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Dietary replacement of artificial feed with bioflocs cultured from aquaculture waste water and its beneficial effect on the growth of fingerlings of *Cirrhinus mrigala*

Arti Sharma, Bandhana*, Radhika Singh and Rashmi Sangotra
Department of Zoology, University of Jammu, (J&K), India
bandhanasharma17@gmail.com

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Abstract

Biofloc technology, the new blue revolution in aquaculture works with minimum or zero water exchange and recycling of nutrients. It converts the nitrogen present in aquaculture waste into microbial flocs which have great nutritional value and act as natural fish food. The bioflocs thus formed can partially or completely replace artificial feed in aquaculture. In the present investigation, the growth performance of Cirrhinus mrigala fingerlings was analysed by replacing commercial feed with bioflocs along with some physicochemical parameters viz. temperature, pH, DO, FCO_2, ammonia, nitrite and nitrate. A 90-day experiment was conducted in tubs having 60 litre capacity, each stocked with 15 fingerlings. The experimental unit consisted of four sets which were provided with different diets having artificial feed @ 2% body weight (Control), 50% artificial feed and 50% bioflocs (Treatment I), 100% bioflocs cultured externally (Treatment II) and 100% in-situ bioflocs grown along with fishes in the same system (Treatment III). The growth of fingerlings was analysed in terms of mean weight gain, percent weight gain and specific growth rate. The results showed maximum growth of fingerlings in Treatment III and minimum in Control thereby revealing that artificial feed in the diet of C.mrigala can be replaced by bioflocs.

Keywords: Bioflocs, *Cirrhinus mrigala*, growth performance, mean weight gain.

Introduction

Aquaculture industry is the major source of high quality protein consumed by the man. In the world, about one billion people rely on fish as the chief source of animal protein[1]. With the enhancement in the world's population, the demand for the fish protein is also increasing but due to slow growth in aquaculture, there exists a wide gap between production and demand. In order to meet the growing demand of fish protein, there is the shift of extensive aquaculture to intensive aquaculture keeping in view the limitations of water and availability of suitable land.

Feeding is one of the important features in aquaculture determines the growth of fishes. Protein forms the chief component of the fish body. So, adequate dietary supply is required for the optimal growth of fish. Fish meal, which is a extensively used and costly protein component acts as the prime source of dietary protein in fish feed[2-4]. Intensive aquaculture mainly relies on artificial feed that comprises of fish meal. In intensive aquaculture system, the feed cost is the major component in the expenditure of fish production. About 50% of the operational cost in intensive aquaculture pond is constituted by the artificial feed so there is a need to reduce the reliance of the aquaculture on fish meal, an important and costly ingredient of artificial feed by maximizing the feed utilization and by developing cheaper and sustainable substitute of fish meal without compromising the growth.

To provide economic stability to the aquaculture industry recently developed biofloc technology was adopted which deals with the recycling of nutrients by maintaining C: N ratio greater than 10 in the water in order to stimulate heterotrophic bacterial growth that converts inorganic nitrogen into microbial biomass[5]. This microbial biomass is the aggregates of algae, heterotrophic bacteria, zooplanktons like protozoans, rotifers and other kinds of particulate matter such as uneaten feed and faeces that remain suspended in water column and are called as bioflocs. These can be used as a protein source in aqua feed thereby replacing the artificial feed. This will reduce the production cost and dependence on fish feed as reduction in feed cost could be a key factor for successful growth of aquaculture industry.

The present work aims at the use of bioflocs as a substitute of artificial feed and see the production performance of fingerlings of *Cirrhinus mrigala* in zero water exchange system using different diet combinations.

Materials and methods

Experimental design: *Cirrhinus mrigala* fingerlings were procured from local fish farm and acclimatized for the period of one week under laboratory conditions. During acclimatization they were fed with artificial feed @ 2% by body weight. After one week, the fingerlings were segregated and stocked in plastic troughs each with 20 fingerlings. The experimental unit

consisted of four plastic troughs of 60 litres capacity each, designated as Control (C), Treatment I (T-I), Treatment II (T-II) and Treatment III (T-III) and the entire set was maintained for a period of 90 days during which no water exchange and constant aeration was done. During experimental period, regular monitoring of the water quality parameters was done.

