Growth performance and biochemical composition of fingerlings of Cirrhinus mrigala fed with bioflocs in zero water exchange culture tanks

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Abstract:- Biofloc Technology serves to increase environmental and economic sustainability, which reduces inorganic nitrogen species and increases feed utilization through recycling. A 90 day experiment was conducted during which the growth performance and biochemical composition of Cirrhinus mrigala fingerlings were assessed using bioflocs as feed in zero water exchange culture tanks. The experimental unit comprised of two plastic tubs of 60 litre capacity each and marked as Control (C) where the fingerlings were fed with artificial feed containing 32% protein @ 2% body weight and Treatment I (T-I) fed with in-situ bioflocs. Water quality parameters i.e. temperature, pH, DO, FCO_2 were monitored regularly. Fish growth was analysed in terms of mean weight gain (MWG), Specific growth rate (SGR) and Percent weight gain (PWG). The data was analysed statistically using one way ANOVA to determine the significance of growth parameters at α= 0.05 using SPSS software. The biochemical composition of fish muscle was also done at the beginning and end of the experiment that showed significant results. Moreover, water quality parameters were also found to be within the permissible limit in both the experimental sets required for the growth of both bioflocs as well as fingerlings of Cirrhinus mrigala.

Keywords :- Bioflocs, growth performance, in-situ bioflocs.

Introduction

In the modern world with health consciousness, fish and fishery products are considered to be the safest food of animal origin (MPEDA, 1992; Prajith and Kurup, 2011). According to FAO (2000), there are about one billion people in the world that rely on fish as their primary source of animal protein. Due to slow growth in aquaculture, there exists a huge gap between production and demand. This growing demand of fish protein has resulted in the shift of extensive aquaculture to intensive aquaculture where fish production relies on supplement feed.

Feeding, which is one of the important features in aquaculture that determines the growth of fishes. The growth of fish at all stages is largely governed by the kind of food, ration, feeding frequency, food intake and its ability to absorb the nutrients (Mollah and Tan, 1982). Sufficient dietary supply is required for the optimum growth of fish. Protein, the chief constituent of fish body and fish meal acts as its primary source in fish feed (Olvera-Novoa *et al.*, 2002). Fish meal is widely used and is cost effective (Pillay, 1990; Carter and Hauler, 2000 and Engin and Carter, 2005). So there is a need to reduce the dependence of the aquaculture industry on fish meal by maximizing the feed utilization and by developing cheaper and sustainable substitute of fish meal without compromising the growth. This will reduce the production cost and dependence on fish feed for successful development of aquaculture.

Keeping in view the above problems and in order to make intensive aquaculture more successful, Biofloc Technology was developed. In zero water exchange system, addition of molasses as carbon source reduces inorganic nitrogen species into aggregates of microbial biomass by heterotrophic bacteria thereby increasing feed utilization through recycling. The microbial biomass thus formed is known as bioflocs can be used as a protein source in aquafeed. Biofloc technology can be considered as a sustainable and environmental friendly aquaculture system.

Keeping in view the constrain on water resources, the growth performance and biochemical composition of muscles of *Cirrhinus mrigala* fingerlings was done in the zero water exchange culture system.

Material and methods

The fingerlings of *Cirrhinus mrigala* were collected from the local fish farm at Gho-manhasan, Jammu which is about 14-15 kms from Jammu city and were transported to the departmental laboratory for the experimental work in polythene bags filled with oxygenated water and were acclimatized for the period of 15 days during which they were fed with artificial feed containing 32% protein.

Experimental set up:

After 15 days, the fishes were segregated in 2 plastic tubs of 60 litre capacity each and stocked with 15 fingerlings per tub with mixed sex ratio. First tub acted as Control (C) and second set as Treatment I and the experimental set up was maintained for the period of 90 days. During the experimental period, in Control set, the fingerlings were fed with artificial feed containing 32% protein @ 2% body weight. In Treatment I (T-I), initially water of the tub was inoculated with pond soil along with cowdung and its ammonia level was measured using standard methodology. In order to maintain C/N ratio more than 10, molasses were added as a carbon source along with continuous aeration and regular addition of alkalinizing substance. Proper monitoring and sludge management was done in Treatment I.

Water quality:

Water quality parameters i.e. Temperature, pH, DO, FCO_2, Ammonia, Nitrite and nitrate were measured using mercury bulb thermometer, pH meter (Hanna), APHA (1985) respectively. Water temperature and pH were measured daily while DO and FCO_2 were measured thrice a week. The ammonia, nitrites and nitrates were monitored twice a week.

