal by-product in fish diet is the quality of these terrestrial animals which depend on the quality of raw material and processing technique.[10,21,22] However, less attention has been paid to use other protein sources such as hydrolysate fish protein products.

HFPC produced from by-products of the fishery industry. HFPCs generally containing high levels of nutritional value and free amino acids are potential alternative ingredients to replace fish meal in fish feeds.[23-25] The chemical compositions of HFPC were usually similar to those of the raw materials used.[25,26] Hydrolysed fish protein products have been extensively studied as dietary ingredients for *Salmosalar* L,[27] cobia *Rachycentron canadum*,[28] pacu *Piaractus mesopotamicus*,[29] African catfish[30,31] and common carp *Cyprinus carpio*.[32] Highly apparent digestibility coefficient [ADC] of protein of sardine silage [95.1%] in the diets for tilapia was similar with ADC of protein of fish meal [95.8%].[33] The fermented silage had higher protein digestibility than acid silage in the diets for pacu *Piaractus mesopotamicus*.[29] The protein digestibility of enzymatic fish silage was higher than acid and bacterial fish silage in the diets for Nile tilapia *Oreochromis niloticus*.[34] However, another concern of using HFPC in fish diet is the deficiency of amino acid such as tryptophan due to prolonged autolysis when making this product.[23,34]

A number of studies are also available on the use of ingredients from terrestrial plant[35] and animal by-products[36-40] in grouper diets to partially or totally replace fish meal. However, there is no study on the utilization of HFPC as a protein source in grouper diet. The purpose of the present study was to evaluate the effects of HFPC as a partial replacement of fish meal protein in the diets on the growth performance and body composition of orange-spotted grouper *Epinephelus coioides*.

Materials and methods

Experimental Diets

Five isonitrogenous [45%] and isolipidic [11%] experimental diets were formulated in the present study. The experimental diets were included 0, 2.35, 4.68, 7.03 and 9.37%HFPC to replace 0% [0FS], 5% [5FS], 10% [10FS], 15% [15FS] and 20% [20FS] of fish meal protein. HFPC was obtained from commercial supplier [Scan Pro 35/4, Scan Bio, Trondheim, Norway]. The hydrolysate was conducted by formic acid treatment. The formulation and proximate compositions of diets are given in Table 1. Proximate composition analyses showed that all experimental diets contained 45.9-47.0% crude protein and 9.4-10.5%

crude lipid. Ash content decreased with increasing HFPC in the diets [16.95-14.50%].

In preparing the experimental diets, all dietary ingredients were first ground to small particle size with hammer mill and then passed through a 250µm mesh sieve. Dry ingredients of experimental diets were mechanically mixed to insure homogeneity. Distilled water was added [approximately 25% of dry weight] and the mixture blended thoroughly by hand until a consistency for extrusion was achieved. Diets were cold-extruded through a chopper [3.0mm die diameter] to produce pellets. The pellets were dried in an air dry oven at 60oC for 12 hours. The experimental diets were stored at 4oC in a refrigerator until used [Table 1].

Feeding Trial

Orange-spotted grouper were obtained from the southern coast of Taiwan and transported by air, in tightly sealed bags, quarter filled with seawater and inflated with oxygen, enclosed in an insulated container. They were acclimatized to laboratory condition for one week prior to the start of feeding trial. During this period, orange-spotted grouper were fed a control diet [45.9% crude protein, and 10.5% crude lipid] three times a day. At the beginning of experiment, fish were starved 24 h and 1.77 g uniform-sized fish randomly distributed into 15 glass aquaria [57x30x35 cm] with 10 fish in each aquarium. Each of the five experimental diets was hand-fed gradually to triplicate groups of fish three times a day at 09:00, 16:00 and 21:00 h until satiation for six weeks. Feces and uneaten feed were removed every day, and lost water during cleaning was replaced. Fish in each aquarium were individually weighed every two weeks of the feeding trial to monitor growth performance, and also individually was weighed at the beginning and the end of the feeding trial.

