Efficacy of *in situ* and *ex situ* Biofloc on Growth, Immune-physiological Responses, Nutrient Composition and Muscle Growth-related Gene Expression of *Etroplus suratensis*

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ABSTRACT

Background: A 90-day trial was carried out to investigate the effects of *in situ* and *ex situ* biofloc on *the immune*-physiological responses, nutrient composition and muscle growth-related gene expression.

Methods: The experimental groups consisted of IB-1, IB-2, IB-3 (*in situ* biofloc), EB-1, EB-2, EB-3 (*ex situ* biofloc). The capacity of 2.4-ton outdoor tanks were stocked with 4.36±0.1 g pearlspot (*Etroplus suratensis*) juveniles @ 150/m^3. Organic jaggery was used as a carbon source to maintain 12:1, 15:1 and 20:1 C/N (IB-1, IB-2, IB-3) ratio for *in situ* biofloc development and *ex situ* biofloc mass production and feed supplementation at 10, 20 and 30% graded level in the diet (EB-1, EB-2, EB-3).

Result: At the end of the experiment, *in situ* biofloc system showed significantly better physiological performances in terms of SOD, RBT, MPO, GLU, CAT and serum protein compared to *ex situ* treatment. In the *ex situ* system, biofloc supplemented at 20% significantly improved nutrient composition. There was a significant effect of *in situ* and *ex situ* biofloc treatments on whole-body amino acids like phenylalanine, leucine, isoleucine, methionine, tryptophan and lysine. However, the targeted muscle-growth-related *myo D* gene was significantly upregulated in IB-3 *in situ* and EB-2 *ex situ* treatments, while the myostatin was downregulated *in* IB-3 and EB-2. Therefore, this present study suggests the adoption of the *in situ* biofloc method to obtain better performance of Pearlspot, *in situ* biofloc at 20:1 C: N level could be used to improve overall health and muscle growth in *Etroplus suratensis*.

Key words: Biofloc, *Ex situ*, *In situ*, Muscle gene expression, Physiological response.

INTRODUCTION

Over the past few decades, the unprecedented world population has explained various plausible scenarios for aquaculture expansion and fish intensification, which may dissipate the limited water resources and increase environmental deterioration (Wetengere, 2011). The major challenge in the sector is the periodical water exchange requirement to maintain optimum water quality, resulting in huge water consumption and thereby the release of nutrient and organic matter-loaded effluent. Biofloc Technology" (BFT) could be a potential and sustainable alternative that can reduce environmental impacts with zero water exchange and less feed input while increasing stocking density and production (Emerenciano *et al*., 2022).

The crucial factor for the ammonia pathway in BFT systems was carbon amendment at a high C: N ratio, heterotrophic bacteria are the most predominant species (Elaiyaraja *et al*., 2020). The jaggery is considered a simple carbon source with a higher dissolving capacity than other complex carbon sources. On the contrary, simple carbon sources can disperse quickly in water and help reduce ammonia compared to complex carbon sources (Dauda *et al*., 2017). Hence, the simple carbon sources like jaggery, glycerol and sugars can be efficiently utilized by heterotrophic bacteria for multiplication resulting in dispersion and quicker floc and paving the way for the heterotrophic bacteria to assimilate ammonia with decreased concentrations of TSS thereby maintaining ideal water quality (Felix *et al*., 2015).

To overcome the demand for aquatic food for the growing population, aquaculture needs to be projected through species diversification and technological advancements. *Lates calcarifer*, *Chanos chanos*, *Etroplus suratensis* and *Mugil cephalus* are already under domestication for the development of commercial aquaculture in India. As a medium to high-value species, the pearl spot, *E. suratensis* is considered one of the most suitable species for diversified freshwater and coastal

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aquaculture in India. This species is mostly produced in Kerala and Goa, although it has recently gained recognition in other parts of India due to high consumer preferences and market prices.

Feed costs in aquaculture can be reduced by the adoption of biofloc technology since the expenses with commercial diets account for more than 50% of the production cost. Biofloc contains up to 30% crude protein and about 2% lipids (Azim and Little, 2008; Ballester *et al*., 2010; Xu and Pan, 2012; Luo *et al*., 2014). The dried biofloc can be used possibly to replace fishmeal or soybean meal as cheaper sources of protein. The major objective of the study aimed to determine the intake of biofloc by different incorporation methods and its impact on animals' immune-physiological and muscle growth performance.

MATERIALS AND METHODS

Ethical statement

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. This study was approved by the ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India

Experimental design

A completely randomized design (CRD) was followed for the experiments. The experiment was designed with *in situ* biofloc developed within the culture system (IB-1, IB-2, IB-3) and *ex situ* biofloc incorporation in diet (EB-1, EB-2, EB-3). The experimental trial was performed in an FRP tank, which consisted of eighteen circular tanks of 2,400 L capacity. The oxygen supply was maintained at an optimum level with a 1.5 HP air blower. Before stocking all the water quality parameters were analyzed.