Growth parameters:

Fish growth was analysed in terms of mean weight gain (MWG), Specific growth rate (SGR) and Percent weight gain (PWG).

Mean weight gain (g):

It is calculated by the formula**:**

$MWG = (W_F - W_I)/100$

Where W_F is the final weight of fishes and

W_I is the initial weight of fishes.

Percent weight gain (%):

It was calculated by using the formula:

$$\%WG = (W_F - W_I)/W_I \times 100$$

Where W_F is the final weight of fishes and

W_I is the initial weight of fishes.

Specific growth rate (SGR):

It was calculated by using formula:

SRG = Final weight – Initial weight/no. of days x100

Statistical analysis:

Fish growth parameters were compared statistically using one way ANOVA to determine the significance of growth parameters at $\alpha = 0.05$ using SPSS software.

Biochemical composition:

Biochemical composition of bioflocs were determined by using standard methods i.e. Lowry et al. (1951), Folch et al. (1957), hot air oven and muffle furnace (AOAC, 1995) respectively. Also, the biochemical composition of fish muscle was analysed at the beginning and end of the experiment. It was done in triplicates. Three fishes were randomly selected for biochemical analysis. The protein, lipid, moisture and ash content were determined by using standard methods i.e. Lowry et al. (1951), Folch et al. (1957), hot air oven and muffle furnace (AOAC, 1995) respectively.

Results and Discussion

During the experimental period of 90 days, the fishes fed with different diets revealed significant results. The water quality when monitored also revealed optimum values favourable for the growth of fishes as well as bioflocs.

Water quality parameters:

Temperature

Water temperature recorded in both the experimental sets showed minor variations. It fluctuated between 14.2-18.2^{0}C and 14.6-18.2^{0}C in Control and Treatment I, respectively (Table 1). Water temperature recorded was within the optimum range (14.2-18.3^{0}C) favourable for the growth of fingerlings as well as bioflocs. In reference to present studies, Shakir *et al.* (2013) recommended that the fingerlings of *Cirrhinus mrigala* can tolerate a minimum temperature of 14^{0}C and thus supports the present results.

pH

During the experimental period, pH varied from 8.0-8.3 in control and 7.9-8.2 in Treatment I, respectively (Table.1). During the present studies, pH remained an important parameter that was maintained for the growth of both fishes as well as bioflocs in Treatment I. However, in T-I due to aerobic respiration of fingerlings of *Cirrhinus mrigala* and bacteria in biofloc system. The addition of molasses in culture system also resulted in wide fluctuation in pH and this was compensated by the addition of sodium bicarbonate (Van wyk and Scarpa, 1999). The most suitable pH range known for Indian major carp fry and fingerlings was found to be 6.12- 8.6 (Wurts and Durborow, 1992; Das *et al.*, 1995; Bhatnagar *et al.*, 2004 and Santhosh and Singh, 2007). While the desirable pH for the optimum growth of bioflocs is 6.94-8.65 (Avnimelech, 1999). During the study period, the pH remained in the range of 7.9-8.3in both the sets and seems to be and this within optimum range for the growth of fingerlings and bioflocs as also authenticated by the growth parameters.

Dissolved oxygen

Dissolved oxygen fluctuated from 5.1-6.7 mg/l in Control and 4.0-6.5 in T-I, respectively(Table.1). A decline in DO level was observed in in-situ system (T-I) and may be ascribed to its utilisation by both the fingerlings of *Cirrhinus mrigala* and the heterotrophic bacteria as both were cultured in the same system. Kuhn and Lawrence (2012) also reported that in-situ biofloc systems have high oxygen demand. The present results are also in conformity to the observations of Phulia *et al.* (2013) who recommended DO level of 2-5 mg/l is highly suitable for adequate development of bioflocs and Martinez- Cordova *et al.* (2015) who stressed DO levels above 4-5 mg/l can be highly suitable for adequate development of bioflocs.

Free Carbon dioxide

In the present studies, the concentration of FCO_2 in experimental units revealed varied fluctuations viz. 6.67-9.0 mg/l in Control and 7.0-11.33 mg/l in Treatment I (Table.1). FCO_2 remains low in Control as compared to T-I, where it remained high and can be attributed to higher respiration rate of both heterotrophic community and fingerlings (Tacon *et al.*, 2002; Wasielesky *et al.*, 2006) and decomposition of sludge that reduces DO and pH levels whilst increasing ammonia and CO_2 (Ebeling and Timmons, 2006). FCO_2 less than 10 mg/l has been recommended as tolerable concentration (Ekubo and Abowei, 2011 and Swann, 1997).

