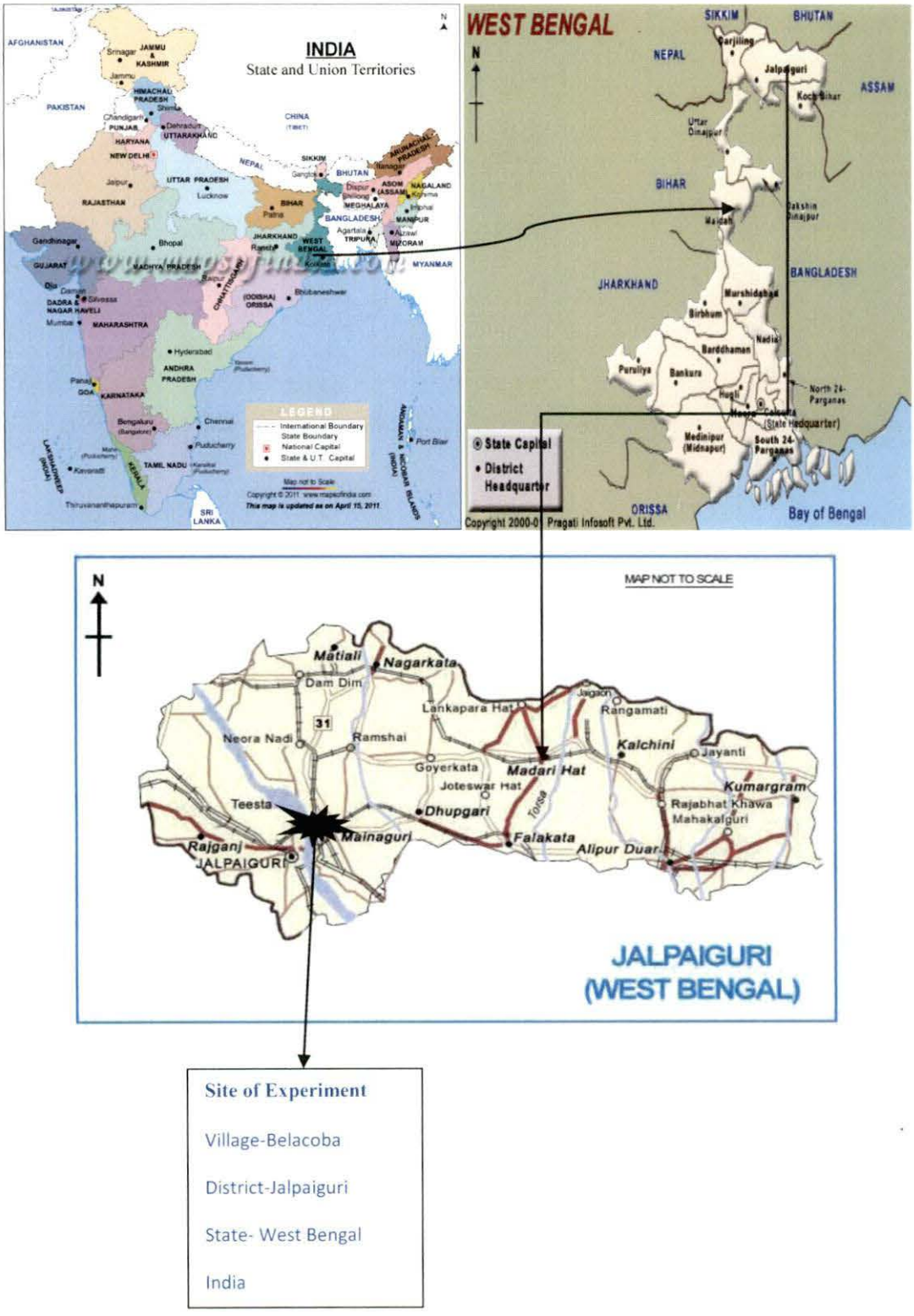
Materials and Methods

3.1 Study Site and Duration of Study

To investigate the potentialities of Crop-Livestock-Fish Integrated Farming, experiments were conducted randomly at selected sites of village Belacoba , Jalpaiguri district (latitude 26°58′N and longitude 88°58′E) within the Terai region of West Bengal (Pic-1). The area is a sub-tropical humid climate and situated 43 m above mean sea level (msl) having sandy-loam soil. The average annual rainfall of the area remained within 2200-2700 mm and average minimum and maximum temperature ranged from 18.5°-20.8°C and 28.5°- 31.5°C, respectively.

Three experiments were conducted from the month of April to September of four consecutive years, 2008 to 2011, as the ponds in this area were mostly seasonal and shrinking in nature. The water in the pond generally stayed from April to September or October depending on two sources of water namely, rainfall and water from the Teesta Barrage. The observations of the said experiment regarding pond soil and water quality, fish growth rate, zooplankton production and total fish production were studied for the period April to September for each of the four consecutive years from 2008 to 2011. The experimental analyses were carried out at Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, Darjeeling District, West Bengal.

Other productions, like production from animal components, was studied round the year from 2008 to 2011 whereas, the productions from turmeric cultivation along the surrounding area of ponds were studied during the period from April to November of the four consecutive years, 2008 to 2011.



Picture – 1: Map showing the Site of Experiment.

3.2 Experimental Design

For determining the potentiality of different Integrated Farming System (IFS) over Non-Integrated Farming System (NIFS) and also developing the location specific IFS packages involving different components, that are locally available from farm resources of marginal farmer, the present investigation was conducted in farmer's field condition considering three different experimental designs. Nine ponds were selected in triplicate for each experiment of same area 0.01 hectare (ha) to carry out the three experiments. Three field experiments, namely Non-Integrated Farming System (NIFS), Integrated Farming System-I (IFS-I) and Integrated Farming System-II (IFS-II) were set up in triplicate. The flow chart of the Experimental Design NIFS, IFS-I and IFS-II are shown in Fig-5, Fig-6 and Fig-7, respectively.

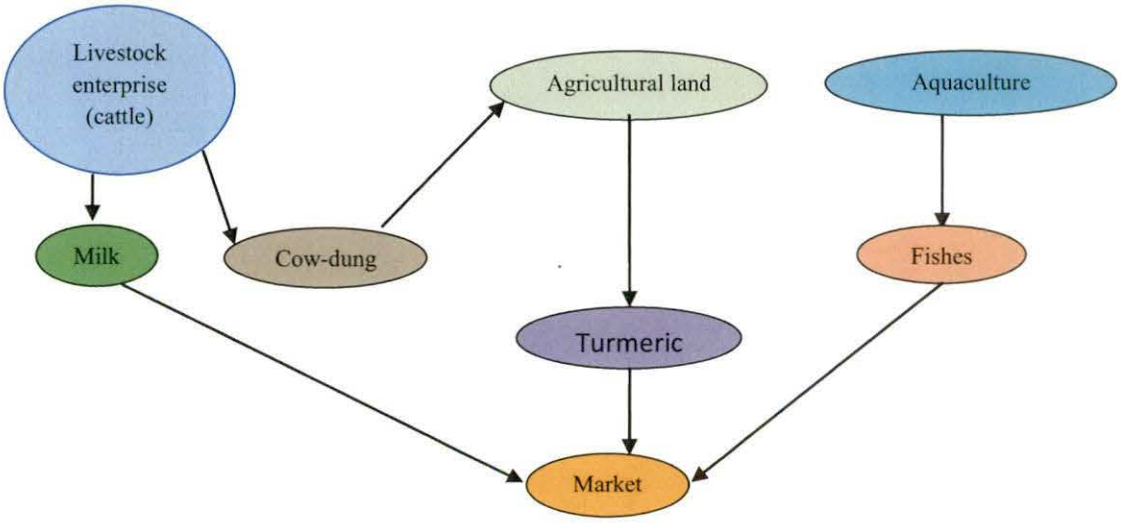


Fig- 5: Flow chart of Experiment-I (Non-Integrated Farming System) showing the non-integration of different components of farming.