Table 1 Formulation and proximate composition of experimental diets for orange-spotted grouper *Epinepheluscoioides*

	Diets				
	0FS	5FS	10FS	15FS	20FS
Ingredients (% dry basis)					
Fish meal[a]	52	49.40	46.80	44.20	41.60
HFPC[b]	0	2.35	4.68	7.03	9.37
Basal mix[c]	35	35	35	35	35
Oil[d]	5.40	5.28	5.15	5.03	4.90
α-cellulose	7.60	7.98	8.37	8.74	9.13
Proximate composition (%)					
Moisture	2.95	3.52	3.84	2.98	2.89
Crude protein[e]	45.86	46.85	46.69	47.00	46.72
Crude lipid[e]	10.52	10.03	10.33	9.40	9.49
Crude fiber[e]	4.42	3.33	3.80	4.39	5.11
Ash[e]	16.95	15.31	14.80	14.68	14.50
NFE[e,f]	22.25	24.48	24.38	24.53	24.18
Gross energy (Kcal/100g)[e]	447.90	454.00	450.25	455.60	455.00

aFish meal : 63.50% crude protein and 8.56% crude lipid.

bHFPC (dry matter): 70% crude protein, 8% crude lipid and 12% ash.

cSquid meal 5%, shrimp meal 10%, yeast 1%, wheat flour 15%, mineral mix 2% (Bernhart-Tomarelli modified[59]) and vitamin mix 2% (0.5% Thiamine HCl, 0.8% Riboflavin, 2.6% Niacinamide, 0.1% D-Biotin, 1.5% Ca-pantothenate, 0.3% Pyridoxine HCl, 0.5% Folic acid, 18.1% Inositol, 12.1% Ascorbic acid, 3% Para-aminobenzoic acid, 0.1% Cyanocobalamine, 0.1% BHT and 60.3% α-cellulose).

dFish oil: corn oil = 2:1.

eExpressed as percent dry weight.

fNFE (Nitrogen free extract): 100 - (% crude protein + % crude lipid + % crude fiber + % ash).

The feeding trial was conducted in a recirculation seawater system and each aquarium was provided with continuous aeration through an air stone connected to a central air compressor. Water temperature and salinity varied from 27 to 31oC and from 32 to 35ppt, respectively. A 12 h light: 12 h dark photoperiod was maintained to simulate the natural light cycle.

After the termination of feeding trial, all fish from each tank were randomly sampled for proximate analysis. Fish were killed by immersing in ice water. Fish muscle were carefully dissected, dried and then homogenized. The homogenates were mixed into one sample for each diet treatment and analyzed in duplicates.

Proximate Analysis

The ingredients, experimental diets and fish muscle were analyzed for proximate composition based on the standard method of.[41] Samples of ingredients, experimental diets and fish muscle were dried to a constant weight at 105oC to determine moisture. Ash was determined by combustion at 540oC in a muffle furnace, protein was measured by nitrogen [N x 6.25] using the Kjeldahl system method after an acid digestion using a Kjeldahl system [Kjeldahl system 1002, Tecator, Sweden], and lipid was determined by chloroform and methanol [C:M ratio 2:1, v/v] extraction procedure according to.[42] Fiber was determined by using the Fiber tec system M 1020 [FossTecator, Sweden]. Nitrogen free extract [NFE] was determined by differences [NFE = 100% – (% protein + % lipid + % ash + % fiber)]. Gross energy of diets was determined by using a bomb calorimeter [IKA calorimeter system C 2000 basic, German].

Data Calculation and Statistical Analysis

Weight gain percentage and specific growth rate [SGR] were calculated according to the following equations: WG (%) = 100 x $[W_t - W_0] / W0$ and SGR [% day^{-1}] = 100 x $[(\ln W_t - \ln W_0)/t]$, where W_0 is the initial mean body weight [g], Wt is the final mean body weight [g] and t [day] is the feeding period.

A one-way analysis of variance [ANOVA] was performed to examine differences in weight gain percentages, SGR and survival among treatments. When a significant difference was observed, a Duncan's new multiple range test was used to compare differences among treatment means. The significant level was set at $p<0.05$and all statistical analyses were conducted using SAS software program for windows [V.9.3., SAS Institute, Cary, North Carolina, USA].

Results

All diets were well accepted by fish and consumed aggressively for the duration of the experiment. Survival of fish was greater than 90% for all treatment diets. Growth performances of orange-spotted grouper fed experimental diets are presented in Table 2. The growth performance of orange-spotted grouper fed 5FS, 10FS and 20FS diets were significantly higher than that of grouper fed diet without supplemented with HFPC. The growth performance of fish fed the control diet was not significantly different from that of fish fed 15FS. However, there were no significant differences in weight gain percentage and SGR among fish fed diets containing different levels of HFPC. Feed conversion ratios [FCR] of grouper fed diets containing HFPC were lower than that of grouper fed the control diet.

