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Growth, biochemical indices and carcass quality of red tilapia reared in zero water discharge based biofloc system in various salinities using inland saline ground water

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ABSTRACT

Biofloc based aquaculture is important in waste recycling in intensive aquaculture and is a promising avenue for inland saline aquaculture. In the present study, growth, biochemical and carcass quality of red tilapia reared in inland saline waters of different salinities based on biofloc technology was estimated during 90 days culture period. Fifteen experimental tanks (500 L each) were filled with inland saline ground water to represent 5 biofloc treatments with variable salinities such as T1 (5 ppt), T2 (10 ppt), T3 (15 ppt), T4 (20 ppt) and control (0 ppt) in triplicates. Red tilapia fry with average body weight of 13.78 ± 0.62 g were stocked at the rate of 150 no.m $^{-3}$ in each biofloc unit. The units maintained at higher salinity (20 ppt) obtained significantly better ($P < 0.05$) growth performances in terms of percentage weight gain (329.89 ± 13.41), specific growth rate (1.65 ± 0.05), protein efficiency ratio (1.18 ± 0.07) and feed conversion ratio (0.93 ± 0.06). Biochemical properties such as serum glucose (118.49 ± 3.73 mg dL^{-1}), serum total protein (4.23 ± 0.04 mg dL^{-1}), total serum albumin (2.08 ± 0.03 mg dL^{-1}), and total serum globulin (2.16 ± 0.04 mg dL^{-1}) contents were the highest in T4 (20 ppt), whereas activities of enzymes, superoxide dismutase and catalase were the highest in control (0 ppt). Treatment T4 (20 ppt) had shown the highest survival rate (95.56 ± 1.28), carcass quality like firmness (121.45 ± 1.91), and whiteness (49.52 ± 0.79) and in addition, overall sensory attributes were also the highest in T4 (20 ppt). Consequently, the findings of the current investigation have shown that growth efficiency, survival, biochemical indices and carcass quality of red tilapia had improved when reared in higher salinity using inland saline groundwater.

1. Introduction

The expansion of aquaculture must be geared towards more production without significantly increasing the use of natural resources and must guarantee ecological, economic and social sustainability (Avnimelech, 2009; Naylor et al., 2000). Water scarcity and climate change are affecting agriculture in general and aquaculture is not an exception. The problem of salt affected soil is a case of global occurrence and it affects developing as well as developed countries. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity. In India, around 8.62 million ha of land has been badly affected by the problem of soil salinity and 1.93 million km^2 area is having inland ground saline water (ISGW). The lands with higher ground water salinity are left barren as they are not useful for agriculture. So, the culture of salt tolerant fish or shellfish provides an opportunity for the diversification, expansion, and potential option for the utilization of ISGW for economic and environmental benefits. At the same time, intensification in this sector in ecologically sensitive areas and fragile lands will further lead to environmental degradation, if effluents are not treated before discharge.

Biofloc technology (BFT) is a sustainable alternative for intensive production in aquaculture as this technology is based on zero or minimal water-discharge system. BFT develops microbial protein in-situ in the rearing unit through assimilating the nitrogenous waste and converting them into the proteinaceous matter. Biofloc is the suspended growth of microbial protein in pond water, which consists of aggregates of living

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and dead organic particulate matter, phytoplankton, bacteria and bacterial grazers, according to Hargreaves (2013). BFT recycles the nitrogenous waste with the help of heterotrophic bacteria which consumes organic substrates and reduces the ammonium concentrations in the water (Avnimelech, 1999; De Schryver et al., 2008). Biofloc thus developed, can serve as fish food and hence facilitate the reuse of water (Crab et al., 2012). So, BFT has the advantage of reducing feed inputs by recycling feed waste and fecal matter into microbial proteins in the system by manipulating carbon: nitrogen ratio (C/N ratio) with external carbon source. Maintenance of C/N ratio to enhance favorable microbial development is the major principle behind BFT. BFT was reported to improve growth, immune response, digestive enzyme activity and feed utilization in fish (Ahmad et al., 2016; Haridas et al., 2017; Ahmad et al., 2017). It also effectively improved stress amelioration, disease resistance and feed conversion ratio in cultured fish (Burford et al., 2004; Moss et al., 2006; Wasielesky et al., 2006; Ballester et al., 2010; Emerenciano et al., 2012; Xu and Pan, 2012; Haridas et al., 2017; Ahmad et al., 2019). The suitable species for BFT should have the tolerance for higher stocking density, high amount of suspended matter, and intermediate levels of dissolved oxygen in the water. Tilapia, with omnivorous habits can assimilate the microbial particles and can tolerate high stocking density and can grow well in brackish water ponds (Al-Harbi and Uddin, 2005; Haridas et al., 2017). The effects of salinity on fish growth have been widely studied (Tseng and Hwang, 2008) and in the case of tilapia, 10 to 20 ppt salinity range was found to be optimum for their growth (Romana-Eguia and Eguia, 1999; Suresh and Lin, 1992). The use of improved varieties for culture is the generally adopted measure to overcome the constraints related to tilapia culture. Improved strain, red tilapia showed higher growth rate, uniform red coloration, high survival and good adaptation to the local environment (Hamzah et al., 2008). It attains marketable size in shorter duration and its preference for brackish or saline water (Balcazer et al., 2006) and omnivorous feeding habit makes it a suitable species for BFT in inland saline aquaculture. In addition, tilapia can be successfully grown in high concentrations of suspended solids in biofloc, and hence tilapia is a good candidate for cultivation in BFT (Emerenciano et al., 2013). Due to the high tolerance to salinity, red tilapia is suitable for the cultivation in both brackish and sea water (Yue et al., 2016; Ahmadi et al., 2016). Keeping all these in view, the present study was designed to evaluate the effect of salinity on growth, biochemical indices and carcass quality of red tilapia in zero-water discharge BFT system using ISGW.

