Dietary leucine requirement of fingerling Indian major carp, *Labeo rohita* (Hamilton).

**Introduction**

Growth is a factor of prime importance in fish culture; understanding growth limitations should be beneficial in terms of productivity, sustainability and profitability of fish culture. Growth involves accretion of body constituents. To ensure maximum economic return, protein and amino acids must be provided in sufficient amounts to meet dietary needs. Protein has been given priority in nutritional requirement studies because it is the principal dietary component for animal growth, and also the highest cost component in commercial feeds (Lim, Sukahwongs & Pascual 1979; Mai, Mercer & Donston 1995). Early research suggest that fish required two to four times more dietary protein than warm-blooded animals like birds and mammals. However, the need for high protein feeds by fish was that they can utilize a significant portion of dietary protein to meet their energy needs if insufficient non-protein energy is available in their feed. (Cowey & Luquet 1983).

Essential amino acids are needed to achieve the optimum growth, feed conversion ratio and desirable carcass quality. Supplemental amino acids represent a way in which the amino acid ratio of a diet can be manipulated to more closely match the animal’s ideal

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amino acid balance. The biological value of a dietary protein is determined by the amount and proportion of essential amino acids it provides. If any of the essential amino acid is not present in sufficient amount or present in excessive amounts relative to other amino acids, protein synthesis will not be supported. Under these circumstances, labile body proteins will be catabolized to provide the amino acids so that the protein synthesis may continue. Building blocks of proteins are twenty amino acids. Of these, ten are considered to be essential in most aquatic species because their carbon skeleton can not be synthesized by the body enzymes, the remaining ten which the animal can synthesize are deemed nutritionally non-essential which can be synthesized endogenously with transfer of amino groups to carbon compounds that are formed as intermediates of glucose and lipid metabolism from gluconeogenic and ketogenic amino acids.

Leucine is essential for growth because it stimulates protein synthesis in muscle tissues. It is a ketogenic amino acid and is specific among the branched-chain amino acids in its ability to stimulate insulin release from the islet cells of the pancreas (Panten, Christians, Kriegstein, Von Poser & Hasselblatt 1974). It is needed for the maintenance of nitrogen balance. The unique ability to regulate protein synthesis in muscle was first documented in the 1970s (Buse & Reid 1975; Fulks, Li & Goldberg 1975; Li & Jefferson 1978). Leucine works with valine and isoleucine to protect and fuel the muscles. It is an important amino acid for the production of hemoglobin. It maintains blood sugar levels and increases growth hormone production and also plays an important role in stress, energy and muscle metabolism. A deficiency of leucine in the diet can cause severe biochemical malfunction including growth retardation.
Indian major carp *Labeo rohita*, commonly known as rohu, is a species with demonstrated aquaculture potential. It has a good demand and potential in most of the tropical markets. It grows to about 800-1000g in less than a year (Jhingran & Pullin 1988). In the past, much emphasis was given to work out its protein requirements (Renukaradhya & Varghese, 1986; Mohanty, Swamy & Tripathy 1990; DeSilva & Gunasekera, 1991; Khan, 1991; Jena, Mukhopadhyay, Sarkar, Aravindakshan & Muduli 1996) but information on its essential amino acid needs are still in its infancy. Amino acid requirements of fishes are usually determined based on the growth rates of fishes fed graded levels of the particular amino acid (Wilson, Allen, Robinson & Poe 1978; Nose 1979; Rodehutscord, Baker, Pack & Pfeffer 1997; Twibell, Wilson & Brown 2000; Abidi & Khan, 2004a; Abidi & Khan, 2004b; Ahmed, Khan & Jafri 2004). The complete ten quantitative essential amino requirements have been established for only a limited number of cultured fish species (Wilson R. P. 2002) Dietary arginine, lysine, methionine and tryptophan (Khan & Jafri 1993), valine requirement (Abidi & Khan 2004a) and histidine requirements (Abidi & Khan 2004b) of *L. rohita* have been worked out. Excepting the work of Murthy & Varghese (1997), no information is available on its essential amino acid leucine requirement. The purpose of present study was, therefore, to estimate the optimum dietary leucine requirements for intensive culture of fingerling *L. rohita*. 
Materials and methods

