CHAPTER I
BIOENERGETICS, GROWTH AND RNA-DNA TURNOVER IN TISSUES OF THE UNDER YEARLINGS OF MAJOR CARP CIRRHINA MIRIGALA FED ARTIFICIAL DIETS

INTRODUCTION

Practical diets for commercial fishes are formulated to contain energy sources besides other nutrients indispensable for promoting growth and ensuring healthy survival. In case of farm animals like fishes the main consideration is to assure maximum possible conversion of quality feeds of low price into superior flesh for human consumption. Inflation in the cost of balanced fish feeds is a serious challenge to the profitability of fish husbandry enterprise. The present work was designed to formulate diets for major carp Cirrhina mrigala by incorporating low priced products, even those which are considered outright waste, and to test their nutritional efficacy. The nutritional data were correlated with dynamics of variation in RNA and DNA in fish tissues. The fish selected forms substantial part of inland catch in many Indo-Pacific Countries and is widely cultured in freshwater ponds. Its consumer appeal is high.

MATERIALS AND METHODS

Young specimens of Cirrhina mrigala (total length 4.4-8.5 cm, body weight 3.3-7.3 g) were procured from a freshwater pond in
University campus at Aligarh (Lat. 27° 37' 30" N, Long. 78° 9' 26" E). The specimens were 8 months old according to authentic record. Fish were reared in tanks supplied with filtered water of the same pond for 7 days and were exposed to natural photoperiod. Water was renewed daily. Specimens were grouped randomly into four duplicate batches, each of 30 individuals. Fish of different batches were maintained in tanks of equal capacity. Temperature, dissolved oxygen and pH of the medium were 26-28°C, 4.5 parts per million and 7.5, respectively.

Four types of diets ('A', 'B', 'C', 'D') were compounded. Their composition has been indicated in Table I. Details of feed processing are given below.

**Frog offal meal**

Frog wastes (offal) including viscera, skin, head, flesh pieces etc. were procured as a mixed lot and washed thoroughly using potable water. The product was then cooked under high pressure for about 1-1\(\frac{1}{2}\) hours and the cooked mass transferred to a pressing machine. The liquid (stickwater) was pressed out. The resulting product was transferred to a thermostat and incubated at 100°C till it was completely dry. This dried matter was placed in muslin cloth bag and dipped in a mixture containing 0.5% glacial acetic acid and equivalent volume of 0.5% aqueous solution of sodium benzoate. The mixture served as a preservative. After 3-5 hours
of treatment at 100°C, the bag was taken out to room temperature, pressed to remove the liquid and spread over metallic trays in the electric thermostat running at 100°C. The process continued for at least 48 hours and the dry product was powdered and stored.

Water hyacinth meal

Water hyacinth was obtained from canals in the university campus where it grows prolifically. The plant was washed with tap water and transferred to thermostat maintained at 100°C till the product was dry. The dry matter was then powdered in a mechanical grinder.

Blood meal

Blood collected from the slaughter house was dried at 100°C in thermostat and stored in sealed containers.

Egg shells

Discarded shells of poultry eggs were procured, washed with water and finely powdered in the grinder.

Mustard oil cake, linseed oil cake, cotton seed, wheat bran, sugarcane bagasse

These products were collected, dried in thermostat at 100°C and powdered separately in the grinder. Various ingredients thus
obtained were mixed in different proportions and the finely ground dry diets were supplied separately to the different batches at the rate 3\% of body weight every day. Size of the ration was decided after carrying out preliminary feeding trials before the experiment with the objective of finding out the food quantity commensurate with the appetite of the fish and avoiding wastage. Experimental duration was limited to 2 weeks in anticipation of abrupt fluctuations in environmental factors in the forthcoming season. On fifteenth day the specimens were taken out of the tanks and measured for their weight.

Instantaneous/Specific Growth Rate (SGR) was calculated by the equation:

\[
SGR = \frac{(\log e W_2 - \log e W_1)}{D} \times 100
\]

where,

- \( W_1 \) = Initial body weight (g)
- \( W_2 \) = Final body weight (g)
- \( D \) = Days of feeding

Gross Food Conversion Efficiency (FCE) was evaluated using the formula:

\[
FCE = \frac{\gamma}{\delta} \times 100
\]
where, $G =$ Gain in body weight ($g$)
$I =$ Amount of food consumed ($g$)

To measure the efficiency of protein utilization, Protein Efficiency Ratio (PER) was derived by the help of the equation:

$$\text{PER} = \frac{\text{Gain in body weight} (g)}{\text{Protein intake} (g)}$$

