MATERIALS
AND METHODS
2.0. MATERIALS AND METHODS

2.1. Experimental animal

To investigate the influence of protein to carbohydrate and lipid ratio on growth response, biochemical changes and nutrient digestibility, one of the commercially important brackish water fish *Mugil cephalus* was selected. The taxonomic position of *M. cephalus* is provided below (Jordan and Verma 1976, Jhingran, 1977).

**Classification:**

- **Phylum**: Chordata
- **Group**: Vertebrata
- **SubPhylum**: Gnathostomata
- **Series**: Pisces
- **Class**: Teleostomi
- **Sub Class**: Actinopterygii
- **Order**: Mugiliformes
- **Sub order**: Mugiloidei
- **Family**: Mugilidae
- **Genus**: *Mugil*
- **Species**: *Cephalus*
2.1.1. Habit and Habitat

Mullets rank next to eels in European fish-culture. In India too, they constitute the most abundant fish found in estuaries and backwaters, with rich species diversity. In confinement, they exhibit in a large degree, the quality of hardiness, they are easy to feed and will live where most other fish species would die of starvation.

Among the brackish water fish species the grey mullet *M. cephalus* is commonly occurring in almost all the Indian Coasts. They are considered as the candidate species for brackish water aquaculture because they are not affected by the variations in temperature and salinity. For example they can tolerate temperature variations from 3 to $35^\circ$C and salinity from 0 to 35 ppt. They also do not have special food requirements, as they feed mainly on plankton, algae and higher plants, which are died off.

Mullet fry feed principally on planktons and are believed to prefer diatoms and epiphytic cyanophyceae. Adult of all species are primarily benthic feeders, consuming algae and vegetable detritus, with an incidental intake of small animals. They feed in a head – down position, moving their heads from side to side so violently that their entire body vibrates. Mullets generally form a school when feeding near the surface. Their intestine, which is three to six times the length of fish, show the vegetable nature of their diet (Iverson, 1976).

Mullets occur in the sea, estuaries and some even migrate into fresh water. Several males and a single female spawn at sea during the cold months, laying
pelagic eggs which hatch within two days. The minute (26 mm in length), heavily pigmented fry move into estuaries and coastal tide pools in late winter or early spring to remain there until moving offshore in the following fall of winter. Mullets apparently prefer warm water. Though most of the mullet species are found at a salinity of 30 ppt or below, they may thrive in areas of very high salinity also. *Mugil cephalus* reaches the length of 50 to 55 cm and weighs 1.2 to 2.0 kg in four to six years (Bose *et al.*, 1991).

### 2.2. Collection and maintenance

Juveniles of *M. cephalus* of about 0.700–1.00 g size were collected from Manakudy estuary situated about 12 km away from Nagercoil towards southern side in Kanyakumari district (E. Longitude 76°, N. Latitude 80°), Tamil Nadu (N. Latitude 80° 5' - 13° - 35'; E. Longitude 76° 15' - 80° 20'), India. The juveniles were collected with the help of a nylon mosquito net of about 1 mm mesh size in the early morning hours. The collected seeds were then transported to the laboratory with least disturbance in polythene bags containing oxygenated brackish water. Then the fish were acclimatized to the ambient laboratory condition for a period of 15 days in three different salinities i.e. 5, 15 and 30 ppt in 100 l glass tanks. Mild aeration was also provided in the rearing tanks to maintain the dissolved oxygen at the optimum level (5.5 to 7.0 mg l⁻¹).

During the period of acclimatization the juveniles were fed *ad libitum* with freshly prepared practical diet containing Fishmeal, Soya meal, Tapioca powder etc. for an initial period of 5 days. Subsequently, the fish were fed with pure diet containing Casein, Gelatin, Dextrin etc as the major ingredients. The feeding was done once in a day at *ad libitum* level and feed was allowed to remain in the tank
for about 5 hours. The water was changed (50%) every day to prevent accumulation of metabolites. After the acclimatization period, the experimental fish were grouped into three according to their rearing salinity viz. 5 ppt, 15 ppt and 30 ppt.

