RESEARCH PAPER | Zoology | Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

The Effect of Organic Manure and Supplementary Diet on Growth Performance of *Heteropneustus fossilis*

KEYWORDS: *Heteropneustus fossilis*, mix feed, cow dung and poultry waste

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ABSTRACT *The present investigation was carried out to study the effect of organic manure such as cow dung and poultry manure with and without feed supplements on growth performance and other biological parameter of Heteropneustus fossilis. Five different treatments such as mix feed, only cow dung, only poultry waste, cow dung and mix feed and poultry waste and mix feed were used. The study was conducted for 35 days. Cow dung and poultry manure were supplemented according to area of experimental tank and mix feed were supplemented according to 3% of body weight of fishes. The results showed increase in body weight of fishes in all the treatments.*

Introduction

Aquaculture in India assumed new dimension during recent years. Through scientific orientation, new technology have been developed and successfully implemented. These effective fish culture technologies have brought a lot of awareness and enthusiasm among the fish culturists in the country. The average production of fish in the country which was mere 0.6 tones / ha / year, prior to the development of the present technologies has now been raised to the tune of 12-15tonnes/ha/year. However, as these technologies are mainly based on species combination and ratio, stocking density, heavy feeding schedule and certain other management measures they could not raise the production levels any further. Also, due to the rising cost of the feed ingredients, which are the main input components in the culture system, the present day fish culture technologies appear not to be cost effective any more.

Among the air breathing cat fishes, stinging cat fish Clariousbatrachus and *Heteropneustus fossilis* is very popular and high priced fish in all over India. *Heteropneustus fossilis* is derived from Greek word in which Heteros means "other", pneo means to breath(atmospheric Air) fossils means "ancient". It is commonly known as singhi. They differ from other teleost by possessing an accessory respiratory organ (Doria and Leonhardt, 1993) for direct aerial respiration by periodically gulping air at the air water interface. It is found in fresh water of India, Pakistan, Ceylon, Burma, Thailand, Bangladesh, sri-lanka, Indonesia, Philippines, loas and china. It is considered to be highly nourishing, palatable and tasty and well preferred because of its fewer spines, less fat and high protein in many parts of Indian subcontinent, Owing to its taste, medicinal value (Gerking, 1966) and can be marketed as live fish. It is having good demand in the domestic market.

Cat fish culture has number of advantage over other culture such as greater survival in oxygen depleted water, tolerance to crowding, high stocking rate on artificial feed, fewer intramuscular bones and tender flesh and delicious taste. It occur in all types of pond and can survive for a very long time when kept in captivity even in a small quantity of water for it has massive paired sac-like pharyngeal lungs as accessory respiratory organs.

Heteropneustes fossilis inhabits muddy bottoms of weed Infested swamps, subsisting on rich benthic fauna and detritus of decaying organic matter (Munsi, J.J.D 1996). It is found in swamps, ponds, beels, tanks, rivers and wetlands. It is able to tolerate slightly brackish water .Its air breathing apparatus enables it to adopt in almost any kind of water. It lives in large shoals and extensively fished because of the reported invigorating qualities of its flesh. During the dry season, singhi lives in semi-liquid and semi-dry mud and even at the bottom of fissures and crevices formed by the cracking of mud. Colour of H.fossilis is Leaden or dark purplish- brown above, lighter below, usually with two lateral yellowish bands. Young one is reddish. Regarding characteristic features body of H. fossilis is elongated, sub cylindrical to pelvic fin base, compressed behind. It poses a pair of long, hollow cyclindrical cavities extending backward on one side of the body from the gill cavity through the muscles of the back. Branchiostegals are 7 in numbers. Gill opening with its membranes not confluent with the skin of the isthmus and separated by deep notch. Head is depressed and is covered over with skin, Mouth is transverse. Teeth in villiform bands on jaws.Barbels four pairs, dorsal shorter with spine, anal fin vary long not confluent with caudal. Skin smooth, leaden, or dark purplish often with two yellowish bands, young ones reddish brown. Fishes from beels and swamps are darker than the pond reared. This fish is much dreaded because of its

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

aggressive behaviors and can inflict painful wounds with its potentially dangerous pectoral spines.