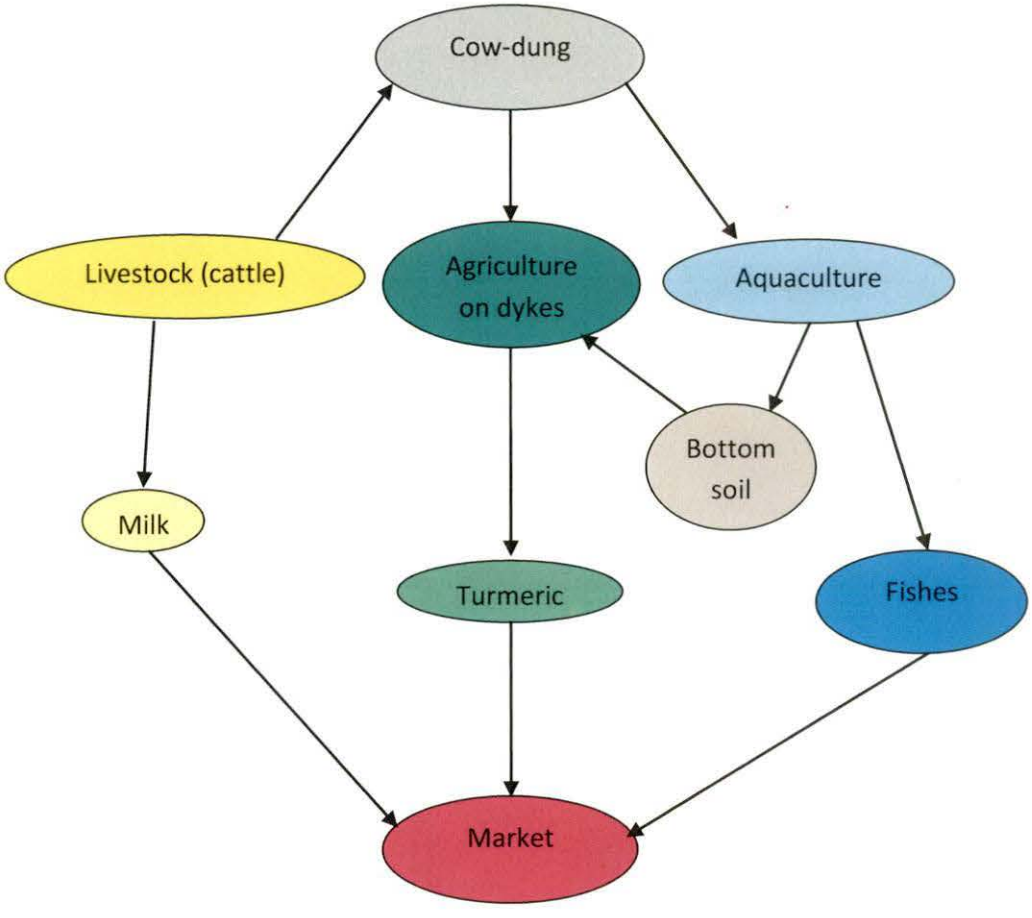


Fig- 6: Flow chart of Experiment-II (Integrated Farming System-I) showing the integration of different components of farming.

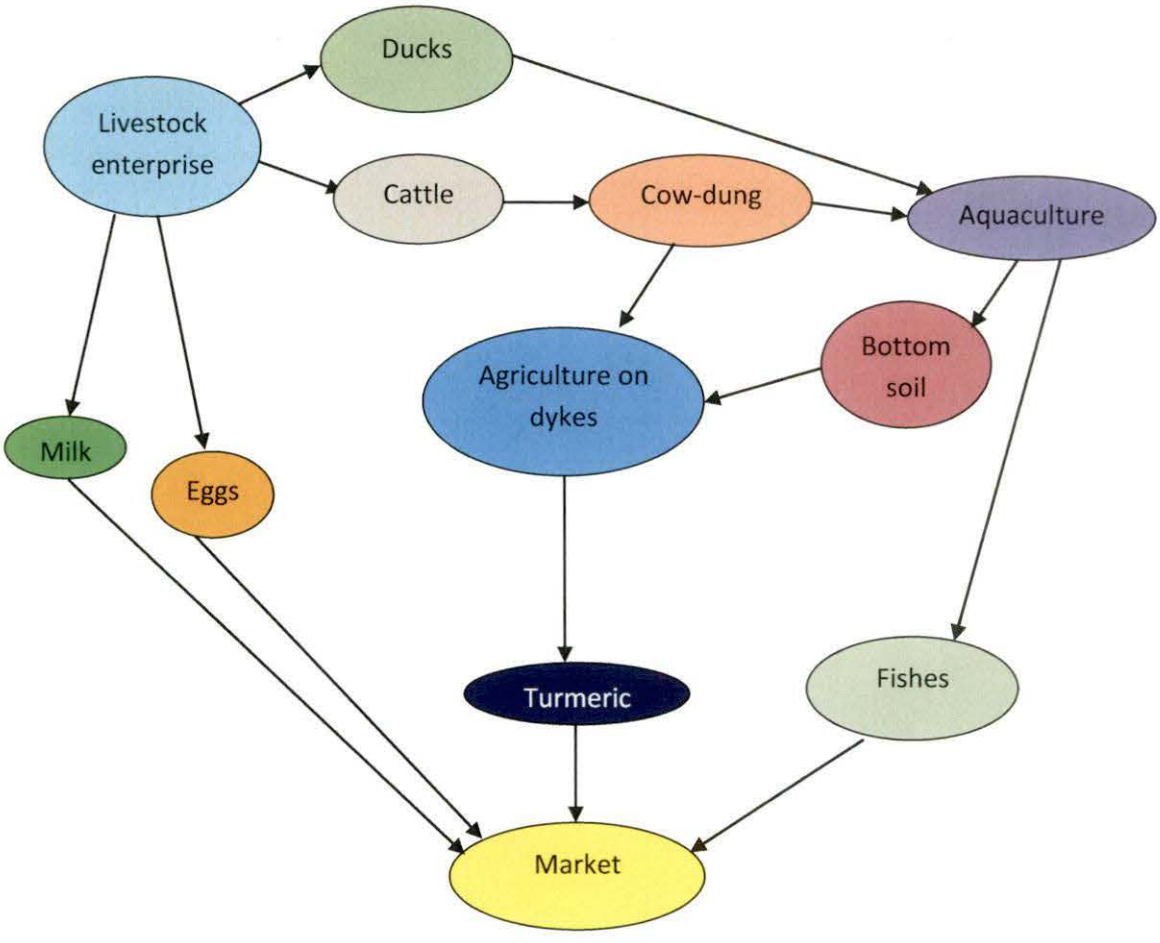


Fig- 7: Flow chart of Experiment-III (Integrated Farming System-II) showing the integration of different components of farming.