2.3. Experimental design

After the period of acclimatization, healthy fish weighing 0.700 ± 0.031 to 1.00 ± 0.049 g were selected. The fish were starved for about 24 hours, prior to the start of the experiment inorder to evacuate their gut contents. Two different sets of experiments were conducted, each one for protein to carbohydrate ratio and protein to lipid ratio, respectively. In each set, three experiments were conducted simultaneously with adequate replicate at 5, 15 and 30 ppt salinities. During the experimental period, the test fish were reared individually in 10.0 l plastic troughs, containing 5 l of water and all the experiments lasted for 21 days. The detailed experimental design was shown in the figure 1.

2.4. Feed formulation

The primary objective of feed formulation is to provide an acceptable diet that meets its nutritional requirements at different life stages of cultivable organisms so as to yield optimum production at minimum cost. In this experiment, tested diets were formulated using purified feed ingredients. The proximate composition i.e. protein, carbohydrate and lipid contents of feed ingredients were estimated prior to the feed formulation, following the methods described by Lowry et al. (1951), Seifter et al. (1957) and Bligh and Dyer (1959), respectively. The calorific value of the ingredients are calculated using the following values
Fig 1. EXPERIMENTAL DESIGN

A. Feed preparation

- Carbohydrate supplementation
  - 0%
  - 5%
  - 10%
  - 15%
  - 20%

- Dietary protein level
  - 25%
  - 30%
  - 35%
  - 40%

- Lipid supplementation
  - 0%
  - 4%
  - 8%
  - 10%
  - 15%

B. Feed Evaluation

Mugil cephalus in different salinities

- 5 ppt
- 15 ppt
- 30 ppt

Growth response
Feed conversion
Body composition
Nutrient metabolism
16.7 KJ g\(^{-1}\) for protein and carbohydrate and 37.7 KJ g\(^{-1}\) for lipid (Lee and Putnam, 1973; Garling and Wilson, 1977; Gholam et al., 1992). In all the experimental diets, protein to energy ratio was maintained at 23 mg KJ\(^{-1}\). By keeping the P : E ratio constant diets having 25 to 40% protein were formulated following the method of New (1987). Thus experimental feed were formulated for both protein to carbohydrate and protein to lipid ratios at each protein level.

In first set, feed with protein to carbohydrate ratio, there were 20 feed (F1 to F20), with various percentage of carbohydrate viz. 0, 5, 10, 15 and 20% at each protein level (Table 1.1). Similarly in the second set of feed with protein to lipid ratio again there were 20 feed (D1 to D20), with 0, 4, 8, 10 and 15% lipid at each protein level (Table 1.2). Altogether forty experimental feed were prepared.

2.4.1. Feed ingredients

Based on the suitability, the following ingredients were selected and used for the formulation of purified diet.

a. **Casein**: Casein is extracted from milk and is constituted known as the milk protein and the protein content of Casein was 84.5%. It was used as the main protein supplement in purified diets.

b. **Gelatin**: Gelatin contains high protein content of 87.7%. It was used as a supplemented protein and as well as a binder to give texture and stability to experimental diets.
Table 1.1 Composition of the feed ingredients (g/100g dry weight) used for the formulation of first set of experimental diets and their biochemical composition.

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Table 1.2 Composition of the feed ingredients (g/100g dry weight) used for the formulation of second set of experimental diets and their biochemical composition.

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<td>23.00</td>
<td></td>
</tr>
</tbody>
</table>

* To compensate the P/I ratio the ingredients were added in excess (>100g)
c. **Dextrin**: Dextrin was used as a carbohydrate supplement. It is a pure form of carbohydrate (100%).

d. **Carboxyl methyl cellulose**: It is commonly known as CMC and is mainly used as the binder in purified diet.

e. **Cellulose**: It has no nutritive value *i.e.* it is neither a protein nor a carbohydrate or lipid. In the present study it was used as a filler.

f. **Cod liver oil**: Cod liver oil purchased from the commercial market was used as lipid source. Moreover, it also acted as a lubricant while extruding the pellet.

g. **Vitamins and Minerals**: Vitamins are chemically diverse group of organic substances. They constitute only a minute fraction of the diet and critical for the maintenance of normal metabolic and physiological functions, resulting in increase of growth and high survival rate of organisms. Minerals are the important constituents of the structural components of tissues and skeletons in the regulations of osmotic pressure, nerve impulse transmission and in muscle contraction. Many of the minerals serves as components of enzymes, vitamins, hormones, pigments and as co-factor in metabolism or as enzyme activators.

