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Dietary amino acid L-tryptophan requirement of fingerling Indian catfish, *Heteropneustes fossilis* (Bloch), estimated by growth and haemato-biochemical parameters

Imtiaz Ahmed

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Abstract An 8-week feeding trial was conducted to determine the dietary tryptophan requirement of fingerling Indian catfish, *Heteropneustes fossilis* (6.10 ± 1.15 cm, 4.44 ± 0.50 g). Six isonitrogenous (40 g 100 g^{-1}) and isoenergetic (17.90 kJ g^{-1}) amino acid test diets were formulated with gradation of 0.1 g 100 g^{-1} containing graded levels of L-tryptophan (0.04–0.54 g 100 g^{-1}, dry diet). Fish were stocked in triplicate groups, in 75-L circular trough with flow-through system and fed experimental diets at 4% BW/day twice daily. Maximum live weight gain (258%), best feed conversion ratio (FCR) (1.54) and protein efficiency ratio (PER) (1.62) were obtained in fish fed diet containing 0.34 g 100 g^{-1} tryptophan. However, quadratic regression analysis of weight gain, FCR, PER and body protein deposition (BPD) data indicated requirements for dietary tryptophan at 0.37, 0.33, 0.32 and 0.33 g 100 g^{-1} of dry diet, respectively. Significantly ($P < 0.05$) higher body protein, minimum moisture and intermediate fat contents were recorded at 0.34 g 100 g^{-1} dietary tryptophan diet. Ash content was not significantly different ($P > 0.05$) among treatments except for diets 0.04 and 0.14 g 100 g^{-1}. Excellent somatic and haematological indices values were obtained at the requirement level. Based on above results, it is recommended that the diet for *H. fossilis* should contain tryptophan at 0.32 g 100 g^{-1}, dry diet, corresponding to 0.80 g 100 g^{-1} dietary protein for optimum growth and efficient feed utilization.

Keywords *H. fossilis* · Dietary tryptophan requirement growth · Haemato-biochemical parameters

I. Ahmed (✉)
DST, Sponsored Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Hazratbal, Srinagar 190006, J&K, India
e-mail: imtiazamu1@yahoo.com

Introduction

The catfishes are widely distributed throughout Southeast Asia, the Indian subcontinent and Africa and are often used as important component of human nutrition (Shirai et al. 2002). However, in Asian catfish, farming is emerging interest especially in India because of their hardy nature and high market demand, which plays a vital role in Indian subcontinent, to bridge the gap of food scarcity prevailing due to the shrinkage of agricultural land because of increase in population size.

The Indian catfish, *Heteropneustes fossilis* (Bloch), is considered to be a highly nutritious, palatable, tasty and well-preferable food fish besides having less spine, low fat and high digestibility and is used as important component of food in many parts of Indian subcontinent as well as in Asia, especially the nutrition of socially weaker sections in India due to it cheapness and taste. The fish because of its omnivorous habitat

with air-breathing organs is widely distributed throughout the Indian subcontinent and found mainly in ponds, ditches, swamps and marshes, but some times occurs in muddy rivers. It lives in large shoals in suitable locations and is the object of an extensive fishing effort, as result of a high demand. It has also been reported that the muscles of the *H. fossilis* contain high quantity of iron (226 mg 100 g^{-1}) and calcium, which are highly useful for human health. Due to its excellent nutritive profile and medicinal value, and can also be available in market as live fish, the fish has a high market demand and is generally propagated on extensive line (Pillay 1990; Jhingran 1991; Thakur 1991). However, due to continuous fall in the catch of this fish from natural resource and lack of information on their nutritional requirements, there is a dire need to intensify its culture by developing least cost, nutritionally balanced dietary formulations for this species.

In intensive culture systems, nutritionally complete feeds have been used and comprised a major portion of fish production costs. Feed cost generally account for 40–60% of total farm production costs, and crude protein content of fish feed is one of the most expensive component of complete feeds (Cheng et al. 2003; Nguyen and Davis 2009), providing essential amino acids which are used for tissue repair and growth (Murillo-Gurrea et al. 2001), besides precursor of protein and also act as an energy sources. Hence, the development of cost-effective feeds is critical to economic success of aquaculture. Dietary protein is utilized in fish for maintenance of normal tissue function and growth as well as energy source (Masumoto et al. 1998). Thus, dietary formulations that meet but do not exceed the essential amino acids requirement for fish are also important for the sustainability of aquaculture. Excessive levels of essential amino acid in diets result in higher levels of ammonia-N excretion (Cai et al. 1996; Yang et al. 2002) and eventually discharged from aquaculture production systems. Therefore, dietary manipulations that minimize ammonia excretion will be important for the sustainability of aquaculture (Twibell et al. 2003).

Amino acids play important and exceptional roles in fish nutrition and metabolism. Balanced dietary amino acid profile is required for optimal growth and feed conversion in fish, as imbalances in the dietary amino acid pattern not only lead to reduction in feed intake and protein utilization for growth (Wilson and Halver 1986; D'Mello 1994; Yamamoto et al. 2000; Berge et al. 2002; Green and Hardy 2002; Gomez-Requeni et al. 2003), but also have effects on nitrogen loading in water (Small and Soares 1999). Generally, the ingredients used for formulation of artificial feeds are limiting in four major essential amino acids such as lysine, arginine, methionine and tryptophan. Therefore, supplementation of these limiting amino acids in the diet as per the optimum requirement of the fish is the first priority of nutritionist for formulating essential amino acid-balanced feeds, which not only improve growth performance and the profitability but also reduce the organic load of the culture system.

The requirement for all 10 indispensable amino acids have been established only for 10 cultured fish species such as chinook salmon *Oncorhynchus tshawytscha,* channel catfish *Ictalurus punctatus*, Japanese eel *Anguilla japonica* (NRC 1993), rainbow trout *O. mykiss* (Ogino 1980), coho salmon *O. kisutch* (Arai and Ogata 1993), chum salmon *O. keta* (Akiyama and Arai 1993), common carp *Cyprinus carpio* (Nose 1979), Nile tilapia *Oreochromis niloticus* (Santiago and Lovell 1988), Indian major carp, catla, *Catla catla* (Ravi and Devaraj 1991), milkfish *Chanos chanos* (Borlongan and Coloso 1993) and recently an other Indian major carp, mrigal, *Cirrhinus mrigala* (Ahmed 2005, 2009).

