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Dietary phenylalanine requirement and tyrosine replacement value for phenylalanine for fingerling *Catla catla* (Hamilton)

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ABSTRACT

Two 12-week experiments were conducted to determine the dietary phenylalanine requirement and tyrosine replacement value for phenylalanine for fingerling *Catla catla*. In experiment I, phenylalanine requirement was determined by feeding six casein–gelatin based amino acid test diets (330 g kg^{-1} CP; 16.72 kJ g^{-1} GE) with graded levels of phenylalanine (3.9, 6.4, 8.7, 11.2, 13.8, 16.2 g kg^{-1} dry diet) at a constant level (10 g kg^{-1}) of dietary tyrosine to triplicate groups of fish (3.95 ± 0.24 cm; 0.68 ± 0.19 g) near to satiation. Live weight gain (LWG%), specific growth rate (SGR % day^{-1}), feed conversion ratio (FCR), protein retention efficiency (PRE%), phenylalanine retention efficiency (PHRE%) and RNA/DNA ratio responded positively with the increasing concentrations of phenylalanine reaching the highest values at 11.2 g kg^{-1} of dry diet. Quadratic regression analysis of LWG, SGR, PRE, PHRE and RNA/DNA ratio at 95% of maximum response against varying levels of dietary phenylalanine exhibited the requirement at 10.3, 10.1, 9.9, 9.7 and 10.6 g kg^{-1} dry diet, respectively. The above analysis revealed that inclusion of phenylalanine at 10.1 g kg^{-1} of dry diet, corresponding to 594 g kg^{-1} of lysine is optimum. In experiment II, six diets with different levels of L-tyrosine (1.9, 3.8, 5.9, 8.1, 9.8, 11.8 g kg^{-1} dry diet) with 10.1 g kg^{-1} phenylalanine (determined in experiment I) fixed in all the test diets were fed to fish (3.85 ± 0.25 cm; 0.66 ± 0.16 g) to determine the tyrosine requirement under identical conditions. Quadratic regression analysis of LWG, SGR, PRE, PHRE and RNA/DNA ratio at 95% of maximum response against dietary tyrosine concentrations indicated the requirement at 6.3, 6.5, 6.5, 7.1 and 7.3 g kg^{-1} dry diet, respectively. Hence, inclusion of tyrosine at 6.8 g kg^{-1} of dry diet, corresponding to 378 g kg^{-1} of lysine is taken as the tyrosine required for optimum utilization of phenylalanine. Based on above data, a total requirement of phenylalanine and tyrosine for fingerling *C. catla* was found to be 16.9 g kg^{-1} (10.1 g kg^{-1} phenylalanine + 6.8 g kg^{-1} tyrosine) of dry diet, corresponding to 939 g kg^{-1} of lysine. Tyrosine replacement value for phenylalanine was computed to be 37% on molar basis.

1. Introduction

Feed is one of the major inputs in aquaculture and the success of fish farming depends primarily on the provision of adequate quantity of nutritionally balanced feeds in a form which is acceptable to fish (Zargar et al., 2012). Hence, the inclusion of optimum quantity of a particular nutrient is necessary for successful aquaculture system. Determining the essential amino acid requirements of cultured fish is extremely important because of significant effects of these nutrients on muscle deposition, feed cost, and nitrogen pollution (Small and Soares, 1999). They are important fuel molecules, signaling factors and major substrates for the synthesis of a wide range of bioactive molecules and proteins (Finn and Fyhn, 2010). Besides being the building blocks of protein synthesis, amino acids in fish are also used in energy production or for other metabolic purposes (Ronnestad et al., 2001). Quantitative dietary requirements for the ten indispensable amino acids have been determined for several fish species (NRC, 2011; Wilson, 2002).

Phenylalanine, an aromatic indispensable amino acid is required for normal growth and metabolic processes. It is the sole precursor of tyrosine. Phenylalanine can be converted to tyrosine by tetrahydrobiopterin-dependent phenylalanine hydroxylase in liver and kidneys but phenylalanine cannot be synthesized back from tyrosine (Li et al., 2009). Thus, adding tyrosine to diets for fish can reduce requirement for phenylalanine. Tyrosine is a common precursor for important hormones and neurotransmitters, including thyroxine (T4), triiodothyronine, epinephrine, nor-epinephrine, dopamine, and melanin (Li et al., 2009). Pinto et al. (2009) reported that dietary requirements for phenylalanine and tyrosine of fish increase substantially during metamorphism. These molecules have important regulatory roles (Chang et al., 2007). Information on the effects of phenylalanine and tyrosine on growth is scarce. Hence, inclusion of sufficient amounts of phenylalanine and tyrosine to optimize the growth, body protein synthesis and also for the other physiological functions in fish is essential.

