

Dietary branched-chain amino acid valine, isoleucine and leucine requirements of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton)

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Three 8-week growth experiments were conducted to quantify the requirements of the fingerling *Cirrhinus mrigala* for the dietary branched-chain amino acids valine (experiment 1), isoleucine (experiment 2) and leucine (experiment 3). Six isonitrogenous (400 g/kg) and isoenergetic (17.90 kJ/g) test diets were formulated with a gradation of 2.5 g/kg for each test amino acid, valine (7.5–20 g/kg), isoleucine (5.0–17.5 g/kg) and leucine (7.5–20 g/kg), and fed to randomly stocked fish in circular troughs. In experiment 1, the maximum weight gain (312%), best feed conversion ratio (FCR; 1.45) and best protein efficiency ratio (PER; 1.72) were obtained in fish fed 15.0 g dietary valine/kg. In experiment 2, the highest weight gain (317%), best FCR (1.47) and best PER (1.70) were recorded at 12.5 g dietary isoleucine/kg, and in experiment 3, the highest weight gain (308%), best FCR (1.46) and best PER (1.71) were noted at 15.0 g dietary leucine/kg. A quadratic regression analysis of weight gain, FCR and PER data showed an optimum requirement at 15.9, 15.0 and 14.8 g/kg for valine, 13.2, 12.3 and 12.1 g/kg for isoleucine and 15.6, 15.4 and 15.1 g/kg for leucine in dry diets. Low body moisture and higher protein were noted in fish fed diets containing 15.5, 12.5 and 15.0 g valine, isoleucine and leucine per kg, respectively. Body fat increased with increasing levels of the branched-chain amino acids. On the basis of a regression analysis of growth data, it is recommended that a diet for *C. mrigala* should contain valine at 15.2, isoleucine at 12.6 and leucine at 15.4 g/kg dry diet, corresponding to 38.0, 31.5 and 38.5 g/kg dietary protein, respectively.

Dietary branched-chain amino acids: Requirement: Body composition: *Cirrhinus mrigala*

Animals, including fish, are of major importance as human food commodities owing to their relatively high content of essential amino acids. Moreover, positive health claims are often attributed to fish consumption in view of the fact that fish contain high amounts of PUFA relative to the amount found in domestic animals. Hence, there is an increasing demand for fish, requiring their intensive culture. The success of intensive fish culture depends to a large extent on artificial feeding. Feed also constitutes the largest production cost for commercial aquaculture (Li & Wang, 2004). Protein is generally considered to be the most expensive component of fish feed. A supply of protein is needed throughout life for maintenance and growth. Ingested proteins are hydrolysed to release amino acids that may be used for the synthesis of tissue proteins. Proteins are composed of amino acids, some of which are dietary essentials; satisfying these essential amino-acid needs in fish and shellfish is therefore one of the highest annual costs in intensive aquaculture production (Twibell *et al.* 2003).

The branched-chain amino acid (BCAA) group comprise 14% of the total amino acids present in human skeletal muscle protein and differ from other indispensable amino acids in that they are oxidised primarily in the skeletal muscle by the BCAA dehydrogenase enzyme complex (Ferrando *et al.* 1995; Mager *et al.* 2003), whereas all of the other amino acids are broken down in the liver. BCAA (valine, isoleucine, leucine) are essential amino acids and

play very important roles in certain biochemical reactions and the growth of monogastric and preruminant terrestrial animals. They are involved in protein synthesis, synthesis of the amine neurotransmitters serotonin and the catecholamines dopamine and norepinephrine, which are derived from the aromatic amino acids tryptophan, phenylalanine, and tyrosine, the production of energy and the compartmentalisation of glutamate (Fernstrom, 2005).

Studies have suggested that BCAA, when ingested as a supplement, play an important role in protein synthesis and inhibiting protein degradation (Holecek *et al.* 2001; Shimomura *et al.* 2006). It has recently been reported that leucine activates mammalian target of rapamycin (mTOR), which results in increased protein synthesis (Norton & Layman, 2006) involving both unbranched amino acids and BCAA. The movement of plasma BCAA across the blood–brain barrier is competitively inhibited by aromatic amino acids. When plasma BCAA concentration rises, which occurs in response to food ingestion, the levels of brain aromatic amino acids decline. Such reductions in brain aromatic amino acids have several functional consequences, including a diminished synthesis and release of neurotransmitters such as serotonin and the catecholamines dopamine and norepinephrine (Fernstrom, 2005).

The BCAA share a common system for transport through cellular membranes and use the same enzyme for degradation (Harper, 1984); the first two steps of the catabolism of these

amino acids also use the same enzymes (Harris *et al.* 2005; Hutson *et al.* 2005). The first step in each case is a transamination using a single BCAA aminotransferase, with α -ketoglutarate as the amine acceptor. As a result, three different α -keto acids are produced and are oxidised using a common branched-chain α -keto acid dehydrogenase, yielding three different CoA derivatives. Valine produces propionyl CoA, isoleucine produces acetyl CoA, and propionyl and leucine produces acetyl CoA and acetoacetyl CoA.

The requirements for all ten indispensable amino acids have been established for only nine cultured fish species: chinook salmon (*Oncorhynchus tshawytscha*), channel catfish (*Ictalurus punctatus*) and Japanese eel (*Anguilla japonica*) (National Research Council, 1993), Coho salmon (*Oncorhynchus kisutch*; Arai & Ogata, 1993), chum salmon (*Oncorhynchus keta*; Akiyama & Arai, 1993), common carp (*Cyprinus carpio*; Nose, 1979), Nile tilapia (*Oreochromis niloticus*; Santiago & Lovell, 1988), Indian major carp, catla (*Catla catla*; Ravi & Devaraj, 1991) and milkfish (*Chanos chanos*; Borlongan & Coloso, 1993).

The dietary requirements of these BCAA have previously been worked out for different fish species such as Japanese eel (Arai *et al.* 1972), chinook salmon (Chance *et al.* 1964), channel catfish (Wilson *et al.* 1980), rainbow trout (*Oncorhynchus mykiss*; Ogino, 1980), lake trout (*Salvelinus namaycush*; Hughes *et al.* 1983), Mossambique tilapia (*Oreochromis mossambicus*; Jauncey *et al.* 1983), rohu (Murthy & Varghese, 1996, 1997a,b), white sturgeon (*Acipenser transmontanus*; Ng & Hung, 1995), red sea bream (*Pagrus major*; Forster & Ogata, 1998), European sea bass (*Dicentrarchus labrax*), gilt-head seabream (*Sparus aurata*) and turbot (*Psetta maxima*) (Kaushik, 1998), Atlantic salmon (*Salmo salar*; Rollin, 1999) and rainbow trout (*Oncorhynchus mykiss*; Yamamoto *et al.* 2004). In view of the existing literature, it is noted that no information is available on any of the BCAA requirement for Indian major carp (*Cirrhinus mrigala*) in order to develop BCAA-balanced diets for its mass production.

Cirrhinus mrigala, commonly known as mrigal, is a fast-growing fish species that is cultured in the Indian subcontinent with other indigenous and exotic species of carp. It grows to over 1 kg over a period of 1 year (Jhingran & Pullin, 1988). Although the dietary protein requirement of fingerling *C. mrigala* has been reported (Singh *et al.* 1987; Swamy *et al.* 1988; Mohanty *et al.* 1990; Das & Ray, 1991; De Silva & Gunasekera, 1991; Khan, 1991), no information is available on its BCAA requirements except for the data available on methionine (Ahmed *et al.* 2003), threonine (Ahmed *et al.* 2004), lysine (Ahmed & Khan, 2004a), arginine (Ahmed & Khan, 2004b), tryptophan (Ahmed & Khan, 2005a) and histidine (Ahmed & Khan, 2005b). The present study was therefore undertaken to estimate the dietary BCAA valine, isoleucine and leucine requirements of this fish species.

Materials and methods

Experimental diet

Six isonitrogenous (400 g crude protein/kg) and isoenergetic (17.90 kJ/g gross energy) diets (I–VI) with graded (by 2.5 g/kg) levels of valine (7.5, 10.0, 12.5, 15.0, 17.5, 20.0 g/kg) including 25.91 g isoleucine/kg and 25.94 g leucine/kg in

experiment 1, isoleucine (5.0, 7.5, 10.0, 12.5, 15.0, 17.5 g/kg) with valine 23.45 g/kg and leucine 28.44 g/kg in experiment 2, and leucine (7.5, 10.0, 12.5, 15.0, 17.5, 20.0 g/kg) including valine 24.17 g/kg and isoleucine 27.90 g/kg dry diet in experiment 3 were formulated using casein (fat-free), gelatin and L-crystalline amino acid premix (Tables 1, 2 and 3). The dietary protein level was fixed at 400 g/kg, reported as being optimum for the growth of *C. mrigala* (Khan, 1991). L-Crystalline amino acids were used to simulate the amino-acid profile of the diets to that of 400 g/kg whole-egg protein (Chance *et al.* 1964), excluding the test amino acids (valine, isoleucine, leucine).