2. Materials and methods

2.1. Experimental setup and design

The experiment was conducted at Rohtak Centre of ICAR- Central Institute of Fisheries Education, Mumbai, India for 90 days using ISGW of various salinities (0 ppt, 5 ppt, 10 ppt, 15 ppt and 20 ppt). The various salinities were obtained by diluting the water from the borewell located in a high saline zone (20 ppt). The stored water of higher salinity was pumped into 1200 L fiber reinforced plastic (FRP) tanks and was diluted by mixing freshwater along with vigorous aeration till they reached the desired salinities of 0, 5, 10, 15 and 20 ppt respectively.

Red tilapia fingerlings (average weight of 0.51 ± 0.04 g) were procured from Madurai, Tamil Naidu and acclimatized them for 25 days in hapas placed in a cemented freshwater pond. The fishe were slowly acclimatized to salinities of 0, 5, 10, 15 and 20 ppt respectively within 20 days, by gradually increasing the salinity at a rate of 1 ppt per day. The red tilapia fingerlings (avg. wt. 13.83 ± 0.63 g) at a stocking density of 150 no. m^{-3} were randomly distributed in 15 FRP tanks (500 L each) following completely randomized design (CRD) with four treatments T1 (5 ppt), T2 (10 ppt), T3 (15 ppt), T4 (20 ppt) and C (0 ppt) in triplicates. The experiment trial need not require any ethical approval.

2.2. Preparation of inoculum

The inoculum was prepared by mixing 10 mg L^{-1} ammonium sulphate (Nitrogen Source) and 200 mg L^{-1} carbon source (Jaggery) with 10 g L^{-1} pond bottom soil (Avnimelech, 1999) collected from the inland saline pond of CIFE, Rohtak centre. The inoculum was prepared for each salinity treatments separately. Fermented jaggery was used as carbon source and the fermentation process was done for 24 h using Baker's yeast with vigorous aeration. The inoculum was developed within 10 days and was distributed equally into the prepared experimental units at the rate of 1 L of inoculum to 100 L of ISGW. The C/N ratio was maintained at 15:1 as per Avnimelech (1999) using fermented jaggery twice a week in the treatment units based on the quantity and protein content in the feed. The C/N ratio was maintained at 15: 1 initially and was reduced to 10:1 towards last month of rearing. Continuous aeration was provided in all the experimental tanks from a centralized aeration unit connected to two Hiblow HP 200 air pumps of 150 W with an output capacity of 200 L min^{-1} and the tanks were aerated well to avoid any settling point.

2.3. Feeding and maintenance

Fish were fed with commercially available feed (32% crude protein, 4% crude fat, 5% crude fiber and 10% moisture) at 8 h and 17 h daily at the rate of 5% body weight initially and was lowered to 2% towards the end of the experiment. The experimental units were maintained under zero-water discharge conditions and were only compensated for the evaporation loss.

Water quality parameters like temperature, salinity, floc volume, pH and Dissolved Oxygen (DO) were measured daily. Other parameters such as hardness, alkalinity, total ammonia nitrogen (TAN), nitrate-nitrogen (nitrate-N), nitrite-nitrogen (nitrite-N) and floc volume were analysed at 5 days interval.

Dissolved oxygen, alkalinity and hardness were measured by standard method (APHA, 2005). TAN, nitrite-N and nitrate-N content of the water samples were determined using TAN, nitrite-N and nitrate-N test kit (Merck, Germany) respectively, according to the manufacturer's instructions (Merck Spectroquant, NOVA 60). Floc volume was measured by using Imhoff cone by allowing the floc to settle down for 30 min.

2.4. Growth parameters and survival

Growth was evaluated by measuring the body weight of fish ($n = 10$) from each tank at a regular interval of 10 days during the culture period of 90 days. The equations used for various parameters are as follows: Feed conversion ratio (FCR) = feed given (DW)/weight gain (WW), specific growth rate (SGR) (% day^{-1}) = (ln final weight – ln initial weight/ number of days) x 100, and protein efficiency ratio (PER) = body weight gain (WW)/crude protein fed (DW). Survival rate at the end of the 90 days experimental period was estimated by formula: Survival rate (%) = (total number of an animal harvested/total number stocked) x 100, Whereas, DW: dry weight, WW: wet weight and ln: natural log.

2.5. Blood collection and bio-chemical indices

Blood was drawn from the tail vein of anesthetized experimental fish ($n = 3$) from each tank using a 3·0 mL syringe with 27 gauge hypodermic needle syringe were allowed to clot, refrigerated overnight and centrifuged at 3000 x*g* for 10 min. Serum was collected and stored at −20 °C until used.

Serum glucose estimation was performed using Erba-Manheim glucose kit (catalog number BLT00027). Optical density (OD) was measured at a wavelength of 490–550 nm. The serum protein was estimated by Biuret method (Reinhold, 1953) using kit (ERBA full details as above) following the manufacturer's protocol.

Albumin was estimated by a method of binding to bromocresol green

(Doumas et al., 1971). The absorbance of the standard (S) and the test (T) were immediately measured relative to blank value (B) in a spectrophotometer at 630 nm.

Globulin was calculated by deducting albumin values from total plasma protein by using the formula: Globulin (g dL^{-1}) = Total protein (g dL^{-1}) - Albumin (g dL^{-1}). Albumin - globulin ratio (A/G ratio) was calculated by using the following formula: A/G ratio = Albumin (g %) / Globulin (g %).

Muscle, intestinal and hepatic tissue were extracted from fish ($n = 3$) from each tank at the end of experimental period and tissues (10 mg each) were then pooled and stored in 0.25 M sucrose solutions at 1:19 (tissue: sucrose) ratio and refrigerated. The tissues were then homogenized, centrifuged at 5700 x*g* for 4 min at 4° C, the supernatant was collected and stored at −80 °C to abstain from denaturing the protein.

The activity of superoxide dismutase (SOD) in liver tissue was estimated using the method of Misra and Fridovich (1972) with minor modifications. The reaction mixture consisted of 50 μL ofe sample, 1.5 mL of phosphate buffer and 0.5 mL of epinephrine. The solution was well mixed and the OD change at 480 nm for 2 min was measured in the UV spectrophotometer. One unit of SOD activity was the amount of protein needed to achieve 50% inhibition of adrenaline auto-oxidation.

