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Effect of different biofloc based culture systems on the growth and immune response of Tilapia (*Oreochromis niloticus*)

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Abstract
The objective of the present study was to assessment the growth performance and some non-specific immunological effects of growing fish Nile tilapia, *Oreochromis niloticus* in substrate based biofloc systems. This research was consisted of three types of biofloc systems in which bioflocs were produced by daily supplementation of three different carbon sources, i.e., T1 (only biofloc), T2 (biofloc + bamboo) and T3 (biofloc + nylon mat substrates) using wheat flour as carbon source along with control T4 (without biofloc and substrate) at an estimated C/N ratio of 15 and a control system without any organic carbon addition. Each biofloc system was stocked with 15 fingerlings (4.40 g±0.05) of *O. niloticus* for growth performance with each group consisted of four replicate aquarium tanks of 70 lit capacity that were reared for a period of 60 days. The present study illustrated the prospects of rearing Nile tilapia, *Oreochromis niloticus*" in a biofloc-based intensive culture tanks without affecting its survival and growth. It enhanced the fish growth and amplified the non-specific immune response and the antioxidant capability.

Keywords: Biofloc technology (BFT); substrate based, *Oreochromis niloticus*; growth parameters Immune and stress parameters

Introduction

Biofloc technology is mainly based on the principle of waste nutrients recycling, in particular nitrogen, into microbial biomass that can be used in situ by the cultured animals or be harvested and processed into feed ingredients [1, 2]. Heterotrophic microbiota is stimulated to grow by steering the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source in the water [3], so that the bacteria can assimilate the waste ammonium for new biomass production. Aquaculture as a food-producing sector offers ample opportunities to alleviate poverty, hunger and malnutrition, generates economic growth and ensures better use of natural resources [4]. Aquaculture production is projected to rise from 40 million tonnes by 2008 to 82 million tonnes in 2050[5].

Tilapia is a farmed species that is being produced intensively everywhere in the universe. In 2012, the world tilapia production reached 4.5 million MT [6] with eminent anticipations to extend more exponentially in the future. Aquatic feeds usually comprise more than 25% protein. Whereas cultured fish solely retain 16-41% of feed nitrogen [7, 8]. Crab *et al.* [9] described ammonia as the prime nitrogenous waste product of protein metabolism and fish feces decomposition as well as the uneaten feed. Due to its toxicity nature (i.e. ammonia), the extent of this product in an aquaculture medium should be sustained at 0.01 mg/L[10] by regular water replacement or by applying water management system.

Bioflocs would have a distinguished priority over traditional fish farming in a low-to-zero exchange of water system that also supplies a complementary and inherently nutritious food for the commercial crop [11, 12]. Also, physiological functions such as immune and antioxidant systems are essential for tilapia in maintaining their health and growth performance, particularly beneath the stressful environmental, e.g. high-density culture [13] and thereby guarantee healthy tilapia culture. Therefore, the current study was intended to estimate the effect of BFT (Bio-Floc Technology) on the growth performance and some non-specific immunological of Nile tilapia, *Oreochromis niloticus* using different biofloc based systems.

Material and methods

Designing the experimental procedure

A 60 day research was conducted in the Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur. 240 numbers of healthy and uniform sized *O. niloticus* fingerlings were selected from the stock population and fifteen fish were randomly collected from these and stocked in each of twelve experimental tanks and four control tanks. The average length and weight of the fingerlings at the start of the experiment was 4.6±0.3 cm and 4.4±0.05 g respectively. Each replicate tank of the treatments T2 and T3 were placed vertically with one bamboo mat and one nylon mesh substrates respectively.

Production of biofloc

Four carbon sources *viz*., wheat flour, rice flour, tapioca flour and molasses were purchased locally and tested before conducting the experiment for the development of best quality biofloc inoculum. The proximate composition of carbohydrate sources used was given in table1.

Table 1: The proximate composition of four carbohydrate sources tested for the experimental study

Carbohydrate Source	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Moisture (%)	NFE (%)
Wheat flour	12.80	1.80	13.30	1.20	11.00	70.90
Tapioca flour	2.10	12.30	0.60	0.89	9.40	84.11
Molasses	5.90	0.06	9.40	0.10	29.60	84.54
Rice flour	7.50	0.80	0.70	0.60	11.00	90.40

Out of four carbon sources tested, good quality biofloc with various groups of microorganisms such as protozoan ciliates and flagellates, rotifers and round worms were observed in wheat flour inoculum (plate 4). Hence, wheat flour was identified as best carbohydrate source for the development of good quality biofloc for the experimental study.