The composition of vitamins (choline chloride 500 mg, thiamine hydrochloride 5 mg, riboflavin 20 mg, pyridoxine hydrochloride 5 mg, nicotinic acid 75 mg, calcium pantothenate 50 mg, inositol 200 mg, biotin 0.5 mg, folic acid 1.5 mg, ascorbic acid 100 mg, menadione (vitamin K) 4 mg, α -tocopheryl acetate (vitamin E) 40 mg, cyanocobalamin (vitamin B₁₂) 0.01 mg (g/100)) and minerals (AlCl₃·6H₂O 15 mg, ZnSO₄·7H₂O 300 mg, CuCl₂ 10 mg, MnSO₄·4-6H₂O 80 mg, KI 15 mg, CoCl₂·6H₂O 100 mg, plus USP #2 (United States Pharmacopeia) Ca(H₂PO₄)₂·H₂O 13.58 g, C₆H₁₀CaO₆ 32.7 g, C₆H₅O₇Fe·5H₂O 2.98 g, MgSO₄·7H₂O 13.20 g, KH₂PO₄ (dibasic) 23.98 g, NaH₂PO₄·2H₂O 8.72 g, NaCl 4.35 g (g/100 g)) were used according to Halver (2002).

A casein:gelatin ratio contributing a minimum quantity of the test amino acids and maximum quantities of other amino acids was maintained. The quantity of valine, isoleucine and leucine were increased at the expense of glycine, proline and serine (2:1:1 by weight) for valine, and glycine, proline, aspartic acid (2:1:1 by weight) for isoleucine and leucine, in order to make the diets isonitrogenous. The levels of test amino acids in the diets were fixed on the basis of information available on the other Indian major carp, *C. catla* (Ravi & Devaraj, 1991) and *Labeo rohita* (Murthy & Varghese, 1996, 1997a,b).

Pre-weighed quantities of L-crystalline amino acids and salt mix were thoroughly stirred in hot water (80°C) in a steel bowl attached to a Hobart electric mixer (Hobart Corporation, Troy, OH, USA). The pH of the resulting mixture was adjusted to neutral with 6M-NaOH solution (Nose *et al.* 1974). Gelatin was dissolved separately in a known volume of water with constant heating and stirring, and then transferred to the above mixture. The mixer bowl was removed from heating, and dextrin was added. Other dry ingredients including vitamins and oil premix, except carboxymethyl cellulose, were added to the lukewarm bowl (40°C) one by one with constant mixing. Carboxymethyl cellulose was added last, and the speed of the blender was gradually increased as the diet started to harden. The dough was passed through a pellet-maker fitted with a 2 mm die to obtain pellets, which were dried in an hot air oven at 40°C to reduce the moisture content to below 100 g/kg. The dry pellets were crumbled, sieved (0.20–0.25 mm) and stored at 4°C until use.

Experimental design and feeding trial

Fingerlings of *C. mrigala* were obtained from the hatchery of College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar, India. These were transported to the wet laboratory in O₂-filled polythene bags, given

Table 1. Composition (g/kg dry diet) of experimental diets used for estimating the dietary valine requirement of fingerling *Cirrhinus mrigala*

Ingredients (g/kg dry diet)	Experimental diets (g/kg)					
	I	II	III	IV	V	VI
Casein*	104.0	104.0	104.0	104.0	104.0	104.0
Gelatin†	26.0	26.0	26.0	26.0	26.0	26.0
Amino acid mix‡	321.1	321.7	322.3	322.9	323.5	324.0
Dextrin	284.1	283.2	282.2	281.4	280.5	279.7
Corn oil	50.0	50.0	50.0	50.0	50.0	50.0
Cod-liver oil	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mix (Halver, 2002)	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin mix (Halver, 2002)§	30.0	30.0	30.0	30.0	30.0	30.0
Carboxymethyl cellulose	100.0	100.0	100.0	100.0	100.0	100.0
α Cellulose	24.80	25.14	25.44	25.75	26.00	26.30
Total	1000	1000	1000	1000	1000	1000
Total valine	7.5	10.0	12.5	15.0	17.5	20.0
Isoleucine	25.91	25.91	25.91	25.91	25.91	25.91
Leucine	25.94	25.94	25.94	25.94	25.94	25.94
Calculated crude protein (g/kg)	400	400	400	400	400	400
Analysed crude protein (g/kg)	396.6	401.2	398.6	400.8	401.1	400.6
Gross energy (kJ/g dry diet)	17.90	17.90	17.90	17.90	17.90	17.90

Crude protein: *80%, †93%; Loba Chemie, India.

‡ Essential amino acids (g/kg): arginine 19.75, histidine 5.50, lysine 19.26, methionine 12.84, phenylalanine 19.54, threonine 13.16, tryptophan 5.17, valine variable. Non-essential amino acids (g/kg): cystine 9.19, tyrosine 12.67, alanine 17.29, aspartic acid 5.78, glutamic acid 3.77, proline, glycine and serine variable; Loba Chemie, India.

§ 10 g vitamin mix and 20 g α-cellulose.

|| Calculated on the basis of fuel values 23.10, 22.21, 24.27, 16.02 and 37.65 kJ/g for casein, gelatin, amino acids, dextrin and fat, respectively, as estimated on a Gallenkamp ballistic bomb calorimeter.

a prophylactic dip in KMnO₄ solution (2.1 × 10⁻⁶ M) to rule out any possible microbial infection and stocked in indoor circular fish tanks (1.22 m × 0.91 m × 0.91 m; water volume, 600 litres) lined with aluminium plastic (Plasticrafts Corporation, Mumbai, India) for 14 d. During this period, the fish were fed to apparent satiation a mixture of soyabean, mustard oil

cake, rice bran and wheat bran in the form of moist cake twice daily at 08.00 and 16.00 hours. Later, these fish were acclimated for 2 weeks on casein–gelatin-based (400 g crude protein/kg) H-440 diet (Halver, 2002).

Cirrhinus mrigala fingerling for determining the valine (4.29 (SD 0.55) cm, 0.62 (SD 0.03) g), isoleucine (4.22 (SD

Table 2. Composition (g/kg dry diet) of experimental diets used for estimating the dietary isoleucine requirement of fingerling *Cirrhinus mrigala*

Ingredients (g/kg dry diet)	Experimental diets (g/kg)					
	I	II	III	IV	V	VI
Casein*	80.0	80.0	80.0	80.0	80.0	80.0
Gelatin†	20.0	20.0	20.0	20.0	20.0	20.0
Amino acid mix‡	351.55	352.25	353.05	353.75	354.45	355.15
Dextrin	280.2	279.1	277.9	276.8	275.7	274.7
Corn oil	50.0	50.0	50.0	50.0	50.0	50.0
Cod-liver oil	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mix (Halver, 2002)	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin mix (Halver, 2002)§	30.0	30.0	30.0	30.0	30.0	30.0
Carboxymethyl cellulose	100.0	100.0	100.0	100.0	100.0	100.0
α Cellulose	28.25	28.65	29.05	29.45	29.83	30.15
Total	1000	1000	1000	1000	1000	1000
Total isoleucine	5.0	7.5	10.0	12.5	15.0	17.5
Valine	23.45	23.45	23.45	23.45	23.45	23.45
Leucine	28.44	28.44	28.44	28.44	28.44	28.44
Calculated crude protein (g/kg)	400	400	400	400	400	400
Analysed crude protein (g/kg)	397.4	401.4	397.6	400.5	401.5	394.6
Gross energy (kJ/g dry diet)	17.90	17.90	17.90	17.90	17.90	17.90

Crude protein: * 80%, † 93%; Loba Chemie, India.

‡ Essential amino acids (g/kg): arginine 21.09, histidine 6.16, isoleucine variable, lysine 21.45, methionine 13.68, phenylalanine 20.84, threonine 14.11, tryptophan 5.36. Non-essential amino acids (g/kg): cystine 9.28, tyrosine 13.90, alanine 18.60, aspartic acid variable, glutamic acid 9.42, proline variable, glycine variable, serine 3.06; Loba Chemie, India.