Catalase activity (CAT) of liver tissue was estimated using the method of Takahara et al. (1960). Up to 2.45 mL phosphate buffer (50 mm, pH 7.0) and 50 μL of tissue homogenate were added and the reaction was started by adding 1.0 mL of H_2O_2 solution and absorbance was measured at 240 nm after 30 s. intervals for 2 min. The enzyme blank was used simultaneously with 1.0 mL of distilled water instead of H_2O_2. CAT activity was expressed in nmol of H_2O_2, broken down min^{-1} $protein^{-1}$.

2.6. Carcass quality of red tilapia

2.6.1. Proximate composition

Proximate composition of the fish tissue ($n = 3$) from each experimental units were estimated at the end of trial period as per AOAC (1995) standard protocols. The samples were weighed and dried in a hot air oven at 105 °C until a constant weight was reached. Total nitrogen was estimated using the Kjeldahl method in an automated nitrogen analyzer (KEL Plus - Classic DX Model VA, Pelican Equipment, India). The percentage of crude protein was obtained by multiplying the total nitrogen by a factor of 6.25. The ether extract was estimated with the Soxhlet apparatus (SOCS PLUS-SCS 08 AS, Pelican Equipment, India). The total ash content was estimated in a muffle furnace (AI-7981, Expo Hi-Tech, Mumbai) at 550 °C for 6 h.

2.6.2. Texture analysis

Raw fillet of 3 fish from different experimental groups with a uniform size of 2.5 cm × 2.0 cm (diameter × height) was used for the texture analysis using a Taxt-plus texture analyzer (Stable Micro Systems Ltd., Surrey, UK) with a 5 kg load cell. A 2 mm diameter compression needle probe was used with a pre and post-speed of 2.0 mm s^{-1} and a test speed of 1.0 mm s^{-1}. Each treatment was tested to obtain six measurements and the average was given for each parameter. A data acquisition rate of 200 pps was used to generate a force-time diagram and texture parameters were calculated using the Taxt-Plus software supplied with the instrument (Dincer and Cakli, 2010).

2.6.3. Colour analysis

The colour of the fillet of red tilapia was determined using HunterLab (Virginia, USA). Whiteness of the raw material was calculated after obtaining lightness (L*), red/greenness (a*) and yellow/ blueness (b*) values through formula: whiteness = 100 - $[(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Surasani, 2017).

2.6.4. Sensory analysis

Sensory analysis was performed for the raw and cooked (smoked) red tilapia fillet with the help of a semi-trained panellist of 20 members. The panel was made up of men and women in the age group of 30 to 40 years. The fillets were presented on a white porcelain plate under natural light with random coding. Panel members were asked to rate all samples on the 9-point hedonic scale (Meilgaard et al., 2006). They were asked to rate sensory properties such as appearance, texture, smell of mud, taste, colour and general acceptance of the raw and cooked fillet. A sensory score was assigned separately for each sensory attribute on a descriptive hedonic scale from 1 to 9, which means the score ranging from dislike extremely (not recommendable) to like extremely (highly acceptable) of the sample.

2.7. Statistical analysis

The analysis was performed using PROC UNIVERATE, PROC GLM and PROC MEANS from SAS version 9.3 (SAS Institute, Cary, NC, 2002). All experimental data (except water quality parameter data) were also analysed statistically using the SPSS version 22 statistical software. All data were analysed using Levene test for homogeneity of variance and Shapiro Wilk's test for normality. One-way ANOVA followed by Tukey's test was used to assess the significance of each parameter among different treatments with a significance level of $P < 0.05$ for the separation of means.

3. Results

3.1. Growth parameters

Body weight gain of fish from BFT units with salinities ranging from 5 to 20 ppt were significantly higher ($P < 0.05$) compared to control (0 ppt) and it increased with increase in salinity (Table 1). The highest growth in terms of percentage body weight was recorded in T4 (329.89 ± 13.41). SGR (1.65 ± 0.05) and PER (1.18 ± 0.07) were recorded to be the highest in T4, while FCR (0.93 ± 0.06) was the lowest.

3.2. Water quality parameter and floc volume

Various water quality parameters and their ranges recorded were; temperature (27.71 ± 0.25 to 27.5 ± 0.34 °C), pH (7.71 ± 0.09 to 7.54 ± 0.09), dissolved oxygen (6.85 ± 0.15 to 6.7 ± 0.12 mg L^{-1}), TAN (0.44 ± 0.04 to 0.23 ± 0.03 mg L^{-1}), nitrite-N (0.47 ± 0.05 to 0.37 ± 0.02 mg L^{-1}), nitrate-N (4.28 ± 0.13 to 2.69 ± 0.10 mg L^{-1}), total

Table 1
Growth parameters of red tilapia in different treatment groups during the experimental period.

Treatments	C (0 ppt)	T1 (5 ppt)	T2 (10 ppt)	T3 (15 ppt)	T4 (20 ppt)
PWG	267.86 ± 9.75^{c}	277.39 ± 9.81^{bc}	314.35 ± 11.98^{ab}	321.27 ± 10.57^{a}	329.89 ± 13.41^{a}
SGR	1.32 ± 0.07^{b}	1.51 ± 0.06^{ab}	1.33 ± 0.06^{b}	1.43 ± 0.06^{ab}	1.65 ± 0.05^{a}
FCR	1.38 ± 0.10^{a}	1.16 ± 0.08^{ab}	1.40 ± 0.10^{a}	1.27 ± 0.10^{ab}	0.93 ± 0.06^{b}
PER	0.84 ± 0.06^{b}	0.96 ± 0.06^{ab}	0.83 ± 0.06^{b}	0.93 ± 0.07^{ab}	1.18 ± 0.07^{a}
Survival	94.07 ± 1.92^{a}	94.07 ± 1.96^{a}	90.37 ± 1.96^{b}	93.33 ± 1.28^{ab}	95.56 ± 1.28^{a}

Different superscript letters within a row designate statistically consequential differences ($P < 0.05$) between the treatments. Data are presented as mean ± SE (range). PWG- Percentage Weight Gain, SGR – Specific Growth Rate, FCR – Feed Conversion Ratio, PER – Protein Efficiency Ratio.

alkalinity (163.67 ± 1.76 to 82.93 ± 5.32 mg L^{-1}) and total hardness (3773.93 ± 110.76 to 460.37 ± 34.42 mg L^{-1}) (Table 2).