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Dietary phenylalanine requirements have been ascertained for a number of fish species such as channel catfish *Ictalurus punctatus*; chinook salmon *Oncorhynchus tshawytscha*; chum salmon *O. keta*; common carp *Cyprinus carpio*; rohu *Labeo rohita*; mrigal *Cirrhinus mrigala*; rainbow trout *O. mykiss*; Nile tilapia *Oreochromis niloticus*; Atlantic salmon *Salmo salar*; European sea bass *Dicentrarchus labrax*; Japanese flounder *Paralichthys olivaceus*; red drum *Sciaenops ocellatus*; Japanese eel *Anguilla japonica*; Red seabream *Pagrus major*; turbot *Scophthalmus maximus* and white sturgeon *Acipenser transmontanus* (Halver, 2002; NRC, 2011).

The Indian major carp, *Catla catla*, is a promising species for aquaculture exploitation with its rapid growth and good market potential. In terms of value-added, processed fish products, this species should have potential as the present market price of this fish is ranging between Rs. 80 to 140 kg^{-1} in Indian markets (Srivastava et al., 2013). Catla, along with the other Indian major carps, also form the mainstay of culture practices, contributing approximately 5.4 million tonnes to the total aquaculture production in 2010 (FAO, 2012). India also dominates the global Catla production by contributing about 71% to the global total of 3.87 million tonnes (FAO, 2012). Its highest growth potential, coupled with high consumer preference, has established Catla as an important freshwater species for aquaculture. It has been known that the successful intensive fish farming depends on the development of low-cost but nutritionally efficient formulated diets and the improvement of production technology. Currently, the lack of data on nutrient requirements of this fish is one of the major constraints for developing low-cost and nutritionally rich diet for this fish. Information on some nutritional aspects of *C. catla* is available (Abidi and Khan, 2014; Biswas et al., 2006; Dars et al., 2010; Khan and Jafri, 1991; Kumar et al., 2011; Ravi and Devaraj, 1991; Seenappa and Devaraj, 1995; Sinha and Sinha, 1994; Srivastava et al., 2013; Sukumaran et al., 2009). There is little information available on dietary phenylalanine requirement of *C. catla* (Ravi and Devaraj, 1991). Data on phenylalanine requirement and tyrosine replacement value for phenylalanine of the fingerling stage of this fish is completely lacking which is the major hindrance in developing phenylalanine balanced feeds for its intensive culture. Therefore, this study was aimed to investigate the dietary phenylalanine requirement and tyrosine replacement value for phenylalanine for fingerling *C. catla*.

2. Materials and methods

2.1. Experimental diets

Two 12 week experiments were conducted to determine the phenylalanine requirement and the tyrosine replacement value for phenylalanine in fingerling *C. catla*. For conducting experiment I, six isonitrogenous (330 g kg^{-1} crude protein, CP) and isocaloric (16.72 kJ g^{-1} gross energy, GE) amino acid test diets (P1, P2, P3, P4, P5 and P6) using casein (fat-free), gelatin and crystalline L-amino acids with graded levels of phenylalanine (4.0, 6.5, 9.0, 11.5, 14.0 and 16.5 g kg^{-1} dry diet) with fixed level (10 g kg^{-1}) of tyrosine were prepared. The level of tyrosine in the amino acid test diets was fixed on the basis of information available on other Indian major carp namely *Cirrhinus mrigala* (Ahmed, 2009). Casein and gelatin served as intact protein sources and provided 3.6 and 0.4 g kg^{-1} phenylalanine, respectively in the basal diet (P1). Incremental levels of L-phenylalanine (2.5, 5.0, 7.5, 10.0 and 12.5 g kg^{-1}) were added to prepare P2, P3, P4, P5 and P6 diets. The analyzed values of phenylalanine in experimental diets were found to be 3.9, 6.4, 8.7, 11.2, 13.8 and 16.2 g kg^{-1} dry diet. The levels of phenylalanine in the amino acid test diets were fixed on the basis of information available on other carps (NRC, 2011). In Experiment II, six diets with different levels of L-tyrosine (2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 g kg^{-1} dry diet) with fixed phenylalanine (10.1 g kg^{-1} dry diet) were formulated to determine the tyrosine requirement. The level of phenylalanine in all the experimental diets was fixed as the requirement determined in experiment I. The diets were marked as T1, T2, T3, T4, T5 and T6. Casein and gelatin served as intact protein sources and provided 1.5 and 0.5 g kg^{-1} tyrosine, respectively in the basal diet (T1). Incremental levels of crystalline L-tyrosine (2.0, 4.0, 6.0, 8.0 and 10.0 g kg^{-1}) were added to prepare T2, T3, T4, T5 and T6 diets. The analyzed values of tyrosine in experimental diets were found to be 1.9, 3.8, 5.9, 8.1, 9.8 and 11.8 g kg^{-1} dry diet. Levels of tyrosine in the amino acid test diets were fixed on the basis of information available on other fish species (Ahmed, 2009; NRC, 2011). The compositions of the basal diets used in experiments I and II are given in Table 1. The analyzed amino acid compositions of the basal diets used in above experiments are presented in Table 2. The dietary protein level fixed in this study is slightly lower than the optimum protein requirement (350 g kg^{-1} dry diet) of fingerling *C. catla* reported by Khan and Jafri (1991) and Dars et al. (2010). This reduction was made to ensure maximum utilization of the limiting amino acid from the diet (Wilson, 2002). Crystalline L-amino acids, excluding the phenylalanine and tyrosine, were used to simulate the amino acid profile of the experimental diets to that of 330 g kg^{-1} whole chicken egg

Table 1
Composition of the basal diets (g kg^{-1}) used in experiments I and II.