Amino acids are required by all fish species, and tryptophan is of particular concern (Ahmed and Khan (2005). After lysine and methionine, tryptophan is one of the most limiting amino acid in plants protein used for fish feed such as corn meal, wheat grain meal and mung bean meal (Kim and Lall 2000; Coloso et al. 2004). Tryptophan along with water soluble vitamin niacin plays an important role in the brain as a precursor of the neurotransmitter serotonin, which has a major effect on the feeding behaviour of animals among its many functions (Blundell and Latham 1978; Tackman et al. 1990; Mullen and Martin 1992; Coloso et al. 2004). Tryptophan along with niacin is also involved in protein and lipid metabolism in fish (Ahmed 2010) and also required for the synthesis of insulin-like growth factor-1 (IGF-1), which is involved in improving the growth rate of fishes (Perez-Sanchez and Le Bail 1999; Dyer et al. 2004). The essentiality of tryptophan has been well established in fish nutrition studies by several workers in the past and suggested that tryptophan is essential for fish as precursor of serotonin (5-hydroxytryptamine, 5-HT) with stress-releasing effect (Winberg et al.

2001; Lepage et al. 2002; Hseu et al. 2003; Papoutsoglou et al. 2005). Further feeding tryptophan-supplemented diet resulted in the inhibition of endogenously derived aggressive behaviour (Winberg et al. 2001; Hseu et al. 2003). Previous studies on rainbow trout have demonstrated dietary tryptophan supplementation resulted in slight basal cortisol increase and considerable decrease in subsequent stress-induced cortisol elevation (Lepage et al. 2002, 2003). Tryptophan is also reported to be an inefficient precursor of niacin in channel catfish (Ng et al. 1997). Recently, Tejpal et al. (2008) reported the effects of tryptophan on mitigation in crowding stress response in *Cirrhinus mrigala,* while tryptophan supplementation enhanced salt water tolerance in common carp (Hoseini and Hosseini 2010).

The dietary tryptophan requirement has been worked out for a limited number of cultured fish species such as sockeye salmon *Oncorhynchus nerka* (Halver 1965), channel catfish (Wilson et al. 1978; Ng et al. 1997), rainbow trout (Poston and Rumsey 1983; Walton et al. 1986; Kim et al. 1987), gilthead sea bream *Sparus aurata* (Luquet and Sabaut 1974; Kaushik 1998), milk fish (Coloso et al. 1992), Indian major carp *Labeo rohita* (Murthy and Varghese 1997), African catfish *Clarias gariepinus* (Fagbenro and Nwanna 1999), Asian sea bass *Lates calcarifer* (Coloso et al. 2004), hybrid striped bass *Morone chrysops* x *M. saxatilis* (Gaylord et al. 2005), and an other Indian major carp, mrigal (Ahmed and Khan 2005).

Some aspects of nutrition of *H. fossilis* have been worked out in the past and have been reviewed in previous study (Ahmed 2010). However, except for the amino acid threonine (Ahmed 2007), no information is available on any of its indispensable amino acid requirements. Due to that, the efforts remained hampering in developing amino acid-balanced practical feeds required for the intensive culture of this nutritionally and commercially valued aquaculture species.

The haematological parameters now-a-days have been proved to be essential tools for analysing the health status and biological manifestations of fish in determining the nutritional status of the fish in response to dietary manipulation (Adhikari et al. 2004; Shah and Altindag 2005; Congleton and Wagner 2006; Mohammed and Sambo 2007; Maheswaran et al. 2008). Therefore, in the present study, haematological parameters have also been analysed in addition to the growth parameters for assessing the effects of dietary tryptophan on growth performance of *H. fossilis*. The present investigation was therefore undertaken to determine the optimum dietary tryptophan requirement of fingerling *H. fossilis* by using growth, biochemical and haematological parameters.

Materials and methods

Experimental diet

Six isonitrogenous (40 g 100 g^{-1}) crude protein (CP) and isoenergetic (17.90 kJ g^{-1}, gross energy) diets (I–VI) with graded levels of tryptophan were formulated using casein (fat-free), gelatin and L-crystalline amino acid premix (Table 1). The dietary protein level was fixed at 40%, which is reported optimum for the growth of *H. fossilis* (Akand et al. 1989; Firdaus 1993). L-crystalline amino acids were used to adjust the amino acid profile of the diets to that of 40% whole egg protein, excluding the test amino acid (tryptophan). The levels of L-tryptophan were in increments of 0.10 g 100 g^{-1}, dry diets. A casein–gelatin ratio, contributing minimum quantity of the test amino acid and maximum quantities of other amino acids, was maintained. The quantity of tryptophan was increased at the expense of glycine, glutamic acid and aspartic acid so as to make the diets isonitrogenous. The levels of tryptophan in the test diets were fixed on the basis of information available on the other catfish species channel catfish (Wilson et al. 1978). Method of preparation of experimental diets used in the present study was same as described earlier (Ahmed 2007), while composition of vitamin and mineral premixes was prepared as per (Halver 2002).

