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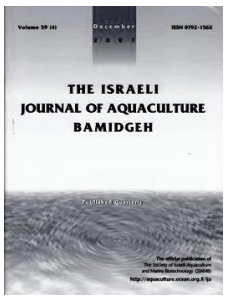

Dietary Histidine Requirement for Juvenile Large Yellow Croaker, *Pseudosciaena crocea* R.

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Abstract

Dietary histidine requirements for large yellow croaker (initial average weight, 6.0 ± 0.10 g) were quantified by feeding isonitrogenous (crude protein 44%) and isocaloric (20 KJ/g) amino acid test diets with graded levels of histidine [0.45% (D1), 0.66% (D2), 0.78% (D3), 0.98% (D4), 1.24% (D5) and 1.40% (D6) of dry diet]. Each diet was randomly assigned to triplicate floating sea cages (1.0 × 1.0 × 1.5 m), 60 fish/cage. At the end of the 51 day experiment, the final weight (FW) and weight gain (WG) of large yellow croaker showed a positive correlation to increasing dietary histidine content (up to 0.78%), and thereafter declined. The growth of fish fed the D6 diet was significantly lower than fish maintained on the D2 and D3 diets; however, there were no significant differences in the growth of large yellow croaker among all the dietary treatments except D6. Fish fed the D3 diet had the highest FW and WG. The shift in feed efficiency (FE) values of fish fed D1 to D5 diets increased as dietary histidine content increased, and significantly decreased in fish fed the D6 diet. Based on the second-degree polynomial regression analyses of the growth data, optimum histidine requirement for juvenile large yellow croaker was 8.7 g/kg dry diet, (18.8 g/kg–20.8 g/kg of dietary protein within 95% confidence interval).

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Introduction

Histidine (HIS) is an essential amino acid (EAA) for fish growth and affects health if present at sub-optimal or super-optimal levels in the diet (Khan and Abidi, 2009). HIS is a precursor of histamine, carnosine and anserine, which have been found to act as antioxidants in the lens of eyes (Glover and Wood, 2008). Previous studies indicated that Atlantic salmon (*Salmo salar* L.) fed a diet with lower HIS content had significantly higher mean cataract scores than individuals in the treatment with a higher level of HIS (Breck et al. 2005). When fed higher than optimal levels of HIS, the growth of fish such as Indian major carp *C. catla* (Ravi and Devaraj, 1991), *Labeo rohita* (Abidi and Khan, 2004), and African catfish *Clarias gariepinus* (Khan and Abidi, 2009), could be inhibited. The reason for the decline in weight gain has not been established but may be due to fish intolerance to amino acids above the optimum dietary level (Millamena et al., 1999; Khan and Abidi, 2009). Determining the optimal HIS requirement for fish is imperative when formulating high efficiency feed.

Large yellow croaker is an economically important food fish in China, and has been widely cultured in recent years. Trash fish is the main food for culturing this fish in sea cages. However, trash fish cannot meet the nutritional requirements of fish, is difficult to store, and pollutes aquaculture environments. The formulation of a nutritionally adequate and cost-effective feed is most important for the successful culture of large yellow croaker (Zhang et al. 2008). One of the prerequisites for developing high efficiency diets for fish requires extensive knowledge of its nutritional requirements, especially the essential amino acids (EAA) requirements (Mai et al. 2006). So far only two EAA have been tested for large yellow croaker, methionine (Mai et al. 2006) and lysine (Zhang et al. 2008). The purpose of the present study was to quantify the dietary histidine requirement for large yellow croaker.

Materials and Methods

Experimental diets: The basal diets contained 44% protein and were comprised of fish meal, corn gluten meal, gelatin and brewer's yeast as the intact protein source (Table 1).

Table 1. Formulation and composition of the test diets used for the histidine requirement for juvenile large yellow croaker (g/kg dry matter).

