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Influence of *in situ* Biofloc Production on Bio Growth Performance, Physiological Immune Response, Digestive Enzyme Activity, Nutrient Composition and Disease Resistance of *Etroplus suratensis*

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ABSTRACT

Backgroud: The present experiment was conducted to investigate the effects of *in situ* jaggery based biofloc on bio growth performance, physiological immune response, digestive enzyme activity, nutrient composition and disease resistance of *Etroplus suratensis*. **Methods:** The fingerlings of pearlspot, *E. suratensis* with an average body weight of 5.67±0.08 g was stocked at the rate of 150/m^3 in treatment and control system. The *insitu* biofloc was produced in 2.4-ton capacity outdoor tanks using jaggery as carbon source at two different C:N ratio *viz,* 10: 1 (B1) and 20:1 (B2), where clear brackishwater system and without carbon sources as control. **Result:** The levels of ammonia nitrogen, nitrite-nitrogen and nitrate-nitrogen were reduced in the *in situ* biofloc compared to control while TSS and TS were increased significantly in all *in situ* biofloc compared to control. The physiological immune responses such as respiratory burst test, myeloperoxidase, serum lysozyme and antioxidant enzymes activity, superoxide dismutase and catalase were found to be significantly higher in B2 at end of the trial. Stress parameters glucose and cortisol were significantly lower in jaggery based biofloc system especially in (B2) C: N 20:1 compared to control. The fishes from the jaggery based biofloc groups possessed significantly ($P<0.05$) higher immune status as compared to control. The digestive enzymes activity such as amylase protease and lipase, of fish was higher in biofloc treatment and lower in control. Compared to control the nutrient composition of *E. suratensis* was significantly higher in the treatment groups. Moreover, jaggery based *in situ* fishes showed lower cumulative mortality rate and enhanced relative levels of protection after experimental challenge with *A. hydrophila* compared to control.

Key words: Biofloc, Disease resistance, *Etroplus suratensis, In situ* floc, Jaggery.

INTRODUCTION

Aquaculture has become one of the fastest-growing food producing sectors. In 2020, production from aquaculture reached 87.5 million tonnes and in that 66% of world fish production was consumed as food (FAO, 2022). The demand for aquatic food is increasing as it has proved to be an option to cope with the world food demand even though there are lot of criticisms arising in this sector due to overutilization of water resources, degradation of natural ecosystem, salinization and acidification of soils, environmental impacts such as eutrophication and nitrification due to effluents discarded into the natural water bodies. To overcome these bottlenecks, adoption of advanced culture methods with a sustainable intensification is needed for Aquaculture sector. The recent advanced "Biofloc Technology" could be a potential and sustainable alternative that can reduce environmental impacts with zero water exchange, less feed input while increasing stocking density and hence the production and crop yield. The external addition of carbon sources to the culture water stimulates the growth of heterotrophic bacteria and its uptake of nitrogen by the production of the microbial protein (Avnimelech, 1999) faster than regular nitrification process (Hargreaves, 2006). The carbon source will trigger the heterotrophic bacterial population by promoting the nitrogen uptake through the production of microbial protein and more rapidly decreases the ammonium concentration (Kasan *et al*., 2015). The selection of carbon source may depend on the accessibility and digestibility of carbohydrates, protein content and cost-effectiveness (Khanjani *et al*., 2017). However, few studies have been carried out on freshwater fishes like Tilapia (Azim *et al*., 2008; Ekasari *et al*., 2015; Long *et al*., 2015; Perez-Fuentes *et al*., 2013; Menaga *et al*., 2019; Elayaraja *et al*., 2020). Diversification

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of aquaculture species can be achieved by the exploitation of new cultured species that offers both biological and economic benefits. Pearlspot, *Etroplus suratensis*, known locally as 'Karimeen' is the largest among Indian cichlids. It is a high-valued food fish endemic to peninsular India and Sri Lanka (Munro, 1955). Keeping in view the above factors, the present study aimed to evaluate the jaggery based biofloc on the bio growth performance, physiological immune response, digestive enzyme activity, nutrient composition and disease resistance of *Etroplus suratensis* reared in *in situ* biofloc system.

