PART I
CHAPTER I

PROTEIN REQUIREMENT OF THE SILUROID,
HETEROPNEUSTES FOSSILIS, BLOCH.

INTRODUCTION

Protein is by far the most expensive fish feed ingredient. Optimized utilization of dietary protein is, therefore, essential for economical feed and fish production. Assessment of the quantitative requirement of dietary protein of fish under culture conditions enables the formulations of well-balanced and cost-effective feeds. Earlier studies indicate that protein requirements vary widely with different fish species (Ogino and Satio, 1970; Nose and Arai, 1972; Page and Andrews, 1973; Anonymous, 1976; Dabrowski, 1977; Mazid et al., 1979; Akiyama et al., 1981; Anderson et al., 1981; Moreno, 1981; Yang et al., 1981; Jauncey, 1982; Millikin, 1982, 1983; Wee, 1983; Wilson, 1984; and Wang et al., 1985a, b). The requirements are tied to factors like amino acid composition of the diet (Mertz, 1969), protein digestibility and protein energy ratio (Fowler et al., 1964; Ringrose, 1971; Lee and Putnam, 1973; Page and Andrews, 1973; Prather and Lovell, 1973; Garling and Wilson, 1976;
Gulbrandsen and Utne, 1977; Austrenge, 1978; Lied and Rosenlund, 1984), water quality and water temperature (Delong et al., 1958), salinity (Zeitoun et al., 1974), fish size (Satia, 1974), and presumably also to the genetic make-up of fish (Austreng and Refstie, 1979; Newman et al., 1979; and Bondari, 1983). Cowey and Luquet (1983), while dealing with physiological basis of protein requirement of fishes, made a critical analysis of protein allowances in fish rations. Utilization of dietary protein by salmonid fish has been reviewed by Pfeffer (1982).

The optimum dietary protein level resulting in maximum growth has been studied in the past for several fish species under different culture conditions (Lovell, 1972; Teng et al., 1978; and Lim et al., 1979).

The siluroid, *H. fossilis*, is an important tropical freshwater food fish. In recent years, the farming of this species has gained considerable importance in India. Although for the economical production of this species conventional feeding strategies, such as feeding combinations of trash fish, oil cakes, etc., are generally adopted (Dehadrai and Thakur, 1980), no scientific information is apparently available on its dietary requirements. The present study was, therefore, conducted to evaluate the protein requirement of young *H. fossilis*, held under laboratory condition, using purified diets, containing varying levels (10-60% by weight) of dietary protein. The
information, it is presumed, would be useful in developing high quality artificial feed for the culture of this siluroid on intensive scale.

MATERIALS AND METHODS

Six different test diets within a range of 10-60% crude protein were formulated (Table I) using casein and gelatin as protein sources (Mertz, 1972). The diets incorporated a balance of essential amino acids similar to that of whole egg protein (Delong et al., 1958). Details of the amino acids mixture have been presented in Table II. The mineral and vitamin premixes, as shown in Tables III and IV, were prepared according to Halver (1970). Diets were made isocaloric by adjusting the dextrin content. The caloric value of each diet was estimated on the basis of energy equivalents quoted elsewhere (see page 25). These diets contained a range of protein:energy ratios from 24.57 to 147.42 mg protein/kcal.

I. Preparation of diet

The calculated quantities of various ingredients (Table I), with the exception of carboxymethyl cellulose, were blended in dry state in high speed electric grinder to obtain a complete homogeneous mixture. To this mixture was added 100 ml distilled water heated previously to 80-90°C. This was stirred constantly
until homogeneous and soluble components were in solution. At this stage, with continuous stirring, carboxymethyl cellulose was slowly added, and as the diet began to solidify, the speed of stirring was gradually increased to incorporate air into the mixture. The final diet, about the consistency of bread dough, was stored in plastic container at -10°C until used. The firmness of the diet increased further upon cooling and enabled easy preparation of moist pellets (Fig. 1). At the time of feeding, the dough was passed through a hand pressed extruder, the strands air-dried for about 20-30 minutes and cut into pellets (2-3 mm diameter).

II. Feeding trial

Live fish (average weight 6.13 ± 0.15 g, total length 11.24 ± 0.77 cm) were procured from previously acclimatized fish stock maintained in the laboratory. Details of acclimatization have been given under 'General Methodology' section. The fish, stocked in 40-l glass aquaria (water volume 30-l) in six duplicate groups at the stocking density of 15 individuals per aquarium, got accustomed to the experimental diets in about a weeks time when initial measurements were made (Table V).

The fish were fed slow-sinking moist pellets till satiation daily at 17.00 h. The feeding schedule was chosen after carefully observing the feeding behaviour of H. fossilis. Preliminary experiments indicated that the fish feeds actively during
the evening time. Care was taken to ensure that the pellets were quickly consumed. Accumulation of pellets at the bottom was avoided to prevent leaching of the nutrients. No food was offered to the fish on the day the weekly measurements were made. The average water temperature and dissolved oxygen over the 6-week feeding trial based on daily measurements were $25 \pm 1^\circ C$ and $6.5 \pm 1$ ppm, respectively.

The requirement of dietary protein was assessed through various nutritional parameters (see pages 28-30).

Survival rates were $100\%$ in all the dietary treatments, excepting the $10\%$ and $20\%$ protein diets where the rate of survival was $80\%$ and $90\%$, respectively.

RESULTS

Summary of the results of 6-week feeding trial conducted on *H. fossilis* to quantify its protein requirement has been shown in Table V.

Data concerning details of the initial and final size and weight of fish in replicate groups for various dietary treatments have been given in Tables VI-VII. The average weight of fish over the experimental period for all the test diets increased linearly on their initial weights (Fig. 2).
These changes were significant \( P < 0.05 \) with correlation coefficient \( r \) varying from 0.833 to 0.834 (Table VIII) in different dietary protein levels. Regression equations of weight obtained over the initial weight of fish for different treatments have been presented in Table IX.

The fish registered a gain in live weight with dietary protein concentrations up to 40% crude protein level. Decreased average gains in weight were noted when the dietary protein level increased from 40% to 60%. The lowest gain was recorded at 10%, followed by 60% level of dietary protein (Fig. 3). Analysis of covariance indicated highly significant \( P < 0.01, F_{5,6} = 31.1438 \) treatment effects (of protein levels) on growth in fish weight, the replication being insignificant. The factorial analysis of data also revealed highly significant \( P < 0.01 \) treatment response. Based on the significance level, the diets containing the various protein levels could be arranged in the following sequence 40%, 30%, 50%, 20%, 60% and 10%.

Growth curve representing the weekly mean live weight gain percent (Fig. 4) for the various dietary treatments over the experimental period showed maximum (75%) gain in fish fed 40% crude protein diet whereas the gain was only about 23% at 10% dietary protein level. The average specific growth rate of fish for the various treatments have been plotted in Fig. 5. The specific growth rate increased almost linearly with
increasing dietary protein content up to the incorporation rate of 40% but declined sharply at higher levels of dietary protein.

In an attempt to compare the actual gains at different treatment levels with the calculated or estimated values, a weight-gain response curve was constructed. Similar data treatment has earlier been carried out for the grouper (Teng et al., 1978) and other fish species (Zeitoun, et al., 1976). To purge away the effect of initial weight of fish on weight-gains at different protein levels, estimated weight-gains were calculated using an averaged initial mean weight ($6.25 \pm 0.15$ g) of fish for all the test diets. Using this initial mean weight and regression equation in Table IX, weight obtained and gain per fish were calculated for all the test diets (Table X). These calculated weight gains were found comparable to the actual gains. A response-curve fitted with second degree polynomial (Snedecor and Cochran, 1974) with 90% confidence limits, along with quadratic equation, has been depicted in Fig. 6. The level of dietary protein that produced the maximum growth of the fish was observed to be at 40% ($X_{\text{max}}$). The maximum weight gain per fish at this level was estimated to be around $3.72 \text{ g} (Y_{\text{max}})$.

Fig. 7 shows the relationship between daily food intake and daily gain in weight per fish. It can be seen that the daily intake of food by the fish was noticeably affected by
the level of protein in the diet. The food intake was minimum (181.58 mg day\(^{-1}\) per fish) at 40\% protein level. Intakes were appreciably more at dietary protein levels lower or higher to 40\% crude protein level. The gain day\(^{-1}\) per fish increased with the protein level of the diet, reaching a maximum (111.904 mg day\(^{-1}\) per fish) at 40\% crude protein level and declining sharply beyond this level.