In the present study, for the feed preparation ‘Supradin’ a multivitamin and mineral tablets were used and its description is given in Table (2).
Table 2. Chemical composition of (Per g) Multivitamin and minerals tablet SUPRADIN used in the experimental diets *.

<table>
<thead>
<tr>
<th>Vitamins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins A. I. P. (as acetate)</td>
<td>10,000 I.u.</td>
</tr>
<tr>
<td>Cholecalciferol I. P. (Vitamin D3)</td>
<td>1,000 I.u.</td>
</tr>
<tr>
<td>Thiamine monozitate I. P.</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>Riboflavine I. P.</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>Pyridoxine Hydrochloride I. P.</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Cyanocobalamine I. P.</td>
<td>15.0 mg</td>
</tr>
<tr>
<td>Nicotinamide I. P.</td>
<td>100.0 mg</td>
</tr>
<tr>
<td>Calcium Pantothenate I. P.</td>
<td>16.3 mg</td>
</tr>
<tr>
<td>Ascorbic Acid I. P.</td>
<td>150.0 mg</td>
</tr>
<tr>
<td>Tocopherol acetate I. P.</td>
<td>25.0 mg</td>
</tr>
<tr>
<td>Biotin U. S. P.</td>
<td>0.25 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minerals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium phosphate I. P.</td>
<td>129.00 mg</td>
</tr>
<tr>
<td>Magnesium oxide light I. P.</td>
<td>60.00 mg</td>
</tr>
<tr>
<td>Dried Ferrous sulphate I. P.</td>
<td>32.04 mg</td>
</tr>
<tr>
<td>Manganese sulphate B. P.</td>
<td>2.03 mg</td>
</tr>
<tr>
<td>Total phosphorus in preparation</td>
<td>25.80 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trace elements</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulphate I. P.</td>
<td>3.39 mg</td>
</tr>
<tr>
<td>Zinc sulphate I. P.</td>
<td>2.20 mg</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>0.25 mg</td>
</tr>
<tr>
<td>Sodium Borate I. P.</td>
<td>0.88 mg</td>
</tr>
</tbody>
</table>

* Values were provided as per the indication given in commercial market.
2.4.2. Preparation of feed

Mixing the different ingredients of diet at various proportions results in feeds of different composition. The experimental feeds were prepared separately using the known quantities of ingredients to maintain the required protein to carbohydrate and lipid ratio as well as protein to energy ratio.

The selected ingredients were powdered and sieved to get fine particles of uniform size. Then the ingredients were weighed according to the quantity and were mixed well in a container. To this, sufficient quantity of distilled water was added and the ingredients were made into a dough. To this, cod liver oil, vitamin and mineral mixture were added and mixed well. Then chromic oxide as an inert marker (0.5%), Butylated Hydroxy Anisole (BHA) as an antioxidant (0.2%), citric acid as preservative (0.2%) and streptomycin sulphate (0.1%) as antibiotic to stabilize the composition as well as to prevent the microbial action were added to the dough. Here the chromic oxide, BHA, Citric acid and Streptomycin sulphate were added in addition to 100 g formulated feed.

The dough was then allowed to pass through a pelletizer having perforation diameter of 1.5 mm in the die. The diets having more than 10% cod liver oil was drawn into spaghetti – like strands after the addition of 20% water because of high fat content (Brauge et al., 1994). The test diets in the form of pellets and strands were dried in an oven till the moisture content was reduced to less than 10% and were then broken manually to a length of about 1 cm. The dried pellets and strands were packed in airtight plastic containers and were temporarily stored in freezer until the commencement of experiment.
2.4.3. Water stability of pelleted feed

The water stability of the pellets was tested over a period of 5 hours by the method of Jayaram and Shetty (1981) and Immanuel (1996) as follows.