	Diet no/Supplementation level					
Ingredients	*D1(0.0)*	*D2(2.0)*	*D3(4.0)*	*D4(6.0)*	*D5(8.0)*	*D6(10.0)*
Fish meal[1]	140.0	140.0	140.0	140.0	140.0	140.0
Corn gluten meal[1]	110.0	110.0	110.0	110.0	110.0	110.0
Gelatin[1]	60.0	60.0	60.0	60.0	60.0	60.0
brewer's yeast[1]	30.0	30.0	30.0	30.0	30.0	30.0
Amino acid mixture[2]	193.0	193.0	193.0	193.0	193.0	193.0
Fish oil	50.0	50.0	50.0	50.0	50.0	50.0
Soybean oil	40.0	40.0	40.0	40.0	40.0	40.0
Mineral mixture[3]	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin mixture[4]	20.0	20.0	20.0	20.0	20.0	20.0
Attractant[5]	3.0	3.0	3.0	3.0	3.0	3.0
Mold inhibitor[6]	1.0	1.0	1.0	1.0	1.0	1.0
Antioxidant[7]	0.5	0.5	0.5	0.5	0.5	0.5
Lecithin	30.0	30.0	30.0	30.0	30.0	30.0
Dextrin	260.0	260.0	260.0	260.0	260.0	260.0
Microcrystalline	32.5	32.5	32.5	32.5	32.5	32.5
Histine	0.0	2.0	4.0	6.0	8.0	10.0
Glutamic acid	10.0	8.0	6.0	4.0	2.0	0.0
Composition analysis (g/kg, on a dry weight basis)						
Crude protein	441.0	437.0	440.0	438.0	443.0	440.0
Crude lipid	136.0	138.0	131.0	135.0	140.0	136.0
Total energy (KJ/g)	202.0	206.0	204.0	206.0	206.0	204.0
Histidine	4.5	6.6	7.8	9.8	12. 4	14.0

[1]Fish meal (white fish meal): obtained from Hangzhou Wensli Biology Science and Technology Corporation (Zhejiang, China), crude protein 675 g/kg dry matter, crude lipid 78 g/kg dry matter; Corn gluten meal: obtained from commercial market, crude protein 655 g/kg dry matter, crude lipid 49 g/kg dry matter; Beer yeast, crude protein 571 g/kg dry matter, crude lipid 35 g/kg dry matter; Gelatin, crude protein 953 g/kg dry matter, crude lipid 20 g/kg dry matter.

[2]Amino acid mixture (g/kg diet): arginine, 2.23; isoleucine, 0.95; leucine, 1.66; lysine, 2.33; methionine, 0.88; phenylalanine, 0.84; threonine, 1.12; aspartic acid, 2.33; serine, 0.85; alanine, 1.26; glycin, 0.28; cysteine, 0.08; tyrosine, 0.61; glutamic acid, 2.91; valine, 0.95.
[3]Mineral premix (mg or g/kg diet), NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1,200 mg; $Ca(H_2PO_4)_2 \cdot H_2O$, 3,000 mg; NaCl, 100 mg; Zoelite, 15.448 g.
[4]Vitamin premix (mg or g/kg diet), thiamin, 25 mg; riboflavin, 45 mg; pyridoxine-HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2000 mg, ethoxyquin, 150 mg, wheat middling, 14.52 g.
[5]Attractant, glycine and betaine.
[6]Mold inhibitor, p-Aminobenzoic acid.
[7]Antioxidant: Ethoxyquin.

The feeds were supplemented with crystalline amino acids premix to simulate the whole body amino acid pattern of large yellow croakers, except for HIS.

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

AA[1]	*FM*[1]	*CGM*[1]	*BY*[1]	*GL*[1]	*Total*[2]	*Addition*[3]	*43%Whole body*[4]
Arg	0.59	0.19	0.08	0.45	1.31	2.23	3.54
His	0.19	0.11	0.03	0.08	0.42	Varied	0.88
Ile	0.41	0.25	0.07	0.08	0.81	0.95	1.76
Leu	0.67	0.95	0.11	0.16	1.88	1.66	3.54
Lys	0.73	0.10	0.12	0.21	1.15	2.33	3.48
Met	0.27	0.14	0.02	0.02	0.45	0.88	1.33
Phe	0.37	0.37	0.07	0.11	0.92	0.84	1.76
Val	0.50	0.29	0.09	0.14	1.02	0.95	1.97
Thr	0.40	0.20	0.07	0.10	0.78	1.12	1.90
Asp	0.90	0.36	0.15	0.32	1.74	2.33	4.07
Ser	0.42	0.30	0.08	0.18	0.98	0.85	1.83
Gly	0.60	0.16	0.07	1.34	2.18	0.28	2.46
Ala	0.55	0.52	0.11	0.51	1.68	1.26	2.94
Cys	0.06	0.05	0.01	0.00	0.12	0.08	0.20
Tyr	0.32	0.30	0.05	0.02	0.70	0.61	1.31
Glu	1.23	1.29	0.17	0.56	3.26	2.91	6.17
Total addition[5]						19.30	