Performance of fish growth condition

During the experimental period fishes were subjected to growth assessment at every fortnight interval. The growth parameters like net weight gain, specific growth rate (SGR), percentage survival, food conversion ratio (FCR) and feed efficiency ratio (FER) was observed during the experimental period.

Analysis of Immune Parameters

The immunological assays *O. niloticus* blood from different experimental groups were collected and anaesthetized using 30 ppm MS 222 for serum assays.

Serum Lysozyme Activity: Serum lysozyme was determined by a turbidimetric method Ellis [14].

Total Serum Protein, Serum Albumin and Globulin: The concentration of total serum protein was analyzed using Bradford [15] method and Serum albumin was estimated by the BCG binding method by Doumas *et al.* [16].

Anti-protease Activity: Anti-protease activity was measured using a modification of the method describedby Bowden *et al.* [17] and Magnadottir *et al.* [18].

Stress Parameters Determination

The serum collected at the end of the experiment was used for assessment of the stress parameters like serum glucose was estimated using blood glucose diagnostic kit (Merck, Germany) and cortisol was estimated using the Caymans cortisol EIA kit; (Caymans chemical, USA).

Statistical Analysis

Statistical analysis was done using SPSS and graphs drawn with Microsoft Excel - 2007. One way analysis of variance (ANOVA) was used to test the significance of differences and Duncan's multiple range test were used for significance <0.05.

Results

An experimental study was conducted for 60 days to assess the "Effect of biofloc technology on growth and immune response of Nile tilapia, *Oreochromis niloticus*" with three treatments and one control viz., T1 (only biofloc), T2 (biofloc + bamboo mat substrate), T3 (biofloc + nylon mat substrate) and control (without biofloc and substrate). The results of growth and immune parameters assessed during the experimental study are shown below.

Performance of fish growth condition

Data on growth performance of *O. niloticus* in all the experimental tanks were observed at fortnightly intervals and are given below.

Average Body Weight and Weight Gain *(g):* The average body weight gain was significantly lower in control in comparison with treatment groups during the entire experimental period. Among the treatments the T2 has shown significantly higher weight gain than T3 and T1, except on 30th day where T2 (6.30±0.18) and T3 (6.78±0.03) were not significantly different.

Net Weight Gain [NWG (g)]: At the end of the experiment, Among the treatment groups, T2 (24.42±0.83) showed higher NWG followed by T3 (21.77±0.74) and T1 (20.35±0.66).

Specific Growth Rate (SGR): The SGR of *O. niloticus* in different experimental groups significantly higher SGR ($P<0.05$) was recorded in T2 (3.12±0.04) followed by T3 (2.97±0.53) and T1 (2.86±0.21) when compared to control (2.01±0.63).

Survival Rate: 100% survival rate of *O. niloticus* fingerlings was recorded in all the three treatments and control.

Food Conversion Ratio (FCR): FCR of *O. niloticus* in different biofloc based treatments and control is significantly better FCR was observed in all the treatments compared to control (1.59±0.67).

Feed Efficiency Ratio (FER): FER of *O. niloticus* in the different experimental groups and FER were observed in the treatment groups compared to control (0.71±.27). Among the biofloc based treatments, T2 (1.08±0.04) and T3 (1.06±0.15) has shown higher FER values than T1 (1.02±0.09).

Analysis of Immune Parameters

The immune parameters like serum protein, albumin, globulin, serum lysozyme activity, anti-protease activity were statistically analyzed and compared between the treatments and control.

Serum Lysozyme and Anti-protease Activity

The values for the Serum lysozyme (0.68±0.01) and Anti-protease activity (57.88±1.28) were observed between the treatments and control. The lowest value was observed in control when compared to the treatments. Among the treatments, significantly higher value was recorded in T2 Serum lysozyme (0.77 ±0.01) and T2 Anti-protease (81.89±2.20) while compared with T1 and T3 and no significant values were observed in between T1 and T3 respectively.

Total Serum Protein, Serum Albumin and Globulin
Consequently lower values were observed in control (4.55±0.22, 3.64±0.44 and 0.91±0.29) for all the three parameters in comparison to treatments. Among the treatments, T2 (7.19±0.20, 4.46±0.10 and 2.65±0.12) and T3 (6.97±.011, 4.37±0.61 and 2.60±0.17) were recorded with significantly higher values of serum protein, albumin and globulin than T1 (5.43±0.22, 3.98±0.76 and 1.45±0.16) respectively.

Stress Parameters determination
Stress parameters like serum glucose and cortisol were statistically analyzed and compared between treatments and control.