The diets prepared were tested for stability in water by placing 1.0g of feed in each of 45 glass bowls, each known capacity (100cc). The 45 bowls were divided into 3 lots of 3 each for 5 different types of feed. The 3 lots were then kept immersed separately in plastic trough containing 10 l of water for a period of 5 hours. Each lot of bowls was removed from the trough after the set time interval without spilling the feed materials. Water from each bowl was drained carefully using No.30 blotting silk cloth and the residue was dried in a hot air oven at 105°C for 30 minutes, followed by further drying at 65°C to a constant weight. The mean weights before immersion and after drying were used to calculate the percentage of dry matter loss, which is a measure of the water stability of the pellets. The mean percentage of leaching of dry matter estimated in the present study was taken into account while estimating the food consumption.

2.5. Experimental setup

2.5.1. Biological evaluation

After the acclimatization period, healthy fingerlings of *M. cephalus* were chosen and starved for 24 hours before commencement of the experiment. Then the fish were weighed individually in a monopan balance to 0.1 mg accuracy. The initial weights of *M. cephalus* were ranged from 0.701 ± 0.03 to 0.995 ± 0.04 g.
2.5.2. Experiments

For the present study two sets of experiments were conducted.

a. Experiment I

In the first set of experiments, the influence of protein to carbohydrate ratio in *M. cephalus* was investigated at 25 to 40% dietary protein level in three different rearing salinities viz. 5, 15 and 30 ppt. The fish were reared individually in triplicate for all the twenty experimental feed (F1 to F20). Mild aeration was also provided in the experimental troughs in order to maintain the dissolved oxygen.

b. Experiment II

In the second set of experiments, the influence of protein to lipid ratio was studied at different protein levels *viz.* 25 to 40% in three different salinities (5, 15 and 30 ppt). The fish were reared on test diet (D1 to D20) similar to that of first set of experiments.

2.5.3. Feeding, collection of unfed and faeces

During the experimental period, test diet were offered to the animals in petridish kept at the bottom of each trough. The animals were fed at *ad libitum* (15% of their body weight) once in a day. It was regulated according to the diet remained uneaten after stipulated feeding time (5 hrs). The leftover diet was then recovered, after collecting the faecal matter 50% of water was exchanged. The
unconsumed food and the faecal pellets were then dried at \(80^0\text{C}\) in hot air oven to weight constancy. Water quality parameters like temperature \((28 \pm 1^0\text{C})\), oxygen \((5.5 \pm 0.5\ \text{mg}\ \text{l}^{-1})\), pH \((8.0 \pm 0.5)\) and ammonia \((0.2 \pm 0.01\ \text{mg}\ \text{l}^{-1})\) were maintained at the optimum level.

At the end of the experiment (21st day) the tested animals were starved for 24 hrs to ensure complete evacuation of the gut. Then the animals were stunned to death, weight was measured individually and dried to estimate the water content. The dry material was also weighed and stored for biochemical estimation.

2.6. Biochemical analysis

In order to ascertain the impact of dietary protein to carbohydrate and lipid levels as well as environmental salinity on tissue composition, the study was also extended to estimate bio-chemical composition of the test fish \(M.\ cephalus\). The contents of water, protein, carbohydrate and lipid were estimated as described below. Feed and faecal samples were also analyzed for protein, carbohydrate, lipid and chromic oxide contents.

2.6.1. Water content

The fish were evacuated of their gut contents, blotted off the surface water and weighed in a monopan balance to an accuracy of 0.1 mg. They were then transferred to a hot air oven maintaining the temperature at \(80^0\text{C}\) and the dry matter left was weighed till weight constancy.
2.6.2. Protein estimation

The protein was estimated following the method described by Lowry et al. (1951) using Bovine Serum Albumin (BSA) as standard. Icecold 10% TCA (Trichloro Acetic Acid) was added to an aliquot (5ml) of the homogenate and kept in 15°C overnight to facilitate complete precipitation of protein. The precipitated protein was centrifuged in 3000 rpm for about 10 min and the protein pellet was dissolved in a known amount of 30% sodium hydroxide (NaOH). To an aliquot of this, 2 ml of sodium carbonate (12.5%) and 0.5 ml of copper sulphate (0.1%) were added. After 10 min, 0.5 ml of folinphenol solution was added and the optical density was measured at 740 nm after two minutes in spectrophotometer using a red filter.

