Aquaculture 433 (2014) 476–480

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Hydroxyproline supplementation on the performances of high plant protein source based diets in turbot (*Scophthalmus maximus* L.)

Yunzheng Liu, Gen He *, Qingchao Wang, Kangsen Mai, Wei Xu, Huihui Zhou

Key Laboratory of Aquaculture Feed, Nutrition Ministry of Agriculture, Ocean University of China Qingdao, 266003, PR China

ARTICLE INFO

Article history:
Received 2 April 2014
Received in revised form 23 June 2014
Accepted 2 July 2014
Available online 19 July 2014

Keywords:
Turbot
Hydroxyproline
Plant protein
Muscle texture

ABSTRACT

Hydroxyproline (hyp) is one of the bioactive molecules rich in fishmeal but low in plant protein sources. With increased utilization of plant proteins in aquafeeds, a better understanding is warranted for the necessity of hyp supplementation in high plant protein based diets. In the present study, isonitrogenous and isoenergetic turbot diets were formulated with 40%, 50% and 60% fishmeal substituted by plant proteins, with (or without) addition of 0.6% hyp. After an 8-week feeding trial in juvenile turbot, hyp supplementation significantly improved specific growth rate (SGR) and feed efficiency ratio (FER) in fish fed diets with 50% or higher fishmeal replaced, but not in the group with 40% fishmeal replaced by plant proteins. Hyp levels in plasma and tissues were reduced after plant protein substitutions and replenished by dietary supplementation. Dietary hyp significantly increased muscle hardness, springiness and chewiness of fish ($P < 0.05$). These results suggested that dietary hyp supplementation is particularly necessary for fishmeal-replaced diet exceeding a certain high level.

1. Introduction

Fishmeal is the most commonly used protein source in aquaculture and indispensable for many farmed fish species. For the sustainability of aquaculture industry and lower cost, many aquafeed manufacturers have turned their focus on plant proteins for fishmeal replacement (Hardy, 2010). However, there are still major challenges remaining for fishmeal replacement especially in diets for carnivorous fish, such as Atlantic salmon (Carter and Hauler, 2000; Storebakken et al., 1998), rainbow trout (Gomes et al., 1995; Kaushik et al., 1995), gilthead sea bream (Gómez-Requeni et al., 2004), Japanese flounder (Kikuchi, 1999), Korean rockfish (Lim et al., 2004) and Atlantic cod (Hansen et al., 2007). Understanding the differences in composition and utilization of fishmeal and plant protein sources is necessary for the development of new protein sources for aquaculture.

Hydroxyproline (hyp) is one of several bioactive factors (such as taurine) identified to be rich in fishmeal but low or none in plant protein sources (Aksnes et al., 2006; Cheng and Hardy, 2004; Gaylord et al., 2007; Lunger et al., 2007). Hyp is crucial for collagen synthesis. The helical region of collagen comprises the repeat of Gly–X–Y, where hyp occurs in the Y position. Hyp is also necessary in many physiological processes. It is a substrate for the synthesis of glycine, pyruvate, and glucose (Wu et al., 2011) and may also scavenge oxidants and regulate the redox state of cells (Phang et al., 2008, 2010). In addition, hyp has been proved to be the only free amino acid in tissues that was positively correlated with the growth rate of juvenile salmon (Kousoulaki et al., 2012; Sunde et al., 2001).

Hydroxyproline is produced by hydroxylation of the amino acid proline by the enzyme prolyl hydroxylase following protein synthesis (Gorres and Raines, 2010). However, it is largely unknown for the ability of hyp biosynthesis in fish. In recent years, scientists have found that hyp might be a conditionally indispensible amino acid for fish (Li et al., 2009). It was reported that dietary hyp inclusion promoted growth in Atlantic salmon fed with high plant protein diets (Aksnes et al., 2008), but not in turbot (Zhang et al., 2013). The application of dietary hyp supplementation, especially to high plant protein based diets, requires further examinations. The present study was conducted to evaluate effects of hyp addition to diets with serial levels of plant proteins on growth performance, muscle texture, hyp content in tissues of juvenile turbot.