§ 10 g vitamin mix and 20 g α-cellulose.

|| Calculated on the basis of fuel values 23.10, 22.21, 24.27, 16.02 and 37.65 kJ/g for casein, gelatin, amino acids, dextrin and fat, respectively, as estimated on a Gallenkamp ballistic bomb calorimeter.

Table 3. Composition (g/kg dry diet) of experimental diets used for estimating the dietary leucine requirement of fingerling *Cirrhinus mrigala*

Ingredients (g/kg dry diet)	Experimental diets (g/kg)					
	I	II	III	IV	V	VI
Casein*	70.0	70.0	70.0	70.0	70.0	70.0
Gelatin†	17.5	17.5	17.5	17.5	17.5	17.5
Amino acid mix‡	363.22	363.62	364.27	365.42	366.07	366.82
Dextrin	285.3	284.7	283.0	281.9	281.0	279.8
Corn oil	50.0	50.0	50.0	50.0	50.0	50.0
Cod-liver oil	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mix (Halver, 2002)	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin mix (Halver, 2002)§	30.0	30.0	30.0	30.0	30.0	30.0
Carboxymethyl cellulose	100.0	100.0	100.0	100.0	100.0	100.0
Alpha cellulose	23.98	24.18	24.78	25.18	25.43	25.88
Total	1000	1000	1000	1000	1000	1000
Total leucine	7.5	10.0	12.5	15.0	17.5	20.0
Isoleucine	27.90	27.90	27.90	27.90	27.90	27.90
Valine	24.17	24.17	24.17	24.17	24.17	24.17
Calculated crude protein (g/kg)	400	400	400	400	400	400
Analysed crude protein (g/kg)	391.6	411.2	395.6	399.2	405.1	398.8
Gross energy (kJ/g dry diet)	17.90	17.90	17.90	17.90	17.90	17.90

Crude protein: * 80%, † 93%; Loba Chemie, India.

‡ Essential amino acids (g/kg): arginine 21.66, histidine 6.44, leucine variable, lysine 22.37, methionine 14.04, phenylalanine 21.39, threonine 14.51, tryptophan 5.44. Non-essential amino acids (g/kg): cystine 9.32, tyrosine 14.42, alanine 19.15, aspartic acid variable, glutamic acid 11.78, proline variable, glycine variable, serine 3.54; Loba Chemie, India.

§ 10 g vitamin mix and 20 g α-cellulose.

|| Calculated on the basis of fuel values 23.10, 22.21, 24.27, 16.02 and 37.65 kJ/g for casein, gelatin, amino acids, dextrin and fat, respectively, as estimated on a Gallenkamp ballistic bomb calorimeter.

0.43) cm, 0.61 (SD 0.05) g) and leucine (4.38 (SD 0.63) cm, 0.64 (SD 0.06) g) requirements were then randomly stocked in triplicate groups in 70-litre circular polyvinyl tanks (water volume 55 litres) fitted with a continuous water flow-through (1–1.5 l/min) system at the rate of twenty fish per tank for each dietary treatment. Fish were fed test diets in the form of dry pellets at a rate of 5% body weight/d. This amount was fixed according to the results obtained in a preliminary feeding trial previously described (Khan *et al.* 2004). The daily ration was divided into two equal halves and fed at 08.00 and 16.00 hours. No feed was offered to the fish on the day the measurements were taken. Initial and weekly weights were recorded on a top loading balance (Precisa 120A; 0.1 mg sensitivity; PAG Oerlikon AG, Zurich, Switzerland) and feed allowances adjusted accordingly.

The feeding trial lasted for 8 weeks. Faecal matter was siphoned before feeding. Uneaten feed, if any, was filtered on a screen soon after active feeding, dried and weighed to measure the amount of feed consumed. Water temperature, pH, free CO₂, dissolved O₂ and total alkalinity over the 8-weeks feeding trial were analysed following standard methods (American Public Health Association, 1992). The values for the above parameters were 26.5–29°C and 7.1–7.8, 9–13, 6.8–8.0 and 76–80 mg/l, respectively. Weight gain and body protein deposition were calculated using the following standard definitions:

$$\text{weight gain (\%)} = \frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}} \times 100$$

$$\text{protein deposition} = \frac{[(\text{BW}_f \times \text{BCP}_f) - (\text{BW}_i \times \text{BCP}_i)]}{[\text{TF} \times \text{CP}]} \times 100$$

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

Chemical analysis

The proximate compositions of casein, gelatin, experimental diets and initial and final body composition were analysed using standard Association of Official Analytical Chemists (1995) methods for DM (oven drying at 105 ± 1°C for 22 h), crude protein (N-Kjeldahl × 6.25), crude fat (solvent extraction with petroleum ether BP 40–60°C for 10–12 h) and ash (oven incineration at 650°C for 4–6 h). Gross energy content was determined on a Gallenkamp ballistic bomb calorimeter (Gallenkamp, Loughborough, UK). An amino-acid analysis of casein and gelatin, as detailed earlier (Ahmed *et al.* 2003), was carried out with the help of an ultrasphere ODS reverse-phase column fitted to a Beckman System Gold HPLC unit (Beckman Instruments, San Ramon, CA, USA).

Statistical analysis

The response of *C. mrigala* fingerling fed graded levels of dietary valine, isoleucine and leucine were measured by live weight gain per cent, feed conversion ratio (FCR), specific growth rate (SGR%) and protein efficiency ratio (PER), and by analysing whole-body composition. The response variables were subjected to one-way ANOVA (Snedecor & Cochran, 1967; Sokal & Rohlf, 1981). To determine significant differences ($P < 0.05$) among the treatments, Duncan's multiple range test (Duncan, 1955) was also employed. Second-degree polynomial regression ($Y = a + bX + cX^2$)

analysis (Zeitoun *et al.* 1976) was applied to growth parameters to predict a more accurate response to the dietary intake. The break-points obtained represented the optimum valine, isoleucine and leucine requirements of the fish.

Results

The weight gain per cent, SGR, FCR and PER of *C. mrigala* fed diets with graded levels of valine over an 8-week feeding trial differed significantly ($P < 0.05$); the values are presented in Table 4.

Among all the diets fed to *C. mrigala*, maximum live weight gain was evident in fish fed the diet containing 15.0 g dietary valine/kg (diet IV). The overall weight gain of the fish at this level of dietary valine was found to be 312 %, which was significantly ($P < 0.05$) higher than that of the other dietary groups. The significantly ($P < 0.05$) highest SGR (2.53) was recorded in fish fed 15.0 g dietary valine/kg compared with the other dietary levels. Fish fed different levels of dietary valine produced significant ($P < 0.05$) differences in FCR values, which ranged between 1.45 and 2.82. The best FCR (1.45) was noted in fish fed 15.0 g dietary valine/kg. The PER (1.72) of fish fed the diet with 15.0 g dietary valine/kg was significantly ($P < 0.05$) higher than those fed the other dietary valine levels.

The fish fed lower levels of dietary valine (<15.0 g/kg) showed a poor growth rate and efficiency of feed utilisation, whereas fish fed higher (>15.0 g/kg) amounts of dietary valine could not produce additional growth. When the live weight gain data and dietary valine levels were subjected to second-degree polynomial regression analysis, a break-point was evident at 15.9 g dietary valine/kg, corresponding to 39.75 g/kg dietary protein. The relationship was described by the equation:

$$Y = -258.5543X^2 + 822.8140X - 348.9644$$

(with $P < 0.05$, r 0.991).

The FCR and PER data were also subjected to second-degree polynomial regression analysis. The relationship of FCR (Y) to dietary valine level (X) was described by the equation:

$$Y = 2.3114X^2 - 6.9439X + 6.7831$$

(with $P < 0.05$, r 0.981).

Similarly, the relationship of PER (Y) to dietary levels of valine (X) was also described by the mathematical equation:

$$Y = -1.4086X^2 + 4.1627X - 1.5122$$

(with $P < 0.05$, r 0.933).

Based on the above polynomial equations, it is evident that the highest weight gain, best FCR and best PER were recorded at 15.9, 15.0 and 14.8 g/kg dry diet, respectively.

