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Research Article

Effect of Pulsed Feeding of GIFT Strain of Tilapia in Biofloc System Using Inland Saline Water

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Feed is one of the major inputs in aquaculture system and constitutes 60%–80% of total production costs of tilapia. Inappropriate selection of feed quality and the feeding strategy affects the feed utilization resulting in high food conversion ratio (FCR). A 60 days experiment was conducted to evaluate the growth performance and immuno-physiological responses of GIFT tilapia, (*Oreochromis niloticus*) by pulsed feeding under biofloc culture system in inland saline water. For the experiment, feeding pattern in pulsed was followed viz., *in situ* biofloc with daily feeding (T1), *in situ* biofloc with alternate day feeding (T2), *in situ* biofloc with every third day feeding (T3), *in situ* biofloc with no feeding (T4), and clear water control with daily feeding (C) each in triplicates. Biofloc based treatment receiving daily feeding (T1) resulted in significantly ($P < 0.05$) higher average body weight, weight gain, and specific growth rate (SGR) compared to control. T1 and C showed a significantly similar feed conversion ratio (FCR) and protein efficiency ratio (PER). Fish maintained in T4 grew the least and survival was lowest (85%). The immunological parameters showed a significant difference ($P < 0.05$) for nitroblue tetrazolium (NBT) and myeloperoxidase content whereas no significant difference ($P > 0.05$) for lysozyme activity was observed. Higher NBT activity was observed in biofloc based treatments compared to control. Activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity were considerably higher ($P < 0.05$) in biofloc based treatments than control. Among biofloc based treatments the antioxidant activities were lower in T1. The carbohydrate metabolism enzymes lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were lower in T1, T2, and T3 compared to control and T4. In conclusion, *in-situ* biofloc with daily feeding is found to be effective in growth improvement and to elicit immune-physiological responses in GIFT tilapia under pulsed feeding using biofloc based system.

1. Introduction

In India, around 8.621 million ha of land has been badly affected with the problem of soil salinity and 1.93 million km^2 areas is under laden with ground saline water [1]. Agricultural farmers abandon these lands as barren fields due to poor agriculture productivity [2], but these lands prove to be a valuable asset for aquaculture [3]. Aquaculture can be the right strategy to reduce the salt content in underground water tables and to generate income through enhanced production of euryhaline and marine fishes with high growth potential [3, 4]. However, its expansion is limited due to scarcity of water sources and competition with other water users such as agriculture and urban activities [5]. Also, intensification in this sector in ecologically sensitive areas and fragile lands lead to environmental degradation, if effluents are not treated before discharge [6]. So alternate and sustainable use of available water resources has become a necessity for production of food especially quality protein to feed the growing population [7].

Biofloc technology (BFT) would be a remunerative and sustainable means to reclaim salt-affected resources for food

production using limited water and land resources [8, 9]. BFT minimize water exchange and water usage in aquaculture systems through maintaining adequate water quality within the culture unit, while producing low-cost bioflocs rich in protein [10]. Manipulation of Carbon and Nitrogen balance stimulates heterotrophic bacterial growth responsible for transforming nitrogenous waste accumulated as uneaten feed and excreta products in the culture system (70–80%) into microbial protein [11, 12] hence minimizing ammonium concentration faster than nitrification process [10, 13 14]. In addition, it acts as a potential extra food source (available 24 hours) for the cultured organism supplying protein and other nutrients required for the culture organism [11, 15, 16]. Considering the long-term sustainability of aquaculture mainly based on feed cost (40–50%), the biofloc system provides an opportunity to reduce this cost [17].

The fact of continuous development in aquaculture sector places the importance of research on the need for new alternatives in diets and feeding strategies [18]. Reference [19] proposed two ways of reducing feed costs i.e., developing low-cost diets or adopting different feeding strategies or good husbandry methods. Pulsed feeding is the short period alteration in feeding strategy. Several studies evaluated different feeding strategies in tilapia including mixed feeding schedules of differing protein content, varying percentage and quality of protein, reducing feeding rate, delayed feeding, and alternate feeding to reduce the production cost [20–23]. BFT too allows implementation of such strategies through adoption of delayed/alternate feeding schedule or through supplementing protein at graded level in diet of aquaculture species reared under BFT [24, 25]. When using BFT for tilapia culture the reduction in the use of artificial feed could be higher and the nutritional demands seem to be modified based on the biofloc diversity, composition and intake ratio enabling greater flexibility in formulations, and inclusion of nonconventional ingredients [26, 27].