Preparation of experimental diets

Six isonitrogenous (40% crude protein) and isoenergetic (17.90 kJ g\(^{-1}\) gross energy) amino acid test diets were formulated (Table 1). The dietary range used to quantify the leucine requirement based upon information available on other Indian major carps. The dietary protein level was fixed at 40%, the reported optimum for growth of *L. rohita* (Khan 1991) and the overall composition of amino acids in the test diets simulated that of 40% whole chicken egg protein excluding the test amino acid leucine. Diets were made isonitrogenous and isoenergetic by adjusting the non-essential amino acids glycine, proline and dextrin. In diets used for determining the leucine requirement, levels of leucine were increased in increments of 0.25 g 100g\(^{-1}\) of dry diet.

L-crystalline amino acid and salt mixtures were stirred mechanically for about 30 min in hot water (80 °C) in a stainless steel bowl attached to a Hobart electric mixer. Gelatin was dissolved separately in a known volume of water and added to the above mix. Other dry ingredients and oil premix were added to the lukewarm bowl one by one with constant mixing at 40 °C. Carboxymethylcellulose was added last and blended thoroughly. The final diet with bread dough consistency was passed through a pelletizer fitted with 2-mm die to obtain pellets which were dried in a hot-air oven at 40 °C to lower moisture below 10%. The dry pellets were crumbled, sieved and stored at 4 °C until used. All the amino acid test diets were pH neutralized according to Nose, Arai, Lee & Hashimoto (1974).

Experimental design and feeding trial
Induced-spawned fry of Indian major carp, *L. rohita*, were obtained from G. B. Pant University of Agriculture & Technology, Pantnagar, Uttaranchal. They were transported to the laboratory in oxygen-filled polythene bags, given a prophylactic dip in KMnO$_4$ solution (1:3000) and stocked in indoor, circular plastic lined (1.22m x 0.91m x 0.91m) fish tanks (water volume 600 L) for a fortnight. During this period, the fish were fed to satiation a mixture of soybean, mustard oil cake, rice bran and wheat bran in the form of moist cake twice a day at 0700 and 1730 h. They were then acclimated for 2 wks to a casein-gelatin based (40% CP) H-440 diet (Halver 2002) and reared to fingerling stage.

*L. rohita* fingerlings (3.50± 0.04 cm.; 0.40± 0.02g.) were stocked in triplicate groups in 70- L circular polyvinyl troughs (water volume 55 L) fitted with a continuous water flow-through system (1-1.5 L min$^{-1}$) at the rate of 20 fish per trough for each dietary treatment level. Fish were fed test diets in the form of crumbles to apparent satiation twice daily at 0700 and 1730h. No feed was offered to the fish on the day they were weighed. Initial and weekly weights were recorded on a top-loading balance (Precisa 120A; 0.1 mg sensitivity, Oerlikon AG, Zurich, Switzerland) and feed allowances adjusted accordingly. The feeding trial lasted for 8 wks. Faecal matter and unconsumed feed, if any, were siphoned off before feeding. The unconsumed feed was filtered on a screen soon after active feeding, dried and weighed to measure the amount of feed consumed. Water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity during the feeding trial were recorded following standard methods (APHA 1992). The average water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity over the 8-week feeding trial, based on daily measurements, were 26.0-27.5 °C, 6.7-7.6 ppm., 5-10 mg L$^{-1}$, 7.1-7.8 and 65-80 mg L$^{-1}$, respectively.
Chemical analysis

