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ISOLEUCINE, LEUCINE, AND VALINE REQUIREMENT OF JUVENILE INDIAN MAJOR CARP, *CIRRHINUS CIRRHOSUS* (BLOCH, 1795)

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Background. Mrigal, *Cirrhinus cirrhosus* (Bloch, 1795)(junior synonym *C. mrigala*), is one of the most widely cultured Indian major carps. The main aim of this study was to quantify the dietary isoleucine, leucine, and valine requirements of mrigal juveniles.
Material and methods. Growth studies were conducted with juvenile mrigal to determine the minimal requirements for the branched-chain amino acids: isoleucine, leucine, and valine. The experimental diets were formulated from purified ingredients to contain 40% crude protein. Casein and gelatin served as protein sources and were supplemented with crystalline L -amino acids to provide an amino acid pattern found in mrigal muscle protein. The amino acids for which the requirements were being determined was were supplemented at varying levels. Dietary inclusion levels for isoleucine ranged from 0.88 to 1.45%, for leucine from 1.36 to 2.05%, and for valine from 1.23 to 1.75%.
Results. The optimum dietary requirements for isoleucine, leucine, and valine for mrigal, estimated using break point analysis were 1.25%, 1.73%, and 1.55% of dry diet, respectively. These values correspond to 3.12% isoleucine, 4.33% leucine, and 3.87% valine when expressed as a percentage of dietary protein. Food conversion rate, specific growth rate, and survival were better in treatments with diets containing 1.25% for isoleucine, 1.73% for leucine, and 1.55% for valine.
Conclusion. : Results of this study clearly demonstrated that *Cirrhinus cirrhosus* could utilize free amino acids for growth. Further, the findings would be useful in formulating isoleucine, leucine, and valine balanced diets in controlled production of mrigal.

Key words: isoleucine, leucine, valine, requirement, fish, mrigal, *Cirrhinus cirrhosus, C. mrigala*

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INTRODUCTION

As with terrestrial animals, fish require the same ten indispensable or essential amino acids (EAA) within their diet. The branched-chain amino acid requirements have been investigated in chinook salmon (Chance et al. 1964), Japanese eel (Arai et al. 1972), channel catfish (Wilson et al. 1978), common carp (Nose 1979), lake trout (Hughes et al. 1983), Mozambique tilapia (Jauncey et al. 1983), Nile tilapia (Santiago and Lovell 1988), catla (Ravi and Devaraj 1991), milkfish (Borlongan and Coloso 1993), and rohu (Murthy 1994), and these studies indicate differences among species (Moon and Gatlin 1991, Borlongan and Coloso 1993).

Cirrhinus cirrhosus (Bloch, 1795) (junior synonym *Cirrhinus mrigala*), popularly known, as "mrigal" is a commercially-important fish species widely cultured in the entire Indian sub-continent. Information on nutritional requirements of a species is a prerequisite for formulation of nutritionally balanced and cost cost-effective feed for its culture. Information on gross protein requirements, without knowledge of the amino acid profile of the diet and requirements is of limited value because a well -balanced mixture of essential and non-essential amino acids is required for optimum growth. There is no published report on the EAA requirements of mrigal, except for lysine, threonine, and tryptophan (Benakappa and Varghese 2002a, b, Benakappa and Verghese 2002c). In the present investigations an attempt has been made to quantify the dietary isoleucine, leucine, and valine requirements of mrigal.

