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Graded levels of fish protein hydrolysate in high plant diets for turbot (*Scophthalmus maximus*): effects on growth performance and lipid accumulation

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ABSTRACT

A 12-week feeding experiment in indoor flowing seawater system was conducted to investigate the effects of graded levels of dietary fish protein hydrolysate (FPH) on growth performance and lipid accumulation of juvenile turbot (*Scophthalmus maximus*) (initial body weight 4.16 ± 0.01 g). Four isonitrogenous and isoenergetic experimental diets with high plant protein were formulated to contain graded levels of FPH, meanwhile the fish meal was replaced correspondingly by 0% (Diet FPH-0, control), 5% (Diet FPH-5), 10% (Diet FPH-10) and 20% (Diet FPH-20) of total dietary protein, respectively. Quadruplicate groups of 25 fish were fed to apparent satiation twice daily during the feeding trial. The results showed that the specific growth rate (SGR), protein efficiency ratio, and protein retention was not significantly different among group FPH-0, FPH-5, and FPH-10. The highest level of dietary FPH (FPH-20) significantly reduced the SGR but increased the feed intake compared to the control group. The viscerosomatic index in group FPH-10 and FPH-20 were significantly lower than that in the control group. Fish fed FPH-20 also showed significantly lower crude lipid concentration in whole body than fish fed the control diet. The concentrations of total and neutral lipid in gut were significantly lower in fish fed FPH-10 compared to the control group. For muscle lipid, the polar lipid concentration significantly decreased while the neutral lipid concentration significantly increased with increasing levels of dietary FPH, but no significant difference in total lipid concentration was observed among experimental groups. The lipid concentrations in liver were not significantly different among dietary treatments. With increasing levels of dietary FPH, serum triacylglycerol and cholesterol concentrations significantly decreased. The influence of dietary FPH on tissue fatty acid compositions generally corresponded with that on tissue lipid concentrations. In conclusion, these results suggested that in high plant protein diets FPH replacing fish meal by 10% of total dietary protein did not compromise the growth of juvenile turbot. However, a higher FPH level (replacing fish meal by 20% of total dietary protein) reduced the growth and feed utilization but it increased the feed intake. In the present experimental conditions, the FPH treatments, especially at high levels, significantly modulated the lipid accumulation and fatty acid compositions in turbot tissues, in a dose- and tissue-dependent manner.

Statement of Relevance: This study provided useful data for the circulatory use of fish processing by-products in fish diets. Also, based on the results of lipid analysis in fish tissues, potential lipid manipulating feed additives could be explored from fish protein hydrolysates.

1. Introduction

As aquaculture expanded over the past decades, the demand for fish protein in feed industry increased rapidly. Improvement of utilization efficiency of marine fish protein is important for sustainable aquaculture. Recycling of fish protein in fish processing by-products in means of enzymolysis is one of the most important ways to improve the utilization efficiency of marine fish protein.

The nutritive value of fish protein hydrolysate (FPH) in fish feed has been demonstrated in a number of fish species, such as Atlantic salmon (Refstie et al., 2004; Hevrøy et al., 2005; Kousoulaki et al., 2012), red sea bream (Bui et al., 2014; Khosravi et al., 2015a, 2015b), European sea bass (Kotzamanis et al., 2007; Skalli et al., 2014; Delcroix et al., 2015), Japanese flounder (Zheng et al., 2012, 2013b), Atlantic cod (Aksnes et al., 2006a; Johannsdottir et al., 2014), olive flounder (Khosravi et al., 2015a), large yellow croaker (Cai et al., 2015), Persian sturgeon

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(Ovissipour et al., 2014), Asian seabass (Chotikachinda et al., 2013), Japanese eel (Masuda et al., 2013), and rainbow trout (Aksnes et al., 2006b). These studies suggested that appropriate levels of fish protein hydrolysate in diets have beneficial effects on the growth, feed intake, nutrient utilization, immune response, oxidative status and disease resistance of fish, especially for larvae and fish fed high plant protein diets. More digestable and absorbable peptide profiles and some bioactive compounds in FPH are suggested to account for these beneficial effects.

Besides these beneficial effects, another potential nutritional value of FPH is the modulation of lipid accumulation. This has been reported in rodent studies, which showed that FPH fed rats/mice had reduced tissue lipids and modulated fatty acid compositions (Shukla et al., 2006; Hosomi et al., 2011; Liaset et al., 2009, 2011; Bjørndal et al., 2013). In accordance with these results in rodents, a study on Atlantic salmon reported that FPH inclusion in the diet reduced the viscera mass (Espe et al., 2012a). Our previous studies on turbot and Japanese flounder also indicated the reduction of fish body lipid and viscerosomatic index by high levels of FPH (Zheng et al., 2013b; Wei et al., 2015). These results indicate the great potential of lipid metabolism-modulating effects of FPH. One aim of the present study is to comprehensively assess the effects of dietary FPH on the lipid accumulation in various fish tissues, which is significant to fish health and fish product quality.

Despite the high nutritive value of FPH, limited information is available regarding the use of FPH in diets for turbot, which is a very important marine aquaculture species in the world and a carnivorous species relying on marine protein. Previous studies in our laboratory have observed the beneficial effects of dietary FPH on growth and nutrient utilization of turbot (Zheng et al., 2013a; Wei et al., 2015), especially the fish meal protein sparing effect of FPH in high plant protein diets. To evaluate the performance of FPH in turbot diets with further higher plant protein diets, the present study was conducted using further lower fish meal protein level in the basal diet.