The proximate composition of the initial whole-body samples of *C. mrigala* and those fed on different dietary valine levels at the end of the 8-week feeding trial is presented in Table 5. The body moisture content of the fish fed diets with different levels of valine varied significantly ($P < 0.05$). A significantly ($P < 0.05$) lower moisture content was noted at 15.0 g dietary valine/kg compared with the other dietary groups. The whole-body protein content was found to be significantly ($P < 0.05$) higher in the fish receiving 15.0 g dietary valine/kg, followed by those fed 17.5 g dietary valine/kg. The whole-body fat content of fish fed varying levels of dietary valine gradually increased with increasing dietary valine concentration and was found to be significantly higher at 20 g dietary valine/kg. Whole-body fat content was, however, significantly ($P < 0.05$) lower with the 15.0 g valine/kg diet compared with those incorporating 12.5, 17.5 and 20.0 g dietary valine/kg.

With the exception of lower dietary valine concentrations (7.5 and 10.0 g/kg), the whole-body ash remained insignificantly ($P > 0.05$) different between the groups. However, fish fed 7.5 and 10.0 g dietary valine/kg recorded a significantly ($P < 0.05$) higher ash content. Body protein deposition was also found to be significantly higher in fish fed the diet with 15.0 g valine/kg, followed by those fed concentrations of 12.5 and 17.5 g dietary valine/kg (Table 4).

The live weight gain (weight gain per cent), SGR and PER of Indian major carp, *C. mrigala*, fed diets with various levels of isoleucine over the 8-week feeding trial are presented in Table 6. Graded concentrations of dietary isoleucine significantly ($P < 0.05$) affected the weight gain, FCR, SGR and PER of fingerling *C. mrigala*. The weight gain (317 %) was significantly ($P < 0.05$) higher among all the dietary groups of fish fed the diet containing 12.5 g dietary isoleucine/kg, followed by fish fed diets with 15.0 and 10.0 g dietary isoleucine/

Table 4. Growth, feed conversion ratio and survival of Indian major carp, *Cirrhinus mrigala*, fed diets containing graded levels of dietary valine (Mean values with their standard errors for three replicates)

	Dietary valine levels (g/kg)											
	7.5	SE	10.0	SE	12.5	SE	15.0	SE	17.5	SE	20.0	SE
Average initial weight (g)	0.619	0.008	0.621	0.007	0.627	0.009	0.630	0.005	0.623	0.006	0.627	0.008
Average final weight (g)	1.405	0.04	1.889	0.04	2.388	0.02	2.600	0.03	2.422	0.03	2.295	0.05
Live weight gain (%)	126.92	3.43 ^e	204.15	4.50 ^d	280.68	4.31 ^b	312.92	4.65 ^a	288.49	4.47 ^b	265.50	4.22 ^c
Specific growth rate*	1.46	0.02 ^e	1.98	0.02 ^d	2.39	0.01 ^b	2.53	0.02 ^a	2.42	0.02 ^b	2.31	0.02 ^c
Feed conversion ratio†	2.82	0.10 ^a	2.28	0.05 ^b	1.68	0.04 ^d	1.45	0.04 ^e	1.81	0.02 ^d	2.12	0.06 ^c
Protein efficiency ratio‡	0.89	0.03 ^e	1.10	0.02 ^d	1.48	0.03 ^b	1.72	0.05 ^a	1.38	0.02 ^c	1.18	0.03 ^d
Protein deposition (%)	13.91	0.43 ^f	19.02	0.47 ^e	27.71	0.62 ^b	34.48	0.74 ^a	26.11	0.48 ^c	22.87	0.61 ^d
Survival	100		100		100		100		100		100	

Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* Specific growth rate = $\frac{(\ln \text{ mean final weight}) - (\ln \text{ mean initial weight})}{\text{number of days}} \times 100$

† Feed conversion ratio = $\frac{\text{dry food fed (g)}}{\text{wet weight gain (g)}}$

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

Table 5. Body composition of Indian major carp, *Cirrhinus mrigala*, fed diets containing graded levels of dietary valine (Mean values with their standard errors for three replicates)

	Dietary valine levels (g/kg)													
	Initial	SE	7.5	SE	10.0	SE	12.5	SE	15.0	SE	17.5	SE	20.0	SE
Moisture (%)	79.21	0.18	77.77	0.32 ^a	76.28	0.20 ^b	75.04	0.27 ^c	73.76	0.33 ^d	74.83	0.16 ^c	74.96	0.28 ^c
Protein (%)	13.11	0.07	14.55	0.06 ^e	15.96	0.08 ^d	17.20	0.04 ^b	18.35	0.11 ^a	17.42	0.09 ^b	16.70	0.06 ^c
Fat (%)	3.06	0.13	3.38	0.08 ^e	3.65	0.07 ^d	4.27	0.05 ^b	4.05	0.04 ^c	4.42	0.06 ^b	4.65	0.04 ^a
Ash (%)	2.93	0.08	2.71	0.05 ^a	2.56	0.03 ^b	2.24	0.05 ^c	2.30	0.03 ^c	2.18	0.04 ^c	2.28	0.06 ^c

Mean values with unlike superscript letters were significantly different ($P < 0.05$).

kg. The lowest weight gain was recorded with fish fed the diet containing 5.0 and 7.5 g dietary isoleucine/kg.

The pattern for SGR was similar to that for weight gain. Significant ($P < 0.05$) differences in FCR were recorded in fish fed diets with various levels of isoleucine, ranging between 1.47 and 2.96, with the best FCR (1.47) in fish fed 12.5 g dietary isoleucine/kg. The PER was also found to be significantly affected by dietary isoleucine level. The PER (1.71) of fish fed diet containing 12.5 g dietary isoleucine/kg was found to be significantly ($P < 0.05$) higher than that of fish fed other dietary levels.

These results indicate that fish fed more than 12.5 g dietary isoleucine/kg could not produce additional growth, whereas fish receiving diets containing less than 12.5 g dietary isoleucine/kg showed reduced weight gain and poor efficiency of feed utilisation. To generate more precise information, however, the growth data were subjected to second-degree polynomial regression analysis to obtain the break-points. When weight gain data and dietary isoleucine levels were subjected to second-degree polynomial regression analysis, a break-point was evident at 13.2 g dietary isoleucine/kg, corresponding to 33.0 g/kg dietary protein. The relationship was described by the equation:

$$Y = -285.6229X^2 + 754.7669X - 193.6463$$

(with $P < 0.05$, $r = 0.991$).

The relationship of FCR (Y) to dietary isoleucine level (X) was described by a second-degree polynomial regression

analysis, the relationship being:

$$Y = 2.6743X^2 - 6.5874X + 5.5837$$

(with $P < 0.05$, $r = 0.996$).

In addition, the relationship between PER (Y) and dietary isoleucine level (X) was described by a second-degree polynomial regression analysis, the relationship being:

$$Y = -1.5457X^2 + 3.7407X - 0.6836$$

(with $P < 0.05$, $r = 0.966$).

Based on the above polynomial regression equations, the best FCR and PER occurred at isoleucine levels of 12.3 and 12.1 g/kg dry diet, respectively.

The effect of dietary isoleucine level on the whole-body composition of *C. mrigala* was found to be significantly affected by various isoleucine treatments (Table 7). The moisture content of fish receiving different levels of dietary isoleucine varied significantly ($P < 0.05$) and was recorded to be lowest at 12.5 g dietary isoleucine/kg. Whole-body protein content was found to be significantly ($P < 0.05$) higher at 12.5 g dietary isoleucine/kg, followed by diets with 10.0 and 15.0 g dietary isoleucine/kg. However, the whole-body protein values for the fish fed diets containing 10.0 and 15.0 g dietary isoleucine/kg were not significantly different ($P > 0.05$). Whole-body fat increased significantly ($P < 0.05$) with increasing dietary isoleucine concentration up to 15.0 g/kg. Further increases in the concentration of dietary isoleucine did not lead to any difference in whole-body fat values.