MATERIAL AND METHODS

Experimental diets

Six isonitrogenous diets with graded levels of isoleucine, leucine, and valine were formulated (Tables 1, 2, 3) to contain 40% crude protein. Casein and gelatin were incorporated as protein sources. Crystalline L-amino acids were supplemented to simulate the amino acid pattern of mrigal muscle protein (Mohanty and Kaushik 1991) except for the test amino acids. The test amino acid content present in casein and gelatin formed the minimal level of that amino acid in the experimental diets. The diets were made isonitrogenous by decreasing the non-essential amino acids as the level of test amino acids increased. Dietary ingredients were mixed, adjusted to a pH of about 7.0 and pelleted as described by Benakappa and Varghese (2002b)

Experimental design and feeding

Induced bred mrigal juveniles were procured from Karnataka State Fisheries Department Fish Seed Farm, Bhadra Reservoir project. Fish were acclimated to laboratory condition for 2 weeks. During the period of acclimatization they were fed diet containing 40% crude protein. The experiments were conducted in 120-l flow--through fibreglass tanks with a flow rate around 500 ml per minute. Continuous aeration was provided and supplemental incandescent lighting was provided with a 12-h light and 12-h dark regime.

Each tank was stocked randomly with 20 fish in each. The initial mean weight of fish ranged from 0.93 ± 0.01 g to 0.95 ± 0.02 g (isoleucine), 1.14 ± 0.01 to 1.16 ± 0.01 g (leucine), and 0.96 ± 0.01 g to 0.97 ± 0.01 g (valine). Each test diet was fed to three replicate groups of fish, at 0900 and 1500 hrs at a rate of 10% of the body weight of the fish for eight weeks. Excess feed and faecal matter were removed daily by siphoning the water form the bottom of each tank. Each group of fish was weighed every week. Water analysis for various physico-chemical parameters, namely temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, and total ammonia were carried out (Anonymous 1995a) at the time of weekly sampling.

Chemical analysis

Proximate analysis of ingredients and diets were performed according to standard methods (Anonymous 1995b). Amino acid compositions of casein, gelatin, and diets were analyzedanalysed by an amino acid analyser (LKB model 4050 alpha plus).

Table 1

Composition [%] of the test diets with varying levels of isoleucine

Ingredients	Diets					
	I	II	III	IV	V	VI
Casein	15.00	15.00	15.00	15.00	15.00	15.00
Gelatin	20.00	20.00	20.00	20.00	20.00	20.00
EAA mix *	6.24	6.24	6.24	6.24	6.24	6.24
Non-EAA mix+	4.81	4.64	4.54	4.44	4.34	4.24
Isoleucine	0	0.17	0.27	0.37	0.47	0.57
Dextrin	25.00	25.00	25.00	25.00	25.00	25.00
Cod liver oil	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower oil	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mix •	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix •	4.00	4.00	4.00	4.00	4.00	4.00
DL- α- tocopherol acetate	0.01	0.01	0.01	0.01	0.01	0.01
BHA	0.02	0.02	0.02	0.02	0.02	0.02
Carboxymethyl Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Cellulose	7.92	7.92	7.92	7.92	7.92	7.92
Total	100.00	100.00	100.00	100.00	100.00	100.00
Total isoleucine	0.88	1.05	1.15	1.25	1.35	1.45
Computed % crude protein	40.00	40.00	40.00	40.00	40.00	40.00
g isoleucine/100 g protein	2.20	2.62	2.87	3.12	3.37	3.62
Crude protein [%] analysed	40.21	40.33	40.41	40.24	40.39	40.44

* EAA mix [g/100 g dry diet] : arginine 0.70; histidine 0.78; leucine 1.13; lysine 1.49; phenylalanine 0.85; threonine 0.68 valine 0.49; tryptophan 0.12

+ Non-EAA mix [g/100 g dry diet] : tyrosine 0.77; alanine 0.63; aspartic acid 1.88; glutamic acid 0.96 serine 0.57

• Benakappa and Varghese (2002b)