The floc volume (mL L^{-1}) had increased with increase in culture days and was significantly different ($p < 0.05$) among the treatments throughout the trial period. At the end of the experiment, the highest floc volume was recorded in control (28.29 ± 0.27) and the lowest floc volume was recorded in T4 (26.68 ± 0.17) (Table 2).

3.3. Proximate composition of biofloc

Proximate composition of biofloc is presented in Table 3. Moisture content was the highest (77.48 ± 0.50) in control (0 ppt) and the least (72.45 ± 0.24) was in T4 (20 ppt). Dry matter content (27.55 ± 0.24), carbohydrate content (3.02 ± 0.06) and ash content (27.41 ± 0.29) were highest in T4 (20 ppt), and were significantly lower than that of control (0 ppt). The highest level of crude protein (29.63 ± 0.35) and ether extract (3.30 ± 0.12) were in control (0 ppt) and the lowest values for the same were in T4 (20 ppt).

3.4. Biochemical indices

Serum glucose (mg dL^{-1}) level was significantly different ($p < 0.05$) among various treatment groups (Table 4). The highest serum glucose level was observed in T4 (20 ppt) group (310.59 ± 2.68), whereas the lowest was in control (210.27 ± 4.45). Total serum protein (mg dL^{-1}) (4.23 ± 0.04), serum albumin (mg dL^{-1}) (2.08 ± 0.03), serum globulin (mg dL^{-1}) (2.16 ± 0.04) levels were also the highest in T4 (20 ppt) and the least in C (0 ppt).

Antioxidant enzyme, SOD activities (7.01 ± 0.67) were highest in control (0 ppt), whereas lowest value (5.51 ± 0.34) was observed in T4 (20 ppt) (Table 4). Catalase (CAT) activity expressed the highest value (15.20 ± 0.55) in T1 (5 ppt) and the least (6.76 ± 0.49) in T4 (20 ppt).

3.5. Carcass quality of red tilapia

The highest moisture content (83.02 ± 0.06) was found in low salinity groups T1 (5 ppt) followed by control (0 ppt). Significantly higher value of dry matter (24.69 ± 0.70), crude protein (15.49 ± 0.80) and ash content (3.31 ± 0.10) were observed in T4 (20 ppt) compared to the control group (Table 5). The carbohydrate value was highest in T2 and T3 (15 ppt) followed by group T1 and T4. The ether extract value was highest in T4 (20 ppt) with significant difference ($P < 0.05$) compared to the control group. Texture analysis indicated highest firmness in T4 (121.45 ± 1.91) and lowest firmness (100.92 ± 2.31) in control (Table 5).

Colour analysis indicated lowest redness/greenness (a) (6.04 ± 0.20) in T3 fishes exposed to 15 ppt salinity, whereas T1 (5 ppt) was having highest redness/greenness (8.49 ± 0.85) (Table 6). T4 (20 ppt) varied significantly ($P < 0.05$) with the control groups (0 ppt) which had registered highest lightness (L) (50.17 ± 1.10), yellow/ blueness (b) (6.89 ± 1.34) and whiteness values (49.19 ± 0.91).

The sensory attributes were assessed viz. appearance, texture, odour, muddy odour, muddy flavour, flavour, colour and overall acceptability by the panelists (Table 7) for raw and cooked fish at the end of the experiment. Only the texture and overall acceptability of the cooked fish showed significant difference ($P < 0.05$) among treatments. Texture scores (8.10 ± 0.20) for the cooked fish of T3 (15 ppt) were significantly higher ($P < 0.05$) than the other treatment groups. The score for overall acceptability in control (8.00 ± 0.30) was significantly higher ($P < 0.05$) than that of T3 (15 ppt) and T4 (20 ppt), but was not different from that

Table 2
Physicochemical parameter of water in different treatment groups during the experimental period.

Treatments	C (0 ppt)	T1 (5 ppt)	T2 (10 ppt)	T3 (15 ppt)	T4 (20 ppt)
DO (mg L^{-1})	6.85 ± 0.15	6.76 ± 0.22	6.82 ± 0.12	6.78 ± 0.06	6.7 ± 0.12
Temperature (°C)	27.51 ± 0.22	27.52 ± 0.22	27.67 ± 0.19	27.5 ± 0.34	27.71 ± 0.25
pH	7.54 ± 0.09	7.61 ± 0.08	7.71 ± 0.09	7.66 ± 0.05	7.66 ± 0.09
TAN (mg L^{-1})	0.23 ± 0.03	0.32 ± 0.03	0.31 ± 0.03	0.38 ± 0.03	0.44 ± 0.04
Nitrite- N (mg L^{-1})	0.37 ± 0.02	0.43 ± 0.06	0.37 ± 0.05	0.42 ± 0.06	0.47 ± 0.05
Nitrate-N (mg L^{-1})	2.69 ± 0.10	3.05 ± 0.16	3.61 ± 0.10	3.85 ± 0.15	4.28 ± 0.13
Alkalinity (mg L^{-1})	82.93 ± 5.32	106.7 ± 1.63	105.04 ± 4.33	146.33 ± 3.06	163.67 ± 1.76
Hardness (mg L^{-1})	460.37 ± 34.42	2467.41 ± 123.94	2955.26 ± 144.20	3350 ± 66.38	3773.93 ± 110.76
Floc Volume (mg L^{-1})	28.29 ± 0.27	27.86 ± 0.15	27.67 ± 0.30	27.49 ± 0.35	26.68 ± 0.17

Different superscript letters within a row designate statistically consequential differences ($P < 0.05$) between treatments. Data are presented as mean ± SE (range).

Table 3
Proximate composition of biofloc of different salinity.