Experimental design and feeding trial

Young catfish *Heteropneustes fossilis* were obtained from Gazipur fish market, New Delhi, and were brought to the laboratory in clay pots. These fishes were given a dip of $KMnO_4$ solution (5 mg/L) to rule out any possible microbial infection and randomly stocked in plastic tanks. During this period, the fish were fed to satiation minced meat twice a day at 0800 and 1700 h for 1 week. Prior to the start of the feeding trials, the *H. fossilis* stocked in plastic tanks were transferred to the wet laboratory and acclimated for

Table 1 Composition of experimental diets used for estimating the dietary tryptophan requirement of fingerling *Heteropneustes fossilis*

Ingredients (g 100 g^{-1}, dry diet)	Experimental diets					
	(I) 0.04	(II) 0.14	(III) 0.24	(IV) 0.34	(V) 0.44	(VI) 0.54
Casein[a]	5.00	5.00	5.00	5.00	5.00	5.00
Gelatin[b]	2.50	2.50	2.50	2.50	2.50	2.50
Amino acid mix[c]	38.875	38.875	38.885	38.882	38.891	38.897
Dextrin	26.08	26.07	26.06	26.06	26.047	26.038
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00
Cod liver oil	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix[d]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[d,e]	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl cellulose	10.00	10.00	10.00	10.00	10.00	10.00
Alpha cellulose	3.545	3.551	3.555	3.557	3.562	3.565
Total	100	100	100	100	100	100
Total tryptophan	0.04	0.14	0.24	0.34	0.44	0.54
Calculated crude protein (g 100 g^{-1})	40	40	40	40	40	40
Analysed crude protein (g 100 g^{-1})	40.25	39.83	40.07	40.12	39.96	40.21
Gross energy[f] (kJ g^{-1}, dry diet)	17.9	17.9	17.9	17.9	17.9	17.9

[a] Crude protein (80%)

[b] Crude protein (93%), Loba Chemie, India

[c] Essential amino acids (g kg^{-1}): arginine, 21.77; histidine, 6.90; isoleucine, 28.94; leucine, 31.25; lysine, 23.72; methionine, 14.68; phenylalanine, 22.25; threonine, 15.12; tryptophan variable, valine, 25.42. Non-essential amino acids: cystine, 9.40; tyrosine, 15.38; alanine, 18.90; aspartic acid and glutamic acid variable, proline, 24.94; serine, 4.20; glycine variable (Loba Chemie, India)

[d] Halver 2002 mineral ($AlCl_3$. $6H_2O$, 15 mg; $ZnSO_4$. $7H_2O$, 300 mg; CuCl,10 mg; $MnSO_4$.4 -$6H_2O$, 80 mg; KI, 15 mg; $CoCl_2.6H_2O$, 100 mg; plus USP # 2 Ca $(H_2PO_4)_2$. H_2O, 13.58 g; $C_6H_{10}CaO_6$, 32.70 g; $C_6H_5O_7Fe.5H_2O$, 2.98 g; $MgSO_4.7H_2O$, 13.20 g; KH_2PO_4 (dibasic), 23.98 g; $NaH_2PO_4.2H_2O$, 8.72 g; NaCl, 4.35 g (g 100 g^{-1})

[e] vitamin mix (choline chloride, 500 mg; thiamine HCL, 5 mg; riboflavin, 20 mg; pyridoxine HCL, 5 mg; nicotinic acid, 75 mg; calcium pantothenate, 50 mg; inositol, 200 mg; biotin, 0.50 mg; folic acid, 1.50 mg; ascorbic acid, 100 mg; menadione, 4 mg; alpha-tocopheryl acetate, 40 mg; cyanocobalamine, 0.01 mg (g 100 g^{-1})

[f] Calculated on the basis of fuel values 23.10, 20.21, 24.27, 16.02 and 37.65 kJ g^{-1} for casein, gelatin, amino acids, dextrin, and fat, respectively, as estimated on Gallenkamp ballistic bomb calorimeter

2 weeks on casein–gelatin-based (40% CP) H-440 (Halver 2002) purified diet.

H. fossilis (6.10 ± 1.15 cm, 4.44 ± 0.50 g) were then stocked in triplicate groups in 75 L circular polyvinyl troughs (water volume 60 L) fitted with a continuous water flow-through (1–1.5 L/min) system at the rate of 20 fish per trough for each dietary treatment level. Fish were fed test diets in the form of dry crumbles at a rate of 4% body weight (Ahmed 2010), twice daily at 0800 and 1800 h. No feed was offered to the fish on the day the weekly measurements were taken. Initial and weekly weights were recorded on a top loading balance (Sartorus CPA-224S 0.1 mg sensitivity, Goettingen, Germany) and feed allowances adjusted accordingly. The feeding trial extended for 8 weeks. Faecal matter was removed by siphoning every day before feeding, and unconsumed feed, if any, was filtered over a screen soon after active feeding dried and weighed in order to measure the exact amount of feed consumed during the trial. At the end of feeding trial, fishes were anaesthetized with tricaine methanesulphonate (MS 222) and final weight was taken.

Water quality analysis

Water temperature, dissolved oxygen, free carbon dioxide, total alkalinity and pH were recorded

following the standard methods (APHA 1992). The average water temperature, dissolved oxygen, free carbon dioxide, total alkalinity and pH over the 8-week feeding trial, based on daily measurements, were 22.0–23.5°C, 6.5–7.6, 8–20, 63–82 mg/L^{-1} and 6.7–7.8, respectively.

Chemical analysis

Proximate composition of casein, gelatin, experimental diet, initial and final carcass was estimated using standard AOAC (1995); (Ahmed 2007) methods for dry matter (oven drying at 105 ± 1°C for 22 h), crude protein (N-Kjeldhal × 6.25), crude lipid (solvent extraction with petroleum ether B.P 40–60°C) using Soxhlet extraction technique (FOSS Avanti automatic 2050 equipment, Sweden), and ash (oven incineration at 650°C for 2–4 h). At the end of the feeding trial, blood samples were drawn from each fish by serving the caudal peduncle with sharp razor, and the pooled blood samples of five fishes in each group were stored in heparinized plastic vials for haemoglobin, haematocrit and erythrocyte sedimentation rate (ESR) estimation using the technique as described by Sandnes et al. (1988).

Statistical analysis

The response variables (weight gain %, SGR%, FCR, protein efficiency ratio and carcass composition) were subjected to one-way analysis of variance (ANOVA) (Snedecor and Cochran 1967; Sokal and Rohlf 1981). To determine significant differences ($P < 0.05$) among the treatments means, Duncan's multiple range test (Duncan 1955) was employed. Quadratic regression analysis (Zeitoun et al. 1976) was used to determine the break point in growth parameters, which represented the optimum tryptophan requirement for the fish. Data were statistically analysed using Matlab (version 7.1, Matlab software, Natick, MA, USA) and Kyplot (version 2.0) beta 5 Koichi Yoshioka, Japan.