MATERIALS AND METHODS

The experiment was conducted in the Pulicat Research Farm Facility, Pazhaverkadu, Ponneri. This study was approved by ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.

Experimental design

The experiment was conducted in the nine outdoor biofloc unit tanks (2.4-ton capacity) with completely randomized design (CRD). The advanced fingerlings of Pearlsport (*Etroplus suratensis*) were used for the experimental study were collected from Pulicat lake, Pazhaverkadu and disinfected with $KMnO_4$ and stocked in 30 ppt brackish water. The fishes were slowly acclimatized to 22 to 25 ppt by adding freshwater. Pearlspot fingerlings weighing 5.68 to 5.79±0.01 g was stocked at the rate of 150/m^3. The development of *in situ* Biofloc within the system and carbon addition was carried out based on the protocol described by Avnimelach 2009. Development of floc was triggered with continuous aeration with help of the air compressor. The tanks were left for 15 days to make it favorable for the growth of microbes to develop the bio floc. Strong aero tube aeration was made to keep floc in constant suspension. These biofloc production tanks were monitored regularly for two different ratios of C:N (10:1) B1 and (20:1) B2 maintained by adding jaggery on the rate of ammonia Nitrogen content of water. Clear brackish water system used for control (C). Fishes were fed with 3 to 5% of body weight. The feeding ration was divided into three equal quantities and given thrice a day *viz.*, 10.00 am, 2.00 pm and 5.00 pm, while the *in situ* biofloc B1and B2 fishes were fed to apparent satiety. Protocol for the development of *in situ* jaggery based Biofloc in the FRP tank were given in Table 1.

Growth parameters and survival

To calculate the bio growth parameters, including the beginning and final growth rates, the absolute and specific growth rates and the feed utilization factors (FER, FCR, PER, BWI and FCE), sampling was carried out every two weeks. The growth parameters in question were computed.

Water quality parameters

Water temperature and dissolved oxygen (DO) in each tank were measured three times a day using a digital thermometer and DO meter respectively. Weekly ammonium (NH_4^+), nitrite (NO_2^-), pH, conductivity, TDS, total alkalinity, total hardness, magnesium hardness and calcium hardness were analyzed as per (APHA, 2005) procedure.

Nutrient composition of *insitu* jaggery based biofloc reared *E. suratensis*

To determine the whole-body nutrient composition of Pearlspot fingerlings the fishes were cut into pieces, minced, homogenized and immediately frozen until further analysis and the proximate analysis was determined using (AOAC, 1995) method.

Digestive enzyme activity

Protease activity (Moore and Stein, 1948) and lipase activity (Cherry and Crandell, 1932) methods were largely modified to yield amylase activity (Rick and Stegbauer, 1974).

Physiological immuno parameters

The Superoxide Dismutase activity was estimated following the modified method of Mishra and Fridovich, (1978). Catalase activity was estimated according to the method of Takahara *et al*. (1960). The serum glucose and cortisol level in the serum using the Semi-automatic blood biochemical analyzer Alpha technologies instrument (Alpha chem. 100i). For the total Cholesterol analysis the serum and ferric chloride was taken in a test tube and incubated at room temperature for a period of 10 min and centrifuged at 3000 rpm and the OD was measured at 560 nm by adding sulphuric acid to the supernatant The total triglycerides were measured using an auto-analyser (MERCK Selectra, Germany).

Disease resistance against *Aeromonas hydrophilla*

At the end of *insitu* experimental days of culture, the experimental fishes were challenged with *Aeromonas hydrophila* pathogen obtained from State Referral Laboratory under TNJFU. The isolate grown in tryptic soy broth (TSB Hi Media) for 24 h (30-31°C) was centrifuged at 10000 rpm for 10 min followed by pellet resuspension in phosphate buffered saline (PBS, pH 7.2). The suspension in sterile PBS was injected intramuscularly (0.1 ml) in healthy *Etroplus suratensis* from all the treatments delivering 10^7 CFU/fish. Following the challenge, the fishes were observed for mortality every day for up to 14 days and the cumulative mortality was recorded. The relative level of protection (RLP) was calculated as:

$$\text{RLP (\%)} = \frac{1 - \text{Mortality (\%) of } \textit{in situ} \text{ biofloc reared group}}{\text{Mortality (\%) of control group}} \times 100$$

Data analysis

Statistical analysis of bio growth and other parameters were analysed by one-way analysis of variance (ANOVA) using SPSS, 20.0. Duncan's multiple range tests was used for post hoc comparison of mean ($P<0.05$) and statistical significance for the test was set at $P<0.05$ between different groups.