2. Materials and methods

2.1. Feeding ingredients and diet formulation

L-hydroxyproline (>99% pure) was obtained from Hengyuan Biotech (Shanghai, China) Co., LTD. Fishmeal, soybean meal, corn gluten meal, wheat gluten meal, peanut meal, and beer yeast were used as the primary protein sources. Fish oil and soybean lecithin were used as the lipid sources. Wheat flour was used as the carbohydrate sources. Seven isonitrogenous and isoenergetic diets were formulated: a reference diet (FM) containing 60% fish meal and three other diets (40I, 50I and 60I) in which 40%, 50% and 60% fish meal was substituted. Based on the supplementation levels used in reports (Asknes et al., 2008; Zhang

* Corresponding author at: No. 5 Yushan Rd, Qingdao, PR China. Tel.: +86 532 82031589; fax: +86 532 82032038.
E-mail address: hegen@ouc.edu.cn (G. He).

http://dx.doi.org/10.1016/j.aquaculture.2014.07.002
0044-8486/

et al., 2013), experimental diets with plant protein substitutions were either supplemented with 0.6% hyp or not. All ingredients were grounded into fine powder through 178 μm mesh. Ingredients were blended with oil and water then added. Pellets (3 mm × 4 mm) were made automatically by pellet-making machine and dried for 12 h in a ventilated oven at 40 °C. The hyp concentrations after diet preparation were quantitated as 0.23% (FM), 0.16%(40I), 0.58%(40I HYP), 0.18% (50I), 0.62% (50I HYP), 0.14% (60I) and 0.57% (60I HYP). DL-Methionine, L-Threonine, L-Histidine, and Lys-H_2SO_4 (Crystalline amino acids) were supplemented to meet the essential amino acid (EAA) requirements of juvenile turbot based on the amino composition of FM diet. All the compositions of experiment diets were shown in Table 1.

Table 1
Formulation and proximate chemical composition of the tested diets (% dry matter).

Ingredients	Treatments						
	FM	40I	40I HYP	50I	50I HYP	60I	60I HYP
Fish meal[a]	60.00	36.00	36.00	30.00	30.00	24.00	24.00
Wheat flour[a]	27.50	12.95	12.35	10.23	9.63	7.51	6.91
Soybean meal[a]	0.00	15.68	15.68	19.60	19.60	23.52	23.52
Corn gluten meal[a]	0.00	8.00	8.00	10.00	10.00	12.00	12.00
Wheat gluten meal[a]	0.00	5.12	5.12	6.40	6.40	7.68	7.68
Peanut meal[a]	0.00	3.20	3.20	4.00	4.00	4.80	4.80
Beer yeast[a]	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin premix[b]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[c]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Attranct[d]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Taurine	0.00	1.00	1.00	1.00	1.00	1.00	1.00
Sodium alginate	0.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine	0.00	0.26	0.26	0.32	0.32	0.38	0.38
L-Threonine	0.00	0.18	0.18	0.22	0.22	0.26	0.26
L-Histidine	0.00	0.19	0.19	0.23	0.23	0.27	0.27
Lys-H_2SO_4	0.00	0.74	0.74	0.92	0.92	1.10	1.10
Fish oil	3.00	5.50	5.50	5.90	5.90	6.30	6.30
Soybean lecithin	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Choline chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25
$Ca(H_2PO_4)_2$	0.00	0.40	0.40	0.40	0.40	0.40	0.40
Phytase	0.00	0.20	0.20	0.20	0.20	0.20	0.20
Y_2O_3	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calcium propionate	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
$FeSO_4 \cdot H_2O$	0.00	0.05	0.05	0.05	0.05	0.05	0.05
$ZnSO_4 \cdot H_2O$	0.00	0.03	0.03	0.03	0.03	0.03	0.03
L-Hydroxyproline (% dry matter)	0.00	0.00	0.60	0.00	0.60	0.00	0.60
Moisture	3.90	4.23	3.84	5.00	4.37	3.94	4.97
Crude protein	50.75	50.97	51.75	51.39	51.51	52.17	51.83
Crude lipid	10.95	11.48	11.14	11.56	11.10	11.75	11.35
Ash	11.64	10.08	9.77	9.44	9.15	8.77	8.60
Gross energy (KJ g^{-1})	20.23	20.57	20.67	20.60	20.66	20.99	20.73

Note: FM, diet fish meal; 40I, replacement of 40% fish meal by plant protein mixture; 40I HYP, replacement of 40% fish meal by plant protein mixture with addition of 0.6% hydroxyproline; 50I, replacement of50% fish meal by plant protein mixture; 50I HYP, replacement of 50% fish meal by plant protein mixture with addition of 0.6% hydroxyproline; 60I, replacement of 60% fish meal by plant protein mixture; 60I HYP, replacement of 60% fish meal by plant protein mixture with addition of 0.6% hydroxyproline.

[a] Supplied by Qihao Biotech. Co., Ltd. (Qingdao, Shandong); red fish meal, crude protein, 73.38%, crude lipid, 10.42%; wheat flour, crude protein, 17.05%, crude lipid, 2.29%; soybean meal, crude protein, 55.04%, crude lipid 2.02%; corn gluten meal, crude protein, 70.45%, crude lipid, 1.67%; wheat gluten meal, crude protein, 80.27%, crude lipid, 1.24%; peanut meal, crude protein, 50.82%, crude lipid, 2.90%; beer yeast, crude protein, 49.78%, crude lipid, 1.61%.