Table 6. Growth, feed conversion ratio and survival of Indian major carp, *Cirrhinus mrigala*, fed diets containing graded levels of dietary isoleucine (Mean values with their standard errors for three replicates)

	Dietary isoleucine levels (g/kg)											
	5.0	SE	7.5	SE	10.0	SE	12.5	SE	15.0	SE	17.5	SE
Average initial weight (g)	0.615	0.009	0.619	0.006	0.612	0.006	0.613	0.007	0.614	0.008	0.615	0.009
Average final weight (g)	1.340	0.04	1.860	0.02	2.298	0.05	2.561	0.04	2.373	0.02	2.179	0.02
Live weight gain (%)	117.61	3.49 ^e	200.74	4.51 ^d	275.30	4.84 ^b	317.49	4.70 ^a	286.23	4.43 ^b	254.08	4.09 ^c
Specific growth rate*	1.39	0.03 ^e	1.96	0.02 ^d	2.36	0.02 ^b	2.53	0.01 ^a	2.41	0.02 ^b	2.26	0.02 ^c
Feed conversion ratio†	2.96	0.09 ^a	2.15	0.07 ^b	1.68	0.05 ^c	1.47	0.04 ^d	1.79	0.05 ^c	2.22	0.04 ^b
Protein efficiency ratio‡	0.85	0.02 ^d	1.16	0.03 ^c	1.49	0.04 ^b	1.70	0.04 ^a	1.39	0.04 ^b	1.13	0.02 ^c
Protein deposition (%)	13.26	0.60 ^d	19.60	0.80 ^c	27.68	0.82 ^b	33.78	0.67 ^a	26.26	0.83 ^b	19.72	0.41 ^c
Survival	96	1.0	98	1.45	100		100		100		100	

Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* Specific growth rate = $\frac{(\ln \text{mean final weight}) - (\ln \text{mean initial weight})}{\text{number of days}} \times 100$.

† Feed conversion ratio = $\frac{\text{dry food fed (g)}}{\text{wet weight gain (g)}}$.

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

Table 7. Body composition of Indian major carp, *Cirrhinus mrigala*, fed diets containing graded levels of dietary isoleucine (Mean values with their standard errors for three replicates)

	Dietary isoleucine levels (g/kg)													
	Initial	SE	5.0	SE	7.5	SE	10.0	SE	12.5	SE	15.0	SE	17.5	SE
Moisture (%)	79.05	0.25	77.48	0.23 ^a	76.11	0.27 ^b	74.82	0.17 ^d	73.26	0.22 ^e	74.28	0.15 ^d	75.40	0.21 ^c
Protein (%)	13.18	0.09	14.54	0.05 ^e	15.61	0.10 ^d	17.18	0.04 ^b	18.27	0.08 ^a	17.36	0.05 ^b	16.29	0.04 ^c
Fat (%)	3.20	0.16	3.50	0.07 ^e	3.81	0.06 ^d	4.25	0.05 ^c	4.45	0.04 ^b	4.58	0.05 ^{ab}	4.66	0.06 ^a
Ash (%)	3.03	0.10	2.82	0.03 ^a	2.63	0.04 ^b	2.30	0.03 ^c	2.28	0.05 ^c	2.34	0.06 ^c	2.39	0.03 ^c

Mean values with unlike superscript letters were significantly different ($P < 0.05$).

Fish fed a diet containing 5.0 and 7.5 g dietary isoleucine/kg showed significantly ($P < 0.05$) higher body ash content compared with the those receiving other dietary isoleucine levels. However, the ash contents of the fish receiving other dietary isoleucine levels remained insignificantly ($P > 0.05$) different from each other. The body protein deposition of fish fed diets with different levels of isoleucine also varied significantly and showed a trend similar to that of whole-body protein content (Table 6).

The live weight gain, FCR and PER response of fingerling *C. mrigala* fed with different levels of dietary leucine for the 8-week growth trial are shown in Table 8. Within the test range, weight gain increased significantly ($P < 0.05$) up to 15.0 g dietary leucine/kg, beyond which it decreased. The overall weight gain at this level of dietary leucine was 308%. Similarly, the highest SGR per cent (2.51) and best FCR (1.47) were noted in fish fed a diet containing 15.0 g dietary leucine/kg. The PER was also found to be significantly ($P < 0.05$) affected by the dietary leucine level and was recorded to be significantly higher (1.71) in fish fed a diet containing 15.0 g dietary leucine/kg compared with those fed other dietary leucine levels.

In light of above results, it is noted that the fish fed more than 15.0 g dietary leucine/kg could not produce additional growth, whereas fish fed lower concentrations of dietary leucine recorded reduced weight gain and efficiency of feed utilisation. However, on subjecting the live weight gain data and dietary leucine levels to second-degree polynomial regression analysis, a break-point was evident at 15.6 g dietary

leucine/kg, corresponding to 39 g/kg dietary protein. The relationship was described by the equation:

$$Y = -274.8771X^2 + 862.9407X - 377.6361$$

(with $P < 0.05$, r 0.997).

The relationship between FCR (Y) and dietary levels of leucine (X) was described by a second-degree polynomial regression analysis, the relationship being:

$$Y = 2.1400X^2 - 6.5959X + 6.6099$$

(with $P < 0.05$, r 0.995).

Similarly, the relationship of PER (Y) to dietary levels of leucine (X) was described by a second-degree polynomial regression analysis, the relationship being:

$$Y = -1.2771X^2 + 3.8767X - 1.3581$$

(with $P < 0.05$, r 0.964).

Based on the above polynomial equations, the highest weight gain and best FCR and PER occurred at leucine levels of 15.6, 15.4 and 15.1 g/kg dry diet, respectively.

Significant ($P < 0.05$) differences were observed in the whole-body composition of fish fed different levels of dietary leucine (Table 9). The moisture content of the fish decreased significantly ($P < 0.05$) with increasing leucine levels up to 15.0 g/kg (diet IV), after which an increase in moisture was noted. The whole-body protein content was found to be significantly ($P < 0.05$) higher in fish receiving the diet containing 15.0 g leucine/kg, followed by those receiving diets containing

Table 8. Growth, feed conversion ratio and survival of Indian major carp, *Cirrhinus mrigala*, fed diets containing graded levels of dietary leucine (Mean values with their standard errors for three replicates)

	Dietary leucine levels (g/kg)											
	7.5	SE	10.0	SE	12.5	SE	15.0	SE	17.5	SE	20.0	SE
Average initial weight (g)	0.646	0.009	0.642	0.010	0.649	0.007	0.631	0.006	0.636	0.008	0.631	0.007
Average final weight (g)	1.405	0.02	1.974	0.03	2.371	0.02	2.576	0.05	2.470	0.04	2.196	0.03
Live weight gain (%)	117.56	4.04 ^f	207.52	4.26 ^e	265.40	4.32 ^c	308.09	4.80 ^a	288.14	4.61 ^b	247.95	4.33 ^d
Specific growth rate*	1.39	0.03 ^f	2.01	0.02 ^e	2.31	0.02 ^c	2.51	0.02 ^a	2.42	0.02 ^b	2.23	0.02 ^d
Feed conversion ratio†	2.87	0.06 ^a	2.14	0.04 ^b	1.75	0.05 ^d	1.46	0.03 ^e	1.68	0.04 ^d	1.96	0.05 ^c
Protein efficiency ratio‡	0.88	0.02 ^a	1.17	0.02 ^d	1.43	0.04 ^b	1.71	0.04 ^a	1.49	0.04 ^b	1.27	0.03 ^c
Protein deposition (%)	13.56	0.38 ^f	19.71	0.35 ^e	26.43	1.00 ^c	33.86	0.66 ^a	28.19	0.57 ^b	22.29	0.54 ^c
Survival	98	1.80	100		100		100		100		100	

Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* Specific growth rate = $\frac{(\ln \text{ mean final weight}) - (\ln \text{ mean initial weight})}{\text{number of days}} \times 100$.

† Feed conversion ratio = $\frac{\text{dry food fed (g)}}{\text{wet weight gain (g)}}$.

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

Table 9. Body composition of Indian major carp, *Cirrhinus mrigala*, fed diets containing graded levels of dietary leucine (Mean values with their standard errors for three replicates)

	Dietary leucine levels (g/kg)													
	Initial	SE	7.5	SE	10.0	SE	12.5	SE	15.0	SE	17.5	SE	20.0	SE
Moisture (%)	78.93	0.23	77.65	0.21 ^a	76.30	0.18 ^b	74.95	0.27 ^d	73.51	0.11 ^f	74.40	0.19 ^e	75.42	0.15 ^c
Protein (%)	12.86	0.13	14.27	0.07 ^e	15.58	0.09 ^d	16.97	0.06 ^c	18.12	0.07 ^a	17.38	0.05 ^b	16.17	0.04 ^c
Fat (%)	3.43	0.07	3.61	0.05 ^e	3.92	0.07 ^d	4.20	0.05 ^c	4.38	0.03 ^b	4.56	0.02 ^a	4.62	0.03 ^a
Ash (%)	2.84	0.06	2.68	0.04 ^a	2.32	0.03 ^b	2.36	0.05 ^c	2.23	0.03 ^c	2.28	0.04 ^c	2.18	0.03 ^c

Mean values with unlike superscript letters were significantly different ($P < 0.05$).