It is a heterosexual fish. Attain maturity at the end of one and a half year of its life. Its fecundity ranges from 2922-20550 eggs. It breeds during rainy season. It moves into rice field to spawn when there is flood and both male and female excavates hollow in the depression (Padlan, 1960). The egg are deposited and guarded by both parents. Parental care continues over a considerable length of time. Eggs are semi-adhesive, demersal and spherical ion form measuring between 1-2 to 2.6mm in diameter with reddish orange blastoderm with green yolk. The yolk is absorbed on 4th day and air breathing habit start from 8th day. Incubation time is 18-26 hrs depending upon temperature. It can breed in captivity (Beaven, 1877).

The young grow rapidly and were 80-100mm long in few months. Spine have been found to be useful for age related studies(Kerns and Roelofs, 1977) .In first year it attain average length of 175mm in two year 209mm and in three year 235mm length. It attains maturity in first year .

Materials and methods
Procurement of fishes:
The sample were collected in plastic container from Chouldhari fish market and brought to wet lab of CARI in battery operated aerated conditions. The fish were given potash treatment (4ppm) for 2-3 minutes and transferred to storage tank. Fishes were acclimatized for 10 days prior to using them for the experimental purpose. During the acclimation period fishes were given same feed which was prepared for the experiment. Continuous aeration was provided during acclimation as well in throughout the culture period.

Preparation of Treatments:
Organic Manure:
(1) Cow Dung:
Cow dung was collected from cow rearing unit of Animal Science Division(CARI) which was powdered manually and stored in clean plastic jar and was supplemented daily as per as experimental design.

(2) Poultry Manure:
Poultry manure was collected from chicken rearing unit of Animal Science Division (CARI). Unwanted matter was removed, which was then powdered manually and was then stored in clean plastic jar and was supplemented daily as per as experimental design.

(3) Mix Feed:
Mix feed was prepared by using rice bran, mustard oil cake and wheat flour at a ratio of (50:25:25).Mix Feed was prepared by mixing all the ingredients with water and was stored in plastic jar in refrigerator and was supplemented according to daily nutritional regime. It contains 3% of protein.

Experiment:
Feed and organic manure were given as per as the experimental design. Ten fishes were selected in each tank for the present experiment. Initial length and weight of the fishes were recorded and at the end of 35 days final length and weight were taken. The size of the tank for the present experiment was 1 sq.m. Weekly 15% water exchange has been done to remove the uneaten feeds and accumulated metabolites.

WATER QUALITY PARAMETERS:
Parameters such as Temperature, pH was also analyzed:

Dissolve Oxygen:
Dissolve oxygen (d02) was measured from different tank once in a week by using Winkler. Method all the Water quality parameters viz. temperature, PH and dissolve oxygen were analyzed by following APHA (1991).

Microbial Analysis: Standard Microbiological techniques were followed for isolation and identification of bacterial species in the selected fish.

Hematological Analysis:
Hematological analysis of fish blood was carried out to know the physiological status of fish in relation to their growth.

The collected blood was subjected to Total Leukocyte Count (TLC), Total Red Blood Cell Count or Total Erythrocyte Count, and Determination of PCV or Haematocrit:

Proximate Analysis:
These analysis is used to know the moisture, crude protein(total nitrogen),crude lipid, and ash content of the sample. A fuller description of these analyses can be found in (Osborne and Voogt, 1978; AOAC,1984).

Calculation: Moisture Content (%) =100(B-A)-(C-A) / (B-A)
Where:A= Weight of clean, dry Petri dish (g), B=Weight of Petri dish+ wet sample (g)

C=Weight of Petri dish+ dry sample (g)

Crude Lipid:
In this method the fats are extracted from the sample with petroleum and evaluated as a percentage of the weight before the solvent is evaporated.

Calculation: Crude Lipid Content (%) =100(B-A/C)
Where A =weight of clean dry flask (g), B=weight of flask with fat (g)and C =weight of sample (g)

Ash Content:
This method is used to determine ash content in fish by calcinations. Ash is considered as the total mineral or inorganic content of the sample.

Calculation:
Ash content (%) =100A-B/C

Where: A = weight of crucible with sample (g); B = weight of crucible with ash (g).

C = weight of sample (g).

Crude Protein:
Analysis of crude protein from fish is done by Kjeldhal's method, which evaluates the total nitrogen content of the sample after it has been digested in sulphuric acid with a mercury or selenium catalyst.

Calculation:
Nitrogen in sample (%)= 100(A×B/C 0.014)

Where; A = Hydrochloric acid used in titration (ml);B = Normality of standard acid. (0.1N) C = Weight of sample (g).