Experimental fish

The pearlspot fingerlings used for the experimental study were collected from Pulicat Lake, Pazhaverkadu and the fishes were disinfected with $KMnO_4$ and stocked in 25 ppt brackish water. The fish were slowly acclimatized to 22 ppt by adding fresh water. Before the experiment, an average size of 4.36±0.1 g juveniles was used for experiments. During the trial, fish were fed @ 5% of fish body weight and feeding was done three times a day. The water quality parameters were monitored daily and the mean values were recorded as per standard procedure (APHA, 2005). The bio-growth sampling was done every fortnight.

Production of biofloc for *ex situ* feed preparation

The development of biofloc was carried out as per the protocol described by Avnimelach (2009). The development of floc was triggered with continuous aeration with the help of an air compressor. The C: N ratio of 20:1 was maintained by adding Jaggery as a carbon source. The amount of carbon required to reduce the ammonia content in the water was calculated according to Avnimelech (1999). The floc samples were collected and checked for floc development using of Imhoff cone. When floc volume reached 30 ml/l, once in two days wet biofloc was collected from the tank with the help of 20 µ mesh size hand net, centrifuged at 2000 rpm and washed twice with filtered freshwater to get rid of the traces of ammonia nitrogen if any, dried under shadow and then powered into fine particles <200 µm and stored in airtight containers for experimental feed preparation.

Experimental diets

The experimental diets were prepared with biofloc powder incorporation at 10,20,30% of the feed *viz*., EB-10, EB-20 and EB-30. The experimental feed comprised fishmeal, biofloc meal, soybean meal, wheat gluten, soy lecithin, vitamins, minerals and fish oil. The formulation of dietary ingredients and chemical composition of experimental diets is shown in Table 1. The wet-formulated feed was dried in sunlight to reduce moisture content (<10%) and stored in an airtight container for *ex situ* feeding trials. 0% without-biofloc incorporation feed was used for the *in situ* experimental trials.

Growth analysis

The bio-growth parameters of fish like percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate were calculated (Ruby *et al*., 2022).

Immune-physiological assay

At the end of the 90-day trial, the fish (n=5) were anesthetized using clove oil to collect blood samples from a caudal vein. The blood was collected in EDTA-coated (anti-coagulant) vials and blood was allowed to clot and centrifuged to separate the serum.

The Respiratory Burst Activity was measured by Nitro blue tetrazolium assay (NBT as modified by Anderson and Siwiki (1995). Myeloperoxidase activity-Total MPO content present in serum was measured according to Quade and Roth (1997). The lysozyme assay was carried out with a few minor adjustments based on (Guardiola *et al*., 2019). The Superoxide dismutase (SOD) activity was estimated following the modified method of (Mishra and Fridovich, 1972). Catalase activity (CAT) was estimated according to the method of Takahara *et al*. (1960). The serum glucose Blood glucose (GLU), Serum cholesterol (CHO) and Triglyceride (TG) were estimated using a semi-automatic blood biochemical analyzer (Alpha chem. 100i). The total triglycerides were measured using an auto-analyzer (MERCK Selectra Junior, Germany).

Nutrient and amino acid analysis

To determine the carcass composition of pearlspot fingerlings (n=8) the fishes were cut into pieces, minced, homogenized and immediately frozen until further analysis

and the Proximate and Amino acid were determined using AOAC (1995) method.

Expression of muscle-related genes by real-time analysis (qRT-PCR)

Consecutively, after the end of the trial all fishes were fasted for 24 hours before the collection of samples (n=5 fish per treatment) of skeletal muscle (for MyoD and myostatin), Total RNA was extracted from muscle tissue using RNA iso-plus (Takara Bio Inc., Otsu, Shiga, Japan) according to the manufacturer's protocol. The gene-specific primers of MyoD, myostatin, 18sRNA and β actin are shown in Table 2. The first strand complementary DNA (cDNA) was synthesized from 2 µg of total RNA using the first strand cDNA synthesis kit (Thermo Scientific, Vilnius, Lithuania) as following the manufacturer's protocol. The Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) consisted of 20 ng of cDNA template, 10 µM of each primer (forward and reverse) and 1× SYBR Green PCR Master Mix Kit (Takara Bio Inc., Otsu, Shiga, Japan), in a 20 µl of total volume (Table 3). The qRT-PCR was carried out in a C00 Touch thermal cycler-CFX96 Real-time PCR (Bio-Rad, Foster City, California, USA) programmed with an initial denaturation at 95°C for 10 min, followed by 40 cycles of 15 s denaturation at 95°C, annealing at 60 to 62°C (depends on the target genes) for 30 s, extension at 72°C for 30 s and ended with dissolution curve. The qRT-PCR was performed in triplicate and the threshold cycle (Ct) values obtained by amplification were measured and a relative expression level of the specific gene was presented as $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). The expression of housekeeping genes 18S rDNA and β-Actin was used to compare the relative expression levels of the genes. PCR amplification was carried out using a 96-well Takara PCR System (Takara, Japan).