Treatments	C (0 ppt)	T1 (5 ppt)	T2 (10 ppt)	T3 (15 ppt)	T4 (20 ppt)
Moisture	77.48 ± 0.50^{a}	76.41 ± 0.80^{a}	75.77 ± 0.78^{ab}	72.84 ± 0.52^{b}	72.45 ± 0.24^{b}
Dry Matter	22.52 ± 0.50^{b}	23.59 ± 0.81^{b}	24.23 ± 0.72^{ab}	27.16 ± 0.50^{a}	27.55 ± 0.24^{a}
Protein	29.63 ± 0.35^{a}	28.96 ± 0.34^{a}	28.46 ± 0.56^{a}	26.95 ± 0.89^{ab}	25.01 ± 0.60^{b}
Carbohydrate	1.63 ± 0.02^{d}	2.16 ± 0.04^{c}	2.15 ± 0.03^{c}	2.49 ± 0.02^{b}	3.02 ± 0.06^{a}
Ether extract	3.30 ± 0.12^{a}	3.23 ± 0.12^{a}	3.07 ± 0.20^{a}	2.97 ± 0.09^{ab}	2.37 ± 0.09^{b}
Ash	19.97 ± 0.08^{c}	20.41 ± 0.29^{c}	22.43 ± 0.43^{b}	27.18 ± 0.36^{a}	27.41 ± 0.29^{a}

Different superscript letters within a row designate statistically consequential differences ($P < 0.05$) between treatments. Data are presented as mean ± SE (range).

Table 4
Biochemical indices of red tilapia reared in different treatment groups.

Treatments	C (0 ppt)	T1 (5 ppt)	T2 (10 ppt)	T3 (15 ppt)	T4 (20 ppt)
Serum glucose (mg dL^{-1})	210.27 ± 4.45^{b}	293.44 ± 3.23^{ab}	294.07 ± 4.40^{ab}	301.66 ± 5.31^{a}	310.59 ± 2.68^{a}
Total serum protein (mg dL^{-1})	2.86 ± 0.02^{d}	3.58 ± 0.01^{c}	3.67 ± 0.01^{c}	4.04 ± 0.05^{b}	4.23 ± 0.04^{a}
Serum albumin (mg dL^{-1})	1.28 ± 0.02^{d}	1.50 ± 0.01^{c}	1.61 ± 0.01^{c}	1.90 ± 0.02^{b}	2.08 ± 0.03^{a}
Serum globulin (mg dL^{-1})	1.58 ± 0.02^{b}	2.09 ± 0.02^{ab}	2.07 ± 0.01^{ab}	2.15 ± 0.03^{a}	2.16 ± 0.04^{a}
SOD (Units mg $protein^{-1}$)	7.01 ± 0.67^{a}	6.50 ± 0.34^{a}	6.69 ± 0.37^{a}	5.60 ± 0.35^{a}	5.51 ± 0.34^{a}
Catalase (Units mg $protein^{-1}$)	13.62 ± 0.74^{a}	15.2 ± 0.55^{a}	10.45 ± 0.58^{ab}	6.97 ± 0.81^{c}	6.76 ± 0.49^{c}

Different superscript letters within a row designate statistically consequential differences ($P < 0.05$) between treatments. Data are presented as mean ± SE (range).

Table 5
Proximate composition and texture analysis of experimental fishes (% dry weight).

Treatments	C (0 ppt)	T1 (5 ppt)	T2 (10 ppt)	T3 (15 ppt)	T4 (20 ppt)
Moisture	81.67 ± 0.60^{a}	83.02 ± 0.60^{a}	77.94 ± 0.80^{b}	75.39 ± 0.70^{c}	75.31 ± 0.71^{c}
Dry matter	18.33 ± 0.65^{c}	16.98 ± 0.60^{c}	22.06 ± 0.82^{b}	24.61 ± 0.75^{a}	24.69 ± 0.70^{a}
Protein	8.37 ± 0.66^{c}	7.39 ± 0.26^{c}	13.62 ± 0.33^{b}	15.23 ± 0.92^{ab}	15.49 ± 0.80^{a}
Carbohydrate	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}	0.04 ± 0.02^{a}	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}
Ether extract	2.23 ± 0.06^{c}	2.47 ± 0.12^{c}	2.44 ± 0.09^{c}	3.09 ± 0.13^{b}	3.91 ± 0.06^{a}
Ash	2.31 ± 0.03^{c}	2.09 ± 0.04^{c}	2.77 ± 0.15^{b}	3.06 ± 0.20^{ab}	3.31 ± 0.10^{a}
Firmness	100.92 ± 2.31^{c}	102.01 ± 1.89^{c}	106.66 ± 0.95^{b}	108.24 ± 1.72^{ab}	121.45 ± 1.91^{a}

Different superscript letters within a row designate statistically consequential differences ($P < 0.05$) between treatments. Data are presented as mean ± SE (range).

Table 6
Colour values of red tilapia fillet of different treatment groups.

Colour values	C (0 ppt)	T1 (5 ppt)	T2 (10 ppt)	T3 (15 ppt)	T4 (20 ppt)
L value	45.20 ± 1.10^{b}	47.20 ± 0.61^{ab}	47.68 ± 0.98^{ab}	48.59 ± 0.36^{a}	50.17 ± 1.10^{a}
a value	8.18 ± 0.81^{a}	8.49 ± 0.85^{a}	8.36 ± 0.40^{a}	6.04 ± 0.20^{b}	6.72 ± 0.53^{ab}
b value	5.6 ± 0.96^{b}	5.27 ± 0.78^{bc}	6.61 ± 0.98^{a}	4.61 ± 0.23^{c}	6.89 ± 1.34^{a}
Whiteness	44.28 ± 1.25^{b}	46.23 ± 0.40^{ab}	46.44 ± 0.94^{ab}	48.03 ± 0.34^{a}	49.19 ± 0.91^{a}

Different superscript letters within a row designate statistically consequential differences ($P < 0.05$) between treatments. Data are presented as mean ± SE (range). L value = lightness; a value = red/greenness; b value = yellow/ blueness.

of T2 (10 ppt) and T1 (5 ppt) for cooked fillet. The sensory scores of appearance, odour, muddy odour, muddy flavour, flavour and colour of the cooked fish were not significantly different ($P < 0.05$) among all the treatments. In the case of raw fish, highest scores for appearance, texture and overall acceptability were reported in the T4 (20 ppt) as 7.90 ± 0.18, 7.90 ± 0.10 and 7.90 ± 0.23 respectively. There were no significant difference ($P < 0.05$) among different treatments in odour, muddy odour, muddy flavour, flavour and colour. The sensory scores of all the samples were within the acceptable range.