Proximate composition of casein, gelatin, experimental diets, and initial and final carcass was estimated using standard methods (AOAC 1995) for dry matter (oven drying at 105±1 °C for 22 h.), crude protein (N-Kjeldhal X 6.25 using Kjeltec Tecator™ Technology 2300, Sweden), crude fat (solvent extraction with petroleum ether B.P 40-60 °C for 12-14 h.) and ash (oven incineration at 650 °C for 4-6 h). Gross energy content was determined on a Gallenkamp ballistic bomb calorimeter-CBB 330 010L (Gallenkamp, Loughbrough, UK). Amino acid analysis of casein, gelatin and experimental diets, as described earlier (Ahmed et al. 2004), was made with the help of an Ultrasphere ODS reverse phase column fitted to a Beckman System Gold HPLC unit (Beckman Instruments, San Ramon, CA, USA). Six subsamples of a pooled sample of 40 fish were analyzed for initial carcass composition. At the end of the experiment, all 20 fish from each replicate of dietary treatments were pooled separately. Six subsamples from each pooled replicate were analyzed for final carcass composition.

Data analysis

Growth was evaluated during the eight week feeding trial. Performance was measured as a function of the weight gain and by calculating following parameters:

\[
\text{% Live weight gain} = \left(\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}}\right) \times 100
\]

\[
\text{PER} = \frac{\text{Weight gain}}{\text{Protein fed}}
\]

\[
\text{FCR} = \frac{\text{Feed fed}}{\text{Weight gain}}
\]
Survival Rate (SR%) = (Final number of fish/Initial number of fish) x 100

Body protein deposition was calculated by the formula:

\[
\text{Body protein deposition} = \frac{[(\text{Final body weight} \times \text{final body crude protein}) - (\text{Initial body weight} \times \text{initial body crude protein})]}{\text{Protein fed}}
\]

Statistical analyses

All growth data were subjected to analysis of variance (Snedecor & Cochran 1968; Sokal & Rohlf 1981). Differences among treatment means were determined by Duncan’s Multiple Range Test at a P<0.05 level of significance (Duncan 1955). All the growth parameters were subjected to a second-degree polynomial regression analysis. To predict more accurate response to the dietary intake of leucine, a break-point for optimum dietary leucine requirement was estimated using second-degree polynomial regression analysis (Y=aX^2+bX+c) as described by Zeitoun, Ullrey, Magee, Gill & Bergen (1976). Statistical analysis was done using Matlab and SPSS.

Results

Graded concentrations of dietary L-leucine significantly (P<0.05) affected weight gain, feed conversion ratio and other growth parameters in *L. rohita* fingerling, although it did not affect survival (Table 2). Weight gain and feed conversion ratio of fish increased as dietary leucine concentrations increased from 0.75 to 1.50% and then decreased slightly thereafter with further increase in dietary leucine concentrations. Fish fed lower concentrations of leucine exhibited significantly lower weight gain and higher FCR. Fish receiving 1.50% dietary leucine reflected a maximum gain in weight (315%). Highest
protein efficiency ratio (1.86), body protein deposition (33.9) and the best feed conversion ratio (1.35) were evident in fish receiving the dietary concentration of 1.50% dietary leucine. Fish fed leucine beyond 1.50% of the diet did not show any extra gain in weight, FCR, PER, and BPD values. On subjecting the live weight gain data to quadratic regression analysis (Zeitoun et al. 1976), a break-point was evident at 1.57% leucine of dry diet, corresponding to 3.92% of dietary protein (Fig. 1). The relationship was described by the following equation:

\[
Y= -341.85X^2+1076.95X-538.76 \quad (r=0.960)
\]

The FCR of *L. rohita* fingerlings fed 1.50% leucine diet differed significantly (P<0.05) from the other levels of leucine inclusion. The FCR (Y) to dietary concentrations of leucine (X) relationship was estimated by the following second-degree polynomial regression equation.

\[
Y= 2.5057X^2-7.7844X+7.3428 \quad (r=0.989)
\]

Based on the above equation, the estimated FCR occurred at a dietary leucine concentration of approximately 1.55% of dry diet, corresponding to 3.87% of the dietary protein (Fig. 2).