17.5 and 12.5 g leucine/kg. Whole-body fat increased gradually with the increase of dietary leucine and was significantly ($P < 0.05$) higher at 17.5 and 20 g leucine/kg compared with other dietary groups. Fish receiving different leucine levels, with the exception of those fed the 7.5 g leucine/kg diet, showed no significant differences in their body ash content. However, the inclusion of 7.5 g dietary leucine/kg produced significantly ($P < 0.05$) high ash values. Body protein deposition was also found to be significantly higher with 15.0 g dietary leucine/kg (Table 8), followed by those receiving diets containing 17.5 and 12.5 g leucine/kg.

Discussion

The BCAA valine plays a very important role in protein synthesis and the optimum growth of fish. It is required for the repair and growth of tissue and for maintaining N balance in the fish body. Valine, along with the other BCAA isoleucine and leucine, has been used as a supplement for body growth and has also been used in the body to produce certain biochemical compounds that help energy production and reduce twitching and tremors in animals. Owing to its important role and the fact that it is an indispensable amino acid, the inclusion of an optimum amount of valine is a prerequisite for formulating a diet balanced in amino acids for the mass production of *C. mrigala*.

One of the common methods of determining the requirement of a species for an essential amino acid involves feeding a diet that is nutritionally balanced except for the essential amino acid in question (De Silva & Anderson, 1995). Maximum live weight gain and best FCR and PER at 15.0 g dietary valine/kg were indicated to be the optimum requirement for *C. mrigala*. Quadratic regression analysis of live weight gain, FCR and PER data showed, however, the valine requirement of *C. mrigala* to be at 15.9, 15.0 and 14.8 g/kg diet, respectively.

The whole-body composition of *C. mrigala* fed various levels of dietary valine was also affected. Significantly low moisture and higher whole-body protein content was recorded at 15.0 g dietary valine/kg. Except for the fish fed the 15.0 g valine/kg diet, the whole-body fat content was found to increase significantly in fish fed all the other diets. Body protein deposition was also found to be significantly higher at this level of dietary valine. In the light of quadratic regression and whole-body composition analysis, it is recommended that 15.2 g dietary valine/kg, corresponding to 38.0 g/kg dietary protein, estimated in the present study is taken as the optimum requirement of *C. mrigala*, which is comparable to the

requirement reported for other fish species (Table 10). The valine requirement varies between 25.0 and 40.0 g/kg dietary protein among species and within a particular species (National Research Council, 1993; Wilson, 2002). The dietary valine requirement of *C. mrigala* estimated in the present study is well within that range.

Isoleucine is a neutral, genetically coded BCAA considered indispensable for the growth of all fish species. The principal products from isoleucine catabolism terminate with production of acetyl CoA and propionyl CoA; thus, isoleucine is both glucogenic and ketogenic. It is involved in many metabolic pathways, for example protein synthesis (Hutson *et al.* 2005) and energy production.

The importance of dietary isoleucine for the growth of *C. mrigala* demonstrated in the present study indicated that 12.5 g dietary isoleucine/kg is optimal for the maximum growth of *C. mrigala*. This level of dietary isoleucine also provided the best FCR (1.47) and highest SGR (2.53) and PER (1.70). Quadratic regression analysis of weight gain data indicated the requirement to be at 13.2 g/kg for dietary isoleucine, corresponding to 33.0 g/kg dietary protein. However, a quadratic regression analysis of FCR and PER data indicated an optimum dietary isoleucine requirement for *C. mrigala* at 12.3 and 12.1 g/kg dry diet, respectively.

The whole-body composition of the fish fed different levels of dietary isoleucine showed some remarkable changes. Body moisture content remained low with the 12.5 g isoleucine/kg diet compared with the other dietary groups, whereas whole-body protein content recorded maximum values at this dietary isoleucine level. The whole-body fat content gradually increased with increasing levels of dietary isoleucine and was significantly higher in fish fed diets with excess isoleucine concentrations. Body ash remained constant among all the dietary groups, with the exception of those fed lower doses (5.0 and 7.5 g/kg) of isoleucine, for which a significantly higher ash content was recorded. Body protein deposition was also noted to be significantly higher at 12.5 g dietary isoleucine/kg.

Based on the present results, the optimum dietary isoleucine requirement for *C. mrigala* is suggested to be 12.6 g/kg dry diet, corresponding to 31.5 g/kg dietary protein. The isoleucine requirement of different fish species varies between 20 and 40 g/kg dietary protein (De Silva & Anderson, 1995; Wilson, 2002), and the isoleucine requirement of *C. mrigala* calculated in the present experiment is well within this range (Table 10).

The highest weight gain, best FCR and highest PER were recorded in fish fed a diet containing 15.0 g dietary leucine/kg and was significantly higher than those of other dietary groups. However, when live weight gain, FCR and PER data

Table 10. Dietary branched-chain amino acid (valine, isoleucine and leucine) requirement of different fish species

Species	Valine		Isoleucine		Leucine		Reference
	(g/kg dry diet)	(g/kg protein)	(g/kg dry diet)	(g/kg protein)	(g/kg dry diet)	(g/kg protein)	
Mrigal	15.2	38.0	12.6	31.5	15.4	38.5	Present study
Rohu	15.0	37.5*	12.0	30.0†	18.4	46.0‡	Murthy & Varghese (1997a)
Common carp	14.0	36.0	9.0	23.4	13.0	33.8	Nose (1979)
Chinook salmon	13.0	32.5	9.0	22.0	16.0	39.0	Chance <i>et al.</i> (1964)
Japanese eel	15.0	40.0	15.0	40.0	20.0	53.0	National Research Council (1993)
Milkfish	14.2	35.5	16.0	40.0	20.4	51.1	Borlongan & Coloso (1993)
Channel catfish	7.1	29.6	6.2	25.8	8.4	35.0	Wilson <i>et al.</i> (1980)
Coho salmon	9.0	22.0	5.0	12.0	13.6	34.0	Arai & Ogata (1993)
Chum salmon	12.0	30.0	10.0	24.0	15.0	38.0	National Research Council (1993)
Lake trout	7.8	22.9	7.2	26.6	13.0	48.1	Hughes <i>et al.</i> (1983)
Nile tilapia	7.8	28.0	8.7	31.1	9.5	33.9	Santiago & Lovell (1988)
Catla	14.2	35.5	9.4	24.0	14.8	37.0	Ravi & Devaraj (1991)
Mossambique tilapia	8.8	22.0	8.1	20.1	13.6	34.0	Jauncey <i>et al.</i> (1983)
Red sea bream		25.0		22.0		42.0	Forster & Ogata (1998)
Japanese flounder		25.0		22.0		39.0	Forster & Ogata (1998)
Rainbow trout		31.0		24.0		44.0	Ogino (1980)
White sturgeon		33.0		30.0		43.0	Ng & Hung (1995)
European sea bass		29.0		26.0		43.0	Kaushik (1998)
Gilthead sea bream		30.0		26.0		26.0	Kaushik (1998)
Turbot		29.0		26.0		46.0	Kaushik (1998)
Atlantic salmon		39.0		32.0		52.0	Rollin (1999)

* Murthy & Varghese (1997a).

† Murthy & Varghese (1996)

‡ Murthy & Varghese (1997b)

were subjected to quadratic regression analysis, the optimum dietary leucine requirement was noted to be at 15.6, 15.4 and 15.1 g/kg diet, respectively. Whole-body composition was also significantly affected in fish fed different levels of dietary leucine. Minimum moisture and maximum protein contents were evident in fish fed 15.0 g dietary leucine/kg. A similar trend was also recorded in body protein deposition. Whole-body fat increased with the increase in dietary leucine and was significantly higher at 17.5 and 20.0 g dietary leucine/kg compared with the other dietary group. Based on the above results, the optimum dietary leucine requirement of *C. mrigala* is recommended to be 15.4 g/kg dry diet, corresponding to 38.5 g/kg dietary protein. The leucine requirement varies from 33.0 to 39.0 g/kg protein within and among species, and the recommended requirement in the present study falls within this range (Table 10).

The large variation observed in levels of valine, isoleucine and leucine among different fish species and within the same species may be due to differences in the methodologies used to determine the requirement, such as the nature of the intact protein sources in the test diet, for example casein, gelatin, zein, gluten, fish meal, soyabean meal and crystalline amino acids in various combinations. Apart from above factors, another very important factor that could affect the requirements is the composition of the test diets in terms of the presence or absence of other BCAA.