The plan of the work conducted in this study is described in Fig-8. The seasonal variation was studied considering the month April and May as summer season, June and July as pre-monsoon season and August and September as monsoon season. The Experimental protocol of the three experiments for the investigation is summarised in Table-2.

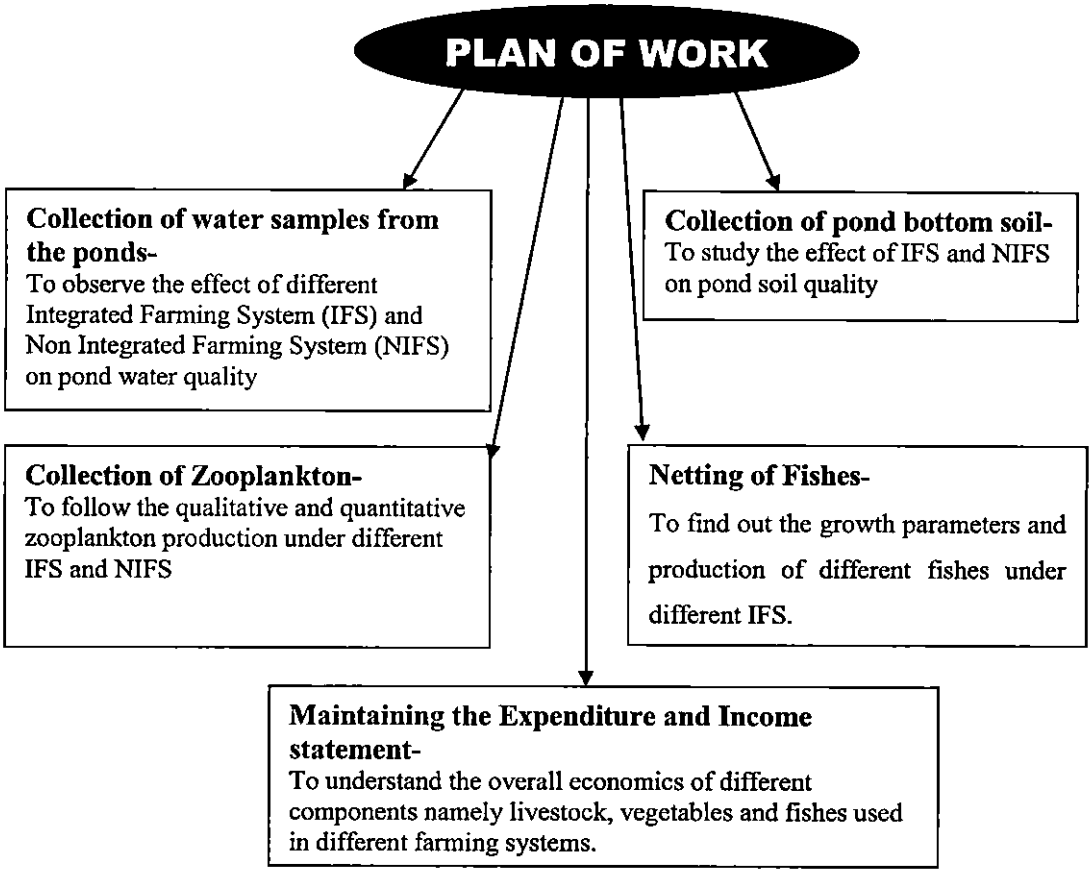


Fig-8: Flow chart explaining the Plan of Work to be executed during the study period (2008 to 2011).

Table-2: Summary of the Experimental Design showing different protocols followed in Experiment-I, II and III.

Protocols	Experiment-I (Control)	Experiment-II	Experiment-III
Components of farming	**Fish , cow and crop**	**Fish , cow and crop**	**Fish ,cow , duck and crop**
Type of farming	**Non-integrated (NIFS)**	**Integrated(IFS-I)**	**Integrated(IFS-II)**
No. of ponds	**3**	**3**	**3**
Average size of pond(ha)	0.01	0.01	0.01
Manuring	**No manuring**	**Manuring @2600kg ha^{-1} 10 d^{-1} with cowdung**	**Manuring @2600kg ha^{-1} 10 d^{-1} with cowdung**
Stocking density of fingerlings/ha	10,000	10,000	10,000
Types of fish stocked	IMC+Grass carp	IMC+Grass carp	IMC+Grass carp
Stocking ratio	3:3:3:1	3:3:3:1	3:3:3:1
Fish feeding schedule(on daily basis)	@ 2% of total body weight with Mustard oil cake and Rice Bran (1:1)	@ 2% of total body weight with Mustard oil cake and Rice Bran (1:1)	@ 2% of total body weight with Mustard oil cake and Rice Bran (1:1)
Type/No. of Cattle per pond	Non descriptive type,1 lactating cow	Non descriptive type,1 lactating cow	Non descriptive type,1 lactating cow
System of cattle rearing	**Extensive**	**Semi extensive**	**Semi extensive**
Feeding schedule of cow	**12 hours grazing with 3-4kg paddy straw**	**6 hours grazing and Concentrate feed and green grass along with paddy straw**	**6 hours grazing and Concentrate feed and green grass along with paddy straw**
System of duck rearing	**Nil**	**Nil**	**Extensive**
Type/No. of ducks per pond	**Nil**	**Nil**	**Non descriptive type, 20 Ducks of 22 wks of age**
Type of crop cultivated	**Turmeric plant(Suranjana Variety)**	**Turmeric plant(SuranjanaVariety)**	**Turmeric plant(Suranjana Variety)**
System of cultivation	**Separately on agricultural field**	**On pond dykes utilizing pond bottom soil**	**On pond dykes utilizing pond bottom soil**
Duration of Study	2008-2011(4 years)	2008-2011(4 years)	2008-2011(4 years)
Harvesting of Fish	After 150 days of stocking	After 150 days of stocking	After 150 days of stocking

Considering the market preferences and judicious exploitation of all the niches available in the ponds, Composite Fish Farming (four species culture) was done using Indian Major Carps (IMC) as *Catla catla, Labeo rohita* and *Cirrhina mrigala* and Exotic Carp as *Ctenopharyngodon idella.* The *Ctenopharyngodon idella* (Grass Carp)

was considered along with IMC as the semi-digested excreta of herbivorous fish could be utilised to fertilise the water and produce plankton for filter-feeding fish to consume (Martin *et al.*, 2005). The seeds (fingerlings) were collected from the available local market of the experimental area.