4. Discussion

Better growth and survival of red tilapia obtained in higher salinity of 20 ppt in our study suggests that the biofloc system of higher salinity would be optimum, if the water quality is maintained for growth of this euryhaline species. BFT reared fish recorded 95% survival similar to the earlier report by Luo et al. (2017). It can be attributed to the activity of heterotrophic microbial population flocculated in the culture system which will effectively break down organic matter, nitrogenous compounds and enhance the water quality to provide a healthier habitat for fish (Wang et al., 2019; Effendi, 2017). The water quality ranges recorded in this study were within the recommended safety limits for red tilapia, as described by Putra et al. (2019) and it is also proved for sustainable management of aquaculture waste.

The lowest FCR was obtained in higher salinity group compared to the other biofloc units in our experiment. Biofloc can influence digestive enzymes and the intestinal microflora, which gives good growth performance as mentioned by Long et al. (2015). Low FCR, high SGR, PER and survival of the red tilapia reared in higher salinity (20 ppt) can be credited to the quality of microbial biofloc aggregates that served as the natural food and physiological wellbeing due to the conducive salinity in our study. Enhanced nutrient utilization, feed efficiency and water quality management using BFT was reported by Ekasari and Maryam (2012) in red tilapia, Anand et al. (2014) in *P. monodon*, Ahmad et al. (2016) in *Labeo rohita*, Haridas et al. (2017) in GIFT strain of tilapia, etc. Nutrient utilization, innate immunity, and biochemical composition are directly linked to the microbial aggregates and their ability to produce and store various bioactive compounds such as carotenoids and fat-soluble vitamins (Ju et al., 2008a) and other immunostimulatory compounds (Crab et al., 2012) in BFT.

At the end of the culture period, the highest growth and the lowest whole body protein were observed in 20 ppt unit. This result could be due to utilization of more energy for growth as the salinity is suitable for proper physiological metabolism of this euryhaline species. The percentage of crude protein and lipid content in fish appeared to decline with rising salinity. The content of dry matter and ash were positively correlated with a rise in salinity. Ju et al. (2008b) and Maica et al. (2012) have also stated to have similar outcomes. The microbial population in BFT are subjected to similar physical conditions of activated sludge treatment such as availability of nutrients, temperature, salinity, energy intake, chemical oxygen demand and oxygen content (Yan and Subramanian, 2007; De Schryver and Verstraete, 2009; Luo et al., 2017). The lipid content of biofloc varied from 2.37 to 3.30% and the highest value in 20 ppt treatment indicates that salinity has influence on lipid metabolism and ability to accumulate lipid by microbial community of BFT. The ash content indicates the ability of the microbial organisms to accumulate minerals and trace elements during their growth and flocculation which might be further influenced by the salinity of the environment as indicated in our study, which is also reported by Tacon et al. (2002).

Among biochemical indices, serum glucose level increased with increase in salinity, as high salinity caused higher energy requirements for various osmoregulatory organs, which was reported in other euryhaline fish species too (Sangiao-Alvarellos et al., 2003; Sangiao-Alvarellos et al., 2005). A strong innate immune status is associated with increasing levels of proteins, albumin and globulin, which are the main proteins in plasma (Rao et al., 2006). In our study, serum glucose level had increased in higher salinity based BFT, which can be attributed to enhanced immune status of this euryhaline fish in those treatments. The total protein was also reported to be linked to a stronger innate immune response in fish (Bakhshi et al., 2018). The study further attributes that salinity has a positive impact on the innate immune status of red tilapia which will make them less susceptible to diseases, when reared in 20 ppt salinity ISGW conditions.

SOD and catalase are enzymes linked to the prevention of lipid peroxidation. SOD catalyses the superoxide anion to form hydrogen peroxide (Tao et al., 2013), which in turn is broken down into water and oxygen by catalase, which prevents the onset of lipid peroxidation (Tavares-Sánchez et al., 2004). Recent studies have shown that changes in salinity can often cause oxidative stress, which affects antioxidant defences. The change in salinity can be toxic due to induction of oxidative stress as it promotes the creation of reactive oxygen species (ROS) and damage to cellular components, known as “oxidative stress” (Liu et al., 2007). The SOD and CAT are the two main radical scavenging enzymes involved in protective mechanisms in tissue damage after the oxidative process and phagocytosis. Their activities are linked to

Table 7
Changes in sensory score of Raw and cooked tilapia reared in different treatment groups.