[1]AA, amino acid; FM, fish meal; CGM, corn gluten meal; BY, brewer's yeast; GL, gelatin.
[2]Total, the sum of each kind of amino acids in FM, CGM, BY and GL.
[3]Addition = the content of each kind of amino acids in 43% Whole body protein – the total of each kind of amino acids in all the protein ingredients.
[4]43% Whole body protein, the amino acids content of large yellow croaker in 43% whole body protein.
[5]Total addition, the sum of each kind of amino acids addition.

The basal diet contained 4.5 g/kg HIS and served as control diet. Crystalline L-HIS was added to the basal diet at six graded levels from 0 g/kg to 10 g/kg, and the HIS content in the diets were 4.5 g/kg, 6.6 g/kg, 7.8 g/kg, 9.8 g/kg, 12.4 g/kg and 14.0 g/kg which were analyzed by an amino acids analyzer (Biochrom Ltd®, England). A combination of fish oil, soybean oil and lecithin was used as a source of lipid. Vitamin and mineral premixes were prepared using the method described in Mai et al., (2006). By adjusting dextrin and microcrystalline, cellulose diets were isonitrogenous (crude protein 44%) and isocaloric (20 KJ/g). The diets were marked as D1, D2, D3, D4, D5, and D6, respectively.

The ingredients were ground into fine powder through 320 μm mesh. All protein ingredients, apart from the amino acids were mixed until homogenous. Amino acids were individually weighed and mixed. The attractant, mold inhibitor, antioxidant, mineral, and vitamin mixture, were mixed separately with microcrystalline cellulose, and then mixed with the above protein mixture. The oils and lecithin were blended and added to the mixture. Water was added to produce stiff dough. The pH of diets was adjusted to 7.0-8.0 by gradually adding 6 mol/l NaOH solution to neutralize the acidity of amino acids (Nose et al. 1974). The homogenous dough was passed through a pelletizer (F-26 (II), South China University of Technology) with two different diameter die (1.5mm and 2.5mm). The moist pellets were dried in an oven at 45 °C for 12 h. The dry pellets were crushed and sieved to obtain suitable pellet sizes (1.5 × 2.0 and 2.5 × 3.0 mm), then sealed in bags and stored at −15 °C until used.

Experimental procedure: Fish were obtained from a commercial farm in Ningbo, China. Prior to the feeding trial, the fish were reared in floating sea cages (3.0 × 3.0 × 3.0 m), and fed the control diet (Diet 1) for 2 weeks for acclimation to experimental conditions.

Juvenile large yellow croaker (initial average weight 6.0 ± 0.10 g) were taken from the above acclimated stock and randomly assigned to 18 floating sea cages (1.0 × 1.0 × 1.5 m) 60 fish/cage. Each diet was randomly assigned to triplicate cages. Fish were fed test diets to satiation twice daily at 05:00 and 17:30. The duration of the feeding trial was 51 days. During the experimental period, the water temperature ranged between 26-32°C, salinity from 26 g/l-30 g/l, and concentration of dissolved oxygen was approximately 7mg/l.

Measurement and analysis: At the end of the experiment, fish were not fed for 24 h prior to harvest. Before weighing and counting, eugenol (1:10000) was used to anesthetize the fish (Shanghai Reagent Corp, China). Five fish from each pooled replicate were sampled and stored frozen (−20°C) for proximate analysis of whole body composition. Proximate analyses on feeds, diets and fish were performed according to the standard methods of AOAC (1995). The samples of whole fish and diets were dried at 105°C to a constant weight to determine % moisture. The dried fish from each replicate were crushed and mixed thoroughly, then sealed in a plastic bag and stored frozen at -20°C until analysis for protein, lipid, and ash content. Crude protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method after acid digestion. Crude lipid was measured by ether extraction using the Soxhlet method. Ash content was placed in a muffle furnace and incinerated at 600°C for 12 h. The feed ingredients or experimental diets were freeze-dried, and then hydrolyzed with 6 N HCl at 110 °C for 22 h and analyzed by amino acids analyzer (Biochrom Ltd®, England).