Water quality monitoring: Water samples were collected from each trough twice a week and analysed for various physicochemical parameters viz. water temperature, pH, DO, FCO_2, ammonia nitrites and nitrates. Water temperature and pH were measured using mercury bulb thermometer and pH meter, respectively. DO, FCO_2, ammonia, nitrite and nitrate were estimated following standard method[6].

Experimental conditions: In Control set (C), fingerlings were fed with commercial feed pellets made up of spirulina purchased from the market under the trade name 'Tokyii'. Artificial feed comprised of 32% protein and 6% lipid. Feed was given @ 2% body weight twice a day.

In Treatment I (T-I): Fingerlings were fed with a combination of bioflocs and artificial feed. The artificial feed was reduced to half of the amount used in control. The reduced feed was compensated using 100 ml of biofloc suspension cultured outside twice a day.

In Treatment II (T-II): No artificial feed was used and fingerlings fed entirely on the bioflocs cultured externally. The amount of bioflocs (ex-situ) were doubled, the amount used in treatment I and fed with 200ml twice daily.

In Treatment III (T-III): The fingerlings were neither fed with artificial feed nor with bioflocs cultured externally. In T-III set, the culture system was initially inoculated with heterotrophic bacteria through cow dung and some pond soil with continuous aeration. C: N ratio was maintained by addition of molasses which resulted in multiplication of bacteria. Heterotrophic bacteria in turn consume the nitrogen from faecal matter and convert it into bioflocs. Since no artificial feed was provided to the system, the fingerlings in this set relied only on the bioflocs as their feed. Bioflocs remained in the system for 24×7 and fingerlings fed on them until apparent satiation.

Fish growth parameters: Fish growth was analysed in terms of Specific growth rate (SGR), mean weight gain (MWG), 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 α=0.05 using SPSS software.

Results and discussion

During the experimental period of 90 days, no water exchange was done. Water quality was monitored regularly for all the experimental sets. Water quality varied with the experimental period but found to be within optimum range. Depending upon the diet combinations in different experimental sets, the physicochemical parameters showed variations. Also, the fingerlings fed with different diets showed significant growth performance.

Water quality: Biweekly monitored water quality parameters in all the four experimental units revealed fluctuation as shown in Table-1. All the physicochemical parameters were within the permissible limits.

Water Temperature: Results obtained from the present study point out that mean water temperature recorded in all the four experimental sets showed minor variations (Table-1). It fluctuated between 14.2-18.2^{0}C, 14.3-18.2^{0}C, 14.6-18.3^{0}C and 14.6-18.3^{0}C in Control, Treatment I, Treatment II, and Treatment III, respectively. Water temperature was found to be higher in Treatment II and Treatment III due to multiplication of heterotrophic bacteria. In both these sets, older bioflocs settle down and resulted in the formation of sludge which upon decomposition increase the water temperature. 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, the fingerlings of *Cirrhinus mrigala* can tolerate a minimum temperature of 14□C[7] and thus supports the present results. Stable biofloc production at 16-20^{0}C and high water temperature (30-35□C) results in bulking of sludge has been reported[8]. Low temperature is favourable for the growth of bioflocs[9].

pH: During the experimental period of 90 days, slight variations in pH was recorded in Control (8.0-8.3), Treatment I (8.0-8.3), Treatment II (7.9-8.2) and Treatment III (7.9-8.2). However, pH remained low in T-II and T-III due to aerobic respiration of fingerlings of *Cirrhinus mrigala* and bacteria present in biofloc system. The addition of molasses in in-situ culture system also resulted in wide fluctuation in pH and this was compensated by the addition of sodium bicarbonate[10]. Decomposition of dead bioflocs accumulated at the bottom in the form of sludge, also

decreased DO level along with pH. This sludge formed at the bottom was continuously removed by siphoning during the experimental period in T-II and T-III. During the study period, the pH value was within the optimum range for the growth of fingerlings and bioflocs[11-14].

Dissolved Oxygen: Perusal of the Table-1 clearly reveals the variations in DO level in all the Treatment units. It varied from 5.1-6.7mg/l in Control, 5.1-6.7mg/l in Treatment I, 4.5-6.7mg/l in Treatment II and 4.0-6.5mg/l in Treatment III. In all the experimental sets least concentration in DO (5.1±0.681mg/l) was observed in T-III viz. in-situ biofloc system followed by T-II (5.4±0.630mg/l), T-I (5.7±0.575mg/l) and Control (5.9±0.578 mg/l). In in-situ system (T-III), the decline in DO may be because of its utilisation by both the fingerlings of *Cirrhinus mrigala* and the heterotrophic bacteria as both were cultured in the same system[15]. DO level remained within the permissible limit in all the sets and seems to support the survival of fingerlings of *Cirrhinus mrigala* and production of high quality bioflocs[16-19].

