CHAPTER- 3

MATERIALS AND METHODS

The experiments was conducted at the Devapudi village near Kiakaluru for studying the survival, growth performance and meat quality of major carps catla (**Catla catla** as a surface feeder), Rohu (**Labeo rohita** as a column feeder), mrigala (**Cirrhinus mrigala** as a bottom feeder) and catfish (**Pangasius hypophthalmus**) under different treatments with triplicate per treatments. Earthen ponds of equal size were used to conduct the experiments.

Each pond size has an area of one hectare. Ponds were drained and dried before conducting the experiments. For the purpose of disinfection and the stabilization of pH values, liming with CaO was applied at the rate of 125 kg/ha. Nylon screen enclosures were installed on the water inlets and outlets pipes of each pond to prevent escape and entry of unwanted fishes and other organisms. Each ponds was filled water up to 1.5 – 2.0 m water level, all the ponds were fertilized with organic manure (Cowdung) as started dose to stimulate the productivity of pond at the rate of 3000 kg/ha 15 days before the stocking of the fish (Javed *et al.*, 1990). After 15 days of manuring each pond were stocked with catla, rohu, mrigala and pangas under 3: 9: 3: 6 ratios. The fish body weight and total length was measured and recorded. In Devapudi pond was started from 1.8.2009 to 1.8.2010.
The amount of fertilizers and supplementary feed was calculated on the basis of nitrogen availability at the rate of 2g N/100 g of the body weight of the fish. Cowdung at the of 0.2 g N/100 g, Nitrophos at the rate of 0.15 g N/100g and Urea at the rate of 0.2 g N/100g were introduced daily. The proximate analysis was carried out for all fertilizers following the analytical procedure of A.O.A.C., (1995). Fertilization was done on weekly basis while feeding was done on daily basis. The supplementary feed was formulated for treatments having 28.5% crude protein (Islam, 2002). The cow dung and nitrophos was added on weekly basis while supplementary feed was added on daily basis. After every one month interval, on the basis of wet fish body weights, amount of organic and inorganic fertilizer and supplementary feed to be added in fish ponds were determined for each treatment.

3.1. FISH GROWTH STUDIES

Fish were sampled once in each month using to record their gain in weight (g).

3.1.1. Sampling of the fish: Fishes were sampled once a month using drags net. Length and weight of ach species were measured separately to assess the health of condition of fish and their growth. The length (mm) and weight (g) of individual of fish were recorded separately on treatment wise with the help of measuring scale and portable sensitive balance. Fishes were captured randomly from each experimental treatment and these fishes were released back into their respective ponds after recording the data.
The experiment was conducted one year after stocking and the fish were harvested by repeated netting by drying of ponds and the final growth and survival of fish were recorded. The following growth parameters were studied during these investigations

1. **Fish wet body weight (g)**

2. **Total length (mm)**

3. **Condition factor (K)**

The value of condition factor (K) was determined by given formula:

\[
K = \frac{W \times 10^5}{L^3}
\]

Where;

- \( W \) = Wet fish body weight (g),
- \( L \) = Wet fish total length (mm)

Number 105 is the factor bringing the pondered index or condition factor (K) near the unity (Carlander, 1970).

4. **Survival rate**

At the final harvesting, the survival rate of three fish species was calculated by this formula.

\[
\text{Survival rate} = \frac{\text{Final number of fish harvested}}{\text{Number of fishes stocked}} \times 100
\]

5. **Specific growth rate (SGR)**

Specific growth rate (SGR) was estimated by the formula given by Dhawan and Kaur (2002a).

\[
\text{SGR} = \frac{\ln(W_2) - \ln(W_1)}{T} \times 100
\]

\( W_1 = \text{Average initial weight (g)}; W_2 = \text{Average final weight (g)}; T = \text{time (days)} \)
6. **Feed conversion ratio (FCR)**

\[
FCR = \frac{\text{Total feed fed (kg)}}{\text{Total wet weight gain (g)}}
\]

3.2. **PROXIMATE COMPOSITION OF FEED/FISH MEAT:**

At the final harvest, the meat samples from culture ponds were examined for the proximate composition of cultured fish species in terms of moisture, crude protein, total fats, total ash and carbohydrates to study the effect of fertilization and supplementary feed on the meat quality of catla, rohu, mrigala and pangas by using Association of Official Analytical Chemist (AOAC, 1995) standard techniques. For this purpose five fishes were randomly selected from each pond. Three meat samples were taken from each specimen. Head, viscera, bones, fins, scales and tails of these fishes were removed and only their flesh was used for analysis. The detailed procedure for each analysis is given as under:

3.2.1. **Moisture:**

One gram sample of meat samples were taken in a weighted Petri dish (W1) and placed it in the oven at 60ºC for 12 hours or until the dried. The dried samples were transferred to desiccators for 5 minutes and weighted. The samples were again kept in oven for one to two hours until constant weight (W2) was obtained. The loss in weight was recorded as moisture.

\[
\text{Moisture (\%)} = \frac{W_1 - W_2}{W_3}
\]
Where,

\[ W_1 = \text{weight of Petri dish + sample before drying}; \]
\[ W_2 = \text{weight of Petri dish + sample after drying}; \]
\[ W_3 = \text{weight of the sample}. \]

Dry matter percentage was calculated by the following.

\[ \text{Dry matter (\%)} = 100 - \text{moisture (\%)} \]

3.2.2. Crude protein:

Crude protein of the meat samples were analyzed by using micro kjeldahl’s method. Nitrogen (\%) calculated as under:

\[
\text{Nitrogen (\%)} = \frac{\text{Volume of } H_2SO_4 \times \text{Normality of } H_2SO_4 \times 0.014 \times 250}{\text{Weight of the sample} \times 10}
\]

Where

0.014 = Standard volume of (0.1 N) H2SO4 used to neutralize 1ml of ammonia.

250 = Dilution of the digested mixture

100 = for percentage of N2.

10 = Volume of the digested and diluted sample used.

Crude protein in sample was calculated by following formula.

\[ \text{Crude protein (\%)} = \% N_2 \times 6.25 \]

Whereby;

6.25 = Assumed factor for equation of N2 % to crude protein.

3.2.3. Total fats:

Total fat contents of meat samples were determined following petroleum ether extraction method through the Soxtec HT2 1045 system. Total fat percentage of the sample was calculated by following formula:
Total Fats (%) = \( \frac{W_2 - W_1 \times 100}{\text{Wt. of sample}} \)

Where: W1 = Weight of empty extraction cup.

W2 = Weight of extraction cup with fat after evaporation

3.2.4. Total ash:

The total ash was determined by burning 2g of dried fish tissue in a pre-weighed China dish and then samples were placed in a muffle furnace for ignition at 550 – 600°C till residue was obtained after 4 – 5 hours. Then the samples residue were placed in desiccators to cool and then weight was recorded. Percentage of ash was obtained by using the following formula:

Total ash (%) = \( \frac{\text{Wt. of ash}}{\text{Wt. of sample}} \times 100 \)

3.2.5. Carbohydrates:

The total carbohydrates were determined as follows:

100 – (Moisture + Crude protein + Total fats + Total Ash).

3.3. LIMNOLOGICAL STUDIES.

a) Collection of water samples

For limnological study, water samples were collected from all the experimental ponds on fortnightly but their average was calculated on the monthly basis. The following parameters of the physico-chemical characteristics of pond water were estimated.
b) Physical factors

I. Water temperature.

The temperature of the water was recorded with the help of Dissolved Oxygen Meter (HI-9146) by fixing the temperature factor at 0°C unit.

ii. Transparency.

The penetration of light into the pond water was measured with the help of “Secchi Disc”.

c) Analysis of chemical factors

The chemical analysis of experimental pond water viz., total alkalinity, carbonates, bicarbonates, total hardness, calcium, magnesium, total solids, total dissolved solids and planktonic biomass were determined on monthly basis by following methods described by Boyd (1981) and American Public Health Association (A.P.H.A., 1998).

iii. pH

To measure the pH (hydrogen ion concentration) the microprocessor pH meter (HANNA-HI-8520) was used after setting its range at “pH” point.

iii. Dissolved oxygen:

The dissolved oxygen was measured by Dissolved Oxygen Meter (HI-9146) after setting its range “ppm” unit. The dissolved oxygen was being measured directly at its original site by dipping the sensor of the meter into the pond water.
iv. **Total alkalinity**