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using SPSS, VERSION 20.0 (SPSS Inc., Chicago, IL, USA). All data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) and transformed when the data did not show normal distribution. The significance of differences among the treatment groups was evaluated using a predetermined threshold *p*-value of <0.05.

RESULTS AND DISCUSSION

Growth performance of pearlspot reared in insitu and *ex situ* biofloc system

The bio-growth parameters of pearlspot reared in *in situ* and *ex situ* biofloc systems are shown in Table 3. The results indicated that *in situ* groups significantly ($P \leq 0.05$) performed well in terms of higher weight gain compared to *ex situ* groups ($P \geq 0.05$). Improved weight gain was observed in the 20:1 *in situ* biofloc system due to the continuous availability of *in situ* biofloc in the system. Similar results were documented by Ezhilarasi *et al*. (2019) and Elaiyaraja *et al*. (2020). Various studies resulted in *in situ* biofloc performing higher growth in biofloc-raised tilapia (Crab *et al*., 2009; Azim and Little, 2008; Elaiyaraja *et al*., 2020), *L. rohita* (Mahanand *et al*., 2013; Ahmad *et al*., 2016), *Carassius auratus* (Wang *et al*., 2015), *Cyprinus carpio* Najdegerami *et al*. (2016) and *Etroplus suratensis* Ruby *et al*. (2022) which indicates that biofloc can substitute aquatic animal feed and can promote the growth of diverse fish species.

Table 1: Formulation and chemical composition of experimental diets (g/kg of diet).

Ingredients	Different inclusion level Biofloc meal g/kg diet			
	0% (IB-1,2,3)	10% (EB-1)	20% (EB-2)	30% (EB-3)
Fish meal	18	18	10	7
Dry biofloc meal	0	10	20	30
Soya bean meal	22	22	22	22
Wheat gluten meal	9	9	9	9
Cassava meal	8	8	6	4
Corn flour	20	10	10	5
Wheat flour	18	18	18	18
Soy lecithin	1	1	1	1
Di calcium phosphate	1	1	1	1
Salt	1	1	1	1
Fish oil	1	1	1	1
Vitamin mix	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5
Proximate composition of feed (g/kg dry matter)				
Crude protein	33.61±0.84	33.56±0.80	33.4±0.02	33.52±0.17
Crude lipid	4.16±0.05	4.15±0.03	4.18±0.04	4.15±0.18
Crude fibre	3.46±0.19	4.37±0.22	4.83±0.21	4.70±0.24
Ash	9.46±0.01	8.37±0.22	9.83±0.02	9.70±0.24

The inclusion of biofloc as a dietary ingredient in the shrimp diet was found to improve the growth performance of *L. vannamei* (Ju *et al*., 2008; Kuhn *et al*., 2010). Among *ex situ* treatments, biofloc at 20% incorporation in the feed (EB-20) was recorded with higher weight gain. In contrast, shrimp fed higher biofloc incorporation in their diet had significantly lower growth performances which agrees with Anand *et al*. (2014) who reported that an increase in biofloc meal dietary supplementation in the diet does not increase proportionately the digestive enzyme activities and thereby the growth of shrimp. Our study revealed that enhanced growth performances were obtained within the biofloc *in situ* system compared to the *ex situ* system.

Immune-physiological responses

Immune-physiological responses in *E. suratensis* reared with *in situ* and *ex situ* biofloc treatments are summarised in Table 4. The significant interaction (P<0.05) indicated that *in situ* treatment enhances the overall immune-physiological function of pearlspot.

Increased respiratory burst activity (RBA) was observed in IB-3 treatment (0.73±0.01) when compared to other *in situ* and *ex situ* groups. Significantly (p<0.05) higher levels of Myeloperoxidase, lysozyme, Catalase and SOD were recorded in fishes reared in *in situ* compared to *ex situ* groups. In addition, decreased glucose (80.30±0.8) and cortisol (65.2±0.9) values were observed in the *in situ IB-3* treatment of *Etroplus suratensis* when compared with *ex situ* and other treatments. Respiratory burst activity is an indicator of oxygen-dependent bactericidal activities. The observed higher respiratory burst activity in the present study IB-3 was reported by Ekasari *et al*. (2014) who found a higher respiratory burst activity in the post-challenge of shrimp reared under molasses-supplemented biofloc compared to

Table 2: Primers used for qRT-PCR analysis of selected genes of *Etroplus suratensis* reared in *in situ* and *ex situ* bio floc system.