The relationship between weight gain and protein consumed over 6-week feeding trial has been depicted in Fig. 8. The fish at 40\% protein diet consumed less than half the protein consumed by fish receiving the maximum (60\%) protein percentage diet. No significant difference in protein consumption was evident between 30\% and 40\% crude protein diets. Although the consumption of protein increased with levels of protein in diets, the maximum (4.7 g) gain in fish weight was obtainable at 40\% protein level. Increased dietary protein level thereafter resulted in decreased live weight of fish. The overall efficiencies of utilization of dietary protein by \textit{H. fossilis} has also been worked out in terms of protein efficiency ratio. The protein efficiency ratios at different dietary protein levels have been plotted in Fig. 9. The maximum (1.535) protein efficiency was noted at 40\% protein diet. A sharp decline in the values occurred with increasing dietary protein content, the minimum (0.295) being at 60\% crude protein level.
The food conversion ratio and gross growth efficiency in fish fed different levels of dietary protein have been shown in Fig. 10. Marked differences were seen in the feed conversion values. The best feed conversion ratio (1.62:1) was noted at 40% protein level. Less efficient feed conversions were obtained with diets having levels higher or lower to 40% crude protein. The gross growth efficiency was likewise maximum (0.616) at 40% and minimum (0.137) at 10% protein levels.

On the basis of the conversion efficiencies obtained, the calories required per kg of fish produced have been calculated (Table XI).

**DISCUSSION**

The results of the present study indicate that in *H. fossilis*, with an initial mean weight 6.13 ± 0.15 g, the maximum growth occurred with 40% dietary protein level and protein:energy ratio of 98 mg protein/kcal at 25 ± 1°C. The conversion efficiencies obtained also point to the most efficient utilization of food at the above level of dietary protein. The optimum protein requirement noted for *H. fossilis* has been found close to the values reported earlier for a number of warm water cultivable fish species (Nail, 1962; Dabrowski, 1977; NAS-NRC, 1977; Yang et al., 1981, Corazon et al., 1982; and Jauncey, 1982).
Hastings and Dupree (1969) noted that channel catfish fingerlings stocked in aquaria registered maximum growth with 40% protein food at 25°C. The protein requirement for optimal growth of this fish was reported to be 35% at 20°C and 40% at 25°C (Dupree and Sneed, 1966). In *C. batrachus*, a closely related freshwater catfish species, maximum growth has been reported at 37.7% dietary protein level (Anonymous, 1976).

The growth depressing effects observed in *H. fossilis* at high protein diets have been pointed out in several other fishes like carp (Ogino and Saito, 1970), grouper (Teng et al., 1978), tilapia (Mazid et al., 1979; Jauncey, 1982), milkfish (Lim et al., 1979) and puffer fish (Kanazawa et al., 1980). The coefficient of condition, considered a measure of the relative robustness of fish (Lagler, 1982), also indicated that fish fed dietary protein levels between 30-40% attained the best 'condition'.

In the present study, although sufficiently high dietary energy level was incorporated for efficient protein utilization, the efficiency with which *H. fossilis* was able to utilize protein, as measured through PER, declined beyond 40% crude protein level. The response pattern was found similar to that of plaice (Cowey et al., 1972), grouper (Teng et al., 1978) and snakehead (Wee and Tacon, 1982). In several other fish species, however, the PER was reported to be highest at the lowest
protein level tested, declining almost linearly with protein content of diet (Ogino and Saito, 1970; Dabrowski, 1977; Mazid et al., 1979; and Jauncey, 1982).

The pattern of essential amino acids provided by the dietary protein is known to influence the utilization of protein (Cowey and Sargent, 1972). Protein that matches the qualitative requirement of essential amino acids of the species is more efficiently utilized. Essential amino acids supplied in excess to the optimum requirement in dietary protein may not necessarily enhance the protein utilization (Halver, 1957; Halver and Shanks 1960; and Mertz, 1972). The utilization of protein by fish may likewise be influenced, to an extent, by caloric content of the diet (Garling and Wilson, 1976), and growth is accomplished only if the ration contains sufficient energy in proper ratio (Cowey and Sargent, 1972).

In may be postulated that decrease in growth response, evaluated through the assessment of specific growth rate, and utilization of protein at level of protein above the optimum may be attributed to the reduction in dietary energy available for normal growth due to energy utilized to deaminate and excrete the excessive amino acids.

It may be noted that the diet at 40% crude protein level which resulted in maximum growth in H. fossilis contained 43.5% calories as protein. Enhancement of calories as protein
resulted in depressed growth also because the diet contained insufficient non-protein energy. Contribution of calories from protein sources vis-à-vis non-protein energy sources, seems important even in the isocaloric diets. Lovell (1976) has recorded that in channel catfish weight gain in fish fed high (42%) protein diet with insufficient non-protein energy was lower compared to those fed a low (36%) protein diet with the same low level of energy. In terms of energy economy, as well, the 40% protein diet was found to be the best for _H. fossilis_, since total calories required per kg of fish produced was minimum (6,593 cal), with best conversion ratio (1.6:1), at this level of dietary protein. Aspects dealing with energy consumption and retention in this species have been dealt with in Chapter II.

**SUMMARY**

The dietary protein requirement of siluroid, _Heteropneustes fossilis_ has been worked out using purified test diets containing varying levels of dietary protein over a 6-week feeding period. The live weight gain for each dietary treatment was found to be linearly dependent on initial weights. Linear regression equations of weight gains on initial weights of fish were calculated for each diet. The level of dietary protein that produced the maximum growth, as estimated through a response
curve, was found to be around 40%. Analysis of data revealed that actual weight gains at different levels were close to the calculated values. The values of food conversion ratio, gross growth efficiency, specific growth rate and protein efficiency ratio were found best at 40% level of dietary protein. The various growth parameters were negatively affected at higher dietary protein intakes. The results thus indicate that the dietary protein requirement of *H. fossilis* was about 40% at 25 ± 1°C.
### TABLE I. Ingredient composition, protein and energy contents of purified diets

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<th>4</th>
<th>5</th>
<th>6</th>
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<td>Gelatin (g/200 g diet)</td>
<td>1.54</td>
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<td>4.62</td>
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<td>9.23</td>
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<td>Casein</td>
<td>6.15</td>
<td>12.30</td>
<td>18.46</td>
<td>24.62</td>
<td>30.77</td>
<td>36.92</td>
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<td>Amino acid mixture</td>
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<td>6.77</td>
<td>10.15</td>
<td>13.54</td>
<td>16.92</td>
<td>20.31</td>
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<td>Dextrin (white)</td>
<td>64.93</td>
<td>53.85</td>
<td>42.77</td>
<td>31.69</td>
<td>20.62</td>
<td>9.54</td>
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<td>Fish oil</td>
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<td>Corn oil (Cornello)</td>
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<td>α-cellulose + Vitamins mix</td>
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<td>Carboxymethyl cellulose</td>
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<td>100.00</td>
<td>100.00</td>
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<tr>
<td>Crude Protein (N x 6.25) g/100 g dry wt.</td>
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<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>60</td>
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<tr>
<td>Gross Energy (kcal/100 g dry feed)</td>
<td>407</td>
<td>407</td>
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<td>Amino acid (L-series)</td>
<td>g/100 g of mix</td>
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<td>Arginine</td>
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<td>Histidine</td>
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<td>Phenylalanine</td>
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<td>Threonine</td>
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<td>Isoleucine</td>
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<td>Valine</td>
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<td>Glycine</td>
<td>23.000</td>
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TABLE III. Composition of mineral mixture

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<tr>
<th>Mineral</th>
<th>mg/100 g dry diet</th>
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<tr>
<td>AlCl₃</td>
<td>15.00</td>
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<tr>
<td>ZnSO₄</td>
<td>300.00</td>
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<tr>
<td>CuCl</td>
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<tr>
<td>MnSO₄</td>
<td>80.00</td>
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<tr>
<td>KI</td>
<td>15.00</td>
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<tr>
<td>COCl₂</td>
<td>100.00</td>
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<td>USP XII No.2</td>
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TABLE IV. Composition of vitamin mixture

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<th>Vitamin</th>
<th>mg/100 g dry diet</th>
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<td>Thiamine HCl</td>
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<td>Riboflavin</td>
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<td>Pyridoxine HCl</td>
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<td>Choline chloride</td>
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<td>Nicotinic acid</td>
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<td>Ca-pantothenate</td>
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<td>Inositol</td>
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<td>Biotin</td>
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<td>L-ascorbic acid</td>
<td>33.333</td>
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<td>Vitamin B_{12}</td>
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<td>Menadione</td>
<td>1.333</td>
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<td>α-tocopheral acetate</td>
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<td>Assessment Parameters</td>
<td>10%</td>
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<tr>
<td>Initial mean individual wet weight (g)</td>
<td>6.267</td>
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<td>Final mean individual wet weight (g)</td>
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<td>Mean live weight gain/fish (g)</td>
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<td>Percentage increase in weight</td>
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<td>Specific growth rate %</td>
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<td>Food conversion ratio</td>
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<td>Gross growth efficiency</td>
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<td>Protein efficiency ratio</td>
<td>1.372</td>
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<td>Condition factor</td>
<td>0.565</td>
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<td>Percentage survival</td>
<td>80</td>
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TABLE VI. Initial size of *H. fossilis* used during the feeding trial