Calculations and statistical analysis: The following variables were calculated:
Weight gain (WG) = (final weight – initial weight) / initial weight × 100.
Feed efficiency (FE) = wet weight gain in g / dry diet fed in g. All data were subjected to analysis of variance (ANOVA) and regression analysis where appropriate, using SPSS 13.0 for windows. Differences between the means were tested by Tukey's multiple range tests. The level of significance was reported at $P<0.05$.

Results

Growth performance: Dietary HIS significantly affected the growth of large yellow croaker ($P<0.05$). Final weight (FW), and weight gain (WG) (%) of fish ranged from 11.8g (96.3%) to 13.5g (125.0%) as presented in Table 3.

Table 3. Effects of dietary histidine on final weight (FW), weight gain (WG), feed efficiency (FE) and survival of juvenile large yellow croaker (*Pseudosciaena crocea*)[1].

Diet no. (HIS content g/kg dry diet)	*FW*[2]	*WG (%)*[3]	*FE*[4]	*Survival (%)*
D1(4.5)	12.4ab	106.7ab	0.43ab	100.0
D2(6.6)	13.2a	120.2a	0.41ab	100.0
D3(7.8)	13.5a	125.0a	0.45ab	100.0
D4(9.8)	12.9ab	115.5ab	0.46a	99.4
D5(12.4)	12.9ab	115.5ab	0.49a	100.0
D6(14.0)	11.8b	96.3b	0.32b	100.0
Pooled S.E.M.[5]	0.20	2.82	0.02	0.10
ANOVA[6]				
F-value	4.648	4.481	3.876	1.000
P-value	0.014	0.016	0.025	0.458

[1]Values are means of three replicate groups (n = 3). Means with different letter in the same column differ significantly ($P<0.05$).
[2]FW: final weight
[3]WG: weight gain
[4]FE: feed efficiency
[5]S.E.M.: standard error of means
[6]ANOVA: one-way analysis of variance

Initially FW and WG showed increasing trends with increasing dietary HIS content up to 0.78%, but thereafter declined. The growth of fish was significantly lower in fish fed D6 with the highest HIS content (14.0g/kg dry diet) than in fish fed the D2 (6.6 g/kg) and D3 (7.8 g/kg) dry diet; however, there were no significant differences in the growth of the fish between all the dietary treatments except D6. Fish fed D3 with 0.78% HIS content had the highest FW and WG. The shift in feed efficiency (FE) values of fish fed D1 to D5 increased with increasing dietary HIS content, and significantly decreased when fish were fed with D6. No significant differences in survival were found between the different dietary treatments.

For WG (y) in relation to dietary HIS levels (x) to second-degree polynomial regression analysis, the equation was y = -5.864x2 + 63.907 x – 24.454. The optimum HIS was the 8.7 g/kg diet (Fig.1), corresponding to 19.7 g/kg of dietary protein for juvenile large yellow croaker.

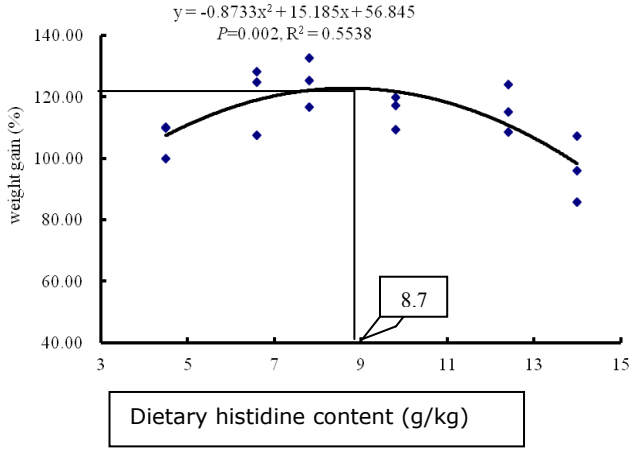

Fig.1. Effects of dietary histidine on weight gain of juvenile large yellow croaker (*Pseudosciaena crocea)* fed experimental diets. Each point represents the mean of three groups of fish within a treatment with all the survival fish per group. Requirements derived with the second-degree polynomial regression analyses for weight gain is 8.7 g/kg diet (19.8 g/kg dietary protein).