Gene name	GenBank number	Primer sequence (5′-3′)
Myogenic factor (*MyoD*)	GU246722	Forward: CCACCTGTCAGACAACCAGA
		Reverse: ACTGCGTTCGCTCTTCAGAC
Myostatin 1	FJ972683	Forward: TCCACATGACCCTGCAGAC
		Reverse: TGCACCACACATACTCCTCATC
18S ribosomal DNA (18SrDNA)	JF698683	Forward: GGACACGGAAAGGATTGACAG
		Reverse: GTTCGTTATCGGAATTAACCAGAC
β-Actin	EU887951.1	Forward: CCACACAGTGCCCATCTACGA
		Reverse: CCACGCTCTGTCAGGATCTTCA

Table 3: Growth performance of *Etroplus suratensis* reared in *in situ* and *ex situ* biofloc system for 90 days.

Treatments	Initial weight	Final weight	Weight gain	SGR	FCR	Survival
IB-1	4.44±0.08	53.26±0.3	48.82±0.26	2.759±0.17	1.12±0.1	92±1.20
IB-2	4.26±0.09	55.04±0.1	50.77±0.17	2.841±0.02	1.06±0.01	95.1±1.6
IB-3	4.39±0.09	57.90±0.11	53.51±0.21	2.865±0.02	1±0.07	94.7±0.8
EB-1	4.46±0.08	45.99±0.21	41.53±0.4	2.591±0.01	1.45±0.06	81.7±1.6
EB-2	4.31±0.18	49.89±0.17	45.58±0.32	2.721±0.04	1.32±0.03	92.4±1.01
EB-3	4.32±0.14	47.04±0.11	42.71±0.6	2.651±0.05	1.55±0.02	92.2±1.01
P value	0.34	0.00	0.00	0.001	0.002	0.00

Values in the same row with different superscripts differ significantly (p<0.05) between the treatments for each parameters.

Table 4: Immune-physiological responses of *Etroplus suratensis* reared in *in situ* and *ex situ* biofloc system for 90 days.

	IB-1	IB-2	IB-3	EB-1	EB-2	EB-3	P value
NBT/RBA	0.59±0.8^{d}	0.67±0.02^{b}	0.73±0.01^{a}	0.67±0.2^{b}	0.58±0.5 ^{d}	0.60±0.5^{d}	0.00
MPO	5.36±0.19^{c}	6.48±0.08^{b}	7.5±0.08^{a}	4.17±0.30^{e}	5.57±0.21^{c}	5.07±0.14^{d}	0.00
Lysozyme (ìg/ml)	11.85±0.4^{c}	15.32±0.8^{b}	18.96±0.6^{a}	9.32±0.4^{d}	9.96±0.2^{d}	8.8±0.6^{e}	0.00
Glucose (mg/dl)	93.33±2^{c}	82.36±1^{d}	80.30±0.8^{d}	108.5±2^{a}	97.9±2.1^{b}	108.8±2.0^{a}	0.00
Catalase (Units mg protein-1)	36.52±0.8^{d}	42.67±0.7^{b}	48.09±0.9^{a}	35.50±0.5^{d}	40.56±0.6^{c}	35.93±0.5^{d}	0.00
SOD (Units mg protein-1)	41±0.7^{c}	45.93±1^{b}	50.86±0.4^{a}	41.49±1.0^{c}	45.64±1.2^{b}	33.59±1.3^{d}	0.00
Total cholesterol (mg/dl)	1.27±0.54^{c}	1.2±1.6^{d}	1.11±2^{e}	1.48±0.9^{a}	1.34±1.5^{b}	1.41±1.2^{a}	0.00
Total glyceride (mg/dl)	2.20±0.64^{c}	2.46±3.1^{b}	2.98±3.5^{a}	1.90±2.1^{d}	2.20±1.2^{c}	2±0.9^{d}	0.00
Cortisol (mg/dl)	77.9±2.01^{c}	67.5±2.03^{d}	65.2±0.9^{d}	96.7±1.1^{a}	93.2±0.56^{b}	96.5±1.5^{a}	0.00

Values in the same row with different superscripts differ significantly (p<0.05) between the treatments for each parameters.

rice bran and tapioca-by-product-supplemented biofloc systems.

Myeloperoxidase (MPO) is an important enzyme having antimicrobial activity. The present study recorded the highest MPO values in the IB-3 (7.5±0.08) groups, followed by IB-2, EB-2, IB-1 and EB-3 least value was documented in the EB-1 (4.17±0.30) group which similar to the inference of (Long *et al*., 2015). The increased level of Lysozyme and catalase enzymes in the IB-3 and IB-2 agreed with the findings of Anantharaja *et al*. (2023) in the *Hypselobarbus pulchellus*. Superoxide dismutase (SOD) is a class of enzymes that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. The SOD values obtained in the present study were in the range (50.86±0.4 to 33.59±1.3) reported in *Carassius auratus* (Wang *et al*., 2015). In the present study, there was a significant increase in SOD levels among *in situ* biofloc-based treatments compared to *ex situ* groups, which indicates the need for SOD activity to scavenge the oxidative radicals produced by higher respiratory activity among the *in situ* biofloc treatments.