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<td>Total length (cm)</td>
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<td>Mean</td>
<td>S.D.</td>
<td>Range</td>
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<td>10-11.8</td>
<td>10.76</td>
<td>0.607</td>
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<td>10-12.0</td>
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<td>10-12.0</td>
<td>10.64</td>
<td>0.727</td>
<td>5-8.0</td>
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<td>Body weight (gm)</td>
<td>Range</td>
<td>Mean</td>
<td>S.D.</td>
<td>Range</td>
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<td>6.13</td>
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<td>10.73</td>
<td>0.841</td>
<td>5-8.0</td>
</tr>
<tr>
<td></td>
<td>10-12.0</td>
<td>10.78</td>
<td>0.739</td>
<td>5-8.0</td>
</tr>
<tr>
<td></td>
<td>10-12.0</td>
<td>11.05</td>
<td>0.579</td>
<td>5-8.0</td>
</tr>
<tr>
<td>Replicate 1</td>
<td>Replicate 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total length (cm)</strong></td>
<td><strong>Body weight (gm)</strong></td>
<td><strong>Total length (cm)</strong></td>
<td><strong>Body weight (gm)</strong></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>Mean</td>
<td>S.D.</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>10.1-12.0</td>
<td>11.08</td>
<td>0.579</td>
<td>8.0-7.0</td>
<td>7.53</td>
</tr>
<tr>
<td>10.1-12.1</td>
<td>10.98</td>
<td>0.668</td>
<td>7.5-9.0</td>
<td>7.86</td>
</tr>
<tr>
<td>10.6-11.9</td>
<td>11.13</td>
<td>0.436</td>
<td>9.0-11.0</td>
<td>9.66</td>
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<tr>
<td>10.4-12.6</td>
<td>11.71</td>
<td>0.608</td>
<td>9.5-12.0</td>
<td>10.86</td>
</tr>
<tr>
<td>10.2-12.2</td>
<td>11.19</td>
<td>0.534</td>
<td>7.5-9.0</td>
<td>8.33</td>
</tr>
<tr>
<td>10.3-12.2</td>
<td>11.71</td>
<td>0.608</td>
<td>7.5-9.0</td>
<td>8.00</td>
</tr>
</tbody>
</table>
TABLE VIII. Correlation coefficient of weight obtained in *H. fossilis* over the experimental period with different levels of dietary protein

<table>
<thead>
<tr>
<th>Dietary protein level</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>0.833</td>
</tr>
<tr>
<td>20%</td>
<td>0.810</td>
</tr>
<tr>
<td>30%</td>
<td>0.832</td>
</tr>
<tr>
<td>40%</td>
<td>0.828</td>
</tr>
<tr>
<td>50%</td>
<td>0.834</td>
</tr>
<tr>
<td>60%</td>
<td>0.834</td>
</tr>
</tbody>
</table>

Significance (*p* < 0.05)
TABLE IX.  Linear regression equations of weight obtained over initial weights of *H. fossilis* fed different levels of dietary protein

<table>
<thead>
<tr>
<th>Level of dietary protein</th>
<th>Replication number</th>
<th>Regression equation</th>
<th>Correlation coefficient's fish</th>
<th>No. of fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1</td>
<td>$Y = 0.3912 + 1.1655 X$</td>
<td>0.8008</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$Y = 3.1640 + 0.7244 X$</td>
<td>0.7005</td>
<td>15</td>
</tr>
<tr>
<td>20%</td>
<td>1</td>
<td>$Y = 1.8092 + 1.0209 X$</td>
<td>0.8659</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$Y = 3.3004 + 0.7951 X$</td>
<td>0.7455</td>
<td>15</td>
</tr>
<tr>
<td>30%</td>
<td>1</td>
<td>$Y = 4.6201 + 0.8505 X$</td>
<td>0.8055</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$Y = 6.7993 + 0.5001 X$</td>
<td>0.7644</td>
<td>15</td>
</tr>
<tr>
<td>40%</td>
<td>1</td>
<td>$Y = 4.3919 + 1.0443 X$</td>
<td>0.7766</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$Y = -4.2109 + 2.4273 X$</td>
<td>0.8658</td>
<td>15</td>
</tr>
<tr>
<td>50%</td>
<td>1</td>
<td>$Y = 4.3751 + 0.6384 X$</td>
<td>0.7514</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$Y = 2.7986 + 0.8891 X$</td>
<td>0.7539</td>
<td>15</td>
</tr>
<tr>
<td>60%</td>
<td>1</td>
<td>$Y = 4.6727 + 0.5425 X$</td>
<td>0.7432</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$Y = 2.5868 + 0.8703 X$</td>
<td>0.8246</td>
<td>15</td>
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</table>
TABLE X. Calculated weight gain of *H. fossilis* of initial averaged initial mean weight of 6.25 g for different protein levels

<table>
<thead>
<tr>
<th>Level of dietary protein</th>
<th>Replication number</th>
<th>Calculated weight (g) obtained/fish</th>
<th>Average weight estimated (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1</td>
<td>7.539</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>7.606</td>
<td>1.306</td>
</tr>
<tr>
<td>20%</td>
<td>1</td>
<td>8.070</td>
<td>1.993</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.176</td>
<td></td>
</tr>
<tr>
<td>30%</td>
<td>1</td>
<td>9.836</td>
<td>3.885</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.866</td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td>1</td>
<td>10.796</td>
<td>4.505</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.675</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>1</td>
<td>8.290</td>
<td>2.175</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.251</td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>1</td>
<td>7.999</td>
<td>1.865</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.924</td>
<td></td>
</tr>
</tbody>
</table>
TABLE XI. Summary effect of protein and calorie levels on the growth efficiency of *H. fossilis*
over the 6-week experimental period

<table>
<thead>
<tr>
<th>Dietary protein level(%)</th>
<th>Calories/kg of diet</th>
<th>% of calories as protein</th>
<th>Average fish weight(g) at the end</th>
<th>Total percentage gain</th>
<th>Total conversion</th>
<th>Calories required/kg of fish produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4070</td>
<td>10.884</td>
<td>7.666</td>
<td>22.5</td>
<td>7.30</td>
<td>29,711.00</td>
</tr>
<tr>
<td>20</td>
<td>4070</td>
<td>21.769</td>
<td>8.100</td>
<td>32.5</td>
<td>5.00</td>
<td>20,350.00</td>
</tr>
<tr>
<td>30</td>
<td>4070</td>
<td>32.658</td>
<td>9.733</td>
<td>62.5</td>
<td>2.65</td>
<td>10,785.00</td>
</tr>
<tr>
<td>40</td>
<td>4070</td>
<td>43.547</td>
<td>10.933</td>
<td>75.0</td>
<td>1.62</td>
<td>6,593.40</td>
</tr>
<tr>
<td>50</td>
<td>4070</td>
<td>54.427</td>
<td>8.233</td>
<td>35.0</td>
<td>5.30</td>
<td>21,571.00</td>
</tr>
<tr>
<td>60</td>
<td>4070</td>
<td>65.316</td>
<td>7.933</td>
<td>30.0</td>
<td>5.61</td>
<td>22,832.70</td>
</tr>
</tbody>
</table>
Fig. 1. Showing the diet in dough, strands and pelleted form.
SYNTHETIC DIET
Fig. 2. Weekly changes in average fish weight (g) at varying protein levels.
Fig. 3. Average weight gain (g) of fish fed varying dietary protein levels over the experimental period.
Fig. 4. Growth curve representing the weekly mean live weight gain percent at different levels of dietary protein.
Fig. 5. Relationship between average specific growth rate (%)/day and dietary protein levels.
Fig. 6. Response curve depicting weight-gains and levels of dietary protein in *H. fossilis*. Curvilinear curve \( \bullet \bullet \bullet \) represents the response-curve with 95% confidence limit \( \bullet \bullet \bullet \bullet \bullet \). \( X_{max} \) is the estimated level of dietary protein that produced the maximum weight gain in the fish \( (Y_{max}) \). \( \Delta \), \( \Delta \) represent the weight-gains of fish at an averaged initial mean weight of 6.25 g.

\[ Y = -1418.399 + 284.60356x - 3.9046028x^2 \]
Fig. 7. Relationship between average food consumed (■) day⁻¹ per fish (mg) and gain in live weight (□) day⁻¹ per fish (mg) at different dietary protein levels.
Fig. 8. Relationship between total protein consumed (■) and total live weight gain (□) in r at different dietary protein levels.
Fig. 9. Effect of dietary protein level on protein efficiency ratio of H. fossilis.
Fig. 10. Relationship between food conversion ratio (•—•) gross growth efficiency (▲—▲) and dietary protein level.
CHAPTER II

EFFECT OF DIETARY PROTEIN LEVELS ON THE PROXIMATE COMPOSITION, NUCLEIC ACIDS AND ENERGETICS OF HETEROPNEUSTES FOSSILIS BLOCH.