Ammonia (NH_3), Nitrite (NO_2) and Nitrate (NO_3)

During the present experimental period of 13 weeks, the level of ammonia, nitrite and nitrate fluctuated from 0.015-0.120 mg/l, 0.011-0.095 mg/l and 0.006-0.080 mg/l in Control and 0.014-0.033 mg/l, 0.003-0.033 mg/l and 0.003-0.031 mg/l in Treatment I, respectively. In Control set with no water exchange, the ammonia, nitrite and nitrate concentration showed an increase throughout the experimental period which may be due to following reasons:-

- No water exchange during the culture period.
- Feeding: - Regular feeding of the fingerlings with the artificial feed and it is a known fact that only 20-30% of the food gets utilized by fish and the rest goes waste (Acosta- Nassar *et al.*, 1994; Avnimelech, 1999; Gross *et al.*, 2000; Avnimelech and Ritvo, 2003 and Davenport *et al.*, 2003) and contribute to increase in ammonia, nitrite and nitrate concentration in control set.
- Decomposition: - Since there was no water exchange, decomposition of the uneaten feed, organic nitrogen in faecal matter and excretion by fingerlings along with lack of organic carbon source to convert inorganic nitrogen to microbial biomass resulted in the increase in all the nitrogen species throughout the experimental period of three months. Widanarni *et al.* (2012) also put forth similar reasons for increase in ammonia level in their experimental studies.

➢ Slow nitrification: - Though, the process of nitrification occured but found to be 10 times less efficient in reducing inorganic nitrogen than heterotrophic bacterial assimilation (Hargreaves, 2013). Moreover, the nitrifying bacteria are slow growers with a generation period in the order of 12 hours versus about 30 hours for heterotrophic bacteria. So, in the Control set, nitrification played a little role in reduction in the ammonia, nitrite and nitrate concentration and thus resulted in increase in their level throughout the experimental period.

➢ Lack of heterotrophic bacterial assimilation.

While in Treatment I, the level of ammonia, nitrite and nitrate initially showed an increase for few weeks after which there was gradual decline. Initial increase in ammonia, nitrite and nitrate concentration occurs due to accumulation of excreta and less growth of heterotrophic bacteria as it took few days for the heterotrophic bacteria to flourish. Once the molasses were added regularly into the culture system and C/N ratio was maintained more than 10, the inorganic nitrogen species depicts gradual decline. Heterotrophic bacteria present in the biofloc system get sufficient carbon thereby multiply using the nitrogen sources from the culture system and form clumps of microbial protein known as bioflocs which were used subsequently as feed by the fingerlings reared in Treatment I set without the supply of artificial feed. In Treatment I, the heterotrophic bacterial assimilation is dominant over nitrification thereby decreasing the ammonia, nitrite and nitrate level in the biofloc culture system.

The present results were confirmed by the observation of Luo *et al.* (2014) who observed sharp decline in total ammonia nitrogen and nitrite in a short time period by adding organic carbon supplement. Samocha *et al.* (2007) also found rapid reduction in ammonia using molasses as carbon source in limited water exchange system. So, the bioflocs maintain water quality and provide feed. Moreover, lack of artificial feed and continuous removal of sludge also resulted in the decline in ammonia, nitrite and nitrate concentration.

During the experimental period, ammonia, nitrite and nitrate remained within the permissible limit for the growth of fish. In present experimental sets, the ammonia level remained less than 0.1 mg/l Meade (1985) and Santhosh and Singh (2007) also recommended 0.1 mg/l as the safe limits of ammonia for aquatic organisms. Swann (1997) recommended 0.02 ppm as safe. While Bhatnagar and singh (2010) recommended less than 0.4 mg/l and 0.2 mg/l, respectively as safe limits for aquatic organisms. Nitrite level was found to be less than 1 mg/l which is optimum for the survival of fishes as reported by Bhatnagar *et al.* (2004) (0.02-1.0 ppm); Santhosh and Singh (2007) (0.5 mg/l). Also, Santhosh and Singh (2007) described the favourable range of 0.1 mg/l to 4 mg/l of nitrate in fish culture water.