Serum cortisol is used as a general index for stress, which can be used to determine primary stress response in animals, including fish (Metwally and Wafeek, 2014). In the current investigation, *ex situ* treatments showed higher cortisol levels indicating more stress in this group. The results agreed with earlier findings in Nile tilapia (Azim and Little, 2008). In the present study, a reduced trend of glucose content was observed in biofloc treated with probiotics, followed by biofloc and the serum glucose content was found highest in *ex situ* which was highest among other treatments.

Whole body nutrient and amino acid composition

The whole-body crude lipid content were not significantly ($p>0.05$) affected by the *in situ* and *ex situ* groups (Table 5) However, higher crude protein content of the whole body was observed in fish reared in IB-3 followed by IB-2 and IB-1. Similar results were reported by (Yuvarajan *et al*., 2023). There were significant ($p<0.05$) differences observed in the whole-body essential amino acids' profiles *viz*., Phenylalanine, lysine, isoleucine, methionine and tryptophane when compared with *ex situ* groups (Table 6). In the present study, *E. suratensis* in *in situ* biofloc showed a higher percentage of protein and lipid compared to *ex situ* groups. The findings of the present study are in agreement with the results reported by, Paul *et al*. (2024) in *Ompok pabda* and Hasbullah *et al*. (2018) in Nile tilapia.

Muscle-related gene expression

The MyoD gene regulates muscle cell proliferation and differentiation during white skeletal muscle growth to create new hyperplastic fibres and hypertrophy (McCarthy *et al*., 2011). The relative expression of muscle-growth-related genes in the white skeletal muscle of *Etroplus suratensis* reared in *in situ* and *ex situ* biofloc with jaggery as a carbon source are illustrated in Fig 1A and B.

Table 5: Whole body nutrient of *Etroplus suratensis* reared in *in situ* and *ex situ* biofloc system for 90 days.

	IB-1	IB-2	IB-3	EB-1	EB-2	EB-3
Protein	18.15±0.48^{c}	19.89±0.5^{b}	21.71±0.28^{a}	15.43±0.47^{d}	18.63±0.4^{c}	16.10±0.5^{d}
Lipid	3.1±0.11^{bc}	3.1±0.27^{bc}	3.9±0.22^{a}	2.56±0.11^{d}	2.95±0.27^{d}	2.96±0.23^{d}
Ash	5.7±0.42^{b}	6.58±0.22^{a}	5.42±0.25^{bc}	4.85±0.6^{c}	6.9±0.9^{a}	5.64±1^{b}
Fiber	2.14±0.04^{c}	1.1±0.9^{d}	1.28±0.01^{d}	3.01±0.61^{a}	2.6±0.31^{b}	2.3±0.5^{b}

Values in the same row with different superscripts differ significantly ($p<0.05$) between the treatments for each parameters.

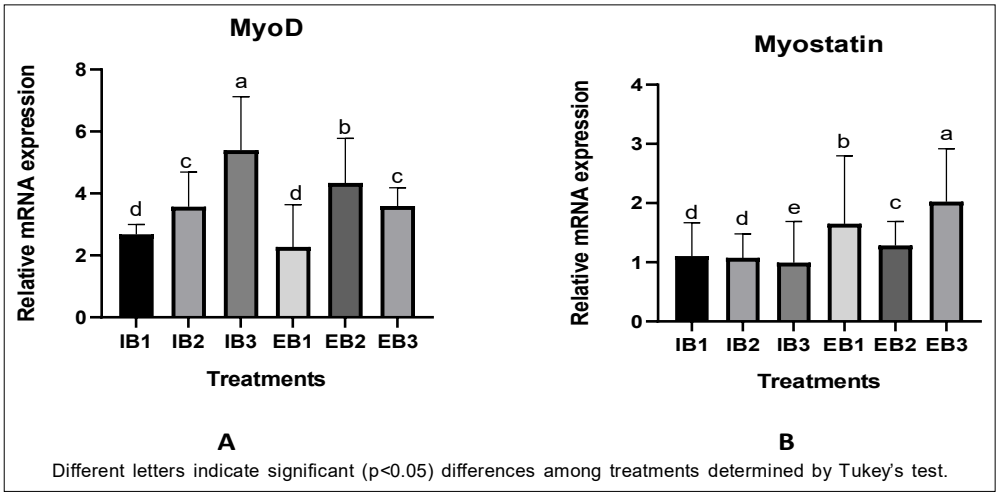


Fig 1: (A) Myo D muscle-related gene, (B) Myostatin in *Etroplus suratensis* reared in *in situ* and *ex situ* biofloc system for 90 days.

Table 6: Amino acid composition of *Etroplus suratensis* reared in in *situ* and *ex situ* biofloc system for 90 days.