Table 2

Composition [%] of the test diets with varying levels of leucine

Ingredients	Diets					
	I	II	III	IV	V	VI
Casein	10.00	10.00	10.00	10.00	10.00	10.00
Gelatin	25.00	25.00	25.00	25.00	25.00	25.00
EAA mix *	6.62	6.62	6.62	6.62	6.62	6.62
Non-EAA mix+	4.84	4.75	4.60	4.45	4.30	4.15
Leucine	0	0.09	0.24	0.39	0.54	0.69
Dextrin	25.00	25.00	25.00	25.00	25.00	25.00
Cod liver oil	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower oil	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mix •	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix •	4.00	4.00	4.00	4.00	4.00	4.00
DL- α - tocopherol acetate	0.01	0.01	0.01	0.01	0.01	0.01
BHA	0.02	0.02	0.02	0.02	0.02	0.02
Carboxymethyl cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Cellulose	7.51	7.51	7.51	7.51	7.51	7.51
Total	100.00	100.00	100.00	100.00	100.00	100.00
Total leucine	1.36	1.45	1.60	1.75	1.90	2.05
Computed % crude protein	40.00	40.00	40.00	40.00	40.00	40.00
g leucine/100g protein	3.40	3.62	4.00	4.37	4.75	5.12
Crude protein [%] analysed	40.27	40.31	40.44	40.39	40.46	40.33

* EAA mix [g/100 g dry diet] : arginine 0.56; histidine 0.88; isoleucine 0.76; lysine 1.69; methionine 0.03; phenylalanine 1.06; threonine 0.78; valine 0.67; tryptophan 0.19

+ Non-EAA mix [g/100g dry diet] : tyrosine 0.84; cystine 0.02; alanine 0.28; aspartic acid 1.87; glutamic acid 1.28; serine 0.55

• Benakappa and Varghese (2002b)

Table 3

Composition [%] of the test diets with varying levels of valine

Ingredients	Diets					
	I	II	III	IV	V	VI
Casein	15.00	15.00	15.00	15.00	15.00	15.00
Gelatin	20.00	20.00	20.00	20.00	20.00	20.00
EAA mix *	6.34	6.34	6.34	6.34	6.34	6.34
Non-EAA mix+	4.81	4.69	4.59	4.49	4.39	4.29
Valine	0	0.12	0.22	0.32	0.42	0.52
Dextrin	25.00	25.00	25.00	25.00	25.00	25.00
Cod liver oil	5.00	5.00	5.00	5.00	5.00	5.00
Sunflower oil	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mix •	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix •	4.00	4.00	4.00	4.00	4.00	4.00
DL-α-tocophherol acetate	0.01	0.01	0.01	0.01	0.01	0.01
BHA	0.02	0.02	0.02	0.02	0.02	0.02
Carboxymethylcellulose	5.00	5.00	5.00	5.00	5.00	5.00
Cellulose	7.82	7.82	7.82	7.82	7.82	7.82
Total	100.00	100.00	100.00	100.00	100.00	100.00
Total valine	1.23	1.35	1.45	1.55	1.65	1.75
Computed % crude protein	40.00	40.00	40.00	40.00	40.00	40.00
g valine/100g protein	3.07	3.37	3.62	3.87	4.12	4.37
Crude protein (%) analysed	40.27	40.36	40.38	40.41	40.29	40.39

* EAA mix (g/100 g dry diet) : arginine 0.70; histidine 0.78; isoleucine 0.59; leucine 1.13; lysine 1.49 ; phenylalanine 0.85; threonine 0.68; tryptophan 0.12

+ Non-EAA mix (g/100 g dry diet) : tyrosine 0.77; alanine 0.63; aspartic acid 1.88; glutamic acid 0.96; serine 0.57

• Benakappa and Varghese (2002b)

Statistical analysis

Mean weight gains of mrigal at the end of experiment were tested using two-way analysis of variance (Snedecor and Cochran 1968). Duncan's multiple range test was employed to determine statistical significance among treatments. Optimum dietary requirements for EAA were determined by fitting the weight gain of fish to break point analysis (Robbins et al. 1979).

RESULTS

The overall average values of water temperature ranged from 27.5°C to 28.9°C. Ranges of other parameters were: pH 6.8–7.9, dissolved oxygen 7.5–10.1 mg · l^{-1}, total alkalinity 44–73 ppm, and total ammonia 0.19–1.77 µg – at N/l.