Total alkalinity was measured by methyl orange indicator method. The water samples for measurement of alkalinity were collected in plastic bottles and analyzed as soon as possible to avoid denaturation. To a subsample of 50 ml in an Erlenmyer’s flask 0.1 ml methyl orange indicator was added and titrated against 0.02N standard sulphuric acid to the end point that is slightly orange. Total alkalinity was estimated by using following formula:

\[
\text{Total alkalinity (mgL}^{-1}\) = \frac{(\text{Volume of acid used}) \times (\text{Normality of acid}) \times (50,000)}{\text{Volume of sample (ml)}}
\]

v. **Carbonates:**

A sample of 50 ml in an Erlenmeyer’s flask, phenolphthalein (0.1) was added as an indicator which appear in pink color and then titrated against H\(_2\)SO\(_4\) (0.02 N) until pink color disappeared. The carbonates were estimated by using following formula.

\[
\text{Carbonates (mgL}^{-1}\) = \frac{(\text{Volume of acid used}) \times (\text{Normality of acid}) \times (50,000)}{\text{Volume of sample (ml)}}
\]

vi. **Bicarbonates**

The bicarbonates were calculated by following formula

\[
\text{Bicarbonates (mgL}^{-1}\) = \text{Total alkalinity – Carbonates}
\]

vii. **Total hardness**

A 50ml of water sample was taken in an Erlenmeyer’s flask and pH was maintained (12-13) by adding appropriate volume of the buffer solution. The reaction mixture was stirred and 0.1 ml of Eriochrome Black T (EBT) indicator was added to it and titrated against Ethylene
Diamine Tetra Acetic acid (EDTA) (0.01 N) to reach the end point which is blue colour. Total hardness was calculated by following formula.

\[
\text{Total hardness (mgL}^{-1}) = \frac{(\text{Volume of EDTA used}) \times 1000}{\text{Volume of sample (ml)}}
\]

viii. Chlorides: Chlorides were estimates using Silver nitrate and potassium chromate as indicator as outlined as APHA (1998).

ix. Nitrate-N: Nitrate-N was estimated colorimetrically by cadmium reduction method using ‘Flow injection analyses in FIA star\textsuperscript{R} of Tecator Company, Sweden.

x. Nitrite-N: Estimation of nitrite was done by calorimetric method as per principle given by Golterman ans Climo (1969).

xi. Ammonia: Ammonia was estimated calorimetrically by gas diffusion method using ‘Flow injection analyses in FIA star\textsuperscript{R} of Tecator Company, Sweden.

xii. Phosphates: the orthophosphates were estimated calorimetrically using molybdate-antimony solution and ascorbic acid as described in Golterman and Clymo (1969).

xiii. Iron: Iron content of the sample water was estimated colorimetrically using 1, 1- phenothroline as indicator (Golterman and Clymo, 1969).

xiv. Total solids

Total solids of water were estimated by evaporation method. A 100 ml of water sample was taken in a pre-weighed beaker and
evaporated in an oven at 103°C. After evaporation, beaker was weighed again and the total solids were calculated by the following equation:

$$\text{Total solids (mgL}^{-1}) = \frac{\text{Increase in weight} \times 100,000}{\text{Volume of sample (ml)}}$$

3.4. Biological parameters:

Quantitative samples of plankton were collected by filtering 100 liters of sub-surface water through plankton net made of a silk bolting cloth N. 25 (mesh size 0.04m). The plankton obtained was fixed in 5% formalin. Enumeration of the individuals’ planktons was done on the lines recommended by Welch (1952) and Wetzel and Linken (1979). Samples volumes were adjusted to 590 ml and three sub-samples of 1.0 ml capacity were removed and counted in a Sedgwick-rafter cell for plankton. The average of the three counts was converted to number of individuals per liter of pond water samples. The computation for the number of planktons per liter of the pond water was worked out by the formula as suggested by Wetzel and Linken (1979).

$$N = \frac{NV_s}{V_f}$$

Where   
- $n =$ number of plankton per liter of original water  
- $N =$ average count per cell +/- S.D of the mean  
- $V_s =$ Volume of sample in ml  
- $V_f =$ Volume of pond water filtered in liters.
3.5. Statistical Analysis

To study the analysis variance among growth of the fish, physico-chemical parameters of water and the influence of the physico-chemical characteristics on the biotic compounds of the water, simple correlation, condition factor, correlation co-efficient were calculated. The data thus obtained was subjected to statistical analysis (Steel et al., 1997; Zar, 1999) and given in tables.