	In situ feed	*Ex situ* feed			Aminoacid composition of pearlspot reared in *in situ* and *ex situ* system						P
	IB-1,2,3	EB-1	EB-2	EB-3	IB-1	IB-2	IB-3	EB-1	EB-2	EB-3	value
Essential amino acid											
Arginine	2.8	2.56	2.8	3.1	7.1±0.2	7.24±0.3	7.82±0.3	7.24±0.5	7.8±0.2	7.23±0.2	0.033
Histidine	0.9	1.32	1.56	1.5	3.1±0.2	3.4±0.3	3.73±0.4	2.87±0.51	3.03±0.14	2.95±0.9	0.063
Isoleucine	1.51	1.52	1.59	1.59	5.99±0.2	7.43±0.4	7.67±0.4	6.74±0.3	6.60±0.2	6.78±0.8	0.001
Leucine	3.02	2.42	2.71	2.5	9.06±0.5	11.09±0.7	10.99±0.4	8.52±0.21	7.75±0.9	7.18±0.3	0.000
Lysine	1.32	2.16	2.42	2.26	10.8±0.3	12.54±0.5	11.88±0.6	9.8±0.3	11.25±0.8	10.25±0.8	0.000
Methionine	0.28	0.42	0.45	0.45	3.08±0.1	4.77±0.46	4.97±0.12	3.69±0.5	4.66±0.5	3.63±0.4	0.000
Phenylalanine	1.85	1.91	1.98	1.94	5.62±0.29	6.15±0.7	6.94±0.33	4.86±0.3	4.52±0.52	4.25±0.6	0.000
Threonine	1.35	1.53	1.62	1.54	5.46±0.4	5.3±0.5	6.52±0.3	5.9±0.9	6.12±1	6.85±1.2	0.001
Tryptophan	0.71	0.56	0.59	0.64	2.15±0.5	2.46±0.8	2.84±1	1.52±0.6	1.36±0.8	2.01±0.8	0.076
Valine	1.62	1.72	1.86	1.73	5.96±08	6.15±1	6.95±0.9	5.62±0.2	6.25±0.5	5.98±0.4	0.017
Non-essential amino acid											
Cysteine	0.55	0.52	0.56	0.55	1.93±0.5	2.15±0.1	2.58±2	1.96±0.5	2.12±0.5	1.95±0.8	0.275
Tyrosine	1.38	1.4	1.39	1.37	2.12±0.5	2.45±0.48	2.59±0.85	2.1±2	2.46±1	2.15±0.6	0.000
Glutamic acid	5.75	4.56	4.74	5.46	15.2±	16.12±1	16.89±0.5	11.25±0.8	12.25±2	12.3±2.1	0.002
Aspartic acid	3.12	3.53	3.8	4.03	8.59±0.5	9.56±0.65	10.25±0.56	9.9±0.2	9.58±0.1	10.01±0.66	0.001
Glycine	1.45	1.17	1.32	1.69	9.58±0.6	10.25±0.56	12.56±0.52	8.45±0.6	8.95±0.9	7.89±0.84	0.001
Serine	1.67	1.67	1.71	1.75	2.63±0.5	3.56±0.8	4.15±0.41	2.56±2.3	3.54±0.9	3.05±0.52	0.002
Alanine	1.51	2.01	2.35	2.32	10.25±0.9	10.56±0.6	13.25±2	10.25±1.2	9.56±1	10.01±2.1	0.74

Values in the same row with different superscripts differ significantly ($p<0.05$) between the treatments for each parameters.

El-Hawarry *et al*. (2021) reported that adding glycerol as a carbon source at Low stoking density downregulated Growth gene expression without affecting MSTN expression. Further, he concluded that the upregulation of both IGF-1 and GHR1 genes 5 and 2.1-fold, respectively and growth-related genes upregulated in fish under LSD with glycerol and molasses as carbon sources. MSTN is a skeletal muscle mass-negative regulator gene that suppresses muscle growth (Rodgers and Garikipati, 2008). To the best of our knowledge, there have been no previous investigations on the expression of muscle growth-related genes in *Etroplus suratensis* grown in an *in situ* and *ex situ* biofloc farming system.

The pattern of the targeted muscle genes expression in pearlspot reared in (IB-3) 5.40-fold change revealed a clear pattern of statistically significant (p<0.05) upregulation of Myo D. Similarly, in the *ex-situ* groups, EB-2 expressed higher Myo D 4.33-fold than other treatments. The MyoD was upregulated in IB-3 and EB-2, respectively. On the contrary, the relative expression of *myostatin* was downregulated in IB-3 0.99 and EB-2 1.28-fold changes. With an increase in the C: N ratio of carbon sources the relative muscle gene expression was upregulated.

However, these findings add to our understanding of the impact of *in situ* and *ex situ* biofloc technology in upregulating muscle growth gene expression which underlies the enhancement of fish growth. Future research should be done to investigate the role of *in situ* and *ex situ* biofloc in the expression and regulation of muscle growth-related genes.