Crude protein (%) = Nitrogen in sample×6.25

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Results

Table 1: Daily Nutritional Regime

Treatment	Items	Organic manure/day /sq. m (g)	Mix feed/day(g)
T0	Mix Feed	------	3%
T1	Only cow dung	100	------
T2	Only poultry wastes	100	------
T3	Cow dung and mix feed	100	3%
T4	Poultry and mix	100	3%

TABLE2: GROWTH OF H.fossilis UNDER DIFFERENT CULTURE CONDITION HAS BEEN ILLUSTRATED.

Treat-ments	Average Initial Length(cm)	Average Final Length(cm)	Growth/day (cm)	Average Initial Weight (g)	Average Final Weight (g)	Growth/day (g)
T_0	9.60±0.235	10.79±0.569	0.03 6.6		7.3	0.02
T1	10.29±0.412	11.00±0.436	0.02	6.6	7.0	0.11
T2	9.90±0.319	10.61±0.46	0.02	6.2	7.1	0.25
T3	9.18±0.176	10.27±0.316	0.03	6.2	7.2	0.28
T4	9.13±0.178	10.73±0.565	0.05	6.4	7.8	0.04

Graph 1: Length and weight increment of *Heteropneustes fossilis.*

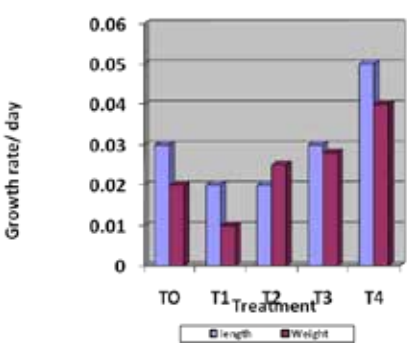


Table3: Water Quality Variability under Different Treatment Condition

Treatments	Water Temperature	pH	Dissolve oxygen(ppm)
T_0	23-27	7.71-7.8	4.2-5.2
T1	23-27	7.18-8.1	3.8-4.8
T2	23-27	7.33-8.1	3.7-4.8
T3	23-27	7.63-7.8	3.9-4.2
T4	23-27	7.8-8.3	4.0-4.8

Table4: Microbial Load On Different Treatments

TREATMENT	1st week (cfu/ml)	2nd Week (cfu/ml)	3rd week (cfu/ml)	4th Week (cfu/ml)
Control(mix feed)	1.9×10^1	2.8×10^2	3.4×10^2	2.1×10^3
Treatment1(only cow dung)	5.3×10^1	7.8×10^2	8.4×10^2	4.0×10^3
Treatment2(only poultry wastes)	2.75×10^2	3.34×10^2	4.32×10^2	3.45×10^3
Treatment3(cow manure and 3%mix feed)	3.67×10^2	4.78×10^2	5.34×10^2	4.67×10^3
Treatment 4 (poultry waste and 3%mix feed)	4.56×10^2	5.34×10^2	6.34×10^2	4.76×10^3

Table 5: Microbiological Analysis

Sample	Colony appearance	Gram Staining	Indole Test	Methyl Red Test	V.P Test	Citrate	Catalase	Oxidase	Nitra-te	Urease	Motility Test	TSI	SugarFermen-tation Test			Probable Miero organisms Identified
													S	L	G	
EMB 1	Blue, Black with Green sheen	-	+	+	-	-	+	-	-	-	+	Acid slant and acid butt with gas	+	+	+	Escherichia coli