In concordance to present results Da silva *et al.* (2013); Liu *et al.* (2014); Wang *et al.* (2015); Yuniartik *et al.* (2015) and Nurhatijah *et al.* (2016) also reported low ammonia, nitrite and nitrate concentration in biofloc Treatment systems than in Control. This occured because the additional organic carbon maintains an appropriate C/N ratio for bacterial transformation of these toxic organic N compounds into single cell protein (Ebeling *et al.*, 2006; Asaduzzaman *et al.*, 2008; Villasenor *et al.*, 2015; Huang *et al.*, 2016 and Lorenzo *et al.*, 2016; Manan *et al.*, 2016; Emerenciano *et al.*, 2017 and Kamilya *et al.*, 2017).

Effect of feed on growth of fingerlings of *Cirrhinus mrigala:*

Fingerlings reared in both Control as well as Treatment I showed variations in the growth parameters viz. final weight, mean weight gain, specific growth rate and percent weight gain. During, the period of 90 days the total weight of fingerlings varied from 4.022 - 4.510 g, and 3.327-3.938 g in Control and Treatment I, respectively (Table 3).

While the average weight gain, percent weight gain, specific growth rate and mean weight gain showed an increase from 0.082-0.508 g, 2.049-12.069%, 0.091-0.564 and 0.00082-0.00508 g, respectively (Table 2) in Control set whereas In Treatment I, the average weight gain, percent weight gain, specific growth rate and mean weight increased from 0.080-0.611 g, 2.405-17.099%, 0.089-0.679 and 0.00080-0.0061 g, respectively (Table 2). Fingerlings fed with In-situ bioflocs system (T-I) showed best growth which may be ascribed to following reasons:-

- Continuous production of bioflocs in the system and their constant availability results in proper harnessing of bioflocs by fingerlings makes them to feed until apparent satiation.
- High nutritional value of bioflocs on which the fingerlings completely rely.
- Along with heterotrophic bacteria, the other important components of bioflocs are also known to act as natural feed for fish.

In reference to the present results, Ogello *et al.*, 2014 reported that Tilapia take up microbial protein twice from the biofloc cultured system.

Azim and Little (2008) reported improved growth rate of Nile Tilapia in biofloc system as compared to Control. Mondal *et al.* (2013) reported best growth of Cirrhinus mrigala with 33% protein rich diet. It has been observed by various researchers that fishes reared in biofloc system show better growth rate, final weight and weight gain in comparison to Control set (Burford, 2004; Wasielesky *et al.*, 2006; Azim and Little, 2008; Kuhn *et al.*, 2009; Xu *et al.*, 2013; Megahed and Mohamed, 2014; Zhao *et al.*, (2014); Choo *et al.*, (2015); Villasenor *et al.*, 2015; Ekasari *et al.*, 2015; Faizullah *et al.*, (2015), Long *et al.*, (2015); Soltan *et al.*, (2015); Brito *et al.*, 2016; Day *et al.*, (2016); Harini *et al.*, (2016); Khanjini *et al.*, (2016); Khatoon *et al.* (2016) and Najdegerami *et al.*, (2016)). Treatment I showed higher final weight of fingerlings whereas lowest final weight was found in Control set which was in concordance with the present results.

Kamilya *et al.* (2017) also attributed enhanced growth and welfare of the cultured fish (rohu) in in-situ maintenance of water quality and presence of microbial flocs within the biofloc system.

Statistical analysis:
The statistical analyses of data of growth parameters i.e. percent weight gain and specific growth rate of Treatment I with Control set revealed that these two parameters were significant between the Control and in-situ biofloc Treatment units at $\alpha = 0.05$.

Biotic and biochemical composition of bioflocs:
The water sample from the culture system was collected in the imhoff cones and allowed to settle for 15 minutes. The filterate when seen under the compound microscope showed the aggregates of heterotrophic bacteria, algae, entangled zooplankton including protozoa, rotifers, diatoms, uneaten feed and other dead organic matter. Biochemical composition of bioflocs in the in-situ Treatment set (T-I) revealed 34% protein, 12.6% lipids, 31% moisture and 15.2% ash when molasses were used as the carbon source. In control set, the artificial feed containing 32% protein and 4% lipids was fed to the fingerlings of *Cirrhinus mrigala*.

Proximate Composition of fish muscle:
The Proximate composition of fish muscle was done at the start and end of the experimental period of 90 days in both the experimental sets.

Moisture
In control set, the moisture content of fish muscle varied from 74.70-73.02% with a fall of 1.68%, and 74.40 to 70.08% with a fall of 4.32% in Treatment I (Table 4,5 and Fig 5,6). The highest decrease was found in Treatment I and lowest in Control as moisture level is known to decrease with the increase in body weight and mainly depends on dietary protein level (Salam *et al.*, 2001). An overall decrease in moisture content during the period of 90 days in all the experimental sets may be due to maximum body weight reported in Treatment I. Moisture percentages are also associated with higher lipid contents in fish (Dempson *et al.*, 2004).