CONCLUSION

This study suggests in terms of an *in situ* system with the use of a 20:1 C: N ratio could improve the biofloc formation and enhance the water quality resulting in higher biogrowth and immune status of *Etroplus suratensis* and it should be an appropriate C: N ratio for biofloc farming of pearlspot. Simultaneously, 20% bio floc meal *ex situ* incorporation in pearlspot feed may potentially improve the health status of fish with sustainability. This study and its findings are the first to know the effect of jaggery-based biofloc intake relating to the enhanced immunological performance of *Etroplus suratensis* with gene expression. These findings concluded that the jaggery carbon source at the C: N (20:1) ratio could be an effective potential source that provides higher production with improved water quality parameters.

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Conflict of interest

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

REFERENCES

Anand, P.S., Kohli, M.P.S., Kumar, S., Sundaray, J.K., Roy, S.D. Venkateshwarlu, G., Sinha, A. and Pailan, G.H. (2014). Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture. 418: 108-115.

Ahmad, I., Verma, A., Rani, A.B., Rathore, G., Saharan, N. and Gora, A.H. (2016). Growth, non specific immunity and disease resistance of *Labeo rohita* against *Aeromonas hydrophila* in biofloc systems using different carbon sources. Aquaculture. 457: 61-67.

Anderson, D.P. and Siwicki, A.K. (1995). Basic hematology and serology for fish health programs. Fish Health Section, Asian Fisheries Society. USGS Publications Warehouse. 185-202.

Anantharaja, K., Barlaya, G., Raghavendra, C.H., Kumar, B.S., Siddaiah, G.M., Chadha, N.K. and Hemaprasanth, K.P. (2023). Growth performance of critically endangered *Hypselobarbus pulchellus* in biofloc systems with various C/N ratios. Indian Journal of Animal Research. 57(12): 1725-1732. doi: 10.18805/IJAR.B-5202.

AOAC, (1995). Official Methods AOAC, 1995. Official Methods of Analysis, 13th (ed). Association of Official Analytical Chemist, Washinton D.C.

APHA, (2005). Standard methods for the Examination of the Water and Wastewater, 22nd edition. American Public Health Association, Washington, D.C.

Avnimelech, Y. (1999). Carbon/Nitrogen ratio as a control element in aquaculture systems. Aquaculture. 176(3-4): 227-235.

Avnimelech, Y. (2009). Biofloc Technology-A Practical guide book, Baton Rouge, LA: World Aquaculture Society. 182 pp.

Azim. M. and Little D.C. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture. 283(1-4): 29-35.

Ballester, E.L.C., Abreu, P.C., Cavalli, R.O., Emerenciano, M., De Abreu, L. and Wasielesky, J.W. (2010). Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero-exchange suspended microbial flocs intensive system. Aquac. Nutr. 16(2): 163-172. https://doi. org/10.1111/ j.1365-2095.2009.00648.

Crab, R., Kochva, M., Verstraete, W. and Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. Aquacult. Eng. 40(3): 105-112.

Dauda, A.B., Romano, N., Ebrahimi, M., Karim, M., Natrah, I., Kamarudin, M.S. and Ekasari, J. (2017). Different carbon sources affects biofloc volume, water quality and the survival and physiology of African catfish *Clarias gariepinus* fingerlings reared in an intensive biofloc technology system. Fisheries Science. 83: 1037-1048.

Ekasari, J., Azhar, M.H., Surawidjaja, E.H., Nuryati, S., De Schryver, P. and Bossier, P. (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish and Shellfish Immunology. 41(2): 332-339.

Elayaraja, S., Mabrok, M., Algammal, A., Sabitha, E., Rajeswari, M.V., Zágoršek, K. and Rodkhum, C. (2020). Potential influence of jaggery-based biofloc technology at different C: N ratios on water quality, growth performance, innate immunity, immune-related genes expression profiles and disease resistance against Aeromonas hydrophila in Nile tilapia (*Oreochromis niloticus*). Fish and Shellfish Immunology. 107: 118-128.

Emerenciano, M.G.C., Rombenso, A.N., Vieira, F.D.N., Martins, M.A., Coman, G.J., Truong, H.H., Noble, T.H., Simon, C.J. (2022). Intensification of penaeid shrimp culture: An applied review of advances in production systems, nutrition and breeding. Animals. 12(3): 236. https://doi.org/10.3390/ani12030236.

El-Hawarry, W.N., Shourbela, R.M., Haraz, Y.G., Khatab, S.A. and Dawood, M.A. (2021). The influence of carbon source on growth, feed efficiency and growth-related genes in Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions and high stocking density. Aquaculture. 542(1): 736919. doi: 10.1016/j.aquaculture.2021.736919.