The livestock, considered in this study, was non-descriptive (local variety with no specific breed character) cows (*Bos indicus*) and ducks (Family-Anatidae), mostly prevalent amongst small and marginal farmers of this area with the production potentiality of 500 L to 800 L / lactation and 150-180 eggs / year , respectively. The average live body weight of adult female ducks was found to be 1.8-2.4 kg and that of male ducks was 2.5-3.0 kg. Pic - 2 and 3 showed the locally available cow and ducks, respectively.

Turmeric (*Curcuma longa* , Linn.) of family , Zingiberaceae having a temperature preference of 20°C - 30°C with considerable quantity of rainfall, was cultivated. It is a perennial rhizomatous herb and a native plant of South Africa. Turmeric being a spice and having medicinal value, the market demand of the same was observed to be always high in the local market. In the present study, the turmeric plant 'Suranjana variety' as shown in Pic - 4, was used for cultivation on the dykes of the pond. The productivity of this variety was 3 tons ha $^{-1}$ yr $^{-1}$.

Picture – 2: Feeding concentrate feed to the locally available non descriptive cow reared in Experiment- II and III.

Picture – 3: Locally available non descriptive ducks reared in Experiment- III.

Picture – 4: 'Suranjana variety' of turmeric cultivated in Experiment- II and III.

3.2.1 Experiment- I: Non Integrated Farming System (NIFS)

In this Experiment, the Non Integrated Farming System (NIFS) was considered as the Control (C). Three ponds were taken for fish farming, livestock and crop in isolation without any integration (Fig-5) for this experiment.

Each of the three ponds under the NIFS (Control) were seasonal and shrinking in nature with 10 to 12m of length, 10 to 12m of breath and depth of 1.5 to 2 m. In the month end of March, all aquatic weeds and existing stock of fishes were removed by repeated netting, and raw cow-dung at 3 tons ha^{-1} was applied as the basal dose 15 days prior to stocking of the fingerlings. Lime @250 kg ha^{-1} was applied three to four days prior to stocking every year. The reason for liming in fish ponds was to

neutralize soil acidity and increase total alkalinity and total hardness concentrations in water.

Ponds were stocked with Indian Major Carps (IMC) (*Catla catla, Labeo rohita* and *Cirrhina mrigala*) and Exotic Carp (*Ctenopharyngodon idella*) in the stocking ratio of 3:3:3:1 (Srivastava, 2009; Jena *et al.* 2007) @ 10,000 fingerlings ha $^{-1}$ having an average weight of 14.75±3.86 grams and average length of 9-10 cm. For the three ponds under the NIFS, supplementary feed was given in the form of Mustard Oil Cake and Rice Bran (1:1 ratio) @ 2% of total body weight of the fishes once daily after stocking the fingerlings. Netting was done twice a month to observe the growth rate parameters of the fishes, whereas desilting of the pond was not followed every year.

Livestock, as cattle, and non-descriptive in nature (without any specific breed characteristics) was selected for isolated practice. The productivity of the cattle, which was local, was found to be 500-600kg per lactation.

The cattle in the NIFS were reared as extensive system, thereby meaning that the cattle were allowed to graze whole day in the open pasture and at night only, night shelter was provided along with some 3-4 kg paddy straw and water. The cow dung was not collected during daytime for integration with aquaculture. Milking of the cow was done twice daily and daily milk records were used for estimation of milk production. De-worming of cattle was done routinely thrice in a year.

Under this experiment, no duck was maintained and the pond dyke was not utilized for any crop cultivation. Dykes were maintained to prevent the overflow of the pond water in the rainy season and thereby protecting the fishes.

A small field of area 100 m^2 (10m of length and 10m of breath) was prepared for the NIFS only to cultivate turmeric in isolation after applying cow dung @ 4 tons ha^{-1}. The recommendations for turmeric cultivation are given in Table – 3 (Kumar *et al.*, 2003). The use of extra fertilizer was not done. All the products namely fishes, turmeric and milk were collected and sold to the wholesaler without any value addition.

Table- 3: The recommendations per year calendar activities for turmeric cultivation.

Date/month	Activities
28 march	3-4 ploughing 8-10 inches deep and leveling properly to make the soil fine.
30 march	For Non- Integrated Farming System adding only cowdung @4 ton bigha $^{-1}$. For Integrated Farming System-I and Integrated Farming System-II Adding whole pond humous along with cowdung @4 ton bigha^{-1}
19 April	Final land preparation, seed bed preparation and seed treatment
20 April	Sowing of seed
21 April	Irrigation followed by Mulching
5 June	Weeding, irrigation if necessary
21 July	Weeding
20 Nov	Harvesting

3.2.2 Experiment-II: Integrated Farming System-I (IFS-I)

The enterprise selected for integration of the experiment IFS-I was livestock (local cow), fish and crop (turmeric). The integration of cow dung with fish culture along with turmeric cultivation on the pond dykes utilizing the bottom soil of ponds was the main objective of the study (Fig-6). For this treatment, three numbers of ponds were selected which were seasonal and shrinking in nature with an average size with 10 to 12m of length, 10 to 12m of breath and depth of 1.5 to 2 m. Single local cow per unit of pond was maintained for integration. No duck was maintained to integrate with the fish culture. Cultivation of turmeric was done considering total 100 m of length and 1 m of breath surrounding the pond dykes (100m X 1m= 100m^2).

In the end of March, ponds were dried to reduce the moisture content of soil so that air could enter the pore spaces among soil particles .The bottom soil along with aquatic weeds were removed (de-silted) and spread over the dykes of the pond to prepare for the cultivation of turmeric. The existing stock of fish in all the three ponds under this experiment were removed for drying and raw cow-dung at 3 tons ha $^{-1}$ was applied as the basal dose 15 days prior to stocking. Lime @250 kg ha $^{-1}$ was applied three to four days prior to stocking of the fingerlings every year. The reason for liming in fish ponds was to neutralize soil acidity and increase total alkalinity and total hardness concentrations in water. This could enhance conditions for productivity of food organisms and increase aquatic animal production (Boyd and Tucker, 1998).

Ponds were stocked with IMC and Grass Carp same as it was mentioned in NIFS. Application of cow-dung @ 2600kg ha $^{-1}$ once in ten days (Jha *et al.,* 2004) was followed for the ponds under this experiment. The supplementary feed recommended was same as followed in the NIFS. Netting was done twice every month to observe the growth rate parameters of the fishes. Desilting of pond was done once in every year so that the pond bottom soil containing organic waste after cultivation of the fish can be utilised for cultivation of turmeric on the pond dykes.

Livestock selected for integration was also local cattle mentioned earlier in NIFS but, under IFS-I one local lactating cow of 3-4 years old was selected for integration with each unit of the pond size area. They were maintained under semi-intensive system and fed with 1kg of concentrate daily for maintenance along with 3-4 kg paddy straw and water. Table-4 describes the composition of concentrate feed (kg ton^{-1}) which was fed to the cattle. The manure obtained was used to fertilize the ponds @ 2600kg ha $^{-1}$ once in ten days. De-worming of the cow was done routinely thrice in a year.