Protein
Protein content during the experimental period showed an increase from 0 to 90 days. The protein content at the beginning of the experiment was 17.06% and 17.29% in control and Treatment I, respectively and reached 18.22% in control and 19.36% in Treatment I (Table 4,5 and Fig 5,6). The protein content was found to be least in fingerling of Control set which can be attributed to the artificial feed (containing 32% protein) fed to the fingerlings. Higher protein content was observed in fingerlings of Treatment I which may be due to their complete dependence on protein rich aggregates viz. bioflocs (34% protein) and their 24 hours availability.

Lipids
The value of lipid content showed an increase of 2% (2 to 4%) in Treatment I whereas it remained same i.e. 2% in Control during the experimental period of 90 days (Table 4, 5 and Fig 5,6). Increase in lipid content of fingerlings in T-I may be due to their dependence on the bioflocs which are rich in lipids (12.6%) as depicted by Table 1 while no change in lipid content observed in Control may be assigned to artificial feed that contains only 4% lipids. According to FAO (1999), the moisture and lipid content in fish are inversely related and their sum is approximately 80% with other components accounting for the remaining 20% which may be one of the reason for increase in the lipid content in Treatment I. Negative correlation exists between percent lipid and percent water (Elliot, 1976; Hartman and Brandt, 1995; Iverson *et al.*, 2002; Love, 1970; Peters *et al.*, 2007; Plante *et al.*, 2005; Rottiers and Tucker, 1982 and Trudel *et al.*, 2005) and this has been revealed by least moisture in T-I after 90 days.

Ash
Ash content depicts the minerals present in the muscle of fish. The ash content showed variations from 0 to 90 days. During the experimental period of three months, the ash content declined in Control set whereas in Treatment I, it remained same. The ash content decreased from 6 to 4% with a decline of 2% in control, While in Treatment III, no fall in ash content (Table 4,5 and Fig 5,6) was observed that can be ascribed to the increase in body weight (Silva *et al.*, 2015) and also due to high mineral content present in bioflocs. Moreover, small sized fish are also known to have high ash content due to higher bone to flesh ratio (Daramola *et al.*, 2007). Ash content remains unaffected by dietary protein content but affected by fish size (Khattab *et al.*, 2000) and also by dietary mineral contents.

Conclusion
Based on the present studies it can be concluded that bioflocs containing 34% protein serves as better feed for the fingerlings of *Cirrhinus mrigala* and in this way we can reduce the feed cost in aquaculture which is one of the important constraints along with the water quality

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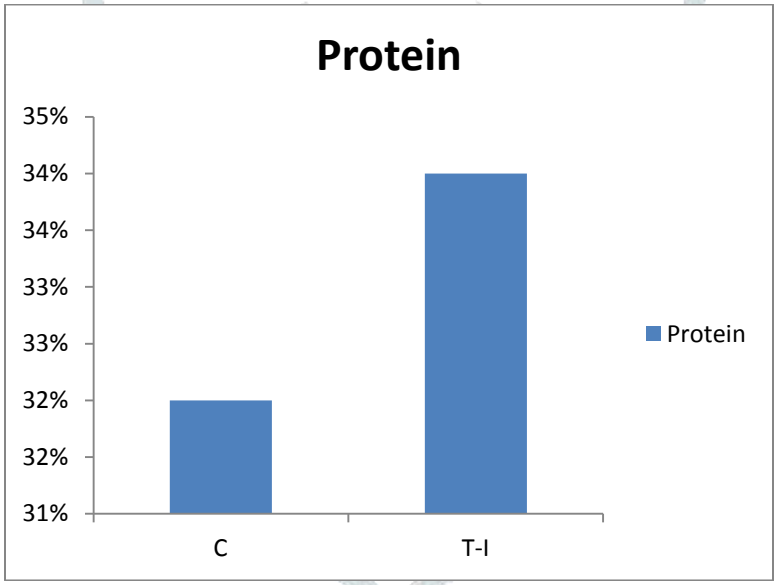

Fig 1:- Variations in the protein content of the two different experimental sets.