Also, the PER of *L. rohita* fingerlings fed 1.50% leucine diet differed significantly (P<0.05) from the other levels of leucine inclusion. The PER (Y) to dietary concentrations of leucine (X) relationship was estimated by the following second-degree polynomial regression equation.

\[
Y= -1.6429X^2+5.0207X-2.035 \quad (r=0.988)
\]
Based on the above equation, the estimated PER occurred at a dietary leucine concentration of approximately 1.52% of dry diet, corresponding to 3.80% of the dietary protein (Fig. 3).

Similarly, the BPD of *L. rohita* fingerlings fed 1.50% leucine diet differed significantly (P<0.05) from the other levels of leucine inclusion. The BPD (Y) to dietary concentrations of leucine (X) relationship was estimated by the following second-degree polynomial regression equation.

\[ Y = -36.177X^2 + 108.688X - 50.636 (r=0.964) \]

Based on the above equation, the estimated BPD occurred at a dietary leucine concentration of approximately 1.50% of dry diet, corresponding to 3.75% of the dietary protein (Fig. 4).

On the basis of above polynomial equations, the maximum live weight gain percent, best FCR, PER and highest BPD occurred at leucine levels of approximately 1.57, 1.55, 1.52 and 1.50% of dry diet, corresponding to 3.92, 3.87, 3.80 and 3.75% of dietary protein, respectively.

Excepting for ash content, which remained almost constant significant (P<0.05) differences were recorded in the carcass composition of *L. rohita* fed different concentrations of leucine (Table 3). Minimum moisture was recorded in fish fed diets containing 1.50% of dietary leucine. Carcass protein was also enhanced significantly with increasing dietary leucine concentrations up to 1.50%; beyond this level a significant fall in carcass protein concentration was evident. A similar trend was found for body protein.
deposition values. Significantly higher (p<0.05) body protein deposition at 1.50% dietary leucine compared to other treatment levels may be due to more efficient utilization of the individual amino acids at this level (Fig. 4). Body fat was also found to be minimum at the above level of dietary leucine inclusion.

Discussion

Growth is the result of intake of the indispensable amino acids resulting in high protein deposition. Among the indispensable amino acids, leucine an essential amino acid is a member of aliphatic side-chain amino acid family that is composed of extremely hydrophobic biochemicals found principally in the interior of proteins and enzymes. Leucine is considered essential for normal growth and reproductive potential of the fish. Dietary leucine imbalance leads to a reduction in growth rate and a decrease in the efficiency of feed utilization. In the present study, excess amount of leucine caused depressive effects such as reduction in weight gain and feed conversion ratio. Probably excessive levels of leucine led to accumulation and oxidation to ketones and other toxic metabolites adversely affecting the growth. It should be incorporated in the diet in that amount which exactly matches the requirement level of fish so as to avoid any deficiency or toxicity symptoms in fish body. Antagonism between branched-chain amino acids generally arises in animals from an excess of leucine over isoleucine and valine because the requirement of branched chain amino acid is affected by each other (De’Mello & Lewis 1970; 71). Data on antagonisms among branched-chain amino acids in fish are not clear-cut and are inconsistent between species. Thus the isoleucine requirement of chinook salmon, \textit{Oncorhynchus tshawytscha} increased slightly with increasing concentrations of dietary leucine (Chance, Mertz & Halver 1964). Hughes, Rumsey &
Nesheim (1983) observed changes in concentrations of branched-chain amino acids in lake trout, *Salvelinus nemaykush*, given diets containing increasing amounts of valine. Yamamoto, Shima & Furutia (2004) have reported an antagonistic effect of branched-chain amino acids induced by excess protein bound leucine in the diet in rainbow trout. Choo, Smith, Cho & Ferguson (1991), however, concluded that growth depression and abnormal morphology of rainbow trout fed excess leucine diet did not result from antagonistic effects of branched-chain amino acids but from toxicity of the excess leucine itself. The existence of an interaction among branched chain amino acids and especially the adverse effects of excess of leucine has been noted primarily in diets that are considered as marginal or deficient in isoleucine and valine for rats (Benton, Harper, Spivey & Elvehjem 1956; Harper, Leung & Yoshida 1964; Harper, Benevenga & Wohlheuter 70), chicks (De’Mello & Lewis 1970; Allen & Baker 1972; Barbour & Latshaw 1992) and turkeys (De’Mello 1975; Tuttle & Balloun, 1976; Jackson & Potter 1984). However, in studies with diets based on practical ingredients meeting minimum requirements for isoleucine and valine, no adverse effects of high levels of leucine were apparent (Burnham, Emmans & Gous 1992; Barbour & Latshaw 1992). Therefore, the lack of an apparent antagonism among leucine with other two branched chain amino acids isoleucine and valine in the present study is in agreement with these cited work. Since we have simulated the amino acid composition of the experimental diets to that of 40% whole chicken egg protein containing an adequate amount of isoleucine and valine, the possibility of antagonism of leucine with these two amino acids is diminished.