Serum Glucose (mg/dL)
The lowest value observed in treatment T2 (84.00±4.55) when compare to the treatments T3 (100.0±2.83), T1 (103.5±4.51) and control (118.75±7.63). Significantly highest value of serum glucose was observed in control.

Serum Cortisol (mg/dL)
Lowest value was observed in treatment T2 (21.58±1.63) than the treatments and T3 (27.73±0.90), T1 (31.58±2.52) and control (57.65±6.49).

Discussion
In the current research, we demonstrated that the application of biofloc technology for Nile tilapia, *Oreochromis niloticus*" culture significantly affects the growth and immune response, an underexplored feature of bioflocs, and may increase the robustness of tilapia to resist infection. These effects seem to be independent of the type of carbon source used to grow the bioflocs. The sustainability of the aquaculture sector depends on efficient management of resources, especially water [19].
Nile tilapia (*O. niloticus*) is a most suitable species to grow in biofloc and substrate based aquaculture systems. More research has been carried out on biofloc- and substrate- based aquaculture systems [1, 9, 20-25].

Performance of fish growth condition
Bioflocs are rich source of many bioactive compounds such as carotenoids, chlorophylls, phytosteroids, bromophenols and antibacterial compounds [26, 27]. Azad *et al*. [28] repoted that, periphyton can acts as an antibiotic or as a probiotic/vaccine. In the present study, higher growth performance and FCR observed in O. niloticus in substrate added biofloc and only biofloc systems might be due to many unknown growth factors or immune-stimulants present in bioflocs and periphyton.
All the treatments recorded significantly (P<0.05) better net weight gain, specific growth rate, food conversion ratio and feed efficiency ratio than control group. Among the treatment groups, the biofloc + bamboo mat substrate treatment has recorded with higher net weight gain, SGR, FCR and FER, though significantly not different from the treatments T3 (biofloc + nylon mat substrate) and T1 (biofloc only). Higher growth performance and better FCR was observed for tilapia [23, 29-31] *L. rohita* [25, 32] reared in biofloc systems. Similarly, use of substrates in biofloc systems has recorded better growth performance in shrimp [24, 33]. Our study revealed that integration of submerged substrates in biofloc based fish culture can improves growth performance in Nile tilapia, *O. niloticus*.

Analysis of Immune Parameters
Non-specific immune parameters viz., total serum protein, lysozyme activity and anti- protease activity of *O. niloticus* reared in biofloc and substrate based biofloc systems were analyzed and compared among different experimental groups.
Serum Lysozyme and Anti-protease Activity
Lysozyme is an important enzyme in blood, which actively lyses bacteria where an increased level of lysozyme has been considered to be a natural protective mechanism in fish [34].Long *et al*. [31] observed considerably higher lysozyme activity in O. niloticus (GIFT) reared in BFT treatment than in control. Ahmad *et al*. [25] reported increased serum lysozyme activity of L. rohita in bioflocsytems using carbon sources (wheat and tapioca) compared to control group.
Anti-protease contributes to the innate immunity of animals by its bactericidal and anti-inflammatory properties [35].The immune-stimulatory effect of bioflocs [26, 27] and periphyton [22] has been well documented. In the present study, the higher anti-protease activity observed might be due to the more suitability of bamboo mat substrate for attachment and proliferation of microbial communities which in turn grazed by tilapia, resulted in enhanced non- specific immunity in fish. The results are in agreement with the observation of Vasudeva and Chakrabarti [36] that the feeding of *L. rohita* with *Achyranthes aspera* (0.5%) mixed diet for 4 weeks enhanced the level of serum anti-protease, which might have provided resistance against the bacterial pathogens. Kaleswaran*et al*. [37] recorded enhancement of serum anti-protease activity in catla fish fed with *Cyanodondactylon*.

Total Serum Protein, Serum Albumin and Globulin: In the current study, control group has shown significantly (P<0.05) lower serum protein, albumin and globulin. Ahmad [38] reported a continuous increase in total serum protein, albumin and globulin in *L. rohita* fingerlings reared in biofloc systems than that of control. Earlier researchers reported positive effects of immune-stimulants like levan [39, 40], β- glucan [41] in enhancing the serum protein, albumin and globulin in fishes.

Stress Parameters determination
The possibility of stress to the fishes in biofloc and biofloc + substrate based systems can be ruled out by the serum cortisol and glucose level obtained in the present study.

Conclusion
This study can be concluded that efficient fish growth may be obtained by reducing the artificial feed sources and utilization of bioflocs as feed. The present study illustrated the prospects of rearing Nile tilapia, *Oreochromis niloticus*" in a biofloc-substrate based intensive culture tanks without affecting its survival and growth. This BFT system would boost the enhanced fish growth will be amplified.

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