INTRODUCTION

The effect of nutritional parameters such as feed intake, dietary composition, energy levels, ratio of nutrients, and nutritional stress on the body composition of fish have been assessed by numerous workers in the past (Stickney and Andrews, 1971; Pandian and Raghurama, 1972; Garling and Wilson, 1976; Marais and Kissil, 1979; Reintz, 1983; Winfree, 1983; Rao and Vijayaraghavan, 1984; and Zeitler et al., 1984). The subject has also been extensively reviewed by Love (1970, 1980).

The performance of diet, on the basis of protein retention and/or turnover, and the total energy required to produce a unit weight of fish have been examined by Cowey (1975), Millward et al. (1976), Ogino, et al. (1976), Pfeffer (1982), and Fauconneau (1984). The response of varying protein:energy ratios in terms of bioenergetics has also been noted for several fish species (Lee and Putnam, 1973; Baird and Hopkins, 1981; Kerr, 1982; Eckhardt et al., 1983; Cho and Kaushik, 1984;
In the present study an attempt has been made to observe the effect of dietary protein levels on the body composition of *H. fossilis*. The relative retention of each nutrient has been worked out and the growth rates of fish fed different dietary protein levels measured using RNA:DNA ratio as a biochemical index.

MATERIALS AND METHODS

The ingredient composition of the diets, details of preparation and feeding trials were the same as described elsewhere (see Chapter I). The calculated caloric contribution of each source of energy and their relative percentages in the diet at different levels of dietary protein have been shown in Table I.

Fish from each dietary group were removed approximately 12 hours after the last feeding, filleted and the fillets analysed for their proximate composition. The estimation of nucleic acids was based on the methods proposed by Schneider (1957) for RNA, and Webb and Levy (1955) for DNA.(see pages 18-25). The caloric contents of the diet and fish tissue were assessed using the energy equivalents as quoted by Hastings.
(1979) for fresh fish. Proximate composition of fish sample analysed from each group before the commencement of the feeding trial was used for comparison (Table II).

RESULTS AND DISCUSSION

The chemical composition of H. fossilis fed isocaloric diets of various protein levels has been given in Table III and depicted graphically in Fig. 1. The protein content of the fish registered an increase over the initial (16.13) percentage with higher protein diets up to 40% level of incorporation when the maximum(17.76%) value was recorded. However, the muscle protein declined in fish fed on protein levels higher than 40%. It is interesting to observe that, in terms of protein deposition, the maximum (2.03%) deposition was noted at 40% dietary protein level (Table IV) with the degree of protein retention declining on either side. Jauncey (1982) observed that juvenile tilapias fed low dietary protein levels tended to have lower protein content. A significant increase in the crude protein content with increasing dietary protein levels have earlier been recorded in the snakeheads (Wee and Tacon, 1982 and Wee, 1983). Millikin (1982), on the other hand, observed no significant effect of dietary protein on the whole body protein, when young striped bass were fed for 6-week period. The same fish, on increasing the duration
of feeding over 10 weeks, showed increased concentration of
whole body protein with higher dietary protein concentration.
Similar observations were evident in plaice (Cowey et al., 1972)
and rainbow trout (Satia, 1974; and Austreng and Refstie, 1979).

Fat content of *H. fossilis* exhibited the identical trend
of increment with dietary protein levels (Table III). The
percentage of fat reached the maximum (2.53%) at 40% dietary
protein inclusion beyond which a significant fall in its
quantity was evident. A corollary to this trend was apparent
in the work of Nose and Arai (1972). Ogino et al. (1976) and
Mazid et al. (1979) have, however, noted decreased fat contents
in fish fed on higher protein diets.

Moisture content was minimum (71%) at 40% dietary protein
level, recording a trend opposite to those shown by protein and
fat contents of the muscle at various dietary protein levels.
The maximum (80.33%) moisture was noted in fish fed 60% dietary
protein level and this was found close to the value observed
in fish sacrificed before the commencement of feeding trial.

Fig. 2 depicts variations in the fat:moisture ratios of
fish muscle at various levels of dietary protein. The signi-
ficance of fat moisture relationship has been well explained
have been observed by Murray et al. (1977), Atack et al. (1979),
and Jauncey (1981) in fishes fed with different levels of
protein in the diet. Reinitz (1983) has recorded that increased dietary protein percentage resulted in lower moisture and higher fat percentages in fish carcass. Zeitler et al. (1984) reported that in *C. carpio* the fat and energy contents of carcass increased while the muscle protein contents decreased upon higher dietary protein intakes.

The carbohydrate content of the muscle of *H. fossilis* was found to increase with higher levels of dietary proteins, attaining the maximum (7.3%) at 40% protein incorporation level (Table III). Garling and Wilson (1977) found no significant change in the deposition of carbohydrate in channel catfish fed different ratios of carbohydrate and lipid diets.

In *H. fossilis*, the ash content of the muscle also registered an increase with dietary protein levels. Fish fed protein at 30-40% levels generally contained higher ash content (Table III). Ogino et al. (1976) and Mazid et al. (1979) found similar gains in the ash content of common carp on higher protein diets. In contrast, Cowey et al. (1974), Adron et al. (1976), Dabrowska and Wajno (1977), Atack et al. (1979), and Millikin (1983) observed no change in the ash content of the fish when fed on different dietary protein contents.

Table III shows the protein fat gains as well as energy retention in fish fed the varying protein level diets. Table IV
shows that the percentage of protein deposited varied with the dietary protein level. A marked increase in the protein deposition was seen with crude protein content of the feed from 10 to 40%, any further increase in the crude protein level of the diet resulted in declining values of the protein deposition. It can be noted that the protein utilization, assessed through protein conversion efficiencies, declined from 105.42 to 16.38% with increasing crude protein intake. However, the maximum (2.03%) protein deposition in fish muscle at 40% dietary protein intake indicated a protein conversion efficiency percentage of 38.81%.

The energy conversion efficiency percentage increased with the increment of dietary protein level up to 40%, followed by a simultaneous increase in energy retention (Table IV). It, therefore, appears that in isocaloric diets the supply of protein markedly influences levels of protein and energy depositions. It can be seen from the data (Table I and IV) that the increase in the protein energy:total energy (%) and protein:energy ratios of the diet was accompanied by higher energy conversion efficiency (%), and energy retained percentage up to 40% dietary protein level, beyond which a decline in the two set of values occurred. This implies that the ratio of calorie contribution from various energy sources have a direct bearing upon protein and energy conversion efficiencies and retention in fish.
In the dietary treatments carried out during the present study (Table I) the non-protein energy contribution from fat was constant, whereas the calories from carbohydrate varied (63.80 to 9.37%) inversely with protein calories (10.89 to 65.32).

Table V gives the data on the distribution of calories deposited in *H. fossilis* at different levels of dietary protein intake, as calculated from its proximate composition.

In terms of energy consumption and fish production, the maximum (29,771.00 cal/kg) calories were consumed at 10% dietary protein level to produce a kilogram of fish. The calorie consumption declined sharply with further increase in dietary protein level, reaching the minimum (6,593.40 cal/kg) at 40% dietary protein level (refer to Table XI, Chapter I). Further enhancement of dietary protein level, to 50% and 60%, respectively, pushed the caloric demand more than three-fold. It is worth noting that the calories consumption by fish at 20% and 50% dietary protein levels were more or less comparable (20,350.00 and 22,832.70 cal/kg, respectively).

One of the important factors altering the energy requirements of fish is the composition of diet and the ratios of food groups in it (Halver, 1970; and Pieper and Pfeffer, 1980). The utilization of protein calories for catabolic or anabolic purposes depends chiefly upon the availability of other calorie
sources such as fat and, to some extent, carbohydrate, to spare protein for tissue production. It is interesting to note that in *H. fossilis* fed lower dietary protein levels the live weight of fish showed no decline over their initial weights (see also Chapter I). It seems that at low (10% to 20%) dietary protein incorporations, the energy (63.80 and 52.92% of total calories) contributed through carbohydrate sources meets the normal maintenance demand of fish, and the available protein energy is spent on tissue production. Another possible reason for depressed growth at lower dietary protein calories could be the lack of balance in amino acids of respective diets. Cowey and Sargent (1972) have pointed out that utilization of dietary protein by fish depends greatly on the pattern of essential amino acids contained in the dietary protein source. On the other hand, poor growth with diets containing very high (54.44 to 65.30%) percentage of calories as protein could be attributed to a greater utilization of protein calories for energy purposes, firstly, due to reduced availability of carbohydrate energy for maintenance, and secondly, due to utilization of protein calories for removing the excess of toxic nitrogenous substances from the body.