Calculations

Weight gain, specific growth rate (SGR%), FCR, protein efficiency ratio (PER) and body protein deposition (BPD) were calculated using following standard definitions:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Specific growth rate (SGR \%)} = \frac{(\text{In mean final weight}) - (\text{In mean initial weight})}{\text{Number of days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry food fed (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g, wet weight basis)}}{\text{Protein intake (g, dry weight basis)}}$$

$$\text{Hepatosomatic Index (HSI \%)} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Survival rate (SR\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Protein deposition} = \frac{[(BW_f \times BCP_f) - (BW_i \times BCP_i)]}{[TF \times CP]} \times 100$$

where BW_i and BW_f = mean initial and final body weight (g), BCP_i and BCP_f = mean initial and final percentage of body protein, respectively, TF = total amount of diet consumed, and CP = percentage of crude protein of the diet.

Results

The results of the present study clearly showed that growth performance and body chemical composition were significantly ($P < 0.05$) affected by varied concentrations of dietary tryptophan. Survival was found to be 100% in higher concentration of tryptophan supplemental diets, excepting at marginal 0.04 and 0.14 g 100 g^{-1} tryptophan diets where 90 and 95% survival rates were recorded. Live weight gain, SGR%, FCR and protein efficiency ratio (PER) of *H. fossilis* fed diets containing graded levels of dietary tryptophan are presented in Table 2. Maximum live weight gain was recorded in fish fed diet containing 0.34 g 100 g^{-1} of dietary tryptophan (diet IV). The overall growth of fish at this level of dietary tryptophan over the 8-week feeding trial was 258%, which was

Table 2 Growth, FCR, protein deposition and percentage survival of Indian catfish, ***Heteropneustes fossilis*** fed diets containing graded levels of tryptophan

	Dietary tryptophan levels (g 100 g^{-1})					
	(I) 0.04	(II) 0.14	(III) 0.24	(IV) 0.34	(V) 0.44	(VI) 0.54
Average initial weight (g)	4.431 ± 0.11	4.397 ± 0.10	4.480 ± 0.13	4.411 ± 0.12	4.515 ± 0.12	4.452 ± 0.11
Average final weight (g)	7.916±0.38	10.893 ± 0.41	14.776 ± 0.40	15.825 ± 0.36	15.396 ± 0.22	13.810 ± 0.26
Live weight gain (%)	78.49 ± 4.16^{f}	147.65 ± 4.32^{e}	229.87 ± 6.27^{c}	258.84 ± 5.53^{a}	241.14 ± 4.36^{b}	210.03 ± 4.71^{d}
Specific growth rate	1.03 ± 0.04^{f}	1.62 ± 0.03^{e}	2.13 ± 0.03^{c}	2.28 ± 0.02^{a}	2.19 ± 0.02^{b}	2.02 ± 0.03^{d}
Feed intake (g DM $fish^{1}$)	10.03 ± 0.67^{f}	13.73 ± 0.69^{e}	17.13 ± 0.40^{d}	17.59 ± 0.76^{c}	19.41 ± 0.18^{b}	20.25 ± 0.83^{a}
Feed conversion ratio	2.88 ± 0.09^{a}	2.11 ± 0.05^{b}	1.66 ± 0.03^{d}	1.54 ± 0.04^{e}	1.78 ± 0.03^{c}	2.16 ± 0.05^{b}
Protein efficiency ratio	0.87 ± 0.03^{e}	1.18 ± 0.03^{d}	1.50 ± 0.02^{b}	1.62 ± 0.04^{a}	1.40 ± 0.02^{b}	1.16 ± 0.02^{d}
Body protein deposition	13.26 ± 0.30^{d}	20.12 ± 0.43^{c}	27.05 ± 0.54^{b}	30.91 ± 0.80^{a}	25.68 ± 0.43^{b}	20.09 ± 0.46^{c}
Survival (%)	90 ± 1.30	95 ± 1.05	100	100	100	100

Mean value of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$)

significantly ($P < 0.05$) higher than the weight gain recorded from other dietary tryptophan levels. The specific growth rate (2.24) followed similar trend as live weight gain. When the utilization and conversion results were statistically compared, it was observed that fish receiving different levels of dietary tryptophan produced significant differences in FCR values, which ranged between 1.54 and 2.88, with the best FCR (1.54) being achieved in fish fed 0.34 g 100 g^{-1} dietary tryptophan level. PER (1.62) of fish fed 0.34 g 100 g^{-1} tryptophan diet was also significantly ($P < 0.05$) higher compared with that fed at other dietary levels. The lower dietary tryptophan concentrations (<0.34 g 100 g^{-1}) produce overall poor growth rate and conversion efficiency compared to the higher concentrations of (>0.34 g 100 g^{-1}) dietary tryptophan. In the light of the above results, it is evident that fish fed >0.34 g 100 g^{-1} dietary tryptophan could not produce additional growth and neither improve conversion efficiency; on the other hand, fish fed lower concentrations <0.34 g 100 g^{-1} dietary tryptophan produced reduced weight gain and poor conversion efficiency and feed utilization. On subjecting the live weight gain data to second-degree polynomial regression analysis, a break point was evident at 0.37 g 100 g^{-1} of dietary tryptophan, corresponding to 0.93 g 100 g^{-1} of dietary protein (Fig. 1). The relationship was described by the following equation:

$$Y = -1.6090x^2 + 1.2095x - 0.0258\,(r = 0.990)$$

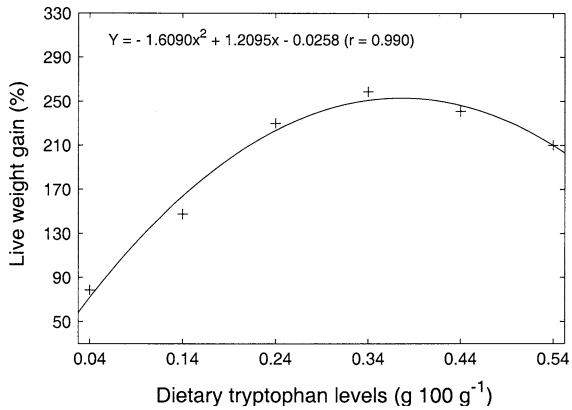

Fig. 1 Second-degree polynomial relationship between dietary tryptophan levels and live weight gain (LWG%) of *H. fossilis* fed with experimental diets for 8 weeks