Table-4: Composition of concentrate feed (kg ton $^{-1}$).

S.No.	Ingredients	Amount (kg ton $^{-1}$)
1	Maize	250
2	Soya bean	100
3	De-oiled Rice Bran	450
4	Mustard Oil Cake	170
5	Salt	10
6	Vitamin and Minerals	5(gram)

Milking of the cow was done twice daily and daily milk records were used for estimation of milk production.

The surrounding pond dykes considered for cultivation of turmeric was 100 m^2 as describe earlier. The dykes of the ponds under this experiment were ploughed 3 to 4 times and leveled properly to make the soil fine by the end of March. After applying whole pond bottom soil with cow dung @ 4 tons ha $^{-1}$ the dyke was made ready for cultivation. The recommendations for turmeric cultivation are described in Table-3. The use of extra fertilizer was not recommended. Only the excavated bottom soil of pond containing manure was used once in a year during the month of March.

All the products namely fishes, turmeric and milk were collected and sold to the wholesale market without any value addition.

3.2.3 Experiment-III: Integrated Farming System-II (IFS-II)

For the experiment IFS-II, integration of livestock (local cow and local ducks) with fish culture and crop (turmeric) were practiced. The main objective of the study was to integrate cow dung and local ducks rearing on the pond with fish culture along with turmeric production on the dykes utilizing the bottom soil of ponds. Three seasonal ponds having an average size 10 to 12m of length, 10 to 12m of breath and

depth of 1.5 to 2 m were selected for IFS-II. Ponds were dried and bottom soil with aquatic weeds were utilized on the dykes for turmeric cultivation same as it was done in IFS-I (Pic - 5, 6 and 7). Raw cow-dung application @ 3 tons ha $^{-1}$ as the basal dose 15 days prior to stocking. Lime @250 kg ha $^{-1}$ three to four days prior to stocking of the fingerlings every year so as neutralize soil acidity and increase total alkalinity and total hardness concentrations in pond water. Ponds were stocked with IMC and Grass Carp in the same way as mentioned earlier in NIFS and IFS-I.

Picture – 5: Dried pond to excavate to pond bottom soil.

Picture – 6: Spreading pond bottom soil on the dykes.

Picture – 7: Preparation of pond dykes for cultivation.

Application of cow-dung for the ponds under IFS –II was also followed similarly as it was followed in IFS-I. The supplementary feeds recommended were the same as mentioned in NIFS and IFS-I. Netting and desilting of pond was done as it was mentioned for IFS-I earlier. Integration of local lactating cattle was also done similarly as was mentioned earlier under experiment IFS-I.

In addition to cattle, in this experiment, about 22 weeks old local ducks having 1200 ± 50 gm were introduced @ 20 ducks pond $^{-1}$ after 2 months of stocking of fish that is, from June to September. After one year, the old ducks were replaced again by 22weeks old ducks in the month of June. The ducks were allowed to go to the ponds under IFS-II in the morning (9.00 a.m) and come back to their habitat (farmer's house) in the evening (5.00 p.m). Scavenging mode or wild grazing practice was adopted. Ducks were fed (average 100g) with fresh kitchen leftovers and agricultural by-products such as rice bran and broken grains. The ducks were housed in cleaned, dried and well-ventilated place. De-worming and vaccination for cattle and ducks were carried out from time to time.

Fig-7 (as shown earlier) describes the flow chart of the Experiment-III (IFS-III) showing the different integration established amongst the different components.

The dykes of the ponds under this experiment were also cultivated as described earlier in IFS-I.

All the products namely fishes, duck eggs, duck meat, turmeric and milk were collected and sold to the wholesale without any value addition.

3.3 Experimental Procedure

3.3.1 Pond Water Sampling

Water samples from each experimental pond under NIFS, IFS-I and IFS-II were collected in sterile bottle during 7am to 8am on a bimonthly (2008 to 2011) basis and the same were transported to the Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, within 2 hours of collection and used for estimation of the water quality parameters.

3.3.2 Water Quality Analysis

The water quality parameters, namely Temperature (°C), pH, Dissolved Oxygen (mgl^{-1}), Free Carbondioxide (mgl^{-1}), Total Alkalinity (mgl^{-1}), Chloride (mgl^{-1}), Total Hardness (mgl^{-1}), Ammonium-N (mgl^{-1}), Nitrite-N (mgl^{-1}), Nitrate-N (mgl^{-1}) and Phosphate-P (mgl^{-1}) from each of the experimental ponds were monitored following the Standard Methods (APHA, 2005).

3.3.2.1 Temperature

Temperatures of air and water were recorded by using mercury Celsius (^{0}C) thermometer on spot.

3.3.2.2 Potential (Puissance) of Hydrogen in concentration (pH)

The pH was measured by using a portable pH meter (Multi-parameter PCS Testr 35, Eutech Instruments, Oakton) immediately after sampling the water in a beaker.

3.3.2.3 Dissolved Oxygen (Modified Winkler's Iodometric Method)

Dissolved Oxygen (DO) levels in natural and wastewaters reflect the physical, chemical and biochemical processes prevailing in the water body. The actual quantity of dissolved oxygen that water can hold under most favourable condition is much less than that constantly present in the atmosphere. The concentration of dissolved oxygen in the water varies in different seasons and reaches its peak in summer season when photosynthesis is high. However, the amount of dissolved oxygen present depends on the factors like sunlight, temperature, transparency, current, eutrophication, phytoplankton and salinity. The analysis for DO is a key test in water pollution and waste treatment process control.

The iodometric method is a titrimetric procedure based on the oxidizing property of DO. The manganous sulphate ($MnSO_4$) reacts with the alkali (KI or NaOH) to form a white precipitate of manganous hydroxide which in the presence of oxygen gets oxidized to a brown colour compound. In the strong acid medium (H_2SO_4) manganic ions are reduced by iodide ions which get converted to iodine equivalent to the original concentration of oxygen in the sample. The iodine can be titrated against N/40 sodium thiosulphate using starch (1%) as an indicator (APHA, 2005).

Water samples were collected in narrow-mouth glass-stoppered BOD bottles of 300 ml capacity, taking all necessary precautions to avoid air bubbles. 2ml of Winkler's A (Manganese sulphate) and Winkler's B (Alkaline potassium iodide) reagents were used to immediately fix the samples. The resultant precipitate thus formed was dissolved by 2ml concentrated Sulpuric Acid. The sample was then titrated against N/40 Sodium thiosulphate ($Na_2S_2O_3$) solution using starch as an indicator. The concentration of Dissolved Oxygen (mgl^{-1}) was calculated using the following formula:

$$\frac{ml\ of\ Na_2S_2O_3 consumed\ X\ 0.025X8X1000}{Volume\ of\ the\ sample\ titrated}$$

Where,

8 = Molecular weight of Oxygen

Volume of the sample titrated = 101.35 ml

As calculated using the formula,

$$\frac{100X300}{(300-4)}$$

When,

Original sample to be taken as per procedure =100ml

Volume of the BOD bottle =300 ml

Total volume of the Winklers'A and Winklers'B = 4 ml.