Fish need this amino acid for maintaining normal health status and for rapid growth. In the present study, dietary leucine requirement was estimated using dose-
response curve which is considered in principal as a method for determining requirements (Cowey 1995). The maximum average weight gain occurred in fingerling \textit{L. rohita} fed the diet containing 1.50\% dietary leucine. However on subjecting the live weight gain, FCR, PER and BPD data to second-degree polynomial regression analysis, the breakpoints were found at 1.57, 1.55, 1.52 and 1.50\% of the dry diet, corresponding to 3.92, 3.87, 3.80 and 3.75\% leucine of the dietary protein.

The data available on the leucine requirements of fish vary between 2.80-5.20\% of the dietary protein. The dietary leucine requirement of \textit{L. rohita} worked out at present study was in the range of 3.75-3.92\% of the dietary protein which is higher than the requirement reported for other fish species including common carp, \textit{Cyprinus carpio} 3.30\% (Nose 1979), coho salmon, \textit{Oncorhynchus kisutch} 3.40\% (Arai & Ogata 1993), channel catfish, \textit{Ictalurus punctatus} 3.50\% (Wilson \textit{et al.} 1980), catla, \textit{Catla catla} 3.70\% (Ravi & Devaraj 1991), approximately equal to the requirement of the fishes including chinook salmon, \textit{Oncorhynchus tshawytscha} 3.90\% (Chance \textit{et al.} 1964), Japanese flounder, \textit{Paralichthys olivaceus} 3.90\% (Forster & Ogata 1998), and lower than the requirement of the fishes including red seabream, \textit{Pagrus major} 4.20\% (Forster & Ogata 1998), white sturgeon, \textit{Acipenser transmontanus} 4.30\% (Ng & Hung 1995), \textit{Cirrhinus cirrhosus} 4.33\% (Benakappa & Varghese 2003), rainbow trout, \textit{Oncorhynchus mykiss} 4.40\% (Kaushik 1998), red drum, \textit{Sciaenops ocellatus} 4.70\% (Moon & Gatlin 1991) and Atlantic salmon, \textit{Salmo salar} 5.20\% of the protein (Rollin 1999). These reported large variations for leucine requirement may be due to differences in fish size, species, age, laboratory conditions including feeding regime, feed allowances, water temperature, stocking density and combination of ingredients used for the preparation of basal diets.
such as casein, gelatin and others. Dietary amino acid requirement is reported to be
influenced by feeding levels adopted (Chiu, Austic & Rumsey 1988). Digestibility, amino
acid profile and energy content bring about variable effects in amino acid requirement
studies (Simmons, Moccia, Bureau, Sivak & Herbert 1999; De Silva, Gunasekera &
Gooley 2000). Variations may also be attributed to differences between phylogenetically
distinct families or species (Akiyama, Oohara & Yamamoto 1997). Maximum body
deposition at this level also supports the requirement and indicates more efficient
utilization of individual amino acids. Significant differences in body composition were