Adaptation to crowded condition, higher density, and physiological adaptations to consume biofloc has made GIFT stain of Tilapia as the preferred species for culture under BFT system [28, 29]. GIFT strain of tilapia gives better growth compared to normal strains and can be cultured in both freshwater and brackish water upto salinity range of 12–15 ppt [29–31] presenting opportunity for diversification of its culture in inland saline water. As GIFT strain of tilapia can consume biofloc, hence would be benefited by nutrition through microbial floc consumption during the period of feed deprivation which might reduce the amount of feed for culture of fish in BFT system. With this in backdrop the current study was planned for 60 days to assess the growth, survival, and immuno-physiological responses of GIFT Tilapia under pulsed feeding in BFT using inland saline water of 10 ppt salinity.

2. Materials and Methods

2.1. Experimental Design and Fish Stocking. The experiment was conducted in 15 fiber reinforced plastic (FRP) circular tanks (500-L capacity with working volume of 300 L) for 60 days at Central Institute of Fisheries Education, Rohtak Centre, Haryana, India. Genetically improved farmed tilapia (GIFT) fry ($n = 1000$) for the experiment were procured from Svara biotechnovations, Therku Pethampatti, Madurai, Tamil Nadu, India. The healthy fish having an average weight of 0.20 ± 0.01 g were acclimatized and held in a nursery pond at 3 ppt for 3 weeks fed with a commercially available feed containing 36% protein (floating feed, Growel growfin fish feed). Then, the fish were held for a week in 1200 L FRP tank for acclimation to 10 ppt inland saline water. The experiment followed completely randomized design (CRD), with four biofloc treatments and one control (clear water) viz., T1 (*in-situ* biofloc with daily feeding), T2 (*in-situ* biofloc with alternate day feeding), T3 (*in-situ* biofloc with every 3rd-day feeding), T4 (*in-situ* biofloc with no feeding), and C (clear water with daily feeding), in triplicates. A total of 600 GIFT fry of mean weight ($6.15\,g \pm 0.02$) were stocked randomly in 15 tanks. The carbon-nitrogen ratio (C/N) in BFT was maintained at 20 : 1 as per Avnimelech [11] using jaggery (39.99% carbon, w/w) added twice a week in the treatment units based on quantity and protein content in the feed. The biofloc inoculum preparation and carbon source requirement calculation were made by following Avnimelech [11]. The fishes were fed with commercially available feed containing 32% protein (floating feed, Growel growfin fish feed) @ 4% body weight split into two equal amounts given at 09:00 am and 05:00 pm.

2.2. Preparation of Inoculum. Biofloc inoculum was prepared in 300 L FRP tank filled with 10 ppt inland saline ground water upto 250 L and continuous aeration was provided. The pond soil was obtained from the dried shrimp pond of CIFE-Rohtak. The carbon source used for inoculums was molasses. Preparation of inoculum was carried out according to Avnimelech [11] using 10 gm/L pond soil, 10 mg/L ammonium sulphate and 200 mg/L molasses. The inoculum developed within 10 days (floc volume, >40 ml/L) and was distributed equally (50 L per tank) into the already prepared experimental tanks. Carbon sources were calculated and added twice a week based on quantity and protein content in the feed used. C/N ratio maintained was 20 : 1. Carbon source used for maintenance of biofloc was jaggery. Continuous aeration was provided in all the experimental tanks from a centralized aeration connected to two air pumps of 150 W (Hiblow HP 200) with an output capacity of 200 $L \cdot min^{-1}$. The aeration pipe in each tank was provided with air stone and a regulator to control the air pressure in all the tanks. There was no removal of floc or sludge throughout the experiment.

2.3. Assessment of Water Quality Parameters. Water quality parameters like temperature, pH, and salinity were monitored daily. Dissolved oxygen (DO) was monitored weekly using Winkler's titrimetric method [32]. Total ammonia nitrogen (NH_4-N), Nitrite-N (NO_2-N), and Nitrate-N (NO_3-N) were measured spectrophotometrically every 7th-day interval according to standard methods [33]. Floc volume was measured by allowing floc to settle down for 30 min. in Imhoff cone without disturbance.

2.4. Growth Parameters. More than 50% of fish of different treatment groups were sampled for length and weight measurement at an interval of 10 days and feeding was adjusted accordingly. Total weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), and survival percentage were calculated using the following formulae:

$$\begin{aligned}
\text{Total weight gain (g)} &= \text{final weight (g)} - \text{initial weight (g)},\\
\text{SGR}\left(\frac{\%}{\text{day}}\right) &= \frac{\log_e \text{ final weight} - \log_e \text{ initial weight}}{\text{number of days}} \text{ X } 100,\\
\text{FCR} &= \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}},\\
\text{FCE} &= \frac{\text{Body weight gain (wet weight)}}{\text{Feed given (dry weight)}},\\
\text{PER} &= \frac{\text{Body weight gain (wet weight)}}{\text{Crude protein fed}},\\
\text{Survival (\%)} &= \frac{\text{Total number of animal harvested}}{\text{Total number of stocked animal}} \text{ X } 100.
\end{aligned} \tag{1}$$