3.3.2.4 Free Carbon dioxide

Free Carbon dioxide (Free CO_2) levels below 10 $mgl^{-1}$are thought to be well tolerated by fish, although sensitivity to the gas varies between species. The level of free CO_2 in the water varies with the respiratory and photosynthetic activity of

animals and plants in incoming water, the level of decomposition of organic material in that water and the respiration of the fish themselves. Free CO_2 can build up to significantly high levels in systems with large numbers of fishes and relatively slow water turnover.

Free CO_2 can be determined by titrating the sample using a strong alkali N/44 NaOH to pH 8.3. At this pH all the free CO_2 is converted into bicarbonates.

The concentration of free CO_2 (mgl^{-1}) in 100 ml sample water was analyzed using 6 drops of 1% Alcoholic phenolphthalein as indicator and N/44 NaOH as titrant as described by Golterman *et al.,* (1978) and Trivedy and Goel (1984). It was calculated using the following formula,

$$= \frac{Vol.of\frac{N}{44}NaOH\ solution\ consumed\ X\ 1000}{Vol.of\ sample\ taken}$$

3.3.2.5 Total alkalinity

Alkalinity of the water is its capacity to neutralize a strong acid and is characterized by the presence of all hydroxyl ions capable of combining with the hydrogen ion. Alkalinity in natural waters is due to free hydroxyl ions and hydrolysis of salts of carbonates, bicarbonates, phosphates, nitrates, borates, silicates etc. formed by weak acids and strong bases. However, most of the waters are rich in carbonates and bicarbonates with little concentration of other alkalinity imparting ions.

Total alkalinity, carbonates and bicarbonates can be estimated by titrating the sample with a strong acid (HCl or H_2SO_4), first to pH 8.3 using phenolphthalein as an indicator and then further to pH between 4.2 and 5.4 with methyl orange or mixed indicator. In the first case, the value is called as phenolphthalein alkalinity (PA) and in second case it is Bicarbonate alkalinity (BA).The sum of total of PA and BA is the

Total Alkalinity. Values of carbonates, bicarbonate and hydroxyl ions can be computed from these two types of alkalinity.

Total alkalinity present in the 100 ml water samples were estimated as mgl^{-1} using 5 drops phenolphthalein and 2drops of methyl orange as indicators and then titrated against N/50 H_2SO_4 (Golterman et al.,1978) and Phenolphthalein alkalinity was estimated only when free carbon-dioxide were found to be absent (Trivedy and Goel ,1984). The concentration (mgl^{-1}) of each alkalinity was calculated by using the following formula,

$$\frac{\textbf{ml of titrant} \times \textbf{1000}}{\textbf{Volume of sample used}}$$

3.3.2.6 Total Hardness

Total Hardness is generally caused by the calcium and magnesium ions present in water. Polyvalent ions of some other metals like strontium, iron, aluminum, zinc and manganese, etc are capable of precipitating the soap and thus contributing to the hardness. However, the concentrations of these ions are very low in natural waters.

Hardness is generally measured as concentration of only calcium and magnesium as calcium carbonate, which are far higher in qualities over other hardness producing ions. Calcium and magnesium form a complex of wine red colour with Eriochrome Black T at pH of 10.0±0.1.The EDTA has got a stronger affinity towards Ca^{++} and Mg^{++} and therefore by addition of EDTA the former complex is broken down and a new complex of blue colour is formed.

EDTA Method (APHA, 2005) was followed to estimate the Total hardness of the 100 ml water samples as mgl^{-1}. It was estimated by titrating the water sample

against Ethylene diamine tetra acetic acid (EDTA) after adding 0.5ml ammonium buffer and 6 drops Eriochrome Black-T as indicator. The end point was indicated by blue colour.

The concentration (mgl^{-1}) was calculated using the following formula,

$$\frac{\textbf{ml of EDTA used} \times \textbf{1000}}{\textbf{Volume of sample used}}$$

3.3.2.7 Chloride

Chloride, in the form of chloride (Cl^-) ion, is one of the major inorganic anions in water and wastewater. The most important source of chlorides in the waters is the discharge of domestic sewage. Man and other animals excrete very high quantities of chlorides together with nitrogenous compounds.

Silver nitrate reacts with chloride to form very slightly soluble white precipitate of AgCl. At the end point when all the chlorides are precipitated, free silver ions react with chromate to from silver chromate of reddish brown colour.

The Chloride content of 100 ml water samples were determined following Argentometric Method, adding 2 ml K_2CrO_4 and titrating it against 0.02N $AgNO_3$ until a persistent red tinge appeared as described by (APHA, 2005) and was expressed as mgl^{-1}.

The concentration (mgl^{-1}) was calculated using the following formula,

$$\frac{\textbf{(ml} \times \textbf{N)of } \mathbf{AgNO_3} \times \textbf{1000} \times \textbf{35.5}}{\textbf{Volume of sample used}}$$

3.3.2.8 Ammonium-N

The most important source of ammonia is the ammonification of organic matter. In this study, the organic matter like cow-dung and duck droppings were utilised to fertilize the pond for fish culture under different integrated farming systems. Hence, disposal of such organic matters tends to increase the ammonia content of the waters. Occurrence of ammonia in the waters indicates the evidence of organic pollution. Ammonia in higher concentration is harmful to fish and other biota. The toxicity of ammonia increases with pH because at higher pH most of the ammonia remains in the gaseous form. The decrease in pH decreases its toxicity due to conversion of ammonia into ammonium ion which is much less toxic than the gaseous form.

An intensely blue compound, indophenols , is formed by the reaction of ammonia, Sodium Oxidising solution (10ml alkaline citrate solution with 2.5ml sodium hypochlorite) , and Alcoholic Phenol catalyzed by Sodium Nitropruside.

Ammonium nitrogen (NH_4-N) of 25ml filtered sample of pond water was estimated by Phenol-hypochlorite method (APHA, 2005). 1ml of each Alcoholic Phenol solution, Sodium Nitropruside solution and Oxidizing solution (10ml alkaline citrate solution with 2.5ml sodium hypochlorite) were added to the samples. The samples were then wrapped with aluminium foil and kept at room temperature (22 – 27 ^{0} C) in subdued light for at least 1 hour. A blue colour appeared which was stable for 24 hours. The NH_4-N concentration of the samples was directly estimated through a double beam UV – Visible Spectrophotometer (Ray Leigh UV-2601) at 640nm wavelength. A standard curve was prepared by using Stock Ammonium Chloride

Solution (0.3819g anhydrous NH4Cl in 100ml DW) to estimate the Ammonium-N concentration of the water samples in mgl^{-1}.