Isoleucine requirement

Mean weight gain, food conversion ratios, specific growth rates, and survival of mrigal fed graded levels of isoleucine are shown in Table 4. The fish fed diets 1 and 2 grew less well than the fish with other dietary treatments. When the weight gains were plotted against levels of isoleucine in the diet (Fig. 1) a break point occurred at approximately 1.25% of the dry diet and this was taken as the dietary requirement level. This requirement based on weight gain, corresponds also with the dietary level, which gave the better food conversion ratio and highest survival rate. This value corresponds to 3.12% of the dietary protein.

Table 4

Weight gain, food conversion ratio (FCR), specific growth rate (SGR) and survival of mrigal fed graded levels of isoleucine

Dietary isoleucine		Mean initial weight [g]	Mean final weight [g]	Mean weight gain [%]	FCR	SGR [%]	Survival [%]
g/100 g dry diet	g/100 g protein						
0.88	2.20	0.95 ± 0.01	1.79 ± 0.04	87.36[a]	8.72	1.13	93.33
1.05	2.62	0.93 ± 0.01	2.23 ± 0.03	138.70[b]	6.06	1.56	86.66
1.15	2.87	0.95 ± 0.02	3.04 ± 0.01	220.00[c]	4.71	2.08	100.00
1.25	3.12	0.94 ± 0.02	3.40 ± 0.02	261.78[d]	4.19	2.30	100.00
1.35	3.37	0.95 ± 0.02	3.00 ± 0.01	215.78[c]	4.89	2.05	90.00
1.45	3.62	0.95 ± 0.02	2.75 ± 0.03	186.31[e]	5.60	1.88	93.33

Values are means of three replicates of 20 fish each
Values with different superscripts differ significantly ($P < 0.5$)

Leucine requirement

The responses of mrigal juveniles to varying levels of leucine are presented in Table 5. Growth of young mrigal increased with increase in leucine concentration up to 1.75% of dry diet and thereafter the growth declined. Fish fed diets deficient in leucine had reduced growth. The diet that gave the best weight gain also resulted the highest survival, feed conversion and specific growth rate. Survival rates ranged from 76.66 to 100%. Broken line regression analysis of growth data revealed a breakpoint in the growth curve at 1.73% of dietary leucine (Fig. 1). This value corresponds to 4.33% of dietary protein.

Table 5

Weight gain, food conversion ratio (FCR), specific growth rate (SGR) and survival of mrigal fed graded levels of leucine

Dietary leucine		Mean initial weight [g]	Mean final weight [g]	Mean weight gain [%]	FCR	SGR [%]	Survival [%]
g/100 g dry diet	g/100 g protein						
1.36	3.40	1.16 ± 0.01	2.03 ± 0.02	75.00	9.43[a]	1.00	76.66
1.45	3.62	1.14 ± 0.01	2.49 ± 0.02	118.42	6.69[b]	1.40	90.00
1.60	4.00	1.16 ± 0.01	3.27 ± 0.04	181.89	5.28[c]	1.85	96.66
1.75	4.37	1.15 ± 0.01	3.56 ± 0.03	208.69	4.94[d]	2.01	100.00
1.90	4.75	1.16 ± 0.01	3.20 ± 0.02	175.00	5.61[c]	1.81	100.00
2.05	5.12	1.15 ± 0.01	2.87 ± 0.01	149.56	5.88[e]	1.63	93.33

Values are means of three replicates of 20 fish each
Values with different superscripts differ significantly ($P < 0.5$)

Valine requirements

Table 6 shows the mean weight gain, food conversion ratio, specific growth rates, and survival of mrigal fed diets containing graded level of valine for 8 weeks. Mean weight gain and specific growth rate increased significantly with increased valine level in the diets. The best feed conversion ratios were obtained with diet, which gave the best growth rates. The poor efficiency of feed utilization by fish fed a valine--deficient diet was evident. Generally, mean survival rates were high and ranged from 76.66 to 100%. Mean weight gain was plotted against level of dietary valine (Fig. 1). A point occurred at 1.55% of dry diet, which is taken as requirement level of mrigal for this amino acid. When expressed as a percentage of the dietary protein, this was equivalent to 3.87%.