2.5. Analysis of Immune Parameters. After completion of the feeding experiment, blood was collected from 9 fish from each treatment groups (3 from each replicate) with and without anticoagulant. The collected blood was allowed to clot for 4 h and then centrifuged at 5000 rpm for 5 min followed by the collection of serum. Serum was stored at −80°C for further analysis. Respiratory burst activity (production of superoxide anion O_2^-) was determined by reduction of nitro blue tetrazolium (NBT) to formazan following the method of [34]. The optical density (OD) was read at 595 nm in an ELISA reader. Serum lysozyme activity was measured using turbidimetric assay utilizing hen egg white lysozyme as standard following Sankaran and Gurnani [35]. The unit of lysozyme activity was described as the amount of enzyme that caused a decrease in absorbance of 0.001 m^{-1}. The total myeloperoxidase of serum was measured following the procedure described by Quade and Roth [36] with some modifications. The optical density was read at 450 nm.

2.6. Antioxidant Enzyme Parameters. At the end of the experiment, liver and muscle tissue were extracted from fish ($n = 3$) from each tank and were then pooled and stored in 0.25 M chilled sucrose solutions at 1 : 19 (tissue: sucrose) ratio and refrigerated. The tissues were then homogenized and centrifuged at 6000 rpm for 10 minutes at 4°C in a refrigerated centrifuge Eppendorf (Germany). The supernatant solution was preserved in the autoclaved tube and stored at −20°C for future use. Protein of different tissues was estimated by the Bradford method [37]. Reading taken at 595 nm against the blank was expressed in mg/g wet tissue.

Superoxide dismutase activity in the liver was assayed as per Misra and Fridovich [38] protocol with slight modifications. The increase in absorbance was recorded at 480 nm at every 30 s for 3 min in UV spectrophotometer and the values are expressed as 50% inhibition of epinephrine auto-oxidation min^{-1} mg $protein^{-1}$. Catalase activity for tissue liver was carried using H_2O_2 substrate (0.03 M in phosphate buffer) according to the method followed by [39]. The decrease in OD was measured at 240 nm at every 30 s for 3 min. and expressed as moles of H_2O_2 decomposed per min. per mg protein. Glutathione peroxidase activity of the serum was assessed by using Cayman Glutathione Peroxidase Assay kit (Cayman Chemical Company, USA) following the specified protocols. The absorbance was measured at 340 nm using ELISA plate reader. Specific activity was expressed as GPxμmol/min/g protein.

2.7. Metabolic Enzymes Assay. The lactate dehydrogenase (LDH) activity was assayed from muscle tissue by the method of Wroblewski and Ladue [40]. OD was recorded at 340 nm at 15 seconds interval for 3 minutes. Enzyme activity was expressed as micromoles of NAD released per mg protein per min at 37°C. Malate dehydrogenase (MDH) activity in muscle was assayed in similar to LDH activity except 0.02 M oxaloacetate was used as the substrate as followed by the Ochoa [41]. Liver, Serum, and muscle protein were estimated by Lowry's method [42] using bovine serum albumin as standard.

2.8. Statistical Analysis. All data were statistically analyzed using software SPSS version 16.0 (Chicago, IL, USA). The significance of each parameter among different treatments was statistically analyzed using one-way ANOVA and significant differences among treatments were coined using Duncan multiple range tests ($P < 0.05$).

3. Results

3.1. Water Quality Parameters. The physicochemical parameters of water quality analyzed during the experimental period are shown in Table 1. Although a significant difference ($P < 0.05$) was observed in DO between control and biofloc treatment with the highest average value in control, no significant difference ($P > 0.05$) was observed in temperature and pH among the different treatment groups. Salinity was maintained at 10 ppt throughout the experimental period.

During the production cycle, nitrogenous compound i.e., ammonia (NH_4^+-N: mg/L), nitrite (NO_2-N: mg/L), and nitrate (NO_3-N: mg/L) showed a significant difference ($P > 0.05$) between the treatments. The lowest value was recorded in control and highest in T1. Ammonia (NH_4^+-N) was in the range of 0.11–0.64 mg/L, nitrite-N was 0.08–0.37 mg/L and nitrate-N was 0.54–9.10 mg/L. All these parameters were significantly higher in biofloc based units as compared to control. During the culture period, floc volume in biofloc tanks ranged from 6.13 to 40.67 ml/L. T4 recorded the lowest floc volume throughout the experimental period.

