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DIETARY THREONINE REQUIREMENT OF INDIAN MAJOR CARP, *CIRRHINUS MRIGALA* (HAMILTON), JUVENILES

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Abstract

A growth study was conducted to determine the dietary threonine requirement of juveniles of the Indian major carp (*Cirrhinus mrigala*) known as “mrigal”. Diets containing casein and gelatin as sources of intact proteins were supplemented with crystalline amino acids to obtain a crude protein content of 40%. Six diets with different levels of threonine (1.0, 1.3, 1.5, 1.7, 1.9 and 2.1%) were fed to triplicate groups of mrigal juveniles twice a day for 56 days. The dietary threonine requirement, estimated by break-point analysis, was 1.66% of the dry diet (4.15% of the dietary protein). The highest growth and specific growth rate were recorded in fish fed the diet containing 1.7% threonine.

Introduction

Threonine is an indispensable amino acid for growth of young fish (Wilson, 1989) and terrestrial animals (Visek, 1984). The required amount of threonine has been investigated in common carp (Nose, 1979), Japanese eel (Arai *et al.*, 1972), channel catfish (Wilson *et al.*, 1978), Nile tilapia (Santiago and Lovell, 1988), milkfish (Borlongan, 1991, catla (Ravi and Devaraj, 1991) and rohu (Murthy and Varghese, 1996).

Cirrhinus mrigala, popularly known as “mrigal”, is one of the most widely cultured of the Indian major carps. The economic success of fish culture depends on the cost of feeds, particularly the cost of protein materials incorporated into the feed. Although gross protein requirements of mrigal have been established (Mohanty *et al.*, 1990), no information is available on the threonine requirement of this species. Hence, the present study

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was undertaken to examine the optimum threonine requirements for juvenile mrigal.

Material and Methods

Experimental diets. Six isonitrogenous diets were formulated to contain 40% crude protein with six graded levels of threonine (Table 1). The diets contained intact protein sources (casein and gelatin) and crystalline amino acids. The casein and gelatin provided threonine at a level of 1% of the dry diet in all the feeds. Threonine supplements were added to reach the desired test level. An essential amino acid mix (EAA mix), which contained no threonine, was added to simulate the amino acid profile of mrigal muscle protein. The diets were kept at 40% protein by decreasing the amount of non-EAA mix as the amount of supplemented threonine increased.

The dry ingredients, except the carboxymethylcellulose (CMC), were mixed homogeneously. Butylated hydroxyanisole and tocopherol were dissolved in oils and then blended with the dry ingredients. The pH of the diet was adjusted to 7-8 by adding a measured quantity of 6N NaOH. The CMC was gelatinized with hot water (80-90°C) and stirred into the dry ingredients. The blended dough was passed through a feed pelletizer to obtain 2-mm diameter pellets, which were dried in an oven at a temperature not exceeding 40°C to reduce the moisture content to below 10%. The dry pellets were ground, sieved and stored at 4°C until used.

Experimental design and feeding. Mrigal juveniles from induced breeding were conditioned for about ten days before the experiment, during which time they were fed a diet containing 40% protein. The experiment was conducted in 18 flow-through flat bottomed plastic tanks of 120 l. Each tank was stocked randomly with 20 fish weighing an average of 1.07 g. Each diet was fed to three replicate groups of fish, at 9:00 and 15:00 at a rate of 10% of the body weight of the fish for eight weeks. Tanks were cleaned daily by siphoning excess feed and fecal matter. The water flow was maintained at a rate of 500 ml/min. Three-fourths of the water was replaced with filtered fresh water daily. This relatively high water replacement was necessary to retrieve all the fecal matter from the flat-bottomed tanks. Continuous aeration was provided, as well as incandescent lighting in a 12 h light/12 h dark regime. All fish were weighed every week to record growth. During the weekly samplings, the tanks were washed thoroughly and filled with fresh water.

Water quality. Water samples from each tank were analyzed every week for dissolved oxygen, free carbon dioxide, total alkalinity, ammonia and pH following standard methods (APHA, 1992). Water temperature ranged 27.5-28.9°C, alkalinity 43-64 ppm., dissolved oxygen 7.5-10.1 mg/l, pH 6.9-7.9 and total ammonia 0.23-1.37 µg N/l. The recorded parameters were within the range suitable for carp growth (Jhingran, 1991).