Whole body composition: The body composition of juvenile large yellow croaker was not significantly affected by dietary HIS content. The range of body moisture of large yellow croaker was 788.2 g/kg - 801.6 g/kg; crude protein was 140.0 g/kg - 146.6 g/kg; crude lipid was 22.7 g/kg - 31.4 g/kg; ash was 40.0 g/kg - 41.2 g/kg (Table 4).

Table 4. Effect of dietary histidine on body composition of juvenile large yellow croaker (*Pseudosciaena crocea*) fed experimental diets [1].

Diet no (histidine Content g/kg dry diet)	*Whole - Body composition (g/kg fresh weight)*			
	Moisture	*Crude protein*	*Crude lipid*	*Ash*
D1 (4.5)	788.2	144.0	25.3	40.6
D2 (6.6)	800.7	141.3	25.4	40.0
D3 (7.8)	801.6	140.0	22.8	40.4
D4 (9.8)	790.7	146.6	31.4	40.3
D5 (12.4)	801.5	143.0	22.7	41.2
D6 (14.0)	796.7	143.3	26.4	41.4
Pooled S.E.M.[2]	2.06	1.07	1.09	0.32
ANOVA[3]				
F-value	1.573	0.688	1.937	0.330
P-value	0.241	0.642	0.168	0.885

[1]Values are means of three replicate groups (n = 3)
[2]S.E.M.: standard error of means
[3]ANOVA: one-way analysis of variance.

Discussion

The data available on the dietary HIS requirements for fish varied between 0.9% and 2.5% of the dietary protein (Abidi and Khan, 2004; Khan and Abidi, 2009). In this study, the optimum dietary HIS requirement for juvenile large yellow croaker was estimated to be 1.97% of dietary protein which falls in the above range. This is probably due to the different requirements of the tested fish. The results were higher than the requirements reported for African catfish fry, *Clarias gariepinus* 1.0-1.05% of dietary protein (Khan and Abidi, 2009), Turbot, *Psetta maxima* 1.5% of dietary protein (Kaushik, 1998), Rainbow trout, *Oncorhynchus mykiss* 1.6% of dietary protein (Ogino, 1980), gilthead sea bream, *Sparus auratus* 1.7% of dietary protein (Kaushik, 1998), European sea bass, *Dicentrarchus labrax* 1.6% of dietary protein (Kaushik, 1998), lower than Japanese eel, *A. japonica* 2.1% of dietary protein (Nose, 1979).

Many previous studies which estimated amino acid requirements for fish also used the second order polynomial regression model (Tibaldi and Tulli, 1999; Ahmed and Khan, 2004; Mai et al., 2006; Khan and Abidi, 2007). Before estimating the amino acid requirements, the significance value (P) and the coefficient of the estimation (R^2) of

different models were compared. The second order polynomial regression model better describes the relationship between dietary HIS levels and WG.

An attempt was made with the basal diet of this experiment to lower the content of HIS. However, while still trying to maintain 42%-44% dietary proteins, many ingredients in the diet did not decrease the HIS content of the basal diet. Thus the values of HIS in diets (0.45% dry diet) were higher than expected. The growth of fish fed the lowest dietary HIS content was not inhibited. However excessive HIS did inhibit the growth of fish in this study. This phenomenon was also found in African catfish *Clarias gariepinus* (Khan and Abidi, 2009), Indian major carp, *C. catla* (Ravi and Devaraj, 1991), and rohu, *Labeo rohita* (Murthy and Varghese, 1995). This may have occurred because excessive HIS disrupted the balance of dietary amino acids, leading to toxicity in the tissues, (Mertz, 1972) or extensive necrosis in the epithelial cells of the hepatopancreas (Recodo, 1991) thereby influencing fish growth.

In conclusion, the optimum HIS requirement for juvenile large yellow croaker was found to be 8.7 g/kg dry diet, corresponding to 18.8 g/kg–20.8 g/kg of dietary protein within 95% confidence interval.

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