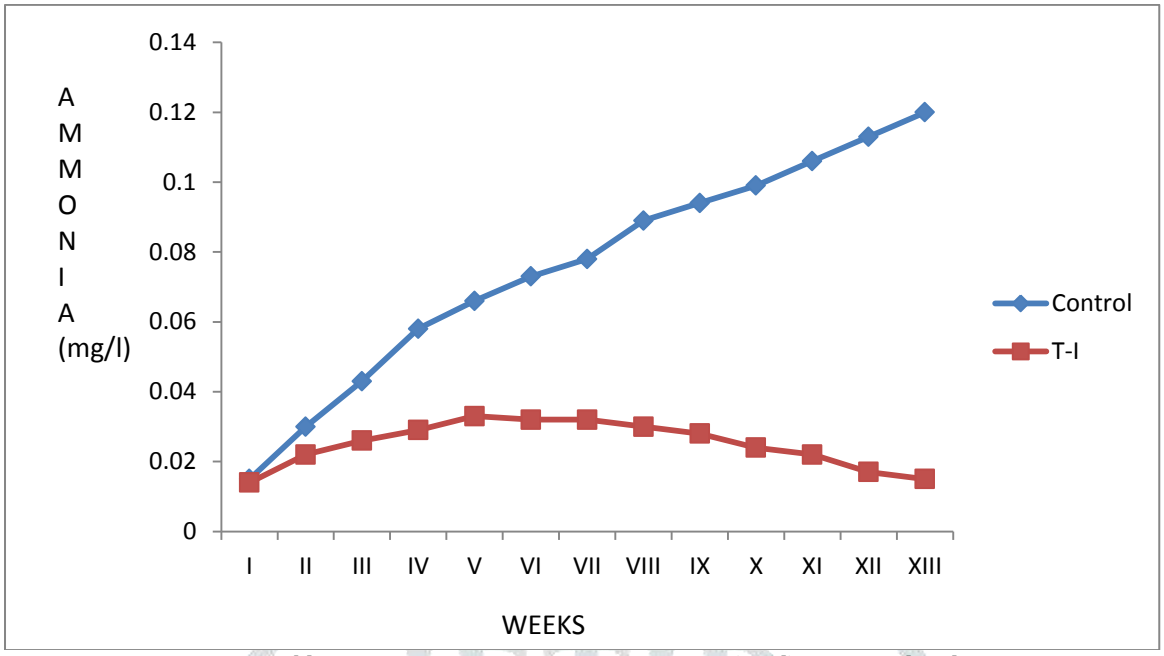

Fig 2 :- Weekly variations in ammonia concentration (mg/l) in Control and T-I.

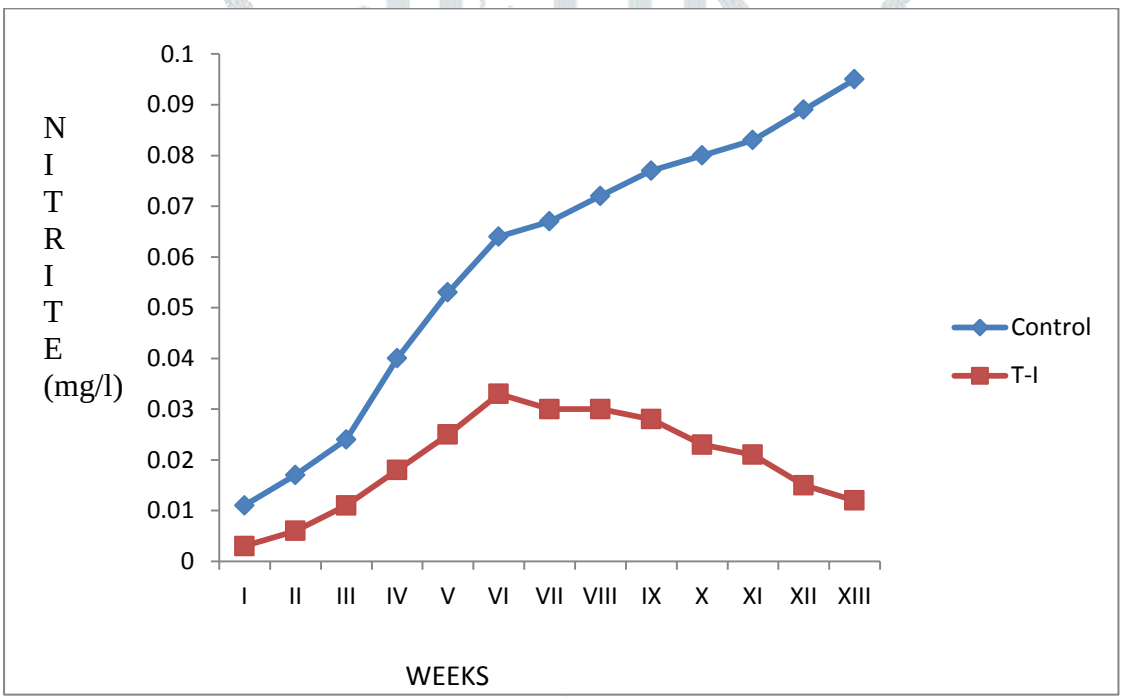

Fig 3:- Weekly variations in nitrite concentration (mg/l) in Control and T-I.