Chemical analysis. The proximate compositions of the casein, gelatin and diets were determined according to standard methods (AOAC, 1995). The amino acid compositions of the ingredients and diets were analyzed employing an amino acid analyzer (LKB model 415 Alfa plus).

Statistical analysis. The average weight gains of the fish in response to the varying levels of threonine were analyzed by two-way analysis of variance (Snedecor and Cochran, 1968). Duncan's multiple range test was employed to determine the statistical significance among treatments. The broken line regression model (Robbins *et al.*, 1979) was used to determine the break-point in the growth curve, which represented the optimum dietary concentration of threonine for the growth of juvenile mrigal.

Results

Mean weight gains, specific growth rates and survival are presented in Table 2. The mean weight gain increased significantly as the threonine increased, up to 1.7% (Diet 4). Fish fed diets deficient in threonine had the poorest growth, indicating that threonine is indeed essential for juvenile mrigal growth. The highest growth was observed with Diet 4 indicating that increasing threonine beyond this level would not improve growth.

When the weight gains were plotted

Table 1. Ingredients of basal and experimental diets (40% protein).

Ingredient	*Content (%)*
Casein	20.00
Gelatin	20.00
EAA mix[1]	4.32
Dextrin	25.00
Cod liver oil	5.00
Sunflower oil	5.00
Vitamin mix[2]	2.00
Mineral mix[2]	4.00
DLα-tocopherol acetate	0.01
BHA	0.02
Carboxymethylcellulose	5.00
Cellulose	7.02
Subtotal	97.37

Variable ingredients	*Diet (%)*					
	1	*2*	*3*	*4*	*5*	*6*
Threonine supplement (% dry diet)	0	0.30	0.50	0.70	0.90	1.10
Non-EAA mix (% dry diet)[3]	2.63	2.33	2.13	1.93	1.73	1.53
Total threonine (% dry diet)	1.0	1.3	1.5	1.7	1.9	2.1
Threonine (% protein)	2.50	3.25	3.75	4.25	4.75	5.25
Crude protein - analyzed (% dry diet)	40.19	40.29	40.37	40.31	40.26	40.44

[1] Essential amino acid mix (g/100 g dry diet): arginine 0.55, histidine 0.66, isoleucine 0.37, leucine 0.74, lysine 1.15, phenylalanine 0.60, valine 0.20, tryptophan 0.05.
[2] Benakappa and Varghese, 2002
[3] Non-EAA mix (g/100 g dry diet): tyrosine 0.47, alanine 0.43, aspartic acid 1.47, serine 0.26

against the threonine levels (Fig.1), the breakpoint occurred at 1.66% of the dry diet, corresponding to 4.15% of the dietary protein. Except for reduced growth, no nutritional deficiency symptoms were observed in the fish fed threonine-deficient diets.

Discussion

Threonine at 4.15% of the dietary protein is comparable to the 4.28% reported for rohu (Murthy and Varghese, 1996). It is lower than the 4.95% reported for catla fry (Ravi and Devaraj, 1991) and the 4.50% reported for milk-

Table 2. Weight gain, specific growth rate (SGR) and survival of mrigal fry fed graded levels of threonine.

Dietary threonine		*Mean initial*	*Mean final*	*Mean*	*SGR*	*Survival*
g/100 g dry diet	*g/100 g protein*	*weight (g)*	*weight (g)*	*weight gain (%)*	*(%)*	*(%)*
1.0	2.50	1.06±0.01	1.50±0.02	41.50[a]	0.62	83.33
1.3	3.25	1.07±0.01	2.12±0.01	98.13[b]	1.22	90.00
1.5	3.75	1.07±0.02	2.96±0.01	176.63[c]	1.82	93.33
1.7	4.25	1.09±0.01	3.42±0.04	213.76[d]	2.04	100.00
1.9	4.75	1.07±0.01	3.02±0.01	182.24[c]	1.85	100.00
2.1	5.25	1.07±0.02	2.60±0.01	142.99[e]	0.89	96.66

Values with different superscripts differ significantly ($p<0.05$).