3.2. Growth Performance. Growth performance of the GIFT strain of tilapia over the experimental period is represented in Table 2. After 60 days of trial, a significant difference ($P < 0.05$) was observed between the treatments in terms of average body weight, body weight gain, length gain, specific growth rate (SGR), feed conversion efficiency (FCE), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate as shown in Table 3. Among the treatment groups highest average body weight, length gain, weight gain, and SGR were observed in T1 compared to control. However, FCR of the group, T1 did not differ significantly ($P > 0.05$) with control. Highest FCE (1.27 ± 0.06) was noticed in T3. T1 and control did not show any significant difference ($P > 0.05$) in FCE. Significantly lowest survival rate was observed in T4 compared to other treatments and control.

3.3. Immune Parameters. Respiratory burst activity (OD at 595 nm) and serum myeloperoxidase activity (OD at 450 nm) differed significantly ($P < 0.05$) (Figures 1(a) and 1(b)). Higher activities were observed in biofloc treatment groups compared to control. Fish reared in T1 showed significantly higher NBT activity while fish in T4 showed higher myeloperoxidase activity. Serum lysozyme activity did not exhibit any significant difference ($P > 0.05$) among the treatments (Figure 1(c)).

3.4. Stress Parameters

3.4.1. Antioxidant Enzymes. A significantly ($P < 0.05$) higher level of liver SOD activity was observed in biofloc groups than control but the highest was in T4 (Figure 2(a)). Liver catalase activity differed significantly with the highest being in T4 and lowest in control (Figure 2(b)). There was no significant difference ($P > 0.05$) in serum GPx among the treatments (Figure 2(c)).

3.4.2. Carbohydrate Metabolic Enzymes. The LDH activity in the muscle of GIFT tilapia showed the highest values in T4 and lowest in T1 group while no significant difference ($P > 0.05$) was observed in T2, T3, and control (Figure 3(a)). However, liver MDH activity did not differ significantly ($P > 0.05$) among the treatments (Figure 3(b)).

4. Discussion

During the experimental period, DO concentrations and pH values were within the acceptable range as reported [43] for the Nile tilapia under biofloc except for the low temperature as the seasonal change directed towards low temperature (Table 1). The study was conducted in the month of October-November that recorded lower temperature. Martinez et al. [44] recognized the growth of fish as a complex process affected by many abiotic factors and temperature is one of the most important factors. Hossain et al. [45] recommended 25°–35°C suitable for raising tilapia. Lower pH and DO in BFT treatment compared to control is likely due to increased C : N ratio which stimulates the growth of heterotrophic bacteria which in turn require oxygen for their growth [46–49]. Salinity was maintained at 10 ppt but a slight increase was noticed in BFT treatment which could possibly due to evaporation [47].

Increasing C : N ratio of 20 : 1 reduces NH_3-N, NO_2-N, and NO_3-N [50] through uptake by the microbial community [51] and maintaining DO enables bacteria to convert ammonium into bacterial biomass [52]. In the present study, inorganic nitrogen concentration was within the range for raising tilapia under BFT [29, 53]. The floc volume in BFT treatments of the present study was recorded as 7.4 ml/L in no feeding treatment to 40.67 ml/L in daily feeding treatment. The volume was sufficient to support growth in all other treatment [54] except no feeding treatment which may be due to feeding on floc which was the only source of food in the system.

Significant variation in average body weight was observed in BFT treatments and control with BFT treatments showing higher ABW than control until 30 days of the culture period (Table 2). Later a similar trend followed till last. Variation in temperature observed in a range of 20°–26.3°C could be the reason for such variation in growth. Weight gain percentage of SGR was significantly higher in BFT with daily feeding compared to other BFT treatments and control (Table 3). Similar enhanced weight gain and SGR of GIFT tilapia were reported in BFT system than the control group [43, 55, 56]. Other biofloc treatments also had enhanced growth parameters indicating that GIFT tilapia fingerlings harvested and assimilated the biofloc effectively, but not sufficient growth was obtained as in T1 and control as they received daily feeding. Eroldogan et al. [57] observed partial compensation in growth when Gilthead Sea Bream, *Sparus aurata* juveniles were subjected to moderate levels of restriction (50%) and short-term restriction (2d) compared

Table 1: Water quality parameters of the biofloc system during the experimental period.