Table 6

Weight gain, food conversion ratio (FCR), specific growth rate (SGR) and survival of mrigal fed graded levels of valine

Dietary valine		Mean initial weight [g]	Mean final weight [g]	Mean weight gain [%]	FCR	SGR [%]	Survival [%]
g/100 g dry diet	g/100 g protein						
1.23	3.07	0.96 ± 0.01	1.50 ± 0.01	56.25[a]	12.07	0.80	76.66
1.35	3.37	0.96 ± 0.01	1.67 ± 0.04	75.00[b]	9.59	0.99	93.33
1.45	3.62	0.96 ± 0.01	2.54 ± 0.03	164.58[c]	5.54	1.73	96.66
1.55	3.87	0.96 ± 0.01	3.02 ± 0.04	214.58[d]	4.49	2.04	100.00
1.65	4.12	0.97 ± 0.01	2.74 ± 0.02	182.47[e]	5.14	1.85	93.33
1.75	4.37	0.97 ± 0.01	2.48 ± 0.01	155.67[c]	5.47	1.67	90.00

Values are means of three replicates of 20 fish each.
Values with different superscripts differ significantly ($P < 0.5$)

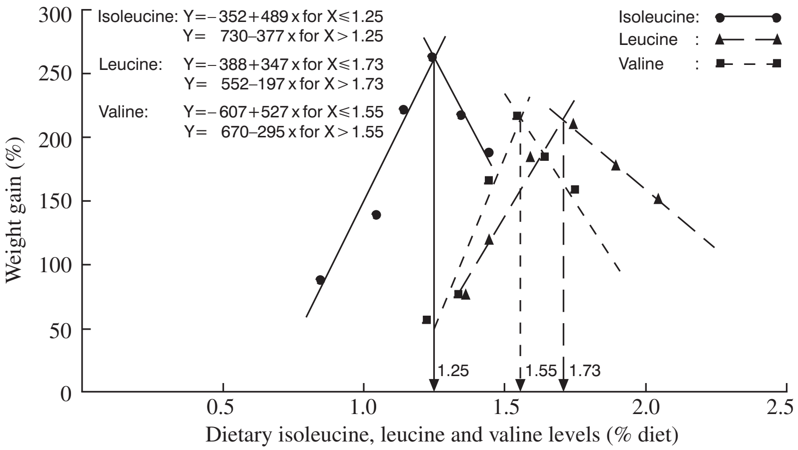

Fig. 1. Relationship between weight gain of mrigal and dietary isoleucine, leucine, and valine, indicating optimum level

DISCUSSION

The recorded water quality parameters were within the ranges suitable for carp growth (Jhingran 1991).

Isoleucine requirement

Isoleucine at 3.12% of the dietary protein is similar to the 3.11% reported for Nile tilapia (Santiago and Lovell 1988) and 3.0% for rohu (Murthy and Varghese 1996). The requirement value obtained was higher than that reported for chinook salmon, 2.2% (Chance et al. 1964) common carp, 2.5% (Nose 1979), channel catfish, 2.6% (Wilson et al. 1980), Mozambique tilapia, 2.01% (Jauncey et al. 1983), lake trout, 2.06% (Hughes et al. 1983) catla, 2.35% (Ravi and Devaraj 1991). However, higher requirements have been reported for eel, 3.6% (Arai et al. 1972), and milkfish, 4.0% (Borlongan and Coloso 1993).

Dietary requirement of isoleucine increased with increased dietary leucine in chinook salmon (Chance et al. 1964). In the present study, the amount of leucine was held constant at 1.13% in the diet and its interrelationship with isoleucine was not studied.