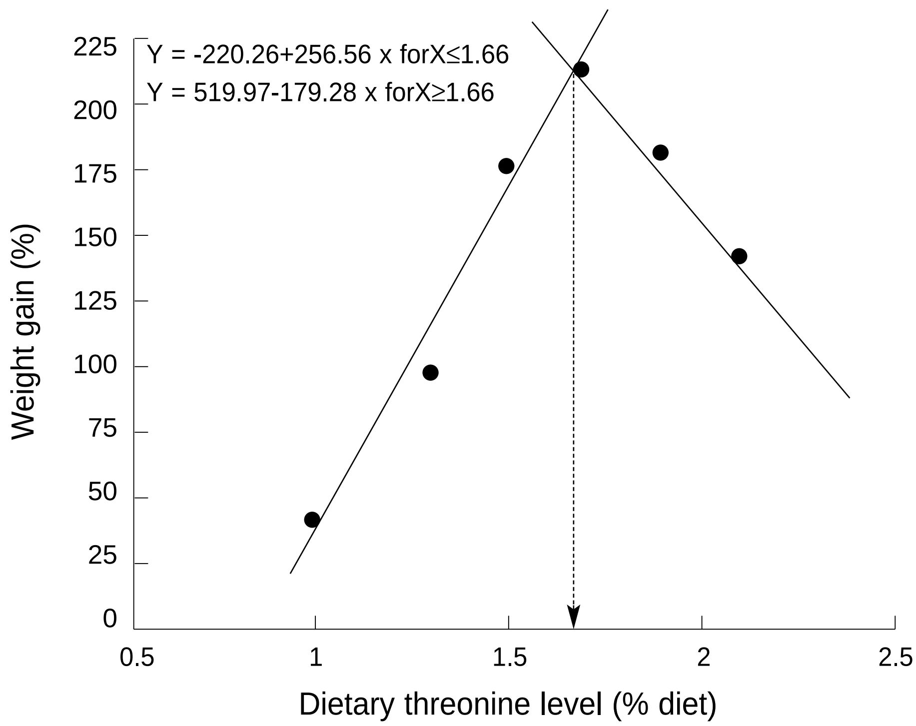

Fig. 1. Optimum dietary threonine level as determined by break-point analysis.

fish (Borlongan, 1991). Lower values have been reported for other species: 3.9% for common carp (Nose, 1979), 3.75% for Nile tilapia (Santiago and Lovell, 1988), 2.21% for channel catfish (Wilson *et al.*, 1978), 3.6% for Japanese eel (Arai *et al.*, 1972), 2.93% for *Oreochromis mossambicus* (Jauncey *et al.*, 1983), 2.25% for chinook salmon (Delong *et al.*, 1962) and 3.0% for chum salmon (Akiyama *et al.*, 1985).

The wide variation in threonine requirements among fish species may be the result of laboratory variances: different basal diets, feeding levels or environmental conditions; fish of different ages, sizes or strains. The growth of the mrigal in the present study was lower than in natural or farm conditions. Slow growth of Indian major carps including mrigal in laboratory conditions has been described by Murthy and Varghese (1995).

A reduction in growth rate would result in an apparent lower amino acid requirement. The reduced growth of mrigal fed a high level of threonine (Diet 6) could be attributed to amino acid toxicity or catabolism. Toxic and adverse effects of excessive amino acids on growth have been attributed to the fact that disproportional intake of amino acids affects the absorption and utilization of the amino acids (Harper *et al.*, 1970; Austic, 1978; Borlongan and Coloso, 1993; Murthy and Varghese, 1995). Choo *et al.* (1991) reported the toxic effect of excess dietary leucine in rainbow trout: growth decreased when the essential amino acids exceeded the requirements. This decrease is attributed to the use of energy for nitrogenous excretion, because amino acids are deaminated and excreted in the form of ammonia (Walton, 1985).

No pathological syndromes such as scoliosis or lordosis were observed in the fish fed threonine-deficient diets. The dietary threonine requirements for juvenile *Cirrhinus mrigala* based on the dose response curve in this study can be used to formulate a threonine-balanced diet for the production of mrigal.

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