It can safely be concluded that the ratio of food groups, amino acid balance, and the ratio of calories contributed by the calorie sources, are of prime importance in maintaining efficiency of the utilization of food calories for tissue
production. The actual calories consumed per fish day$^{-1}$ has been found to be minimum (0.739) at 40% dietary protein level (Table VI), and it was at this level that the fish showed the maximum (0.195/fish day$^{-1}$) calorie retention. In terms of food value, 40% protein level diet produced the best results (Table V). The protein to total energy ratio, with total energy level at 407 kcal/100 g dry diet, was 98.28 kcal/100 g at this level of dietary treatment. The calorie ratios with other energy sources at 40% protein diet were protein:carbohydrate 1.398; protein:fat 2.8133; carbohydrate:fat 2.0120; and carbohydrate:protein 7.151.

Table VII shows the effect of feeding isocaloric diet at different protein levels on the nucleic acids (RNA and DNA) concentrations of the muscle of *H. fossilis*. The RNA increased linearly from 1040.33 to 1156.00 μg/100 mg up to 40% dietary protein level but showed a sharp decline with further increment in the level of protein in diet. The pattern of quantitative changes in RNA noted in *H. fossilis* indicates not only enhanced growth with protein enriched diet but also points to the fact that there are limits to the amount of protein that a fish can convert to its body material. Brown and Roll (1965) have indicated increase in RNA content of the liver of *Pleuronectes platassa* with higher intake of dietary protein. The work of Mustafa and Jafri (1977) on growth and feeding relation to protein and RNA turnover in *C. punctatus* strengthens the
present findings. Since RNA is associated with protein biosynthesis or growth, changes in quantity of RNA observed in *H. fossilis* have also been compared with the 'condition' of fish through the assessment of condition coefficient. The best (0.697) condition coefficient has been found at 40% dietary protein intake and the poorest (0.565) at 10% dietary protein level. Mustafa (1979) has earlier pointed to the RNA and protein synthesis in relation to biological condition of *C. punctatus*. The relation of nucleic acid to condition factor in *H. fossilis* has also been indicated by Mustafa and Zofair (1983) who measured such changes by feeding fresh minced meat to the fish at 3% intake. Buckley (1979) and Smigielski (1980) noted increase, both in RNA and protein concentration of fish tissue, with increasing feeding rates, emphasizing the role of RNA as chief organizer of protein biosynthesis.

In *H. fossilis* fed varying levels of protein the DNA value was found to decline from 240 μg/100 mg in fish fed 10% protein diet to 202 μg/100 mg in those fed 40% protein diet. Thereafter, the DNA content increased with further increment in dietary protein level (Table VII). Since DNA carries the genetic material in each cell and is present in nucleus in fixed quantity (Love, 1970, 1980), it is considered as an index of cell number contributing to per unit weight of tissue. In a lean fish, the number of cells contributing to unit weight
of tissue increases, enhancing the number of nucleus and thereby elevating the DNA content. In a robust fish, on the other hand, the DNA content apparently gets diluted with larger volume of cells per unit weight.

In the present study, the group with best coefficient of condition value showed the lowest value of DNA compared to one (lean fish) with a poor condition coefficient. Lied and Rosenlund (1984), who observed slightly higher average values of DNA in muscle tissue of the Atlantic cod (Gadus morhua) fed low level of protein, compared to fish fed higher dietary protein level, have ascribed the change to difference in cell diameter due to reduced synthesis of myofibrillar protein.

The RNA:DNA ratio is considered a sensitive measure of the growth rate of fish (Love, 1980). Fig. 3 represents the changes in the RNA:DNA ratios of H. fossilis fed different dietary protein levels. The ratios ranged from 3.920 to 5.722. The fish sacrificed before the commencement of feeding trial showed the poorest (3.692) ratio, indicating a poor growth which improved proportionally up to 40% dietary protein incorporation when the best (5.722) ratio was observed. The data indicates that fish with high RNA:DNA ratios at 40% dietary protein level, were actively synthesizing and accumulating protein, resulting in faster growth, compared to groups with low RNA:DNA ratios receiving either low or very high
protein diets. It may be pointed out that increment of protein energy:total energy ratio (%) from 9.8 to 58.87, calculated from the quantity of diet consumed by the fish, had no marked effect on the RNA:DNA ratio throughout the feeding regime (Table VII). However, the RNA:DNA ratio reached the maximum (5.722) when the protein energy:total energy ratio was 39.31. In recent years, considerable interest has been generated in the study of RNA:DNA ratios in fish as a tool for the measurement of growth and/or protein synthesis (Bulow et al. 1981; Fauconneau, 1984; and Lied and Rosenlund, 1984). The RNA:DNA ratio as indicator of growth rates in fish has earlier been worked out by Bulow (1970) and Haines (1973). It has been correlated with prey density and growth rate in Gadus larvae by Buckley (1979), Bulow et al. (1981) have measured this ratio in bluegill, Lepomis macrochirus, for assessing the feeding, reproduction, energy storage and condition of this species in a population.

Thus, in terms of energetics, the 40% protein diet appeared to be the most economical accomplishing the maximum growth in H. fossilis. The fact is also evident from energy and protein intake per fish day\(^{-1}\) (Table VI).
SUMMARY

The effect of isocaloric diets of varying protein levels on the body composition of *Heteropneustes fossilis* was examined. The maximum protein, fat and carbohydrate deposition in the muscle occurred in fish fed 40% dietary protein beyond which a marked depletion was evident. The moisture content showed an inverse relationship with the above nutrient. The protein conversion efficiency improved with increasing protein intakes. The ratio of calorie contribution from various energy sources were found to have a direct bearing upon protein and energy conversion efficiencies, and retention in fish. Minimum calories were required to produce a kilogram of fish at 40% dietary protein intake. The pattern of quantitative changes in RNA concentration indicates not only enhanced growth with protein-enriched diet, up to 40% level of incorporation, but also points to the fact that there are limits to the amount of protein that a fish can convert to its body material. The DNA concentration declined up to 40% dietary protein level. Assessment of RNA:DNA ratio and coefficient of condition of fish have also been made and their significance discussed.
TABLE I. Calorie contribution from different energy sources, with relative percentages in diets, at different levels of dietary protein

<table>
<thead>
<tr>
<th>DIETARY PROTEIN LEVELS</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy through protein source</td>
<td>44.30</td>
<td>88.60</td>
<td>132.92</td>
<td>177.24</td>
<td>221.52</td>
<td>265.84</td>
</tr>
<tr>
<td>% of calories as protein</td>
<td>10.89</td>
<td>21.77</td>
<td>32.66</td>
<td>43.55</td>
<td>54.44</td>
<td>65.32</td>
</tr>
<tr>
<td>Energy through carbohydrate</td>
<td>259.70</td>
<td>215.90</td>
<td>171.08</td>
<td>126.76</td>
<td>82.48</td>
<td>38.16</td>
</tr>
<tr>
<td>% of calories as carbohydrate</td>
<td>63.80</td>
<td>52.92</td>
<td>42.03</td>
<td>31.14</td>
<td>20.26</td>
<td>9.37</td>
</tr>
<tr>
<td>Energy through fat (Non-protein energy)</td>
<td>63.00</td>
<td>63.00</td>
<td>63.00</td>
<td>63.00</td>
<td>63.00</td>
<td>63.00</td>
</tr>
<tr>
<td>% of calories fat</td>
<td>15.48</td>
<td>15.48</td>
<td>15.48</td>
<td>15.48</td>
<td>15.48</td>
<td>15.48</td>
</tr>
<tr>
<td>Total energy (kcal/100 g)</td>
<td>407</td>
<td>407</td>
<td>407</td>
<td>407</td>
<td>407</td>
<td>407</td>
</tr>
<tr>
<td>Protein energy: Total energy (%)</td>
<td>10.88</td>
<td>21.77</td>
<td>32.66</td>
<td>43.55</td>
<td>54.43</td>
<td>65.32</td>
</tr>
<tr>
<td>Protein:Energy ratio</td>
<td>24.57</td>
<td>49.14</td>
<td>73.71</td>
<td>98.28</td>
<td>122.85</td>
<td>147.42</td>
</tr>
</tbody>
</table>
TABLE II. Proximate composition (g/100 g), energy and nucleic acid (µg/100 mg) content of *H. fossilis* at the commencement of feeding trial

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (g/l00 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>16.830</td>
</tr>
<tr>
<td>Fat</td>
<td>1.133</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.740</td>
</tr>
<tr>
<td>Moisture</td>
<td>80.330</td>
</tr>
<tr>
<td>Ash</td>
<td>0.966</td>
</tr>
<tr>
<td>Energy (cal/100 g)</td>
<td>80.477</td>
</tr>
<tr>
<td>RNA</td>
<td>1005.600</td>
</tr>
<tr>
<td>DNA</td>
<td>272.330</td>
</tr>
<tr>
<td>RNA:DNA ratio</td>
<td>3.692</td>
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</tbody>
</table>
TABLE III. Proximate composition (g/100 g) and energy content of *H. fossilis*, fed different levels of dietary protein, at the end of feeding trial