3.3.2.9 Nitrite-N

Nitrite represents an intermediate form during denitrification and nitrification reactions in nitrogen cycle. Nitrite is very unstable ion and gets converted into either ammonia or nitrate depending upon the conditions prevailing in the water. Presence of even a small quantity of nitrite will indicate the organic pollution and the availability of partially oxidized nitrogenous matter. Lethal effects of nitrite in pond were studied by Tilak *et al.*, (2007) and obtained the value to be 171.06 ppm (0.17 mgl^{-1}) for 24 hr.

Nitrite forms a diazonium salt with sulphanilic acid in acid medium (2.0-2.5 pH), which combines with ά-naphthylamine hydrochloride to form a pinkish dye. The colour so produced obey Beer's Law and can be determined spectrophotometrically at 520 nm.

Nitrite- nitrogen (NO_2-N) content of 50ml filtered sample water was estimated using the α-Napthalamine and Sulphanilic Acid Method as described in APHA (2005). The estimation was done using a double beam UV – Visible spectrophotometer (Ray Leigh UV-2601). 1ml of each Ethylene diamine tetra acetic acid (EDTA), Sulphanilic-acid and α-naphthalamine hydrochloride were added to the sample in sequence. A pinkish colour developed and after 10 minutes, observations were taken through a double beam UV – Visible Spectrophotometer at 520 nm wavelength. The concentration was obtained through a standard curve prepared by dissolved $NaNO_2$ in 1 litre of distilled water.

3.3.2.10 Nitrate-N

Nitrate represents the highest oxidized form of nitrogen. The most important source of the nitrate is biological oxidation of organic nitrogenous substances which come in sewage and industrial wastes or produced indigenously in the waters. In the waste treatment systems, high amounts of nitrate denote the aerobic conditions and high stability of the wastes. Although high concentration are useful in irrigitation but their entry into the water resources increases the growth of nuisance algae and triggers eutrophication.

Nitrate and Brucine react to produce a yellow colour, the intensity of which can be measured at 410nm. The reaction is highly dependent upon the heat generated during the test. However, it can be controlled by carrying out the reaction for a fixed time at a constant fixed temperature. The method is suitable for the samples having a very wide range of salinity.

Brucine Method was followed to estimate the Nitrate-nitrogen (NO_3-N) concentration present in the water samples in mgl^{-1} (Trivedy and Goel ,1984). After proper filtration, the residual chloride was removed from the water samples by adding one drop of Sodium Arsenite solution. 10ml of water sample was then placed in cool water bath and 2 ml 30% NaCl solution was added. 10ml H_2SO_4 solution (500ml concentrated H_2SO_4 in 125ml distilled water) was added after mixing the contents thoroughly swirling by hand. 0.5 ml Brucine reagent (Brucine- Sulfanilic acid solution) was added to the samples and then placed in hot water bath for 20 minutes. After cooling the NO_3-N of the water samples were directly estimated through a double beam UV – Visible Spectrophotometer (Ray Leigh UV-2601) at 410 nm wave length. A standard curve was prepared using standard KNO_3 solution in distilled

water and the NO_3-N concentration of the water samples were estimated from the standard curve.

3.3.2.11 Phosphate-P

Phosphorus in the natural freshwaters is present mostly in inorganic forms such as $H_2PO_4^-$, HPO_4^{-2} and PO_4^{-3}. Phosphorus being an important constituent of biological systems, may also be present in the organic forms. The phosphorus content of natural freshwaters is low. The major sources of phosphorus are domestic sewage, detergents, agricultural effluents with fertilizers and industrial waste waters. The higher concentration (greater than 0.5 mgl^{-1}) of phosphorus, therefore, is indicative of pollution.

The phosphate in water react with Ammonium Molybdate and form a complex heteropoly acid (MolybydoPhosphoric Acid), which gets reduced to a complex of blue colour in the presence of Stannous chloride ($SnCl_2$). The absorption of light by this blue colour can be measured at 690nm to calculate the concentration of phosphates.

The Phosphate (PO_4-P) content of water samples were determined by following Stannous Chloride Method (APHA, 2005). 2 ml Ammonium Molybdate solution(25.0 g Ammonium Molybdate + 280 ml of conc H_2SO_4+ upto 1000ml distilled water) and 5 drops Stannous Chloride in glycerol were subsequently added to the properly filtered 50 ml water samples. A blue colouration appeared. The samples were estimated through a double beam UV – Visible Spectrophotometer (Ray Leigh UV-2601) at 690 nm wavelength in between 10 to 12 minutes. A standard curve was prepared through known concentrations of PO_4-P solution (10mg P/lml) and the PO_4-P concentration of the water samples were determined and expressed in mgl^{-1}.

3.3.3 Pond Soil Quality Analysis

In aquatic ecosystems, the sediments are in a complex milieu with the overlying water, they affect water chemistry and are being affected by it. Using an Ekmann's dredger, the pond bottom soil was collected from 10 places and mixed well. The soil samples were then air dried, pulverized with pestle and mortar and sieved through 150μm mesh size sieve and stored in labelled polythene bags before estimation of different parameters.

3.3.3.1 Soil pH

pH of soil is the measure of the 'hydrogen ion activity' and depends largely on relative amounts of the absorbed hydrogen and metallic ions. Thus, it is a good measure of acidity and alkalinity of a soil-water suspension and provides a good identification of the soil chemical nature. pH of soil suspension highly depends on the soil : water ratio and increases with dilution. The soil pH was measured with electrically operated pH meter using soil: water (1:1) suspension (Jackson, 1967).

3.3.3.2 Organic Carbon

The Organic matter present in the soil is digested with excess of potassium dichromate and sulphuric acid, and the residual unutilized dichromate is then titrated with ferrous ammonium sulphate. One ml of 1N $K_2Cr_2O_7$ solution is approximately equal to 0.003g of carbon. It has been estimated, only 77% carbon of the organic compound in soil was oxidized by normal potassium dichromate solution. So, 0.003 should be multiplied by 1.3 to get the total % of carbon present in the soil.

For estimation of Organic Carbon, by Walkley-Black Oxidation Method (Walkley and Black, 1934), 2 gram of air-dried powdered sediment sample treated

with 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of concentrated Sulphuric acid was mixed well. The sample was then agitated for 30 minutes in a mechanical shaker. The agitated sample was then diluted with 170 ml distilled water and then 10 ml Phosphoric acid and 1 ml Diphenyl amine indicator were added. This was then titrated against 0.4 N Ferrous Ammonium Sulphate (Mohr's salt) solution until brilliant green colour appeared (Jackson, 1967). A blank with same quantity of chemicals but without soil was also run simultaneously. The % of the Organic Carbon was calculated using the following formula:

$$\% \text{ of Organic Carbon} = \frac{(B-T)X\ FX0.003X\ 100}{W}\ X\ 1.3$$

Where, B= ml $FeSO_4$ solution required for Blank

T= ml $FeSO_4$ solution required for soil sample.

W= weight of the soil sample

F= normality factor (0.4)

1.3.3.3 Total Nitrogen

Nitrogen in the soil is present mostly in the organic form, together with small quantities of ammonium and nitrates. The total nitrogen of the soil was determined by Kjeldahl Method (Kjeldahl, 1883) modified by Cope (1916).