Deficiencies in valine, isoleucine and leucine (BCAA) cause loss of weight and poor feed conversion (Borlongan & Coloso, 1993; Ravi & Devaraj, 1991; Murthy & Varghese, 1996, 1997a,b). Except for loss of appetite and poor feed efficiency, which resulted in a depressed growth rate in *C. mrigala* fed a diet containing less than the optimum amount of these amino acids, no diet-related mortality or morphological signs of BCAA deficiency were observed. In the present study,

however, excess amounts of these amino acids caused depressive effects such as reduction in growth and efficiency of feed utilisation. A similar observation has also been made for the other Indian major carp, catla (Ravi & Devaraj, 1991) and rohu (Murthy & Varghese, 1996, 1997a,b).

The reduction in the growth of *C. mrigala* fed excess doses of valine, isoleucine and leucine may be due to the result of antagonism between BCAA. The existence of an interaction between BCAA, especially the adverse effects of excess leucine, has been noted primarily in diets that are considered to be marginal or deficient in isoleucine and valine for human subjects (Hambraeus *et al.* 1976), rats (Harper *et al.* 1983; Block, 1989), poultry (D'Mello & Lewis, 1970; Barbour & Latshaw, 1992) and turkeys (Jackson & Potter, 1984).

Antagonism between BCAA in mammals generally arises from an excess of leucine over isoleucine and valine (National Research Council, 1993). However, experimental results on antagonisms among BCAA in fish are not clear-cut and are inconsistent between species. Harper *et al.* (1954) found that growth depression induced by feeding excess leucine could be alleviated by dietary supplements of isoleucine and hypothesised that an antagonism existed between these two structurally similar amino acids. Chance *et al.* (1964) reported that the requirement of isoleucine increased with increasing dietary leucine in chinook salmon. He also mentioned in the same study that the valine requirement might be affected by relatively higher levels of isoleucine and leucine. Nose (1979) noted that fish growth was suboptimal when leucine was presented in excess in the basal diet.

Hughes *et al.* (1983) reported that antagonistic effects were clearly observed in the case of excess leucine in the diet in lake trout, and valine supplementation of these excess-leucine diets relieved the depression of growth and feed utilisation. Yamamoto *et al.* (2004) concluded that there were antagonist

effects owing to excess leucine derived from intact proteins in rainbow trout. Robinson *et al.* (1984) reported an interrelationship between the BCAA in channel catfish. He also described how the antagonism between BCAA did not occur when the levels of the three BCAA in the diet met their requirements.

In the present study, except for the BCAA under study, the amounts of other BCAA were held constant in all the diets as per the reference amino-acid profile (400 g/kg whole egg protein; Chance *et al.* 1964) in each feeding trial, and their interrelationships with each other were not studied.

The other reason for reduced weight gain and poor feed efficiency in *C. mrigala* during the present study may be the disproportionate amounts of isoleucine, leucine and valine in the amino-acid test diets of each feeding trial as a disproportionate amount of one amino acid affects the utilisation of other amino acids (Walton, 1985; Coloso *et al.* 1999). Hughes *et al.* (1984) reported that excess dietary valine does not increase the dietary requirement of lake trout for either leucine or isoleucine. For this reason, the effects of dietary excess valine on lake trout are interpreted as amino-acid toxicity rather than an antagonism. Choo *et al.* (1991) also reported that the growth depression and abnormal morphology of rainbow trout fed an excess-leucine diet did not result from antagonist effects of BCAA but from toxicity of the excess leucine itself. Reduced growth and poor FCR in *C. mrigala* may also be due to stress in the fish body caused by feeding an excess amount of one amino acid, leading to extra energy expenditure directed towards the deamination and excretion of this amino acid.

On the basis of data generated during the present study, we conclude that diets formulated for *C. mrigala* should contain the BCAA valine, isoleucine and leucine at 15.2, 12.6 and 15.4 g/kg dry diet, corresponding to 38.0, 31.5 and 38.5 g/kg dietary protein, respectively, for optimum growth of this fish.

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References

- Ahmed I & Khan MA (2004a) Dietary lysine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquaculture* **235**, 499–511.
- Ahmed I & Khan MA (2004b) Dietary arginine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult Nutr* **10**, 25–30.
- Ahmed I & Khan MA (2005a) Dietary tryptophan requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult Res* **36**, 687–695.
- Ahmed I & Khan MA (2005b) Dietary histidine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult Nutr* **11**, 359–366.
- Ahmed I, Khan MA & Jafri AK (2003) Dietary methionine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult Int* **11**, 449–462.
- Ahmed I, Khan MA & Jafri AK (2004) Dietary threonine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult Res* **35**, 162–170.
- Akiyama T & Arai S (1993) Amino acid requirements of chum salmon fry and supplementation of amino acids to diet. In *Proceedings of the Twentieth U.S.–Japan Symposium on Aquaculture Nutrition*, pp. 35–48 [MR Collie and JP McVey, editors]. Newport, OR: UJNR Department of Commerce.
- American Public Health Association (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th ed. Washington, DC: American Public Health Association.
- Arai S, Nose T & Hashimoto Y (1972) Amino acid essential for the growth of eels *Anguilla anguilla* and *Anguilla japonica*. *Bull Jpn Soc Sci Fish* **38**, 753–759.
- Arai S & Ogata H (1993) Quantitative amino acid requirements of fingerling coho salmon. In *Proceedings of the Twentieth US–Japan Symposium on Aquaculture Nutrition*, pp. 19–28 [MR Collie and JP McVey, editors]. Newport, OR: UJNR Department of Commerce.
- Association of Official Analytical Chemists (1995) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th ed., [P Cunniff, editor]. Arlington, VA: Association of Official Analytical Chemists.
- Barbour G & Latshaw JD (1992) Isoleucine requirement of broiler chicks as affected by the concentrations of leucine and valine in practical diets. *Br Poult Sci* **33**, 561–568.
- Block KP (1989) Interactions among leucine, isoleucine and valine with special reference to the branched-chain amino acid antagonism. In *Absorption and Utilization of Amino acids*, vol.1, pp.229–244 [M Friedman, editor]. Boca Raton, FL: CRC Press.
- Borlongan IG & Coloso RM (1993) Requirements of juvenile milkfish (*Chanos chanos* Forsskal) for essential amino acids. *J Nutr* **123**, 125–132.
- Chance RE, Mertz ET & Halver JE (1964) Nutrition of salmonid fishes. XII. Isoleucine, leucine, valine and phenylalanine requirements of chinook salmon and interrelations between isoleucine and leucine for growth. *J Nutr* **83**, 177–185.
- Choo P, Smith TK, Cho CY & Ferguson HW (1991) Dietary excesses of leucine influence on growth and body composition of rainbow trout. *J Nutr* **121**, 1932–1939.
- Coloso RM, Murillo-Gurrea DP, Borlongan IG & Catacutan MR (1999) Sulphur amino acid requirement of juvenile Asian Sea bass *Lates calcarifer*. *J Appl Ichthyol* **15**, 54–58.
- Das I & Ray AK (1991) Growth response and feed conversion efficiency in *Cirrhinus mrigala* (Ham.) fingerlings at varying protein levels. *J Aqua Trop* **6**, 179–185.
- De Silva SS & Anderson TA (1995) *Fish Nutrition in Aquaculture*. London: Chapman & Hall.
- De Silva SS & Gunasekera RM (1991) An evaluation of the growth of Indian and Chinese major carps in relation to the dietary protein content. *Aquaculture* **92**, 237–241.
- D’Mello JPF & Lewis D (1970) Amino acid interactions in chick nutrition. 2. Interrelationships between leucine, isoleucine and valine. *Br Poult Sci* **11**, 313–323.
- Duncan DB (1955) Multiple range and multiple ‘F’ tests. *Biometrics* **11**, 1–42.
- Fernstrom JD (2005) 4th amino acid assessment workshop: Branched-chain amino acids and brain function. *J Nutr* **135**, 1539S–1546S.
- Ferrando AA, Williams BD, Stuart CA, Lane HE & Wolf RR (1995) Oral branched chain amino acids decrease whole-body proteolysis. *J Parenter Enteral Nutr* **19**, 47–54.
- Forster I & Ogata HY (1998) Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea beam *Pagrus major*. *Aquaculture* **161**, 131–142.
- Halver JE (2002) The vitamins. In *Fish Nutrition*, 3rd ed. pp. 61–141 [JE Halver and RW Hardy, editors]. San Diego, CA: Academic Press.
- Hambraeus L, Bilmazes C, Dippel C, Scrimshaw N & Young VR (1976) Regulatory role of dietary leucine on plasma branched-chain amino acid levels in young men. *J Nutr* **106**, 320–340.