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

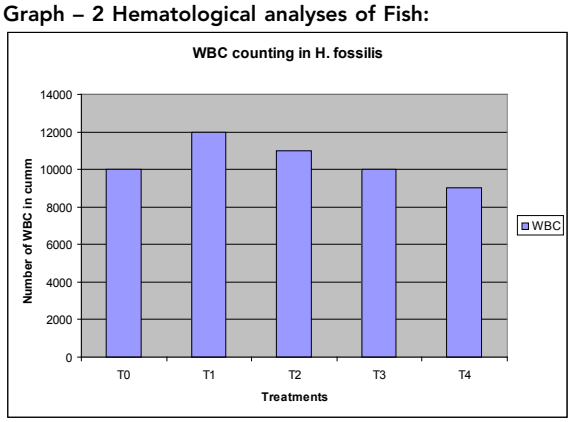
Sample	Colony appearance	Gram Staining	Indole Test	Methyl Red Test	V.P Test	Citrate	Catalase	Oxidase	Nitra-te	Urease	Motility Test	TSI	SugarFermen-tation Test			Probable Miero organisms Identified
EMB 2	Pink	-	-	-	-	+	+	+	-	+	+	Acid slant and acid butt with no gas	-	-	-	Pseudomonas aeruginosa
EMB 3	Pink	+	-	+	+	-	+	-	+	-	+	Acid slant and acid butt with no gas	+	+	+	Staphylococcus aureus
EMB 4	Pink	-	-	-	+	+	+	+	+	-	+	Acid slant and acid butt with gas	+	-	+	Enterobacter aerogenes
TCBS 1	Yellow	-	+	-	+	-	+	+	+	-	+	Acid slant and acid butt	+	-	+	Vibrio alginolyticus
TCBS 2	Green	-	+	+	-	-	+	+	+	-	+	Acid slant and acid butt	-	-	+	Vibrio parahemolyticus
RHM 1	Blue	-	+	+	-	-	+	-	-	-	+	Acid slant and acid butt with gas	+	+	+	Escherichia coli
EMB 5	Dark centered mucoid colonies	-	-	+	-	+	+	-	-	-	+	Acid slant and acid butt with gas	+	+	-	Klebsiella pneumoniae
EMB 6	Pink	+	+	+	-	-	-	-	+	-	+	Acid slant and acid butt with no gas	-	-	-	Proteus vulgaris

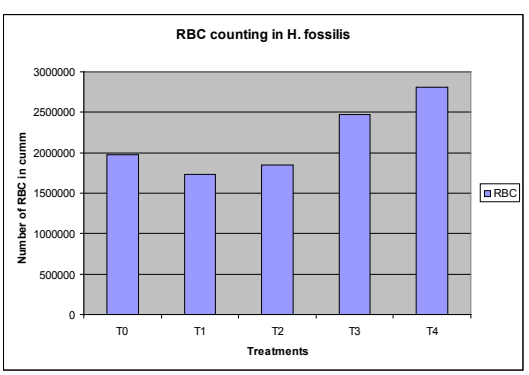
EMB– Eosin Methylene Blue, TCBS –Thiosulphate Citrate Bile salt Sucrose, RHM - Rice Hull Media

Table 6: Hematological Analysis of Fish:

Treatment	Total leucocytes count (WBCs) in cu mm	Total erythro-cytes count (RBCs) in mm^3	Hematocrit (PCV)/100ml
T_0	10×10^3	1.98×10^6	20
T1	12×10^3	1.73×10^6	25
T2	11×10^3	1.85×10^6	25
T3	10×10^3	2.47×10^6	30
T4	9×10^3	2.81×10^6	30

Graph – 2 Hematological analyses of Fish:





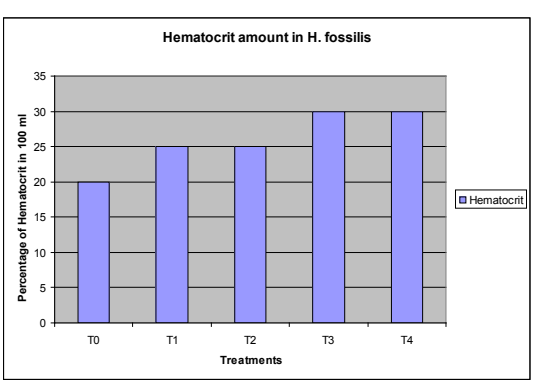


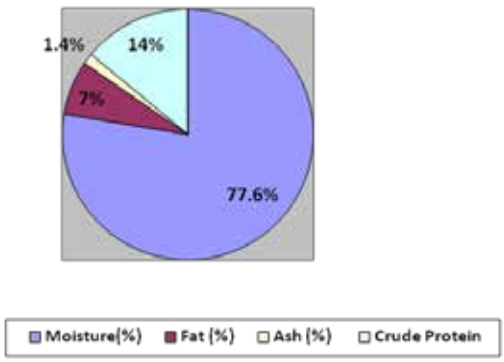
Table 7: Proximate Analysis of H.fossilis.