Parameters	C	T1	T2	T3	T4
DO (mg/L)	6.95 ± 0.24^{a}	6.66 ± 0.27^{ba}	6.82 ± 0.19^{ba}	6.23 ± 0.08^{b}	6.46 ± 0.17^{ba}
Temperature (°C)	24.40 ± 0.64^{a}	23.71 ± 0.71^{a}	23.70 ± 0.72^{a}	23.91 ± 0.72^{a}	23.95 ± 0.80^{a}
pH	8.10 ± 0.06^{a}	7.69 ± 0.04^{a}	7.72 ± 0.08^{a}	7.70 ± 0.06^{a}	7.76 ± 0.06^{a}
Salinity (ppt)	10	10	10	10	10
Ammonia (NH_4^+-N: mg/L)	0.24 ± 0.03^{c}	0.56 ± 0.02^{a}	0.35 ± 0.04^{b}	0.37 ± 0.04^{b}	0.32 ± 0.02^{cb}
Nitrite (NO_2-N: mg/L)	0.16 ± 0.01^{c}	0.31 ± 0.02^{a}	0.28 ± 0.01^{a}	0.30 ± 0.02^{a}	0.22 ± 0.01^{b}
Nitrate (NO_3-N: mg/L)	0.71 ± 0.06^{b}	5.37 ± 1.28^{a}	4.92 ± 1.26^{a}	4.47 ± 1.16^{a}	4.06 ± 1.09^{a}

Values in the same row with different superscripts differ significantly ($P < 0.05$) for each parameter. One-way ANOVA was used following Duncan multiple range test in SPSS-16.0.

Table 2: Average body weight (gm) of GIFT tilapia fingerlings during the experiment in biofloc based treatments with pulsed feeding.

Day	Control	T1	T2	T3	T4
1	6.14 ± 0.02^{a}	6.12 ± 0.01^{a}	6.15 ± 0.01^{a}	6.16 ± 0.03^{a}	6.18 ± 0.02^{a}
10	8.45 ± 0.12^{a}	10.81 ± 0.12^{d}	10.09 ± 0.08^{c}	9.47 ± 0.19^{b}	8.40 ± 0.01^{a}
20	11.18 ± 0.20^{bc}	14.87 ± 0.16^{d}	11.63 ± 0.27^{c}	10.78 ± 0.15^{b}	8.49 ± 0.02^{a}
30	13.93 ± 0.32^{c}	18.59 ± 0.12^{d}	13.30 ± 0.28^{c}	11.08 ± 0.25^{b}	8.67 ± 0.06^{a}
40	17.39 ± 0.34^{d}	23.10 ± 0.08^{e}	15.65 ± 0.12^{c}	12.65 ± 0.58^{b}	8.92 ± 0.08^{a}
50	23.79 ± 0.16^{d}	29.94 ± 0.09^{e}	18.94 ± 0.29^{c}	14.33 ± 0.50^{b}	9.23 ± 0.26^{a}
60	31.37 ± 0.30^{d}	38.29 ± 0.38^{e}	22.71 ± 0.19^{c}	16.94 ± 0.57^{b}	9.89 ± 0.09^{a}

Values in the same row with different superscripts differ significantly ($P < 0.05$) for each parameter. One-way ANOVA was used following Duncan multiple range test in SPSS-16.0.

Table 3: Growth performance of GIFT tilapia fingerlings during the experiment in biofloc based treatments with pulsed feeding.

Parameter	Control	T1	T2	T3	T4
Length gain (cm)	3.76 ± 0.07^{d}	4.54 ± 0.10^{e}	2.25 ± 0.13^{c}	2.03 ± 0.09^{b}	0.23 ± 0.02^{a}
Weight gain (g)	25.24 ± 0.30^{d}	32.17 ± 0.37^{e}	16.55 ± 0.19^{c}	9.78 ± 0.59^{b}	3.70 ± 0.70^{a}
SGR (% day^{-1})	2.72 ± 0.02^{d}	3.06 ± 0.01^{e}	2.17 ± 0.04^{c}	1.58 ± 0.06^{b}	0.78 ± 0.02^{a}
FCR	1.28 ± 0.03^{d}	1.29 ± 0.01^{d}	0.92 ± 0.01^{c}	0.79 ± 0.4^{b}	0.00 ± 0.00^{a}
FCE	0.78 ± 0.02^{b}	0.78 ± 0.01^{b}	1.09 ± 0.01^{c}	1.27 ± 0.06^{d}	0.00 ± 0.00^{a}
PER	2.44 ± 0.05^{b}	2.43 ± 0.02^{b}	3.14 ± 0.01^{c}	3.96 ± 0.17^{d}	0.00 ± 0.00^{a}
Survival rate (%)	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}	100.0 ± 00.00^{b}	99.17 ± 0.83^{b}	85.00 ± 1.44^{a}

The means with no superscript letter in common per factor indicates significant difference. If the effects were significant, ANOVA was followed by Duncan multiple range test. $P < 0.05$. Values are presented as mean ± SE.