2.6.3. Carbohydrate estimation

The carbohydrate content was estimated by the Anthrone method described by Seifter et al. (1950). To an aliquot of tissue homogenate, 4 ml of anthrone reagent (0.2% in sulphuric acid) was added and is incubated in a boiling water bath for 15 min. Tubes were then cooled to room temperature at dark condition. Then the optical density was measured at 750 nm in a spectrophotometer. Here glucose (10mg/100 ml distilled water) was used as the standard.

2.6.4. Lipid estimation

Following the method of Bligh and Dyer (1959) the total lipids (fats) in the experimental sample was estimated by gravimetric method. About 50 mg test samples were weighed to an accuracy of 0.1 mg and is homogenized in 5 ml of
Tris buffer (pH 7.4) using a ground glass homogenizer (capacity 10 ml). The homogenate was transferred to a 10 ml conical flask. To each, 5 ml of methanol followed by 2.5 ml of chloroform were added. The contents were sealed and kept in an oven at 50°C and at regular intervals, the flask were shaken well. After incubation, water (2.5 ml) and chloroform (2.5 ml) were added and centrifuged at 3000 rpm in a tabletop centrifuge for 10 min. The precipitate alone at the bottom of test tube was carefully transferred to the preweighed (0.01 mg) aluminium foil cup. The content of the foil cup was evaporated to dryness and weighed. The difference in initial and final weights represented the lipid value. The lipid content was expressed as mg lipid/g dry tissue.

2.7. Chromic oxide estimation

To determine the nutrient digestibility, an inert marker chromic oxide was used in the feed. To calculate the digestibility, the chromic oxide present in the feed and in the faeces was estimated according to the procedure of Ahamad Ali and Gopal (1995).

Exactly 50 mg of sample was weighed and placed in a 20 ml Kjeldahl flask. Then 5 ml of concentrated nitric acid was added and the contents were boiled gently in a fume cupboard on a digestion mantle for about half an hour. To prevent the contents becoming dry, additional amount of acid was also added. After cooling, 3 ml of 60% Perchloric acid was added and it was again heated until all the nitric acid was expelled. When the fumes stop evolving from the flask, the solution turned to bright golden yellow colour. The content was then cooled and transferred to 50 ml volumetric flask by repeated washing and made up to the mark. From this, 2 ml of solution was transferred into a 50 ml flask and 1 ml of
diphenyl carbazide reagent was added. The mixture was allowed to stand undisturbed for 10 min for a bright pink colour to develop and then made up to the mark. The optical density was then measured using a spectrophotometer at 530 nm against a reagent blank. Here A.R. Grade Potassium-di-chromate (96.77 mg/100 ml) was used as the standard. The concentration of chromic oxide in the test samples was obtained from the standard graph.

\[
\% \text{ Chromic oxide} = \frac{\text{Chromic oxide concentration} \times \text{dilution of sample} \times 100}{\text{ml of sample used for colour} \times \text{wt. of sample taken development}}
\]

2.8. Nutrient Budget

To have a better understanding on the influence of the nutritional and environmental adaptation on utilization of different components of food by the fish, the mass budget was prepared using the dry weight following the IBP formula of Petrulewicz and Mac Fadyen (1970). Then by using the respective nutrient content of relevant sample, the macronutrient budgets were also prepared. The formula used in the present study was given below.

\[
\begin{align*}
\text{Food consumed} & = \text{Feed given} - \text{Unfed leftover} \\
\text{Production} & = \text{Initial wt.} - \text{Final wt.}
\end{align*}
\]
Growth (% increase) = \frac{\text{Final wt.} - \text{Initial wt.}}{\text{Initial wt.}} \times 100

Food Conversion Efficiency (FCE) was calculated using the following formula,

\[ \text{FCE} = \frac{\text{Wet wt. of the fish produced (g)}}{\text{Dry wt. of the feed given (g)}} \times 100 \]

Specific Growth Rate (SGR) was calculated as follows:

\[ \text{SGR} = 100 \frac{(\ln \text{final wet wt.} - \ln \text{initial wet wt.})}{\text{Experimental duration}} \]

Food Conversion Ratio (FCR) was calculated according to Heut (1975) as follows:

\[ \text{FCR} = \frac{\text{Dry wt. of feed given (g)}}{\text{Increase in wt. of fish (g)}} \]