Free Carbon dioxide: In the present studies, the concentration of FCO_2 in experimental units revealed varied fluctuations viz. 6.67-9.0mg/l in Control, 7.0-9.0mg/l in Treatment I, 6.67-9.67mg/l in Treatment II and 7.0-11.33mg/l in Treatment III. At the beginning of experiment the concentration of FCO_2 was low and increased gradually. In T-II and T-III, the FCO_2 level was recorded to be slightly higher than rest of the experimental sets which may be attributed to Higher respiration rates of both heterotrophic community and fingerlings[20, 21] and decomposition of sludge that reduces DO and pH levels whilst increasing ammonia and CO_2[22].

Ammonia, Nitrite and Nitrate: Nitrogenous waste products such as ammonia, nitrite and nitrate showed fluctuations as revealed by Table-1. During the present experimental period of 13 weeks, the variations in the ammonia concentration was recorded as 0.015-0.120mg/l in Control, 0.014-0.044mg/l in Treatment I, 0.016-0.040mg/l in Treatment II and 0.014-0.033mg/l in Treatment III. Ammonia level was found to be highest in Control set and lowest in Treatment III. In Control set (C), ammonia level showed an increasing trend throughout the experimental period i.e. from 0.015-0.120mg/l while its concentration increased upto sixth week in T-I and fifth week in T-II and T-III and after which there was decrease in the level of ammonia (Figure-1).

This may be due to ammonia utilization by the heterotrophic bacteria to produce microbial protein in biofloc systems. Nitrite and nitrate level also depicted the similar trend in all experimental units as followed by ammonia. Nitrite concentration varied from 0.011-0.095mg/l in Control, 0.008-0.037mg/l in Treatment I, 0.005-0.035mg/l in Treatment II, 0.003-0.033mg/l in Treatment III. In control set, the nitrite level indicated an increase throughout the culture period while the concentration of nitrite increased upto sixth week in T-I, T-II and T-III and after attaining peak value it gradually started declining upto week 12^{th} in T-I and T-II. In T-III, it slightly decreased from 6^{th} week till the end of the experimental period (Figure-2). In the present research, the level of nitrate fluctuated from 0.006-0.080mg/l in Control set, 0.004-0.087mg/l in Treatment I, 0.003-0.062 mg/l in Treatment II, 0.003-0.031mg/l in Treatment III. Nitrate level increased throughout in Control but in T-I its level increased upto 9^{th} week and in T-II and T-III it rise upto 8^{th} week. After acquiring the maximum value, there was slight decline in nitrate level upto the end of the culture period (Figure-3).

Table-1: Variations in water quality parameters recorded during the culture period of 90 days.

Treatments	Control (C)			Treatment I (T-I)			Treatment II (T-II)			Treatment III (T-III)		
Parameters	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Temperature (^{0}C)	14.2	18.2	16.29± 1.175	14.3	18.2	16.33± 1.194	14.6	18.3	16.54± 1.151	14.6	18.3	16.44± 1.078
pH	8.0	8.2	8.1± 0.095	8.0	8.3	8.1± 0.107	7.9	8.2	8.1± 0.086	7.9	8.2	8.1± 0.099
DO (mg/l)	5.1	6.7	5.9± 0.578	5.1	6.7	5.7± 0.575	4.5	6.7	5.4± 0.630	4.0	6.5	5.1± 0.681
FCO_2 (mg/l)	6.67	9.0	8.025± 0.787	7.0	9.0	7.948± 0.679	6.67	9.67	8.642± 0.844	7.0	11.33	9.924± 1.328
Ammonia (mg/l)	0.015	0.120	0.0682± 0.029	0.014	0.044	0.0324± 0.008	0.016	0.040	0.0282± 0.079	0.014	0.033	0.0250± 0.006
Nitrite (mg/l)	0.011	0.095	0.0593± 0.0281	0.008	0.037	0.0257± 0.0093	0.005	0.035	0.0226± 0.0089	0.003	0.033	0.0196± 0.0097
Nitrate (mg/l)	0.006	0.080	0.043± 0.0252	0.004	0.087	0.042± 0.0309	0.003	0.062	0.0350± 0.0207	0.003	0.031	0.0172± 0.0108