Sensory attributes	Raw tilapia					Cooked tilapia				
	C(0 ppt)	T1(5ppt)	T2(10ppt)	T3(15ppt)	T4(20ppt)	C(0 ppt)	T1(5ppt)	T2(10ppt)	T3(15ppt)	T4(20ppt)
Appearance	7.45 ± 0.16^{b}	7.50 ± 0.17^{b}	7.60 ± 0.16^{ab}	7.56 ± 0.18^{a}	7.90 ± 0.18^{a}	8.00 ± 0.16^{a}	8.09 ± 0.19^{a}	8.22 ± 0.20^{a}	7.90 ± 0.18^{a}	7.40 ± 0.21^{a}
Texture	7.60 ± 0.18^{ab}	7.55 ± 0.21^{b}	7.67 ± 0.17^{ab}	7.90 ± 0.23^{a}	7.90 ± 0.10^{a}	7.90 ± 0.21^{ab}	7.45 ± 0.16^{b}	7.89 ± 0.20^{ab}	8.10 ± 0.20^{a}	7.30 ± 0.18^{b}
Odour	8.80 ± 0.12^{a}	8.20 ± 0.20^{a}	8.10 ± 0.20^{a}	8.20 ± 0.25^{a}	8.00 ± 0.24^{a}	8.00 ± 0.16^{a}	7.50 ± 0.19^{a}	7.78 ± 0.15^{a}	7.70 ± 0.20^{a}	7.45 ± 0.16^{a}
Muddy Odour	8.40 ± 0.16^{a}	8.20 ± 0.20^{a}	7.90 ± 0.18^{a}	7.89 ± 0.20^{a}	7.73 ± 0.19^{a}	8.20 ± 0.21^{a}	8.00 ± 0.19^{a}	8.11 ± 0.20^{a}	7.90 ± 0.23^{a}	7.90 ± 0.21^{a}
Muddy Flavour	8.20 ± 0.20^{a}	8.20 ± 0.20^{a}	7.45 ± 0.23^{a}	7.89 ± 0.20^{a}	7.90 ± 0.21^{a}	8.30 ± 0.26^{a}	8.27 ± 0.14^{a}	8.22 ± 0.15^{a}	7.90 ± 0.28^{a}	8.00 ± 0.21^{a}
Flavour	7.70 ± 0.21^{a}	8.00 ± 0.19^{a}	7.89 ± 0.20^{a}	8.00 ± 0.21^{a}	7.90 ± 0.23^{a}	7.40 ± 0.19^{a}	7.54 ± 0.18^{a}	8.33 ± 0.26^{a}	7.70 ± 0.17^{a}	8.10 ± 0.30^{a}
Colour	8.20 ± 0.19^{a}	8.10 ± 0.16^{a}	8.00 ± 0.24^{a}	8.00 ± 0.21^{a}	8.00 ± 0.20^{a}	8.20 ± 0.26^{a}	8.00 ± 0.19^{a}	8.11 ± 0.18^{a}	7.60 ± 0.16^{a}	7.60 ± 0.27^{a}
Overall Acceptability	7.45 ± 0.16^{b}	7.56 ± 0.18^{b}	7.60 ± 0.16^{ab}	7.50 ± 0.17^{b}	7.90 ± 0.18^{a}	8.00 ± 0.30^{a}	7.91 ± 0.21^{ab}	7.89 ± 0.26^{ab}	7.80 ± 0.25^{b}	7.70 ± 0.23^{b}

Different superscript letters within a row and column designate statistically consequential differences ($P < 0.05$) between treatments. Data are presented as mean ± SE (range).

different factors, including diet, environmental factors, etc., that affect the status of organisms (Winston and Giulio, 1991). In general, higher SOD and CAT activities indicate that more radicals need to be converted (Chien et al., 2003). Therefore, significantly higher SOD and CAT activities in fish at lower salinity could indicate that stress at low salinity which led to build-up of radicals at higher levels in red tilapia. If these radicals were not trapped, organisms would suffer severe oxidative damage (Winston and Giulio, 1991). Therefore, the increased activities of SOD and CAT at lower salinity in the present study can allow them to maintain health by removing the radicals produced. It can be attributed further that the BFT system induce certain stress to the organisms due to higher density and zero water discharge conditions which can be further influenced by the salinity of the culture environment. Similar result was reported in *Litopenaeus vannamei* by Li et al. (2008).

Various factors such as seasonal, environmental and biological fluctuations influence the body composition of fish (Desai and Singh, 2009; Tao et al., 2012). The higher levels of dry matter, crude protein, ether extract, carbohydrates and ash content of red tilapia in this experiment were recorded from salinity based BFT units compared to freshwater BFT unit . It was reported in many species that environmental factors like salinity affect the carcass composition (Fallah et al., 2013; Jalali et al., 2013; Kumar et al., 2016). The moisture content in 0 ppt biofloc control units was recorded to be the highest compared to other salinity units. The higher moisture content with higher salinity in the present study may be correlated with the fact that fish drink more water to compensate for dehydration. Similar results have been reported by Xu et al. (2010); Barman et al. (2005) and Jalali et al. (2013). We know that the lipid content of fish is inversely proportional to the water content (Shearer, 1994). Ohta and Watanabe (1996) also reported that as the salinity increased, the percentage of lipid declined which is consistent with the results of this study. The reduction in lipid levels of fishes reared in higher salinity level indicated the higher energy expenditure in maintaining osmoregulation. Jarvis and Ballantyne (2003) reported that lipids in short nose sturgeon decreased with increasing salinity during salinity acclimatization, which indicated the effective use of lipids in osmoregulation. In this experiment, the crude protein content of the biofloc ranged from 25 to 29%. Some authors say that 25–30% crude protein in diets is appropriate for tilapia growth (Chou and Shiau, 1996; Jauncey, 2000), which suggests that in our experiment, the crude protein level of biofloc was appropriate for supplementing protein nutrition for fishes. In the present study, it was found that the ash content of the fish was higher with an increase in salinity, which could be due to the deposition of additional ions in saline water as reported by Jalali et al. (2013). Further, the deposition of beneficial ions will be adding value to the final product from culture and need to be further researched to understand the ionic composition of carcass and the beneficial ions out of them.

Freshness is one of the most important aspects to assess the quality of farmed fish, as freshness is directly related to the texture, appearance and taste of the consumer's perception. Texture concerns the sensory interpretation and expression of the structure or internal construction of products, which are related to their stress response and haptic properties (Coppes et al., 2002; Lepetit, 2007). Colour being a powerful feature of appearance, can cognitively alter the perception of smell and taste, although not visualizing food does not eliminate the perception and classification of smell and taste (Delwiche, 2004). In texture analysis, firmness means the force necessary to deform the fish fillet between the tongue and the palate. In this experiment, the firmness of fish fillet increased with increase in salinity. The carcass from 20 ppt salinity groups were having the highest value of firmness, indicating the presence of muscle fat in raw red tilapia fillet, diluting and lubricating the structural elements of the muscle and reducing fillet firmness as reported by Aussanasuwannakul et al. (2011) and Aussanasuwannakul et al. (2012).