<table>
<thead>
<tr>
<th></th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>1.633</td>
<td>1.833</td>
<td>2.300</td>
<td>2.533</td>
<td>1.466</td>
<td>1.233</td>
</tr>
<tr>
<td>Moisture</td>
<td>79.660</td>
<td>77.330</td>
<td>74.000</td>
<td>71.000</td>
<td>74.660</td>
<td>80.330</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>1.630</td>
<td>3.030</td>
<td>5.030</td>
<td>7.310</td>
<td>6.430</td>
<td>2.130</td>
</tr>
<tr>
<td>Ash</td>
<td>1.060</td>
<td>1.260</td>
<td>1.400</td>
<td>1.400</td>
<td>1.260</td>
<td>1.030</td>
</tr>
<tr>
<td>Energy (cal/100 g)</td>
<td>85.737</td>
<td>94.737</td>
<td>109.740</td>
<td>123.050</td>
<td>103.550</td>
<td>80.657</td>
</tr>
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</table>
TABLE IV. Retention and conversion efficiency of protein and energy in *H. fossilis* at different levels of dietary protein

<table>
<thead>
<tr>
<th>DIETARY PROTEIN LEVELS</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein deposited (%)</td>
<td>-4.575</td>
<td>-1.016</td>
<td>0.885</td>
<td>2.032</td>
<td>-0.469</td>
<td>-1.685</td>
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<tr>
<td>Protein conversion efficiency (%)</td>
<td>105.424</td>
<td>56.033</td>
<td>38.161</td>
<td>38.811</td>
<td>20.520</td>
<td>16.382</td>
</tr>
<tr>
<td>Energy retained (%)</td>
<td>0.851</td>
<td>2.382</td>
<td>4.783</td>
<td>9.149</td>
<td>3.605</td>
<td>0.034</td>
</tr>
</tbody>
</table>

* as calculated from initial body composition (see Table II)
TABLE V. Distribution of calories deposited in *H. fossilis* at different levels of dietary protein

<table>
<thead>
<tr>
<th>Dietary Protein Levels</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>64.520</td>
<td>66.12</td>
<td>68.920</td>
<td>71.040</td>
<td>64.640</td>
<td>61.040</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>5.520</td>
<td>12.120</td>
<td>20.120</td>
<td>29.240</td>
<td>25.810</td>
<td>8.600</td>
</tr>
<tr>
<td>Total calories</td>
<td>85.737</td>
<td>94.737</td>
<td>109.780</td>
<td>123.050</td>
<td>103.550</td>
<td>80.650</td>
</tr>
</tbody>
</table>
TABLE VI. Relationship between total calories consumed and retained by *H. fossilis* (per fish day$^{-1}$) at various dietary protein levels

<table>
<thead>
<tr>
<th>Dietary protein level (%)</th>
<th>Actual calories consumed per fish day$^{-1}$</th>
<th>Calories retained per fish day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.988</td>
<td>0.136</td>
</tr>
<tr>
<td>20</td>
<td>0.952</td>
<td>0.150</td>
</tr>
<tr>
<td>30</td>
<td>0.972</td>
<td>0.174</td>
</tr>
<tr>
<td>40</td>
<td>0.739</td>
<td>0.195</td>
</tr>
<tr>
<td>50</td>
<td>1.018</td>
<td>0.164</td>
</tr>
<tr>
<td>60</td>
<td>1.004</td>
<td>0.128</td>
</tr>
<tr>
<td>Dietary protein level</td>
<td>PE/TE</td>
<td>RNA (μg/100 mg dry weight)</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>10%</td>
<td>9.80</td>
<td>1040.33</td>
</tr>
<tr>
<td>20%</td>
<td>19.65</td>
<td>1050.66</td>
</tr>
<tr>
<td>30%</td>
<td>29.46</td>
<td>1105.00</td>
</tr>
<tr>
<td>40%</td>
<td>39.31</td>
<td>1156.00</td>
</tr>
<tr>
<td>50%</td>
<td>49.14</td>
<td>1079.00</td>
</tr>
<tr>
<td>60%</td>
<td>58.87</td>
<td>1045.00</td>
</tr>
</tbody>
</table>
Fig. 1. Effect of dietary protein level on the proximate composition of *H. fossilis*, protein: \( \bullet \) — \( \bullet \), fat: \( \bullet \) — \( \bullet \), carbohydrate: \( \Box \) — \( \Box \), ash: \( \triangle \) — \( \triangle \), moisture: \( \bigcirc \) — \( \bigcirc \).
Fig. 2. Variations in the fat:moisture ratio of *H. fossilis* at varying dietary protein levels.
Effect of dietary protein level on RNA:DNA ratio of *H. fossilis*.
CHAPTER III

QUALITATIVE AMINO ACID REQUIREMENT OF HETEROPNEUSTES FOSSILIS BLOCH.

INTRODUCTION

The prime objective of fish husbandry is to produce fish flesh mainly to obtain protein. The rate of protein turnover in fish is governed chiefly by the quality of dietary protein (Halver, 1961; Mertz, 1972; Love, 1980; and Wilson, 1984), the most effective protein matching the exact qualitative and quantitative amino acid requirements of the fish. The amount of protein to be provided in practical diets, besides depending on the amino acid composition, depends largely on digestibility of the diet (Wilson et al., 1981; Yamada et al., 1981; and Boge et al., 1982).

The development of standard reference protein (amino acid) diet for fish by Halver (1957) enabled a number of workers to identify and quantify the amino acids indispensable to various fish species (Mertz, 1969, 1972; Nose, 1974, 1979b; Nose et al., 1974; Cowey, 1979; Arai et al., 1982; Hughes et al., 1983; and Wilson, 1984). Most of the studies concerning the amino acid
requirements of fish have been limited to species like chinook salmon, common carp, Japanese eel and channel catfish, etc. Some information is also available on selected amino acid requirements of coho salmon, rainbow trout, lake trout, gilt-head bream, and tilapia. An interesting review on the amino acid nutrition of fishes has recently been published by Ketola (1982).

It has now been fairly well established that all fish apparently require the same ten indispensable or essential dietary amino acids as required by most other animals (Cowey et al., 1972; Mertz, 1972; NAS-NRC, 1977; and Halver, 1979\textsuperscript{b}). These indispensable amino acids in proper proportion are determinants for the success and failure of any fish husbandry programme. Fish that are fed diets devoid of any indispensable amino acid fail to grow normally and may develop other deficiency symptoms (Halver, 1979\textsuperscript{b}). Arginine has been found to be indispensable for salmonids, ictalurids and cyprinids. Lysine and tryptophan in rations also determined the rate of growth in several fish species. Low levels of tryptophan resulted in scoliosis and lordosis in fish (Dupree and Halver, 1970).

Since arginine, lysine and tryptophan are 'limiting' in many of the commonly used plant protein dietary ingredients, and are presumably required by most warmwater fish in
considerable quantities, these have exclusively been selected for the present investigations on the siluroid, *H. fossilis*.

Studies concerning the amino acid requirements of Indian cultivable fish species are not well documented. In the present study on *H. fossilis* an attempt has been made to examine the growth response of fish to diets deficient in the above mentioned amino acids. The study could be a logical sequence to future studies on the quantitation of these and other indispensable amino acids, as well as to the formulation of practical diet, with least-cost and improved efficiency, for this species.

**MATERIALS AND METHODS**

**Preparation of experimental diets**

The composition of amino acid test diets have been shown in Table I.

The amino acid balance in the test diets was based on the casein-gelatin pattern developed by Halver (1957) for chinook salmon and adopted later by Nose (1974) for *C. carpio*. The protein level in the diets used during the present investigation was adjusted to 40% by weight as per the estimated protein requirement of *H. fossilis* (see Chapter I). Five
isoprotein purified diets were prepared for the present study. The diet containing the full compliment of crystalline amino acids has been referred to as the amino acid (control) test diet. In the deficient diets, three amino acids, namely, arginine, lysine and tryptophan, were replaced with α-cellulose on equal weight basis. The levels of inclusion of other ingredients were, however, identical in all the test diets. A 40% protein test diet simulating the amino acid balance of the control test diet was likewise prepared for comparison using casein and gelatin as protein sources. Details of the mineral and vitamin mixtures used have been indicated in Tables III-IV of Chapter I. All amino acids used were of L-series. The method of diet preparation was the same as described in the previous Chapter (pages 33-34).

The recovery test diets used during the second phase of the experiment were prepared in the same manner by adding the deleted amino acid to each formulation.

