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DIETARY HISTIDINE REQUIREMENT OF FINGERLING INDIAN MAJOR CARP, *LABEO ROHITA* (HAMILTON)

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Abstract

A 6-week growth trial was conducted to assess the dietary histidine requirement of fingerling Indian major carp, *Labeo rohita* (3.50±0.04 cm; 0.40±0.02 g) by using amino acid test diets (40% crude protein; 4.28 kcal/g) containing casein, gelatin, and L-crystalline amino acids. Diets with graded levels of histidine (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50% of the diet) were fed to triplicate groups of fingerlings at 5% of their body weight divided into two feedings (07:00 and 17:30). Live weight gain, specific growth rate, protein efficiency ratio, and feed conversion ratio were significantly (p<0.05) affected by dietary histidine concentration. Weight gain and conversion efficiencies were best at 0.75% dietary histidine. Whole body protein content was highest and moisture and fat were lowest in the 0.75% dietary histidine treatment while whole body ash was the same at all treatment levels. Second-degree polynomial regression analysis of the live weight gain and feed conversion ratio resulted in more accurate histidine requirement estimates of 0.90 and 0.82% of the dry diet, respectively, corresponding to 2.25 and 2.05% of the dietary protein. It is recommended that dietary histidine be included at a level of 0.82% of the feed, corresponding to 2.05% of the dietary protein, for optimal growth in *L. rohita* fingerlings.

Introduction

The economic success of controlled fish production depends mainly on the cost of feed, especially protein since protein is the major and most expensive feed component (Borlongan, 1991). Fish culture is an efficient means of producing proteins for human consumption and an excellent source of all essential nutrients of biological value including vitamins and minerals. The major function of dietary protein is to supply amino acids required by the fish for growth and reproduction. Although high levels of dietary protein

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are used in feed formulations for most fish species (NRC, 1993), individual amino acid requirements may not be correspondingly high because fish use a significant portion of dietary protein as a source of energy (Kim et al., 1991) if the energy content of the diet is low. The gross dietary protein requirement is directly influenced by the amino acid composition of the diet. Proteins with a poorly balanced amino acid profile are of low nutritional value while a well-balanced, complete protein has high nutritional value.

Fishes do not have an absolute requirement for protein but they have requirements for a well-balanced mixture of indispensable and dispensable amino acids (Wilson and Halver, 1986). The protein requirements of *Labeo rohita* have been worked on by several authors (Renukaradhya and Varghese, 1986; Mohanty et al., 1990; De Silva and Gunasekara, 1991; Khan, 1991; Jena et al., 1996). Dietary arginine, lysine, methionine, and tryptophan requirements of fingerling *Labeo rohita* have been quantified (Khan and Jafri, 1993) but, except for the work of Murthy and Varghese (1995), no information on the dietary histidine requirement of *Labeo rohita* is available. The present investigation was aimed at determining the dietary histidine requirement of fingerling *L. rohita*.

Materials and Methods

Preparation of experimental diets. Six isonitrogenous (40% crude protein) and isoenergetic (4.28 kcal/g gross energy) amino acid test diets were formulated (Table 1). The dietary range necessary to quantify the histidine requirement was based on information available for this species (Murthy and Varghese, 1995). L-crystalline amino acid mixtures were prepared taking into account the amount of amino acids contributed by casein and gelatin. The dietary protein level was fixed at 40%, the reported optimum for the growth of *L. rohita* (Khan, 1991), and the overall composition of amino acids in the test diets was simulated to that of whole chicken egg protein excluding the amino acid histidine. Diets were made isonitrogenous and isoenergetic by adjusting the non-essential amino acids glycine and proline, and dextrin. Histidine levels were increased at increments of 0.25 g/100 g of the dry diet, starting with an initial content of 0.25 g/100 g of the dry diet.

The calculated quantities of L-crystalline amino acids and salt mixtures were stirred mechanically for about 30 min in hot water (80°C) in the stainless steel bowl of a Hobart electric mixer. Gelatin was dissolved separately in water and added to the mixture. Other dry ingredients, oils, and premixes were added to the lukewarm bowl one by one, constantly mixing at 40°C. Carboxymethylcellulose was added in the end and blended thoroughly. The final diet had the consistency of bread dough and was poured into a Teflon coated pan and stored in a refrigerator at -20°C until used. The test diets were neutralized according to Nose et al. (1974).