The nutrient metabolism and digestibility of this experiment was calculated as follows,

\[ \text{Nutrient consumption (mg g}^{-1}\text{day}^{-1}) = \frac{\text{Nutrient consumed (g)}}{\text{Initial wt of fish(g) x duration(days)}} \times 1000 \]
Nutrient production (mg g⁻¹ day⁻¹) = \( \frac{\text{Nutrient production (g) x 1000}}{\text{Initial wt. of fish (g) x duration (days)}} \)

Nutrient efficiency ratio = \( \frac{\text{g of wet wt. gained}}{\text{g of dry protein intake}} \)

Productive nutrient value = \( \frac{\text{Nutrient production (g) x 100}}{\text{Nutrient consumption (g)}} \)

The nutrient digestibility in diet is calculated using the chromic oxide content in the test diet and faeces. The formula is as follows:

Nutrient digestibility in diet (%) = \( 100 - \frac{\% \text{Cr}_2\text{O}_3 \text{ in diet}}{\% \text{Cr}_2\text{O}_3 \text{ in faeces}} \times \frac{\% \text{Nutrient in faeces}}{\% \text{Nutrient in diet}} x 100 \)

2.9. Statistical Analysis

Results obtained in the present study were subjected to the following statistical analysis.
a. **Standard Deviation (SD)**

\[
SD = \sqrt{\frac{\sum d^2}{n-1}}
\]

Where 'd' represent the deviation of each score from mean and 'n' is the total number of samples.

b. **Standard Error (SE)**

\[
SE = \frac{SD}{\sqrt{n-1}}
\]

Where 'SD' is the standard deviation and 'n' number of samples.

c. **Students ‘t’ test**

Student ‘t’ test was made to compare two means by applying the following formula.

\[
t = \frac{X_1 - X_2}{\sqrt{SE_1^2 + SE_2^2}}
\]

Where ‘X_1’ and ‘X_2’ represent the mean values of comparable data, SE_1 and SE_2 are their respective standard errors. The level of significance for ‘t’ at corresponding degrees of freedom (df = n-2) was read from probability table given in Zar (1974), where ‘n’ is the total number of scores in both the experiments.
d. Student's Neumann – Keuls Test (SNK Test)

For the comparison of a series of mean, a multiple range test called SNK test was performed following the procedure described by Daniel (1987).

e. Two-way analysis of variance

Partitioning of total variance into variance due to different factors was carried out following the procedure described by Zar (1974). Values detained at the different experimental conditions were tabulated in different columns and rows. For each column, \( x \) and \( x^2 \) were calculated. Sum of \( X \) of all columns was squared and divided by the number of tabulated values and a correction factor ‘\( c \)’ was obtained.

\[
\text{Correction factor (c ) } = \frac{\text{Grand sum of all } x^2/n}{(n = \text{total number of observation})}
\]

\[
\text{Total Sum of Square (TSS) } = \sum x^2 \text{ for all columns } - C
\]

\[
\text{Between Columns SS (CSS )} = \sum \text{Sum of all values in each column } x \ 2/n \ (n = \text{number of values in the column})
\]

\[
\text{Between row SS (RSS) } = \sum \text{Sum of all values in each row } x \ 2/n \ (n = \text{number of values in the row})
\]
Considering the degrees of freedom for each source of variance, Mean sum of Square (MS) was calculated.

**Degrees of freedom (DF)**

\[
\text{Total DF} = \text{Total number of values} - 1
\]

\[
\text{Between columns DF} = \text{Number of columns} - 1
\]

\[
\text{Between row DF} = \text{Number of rows} - 1
\]

\[
\text{Remainder DF} = \text{Total Df} - (\text{Columns DF} + \text{rows DF})
\]

\[
\text{F-value for the variance between columns} = \frac{\text{MS between columns}}{\text{Remainder MS}}
\]

\[
\text{F-value for the variance between rows} = \frac{\text{MS between rows}}{\text{Remainder MS}}
\]

Significant level at the corresponding DF was read from the standard F-distribution Table given in Zar (1974).

**f. Correlation and Regression**

To find out the relationship between two variables the correlation and regression analysis was calculated by the method described by Zar (1974).