Feeding trial

150 fish (average weight 3.63 ± 0.203 g; average total length 10.38 ± 0.190 cm) were selected from the acclimatized fish stock (see page 17). The individuals were weighed to the nearest mg and transferred to 40-l glass aquaria (water volume 30-l), at the stocking rate of 15 fish per aquarium, in duplicate groups. Prior to the commencement of observation, each group
was acclimatized for a further period of 2-week on specific isoprotein test diet.

Recovery test was conducted after a period of 4 weeks. One replicate from each group continued to receive the amino acid-deficient diet, while the fish in the other replicate were switched over to the feed containing the appropriate amount of deleted amino acid.

The fish were fed to satiation daily (17.00 h) with slow sinking soft pellets. The fish were fed 6 days a week, no food being given on the day the weekly measurements were made. The diets were dispersed with care at the surface layer of water in each aquarium. Accumulation of diet at the bottom was avoided to prevent possible leaching of nutrients. The average daily water temperature over the course of feeding trial was $25 \pm 1^\circ C$. The average dissolved oxygen was $7.3 \pm 1$ ppm. A record of the quantity of food consumed over the experimental period has also been carefully maintained. Details of the measurement, calculation of various parameters and the general experimental precautions have been outlined under 'General Methodology' section.

RESULTS AND DISCUSSION

The result of 8-week trial conducted to evaluate the effect of feeding the amino acid test diets deficient in lysine,
tryptophan and arginine, with recovery tests, and the comparative response of *H. fossilis* fed casein-gelatin diet have been presented in Table II and depicted graphically in Figs. 1-3.

The mean individual live weight of fish fed the amino acid (control) test diet increased from 3.3 g to 4.5 g, corresponding to an average gain of 36%, and specific growth rate of 0.642, over the experimental period. The calculated daily gain was 21 mg day\(^{-1}\) per individual. The food conversion ratio observed with this diet was 3.55:1, the gross growth efficiency and protein efficiency ratio being 0.281 and 0.703, respectively.

Fish fed the lysine-deficient diet increased from 3.5 g to 4.0 g, registering about 15.6% gain in weight. The low daily increment in live weight (9.5 mg day\(^{-1}\) per individual) reduced the specific growth rate to 0.274% over the experimental period. The corresponding values of food conversion ratio, gross growth efficiency and protein efficiency ratio in fish group fed the lysine-deficient diet were found to be 3.7:1, 0.27 and 0.67, respectively.

The mean individual live weight of fish fed the tryptophan-deficient diet increased from 3.8 g to 4.2 g, encountering an overall gain of 10.5%, over the period of feeding trial. The daily weight increment in the fish declined further to
7 mg day^{-1} per individual. A fall in the specific growth rate (0.187%) was likewise evident. Poor food conversion ratio (6.16:1), protein efficiency ratio (0.405) and gross growth efficiency ratio (0.16) were also noticeable.

The performance of arginine-deficient diet, in terms of conversion efficiencies, was found to be the poorest, though the average live weight of the fish fed this diet increased from 3.6 g to 4.0 g over the 8-week experimental period, maintaining a gain (7 mg day^{-1} per individual) almost similar to the fish fed the tryptophan-deficient diet. A slightly higher (0.198) value for the specific growth rate was, however, noted for the fish lot fed the arginine-deficient diet. The values of food conversion ratio, gross growth efficiency and protein efficiency ratio were 7.8:1, 0.128 and 0.319, respectively.

Thus, in order of dietary performance, the lysine-deficient diet was followed by tryptophan- and arginine-deficient diets, the depressing effect on growth being in the reverse order.

Recovery test initiated from the beginning of the 5th week with the fish groups showing the depressed growth, incorporating the deficient amino acids in the diets, indicated marked improvement in growth. The values of the various parameters obtained on recovery have been shown in Table II. The
changes in live weight gain percent have been depicted in Fig. 1-3. The maximum (25%) recovery in terms of live weight gain percent was recorded for lysine followed by tryptophan and arginine.

Growth of fish fed the casein-gelatin diet was much distinct maintaining almost a linear pattern. The mean live weight of the fish on this diet increased from 3.73 g to 7.07 g, over the investigation period, corresponding to a gain of 89% and specific growth rate of 1.59%. Maximal (0.059 g) value was also observed for the live weight gain day\(^{-1}\) per fish. The food conversion ratio, gross growth efficiency and protein efficiency ratio recorded were 2.02:1, 49.50 and 1.237, respectively.

Table III summarizes the data pertaining to the average daily intake of food and corresponding gains in live weight over the experimental period, in fish of various groups. The intake of amino acid (control) test diet was 76 mg day\(^{-1}\) per fish. The consumption of food was minimum (35 mg day\(^{-1}\) per fish) in group fed the lysine-deficient diet, whereas the tryptophan-and arginine-deficient diets were consumed to the extent of 44 mg and 55 mg day\(^{-1}\) per individual. During the recovery test the intake showed a positive increase with all the diets except with arginine incorporated diet. Maximum (120 mg day\(^{-1}\) per fish) intake was, however, noted for the casein-gelatin diet.
The analysis of the experimental data indicate that growth of *H. fossilis* fed the various test diets was significant ($P < 0.05$) over the experimental period, with marked differences in conversion efficiencies.

Depressed growth, low food consumption and sluggishness generally characterized the fish fed on diets devoid of any of the three amino acids. The addition of the deficient amino acid improved the feed intake as well as growth indicating towards the indispensability of these amino acid to the fish. Similar signs of deficiency of dietary amino acid in fishes have earlier been pointed out by Ketola (1982).

Arginine has been reported to be indispensable amino acid for salmonids (Halver, 1957; Halver and Shanks, 1960; Shanks et al., 1962), ictalurids (Dupree and Halver, 1970) and cyprinids (Aoe et al., 1970). It has been emphasized that requirement for this amino acid in fish may be considerably higher (NAS-NRC, 1977). The minimum requirement for arginine noted for trout varied between 5.4-5.9% of dietary protein (Ketola, 1982). Lysine also determines the rate of growth in fish and has been a major component of dietary mixture essential for promoting growth in several fish species (Halver, 1979). The minimum requirement of this amino acid, as high as 6 percent of dietary protein, has been quoted in fish like rainbow and lake trout (Ketola, 1982). The lysine
requirement of catfish is reported to be higher than that of eel and chinook salmon (NAS-NRC, 1977).

The requirement of fish for tryptophan is reported to be relatively low. Halver (1965) has indicated that salmon requires only about 0.5% of protein as tryptophan.

A comparison of the dietary performance of amino acid (control) and casein-gelatin diets indicates that the fish fed the amino acid (control) test diet recorded 53% less gain in live weight over the fish fed the casein-gelatin diet.

The fish receiving the amino acid deficient diets, on the other hand, registered 20 to 26% retardation in growth over those receiving the amino acid (control) diet. H. fossilis, like several other warmwater fishes (NAS-NRC, 1977), do not appear to utilize amino acids in crystalline form in diet efficiently, the amino acid balance obtainable with combinations of natural protein sources such as casein and gelatin being more effectively utilized. Wilson et al. (1980) have also pointed to poor utilization of crystalline amino acids by catfish. Andrews (1977) observed significant increase in weight gain in catfish with the addition of gelatin to purified diets. Amino acid test diet could not also sustain the growth of young C. carpio even when modified in various ways (Aoe et al., 1970). Nose et al. (1974), however, succeeded
in growing *C. carpio* to some extent on amino acid test diet neutralized with NaOH. Test diets composed of crystalline amino acids, however, resulted in satisfactory growth for eel, red seabream and shrimp (NAS-NRC, 1977). It is evident that, besides the amino acid pattern in protein component of diet, the source of nutrient is an important factor in optimizing the utilization of dietary protein by fish. The gross ability of the fish to utilized fully the indispensable amino acid from the source could also be attributed to digestive physiology of fish (Dabrowski, 1983).