evident in *L. rohita* fed diets containing various levels of leucine. Lower body protein
was noted in fish fed diets containing low levels of leucine. Body protein deposition was
maximum in fish fed diet with 1.50% leucine. Body fat increased significantly (*P*<0.05)
in fish fed with different leucine concentrations except those fed 1.50% leucine diet
where a significantly low fat content was noted. Based on the quadratic regression
analysis of growth and conversion efficiency data generated during the present study on
fingerling *L. rohita*, we recommend an inclusion of leucine in the range of 1.50-1.57% of
the dry diet, corresponding to 3.75-3.92% of dietary protein for optimum growth of this
fish. Except for loss of appetite and low feed utilization which resulted in depressed
growth in *L. rohita* fed diets containing less than 1.50% dietary leucine, no diet related
mortality or any other nutritional pathologies of leucine deficiency were observed. Data
generated during the present study would be useful in developing leucine balanced
practical diets for the mass production of this fish.
An 8-week feeding experiment was conducted to evaluate the dietary leucine requirement of fingerling Indian major carp, *Labeo rohita* (3.50± 0.04 cm; 0.40± 0.02 g) using amino acid test diets (40% crude protein; 17.90 kJ g⁻¹ gross energy) containing casein and gelatin as intact protein sources and L-crystalline amino acids. Growth performance and biochemical parameters were assessed by feeding six amino acid test diets supplemented with graded concentrations of leucine (0.75, 1.0, 1.25, 1.50, 1.75 and 2.0 g 100g⁻¹) triplicate groups of fingerlings to apparent satiation divided over two feedings at 0700 and 1730 h. Performance of the fish was evaluated on the basis of live weight gain, feed conversion ratio, protein efficiency ratio and body protein deposition data. Maximum live weight gain (315%), best feed conversion ratio (FCR) (1.35), highest protein efficiency ratio (PER) (1.86) and body protein deposition (BPD) (33.9) were recorded at 1.50 g 100g⁻¹ dietary leucine. Statistical analysis of live weight gain, FCR PER and BPD data reflected significant differences (p<0.05) among treatments. Live weight gain, FCR, PER and BPD data were also analysed using second-degree polynomial regression analysis to obtain more accurate leucine requirement estimate which was found to be at 1.57, 1.55, 1.52 and 1.50 g 100g⁻¹ of dry diet, corresponding to 3.92, 3.87, 3.80 and 3.75 g 100g⁻¹ of dietary protein, respectively. Based on the quadratic regression analysis of the live weight gain, FCR, PER and BPD data, the optimum requirement of fingerling *Labeo rohita* for
leucine is estimated to be in the range of 1.50-1.57 g 100g$^{-1}$ of the dry diet, corresponding to 3.75-3.92 g 100g$^{-1}$ of dietary protein.
Table 1 Composition of experimental diets used for estimating the dietary leucine requirement of fingerling *Labeo rohita*.