RESULTS AND DISCUSSION

Growth performances and feed utilization

Growth performances and feed utilization parameters of *E. suratensis* after 90 days of growth trial are given in Table 2. The final body weight of the *insitu* biofloc B1 and B2 fishes differed significantly with highest growth 53.26±0.18 and 57.90±0.06 respectively. The better feed utilization in terms of FCR (0.55±0.01), FER (1.83±0.01) and PER (1.93±0.01) were observed in (B2) compared to control and (B1). The *in situ* biofloc reared fishes obtained higher survival rate compared to control. The highest biomass was observed in B2 (8260.97±0.05 g) and the lowest biomass was observed in control (5630.08±0.05 g). The earlier studies reported that Biofloc technology (BFT) improves growth performance and feed utilization of cultured fish (Hari *et al*., 2004; Azim and Little, 2008; Kuhn *et al*., 2009; Ahmad *et al*., 2017; Luo *et al*., 2014; Zhang *et al*., 2016). Previous studies concluded that *insitu* floc in various carbon sources served as an incremental food which continuously provide additional protein (EAA), polyunsaturated fatty acids, vitamins and minerals (Avnimelech, 1999; Avnimelech, 2007; Azim and Little, 2008; De Schryver *et al*., 2008; Luo *et al*., 2014).

Table 1: Protocol for the development of *insitu* jaggery based biofloc in the FRP tank.

Day	Activity (Application in g/2.4 ton)
1	Urea - 38.4 g, TSP*-3.6 g, Grain pellet - 144 g, Dolomite - 240 g
2	Tea seed cake 15 ppm
4	Grain pellet - 144 g, dolomite - 240 g
6	Grain pellet - 144 g, dolomite - 240 g
8	Grain pellet - 240 g, Jaggery - 38.4 g
10	Grain pellet - 240 g
12	Tapioca - 240 g

*TSP= Triple super phosphate.

Water quality parameters in *in situ* biofloc system

Water quality parameters were measured throughout the *in situ* biofloc culture period as shown in Table 3. The temperature varied slightly throughout the *in situ* floc production system and control tank further it varied between 26.5-31°C. DO concentration in *insitu* biofloc tanks (B1 and B2) was within a range of 4.4-6.8 mg/l and did not show much variation throughout the experimental period, but it did fluctuate slighlty with increased biodiversity of floc associated organisms. While in control group, DO was in the range of 6.1-7.1 mg/l due to continuous exchange of water. In comparison to the control, the CO_2 detected in the *in situ* treatments was considerably ($P<0.01$) greater. Water pH was in the range of 8.3 to 8.5 in all *in situ* biofloc production tank and 7 to 8 in control tank. Significantly ($P<0.05$) higher pH was observed in B1 and B2 than control. During the culture period ammonia concentration was in range of 0.001 to 0.008 mg/l in *insitu* biofloc and 0.01 to 0.020 mg/l in control. Lower concentration of ammonia was observed in *in situ* biofloc than control. Nitrite- ×100 nitrogen concentration was significantly ($P<0.05$) lower in treatments than control. The results of the water quality parameters on biofloc development with the jaggery as a carbon source was agreed well with findings of (Sakkaravarthi *et al*., 2015; Ruby *et al*., 2022; Elaiyaraja *et al.,* 2020; Susitharan *et al.,* 2021).