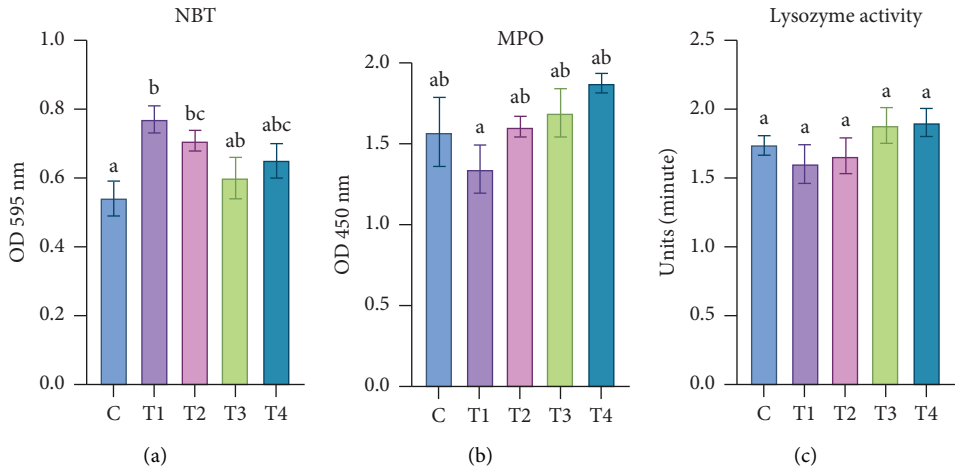

Figure 1: Immunological enzymes activity of GIFT Tilapia in pulsed feeding under biofloc based treatment groups. NBT = respiratory burst activity; MPO = myeloperoxidase activity (serum); lysozyme activity (serum).

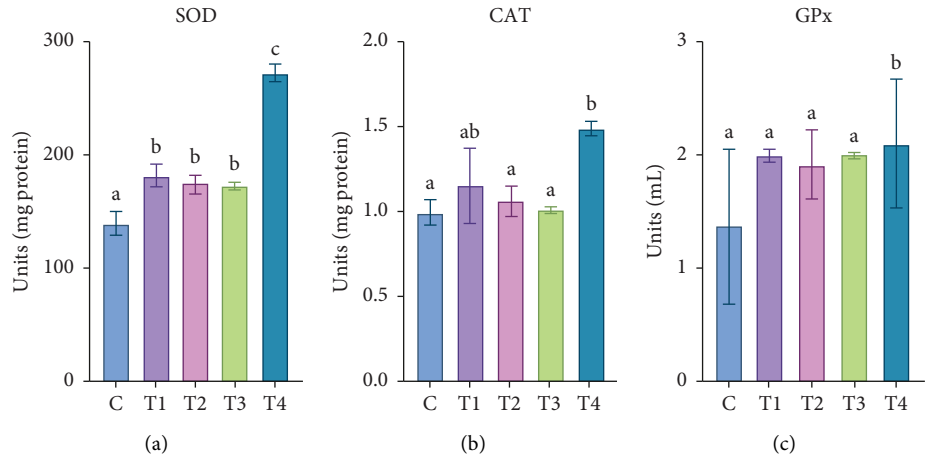

Figure 2: Antioxidant enzymes activity in of GIFT Tilapia in pulsed feeding under biofloc based treatment groups. SOD, superoxide dismutase (liver); CAT, catalase (liver) GPx, glutathione peroxidase (serum).

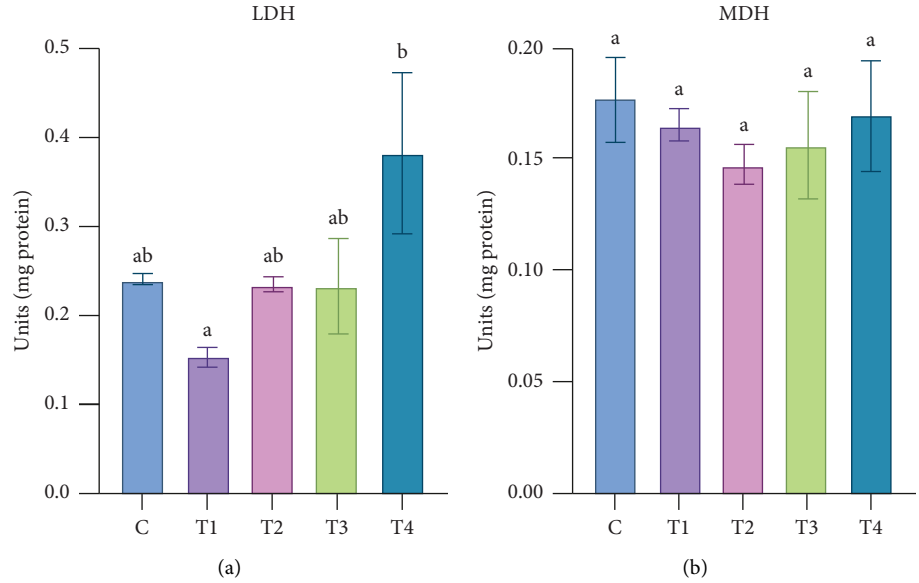

Figure 3: Carbohydrate metabolism enzymes activity in muscle of GIFT Tilapia in pulsed feeding under biofloc based treatment groups. LDH, lactate dehydrogenase; MDH, malate dehydrogenase.