Experimental design and feeding trial. Indian major carp, *L. rohita*, fry from an induced breeding were obtained from a State Government Fish Hatchery. They were transported to the laboratory in oxygen-filled polythene bags, given a prophylactic dip in $KMnO_4$ solution (1:3000), stocked in an indoor circular aluminum plastic lined tank with a water volume of 600 l, and held for a fortnight. During this period, the fish were fed to satiation a mixture of soybean, mustard oil cake, rice bran, and wheat bran in the form of moist cakes twice a day at 07:00 and 17:30. They were acclimated for two weeks on a casein and gelatin based (40% crude protein) H-440 diet (Halver, 2002) and reared to fingerling stage. The feeding regime and ration size were carefully determined in a preliminary feed trial.

L. rohita fingerlings (3.50±0.04 cm; 0.40±0.02 g) were stocked in triplicate groups in 70-l circular polyvinyl troughs (water volume 55 l) supplied with a continuous water flow (1-1.5 l/min) at 20 fish per trough for each dietary treatment level. Fish were fed test diets in the form of moist cakes at 5% of their body weight at 07:00 and 17:30. No feed was offered to the fish on days the measurements were taken. Initial and weekly weights were recorded on a top loading balance and feed allowances adjusted accordingly. The feeding trial lasted six weeks. Fecal matter and unconsumed feed, if any, were siphoned before feeding. Water

Table 1. Experimental diets (40% crude protein, gross energy 4.28 kcal/100g dry diet[1]) used to estimate dietary histidine requirements of *Labeo rohita* fingerlings.

Ingredients in all diets	*g/100 g dry diet*
Casein[2]	8.8
Gelatin[3]	2.2
Corn oil	5.0
Cod liver oil	2.0
Mineral mix[4]	4.0
Vitamin mix[4,5]	3.0
Carboxymethyl cellulose	10.0

	Diet (g/100 g dry diet)					
Ingredient	*I*	*II*	*III*	*IV*	*V*	*VI*
Amino acid mix [6]	36.1	35.9	35.7	35.4	35.2	35.0
Dextrin	25.2	25.5	25.8	26.2	26.5	26.9
α-Cellulose	3.7	3.6	3.5	3.4	3.3	3.1
Total histidine	0.25	0.5	0.75	1.0	1.25	1.50
Analyzed crude protein (%)	39.80	39.85	39.96	40.0	39.95	40.02

[1] Calculated on the basis of 5.52, 4.83, 5.80, 3.83, and 9.00 kcal/g for casein, gelatin, amino acids, dextrin, and fat, respectively, as estimated by Gallenkamp ballistic bomb calorimeter.
[2] Crude protein (80%), Loba Chemie, India.
[3] Crude protein (93%), Loba Chemie, India.
[4] Halver (2002).
[5] 1 g vitamin mix + 2 g α-cellulose.
[6] Loba Chemie, India.

temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity were measured by standard methods (APHA, 1992). The average water temperature was 26.0-27.5°C, dissolved oxygen 6.7-7.6 ppm, free carbon dioxide 5-10 mg/l, pH 7.1-7.8, and total alkalinity 65- 80 mg/l over the six week trial.

Chemical analysis. The proximate composition of casein, gelatin, the experimental diets, and the initial and final whole body was estimated using standard methods (AOAC, 1995) for dry matter (oven drying at 105±1°C for 22 h), crude protein (N-Kjeldhal x 6.25), crude fat (solvent extraction with petroleum ether BP 40-60°C for 12-14 h), and ash (oven incineration at 650°C for 4-6 h). Gross energy content was determined on a Gallenkamp ballistic bomb calorimeter (Loughbrough UK). Amino acids of the casein, gelatin, and experimental diets were analyzed with an Ultrasphere ODS reverse phase column fitted to a Beckman System Gold HPLC unit (Ahmed et al., 2003). Whole body protein deposition was calculated by the formula: whole body protein deposition

= (final body weight x final body crude protein) - (initial body weight x initial body crude protein)/protein fed.

Statistical analysis. All growth data were subjected to analysis of variance (Snedecor and Cochran, 1968; Sokal and Rohlf, 1981). Differences among treatment means were determined by Duncan's Multiple Range Test at $p<0.05$ level of significance (Duncan, 1955). The break-point for the optimum dietary histidine requirement was estimated using quadratic regression analysis, as described by Zeitoun et al. (1976).

Results

Significant differences were observed in live weight gain of *L. rohita* fingerlings fed diets containing different levels of histidine (Table 2). Fish receiving 0.75% dietary histidine had the highest gain (148%); fish fed diets with lower histidine levels had a lower weight gain and efficiency of feed utilization, indicating that histidine is indeed essential for growth of this fish. The 0.75% diet also yielded the best specific growth rate (2.17%), feed conversion ratio (1.70) and protein efficiency ratio (1.46). However, on subjecting the live weight gain data to quadratic regression analysis (Zeitoun et al., 1976), a break-point was evident at 0.90% dietary histidine (Fig. 1), corresponding to 2.25% of dietary protein.