The FCR (Y) to dietary levels of tryptophan (X) relationship was best described by a second-degree polynomial regression analysis (Fig. 2). The relationship being

$$Y = 15.1964x^2 - 10.1596x + 3.2467\,(r = 0.997)$$

The PER (Y) to dietary levels of tryptophan (X) relationship was best described by a second-degree polynomial regression analysis (Fig. 3). The relationship being

$$Y = -8.7679x^2 + 5.7225x - 0.6219\,(r = 0.983)$$

Based on the above polynomial equations, the best FCR and PER occurred at tryptophan levels of

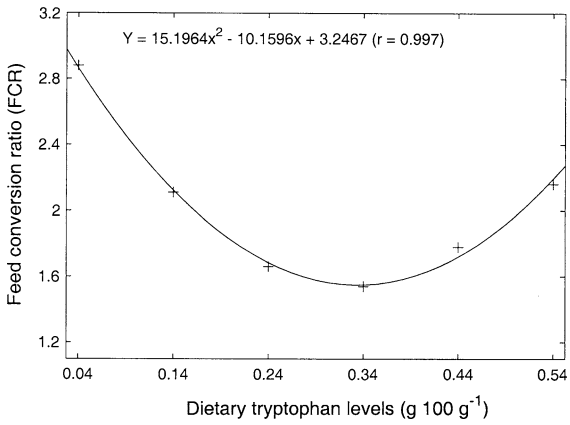

Fig. 2 Second-degree polynomial relationship between dietary tryptophan levels and feed conversion ratio (FCR) of *H. fossilis* fed with experimental diets for 8 weeks

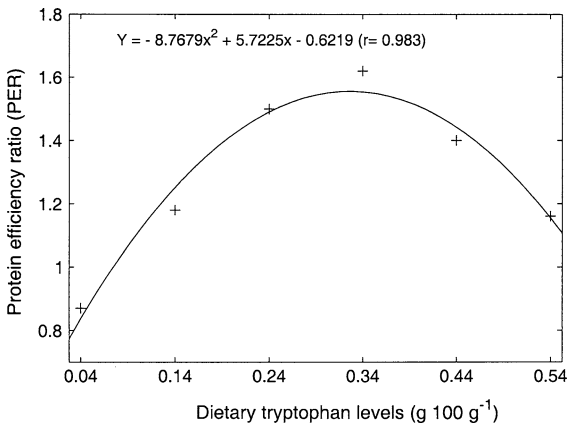

Fig. 3 Second-degree polynomial relationship between dietary tryptophan levels and protein efficiency ratio (PER) of *H. fossilis* fed with experimental diets for 8 weeks

approximately 0.33 and 0.32 g 100 g^{-1} of the dry diet, respectively.

Somato-haematological indices have also shown significant differences (Table 3). The fish fed tryptophan-deficient diet, that is <0.24 g 100 g^{-1}, produced significantly higher hepatosomatic index (HSI) values compared to those fed >0.24 g 100 g^{-1} tryptophan inclusion in the diet. The haemoglobin (Hb) concentration in the blood was increased with the increase in dietary tryptophan concentration up to 0.34 g 100 g^{-1}, and thereafter, a slightly decline in Hb was noticeable. Significantly ($P < 0.05$) higher haematocrit value (44.50%) was noted for the fish fed diet containing 0.34 g 100 g^{-1} tryptophan while the lowest haematocrit value (28.24%) was recorded at marginal tryptophan containing diet. However, erythrocyte sedimentation rate (ESR) gradually declined with the increase in dietary tryptophan concentration up to 0.34 g 100 g^{-1} where minimum ESR value was noted.

Remarkable, significant differences were observed in the whole-body composition of fish and are presented in Table 4. Dietary tryptophan levels significantly ($P < 0.05$) affected the proportions of moisture, protein, fat and ash contents. The moisture content decreased significantly ($P < 0.05$) with increasing levels of dietary tryptophan up to fish fed diet containing 0.34 g 100 g^{-1} tryptophan, and thereafter, a numerical increase in moisture content was noted. Protein content of fish fed diet containing 0.34 g 100 g^{-1} tryptophan was significantly ($P < 0.05$) higher, followed by those receiving diets containing 0.44 g 100 g^{-1} and 0.24 g 100 g^{-1} dietary tryptophan, respectively. Body fat gradually increased with the increase in dietary concentrations and was found to be significantly ($P < 0.05$) higher at maximum concentration 0.54 g 100 g^{-1} tryptophan (diet VI) compared to all the dietary groups. Ash content of fish receiving diets with different tryptophan levels was not significantly ($P > 0.05$) different and remained low, except at 0.04 and 0.14 g 100 g^{-1} where significantly higher ash was noticed. Fish fed diet containing 0.34 g 100 g^{-1} tryptophan also resulted significantly ($P < 0.05$) higher protein deposition compared to that fed at other dietary levels. In order to further strengthen the estimated values, the body protein deposition values were also subject to second-degree polynomial regression analysis.