to fish fed to satiation. A similar report on reduced growth on skip feeding was reported by Cuvin–Aralar et al. [58] in Lake Bato indicating only partial compensation by natural food available in cages. These results are distinctly different from reports of Bolivar et al. [23] on an alternate day feeding strategy for Nile Tilapia in which the mean growth performance of fish in the daily and alternate day feeding groups lacked any significant difference in a pond ecosystem. Qiang et al. [59] observed SGR and FE in the range 2.01–2.38%/day and 0.78–0.86, at water temperature 27.5°C and 10 ppt salinity. In the present study, the weight gain and SGR decreased with the increased gap in feeding days, this may be due to competition for food. In T4 lowest average body weight, SGR and weight gain were attained in GIFT fingerlings as artificial feed was not used and fingerlings were fed purely on bioflocs. Sharma et al. [60] in the *Labeo rohita* fingerlings reared in biofloc suspension supporting that the role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements of fish depend upon the feed supplied and natural productivity of the system that was in the form of biofloc in the present experiment. In the absence of external feed source, they shifted to biofloc that resulted in good growth but feeding on biofloc alone was not suitable for efficient growth as mixed biofloc and artificial feeding also resulted in comparatively less growth.

As expected, FCR was lowest in T4. The decrease in FCR from 1.29 ± 0.01 to 0.79 ± 0.4 indicates that in biofloc treatments with alternate day and every 3rd-day feeding, fingerlings grazed on the biofloc. According to Bolivar et al. [23], grazing undoubtedly contributes to low FCR in Nile tilapia grazing on plankton in ponds which is an important component of the diet of the fish. Wasielesky et al. [61]

observed a decrease in FCR from 1.39 : 1 to 1.03 : 1 when culture subsisted on natural productivity, indicating the potential to reduce the amount of feed in the presence of biofloc. PER was lower for control group as compared to biofloc treatment but was comparable with biofloc with daily feeding, indicating microbial protein utilization as an alternative food source apart from the artificial feed by the fish [29].

The total absence of artificial feed affected the survival rate (85%) in biofloc with no feeding treatment. A period of 7.86 days was sufficient to obtain 50% mortality in *Fenneropeneaus chinensis* juveniles under starvation [62]. Lara et al. [24] observed 37.50% survival in *Litopenaeus vannamei* under starvation for 21 days in a biofloc culture system. Intermittent feeding did not affect the survival rate in juvenile *L.vannamei* as reported by Zhu et al. [63]. The only source of feed to fish in T4 group of the present study was biofloc, reflecting the contribution of heterotrophic bacteria to survivability upto 85% of the starved fish during 60 days of experiment period. Also, lower survival can be related to cannibalism, indicating fish did not survive when fed solely on biofloc and require food to maintain basic metabolic activities.

The general feature of biofloc is immunostimulation effect and considering the immunological factor lower weight and survival is expected in fish under food restriction than fish that did not experience any feeding stress [24] but the immune-stimulation extent is affected by feed restriction in in-situ biofloc. The nitroblue tetrazolium (NBT) assay is indicative of oxidative radical production from neutrophils and monocytes for use in defense against pathogens [64]. In the present study, NBT activity was higher in biofloc based treatments compared to control (Figure 1(a)). Xu and Pan [65] and Ekasari et al. [66] reported increased respiratory burst activity of shrimp in biofloc based culture system. The earlier finding reported enhanced NBT activity in *L. rohita* and MPO values in GIFT tilapia cultured in BFT, supporting the immunostimulatory potential of microbial floc [29, 53]. Among the biofloc based treatments, the NBT activity decreases with the increased days of pulsed feeding indicating stressful condition and requirement of feed by the fish. Lysozyme is an important defense molecule of the innate immune system, which is important in mediating protection against microbial invasion [67] and breaks down β-1, 4 glycosidic acids and N-acetyl-glucosamine in the peptidoglycan of bacterial cell walls. No significant difference was observed in lysozyme activity due to pulsed feeding in biofloc based system (Figure 1(c)). Caruso et al. [68] observed no effect of lysozyme in plasma in 58 days starved European eel. Luo et al. [52] reported no difference in serum lysozyme activity in BFT and RAS in GIFT. MPO is an important enzyme having antimicrobial activity. It utilizes hydrogen peroxide during the respiratory burst to produce hypochlorous acid [69]. Reduced activity may indicate the presence of contaminants or stress [64]. In the present study highest MPO activity in biofloc based treatments in GIFT, tilapia signifies well-developed immune status compared to control (Figure 1(b)). The results of the present study are in agreement with Ahmad *et al.* [53]. who reported the highest MPO activity in biofloc based treatments in *L. rohita*. Wu et al. [70] observed increased myeloperoxidase content after oral administration of *Sophora flavescens* in GIFT tilapia.