Raw fish fillets were firmer than cooked one as also reported by Aussanasuwannakul et al. (2012). Stress also had an impact on the

firmness of the fillet because the stressed fish had a softer texture, for example, handling stress had an influential effect on the fillet firmness (Sigholt et al., 2006; Cheng et al., 2013). The finding of our study can be correlated to the fact that possible stress amelioration in higher salinity group might have led to the firmness of the muscle. The physiological adaptation of these fishes when reared in inland saline BFT, might have contributed to the measures to counteract stress from zero water discharge system with higher suspended solids. This effect can be further correlated with the lower activity of antioxidant defence enzymes like catalase and SOD.

The colour analysis is normally used as supporting information to determine the effect of biofloc of different salinity on the quality of the raw fish fillet in the sensory analysis. In the present study, lightness (L* value), yellowness (b* value) and whiteness increased with increasing salinity. The redness (a *) was significantly higher in the 0 ppt biofloc group. Previous research has shown that biofloc contains various bioactive compounds, including carotenoids, PHB, phytosterols, bromophenols, and amino sugars (De Schryver et al., 2008; Ju et al., 2008b). Carotenoids have been reported to provide essential nutrients and many bioactive physiological functions in animal tissues, including stimulation of the immune system of animals, increasing stress tolerance and growth (Zhao et al., 2012; Linan-Cabello et al., 2002). Carotenoids improve the quality of edible fish by improving muscle colour and pigmentation (Chavarria and Maurilio, 2013). The present study pointed out to the fact that the microbial communities in fresh water accumulate more carotenoid compounds compared to saline water. The science behind this process needs to be thoroughly studied by evaluation of the structure of microbial community and their functional metabolism.

The sensory properties according to the participants were peculiar and showed the lower influence of the higher salinity as studied by Silva and Piana (2020) in the case of the cooked fillet. Similar type of sensory analysis of the uncooked and cooked black tilapia fillets was reported by Mohsin et al. (1999). The highest scores obtained for texture, appearance and overall acceptability of raw tilapia fillet from 10 to 20 ppt salinity reared group indicated that the salinity influenced the basic physiology of this euryhaline species, which can be correlated with the other biochemical indices like firmness, proximate composition etc. Muddy odour, muddy flavour and colour did not impart any visual variation as evidenced through sensory attributes lead to the fact that biofloc system does not create any off flavour or odour with respect to salinity. The heterotrophic bacteria developed in the respective salinities does not impart any changes in the sensory qualities of the fish and can be considered as the positive impact of biofloc system in case of both raw and cooked condition. In the case of cooked fillet, no visible trend was observed as per the variable salinities for appearance and texture upto 15 ppt. But texture and appearance obtained significantly lower score for 20 ppt reared group. In addition, the overall acceptability was also significantly lower in the 15 and 20 ppt group. The lowest scores in appearance, texture and acceptability of cooked tilapia fillet can be attributed to influence of salinity on cooking quality of fish. It will be much early to come to a conclusion in this regard, as the fish were smaller in size and were utilizing much of their energy for osmoregulation than muscular development, which can further be correlated with the proximate composition of the fish. The study can be considered as a preliminary observation about the same for biofloc reared fish in variable salinity and further investigation on table sized fish is required to draw a valuable conclusion. This result of all the organoleptic properties of raw and cooked red tilapia fillets shows that as the salinity of biofloc increases, various organoleptic qualities which determine the acceptance also increase. Further it can be attributed that as salinity increases, the muddy flavour of red tilapia fillet decreases. Green and Schrader (2015) reported that the incidence of geosmin- and 2-methylisoborneol-related off-flavour is low in the BFT production system.

Geosmin and 2-methylisoborneol (MIB) are primarily responsible for the “earthy” and “musty” off-flavours, respectively detected in fish. BFT system is reported to be having positive impact on muddy odour reduction of cultured fishes. The channel catfish reared in the BFT production system with reduced frequency of off-flavour compounds is reduced by BFT (Green et al., 2014) and in our study it is further delineated that the BFT system maintained at higher salinity has positive influence on the organoleptic qualities. This can be further correlated to the fact that bacterial community in BFT may differ at varying salinities and the metabolic activities of flocculating bacteria will be influencing the carcass quality of the cultured fish. Earlier reports indicated that bioflocculating organisms accumulate vitamins, minerals, lipids, carotenoids and presence of these metabolites and bioactive compounds may vary according to the bacterial communities present in BFT system (Emerenciano et al., 2017). From a nutritional point of view, biomass of flocs can provide a source of food as well as various bioactive compounds (Farooqi and Qureshi, 2018; Akiyama et al., 1992). So, the variation in the carcass quality especially the sensory attributes can lead to the fact that the red tilapia gives better quality finished product, when reared in the physiologically optimum salinity rather than culturing them in freshwater. BFT culture system converts nitrogenous metabolites into microbial protein which in turn also serve as fish food is also influenced by the rearing salinity. The current observation is opening up an avenue for in depth studies on the microbial community and their contribution to organoleptic qualities and other physiological functions of the species under culture.

5. Conclusion

The study concluded with the fact that BFT with increasing salinity can enhance the productivity of inland saline water through zero-water exchange mode. The technology is proved to enhance growth, biochemical enzymes and carcass quality of red tilapia reared in inland saline waters. The technology also indicated higher salinity biofloc has stress mitigation effect, maintains the appearance, texture and overall acceptability of red tilapia using environmental manipulation through conversion and assimilation of nitrogenous waste from the system. So, it provides a sustainable method of red tilapia farming in inland saline areas.

Authors statement

Sl. No.	Name of Authors	Contribution of authors
1	SANGEETA KUMARI	Execution of experiment, analysis and manuscript writing (Student author)
2	V. HARIKRISHNA	Overall monitoring of experiment
3	V. K. R. SURASANI	Lab analysis and result interpretation of the experiment related to Carcass quality and biochemical analysis
4	A. K. BALANGE	Experiment monitoring and interpretation of results.
5	BABITHA RANI A.M	Conceived the study, experimental planning and designing, interpretation of data and statistical analyses, Ph.D. advisor (major) of first author

Declaration of Competing Interest

Authors are not having any conflict of interest with this manuscript.

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