[b] Vitamin premix (mg kg^{-1} diet): retinal palmitate, 32; cholecalciferol, 5; DL- -to-copherol acetate, 240; menadione, 10; thiamin-HCl, 25; riboflavin, 45; pyridoxine-HCl, 20; cyanocobalamin, 10; D-calcium pantothenate, 60; amine nicotinic acid, 200; folic acid, 20; biotin, 60; mesoinositol, 800; ascorbyl polyphosphate (contained 35% ascorbic acid), 2000; microcrystalline cellulose, 16473.

[c] Mineral premix (mg kg^{-1} diet): $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 80; ZnSO4 · H_2O, 50; $MnSO_4 \cdot H_2O$, 45; $CoCl_2 \cdot 6H_2O$ (1%), 50; Na_2SeO_3 (1%), 20; calcium iodine, 60; zoelite, 8485.

[d] Attractant: betaine: dimethyl-propiothetin: glycine: alanine: 5-phosphate inosine = 4:2:2:1:1.

2.2. Fish and experimental procedure

Juvenile turbot were obtained from Haiyang fish farm (Haiyang, Shandong, China). Fish were acclimated to the system and fed with commercial diet for 2 weeks before the trials. Juvenile turbot (initial body weight: 8.63 ± 0.03 g) were randomly distributed into 28 tanks (60 cm * 60 cm * 60 cm), and each of the 7 experimental diets was assigned to 4 tanks with 30 fish per tank. Seawater, continuously pumped from the coast adjacent to the experimental station, passed through sand filters into each tank at approximately 1.5 l/min. The experimental fish were fed to apparent satiety twice a day at 7:00 and 19:00 for 9 weeks. The consumption of each tank was recorded every day. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70 °C and reweighed. During the whole experimental period, water temperature was ranged from 19.0 to 22.0 °C; pH from 7.5 to 8.0; salinity from 30‰ to 33‰; ammonia nitrogen was lower than 0.1 mg/l; nitrite was lower than 0.1 mg/l; dissolved oxygen was higher than 6.0 mg/l.

2.3. Sample collection

Before the feeding, 20 fish from the same population were randomly selected for determination of initial whole-body proximate composition. At the end of the feeding trial, the experimental fish in each tank were anesthetized with eugenol (1:10,000) (99% purity, Shanghai Reagent, China). Total number and mean body weight of fish in each tank were measured. Five fish were randomly sampled from each tank and stored frozen at −20 °C for whole body composition analysis. Six fish from each tank were sampled to measure individual body weight, body length, liver weight and visceral weight so as to calculate condition factor, hepatosomatic index and viserosomatic index. Meanwhile, the liver and back muscle were sampled and frozen in liquid nitrogen. Blood samples were taken from the caudal vein of another five fish from each tank using heparinized syringes to obtain plasma samples after centrifugation (4000 *g* for 10 min) at 4 °C and immediately stored at −20 °C until analysis.

2.4. Chemical analyses

2.4.1. Body composition

Dry matter, crude protein, crude lipid, ash and energy were analyzed for ingredients, experimental diets and fish samples using standard Association of Official Analytical Chemist (AOAC) methods (1995). Dry matter was analyzed by drying the samples to constant weight at 105 °C. Crude protein was determined by using the Kjeldahl method (Kjeltec TM 8400, FOSS, Sweden) and estimated by multiplying nitrogen by 6.25. Crude lipid was measured after diethyl ether extraction using Soxhlet method (Buchi 36680, Switzerland). Ash was examined after combustion in a muffle furnace at 550 °C for 16 h. Gross energy was determined with Parr1281 Automatic Bomb Calorimeter (Parr, Moline, IL, USA).

2.4.2. Digestibility determination

Y_2O_3 (0.1%) was supplemented as the indicator for the dry matter and crude protein digestibility determination following previous studies (Glencross et al., 2007; Regost et al., 2003). Fecal samples were collected for 2 weeks from each tank, using an automatic fecal collector by siphoning 1 h after feeding. Collected fecal samples were stored at −20 °C. Yttrium oxide and phosphorus content in the diet and feces were determined by inductively coupled plasma-atomic emission spectrophotometer (ICP-OES, VISTA-MPX).

2.4.3. Texture profile analysis

The flesh of back was obtained from the same position of each fish with similar size (40.10 ± 2.51 g). The texture profile analysis (TPA) was carried out with a texture analyzer immediately after the flesh