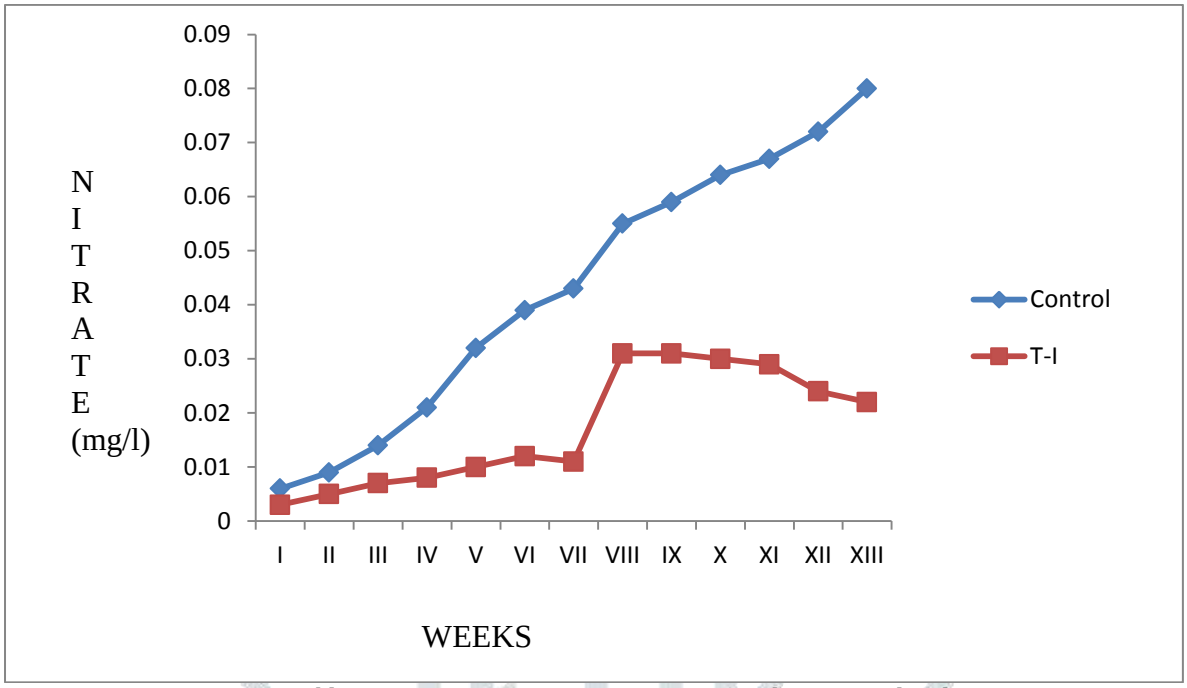

Fig 4:- Weekly variations in nitrate concentration (mg/l) in Control and T-I.

Table1:- Variation in the physicochemical parameters in both the experimental sets.

TREATMENTS / PARAMETERS	**CONTROL (C)**	**TREATMENT I (T-I)**
Temperature (^{0}C)	14.2-18.2	14.6-18.2
pH	8.0-8.3	7.9-8.2
FCO_2 (mg/l)	6.67-9.0	7-11.33
DO (mg/l)	5.1-6.7	4.0-6.5

Table 2:- Variations in the growth parameters of *Cirrhinus mrigala* fingerlings from 0-90 days in both the experimental sets.

TREATMENTS / PARAMETERS	CONTROL (C)	TREATMENT I (T-I)
Initial weight gain (gm)	4.022	3.327
Final weight gain (gm)	4.510	3.938

TREATMENTS / PARAMETERS	CONTROL (C)		TREATMENT I (T-I)	
	0 day	90 days	0 day	90 days
Average weight gain(gm)	0.082	0.508	0.080	0.611
Percent weight gain (gm)	2.049	12.069	2.405	17.099
Specific growth rate	0.091	0.564	0.089	0.679
Mean weight gain (gm)	0.00082	0.00508	0.00080	0.00611

Table 3:- Variations in the weight of fingerlings during the period of 90 days in Control (C) and Treatment-I (T-I).