Fish were decapitated and sample of skeletal muscle excised from a fixed location in trunk region. Known weight of the tissue samples were processed for quantitation of RNA and DNA. Dry fat-free tissue was processed following the technique of Webb and Levy (1955). Known weight of the sample was homogenized in measured volume of distilled water and treated with two volumes of 10% trichloroacetic acid (TCA). The contents were centrifuged at 3000-4000 rpm for 15-20 minutes and the supernatant discarded. The process was repeated thrice to ensure complete removal of acid-soluble and chromogenic substances. Pellet was treated with 95% ethyl alcohol and the contents centrifuged at 3000-4000 rpm for 15-20 minutes. The supernatant was removed and the process carried out 3-5 times for elimination of lipoidal matter. The tissue residue was treated and washed a couple of times with solvent ether and the resulting product incubated for 48 hours in a thermostat running at $100^\circ C$. Processing of the sample this way yielded a white fat-free dry and powdered matter which was
used for determination of RNA and DNA. RNA was extracted and
determined quantitatively according to the technique of Schneider
(1957). A sample of 20 mg obtained through the procedure detailed
above was suspended in 2 ml of 1 N potassium hydroxide and incubated
for 20-24 hours at 37°C. Contents then received 0.4 ml of 6 N
hydrochloric acid and 2 ml of 5% TCA and mixed thoroughly before
centrifugation at 3000-4000 rpm for 10-15 minutes. The centrifuged
solution was filtered and RNA determined in the filtrate by the
standard orcinol reaction. Oracinol was purified by dissolving in
boiling benzene and decolourising with activated charcoal. The
purified product was recrystallized with hexane at room temperature.
A solution of 1% orcinol was prepared in concentrated hydrochloric
acid. The reagent was mixed with equal volume of diluted RNA
aliquot. Mixture was heated in boiling water for 30 minutes,
cooled to room temperature. Intensity of the greenish colour
developed in the solution was read at 660 nm wavelength on
Beckman microprocessor model DU-5 spectrophotometer (UV-VIS-NIR).
'Blank' was prepared by substituting RNA aliquot with distilled
water and processing the contents in the same way. RNA concen-
tration (µg/100 mg) of tissue sample was worked out by the help of
a standard curve (Fig. 1) prepared using purified yeast RNA.

For estimating DNA, 50 mg of dry, fat-free tissue sample
was suspended in 5 ml of 5% TCA. The contents were transferred
to a boiling waterbath and heated continuously for 30 minutes.
After cooling to room temperature, loss of volume of the contents during boiling was made up by addition of 5% TCA. The contents were filtered and DNA determined in the filtrate by the method of Ashwell (1957). DNA aliquot (0.5 ml) was mixed with 0.05 ml of 5% aqueous solution of cysteine, 5.0 ml of 70% sulphuric acid and the contents dispersed uniformly by effective shaking. Mixture was allowed to stand at room temperature for 15 minutes and colour developed in the sample was read at 490 mμ wavelength on the Beckman DU-5 spectrophotometer. Using purified, highly polymerized calf-thymus DNA as the standard, a calibration curve was prepared (Fig. 2) to calculate the DNA content of experimental samples. Values have been recorded as μg/100 mg dry fat-free sample. Since tissue was obtained from a fixed location in trunk to avoid effect of anatomical variation on the biochemical data, the sample from individual specimen was not adequate to process for both RNA and DNA estimation. As such, the two nucleic acids were determined in tissues dissected out from different specimens, and RNA/DNA ratio was computed using mean value of separate sets of RNA and DNA.

Energy content of tissue was determined by the help of a bomb calorimeter. Known weight of dry sample was pressed in a 'pellet press' and the pellet was weighed and placed in a stainless steel crucible. Nichrome wire and prescribed thread of measured length were tied to a hook adjusted in a way as to touch the sample in crucible. The entire assembly was transferred to the 'BOMB' of
the calorimeter. Pure oxygen in the prescribed quantity was supplied to the bomb and electric current passed. The thread and wire initiated the fire and sample ignited electrically. Rise in the temperature of water surrounding the bomb was recorded. Energy content of sample in small calories (c) was calculated as:

\[
\text{Sample Calories} = \frac{(\text{water equivalent} \times \text{difference in temperature, } ^\circ\text{C}) - (\text{Thread cal.} - \text{Wire cal.})}{\text{Weight of sample (g)}}
\]

where, water equivalent = 2200

Difference in temperature, \( ^\circ\text{C} \) = Final temperature - Initial temperature.