Treatment	Moisture (%)	Fat (%)	Ash (%)	Crude protein(%)
T_0	77.6	7.0	1.4	14.00
T_1	77.77	5.5	2.5	14.23
T_2	79.00	7.0	2.0	12.00
T_3	74.47	8.26	1.27	16.00
T_4	72.05	8.44	2.51	17.00

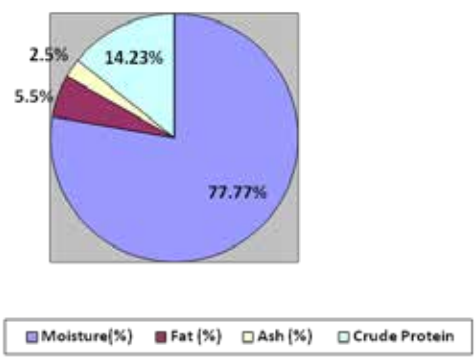
Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Graph – 3 Proximate Analysis of *Hetropneustes fossilis* under different treatment conditions

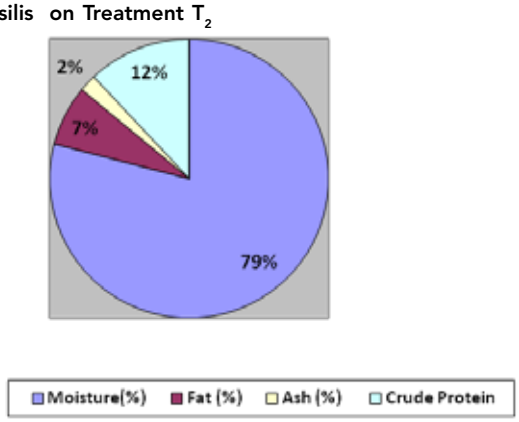
Graph - 3.1Proximate composition of Hetropneustes-fossilis on Treatment T_0



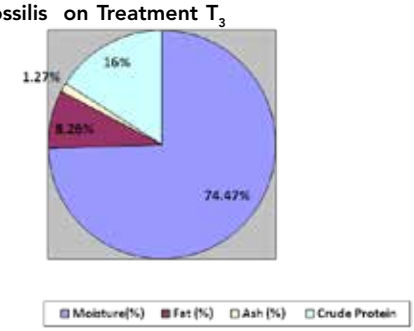
Graph - 3.2Proximate composition of *Hetropneustes fossilis* on Treatment T_1



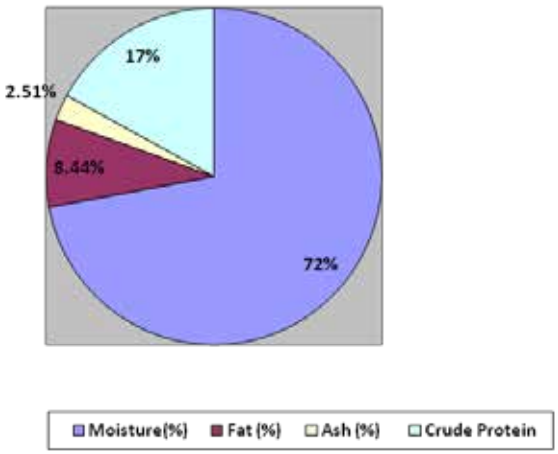
Graph - 3.3Proximate composition of Hetropneustes-fossilis on Treatment T_2



Graph - 3.4Proximate composition of Hetropneustes-fossilis on Treatment T_3



Graph - 3.5Proximate composition of *Hetropneustes fossilis* on Treatment T_4



Discussion

Air breathing fish culture in Andaman and Nicobar island is not well develop, Only recently some initiation has been taken by Central Agriculture Research Institute(CARI) to propagate the culture technique of air breathing fishes specially H.fossilisand Clariousbatrachus with an objective to develop a standard cultivable practice. In composite fish culture practice cow dung is most extensively used throughout India. Next to cow dung poultry manure are most widely used manure for fish culture.