<table>
<thead>
<tr>
<th>Ingredients (g 100 g⁻¹ dry diet)</th>
<th>Diets</th>
<th>(I) 0.75</th>
<th>(II) 1.0</th>
<th>(III) 1.25</th>
<th>(IV) 1.50</th>
<th>(V) 1.75</th>
<th>(VI) 2.0</th>
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<tbody>
<tr>
<td>Casein¹</td>
<td></td>
<td>7.0</td>
<td>7.0</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Gelatin²</td>
<td></td>
<td>1.75</td>
<td>1.75</td>
<td>17.5</td>
<td>17.5</td>
<td>17.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Amino acid mix³</td>
<td></td>
<td>29.12</td>
<td>29.20</td>
<td>292.9</td>
<td>293.8</td>
<td>294.6</td>
<td>295.5</td>
</tr>
<tr>
<td>Dextrin</td>
<td></td>
<td>35.29</td>
<td>35.39</td>
<td>354.7</td>
<td>355.6</td>
<td>356.5</td>
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<td>Corn oil</td>
<td></td>
<td>5.0</td>
<td>5.0</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Mineral mix⁴</td>
<td></td>
<td>4.0</td>
<td>4.0</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mix⁴,⁵</td>
<td></td>
<td>3.0</td>
<td>3.0</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>α- Cellulose</td>
<td></td>
<td>2.84</td>
<td>2.66</td>
<td>2.49</td>
<td>2.31</td>
<td>2.14</td>
<td>1.96</td>
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<tr>
<td>Carboxymethyl cellulose</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
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<tr>
<td>Total</td>
<td></td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total leucine</strong></td>
<td></td>
<td>0.75</td>
<td>1.0</td>
<td>1.25</td>
<td>1.50</td>
<td>1.75</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Analyzed leucine</strong></td>
<td></td>
<td>0.74</td>
<td>0.999</td>
<td>1.23</td>
<td>1.49</td>
<td>1.74</td>
<td>1.99</td>
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<tr>
<td>Analyzed crude protein</td>
<td></td>
<td>39.80</td>
<td>39.85</td>
<td>39.96</td>
<td>40.00</td>
<td>39.95</td>
<td>40.00</td>
</tr>
<tr>
<td>Gross energy⁶ (kJ g⁻¹, dry diet)</td>
<td></td>
<td>17.90</td>
<td>17.90</td>
<td>17.90</td>
<td>17.90</td>
<td>17.90</td>
<td>17.90</td>
</tr>
</tbody>
</table>
1Crude Protein (80%), Loba Chemie, India; 2Crude Protein (93%), Loba Chemie, India.; 3Loba Chemie, India.; 4Halver (2002); 4,51g Vitamin mix +2g α-cellulose; 6Calculated on the basis of fuel values 23.08, 20.199, 24.26, 16.02 and 37.64 kJ for casein, gelatin, amino acids, dextrin, and fat, respectively, as estimated on Gallenkamp ballistic bomb calorimeter.
Table 2 Growth, conversion efficiencies and percentage survival of fingerling *Labeo rohita* fed diets containing graded levels of dietary leucine

<table>
<thead>
<tr>
<th>Dietary leucine levels g 100 g(^{-1})</th>
<th>(I) 0.75</th>
<th>(II) 1.0</th>
<th>(III) 1.25</th>
<th>(IV) 1.50</th>
<th>(V) 1.75</th>
<th>(VI) 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average initial weight (g)(^1)</td>
<td>0.61±0.02</td>
<td>0.59±0.01</td>
<td>0.59±0.01</td>
<td>0.58±0.03</td>
<td>0.58±0.01</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>Average final weight (g)(^1)</td>
<td>1.06±0.04</td>
<td>1.65±0.03</td>
<td>1.83±0.02</td>
<td>2.32±0.03</td>
<td>2.06±0.03</td>
<td>1.92±0.02</td>
</tr>
<tr>
<td>Live weight gain (%)(^1,2)</td>
<td>75.3±3.16(^f)</td>
<td>199±8.1(^e)</td>
<td>269.3±2.3(^d)</td>
<td>315±13.4(^a)</td>
<td>290.3±1.3(^b)</td>
<td>251.0±7.8(^c)</td>
</tr>
<tr>
<td>Food conversion ratio(^1,2,4)</td>
<td>2.98±0.06(^a)</td>
<td>1.98±0.04(^b)</td>
<td>1.46±0.12(^d)</td>
<td>1.35±0.01(^c)</td>
<td>1.51±0.02(^d)</td>
<td>1.72±0.04(^c)</td>
</tr>
<tr>
<td>Protein efficiency ratio(^1,2,5)</td>
<td>0.84±0.01(^e)</td>
<td>1.26±0.02(^d)</td>
<td>1.71±0.1(^b)</td>
<td>1.86±0.1(^a)</td>
<td>1.66±0.01(^b)</td>
<td>1.45±0.03(^c)</td>
</tr>
<tr>
<td>Percentage survival</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^1\) Mean values of 3 replicates ± SEM; \(^2\) Mean values sharing the same superscripts are insignificantly different (P>0.05; n=3, Duncan’s New Multiple Range Test).
Table 3 Carcass composition of fingerling *Labeo rohita* fed graded levels of dietary leucine