Interestingly a break point was also evident at 0.33 g 100 g^{-1} tryptophan of the dry diet (Fig. 4). The relationship being

$$Y = -198.0179x^2 + 130.4760x - 7.4424\,(r = 0.978).$$

Discussion

Determining the essential amino acid requirements of cultured fishes is of extreme importance due to the significant effects of these nutrients on muscle deposition, feed cost and nitrogen pollution (Small and Soares 1999). Therefore, the optimization of fish growth with economical diets containing all essential

Table 3 Hepatosomatic and haematological indices of Indian catfish, *Heteropneustes fossilis*, fed diets containing graded levels of tryptophan

	Dietary tryptophan levels (g 100 g^{-1})					
	(I) 0.04	(II) 0.14	(III) 0.24	(IV) 0.34	(V) 0.44	(VI) 0.54
Hepatosomatic indices (HSI)	2.65 ± 0.21^{a}	2.28 ± 0.18^{b}	1.60 ± 0.15^{c}	1.42 ± 0.22^{c}	1.68 ± 0.30^{c}	1.76 ± 0.25^{c}
Haemoglobin (g dL^{-1})	5.80 ± 0.16^{e}	6.20 ± 0.21^{d}	7.80 ± 0.11^{c}	8.50 ± 0.14^{a}	8.05 ± 0.09^{b}	7.70 ± 0.13^{c}
Haematocrit (%)	28.42 ± 0.40^{d}	30.45 ± 0.55^{c}	34.70 ± 0.85^{b}	44.50 ± 0.46^{a}	37.60 ± 0.58^{b}	32.40 ± 0.65^{c}
ESR (mm h^{-1})	2.34 ± 0.08^{c}	2.21 ± 0.06^{c}	2.05 ± 0.11^{b}	1.75 ± 0.12^{a}	1.86 ± 0.09^{ab}	1.90 ± 0.11^{ab}

Mean values of 3 replicates ± SEM. Mean values with the same superscript letters are insignificantly different ($P > 0.05$)

Table 4 Carcass composition of Indian catfish, *Heteropneustes fossilis,* fed diets containing graded levels of tryptophan

	Initial	Dietary tryptophan levels (g 100 g^{-1})					
		(I) 0.04	(II) 0.14	(III) 0.24	(IV) 0.34	(V) 0.44	(VI) 0.54
Moisture (%)	79.05 ± 0.63	77.36 ± 0.33^{a}	75.98 ± 0.25^{b}	74.86 ± 0.16^{c}	74.24 ± 0.19^{d}	74.45 ± 0.17^{d}	74.62 ± 0.18^{c}
Protein (%)	12.75 ± 0.05	13.86 ± 0.06^{f}	15.29 ± 0.05^{e}	16.41 ± 0.04^{c}	17.28 ± 0.05^{a}	16.69 ± 0.04^{b}	15.88 ± 0.06^{d}
Fat (%)	3.11 ± 0.04	3.40 ± 0.07^{f}	3.85 ± 0.06^{e}	4.16 ± 0.04^{d}	4.44 ± 0.05^{c}	4.68 ± 0.04^{b}	4.92 ± 0.05^{a}
Ash (%)	2.90 ± 0.06	3.12 ± 0.04^{a}	2.68 ± 0.03^{b}	2.47 ± 0.05^{c}	2.56 ± 0.04^{c}	2.62 ± 0.03^{c}	2.51 ± 0.04^{c}

Mean values of 3 replicates ± SEM. Mean values with the same superscript letters are insignificantly different ($P > 0.05$)

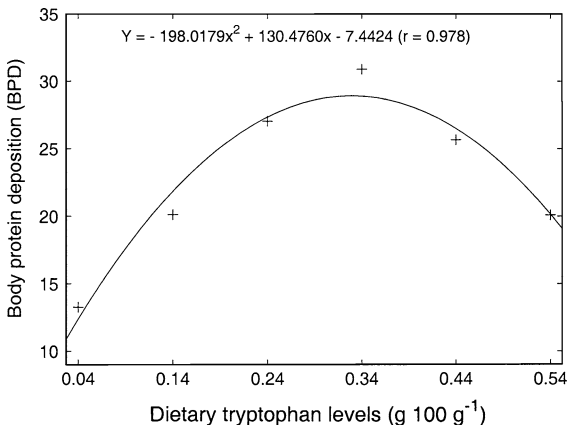

Fig. 4 Second-degree polynomial relationship between dietary tryptophan levels and body protein deposition (BPD) of *H. fossilis* fed with experimental diets for 8 weeks

nutrients above the minimum requirements is the subject of concern for the last few decades, and significant results have been achieved so far.

The growth performance of *H. fossilis* improved in response to supplementary L-tryptophan thus confirming the essentiality of tryptophan to this species for maximum growth. Thus, inclusion of an optimum amount of tryptophan is a prerequisite to the formulation of nutritionally adequate artificial diet for *H. fossilis* culture. The present finding indicates that 0.34 g 100 g^{-1} dietary tryptophan, corresponding to 0.85 g 100 g^{-1} of the protein, is optimum for maximum growth of fingerling *H. fossilis.* Best feed efficiency at this level of dietary tryptophan is reflected by excellent values obtained for FCR, specific growth rate and PER. The feed intake (FI) was started progressively increased with the increase in dietary tryptophan concentration, and a maximum feed intake values was noted at higher concentrations 0.54 g followed by 0.44 g 100 g^{-1} dietary tryptophan containing diets, respectively. However, a slightly impaired FCR was noted at these dietary levels. This may be due to the presence of excess amount of tryptophan at diet V and diet VI that could cause imbalance in the normal functioning of the other essential amino acids in the brain especially the large neutral amino acids (LNAA), which presumably might be the reason for poor FCR/high feed intake in the present study beyond the threshold level. Since tryptophan produced 5-hydroxytryptamine (5-HTP) that is converted into serotonin in the brain, it is involved in feeding behaviour of the fish. However, carrier that transporting tryptophan across the blood–brain barrier is non-specific, also transporting the other LNAA via the same route (Winberg et al. 2001;

Lepage et al. 2002). Therefore, the competition between tryptophan and these LNAA for uptake into the brain is increased across the blood–brain barrier for passage during imbalance like situation (Aldegunde et al. 1998, 2000), which could affect the individual performance of these amino acids and also put stress on the other physiological functioning of the fish including hormonal imbalance, due to which the fish either increased energetic demands or for the synthesis of stress-related proteins and other compounds related with the stress response (Aragao et al. 2008), which can be obtained from the additional feed intake in order to overcome the stress-related problems. Henry et al. (1992) reported the existence of anorexic effect of too severely depleted brain 5-HTP due to dietary tryptophan: LNAA imbalance. Johnston and Glanville (1992) suggested that tryptophan competes for uptake into brain with other LNAA, such as leucine, valine and phenylalanine. This occurs because tryptophan and aromatic amino acids share common transporter at the blood–brain barrier. The uptake of tryptophan into the brain is stereospecific, reaches the saturation level and thereafter inhibited by other aromatic amino acids, such as tyrosine (Aldegunde et al. 1998), which could also resulted poor FCR beyond the threshold level. The similar results related to high feed intake and poor FCR beyond the requirement level have also been reported in the past by several workers in their tryptophan requirement studies among different fish species (Khan and Jafri 1993; Murthy and Varghese 1997; Borlongan and Coloso 1993; Ahmed and Khan 2005).