Digestive enzyme activity

The digestive enzymes *viz,* amylase, protease and lipase activity were significantly affected by biofloc produced by *insitu* manner. The results of digestive enzyme analysis were given in Table 4. Biochemical composition of the diet plays an important role in the digestive enzyme profile of fish and shrimp. The specific activity of digestive enzymes was significantly ($P<0.05$) improved in the current study when compared to control B2 and B1. Treatment B2's increased digestive enzyme activity may have improved digestion and nutrition absorption, as evidenced by this group's much greater ($P<0.05$) growth rate. The study by Xu and Pan

Table 2: Biogrowth and feed utilization of *in situ* jaggery based biofloc reared *E. suratensis.*

Growth parameters	C	B1	B2
Mean initial weight (g)	5.72±0.03^a	5.59±0.06^a	5.70±0.01^a
Mean final weight (g)	47.17±0.14^a	53.26±0.18^b	57.90±0.06^c
Mean weight gain (g)	41.45±0.23^a	47.67±0.41^b	52.20±0.11^b
DGR (g/day)	0.52±0.01^a	0.59±0.001^b	0.64±0.01^c
SGR (%) / day	2.34±0.01^a	2.50±0.02^b	2.58±0.01^c
FCR	1.58±0.01^a	0.81±0.01^b	0.55±0.01^c
FER	0.63±0.01^a	1.24±0.01^b	1.83±0.01^c
PER	1.57±0.01^a	1.77±0.01^b	1.93±0.01^c
FCE	63.13±0.6^a	124.10±1.56^b	182.57±1.11^c
Survival %	89.33±0.77^a	92.44±0.58^b	95.1±0.80^c
Initial biomass	858.61±0.5^a	838.50±0.05^a	855.63±0.02^a
Final biomass	5630.08±0.05^a	7386.1±0.06^b	8260.9±0.05^c

Values in the same row with different superscript differ significantly ($p<0.05$) between the treatments.

(2012) in biofloc based system reported the similar results in *P. vannamei*. Growing performance may be enhanced by greater nutrient consumption brought about by higher digestive enzyme activity (Ezhilarasi *et al*., 2019). Similarly, an enhanced digestive enzyme activity was reported in *L. vannamei* (Xu and Pan, 2012), *P. monodon* (Anand *et al*., 2013).

Nutrient composition (% wet basis) of *Etroplus suratensis*

The whole-body nutrient composition of *E. suratensis* such as protein, lipid and ash are shown in Table 5. The highest crude protein in fish body was found in B2 (20.97±0.07) which differed significantly with C (15.43±0.27). The highest crude lipid recorded was (3.34±0.11) in B1, followed by B2 (3.21±0.05). Similar results with enriched nutritional value were also reported by Ray *et al*. (2011); Xu and Pan (2012) reported that differences in proximate composition may affect nutritional value, sensory qualities and shelf-life of the fish.

Physiological immuno parameters of *Etroplus suratensis*

In the present study, fishes reared in *insitu* biofloc treatments, the non-specific immune parameters NBT, serum lysozyme, myeloperoxidase (Fig 1,2,3) showed higher values as compared to control. Biofloc reduced the physiological stress in GIFT which agrees with the studies of Verma *et al*. (2016) who reported the reduced levels of Cortisol and Glucose (Table 5) in *Labeo rohita* when reared in biofloc systems. SOD and Catalase are two important enzymes in the cellular antioxidant defence system, dealing with oxidative stress. Lower levels of SOD and Catalase are indication for cell damage due to the accumulation of the high-level of free radical affecting the heath of fishes. The results from the present study revealed that the supplementation of *insitu* biofloc increased SOD (Fig 5) and catalase (Fig 4) level in *in situ Etroplus suratensis* than control. Similar studies were done by

Table 3: Water quality parameters during the culture period of 90 days.

Parameters	C	B1	B2
Temperature (°C)	26.24±0.17^{a}	28.81±1.22^{a}	28.6±0.11^{a}
DO (ppm)	6.4±0.92^{a}	5.71±0.17^{b}	5.9±0.05^{cb}
CO_2 (ppm)	3.7±0.13^{c}	5.86±0.35^{ba}	6.12±0.15^{a}
pH	7.7±0.2^{b}	8.54±0.07^{a}	8.2±0.28^{a}
Salinity (ppt)	23.50±1.02^{a}	23.50±1.02^{a}	23.56±1.05^{a}
Alkalinity (mg/L)	95.77±2.5^{a}	80.25±3.46^{b}	82.45±2.65 ^{b}
Hardness (mg/L)	253.3±4.5^{a}	259.2±3.6^{a}	284±4.13^{a}
Magnesium (mg/L)	72.04±2.8^{a}	65 ±2.62^{c}	73.56±3.12^{b}
Calcium (mg/L)	49.72±3.6^{c}	120.66 ±17.03^{b}	165.02±4.56^{a}
TSS (mg/l)	1331±2.1^{c}	1629.61±3.6^{b}	1742±8.1^{a}
TDS mg/l	112.62±2.8^{c}	718.95±11.38^{b}	766.51±19.89^{a}
Ammonia nitrogen (mg/L)	0.05±0.5^{b}	0.003±0.011^{a}	0.003±0.15^{a}
Nitrite N-NO_2 (mg/L)	0.016±0.0^{a}	0.008±0.0^{b}	0.005±0.23^{c}
Nitrate N-NO_3 (mg/L)	0.12±0.01^{b}	0.19±0.01^{a}	0.18±0.03^{a}