No signs of fin erosion, scoliosis, and eye cataracts were noticed in mrigal fed isoleucine isoleucine-deficient diets.

Leucine requirement

The inferred leucine requirement of mrigal i.e. 4.33% of dietary protein is similar to the 4.4% reported for rainbow front (Ogino 1980). It is however higher than the requirements reported for chinook salmon, 3.9% (Chance et al. 1964), juvenile common carp, 3.9% (Nose et al. 1974), young eel, *Anguilla japonica*, 4.1% (Nose and Arai 1972), channel catfish, 3.5% (Wilson et al. 1980), Mozambique tilapia, 3.4% (Jauncey et al. 1983), lake trout, 3.66% (Hughes et al. 1983), Nile tilapia, 3.39% (Santiago and Lovell 1988), and catla, 3.70% (Ravi and Devaraj 1991). Higher requirement values have been reported for Japanese eel, 5.3% (Arai et al. 1972), milkfish, 5.11% (Borlongan and Coloso 1993) and rohu, 4.63% (Murthy and Varghese 1997).

Excess dietary isoleucine reduced the growth rate of chinook salmon fingerling when fed with sub-optimal levels of leucine (Chance et al. 1964). Further, elevated levels of isoleucine in the diet increased the leucine requirement. In the present study, the interrelationships of branched-chain amino acids were not studied.

Other than reduced growth, no gross deformity or nutritional deficiency was observed in mrigal fed leucine deficient diets.

Valine requirement

Feed efficiency, expressed as feed conversion ratio, reflects the weight gain. The dietary requirement of mrigal for valine (3.87% of protein), determined in this study, is higher than that reported for most other fish. Valine requirement, as a percentage of protein, has been reported as 2.96% for channel catfish (Wilson et al. 1980), 3.1% for rainbow trout (Ogino 1980), 3.2% for chinook salmon (Chance et al. 1964), 2.2% for Mozambique tilapia (Jauncey et al. 1983), and 2.9% for Nile tilapia (Santiago and Lovell 1988). However, the valine requirement estimated in this study is nearly equal to that reported for (rohu, (3.75%; Murthy 1994), for eel, (4.0%; Anonymous 1983), for catla (3.55%; Ravi and Devaraj 1991), and for milkfish, (3.55%; Borlongan and Coloso 1993).

Chance et al. (1964) opined that valine requirement might be affected by relatively high levels of isoleucine and leucine (3.20 and 3.68%, respectively) in the egg protein pattern control diet, since valine, isoleucine, and leucine are structurally similar. Thus, the valine requirement of salmon may be less than 1.3% of the dry diet when a more optimal combination of isoleucine and leucine was fed. Additional studies have to be conducted to investigate these apparent interactions in fishes.

The wide variations observed in the requirement levels for isoleucine, leucine, and valine among species, may be due to differences in the methodologies used such as the nature of dietary protein sources in the test diets, the reference protein whose amino acid pattern is being mimicked and the culture conditions. These variations may also be due to fish of different ages, sizes or strains (Cowey 1994).

In general, the growth rate of juvenile mrigal recorded in the present study was low when compared with its growth in natural or farm conditions. This trend is also true with other nutritional studies conducted earlier on Indian major carps including mrigal (Murthy and Devaraj 1991, Ravi and Devaraj 1991). A possible explanation could be that Indian major carps are sensitive to environmental conditions and do not realize maximum growth in a confined environment compared with other hardy species such as tilapia and common carp. Growth of mrigal decreased when the test EAA exceeded the requirement; this decrease is attributed to use of energy in nitrogenous excretion, because excess amino acids are eliminated and excreted in the form of ammonia (Walton 1985). It is concluded that in the absence of other information on quantitative requirements, the estimated dietary requirements of isoleucine, leucine and valine for the Indian major carp, *Cirrhinus cirrhosus*, based on dose -response curve in the present study, would be useful in formulating isoleucine, leucine and valine balanced diets in the culture of mrigal particularly under controlled conditions.

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