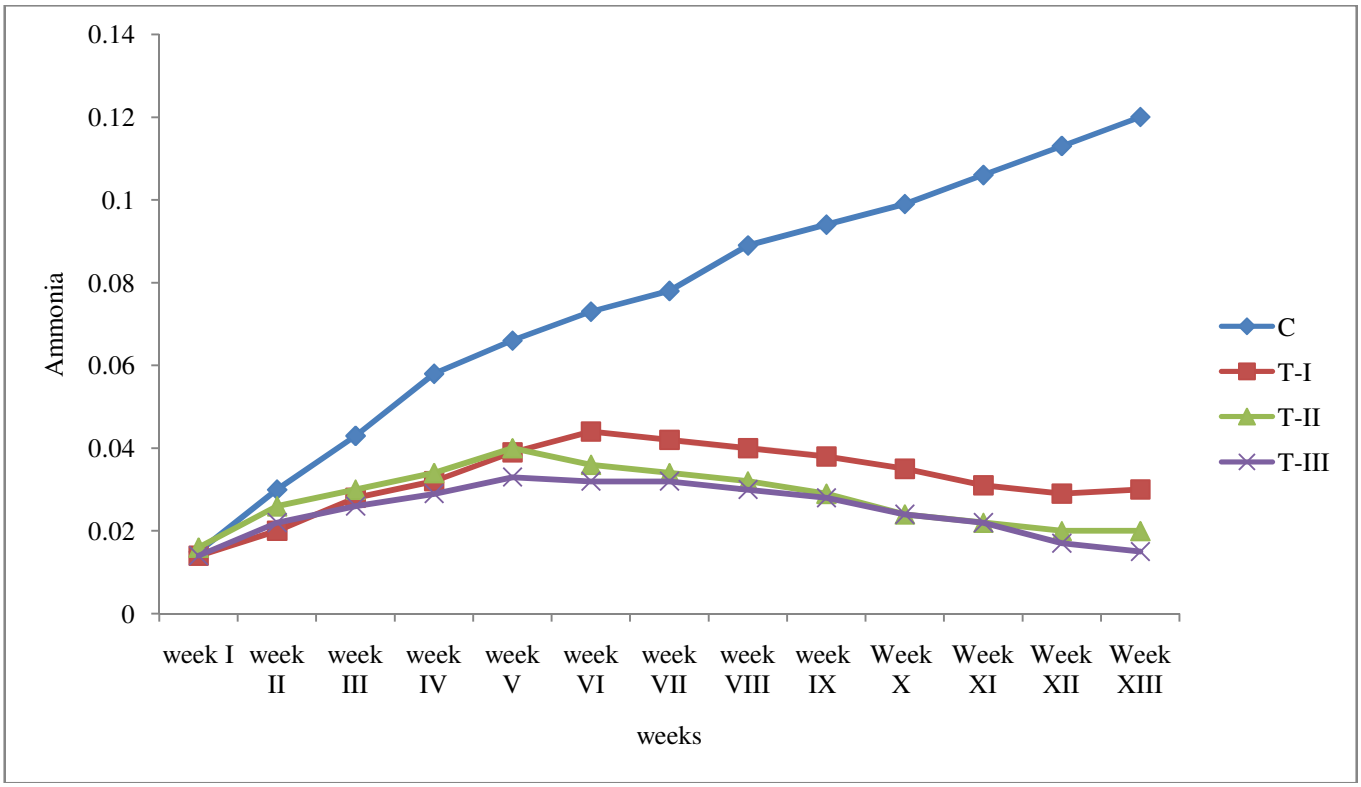

Figure-1: Weekly variations in ammonia concentration (mg/l) in four different experimental sets.