Values in the same row with different superscript differ significantly ($p<0.05$) between the treatments.

Table 4: Digestive enzyme activity of *E. suratensis* reared in jaggery based biofloc system.

Parameter	C	B1	B2
Amylase (U/mg protein/min)	0.022±0.01^{a}	0.024±0.02^{b}	0.029±0.01^{c}
Protease (U/mg protein/min)	0.131±0.02^{a}	0.177±0.33^{b}	0.197±0.01^{c}
Lipase (U/mg protein/min)	0.293±0.01^{a}	0.385±0.02^{b}	0.486±0.01^{c}
	Physiological immuno parameters of *Etroplus suratensis*		
Glucose (mg/dl)	108.58±1.5^{a}	82.36±0.59^{b}	80.61±0.31^{b}
Cortisol (mg/dl)	96.74±0.58^{a}	77.90±1.16^{b}	67.51±1.19^{c}
Total cholesterol (mg/dl)	143.70 ±0.83^{a}	127.42±0.31^{b}	120.0±0.92^{c}
Total glyceride (mg/dl)	196.08±1.17^{a}	220.86±0.37^{b}	246.56±2.12 ^{c}

Values in the same row with different superscript differ significantly ($p<0.05$) between the treatments.

Table 5: Nutrient composition (% Wet basis) of *Etroplus suratensis reared* in *in situ* biofloc system.

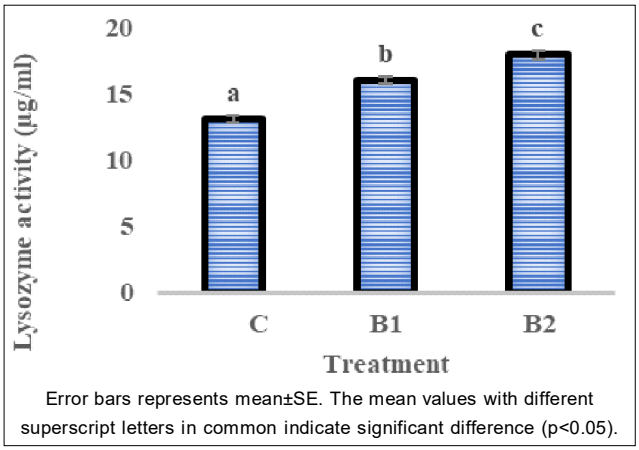
Treatment	Moisture %	Crude protein%	Crude lipid%	Total ash %
C	72.86±0.22^{a}	15.43±0.27^{a}	2.97±0.06^{a}	4.31±0.18^{a}
B1	72.29±0.60^{a}	18.15±0.28^{b}	3.34±0.11^{b}	2.94±0.36^{b}
B2	70.96±0.12^{b}	20.97±0.07^{c}	3.21±0.05 ^{ab}	3.15±0.11^{b}

Values in the same row with different superscript differ significantly ($p<0.05$) between the treatments.

Ruby *et al*. (2022); Menaga *et al*. (2019) and Elaiyaraja *et al*. (2020). Increased bacterial pathogen killing ability of phagocytes can be inferred from increased respiratory burst activity which is a most important bactericidal mechanism in fishes. Verma *et al*. (2016) observed an improvement in the immune parameters of Rohu fingerlings grown in tapioca-based biofloc. This could be connected to the fact that the culture animals' consumption of biofloc improves their nutrition and activates the fish's cellular defense mechanisms through phagocytosis and respiratory burst.