- Harper AE (1984) Interrelationships among the branched chain amino acids. In *Branched Chain Amino and Keto Acids in Health and Disease*, pp. 81–89 [SA Adibi, W Fekl, U Langenbeck and P Schauder, editors]. Basel: Karger.
- Harper AE, Benton DA, Winje ME & Elvehjem CA (1954) Leucine-isoleucine antagonism in the rat. *Arch Biochem Biophys* **51**, 523–524.
- Harper AE, Block KP & Cree TC (1983) Branched-chain amino acids: Nutritional and metabolic interrelationship. In *Proceedings of the IVth Symposium on Protein Metabolism and Nutrition*, vol.1, pp. 159–181 [R Pion, M Arnal and D Bonin, editors]. European Association of Animal Production Publication no. 31. Paris: INRA.
- Harris RA, Joshi M, Jeoung NH & Obayashi M (2005) Overview of the molecular and biochemical basis of branched-chain amino acid catabolism. 4th amino acid assessment workshop. *J Nutr* **135**(6), 1527S–1530S.
- Holecek M, Sprongl L & Tilser I (2001) Metabolism of branched-chain amino acids in starved rats: The role of hepatic tissue. *Physiol Res* **50**, 25–33.
- Hughes SG, Rumsey GL & Nesheim MC (1983) Dietary requirements for essential branched-chain amino acids by lake trout. *Trans Am Fish Soc* **112**, 812–817.
- Hughes SG, Rumsey GL & Nesheim MC (1984) Effects of dietary excesses of branched-chain amino acids on the metabolism and tissue composition of lake trout (*Salvelinus namaycush*). *Comp Biochem Physiol* **78A**, 413–418.
- Hutson SM, Sweatt AJ & LaNouse KF (2005) Branched-chain amino acid metabolism: implications for establishing safe intake. 4th amino acid assessment workshop. *J Nutr* **135**(6), 1557S–1564S.
- Jackson S & Potter LM (1984) Influence of basic and branched-chain amino acid interactions on the lysine and valine requirements of young turkeys. *Poult Sci* **63**, 2391–2398.
- Jauncey K, Tacon AGJ & Jackson AJ (1983) The quantitative essential amino acid requirements of *Oreochromis (Sarotherodon) mossambicus*. In *International Symposium on Tilapia in Aquaculture, Ist Proceedings*, pp. 328–337 [L Fishelson and Z Yaron, editors]. Nazareth, Israel: Tel Aviv University.
- Jhingran VG & Pullin RSV (1988) *A Hatchery Manual for Common, Chinese and Indian Major carps*. ICLARM Studies and Reviews 11. Philippines: Manila: ICLARM.
- Kaushik SJ (1998) Whole body amino acid composition of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. *Aquat Liv Res* **11**, 355–358.
- Khan MA (1991) Protein and amino acid requirements and efficacy of dietary formulations in some cultivable finfish species. PhD Thesis, Aligarh Muslim University, India.
- Khan MA, Ahmed I & Abidi SF (2004) Effect of ration size on growth, conversion efficiency and body composition of fingerling mrigal, *Cirrhinus mrigala* (Hamilton). *Aquacult Nutr* **10**, 47–53.
- Li P & Wang X (2004) Nutritional value of fisheries by-catch and by-product meals in the diet of red drum (*Sciaenops ocellatus*). *Aquaculture* **236**, 485–496.
- Mager DR, Wykes LJ, Ball RO & Pencharz PB (2003) Branched-chain amino acid requirements in school-aged children determined by indicator amino acid oxidation (IAAO). *J Nutr* **133**, 3540–3545.
- Mohanty SN, Swamy DN & Tripathi SD (1990) Protein utilization in Indian major carp fry, *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) fed four protein diets. *J Aqua Trop* **5**, 173–179.
- Murthy HS & Varghese TJ (1996) Quantitative dietary isoleucine requirement for growth and survival of Indian major carp, *Labeo rohita*, (Hamilton) fry. *Indian J Exp Biol* **34**, 1141–1143.
- Murthy HS & Varghese TJ (1997a) Quantitative dietary requirements of the Indian major carp *Labeo rohita* for the essential amino acid valine. *Indian J Anim Sci* **67**, 1028–1030.
- Murthy HS & Varghese TJ (1997b) Dietary requirement of the Indian major carp, *Labeo rohita*, for the essential amino acid leucine. *Mysore J Agricult Sci* **31**, 348–351.
- National Research Council (1993) *Nutrient Requirement of Fish*. Washington, DC: National Academy Press.
- Ng WK & Hung SSO (1995) Estimating the ideal dietary essential amino acid pattern for growth of white sturgeon, *Acipenser transmontanus* (Richardson). *Aquacult Nutr* **1**, 85–94.
- Norton LE & Layman DK (2006) Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *J Nutr* **136**, 533S–537S.
- Nose T (1979) Summary report on the requirements of essential amino acids for carp. In *Finfish Nutrition and Fishfeed Technology*, pp. 145–156 [JE Halver and K Tiews, editors]. Berlin: Heinemann.
- Nose T, Arai S, Lee DL & Hashimoto Y (1974) A note on amino acids essential for growth of young carp. *Bull Jpn Soc Sci Fish* **40**, 903–908.
- Ogino C (1980) Requirements of carp and rainbow trout for essential amino acids. *Bull Jpn Soc Sci Fish* **46**, 171–174.
- Ravi J & Devaraj KV (1991) Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). *Aquaculture* **96**, 281–291.
- Robinson EH, Poe WE & Wilson RP (1984) Effects of feeding diets containing an imbalance of branched-chain amino acids on fingerling channel catfish. *Aquaculture* **37**, 51–62.
- Rollin X (1999) Critical study of indispensable amino acids requirements of Atlantic salmon (*Salmo salar* L.) fry. PhD Thesis, Université catholique de Louvain, Belgium.
- Santiago CB & Lovell RT (1988) Amino acid requirements for growth of Nile tilapia. *J Nutr* **118**, 1540–1546.
- Shimomura Y, Yamamoto Y, Bajotto G, Sato J, Murakami T, Shimomura N, Kobayashi H & Mawatari K (2006) Nutraceutical effects of branched-chain amino acids on skeletal muscle. *J Nutr* **136**, 529S–532S.
- Singh BN, Sinha VRP & Kumar K (1987) Protein requirements of Indian major carp, *Cirrhinus mrigala*. *Int J Acad Ichthyol* **8**, 71–75.
- Snedecor GW & Cochran WG (1967) *Statistical methods*, 6th ed. Iowa: Iowa State University Press.
- Sokal RR & Rohlf FJ (1981) *Biometry*. New York: WH Freeman.
- Swamy DN, Mohanty SN & Tripathi SD (1988) Growth of mrigal (*Cirrhinus mrigala* Ham.) fingerlings fed on fish meal based formulated diets. In *The First Indian Fisheries Forum, Proceedings of Asian Fisheries Society*, pp. 81–83 [MM Joseph, editor]. Mangalore: Indian Branch, Asian Fisheries Society.
- Twibell RG, Griffin ME, Martin B, Price J & Brown PB (2003) Predicting dietary essential amino acid requirements for hybrid striped bass. *Aquacult Nutr* **9**, 373–381.
- Walton MJ (1985) Aspects of amino acid metabolism in teleost fish. In *Nutrition and Feeding in Fish*, pp. 47–68 [CB Cowey, AM Mackie and JG Bell, editors]. London: Academic Press.
- Wilson RP (2002) Amino acids and protein. In *Fish nutrition*, 3rd ed. pp. 143–179 [JE Halver and RW Hardy, editors]. San Diego: Academic Press.
- Wilson RP, Poe WE & Robinson EH (1980) Leucine, isoleucine, valine and histidine requirements of fingerling channel catfish. *J Nutr* **110**, 627–633.
- Yamamoto T, Shima T & Furuita H (2004) Antagonistic effects of branched-chain amino acids induced by excess protein-bound leucine in diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **232**, 539–550.
- Zeitoun IH, Ullrey DE, Magee WT, Gill JL & Bergen WG (1976) Quantifying nutrient requirements of fish. *J Fish Res Board Can* **33**, 167–172.