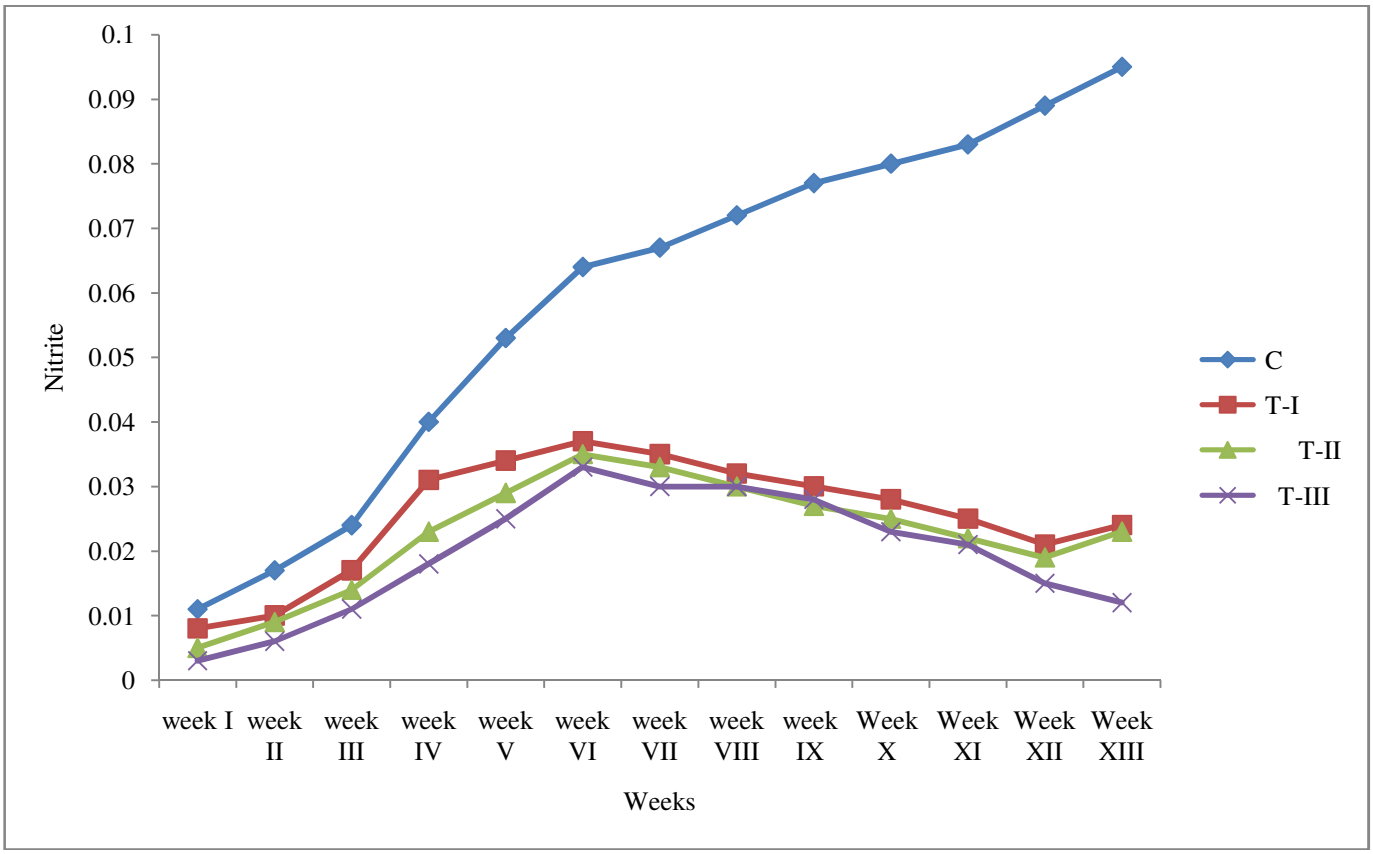

Figure-2: Weekly variations in nitrite concentration (mg/l) in four different experimental sets.

In the present investigation, the level of ammonia (<0.1mg/l), nitrite (0-1mg/l) and nitrate (0.1-4mg/l) were found to be within the optimum range for the fishes[14,23,24]. During the experimental period, the level of inorganic nitrogen such as ammonia, nitrite and nitrate was found to be comparatively higher in Control set than in biofloc treatment systems which may be due to artificial feed addition and absence of heterotrophic bacteria that mobilize inorganic nitrogen into microbial flocs. In reference to the present results, sharp decline in total ammonia nitrogen and nitrite in a short time period was observed by adding organic carbon supplement[25]. Rapid reduction in ammonia using molasses as carbon source was also found in limited water exchange system[26]. Low ammonia, nitrite and nitrate concentration in biofloc tanks in comparison to Control was observed[27-33].