Thread calories = 2.1 x thread used (cm)

Wire calories = 2.33 x length of wire left after burning (cm)

The values were divided by 1000 to get the energy content in terms of dietary (kilo) calories (Kcal).

**Diet analysis**

Protein content of the diet was determined by the procedure of Lowry et al. (1951). Ten mg of dry sample of diet was dispersed in 1 ml of 1 N sodium hydroxide. The contents were treated for 10-15 minutes in a boiling waterbath, cooled to room temperature in running tap water, raised to 10 ml by distilled water and mixed
thoroughly. One tenth (0.1) ml of the aliquot was removed in clean, dry test tube, diluted to 2 ml by distilled water and mixed with 1 ml copper carbonate solution. The contents were incubated at room temperature for 10 minutes and 0.1 ml of Folin-phenol reagent was added. After standing at room temperature for 30 minutes, the intensity of colour was recorded at 500 m\(\mu\) on the Beckman model DU-5 spectrophotometer. A blank was prepared, taking 0.1 ml of a mixture of 1 part of 1N sodium hydroxide and 9 parts of distilled water and proceeding in similar way. Protein content of sample was derived by the help of a calibration curve (Fig. 3) prepared taking bovine serum albumin as the standard.

Energy content of the diet was determined bomb calorimetrically. The technique has been detailed earlier.

RESULTS AND DISCUSSION

It is evident from the data that diet 'A' was nutritionally superior compared to the remaining types of diets ('B', 'C', 'D'). Of all the feeds, 'D' failed to satisfy even the maintenance requirements. This nutritional efficacy of different feeds can be judged from the data pertaining to the percentage gains in body weight, instantaneous growth rate, food conversion efficiency and protein conversion ratio (Table II). Evidently, the diet 'B'
was deficient in some ingredients indispensable for normal growth. Qualitatively 'B' was same as 'A' except that it was devoid of egg shell and blood meal, the important sources of calcium and protein, respectively, and the cotton seed that mainly supplies energy nutrients. Their proportion (10\%) was compensated by additional quantity of sugarcane roughage in feed 'B'.

The study signifies the use of linseed oil cake as a substitute of mustard oil cake. Mustard oil cake is widely used as an essential component of the artificial diet of several major carps. Results of our study emphasize that performance of linseed oil cake is better of the two. The conventional concept of using oil cake of mustard be reconsidered in favour of that of the linseed. The data indicated that the gains in body weight/unit body length, efficiency of the conversion of food into flesh, as well as assimilation of dietary protein in the body were high in specimens reared on a diet having wheat bran and linseed oil cake (50:50) compared to the diet formulated to contain mustard oil cake instead of the linseed oil cake in the same proportion. Fish supplied the latter combination failed to maintain their original body weight.

The feeding trials elaborated that a part of the qualitatively and quantitatively superior food is diverted in the body to life sustaining and metabolic functions, and the other towards
growth. Further, a change in the nature of the diet that reduces its nutritional value alters the ratio of the maintenance/growth food in the direction of a relative increase in the former even when the ration size remains constant.

The data also revealed that food efficiency and PER increased with increase in the protein level of the diet. Concordant results have been obtained by Jobling and Spencer (1980) on plaice, Pleuronectes platessa. However, Jauncey (1982) observed decline in PER with increasing dietary protein content in case of tilapias. It was obvious that food assimilation, PER and gains in body weight were directly related to protein intake. Fowler (1980) noticed that weight gain in chinook salmon fry improved as the energy level of diet increased. Rychly (1980) recorded that food conversion efficiency in trout depended on consumption of protein. Pfeffer (1982) working on salmonid fish concluded that PER was determined by level of dietary protein and metabolizability of non-protein energy.