Table 4:- Variations in the biochemical composition of *Cirrhinus mrigala* fingerlings in Control set (C) during the experimental period.

Variables / Days of Culture	Moisture	Crude protein	Crude lipid	Ash
0 Days	74.07%	17.06%	2%	6%
90 Days	73.02%	18.22 %	2%	4%

Table 5:- Variations in the biochemical composition of *Cirrhinus mrigala* fingerlings in Treatment I (T-I) during the experimental period.

Variables / Days of Culture	Moisture	Crude protein	Crude lipid	Ash
0 Days	74.40%	17.29%	2%	5%
90 Days	70.08%	19.36 %	4%	5%

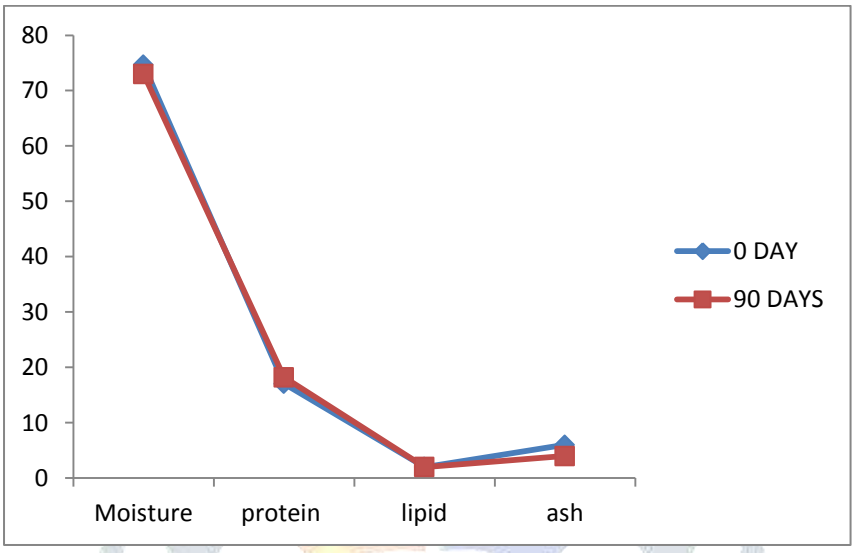

Fig 5:- Variations in the biochemical composition of *Cirrhinus mrigala* fingerlings in Control during the experimental period.

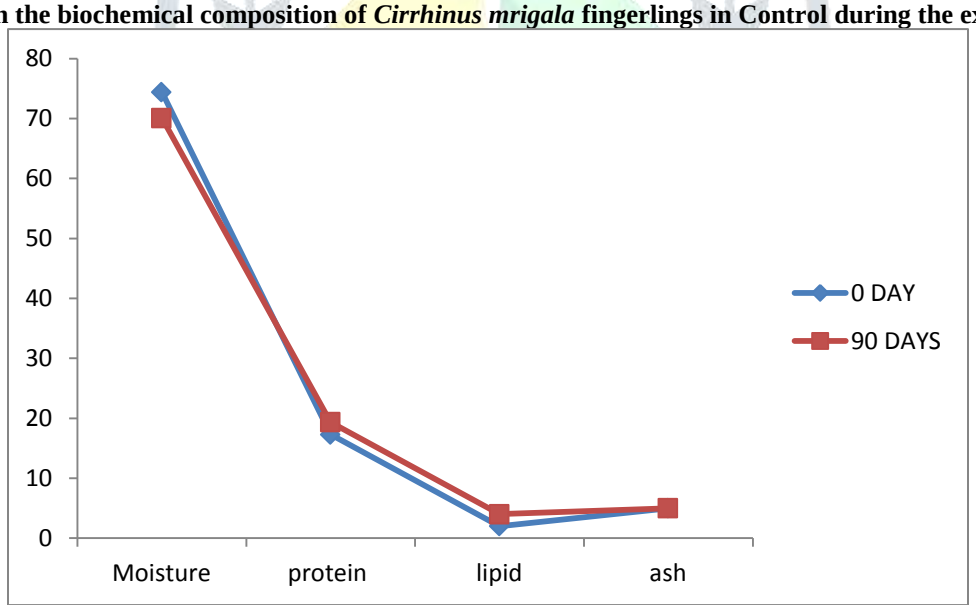

Fig 6:- Variations in the biochemical composition of *Cirrhinus mrigala* fingerlings in Treatment I (T-I) during the experimental period.