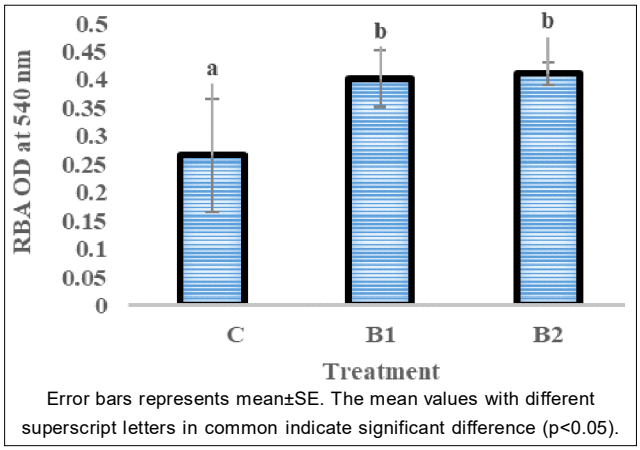
Disease resistance against *Aeromonas* infection of *Etroplus suratensis* reared in *in situ* biofloc system

In this study, fish injected with sterile saline (C-ve) showed no mortalities or pathological lesions, while those challenged with *A. hydrophila* displayed pathological alteration on the third day post infection. The cumulative



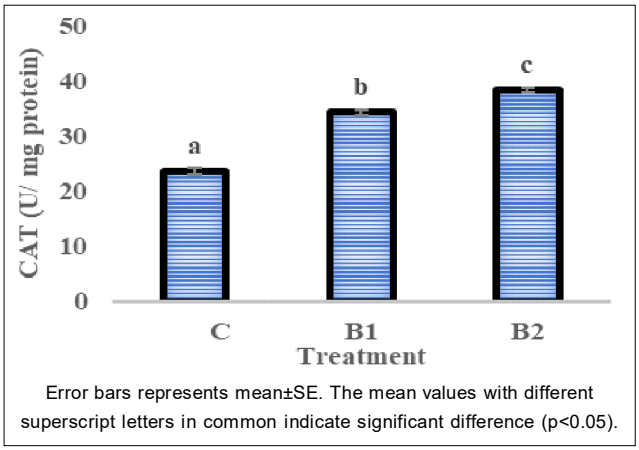
Error bars represents mean±SE. The mean values with different superscript letters in common indicate significant difference (p<0.05).

Fig 3: Lysozyme activity (µg/ml) of *E. suratensis* reared in *in situ* jaggery based biofloc system.



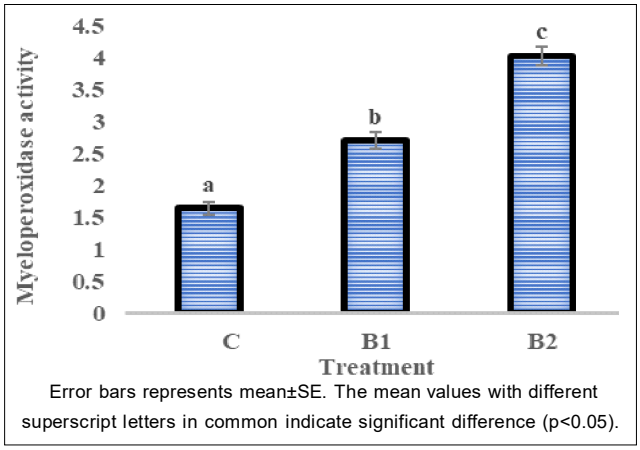
Error bars represents mean±SE. The mean values with different superscript letters in common indicate significant difference (p<0.05).

Fig 1: Respiratory burst activity (OD at 540 nm) of *E. suratensis* reared in *in situ* jaggery based biofloc system.