The procedure involves three main steps namely, digestion, distillation and titration as discussed latter.

a) Digestion

10 grams of sample was digested in 30 ml concentrated Sulphuric acid in the presence of 1g Salicylic Acid. 5g of Sodium thiosulphate was then added and heated

for 5 minutes. After cooling, 20ml of water was added along with 10 g of catalyst mixture (100g K_2SO_4 + 10g $CuSO_4.5H_2O$+1g selenium powder) for digestion until a blue colour appears.

b) Distillation

After cooling the contents, 50ml water was added to the digestion flask and swirled for 2 minutes. The supernatant liquid was then collected in a distillation flask. This process was repeated four times. 10ml of Sodium Sulphide was then added followed by 135ml of 40% Sodium Hydroxide solution. Ammonia gas was then distilled and collected in a receiver flask containing 25 ml Boric Acid and mixed indicator (Bromocresol green and 0.1 g of methyl red in 100ml).

c) Titration

The unreacted Boric Acid solution was back titrated with standard sulphuric acid till the blue colour disappeared. A blank without the soil sample was also carried out.

The % of Nitrogen was calculated using the following formula:

$$\% \text{ of Nitrogen} = \frac{\mathbf{a-b}}{\mathbf{S}} \times \mathbf{N} \times \mathbf{1.4}$$

Where, **a**= ml H_2SO_4 required for sample

b = ml H_2SO_4 required for sample

S = Sample weight in grams

N= Normality of H_2SO_4

3.3.3.4 Carbon Nitrogen ratio (C: N ratio)

Carbon to Nitrogen ratio in balance is a term used to describe a type of nitrogen deficiency in soil. When an organic material is applied to a field, it adds nutrients and organic matter to the soil. The organic matter contains about 60%

organic carbon. The Carbon: Nitrogen (C:N) ratio shows the proportion of organic carbon to total nitrogen of a manure or organic material.

In this present study, cow dung and duck excreta were utilized for fish production. The C: N ratio of pond bottom soil was analyzed because it was utilized to cultivate turmeric on the pond dykes.

Nitrogen is a food source for the micro-organism while they breakdown organic carbon. The nitrogen can come from the added organic material or it can come from the soil. During the process of carbon breakdown soil microbes die and decomposed. The microbial nitrogen is then returned to the soil and becomes available to the plants. This adds to the organic nitrogen pool within the soil along with the added organic material. How long the carbon breakdown process takes depends on the ratio of carbon to nitrogen in the material and in the soil.

The carbon to nitrogen ratio of soil is about 10:1. Solid cattle manure has 20:1 to 40:1 C:N ratio range and solid poultry manure has 5:1 to 10:1 C:N ratio range. When solid manure or other organic material having C:N ratio of greater than 30:1 is added which arises higher risk that soil microbes will steal nitrogen from the soil and tie it up so that the nitrogen become unavailable to the plant.

The Carbon Nitrogen ratio was calculated in this study, by dividing the value of carbon content of the pond bottom soil with the value of the nitrogen content of the same pond bottom soil (Boyd, 1995).

3.3.4 Zooplankton Analysis

Samples of zooplankton were collected with plankton net made of standard bolting silk cloth (No.21 with 77mesh/cm²). About 10 litres of water was collected randomly from selected locations and pooled together for filtering through the plankton net. Collected plankton were concentrated to 20ml, and preserved in 4% formalin for further estimation. Qualitative and quantitative determinations of zooplanktons were made under binocular compound microscope (Magnus make) using 1 ml concentrated solution preserved sample. Sedgwick-Rafter Counter Cell was used to count the planktons. The numbers were expressed in numbers per litre.

It was reported, that irrespective of different feed habits, the IMC and Grass Carp prefer to feed on zooplanktons (Rahman *et al.*, 2006). Jha *et al.*, (2006) also observed that introduction of zooplankton into a fish culture increases the growth rate of carp species. Hence, in the present study, only the zooplanktons were identified with the aid of Needham and Needham (1962), Edmondson (1992) and Battish (1992).

The diversity of individual zooplankton species was calculated using Shannon- Wiener Diversity index (Shannon and Weaver, 1949; after Krebs, 1999) which is expressed as (H').

$$(\mathbf{H'}) = -\sum_{i=1}^{S}(\mathbf{pi})(\mathbf{Log2pi})$$

Where,

H' = Index of species diversity (bits/individual)

S= Number of species

pi= proportion of total sample belonging to ith species (ni/N)

ni=number of individuals of species i in the sample

N= Total number of individuals in the sample= $\sum ni$

The evenness of the zooplankton was calculated using Index of Evenness (Pielou, 1966) as follows:

$$J' = \frac{H'}{H'Max}$$

Where

J′= Evenness measure (range 0-1)

H′= Shannon-Wiener function

H'_{MAX} = Maximun value of H′= $\log_2 S$

3.3.5 Fish Growth

Fishes were randomly sampled from each pond at an interval of 30 days for studying the fish growth parameters and then returned to the pond. Before netting, fishes were starved for 24 hours to avoid stressful effect of netting and handling (Ricker, 1968).

All the fishes from the ponds under the three experimental schedules were harvested after 150 days, which was at the end of September of each year (2008-2011) with stocking of fingerlings in April of each year too.

The Absolute Growth of fish, Growth Increment of fish/day and Total weight gain by fish were calculated using the following formulae:

(a) The Absolute Growth = $W_2 - W_1$

where, W_2 = Initial body weight and

W_1 = Final body weight

(b) The Growth Increment in fish per day= $\frac{W_2 - W_1}{Number\ of\ culture\ days}$

where, W_1= Initial body weight and

W_2 = Final body weight

(c) The Total Weight Gain by fish = $\frac{W_2 - W_1}{W_1}$

where, W_1 = Initial body weight and

W_2 = Final body weight

3.3.6 Economics

The expenditure incurred during each farming activity was recorded and was compiled for calculation of average expenditures incurred on different components of the farming system for assessment. Similarly, the income received by selling each farm produce was recorded and compiled for calculation of average income from different component of the farming system.

Cost-benefit analysis of the data was carried out on the basis of current market prices for the procurement of advanced fry of fish and returns of fish species following the simple procedure as suggested by Jolly and Clonts (1993).

Profit (Benefit) = Gross Output –Total Cost

Benefit achieved per unit expenditure, that is, Benefit (B) : Cost (C) ratio, from the different farming systems was calculated as per the following formula :

B: C ratio = Benefit : Cost

3.4 Statistical Analysis

The value of Mean and Standard Deviation of all the studied parameters under different treatment was done. To verify the significant differences of the data between the three treatments, One-way Analysis of Variance (ANOVA) was done as described by Gomez and Gomez, (1984). If the main effect was found significant, the ANOVA was followed by a Least Significant Difference (LSD) using Duncan's Multiple Range Test (DMRT). Pearson's correlation was used to establish the relation between the different parameters. All statistical tests were performed at a 5% probability level using the statistical package SPSS (Version-18).