<table>
<thead>
<tr>
<th>Dietary leucine levels g 100 g⁻¹</th>
<th>Initial</th>
<th>(I) 0.75</th>
<th>(II) 1.0</th>
<th>(III) 1.25</th>
<th>(IV) 1.50</th>
<th>(V) 1.75</th>
<th>(VI) 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>81.8±0.1</td>
<td>79.3±0.2ᵃ</td>
<td>78.1±0.06ᶜ</td>
<td>77.6±0.02ᵈ</td>
<td>76.6±0.23ᶠ</td>
<td>77.2±0.2ᵉ</td>
<td>78.9±0.12ᵇ</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>11.69±0.25</td>
<td>12.4±0.05ᶜ</td>
<td>14.12±0.1ᵈ</td>
<td>15.55±0.07ᵇ</td>
<td>16.66±0.3ᵃ</td>
<td>14.89±0.51ᵇᶜ</td>
<td>14.41±0.25ᵇᶜᵈ</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.8±0.09</td>
<td>3.4±0.07ᵃ</td>
<td>3.1±0.07ᵇ</td>
<td>2.9±0.1ᵉᵈ</td>
<td>2.5±0.07ᵉ</td>
<td>2.9±0.04ᵈ</td>
<td>3.04±0.05ᶜ</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.09±0.14</td>
<td>3.28±0.01ᵃ</td>
<td>3.15±0.03ᵇ</td>
<td>2.89±0.1₁ᵇ</td>
<td>2.8±0.13ᶜ</td>
<td>2.9±0.19ᵇ</td>
<td>2.9±0.11ᵇ</td>
</tr>
<tr>
<td>Body protein deposition</td>
<td>-</td>
<td>11.6±0.2ᵉ</td>
<td>19.4±0.5ᵈ</td>
<td>29.04±1.6ᵇ</td>
<td>33.9±2.45ᵃ</td>
<td>26.5±0.90ᵇ</td>
<td>22.5±0.06ᶜ</td>
</tr>
</tbody>
</table>

Mean value of 3 replicates ± SEM. Mean values with the same superscripts are insignificantly different (P>0.05; n=3, Duncan’s New Multiple Range Test).
Y = -341.857X^2 + 1076.95X - 538.76 (0.960)

**Live weight gain %**

**Dietary leucine levels (% dry diet)**

Xmax = 1.57
Fig. 1. Second-degree polynomial relationship of live weight gain to dietary leucine levels
Dietary leucine levels (% dry diet)

\[ Y = 2.5057X^2 - 7.7844X + 7.3428 \quad (r=0.989) \]

Feed conversion ratio

\[ X_{\text{min}} = 1.55 \]
Fig. 2.  Second-degree polynomial relationship of food conversion ratio to dietary leucine levels
$Y = -1.6429X^2 + 5.0207X - 2.035 \ (0.988)$

$X_{max} = 1.52$
Fig. 3. Second-degree polynomial relationship of protein efficiency ratio to dietary leucine levels
Y = -36.177X^2 + 108.688X - 50.636 (r=0.964)

Body protein deposition

Dietary leucine levels (% dry diet)

Xmax = 1.50
Fig. 4. Second-degree polynomial relationship of body protein deposition to dietary leucine levels