Ingredients (g kg^{-1} dry diet)	Basal diet (Experiment I)	Basal diet (Experiment II)
Casein[a] (fat-free)	71.5	31
Gelatin[b]	23.8	90
Amino acid mixture	262.4[c]	235.1[d]
Dextrin	348.7[e]	365.1[f]
Corn oil	50	50
Cod liver oil	20	20
Mineral mix[g,i]	40	40
Vitamin mix[h,i]	30	30
α-Cellulose	53.5	38.8
Carboxymethyl cellulose	100	100
Total	1000	1000
Analyzed crude protein (g kg^{-1} dry diet)	328.3	329.1
Analyzed crude lipid (g kg^{-1}dry diet)	71	70
Calculated gross energy (kJ g^{-1}, dry diet)[j]	16.72	16.72
Analyzed gross energy (kJ g^{-1}, dry diet)	16.68	16.70
Digestible energy (kJ g^{-1}, dry diet)[k]	13.81	14.04

[a] Crude protein (760 g kg^{-1}).
[b] Crude protein (960 g kg^{-1}).
[c] Amino acid mixture (g kg^{-1}) arginine 16.61, histidine 4.88, isoleucine 22.14, leucine 22.73, lysine 16.95, methionine 11.06, cystine 7.63, phenylalanine 0, tyrosine 6.31, threonine 11.33, tryptophan 4.37, valine 18.85, alanine 14.43, aspartic acid 5.54, glutamic acid 5.95, proline 17.03, serine 2.13, glycine 42.68 (Loba Chemie, India). Each mixture was made isonitrogenous with the addition of reduced amounts of glycine 74.31, 73.22, 72.08, 70.94, 69.81 and 68.67 in P1, P2, P3, P4, P5 and P6 diets, respectively.
[d] Amino acid mixture (g kg^{-1}) arginine 12.67, histidine 5.40, isoleucine 23.72, leucine 24.98, lysine 17.66, methionine 12.11, cystine 7.79, phenylalanine 6.93, cystine 0; threonine 11.76; tryptophan 4.70, valine 20.58; alanine 8.412, aspartic acid 4.626, glutamic acid 8.796, proline 11.73, serine 2.24; glycine 51.02 (Loba Chemie, India). Each mixture was made isonitrogenous with the inclusion of reduced amounts of glycine 51.02, 50.64, 49.83, 49.01, 48.24 and 47.39 in T1, T2, T3, T4, T5 and T6 diets, respectively.
[e] The experimental diets were made isocaloric with inclusion of dextrin at 348.7, 346.7, 344.6, 342.6, 340.5 and 338.4 g in P1, P2, P3, P4, P5 and P6 diets, respectively.
[f] The experimental diets were made isocaloric with inclusion of dextrin at 365.1, 363.2, 361.3, 359.5, 357.7 and 355.9 g in T1, T2, T3, T4, T5 and T6 diets, respectively.
[g] Mineral mixture (g 100 g^{-1} of mineral premix) calcium biphosphate 13.57; calcium lactate 32.69; ferric citrate 2.97; magnesium sulphate 13.20; potassium phosphate (dibasic) 23.98; sodium biphosphate 08.72; sodium chloride 04.35; aluminium chloride. $6H_2O$ 0.0154; potassium iodide 0.015; cuprous chloride 0.010; manganous sulphate. H_2O 0.080; cobalt chloride. $6H_2O$ 0.100; zinc sulphate. $7H_2O$ 0.40.
[h] Vitamin mixture (10 g vitamin mix + 20 g α-cellulose) choline chloride 5.0; inositol 2.0; ascorbic acid 1.0; niacin 0.75; calcium pantothenate 0.5; riboflavin 0.2; menadione 0.04; pyridoxine hydrochloride 0.05; thiamine hydrochloride 0.05; folic acid 0.015; biotin 0.005; alpha-tocopherol 0.4; vitamin B_{12} 0.0001.
[i] Halver (2002); Loba Chemie, India.
[j] Calculated on the basis of fuel values 23.07, 20.19, 16.01, 24.28 and 37.62 kJ g^{-1} for casein, gelatin, dextrin, amino acid and fat, respectively, as estimated on Gallenkamp ballistic bomb calorimeter-CBB 330 010L (Gallenkamp, Loughbrough, UK).
[k] Digestible energy was calculated on the basis of physiological fuel values 18.83, 14.64 and 35.56 kJ g^{-1} for protein, carbohydrate and fat, respectively (Jauncey, 1982).