If the data on food assimilation and weight gain in fish fed proteinaceous diets ('A' and 'B' in the present case) are compared, it becomes apparent that nutrients other than protein become critically important. That some ingredients are more limiting for fish in comparison with pigs and poultry has been outlined by Pfeffer (1982). Diet 'A' contained 22.2% protein
and 'B' had 19.6% protein, but the latter was devoid of calcium rich egg shell and cotton seed. Diets 'C' and 'D' were poor in protein (9.3% and 9.3%, respectively). This dietary manipulation mattered seriously and changed the nutritional picture. Halver (1976) and Maslova (1977) have rightly stressed the need of mineral supplementation in the diet for the same reason. Although this work in not basically concerned with protein requirements, nevertheless, the related information will not preclude useful conclusions on this aspect. It is well known that protein requirements of fry are much higher than those of larger specimens (NAS — NRC, 1973). The fish used in the work were 8 months old and thus must thrive if protein percentage in diets is around that value. Nose (1971) indicated that utility of dietary protein for body gains remains fairly constant when concentration of this nutrient in the diet varies upto 35%. Second, in nature, Cirrhina mrigala feeds predominantly on sand, mud, detritus, besides smaller quantity of plankton (Khan and Siddiqi 1973) and subsists on low protein foods. Its digestive equipment is not suited to deal with a very high protein diet according to the views of Mustafa (1976).

Regarding bioenergetics, it is obvious that different diets do not necessarily produce resultant increase in calorific value of the flesh in the order of their own energy content. Energy contents per gram of the diets 'A', 'B', 'C', 'D' were 5.798, 6.280, 6.742 and 6.170 kilocalories, whereas equal weights of fish
muscle fed the diets contained 6.697, 7.047, 5.213, 6.062 kilocalories, respectively. Thus feed 'C' was richest source of energy but muscle of the fish supplied diet 'B' had the highest level of energy. Perhaps, the type of energy entering the body (Protein/fat/carbohydrate calories) is the deciding factor. A calorific diet may not essentially be nutritionally as superior inasmuch as proportion of fat whose fuel value exceeds twice that of the equal weights of proteins and carbohydrates combined, may abound in a diet and increase its energy value. Out of the diets tested, 'A' was most proteinaceous as well as most nutritive and gave a high PER but 'C' was poor in protein, rich in calories and yet of lower nutritional efficacy. It vindicates fish's lower requirements for metabolizable energy for producing protein. Indeed, this is a positive aspect if viewed in comparison with other commercial animals including poultry and industrial mammals (Pfeffer 1932). Consideration of protein level of different diets and its correlation with nutritional data summarized in Table I indicates explicitly that a larger part of net energy gain following digestive hydrolysis is laid down in the body chiefly as protein, irrespective of the total energy intake.

It is important to point out that overall poor weight gains in fish stocks reared on the compounded diets used are due to the fact that the fish were already stunted. They were obtained from
a derelict body of water with extremely poor productivity. Their body weight on the average represented growth in 8 months. Normally growing individuals of this age attain on the average a length of 20-22 cm and body weight 100-130 g. Obviously, fish passed a considerable period of its growth phase in an impoverished environment. Results impress upon the need of artificial feeding in the early life of the fish which is characterized by high potential for growth and biomass production.

Nucleic acid analysis of the muscle (Table III) of fish supplied different diets yielded interesting results. RNA seemed quite responsive to quality of diet. Variation in the DNA concentration of tissue was related to lability of cytoplasmic constituents including RNA and protein, and did not imply actual change in the quantity of DNA in cells. Alteration of RNA/DNA ratio thus reflected changes in the cytoplasmic reserves. These changes assume special significance when considered in the light of nutritional and growth data. Mustafa and Jafri (1979), Mustafa and Mittal (1982b), and Mustafa and Zofair (1983) have reviewed the work on this aspect and stressed the accuracy of identifying the nucleic acid changes with growth pulses in fish through regression models.

RNA concentration and RNA/DNA ratio were highest in muscle of fish fed diet 'A'. Next in order were the specimens supplied diets 'B', 'C', 'D'. The ratios, deducible from the data shown in
Table III, were 9.58 (A), 6.49 (B), 5.25 (C), 4.64 (D). Diet 'A' was richest in protein, and the FCE, PER, 3GR and energy content of the muscle of the fish maintained on it were higher than those of other batches. Clearly, level of dietary protein influences RNA in the body. Such a positive correlation between protein intake and tissue concentration of RNA in fish can be deduced from earlier work (Mustafa and Jafri 1977; Mustafa and Mittal 1982a) to substantiate the present findings. Since RNA organizes protein biosynthesis, increase in the concentration of this nucleic acid invariably results in synthesis of larger quantity of protein. It is an established fact that growth and robustness of fish are mainly a function of protein synthesis. Protein so arising epigenetically is laid down in the cell cytoplasm, thus 'diluting' the DNA and manifesting in more voluminous, heavier cells. A lesser number of such cells can form a unit weight of tissue sample compared to cells of smaller weight. It is to this factor that the decline of DNA can be attributed. That the DNA concentration in tissue is related to the number of cells/unit weight of sample has been amply demonstrated (Hotchkiss 1955; Leslie 1955; Sulow 1970; Mustafa and Jafri 1976; Mustafa 1977a,b, 1978; Mustafa and Shams 1982; Mustafa and Zofair 1983). Metabolic stability of DNA, the genetic material, observed earlier in fish even under critical conditions of 'physiological urgency', (Mustafa and Mittal 1982a) warrant against considering this change as owing to the decrease of cellular DNA.
These biochemical assays provide a reliable tool of evaluating the performance of artificial fish feeds within short periods. This information is of practical utility for those concerned with the formulation of balanced and growth promoting rations for cultivated fishes.