2. Materials and methods

2.1. Preparation of fish protein hydrolysate

Fish protein hydrolysate was prepared from by-products of Pollock (*Theragra chalcogramma*) processing as described in our previous studies (Zheng et al., 2013b). The hydrolysate was filtered through Pellicon 2 Ultrafiltration Modules PLAC (Millipore, Billerica, MA, USA) with a filter of 1000 Da to obtain the small molecular weight compounds. The permeate after ultrafiltration was concentrated, freeze dried and stored at −20 °C prior to use. The molecular weights of the hydrolysate were analyzed by HPLC size exclusion chromatography using a TSK G2000 column, according to Boza et al. (1994). The peptide molecular weight distribution of the fish protein hydrolysate was presented in Table 1.

2.2. Experimental diets

Four isonitrogenous and isoenergetic (52% crude protein and 11% lipid) experimental diets with high levels of plant protein (only 15% fish meal in the basal diet) were formulated, differing in the supplementation levels of FPH and fish meal (Table 1). The experimental diets were prepared containing graded levels of FPH (0, 3.1%, 6.2% and 12.4%), meanwhile the fish meal was replaced correspondingly by 0% (Diet FPH-0, control), 5% (Diet FPH-5), 10% (Diet FPH-10) and 20% (Diet FPH-20) of total dietary protein, respectively. The amino acid compositions of the experimental diets were presented in Table 2. The fatty acid composition was constant among the experimental diets (full data not shown).

Ingredients were ground into fine powder through 200 μm mesh. All ingredients were thoroughly mixed with the oils, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill and dried for 12 h in a ventilated oven at 55 °C. After drying, the diets were broken up and sieved into proper pellet size (3.0 × 3.0 mm), and were stored at −20 °C before usage.

Table 1
Formulation and proximate composition of the experiment diets (g kg^{-1}).

Ingredients	Diet			
	FPH-0	FPH-5	FPH-10	FPH-20
Fish meal[1]	150.0	115.0	80.0	10.0
Soybean meal[1]	240.0	240.0	240.0	240.0
Corn gluten meal[1]	120.0	120.0	120.0	120.0
Wheat gluten[1]	180.0	180.0	180.0	180.0
FPH[2]	0.0	31.0	62.0	124.0
Wheat meal[1]	123.0	125.0	126.0	129.0
Fish oil	87.0	89.0	92.0	97.0
Soy lecithin	10.0	10.0	10.0	10.0
Vitamin premix[3]	15.0	15.0	15.0	15.0
Mineral premix[4]	15.0	15.0	15.0	15.0
Choline chloride	20.0	20.0	20.0	20.0
Monocalcium phosphate	20.0	20.0	20.0	20.0
L-ascorbyl-2-polyphosphate	5.0	5.0	5.0	5.0
L-lysine	8.0	8.0	8.0	8.0
D/L-methionine	4.0	4.0	4.0	4.0
L-arginine	3.0	3.0	3.0	3.0
Proximate composition				
Crude protein	518.1	524.2	522.4	521.6
Crude lipid	105.6	107.1	107.5	107.8
Ash	72.4	70.6	70.0	68.3

[1]Fish meal: anchovy meal, crude protein 72.1% dry matter, crude lipid 7.9% dry matter; soybean meal: crude protein 53.5%, crude lipid 0.5%; Corn gluten meal: crude protein 65.1%, crude lipid 1.5%; Wheat gluten: crude protein 81.4%, crude lipid 0.5%; Wheat meal: crude protein 21.3%, crude lipid 1.4%. All these ingredients were purchased from Qingdao Great Seven Bio-tech Co., Ltd. (Qingdao, China).
[2]The peptide molecular weight distribution of fish protein hydrolysate (FPH): 10,000–5000 Da, 0.2%; 5000–2000 Da, 1.14%; 2000–1000 Da, 5.21%; 1000–500 Da, 21.34%; 500–200 Da, 56.64%; 200–100 Da, 3.66%; <100 Da, 11.82%.
[3]Vitamin premix (mg or g/kg diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin, 1.2 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; wheat middling, 13.67 g.
[4]Mineral premix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; $Ca(IO_3)_2 \cdot 6H_2O$ (1%), 60 mg; zoelite, 13.485 g.

Table 2
Amino acid composition of experimental diets (% dry matter).

Amino acid	Diet			
	FPH-0	FPH-5	FPH-10	FPH-20
Aspartic acid	2.86	2.58	2.47	2.77
Threonine	1.43	1.34	1.28	1.36
Serine	1.92	1.78	1.75	1.86
Glutamic acid	10.54	9.61	9.59	10.38
Glycine	1.67	1.58	1.54	1.65
Alanine	2.07	1.98	1.93	2.06
Cysteine	0.53	0.58	0.49	0.55
Valine	1.81	1.74	1.63	1.75
Methionine	0.89	0.62	0.84	0.92
Isoleucine	1.61	1.61	1.50	1.60
Leucine	3.56	3.50	3.38	3.59
Tyrosine	1.58	1.47	1.33	1.43
Phenylalanine	2.72	3.06	3.72	3.42
Lysine	2.31	2.45	2.32	2.38
Histidine	1.02	1.06	0.96	0.89
Arginine	1.93	2.24	1.88	1.90
Taurine	0.14	0.15	0.15	0.16
TAA	38.59	37.35	36.76	38.67
IAA	17.28	17.62	17.51	17.81
DAA	21.31	19.73	19.25	20.86
IAA/TAA	44.78	47.18	47.67	46.06
IAA/DAA	81.09	89.31	90.96	85.38

TAA: total amino acid; IAA: Indispensable amino acid; DAA: Dispensable amino acid.