The apparent health status of *H. fossilis* fed various diets have been depicted in Fig. 4. Although no specific deficiency syndrome, except those mentioned earlier, appeared in fish receiving the various diets, fish fed the arginine-deficient diet were observed to become more sluggish, showing stressed and slow movement to the surface, and were frequently found to rest with their head horizontal to the water surface and remainder of the body dropping down the water column (Fig. 5a) in contrast to active fish (Fig. 5b). No signs of lordosis and scoliosis appeared in any of the individuals fed the tryptophan-deficient diet during the present study. Though there are reports of these tryptophan deficiency symptoms in fishes like chinook salmon and rainbow trout (NAS-NRC, 1977), no such deficiency symptoms have been reported in catfish (Wilson *et al.*, 1978).
SUMMARY

The qualitative requirement of selected indispensable amino acids have been assessed for Heteropneustes fossilis fed isoprotein purified amino acid test diets at 40% dietary protein level. Depressed growth, low food consumption and sluggishness generally characterized the fish fed diets devoid of test amino acids. In terms of performance, the arginine-deficient diet gave the poorest results. This was followed by tryptophan and lysine-deficient diets. Incorporation of the deficient amino acids in the diets indicated marked improvement in growth. Ariginine, tryptophan and lysine thus appeared indispensable to H. fossilis. A comparison of the dietary performance of amino acid (control) test diet with casein-gelatin diet, however, showed poor utilization of crystalline amino acids by the catfish.
<table>
<thead>
<tr>
<th></th>
<th>Casein-Gelatin Diet</th>
<th>Amino-Acid (control) Test diet</th>
<th>Arginine-deficient diet</th>
<th>Lysine-deficient diet</th>
<th>Tryptophan-deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>-</td>
<td>2.860</td>
<td>-</td>
<td>2.860</td>
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<td>Histidine</td>
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<td>1.430</td>
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<td>2.300</td>
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<td>Lysine</td>
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<td>2.850</td>
<td>-</td>
<td>2.850</td>
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<td>2.280</td>
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<td>Tryptophan</td>
<td>-</td>
<td>0.610</td>
<td>0.610</td>
<td>0.610</td>
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<tr>
<td>Tyrosine</td>
<td>-</td>
<td>2.300</td>
<td>2.300</td>
<td>2.300</td>
<td>2.300</td>
</tr>
<tr>
<td>Valine</td>
<td>-</td>
<td>2.300</td>
<td>2.300</td>
<td>2.300</td>
<td>2.300</td>
</tr>
<tr>
<td>Glycine</td>
<td>-</td>
<td>2.900</td>
<td>2.900</td>
<td>2.900</td>
<td>2.900</td>
</tr>
<tr>
<td>Alanine</td>
<td>-</td>
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<td>2.000</td>
<td>2.000</td>
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</tr>
<tr>
<td>Aspartic acid</td>
<td>-</td>
<td>2.900</td>
<td>2.900</td>
<td>2.900</td>
<td>2.900</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-</td>
<td>0.300</td>
<td>0.300</td>
<td>0.300</td>
<td>0.300</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>-</td>
<td>4.600</td>
<td>4.600</td>
<td>4.600</td>
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<tr>
<td>Proline</td>
<td>-</td>
<td>2.900</td>
<td>2.900</td>
<td>2.900</td>
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</tr>
<tr>
<td>Serine</td>
<td>-</td>
<td>1.700</td>
<td>1.700</td>
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<tr>
<td>Leucine</td>
<td>-</td>
<td>3.400</td>
<td>3.400</td>
<td>3.400</td>
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</tr>
<tr>
<td>Vitamin free casein</td>
<td>31.428</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin</td>
<td>8.571</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn oil (Cornello)</td>
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<td>08.000</td>
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</tr>
<tr>
<td>Cod liver oil</td>
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<td>02.000</td>
<td>02.000</td>
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<tr>
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<td>21.000</td>
<td>21.000</td>
<td>21.000</td>
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</tr>
<tr>
<td>Mineral mix</td>
<td>04.000</td>
<td>04.000</td>
<td>04.000</td>
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<tr>
<td>Vitamin mix</td>
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<td>03.000</td>
<td>03.000</td>
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<tr>
<td>α-Cellulose</td>
<td>12.000</td>
<td>12.000</td>
<td>12.000</td>
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<td>12.000</td>
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<tr>
<td>Carboxymethyl cellulose</td>
<td>10.000</td>
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<td>10.000</td>
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</tr>
</tbody>
</table>
### TABLE II. Results of feeding crystalline amino acid (control), casein-gelatin and amino acid deficient diets to *H. fossilis*

<table>
<thead>
<tr>
<th></th>
<th>Amino acid (control) test diet</th>
<th>Casein-Gelatin diet</th>
<th>Arginine-deficient diet</th>
<th>Recovery test diet</th>
<th>Lysine-deficient diet</th>
<th>Recovery test diet</th>
<th>Tryptophan-deficient diet</th>
<th>Recovery test diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mean individual wet weight (g)</td>
<td>3.330</td>
<td>3.733</td>
<td>3.600</td>
<td>3.730</td>
<td>3.460</td>
<td>3.460</td>
<td>3.800</td>
<td>3.933</td>
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<tr>
<td>Final mean individual wet weight (g)</td>
<td>4.530</td>
<td>7.066</td>
<td>4.000</td>
<td>4.400</td>
<td>4.000</td>
<td>4.300</td>
<td>4.200</td>
<td>4.660</td>
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<tr>
<td>Gain %</td>
<td>36.360</td>
<td>89.300</td>
<td>11.110</td>
<td>17.962</td>
<td>15.600</td>
<td>25.140</td>
<td>10.520</td>
<td>18.650</td>
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<tr>
<td>Specific growth rate (%)</td>
<td>0.642</td>
<td>1.590</td>
<td>0.198</td>
<td>0.380</td>
<td>0.274</td>
<td>0.446</td>
<td>0.187</td>
<td>0.332</td>
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<tr>
<td>Gain/Fish/Day</td>
<td>0.021</td>
<td>0.059</td>
<td>0.007</td>
<td>0.011</td>
<td>0.009</td>
<td>0.015</td>
<td>0.007</td>
<td>0.013</td>
</tr>
<tr>
<td>Food/Fish/Day</td>
<td>76.190</td>
<td>0.120</td>
<td>0.055</td>
<td>0.055</td>
<td>0.035</td>
<td>0.050</td>
<td>0.044</td>
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<tr>
<td>Food conversion ratio</td>
<td>3.55:1</td>
<td>2.02:1</td>
<td>7.8:1</td>
<td>4.7:1</td>
<td>3.7:1</td>
<td>3.23:1</td>
<td>6.16:1</td>
<td>4.18:1</td>
</tr>
<tr>
<td>Gross growth efficiency</td>
<td>0.281</td>
<td>0.495</td>
<td>0.128</td>
<td>0.213</td>
<td>0.266</td>
<td>0.309</td>
<td>0.162</td>
<td>0.239</td>
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<tr>
<td>Protein efficiency ratio</td>
<td>0.703</td>
<td>1.237</td>
<td>0.319</td>
<td>0.531</td>
<td>0.666</td>
<td>0.773</td>
<td>0.405</td>
<td>0.597</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Duration (week)</td>
<td>Amino Acid (control) test diet Food intake</td>
<td>Casein-Gelatin diet Food intake</td>
<td>Arginine-deficient diet Food intake</td>
<td>Recovery test diet Food intake</td>
<td>Lysine-deficient diet Food intake</td>
<td>Recovery test diet Food intake</td>
<td>Tryptophan-deficient diet Food intake</td>
<td>Recovery test diet Food intake</td>
</tr>
<tr>
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<tr>
<td>I</td>
<td>66.0</td>
<td>19.0</td>
<td>123.0</td>
<td>38.0</td>
<td>47.0</td>
<td>9.5</td>
<td>42.0</td>
<td>9.5</td>
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<td>14.0</td>
<td>128.0</td>
<td>38.0</td>
<td>52.0</td>
<td>4.0</td>
<td>45.0</td>
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<tr>
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<td>41.0</td>
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<td>IV</td>
<td>71.0</td>
<td>14.0</td>
<td>111.0</td>
<td>50.0</td>
<td>50.0</td>
<td>5.9</td>
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<td>V</td>
<td>76.0</td>
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<td>104.0</td>
<td>49.0</td>
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<td>VI</td>
<td>84.0</td>
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<td>7.4</td>
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<tr>
<td>VII</td>
<td>78.9</td>
<td>19.0</td>
<td>117.0</td>
<td>58.0</td>
<td>53.0</td>
<td>9.25</td>
<td>51.7</td>
<td>14.4</td>
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<tr>
<td>VIII</td>
<td>76.0</td>
<td>21.0</td>
<td>120.0</td>
<td>59.0</td>
<td>55.0</td>
<td>7.0</td>
<td>55.0</td>
<td>11.0</td>
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</tbody>
</table>
Fig. 1. Growth of *H. fossilis* fed —,—, casein-gelatin, diet; ——, complete amino acid (control) test diet, and (Δ) lysine-deficient diet.
Fig. 2. Growth of *H. fossilia* fed (••••) casein-gelatin diet; (○○○○), complete amino acid (control) test diet, (△△△△); and tryptophan-deficient diet.
Fig. 3. Growth of *H. fossilis* fed ( ) casein gelatin diet; ( ) control amino acid (control) test diet; and ( ) arginine-deficient diet.
Fig. 4. The health status of fish fed various purified diets.
CONTROL

CASEIN-GELATIN

L-ARGININE DEFICIENT

L-LYSINE DEFICIENT

L-TRYPTOPHAN DEFICIENT
Fig. 5\textsuperscript{a}. Arginine-deficient fish under acute stress.

Fig. 5\textsuperscript{b}. An active fish feed on casein-gelatin test diet.