The present investigation was carried out to see the effect of organic manure and organic manure supplemented diet on growth performance of H.fossilis under different culture conditions. Five different treatments were selected they are mix feed, cow manure, poultry manure, cow manure and mix feed and poultry manure and mix feed to carry out present investigation.. In present study it is clear that maximum growth in terms of length and weight was observed in poultry waste and mix feed (T4) (Devaraj and Krishna 1981; Javed et al.,1993) recorded the similar result and concluded that poultry manure and supplementary feed exerted a significant effect on the body weight, fork length and total length of fish species, Labeorohita, cirrhinusmrigala and catlacatla in the treated ponds. According to (Schroeder 1974,1978 and 1979) the increases in daily and weight of fish in poutry waste supplemented treatment are due to releasing nitrogen and phosphorus from poutry waste, improvement of the biological condition of pond water and chicken litter contained organic matter that used by fishes as food source in higher amount, all this factor improve the daily length, weight growth, survival rate and fish yield. Poultry manure is a complete fertilizer with characteristic of both organic as well as inorganic fertilizer, this might have resulted in increased growth of fish (Banerjee et al., 1979).The organic nutrients sources are more productive than inorganic sources(Schroeder,1975;Norienge-curtis,1979). The higher growth can also be related to maximum feed intake in one meal and emptying rate of stomach (Vahl, 1979). Increase length and weight can be due to higher amount of protein in diet (Hepher, 1962). (Yakupitiyage et al., 1991)also recorded similar result when he supplemented poutry manure and mix feed (2% of body weight) While comparing cow dung and poultry manure supplemented treatments higher growth was recorded in poultry manure(Green et al.,1989)reported that the final length and weight of fish (Oreochromusniloticus) was significantly greater on poultry

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

manure when compare to cow dung supplemented treatment. Lower growth in cow dung treatment can be due to low amount of nitrogen and phosphorus in the diet fish (Saha et al .,1980).

Water Quality Parameter:
In water quality parameter not many variations among the treatments were recorded. Slight variation in water quality parameter, may not have significant effect on growth of H.fossilis.

Microbial Analysis:
Gram positive bacteria such as Staphylococcus aureus(Gram positive cocci in bunches), and gram negative rods such as Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes,Vibrio alginolyticus, Vibrio parahemolyticus, Klebsiella pneumoniae, Proteus vulgariswere isolated and identified. But the pathogenicity of those isolates was not studied.

Hematological Analysis:
Hematological analysis provide valuable information for monitoring health condition of both wild and cultured fishes It is used for evaluation of fish physiological status and to determine the effect of external stressors and toxic substance(Campos and Sampio, 1976) .WBC count was found highest in cow dung supplemented treatment, this may be due to any infection or due to stress(Sherwani and Parwez, 2000)or this can be due to metabolic disturbance(Aziz et al., 2002; Prowise, 2001)concluded that immune system of fish display similar response to unfavorable condition reported an increase in leukocyte cells of fish infected with parasite (Anguilla anguila)in Europe.. .Higher R.B.C count is a sign of good health condition of fishes. Higher R.B.C count was seen in poultry and mix feed, this can be due to age of fish, cycle of sexual maturity and good health condition (Blaxhall, 1972; Hepher, 1962). Lower R.B.C count was observed in treatment where only cow dung was supplemented (Grizzell, 1975) and Hematocrit level was in normal range for catfish(Clark et al., 1979) reported no significant difference between fish infected with parasite and non-infected ones in terms of hematocrit level.

Proximate Composition:
In the present investigation not much variation was recorded in moisture content of fish except for poultry manure and mix feed supplemented treatment. Regarding ash content poultry manure and mix feed (T4) showed increase ash content this can due to increase amount of phosphorus in poultry manure which directly influence the ash content of fish(Stickney and Simmons, 1977). In terms of fat poultry manure and mix feed treatment has higher level of fat content this could be due to absence of growth inhibiting factor, which decrease the thyroid function resulting in higher total lipid content. Lower lipid content may be due to presence of growth inhibiting factor ,which affect thyroid gland causing lipid immobilization (Roales, 2006). Higher protein level was found in poultry manure and mix feed (T4),This can be due to fish size and life cycle stage which directly influence the protein content of fish. Higher protein content can be due to temperature, dietary protein and energy which have direct influence on body composition (Andrews, 1972).

Conclusion
The present investigation was taken in order to develop a strategy for higher production by manipulating organic manure and mix feed, from the present investigation, it is clear that, by applying poultry manure and mix feed higher growth or production can be achieved. Applying of this two also prove that, they are neither deteriorating water quality of culture medium, nor human pathogen was encountered, blood parameter showed improvement in their physiological status and there was increase in protein and fat content of fish. Hence it can be recommended that by applying poultry manure and mix feed in the farmer field, higher return can be obtained by farmers. However the present experiment has to be further refining to derive those dependent effects of the treatment material with production.

Acknowledgements
The authors are very grateful from the support of Central Agricultural Research Institute and Microlabs, Institute of Research and Technology, Arcot, Tamilnadu, India

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