Table 2 Initial weight, final weight, weight gain, specific growth rate (SGR) and survival of orange-spotted grouper *Epinephelus coioides* 1

Diets	Initial weight (g)	Final weight (g)	Weight gain (%)	SGR (% day^{-1})
0FS	1.77 ± 0.12	16.91 ± 0.74^{b}	858.04 ± 35.63^{b}	5.38 ± 0.09^{b}
5FS	1.77 ± 0.08	20.25 ± 1.91^{a}	1044.03 ± 58.17^{a}	5.80 ± 0.12^{a}
10FS	1.77 ± 0.06	19.76 ± 1.99^{ab}	1013.00 ± 83.49^{a}	5.73 ± 0.18^{a}
15FS	1.77 ± 0.02	19.44 ± 1.67^{ab}	981.54 ± 103.28^{ab}	5.66 ± 0.22^{ab}
20FS	1.77 ± 0.01	19.64 ± 1.15^{ab}	1011.60 ± 66.61^{a}	5.73 ± 0.14^{a}

[1]Values are mean ± SD, obtained from three replicates (n = 3) with 10 fish for each group.

[2]FCR (feed conversion ratio) FCR = feed supply [g]/[final body weight [g] – initial body weight [g]]

Different superscripts in each column indicate significantly different mean values ($p < 0.05$).

Table 3 Muscle proximate analysis of orange-spotted grouper *Epinepheluscoioides*

Muscle composition	Diets			
	0FS	5FS	10FS	15FS
Moisture	78.09	77.64	77.65	77.39
Crude protein*	76.35	74.83	74.40	74.95
Crude lipid*	12.44	11.99	10.29	14.13
Ash*	5.84	5.41	5.15	5.26

*Expressed as percent of dry weight basis.

Partial replacement of fish meal protein by HFPC also affected muscle proximate composition of fish. The ash and water content of muscle of fish fed diets included different levels of HFPC was lower than those of fish fed the control diet. The levels of crude protein and crude lipid of muscle decreased with increasing dietary HFPC levels; however, the trend was slightly fluctuation [Table 3].

Discussion

The growth performance of orange-spotted grouper fed diets supplemented with HFPC to replace fish meal protein can be improved in this study. The positive effects of inclusion acidic and enzymatic hydrolysate fish protein in the diets have also been reported for Atlantic salmon *Salmosalar*,[43-45] Japanese sea bass *Lateolabrax japonicas*,[46] red seabream *Pagrus major*,[47] black bass *Micropterus salmoides*[48] and common carp *Cyprinus carpio*.[32]

In the present study, up to 20% fish meal protein in the diet for *Epinephelus coioides* could be replaced by HFPC. This finding was similar with[49] who reported that fish meal protein could be replaced by enzymatic hydrolyzed fish protein up to 25% in the diet without negative effect on the growth performance of turbot *Scophthalmus maximus*. The hydrolyzed silage head shrimp meal could replace fish meal protein up to 20% in African catfish *Clarias gariepinus* diets.[50] Tilapia and African catfish fed diets supplemented with 15 to 30% hydrolysate protein products to replace fish meal protein showed no adverse effects on growth performance.[51-53] It is referred that hydrolysate fish proteins can be good alternative protein sources to replace dietary fish meal protein.

The growth performances of Atlantic salmon[44,45] and red seabream[47] fed diets containing hydrolysate fish protein-based diets were better than those of fish fed the control diet and feed intakes increased with increasing dietary hydrolysate fish protein. In the present study, grouper fed diets containing HFPC showed low levels of FCR. Therefore, we can see that the more diets were fed by grouper, the higher growth performances of grouper were. This indicates that hydrolysate fish protein could act as an attractant to improve palatability of diet. It is well known that hydrolysate fish proteins contain small molecular-weight compounds which have beneficial effects to enhance fish growth and feed utilization.[54-57] These were also supported by[58] who reported that di- and tripeptides included in the diets could improve growth and survival of sea bass *Dicentrarchus labrax* larvae.

The crude protein, crude lipid and ash contents of muscle of orange-spotted grouper fed different level of HFPC were lower than those of muscle of fish fed the control diet. The low level of ash content of muscle of fish fed HFPC was similar to the previous finding in cobia,[28] red seabream,[47] black bass,[48] African catfish[50,52] and Nile tilapia.[53] However, studies reported that increasing dietary hydrolyzed fish protein increased ash level[31,51] and lipid contents [45,47,52,53] of fish muscle. The differences in proximate composition of muscle of fish fed hydrolysate protein-based diets might be due to the different raw materials used for those studies.

In conclusion, the results of the present study showed that diets supplemented HFPC to replace 20% fish meal protein could improve growth performance of orange-spotted grouper *Epinephelus coioides*.

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Conflicts of interest

None.

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