Growth performance of fingerlings of *Cirrhinus mrigala*: Growth parameters like final weight, average weight gain, mean weight gain, specific growth rate and percent weight gain revealed significant variations with different diet combination when fed to fingerlings of *Cirrhinus mrigala*. During the period of 90 days the total weight of fingerlings varied from 4.022-4.510g, 3.787-4.359g, 5.263-5.804g and 3.327-3.938g in Control, Treatment I, Treatment II and Treatment III, respectively (Table-2).

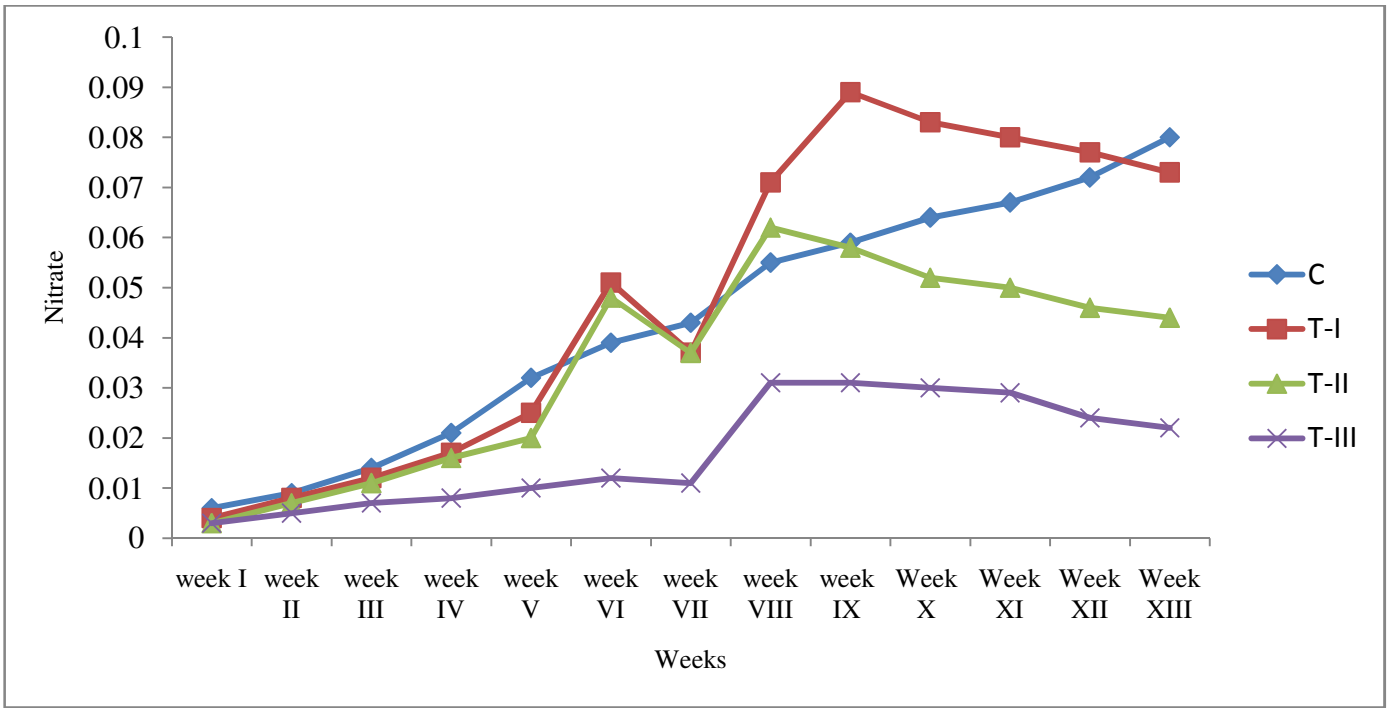

Figure-3: Weekly variations in nitrate concentration (mg/l) in four different experimental sets.

Table-2: Growth parameters recorded in different Treatment sets during the culture period.