Ezhilarasi, V., Verma, A.K., Rani, A.M.B., Harikrishna, V., Chandrakant, M.H., Ahmad, I. and Nageswari, P. (2019). Effect of different carbon sources on growth, non-specific immunity and digestive enzyme activity of amur carp (*Cyprinus rubrofuscus* Lacepede 1803) fingerlings in biofloc based rearing system using inland saline groundwater. Indian Journal of Fisheries. 66(3): 87-94.

Felix, S., Antony Jesu Prabhu, P., Cheryl Antony, Gopalakannan, A., Rajaram, R. (2015). Studies on nursery rearing of Genetically improved farmed tilapia (GIFT) in Biosecured raceway systems in India. J. Indian Fish. Assoc. 42: 1-10.

Guardiola, F.A., Mabrok, M., Machado, M., Azeredo, R., Afonso, A., Esteban, M.A. and Costas, B. (2019). Mucosal and systemic immune responses in *Senegalese sole* (*Solea senegalensis* Kaup) bath challenged with *Tenacibaculum maritimum*: A time-course study. Fish and Shellfish Immunology. 87: 744-754.

Ju, Z.Y., Forster I., Conquest L., Dominy W., Kuo W.C. and Horgen F.D. (2008). Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquaculture Research. 39(2): 118-133.

Hasbullah, D., Karim, M.Y., Trijuno, D.D. (2018). Physiological performance of osmotic work, survival and growth rate of hybridized brackish Nile tilapia (*Oreochromis niloticus*) juveniles at various salinities. Aquacult. Aquari.Conser. Legisl. 11(2): 327-332.

Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, G.J. (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture. 303: 28-33.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 25(4): 402-408.

Long, L., Yang, J., Li, Y., Guan, C. and Wu, F. (2015). Effect of biofloc technology on growth, digestive enzyme activity, hematology and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture. 448: 135-141.

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L. and Tan, H. (2014). Growth, digestive activity, welfare and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquacult. 422: 1-7.

Mahanand, S.S., Moulick, S. and Rao, P.S. (2013). Optimum formulation of feed for rohu, *Labeo rohita* (Hamilton), with biofloc as a component. Aquaculture International. 21(2): 347-360.

McCarthy, J.J., Mula, J., Miyazaki, M., Erfani, R., Garrison, K., Farooqui, A.B., Srikuea, R., Lawson, B.A., Grimes, B., Keller, C. and Van Zant, G. (2011). Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. Development. 138(17): 3657-3666.

Metwally, M.A.A. and Wafeek, M. (2014). Effect of ammonia toxicity on carbohydrate metabolism in Nile Tilapia (*Oreochromis niloticus*). World J. Fish and Marine Sci. 6(3): 252-261.

Mishra, H.P. and Fridovich, I. (1972). The univalent reduction of oxygen by reduced flavins and quinines. J. Biol. Chem. 247: 188-192.

Najdegerami, E.H., Bakhshi, F. and Lakani, F.B. (2016). Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. Fish Physiology and Biochemistry. 42: 457-465.

Paul, P., Islam, M.S., Hasanuzzaman, A.F.M. (2024). Water quality, nutritional, hematological and growth profiles of *Ompok pabda* fish fry reared in biofloc technology and traditional culture system with different stocking densities. Animals. 14(1): 90. https://doi.org/10.3390/ani14010090.

Quade, M.J. and Roth, J.A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Veterinary Immunology and Immunopathology. 58(3): 239-248.

Rodgers, B.D., Garikipati, D.K. (2008). Clinical, agricultural and evolutionary biology of myostatin: A comparative review. Endocr. Rev. 29: 513-534.

Ruby, P., Ahilan, B., Antony, C., Manikandavelu, D., Selvaraj, S. and Moses, T.L.S. (2022). Evaluation of effect of the different stocking densities on growth performance, survival, water quality and body indices of pearlspot (*Etroplus suratensis*) fingerlings in biofloc technology. Indian Journal of Animal Research. 56(8): 1034-1040. doi: 10.18805/IJAR.B-4922.

Takahara, S., Hamilton, H.B., Neel, J.V., Kobara, T.Y., Ogura, Y. and Nishimura, E.T. (1960). Hypocatalasemia: A new genetic carrier state. Journal of Clinical Investigation. 39(4): 610-619.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L., Zheng, Z. (2015). Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. Aquaculture. 443: 98-104.

Wetengere, K. (2011). Socio-economic factors critical for intensification of fish farming technology. A case of selected villages in Morogoro and Dar es Salaam regions, Tanzania. Aquaculture International. 19(1): 33-49.

Xu, W.J., Pan, L.Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture. 357: 147-152.

Yuvarajan, P., Cheryl, A., Gopalakannan, A. and Mahadevi, N. (2023). Effect of distillery spent wash as carbon source in biofloc system on nutrient profile of GIFT Tilapia. Indian Journal of Animal Research. 57(2): 249-253. doi: 10.18805 /IJAR.B-4321.