The FCR increases with the increase in dietary tryptophan concentrations from 0.04 to 0.34 g 100 g^{-1}, which may be a result of a deficiency of tryptophan for protein synthesis in the low tryptophan diets and of the roles of tryptophan as precursor of serotonin or other bioactive molecules. Tackman et al. (1990) and Mullen and Martin (1992) reported that the consumption of feed was increased with the increase in dietary tryptophan concentration up to certain levels and reason cited that tryptophan produce serotonin which is formed in brain, influenced feed consumption of animals. In the present study, quadratic regression analysis of weight gain data indicated the dietary requirement to be at 0.37 g 100 g^{-1} of dry diet. However, quadratic regression analyses of FCR, PER and BPD data indicated the optimum requirement at 0.33, 0.32 and 0.33 g 100 g^{-1} of the dry diet, respectively. Based on the above results, the optimum dietary tryptophan requirement of *H. fossilis* is recommended at 0.32 g 100 g^{-1} tryptophan of the dry diet, corresponding to 0.80 g 100 g^{-1} of dietary protein. The tryptophan requirement varies from 0.40 to 1.10 g 100 g^{-1} of dietary protein among species and within the species (NRC 1993; De Silva and Anderson 1995; Wilson 2002). The recommended tryptophan requirement for current experiment with *H. fossilis* (Table 5) fall well within the reported range.

The hepatosomatic index of fish (ranging 2.65–1.42) was not significantly different among 0.24–0.54 g 100 g^{-1} tryptophan levels. However, at marginal tryptophan concentrations, that is 0.04 and 0.14 g 100 g^{-1}, tryptophan dry diets produced significantly HSI. The higher HSI values along with the poor growth and feed conversion efficiency at marginal levels of dietary tryptophan might be due to the unstable liver function at these tryptophan-deficient diets. Kim et al. (1987) reported that when tryptophan is limiting or deficient, more of the other amino acids will be oxidized, resulting in more ammoniogenesis and lower nitrogen retention, while an increase in HSI values below the methionine and tryptophan requirements in fish has also been reported in Asian sea bass (Coloso et al. 1999, 2004). Berge et al. (2002) reported that diet containing marginal arginine and lysine resulted poor growth, FCR, PER and high HSI values. Contrary to this, Walton et al. (1984) reported that HSI value was low in tryptophan-deficient diets in rainbow trout.

The blood parameters are generally considered health condition indicators of the whole body of fish and therefore are important tools in diagnosing the structural and functional status of fish (Adhikari et al. 2004). These haematological indices are also used to assess the functional activities of the oxygen carrying capacity of the bloodstream and have been widely used as an indicator of metal pollution in the aquatic environment (Shah and Altindag 2005; Maheswaran et al. 2008). Besides these, they are also useful for determining the health status of the fish in response to dietary supplements (Klinger et al. 1996; Congleton and Wagner 2006; Mohammed and Sambo 2007). In the present study, the haemoglobin (Hb) and haematocrit (HT%) values were also affected by increasing tryptophan concentrations and were found to be significantly higher at the requirement level 0.34 g 100 g^{-1} followed by those fed at 0.44 g 100 g^{-1} tryptophan containing diet. Maximum Hb and haematocrit values obtained in fish fed diet at 0.34 g 100 g^{-1} tryptophan levels could

Table 5 Quantitative tryptophan requirements of various cultivated fish species compared with *H. fossilis*

Fish species	Tryptophan (g 100 g^{-1}, dry diet)	Tryptophan (g 100 g^{-1}, dietary protein)	Crude protein (%)	References
Indian catfish, Singhi	0.32	0.80	40.0	Present study
Channel catfish	0.12	0.50	24.0	Wilson et al. (1978)
African catfish	0.44	1.10	40.0	Fagbenro and Nwanna (1999)
Mrigal	0.38	0.95	40.0	Ahmed and Khan (2005)
Catla	0.38	0.95	40.0	Ravi and Devaraj (1991)
Rohu	0.45	1.13	40.0	Murthy and Varghese (1997)
Common carp	0.30	0.80	38.5	Nose (1979)
	0.24	0.60	40.0	Ogino (1980)
Japanese eel	0.40	1.10	38.0	Nose (1979)
Milkfish	0.31	0.60	40–45	Borlongan and Coloso (1993)
Coho and Chinook salmon	0.20	0.50	40.0	Halver (1965), Arai and Ogata (1993)
Chum salmon	0.30	0.70	40.0	NRC (1993)
Rainbow trout	0.60	1.40	42.0	Poston and Rumsey (1983)
	0.30	0.50	55.0	Walton et al. (1984)
	0.20	0.60	35.0	Kim et al. (1987)
	-	0.50	44.0	Ogino (1980)
Nile tilapia	0.48	1.00	28.0	Santiago and Lovell (1988)
Asian sea bass	0.21	0.41	52.0	Coloso et al. (2004)
Hybrid striped bass	0.21–0.25	0.6–0.70	35.0	Gaylord et al. (2005)
Gilthead sea bream	0.20	0.60	34.0	Luquet and Sabaut (1974)
		0.60	(A/E ratio)	Kaushik (1998)
European sea bass	–	0.60	(A/E ratio)	Kaushik (1998)
Turbot	–	0.60	(A/E ratio)	Kaushik (1998)
Japanese flounder	–	0.50	(A/E ratio)	Forster and Ogata (1998)
Red sea bream	–	0.60	(A/E ratio)	Forster and Ogata (1998)
Striped bass	–	0.98	(A/E ratio)	Small and Soares (1998)

be related to enhanced fish growth, providing for an efficient level of the blood oxygen carrier system and also confirmed better utilization of available nutrients at this level of tryptophan. The ESR value declined with the increase in dietary tryptophan concentrations and was found to be lowest at 0.34 g 100 g^{-1} tryptophan inclusion in the diet. A decline in ESR at this level of tryptophan could be due to higher blood viscosity resulting in an increase in erythrocytes count.