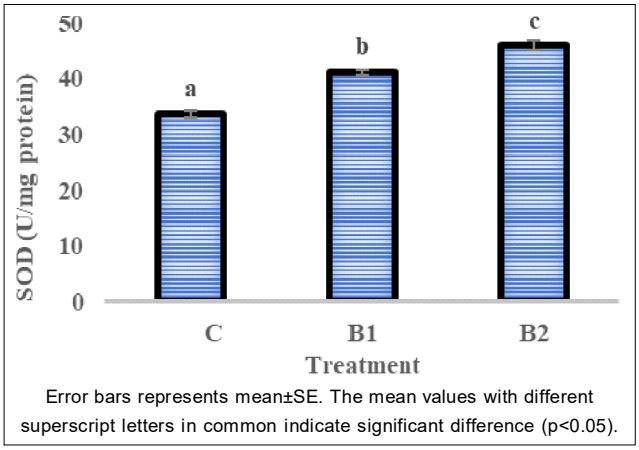
Error bars represents mean±SE. The mean values with different superscript letters in common indicate significant difference (p<0.05).

Fig 4: Catalyse (U/ mg protein) of *E. suratensis* reared in *in situ* jaggery based biofloc system.



Error bars represents mean±SE. The mean values with different superscript letters in common indicate significant difference (p<0.05).

Fig 2: Myeloperoxidase activity (OD at 640 nm) of *E. suratensis* reared in *insitu* jaggery based biofloc system.



Error bars represents mean±SE. The mean values with different superscript letters in common indicate significant difference (p<0.05).

Fig 5: Superoxide dismutase activity (U/ mg protein) of *E. suratensis* reared in *insitu* jaggery based biofloc system.

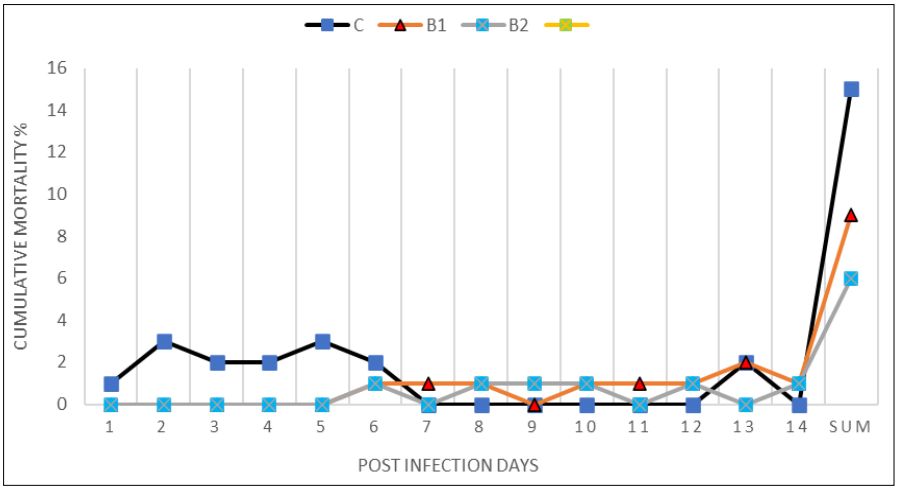


Fig 6: Cumulative mortality rate of *in situ* biofloc reared and control fishes.

Table 6: Mortality percentage and RLP percentage of *Etroplus suratensis* reared *in in situ* jaggery based biofloc system with control.

Treatment	No. of infected fish	No. of dead fish	Mortality %	RLP %
Negative control	10	0	0	-
C	20	15	75	0
B1	20	9	45	40
B2	20	6	30	60

mortality rate (Fig 6) was considerably lower in the *in situ* biofloc groups than control and the maximum relative protection (Table 6) was recorded in the B2, it was noted that all control fish died within 3-5 days post-challenge, while *in situ* biofloc reared fish required a long time 5 to 9 days. The major organ manifestations were observed with the higher degree of infection in control (Abraham *et al*., 2007). Since heterotrophic bacteria in the biofloc, created in the culture system produced immunostimulatory chemicals, the infection in B2 and B2 fish was shown to be less severe.

CONCLUSION

From the present study, it is concluded that when the pearlspot is reared in the *in situ* biofloc system with jaggery as a carbon source an enhanced growth rate and immune response was observed. Therefore, the application of this technology may be helpful in aquaculture to promote the growth and immunity of the *E. suratensis*. With C: N 20: groups thought to be more suitable for pearlspot culture, this work has provided a good understanding of the *insitu* jaggary based biofloc system.

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Conflict of interest

Every step taken was compliant with the responsible parties' ethical standards, the authors declare that they have no conflict of interest.

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