The feed conversion ratio in fish fed the 0.75% histidine diet differed significantly ($p<0.05$) from that of groups fed other histidine levels and, in relation to the dietary histidine level, was best described by a second-degree polynomial regression analysis (Fig. 2). Based on the regressions equation, the best estimated dietary histidine level is approximately 0.82%.

Final whole body composition (Table 3) differed significantly ($p<0.05$) except for whole body ash. Significantly ($p<0.05$) lower moisture and body fat were recorded in fish receiving the 0.75% histidine diet. Whole body protein was significantly higher at 0.75% histidine because of the more efficient utilization of the indispensable amino acids than in groups receiving the other dietary treatments (Fig. 3).

Survival was 100% in all treatments.

Discussion

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). The present finding indicates that 0.75% dietary histidine is required for maximum growth of fingerling *L. rohita*. The best feed efficiency, also obtained with this histidine level, supports this finding. However, second-degree polynomial regression analysis of the weight gain and feed conversion data indicate that the optimum histidine level is 0.90% and 0.82%, respectively. Based on these quadratic regression analyses, it is recommended that 0.82% dietary histidine, corresponding to 2.05% of the dietary protein, is the optimum level for growth of fingerling *Labeo rohita*.

Histidine inclusion values for other species are shown in Table 4. The large variation may be due to differences in fish size or age, or laboratory conditions including feeding regime, feed allowance, water temperature, stocking density, or combination of ingredients (e.g., casein, gelatin) used to prepare basal diets. Digestibility, amino acid profile, and energy content may affect amino acid requirements (Simmons et al., 1999; De Silva et al., 2000) and variations may be attributed to the differences between phylogenetically distinct families or species (Akiyama et al., 1997). Except for poor growth and low feed efficiency, no pathological symptoms were observed in the fingerlings fed low levels of dietary histidine.

Data generated during the present study on the quantitative dietary histidine requirement of *L. rohita* may be useful in developing histidine balanced practical diets for the intensive culture of this species. However, further studies should be carried out using practical diets to confirm the histidine requirement of *L. rohita* determined in this study.

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Table 2. Growth and conversion efficiencies (means±SEM) of fingerling *Labeo rohita* fed diets containing graded levels of dietary histidine (n = 3).

	Dietary histidine level (% of diet)					
	0.25	*0.50*	*0.75*	*1.00*	*1.25*	*1.50*
Average initial weight (g)	0.43±0.006	0.41±0.002	0.42±0.003	0.43±0.003	0.42±0.003	0.42±0.002
Average final weight (g)	0.71±0.003	0.90±0.006	1.04±0.009	0.997±0.007	0.960±0.006	0.900±0.012
Live weight gain (%)	77.49±1.8^{e}	126.71±3.50^{b}	148.83±3.91^{a}	129.47±2.91^{b}	113.30±3.23^{c}	101.97±3.46^{d}
Specific growth rate[1]	1.36±0.03^{e}	1.95±0.04^{b}	2.17±0.04^{a}	1.97±0.03^{b}	1.79±0.03^{c}	1.67±0.04^{d}
Food conversion ratio[2]	2.83±0.08^{a}	2.04±0.03^{c}	1.70±0.11^{d}	2.13±0.06^{c}	2.55±0.06^{b}	2.87±0.06^{a}
Protein efficiency ratio[3]	0.88±0.03^{c}	1.26±0.06^{b}	1.46±1.73^{a}	1.18±0.04^{b}	0.98±0.02^{c}	0.87±0.02^{c}

Mean values in a row with different superscripts are significantly different (p<0.05).

[1] SGR = [(In mean final weight) – (In mean initial weight)/no. days] x 100.

[2] FCR = dry food fed/wet weight gain.

[3] PER = weight gain (g on wet weight basis)/protein intake (g on dry weight basis).

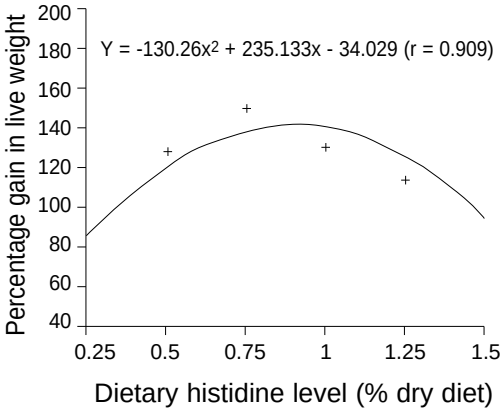

Fig. 1. Live weight gain data for *Labeo rohita* fingerlings fed different levels of L-histidine subjected to quadratic regression analysis.