Significant differences in whole-body composition were evident in *H. fossilis* fed diets containing various levels of tryptophan. Low body protein content was noted in fish fed diets containing lower levels of tryptophan, while body protein content was maximum in fish fed diet with 0.34 g 100 g^{-1} tryptophan followed by 0.44 g 100 g^{-1} dietary concentration. Gaylord et al. (2005) reported that protein utilization might be dramatically compromised if tryptophan is not sufficiently available in conjunction with other essential amino acids in the diet to maintain normal physiological function. In the present study, similar results were noted in body protein content and protein deposition values, as in fish fed at lower levels of dietary tryptophan diets produced less protein utilization as indicated by poor body protein deposition and whole-body protein content. Body fat increased significantly ($P < 0.05$) with the increase in dietary tryptophan concentrations and was found to be significantly higher at 0.54 g 100 g^{-1} (diet VI). Poston and Rumsey (1983) reported that feeding

graded levels of tryptophan caused increased deposition of fat and dry matter and decrease protein content. In the present study, a similar trend was evident in body fat and dry matter, but trend of body protein obtained in present study did not show similar result. Rodehutscord et al. (1997) also reported a similar result in body fat content and mentioned that fat content increased with the increase in dietary tryptophan concentration and was found maximum at higher concentration of dietary tryptophan. Higher body moisture and lower fat content were noted in diets containing lower levels of tryptophan. Kim et al. (1992) also reported similar results in body composition of trout dealing with amino acid requirement study. Body protein deposition was increased significantly with the increase in dietary tryptophan concentration, and maximum protein deposition was also recorded at the requirement level.

The real disparity and reliability of essential amino acids requirements of the fishes studied to date have often being questioned. The variation observed in the requirement level for tryptophan among fish species may be due to the differences in the methodologies used such as the nature of the dietary protein sources in the test diets, the reference protein, which dietary amino acid pattern is chosen and the culture conditions (Luzzana et al. 1998). The variation may also be related to real species differences. Kim et al. (1992) pointed out that large variation in the values for amino acid requirements may be due to differences in the composition of the basal diet used for different experiments. It has also been suggested that the wide variability and the reliability of tryptophan requirements of fish may be affected by fish size and age, feeding regime, feed allowance, adequate levels of other nutrients, water temperature, flow rate, stock density, environmental as well as culture conditions adopted in different laboratories (Cowey and Luquet 1983; Tacon and Cowey 1985; Chiu et al. 1988; Coloso et al. 2004). Digestibility, amino acid profile and energy content may also bring about variable effects in amino acid requirement studies (Simmons et al. 1999; De Silva et al. 2000). Variations may also be attributed due to the true differences between genetically distinct families or species (Akiyama et al. 1997).

The deficiencies of most amino acids cause certain pathological symptoms (Walton 1985) and failure in weight gain and loss of appetite. Tryptophan deficiency has been reported to cause morphological abnormalities, for example, scoliosis and lordosis, in sockeye salmon (Halver 1957; Halver and Shanks 1960) and rainbow trout (Shanks et al. 1962; Poston and Rumsey 1983). Kloppel and Post (1975) noticed that tryptophan-deficient rainbow trout suffered from scoliosis, and histological examinations revealed calcium deposits in the kidney. Scoliosis was also reported in chum salmon feeding with tryptophan-deficient diets (Akiyama et al. 1986). However, these symptoms were not seen in tryptophan-deficient catfish (Wilson et al. 1978). Coloso et al. (1992, 2004) reported impaired growth and low feed conversion in milkfish and Asian sea bass, fed tryptophan-deficient diet. High incidence of skeletal deformities, especially at vertebral column in white seabream, *Diplodus sargus,* was also reported in fish fed tryptophan-deficient diets by (Saavedra et al. 2006a, b). Except for poor growth and low feed efficiency, no gross pathological symptoms in general were observed in *H. fossilis* fed tryptophan-deficient diet. However, in lower tryptophan dose, that is 0.04 g 100 g^{-1}, symptoms of lordosis and spinal curvature were seen in some fishes those died between 7th and 8th weeks. Weight gain of the *H. fossilis* decreased when optimum tryptophan concentration was exceeded. The reduction in growth rate beyond the optimum dietary tryptophan requirement levels could be attributed to amino acid toxicity and catabolism of excessive tryptophan inclusion in the diets, as stress caused by excess amount of amino acid in the body of the fish leading to extra energy expenditure towards deamination and excretion of the same (Walton 1985). Also the accumulation of amino acids or its degraded products in the body pools may stress enzymatic systems of fish, which leads to further accumulation and possible toxicity (Alam et al. 2002). It has also been reported that the excessive levels of amino acids may become toxic and may have an adverse effect on growth because the imbalance intake of one amino acid affects the absorption and utilization of other amino acids (Harper et al. 1970; Borlongan and Coloso 1993; Ahmed and Khan 2005). The major portion of the essential amino acids is used for protein synthesis while amino acid in excess will be more available for oxidation (Anderson et al. 1993; Gahl et al. 1996), which could be the cause of growth depression at higher dietary tryptophan levels. Similar growth depressing effect of feeding higher amounts of tryptophan than optimum was also evident in rainbow

trout (Poston and Rumsey 1983); Nile tilapia (Santiago and Lovell 1988), Indian major carp, catla (Ravi and Devaraj 1991), rohu (Murthy and Varghese 1997) and mrigal (Ahmed and Khan 2005).

Based on the quadratic regression analysis of FCR and PER data, and also the highest body protein deposition value, an inclusion of 0.32 g 100 g^{-1} dietary tryptophan, corresponding to 0.80 g 100 g^{-1} dietary protein, is recommended for optimum growth of *H. fossilis*. The data generated in the present study on the quantitative dietary tryptophan requirement of *H. fossilis* would be useful in developing tryptophan-balanced practical diets for the intensive culture of this species.

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