Parameters \ Treatments	Control (C)	Treatment-I (T-I)	Treatment-II (T-II)	Treatment-III (T-III)
Initial weight (gm)	4.002 ±0.228	3.787 ±0.192	5.263 ±0.221	3.327 ±0.186
Final weight (gm)	4.510 ±0.252	4.359 ±0.188	5.804 ±0.236	3.938 ±0.183
Mean weight gain (gm)	0.00508 ±0.008	0.00571±0.006	0.00541 ±0.012	0.00611 ±0.012
Specific growth rate	0.564 ±0.0089	0.635 ±0.0066	0.602 ±0.00132	0.679 ±0.0130
Percent weight gain (%)	12.069 ±0.2172	14.212 ±0.1062	9.866 ±0.1841	17.099 ±0.2548
Average weight gain (gm)	0.508 ±0.0080	0.571 ±0.006	0.541 ±0.012	0.611 ±0.012

The average weight gain, percent weight gain, specific growth rate and mean weight gain showed an increase from 0.082-0.508g, 2.049-12.069%, 0.091-0.564 and 0.00082-0.00508g, respectively in Control set (Table-2) and in Treatment I, it varied from 0.090-0.571g, 2.377-14.212%, 0.100-0.635 and 0.00090-0.00571g, respectively whereas in Treatment II, the average weight gain, percent weight gain, specific growth rate and mean weight gain in Treatment II varied from 0.069-0.541g, 1.311-9.866%, 0.077-0.602 and 0.00069-0.00541g, respectively and from 0.080-0.611g, 2.405-17.099%, 0.089-0.679 and 0.00080-0.0061g, respectively in Treatment III.

Perusal of Table 2 further revealed that all the growth parameters studied showed its highest values in the Treatment III followed by Treatment I, Treatment II and Control. Least growth of fingerlings observed in Control set may due to the artificial feed fed to the fingerlings containing 32% protein. However, in Treatment I, Treatment II where the fingerlings were fed with 50% artificial feed + 50% ex-situ bioflocs and 100% ex-situ bioflocs showed better growth than control. Out of these two sets Treatment I proved to be beneficial which may be due to the preference of fingerlings to the bioflocs as feed over artificial feed. Similar type of results have also been reported with higher weight gain, final weight and length in biofloc + commercial feed in comparison to Control in post larvae of *Farfantepenaeus paulensis*[34]. Dietary protein level could be minimize to 25% without affecting the growth of shrimp, when *L. vannamei* juveniles were reared in zero- water exchange bioflocs-based tanks[35]. Commercial diet replacement with waste biofloc at 50% level has positive effect on the growth of *Litopenaeus vannamei* post larvae[36]. Optimum growth of *Labeo rohita* was found in the system containing 50% artificial feed and 50% bioflocs[37].

During 90 days experimental period, in-situ biofloc system (T-III) showed highest growth of fingerlings of *Cirrhinus mrigala* as compared to other sets which may be ascribed to continuous production of bioflocs in the system by maintaining C: N ratio above 10 by addition of molasses. In tilapia production, the biofloc technology resulted in the formation of microbial biomass by maintaining proper C/N ratio making it possible for protein to be eaten twice by cultured fish[38]. The constant production of bioflocs in the system makes them available 24×7 hours as feed to the fingerlings of *Cirrhinus mrigala*. The bioflocs which are the aggregate of heterotrophic bacteria along with other biotic components remain suspended in the water column and the fishes can readily take them as their food by properly harnessing these bioflocs as their food when they share the same system. The heterotrophic bacterial population also utilize the ammonium in addition to the organic nitrogenous wastes to produce single cell microbial protein[39] which also act as natural feed for fish[40]. Moreover, the water quality maintenance within the biofloc system along with fishes throughout the experimental period also seems to be responsible for enhanced growth of fingerlings of *Cirrhinus mrigala* in this treatment set and same observation[41] also support the present results.

Improved growth rate of Nile Tilapia was reported in biofloc system as compared to Control[42]. 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[21, 40-46].

The statistical analyses of data of growth parameters i.e. percent weight gain and specific growth rate of all the biofloc treatments with Control set revealed that these two parameters were significant between the Control and biofloc Treatment units at α=0.05. The significance level was found to be highest in Treatment III followed by Treatment II and Treatment I.

Conclusion

Bioflocs are the protein rich aggregates of algae, planktons, uneaten feed and other particulate matter that remain suspended within the water column. Biofloc technology unites the nutrients removal from water with the production of microbial biota, which can be used as food by fishes. It is a more environmentally friendly aquaculture production system. It provides environmental as well as economic benefits by reducing water replacement, waste matter discharge, commercial feed supply and improve biosecurity. In the present investigation, bioflocs were found to maintain the water quality and act as substitute of the artificial feed without affecting the growth performance of fingerlings of *Cirrhinus mrigala* thereby reducing the feed cost in aquaculture that assist towards the achievement of blue revolution in aquatic system.

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