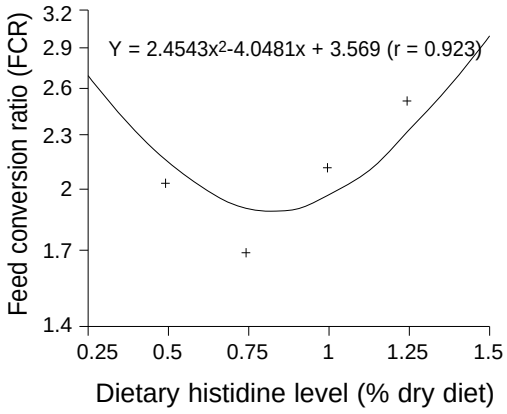

Fig. 2. Feed conversion ratio of *Labeo rohita* fingerlings fed different levels of L-histidine subjected to second-degree polynomial regression analysis.

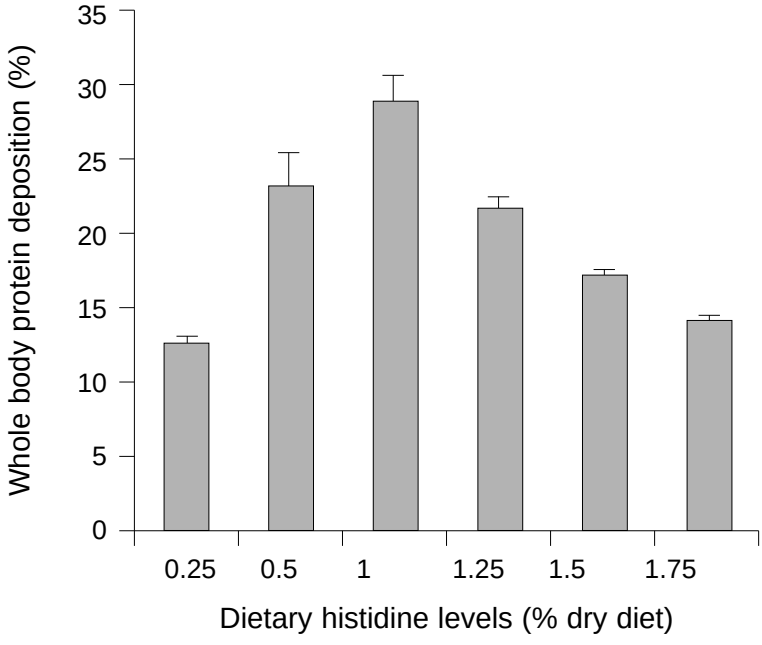

Fig. 3. Whole body protein in *Labeo rohita* fingerlings fed different levels of L-histidine.

Table 3. Whole body composition (%, means±SEM)) of fingerling *Labeo rohita* fed graded levels of dietary histidine (n = 3).

	Dietary histidine level (% of diet)						
	Initial	*0.25*	*0.50*	*0.75*	*1.00*	*1.25*	*1.50*
Moisture	81.33±0.05	80.91±0.10^{a}	79.94±0.15^{d}	79.20±0.09^{e}	79.62±0.06^{de}	80.41±0.26^{bc}	80.78±0.07^{ab}
Protein	11.84±0.13	12.92±0.17^{e}	14.83±0.18^{c}	16.42±0.08^{a}	15.54±0.03^{b}	14.81±0.09^{c}	13.99±0.03^{d}
Fat	3.45±0.25	3.17±0.01^{e}	3.89±0.03^{bc}	3.19±0.10^{e}	3.86±0.02^{bcd}	3.96±0.09^{b}	4.12±0.05^{a}
Ash	3.43±0.13	2.30±0.23	2.20±0.06	2.10±0.15	2.33±0.09	2.05±0.03	2.30±0.06

Mean values in a row with different superscripts are significantly different (p<0.05).

Table 4. Dietary histidine requirements of various fish species (g/100 g dietary protein).

Species	*Histidine (g/100 g protein)*	*Crude protein (g/100 g diet)*	*Reference*
Labeo rohita	2.05	40	Present study
Catla catla	2.5	40	Ravi and Devaraj (1991)
Cyprinus carpio	2.1	38.5	Nose (1979)
	1.4	38.5	Ogino (1980)
Ictalurus punctatus	1.5	24	Wilson et al. (1980)
Oncorhynchus keta	1.6	40	Akiyama et al. (1985)
	1.6	40	Akiyama and Arai (1993)
O. kisutch	1.8	40	Klein and Halver (1970)
	0.9	40	Arai and Ogata (1993)
O. tshawystscha	1.8	40	NRC (1993)
O. mykiss	1.6	35	Ogino (1980)
Anguilla japonica	2.1	38	NRC (1993)
Chanos chanos	2.0	45	Borlongan and Coloso (1993)
Oreochromis niloticus	1.7	28	Santiago and Lovell (1988)

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