Reduction in feeding depletes organ antioxidant storage which is the defense system against oxidative stress [71]. The present study recorded higher antioxidant enzymes activity in the biofloc based treatment units as compared to the control (Figure 2). However, within the biofloc based treatment groups, the enzyme's activity decreases with increase in nonfeeding intervals in pulsed feeding and the highest value was observed in no feeding treatment. The results are in agreement with Kumar et al. [72] who noticed higher SOD and catalase activities in muscle and serum of shrimp fed with periphyton-incorporated diets. According to Luo et al. [52], SOD and CAT activity in GIFT tilapia was observed higher in fish cultured in the BFT than RAS. The antioxidative enzymes, SOD and CAT increased in the 2 and 3 days per week feeding groups of *L. rohita* in an adaptive response to cope with the oxidative stress caused due to feed deprivation [73] which is in contradiction with the present study which may be due to the biofloc being consumed in the absence of artificial feed. Biofloc serves as a potential source of antioxidant containing an appropriate amount of carotenoids [65] and fat-soluble vitamins [74]. This agrees with the earlier reports where the higher immune response in terms of SOD and catalase activities were recorded in shrimps fed β-glucan, carotene [75], microalgae [76], and macroalgal supplements [77]. These suggest that in situ biofloc with pulsed feeding can elicit the antioxidative enzymes in fish and enhance the defense potential against oxidative stress. Prolonged starvation leads to enhanced oxidation and oxidative stress in the liver of *D. dentex* despite activation of some antioxidant defense mechanisms [78]. Hence, biofloc with no feeding can be the result of oxidative stress which is also concerned with the decreased survival rate observed in the treatment. Though there was no significant difference in GPx among the treatments but followed the same trend as that of SOD and CAT activity (Figure 2(c)).

Metabolism is a physiological process reflecting the energy expenditure of living organisms. Lactate dehydrogenase (LDH) assay serves as a useful stress indicator [79]. In fish, there is a negative relationship between growth rate and LDH and MDH activity [80]. The lower level of these enzymes was recorded when fish and shrimps were fed with dietary supplements like tryptophan [81], pyridoxine [82], or periphyton [83]. Kumar et al. [72] observed lower LDH activity in *L. rohita* fed with high protein feed compared to low protein fed groups suggesting that higher dietary protein helps in reducing stress. Generally, the LDH activity increase during temperature stress [84], starvation stress [85], and confinement stress [79]. In the present study, significantly higher LDH activity was noticed in T4 indicating the starvation stress (Figure 3(a)). Lower LDH activity in the biofloc receiving pulsed feeding groups

suggested that the supplementation of biofloc with feed helps in reducing stress in GIFT tilapia.

MDH is an enzyme which catalyzes the NAD/NADH dependent interconversion of the substrates malate and oxaloacetate. The MDH enzyme activity decreases in proportion to the feeding rate. Hence, MDH plays an important role in the generation of NADPH for fatty acid synthesis [86]. Shikata and Shimeno [87] observed that the fatty acid synthesis in the hepatopancreas is markedly depressed by feed restriction including starvation due to the low reproduction rate of NADPH which may cause the decreased fatty acid synthesis. In the present study, the muscle lipid content was not affected and hence the MDH activity was not affected by the pulsed activity in GIFT tilapia under biofloc culture (Figure 3(b)). Kumar et al. [72] noticed no significant difference in MDH activity in the hepatopancreas of treatment groups compared with control indicating that dietary supplementation of biofloc helps to maintain shrimps in a less stressed condition and reduces energy demand in shrimps. Shimeno et al. [88] observed that MDH significantly decreases with decreasing feeding rates in metabolic response in common carp, *Cyprinus carpio*. The fish deprived of feed for 3 DPW had a lower activity of the metabolic enzymes MDH suggesting reduced metabolic activity in these groups, which might be a metabolic adaptation to cope with longer periods of feed deprivation [76].

5. Conclusion

The present study demonstrates that biofloc reared GIFT tilapia under pulsed feeding enhances growth and improves immunity, hence this culture method will cut the feed cost without diminishing production and has obvious potential for production without compromising the survival. But the need for artificial feed cannot be ignored in the intensive culture for higher fillet, growth performance and final production. Further research can be carried out on the identification of gut microbial content, higher salinity culture in inland saline waters and biofloc composition in inland saline water so as to utilize the resources in the arid zones.

Data Availability

The data that support the findings of the study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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