SUMMARY

Performance of four artificial diets with different chemical composition and energy value was studied in major carp Cirrhina mrigala. Nutritional parameters selected include instantaneous growth rate (IR), food conversion efficiency (FCE), protein efficiency ratio (PER). The relation of the ratio of maintenance/growth food with nutritional quality of diet was evident from the data. SGR, FCE and PER varied with protein intake. Marked difference in nutritional data of fish fed diets with comparable quantity of protein revealed the critical value of other nutrients, especially minerals. Efficiency of diet to increase calorific value of flesh seemed to depend on relative proportion of its different energy sources. Nature of diet influenced protein and nucleic acid concentrations in muscle tissue of fish. While protein and RNA increased by feeding diet more effective in growth promotion and of high assimilation, the DNA maintained a reciprocal relation. RNA/DNA ratio was found to be a sensitive and quick parameter for evaluating the nutritional efficacy of artificial feeds.
Fig. 1. Standard curve of RNA
Fig. 2. Standard curve of DNA
Fig. 3. Standard curve of Protein.
Fig. 4. Concentrations of RNA (white bars) and DNA (black bars) in the muscle of *Cirrhina mrigala* fed different diets. Vertical lines indicate standard error of mean.
<table>
<thead>
<tr>
<th>Dietary ingredients</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mustard oil cake</td>
<td>25.0</td>
<td>25.0</td>
<td>-</td>
<td>50.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>25.0</td>
<td>25.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Sugarcane roughage</td>
<td>20.0</td>
<td>30.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Frog offal meal</td>
<td>20.0</td>
<td>20.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood meal</td>
<td>03.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Egg Shell</td>
<td>02.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>05.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linseed oil cake</td>
<td>-</td>
<td>-</td>
<td>50.0</td>
<td>-</td>
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TABLE - II. Food assimilation and growth of *Cirrhina mrigala* fed different artificial diets

<table>
<thead>
<tr>
<th>No. of specimen</th>
<th>Diet supplied</th>
<th>Percentage gain in body weight, g</th>
<th>Food conversion efficiency (%)</th>
<th>Protein efficiency ratio</th>
<th>Instantaneous growth rate(%)</th>
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</thead>
<tbody>
<tr>
<td>30</td>
<td>A</td>
<td>16.35</td>
<td>43.023</td>
<td>1.933</td>
<td>1.08</td>
</tr>
<tr>
<td>30</td>
<td>B</td>
<td>3.44</td>
<td>8.619</td>
<td>0.44</td>
<td>0.24</td>
</tr>
<tr>
<td>30</td>
<td>C</td>
<td>1.02</td>
<td>3.119</td>
<td>0.314</td>
<td>0.07</td>
</tr>
<tr>
<td>30</td>
<td>D</td>
<td>Nil*</td>
<td>-25.19</td>
<td>-2.68</td>
<td>-0.61</td>
</tr>
</tbody>
</table>

*Specimens lost 10.68% of their original body weight.*
TABLE - III. Nucleic acid concentrations in the muscle of *Cirrhina mrigala* supplied artificial feeds (A, B)

<table>
<thead>
<tr>
<th>Diets</th>
<th>RNA, μg/100 mg</th>
<th>DNA, μg/100 mg</th>
<th>RNA/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>843.25 ± 4.865</td>
<td>83.66 ± 4.32</td>
<td>9.58</td>
</tr>
<tr>
<td>B</td>
<td>801.96 ± 7.560</td>
<td>123.5 ± 6.72</td>
<td>6.49</td>
</tr>
<tr>
<td>C</td>
<td>732.93 ± 7.230</td>
<td>143.33 ± 3.14</td>
<td>5.25</td>
</tr>
<tr>
<td>D</td>
<td>735.1 ± 3.330</td>
<td>159.33 ± 29.34</td>
<td>4.64</td>
</tr>
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