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Application of Substrate based Biofloc Systems on Water Quality and Hematological Parameters of Nile Tilapia *Oreochromis niloticus*

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A B S T R A C T

The present study evaluated the effect of different substrate based biofloc technology (BFT) application on water quality and Hematological Parameters of Nile tilapia *Oreochromis niloticus* for 60 day's indoor experiment at Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Muthukur. The results indicated that tilapia reared in different substrate based biofloc treatment showed significantly ($p<0.05$) higher performance, than the control (without biofloc and substrate). At the end of the study period blood and serum samples were collected for the evaluation of haematological parameters. Increased levels of haematological parameters viz., total RBC, WBC, Platelet count, Hb, HCT, MCV, MCH and MCHC were observed in Nile tilapia fingerlings reared in different substrate based biofloc and biofloc treatments than that of control. Among treatments, biofloc + bamboo mat substrate (T2) treatment has shown better haematological response.

Introduction

The world demand of tilapia has been steadily increasing, especially in the United States and European countries. This is followed by the progressively growth of world tilapia and other cichlids production have remained stable at between 0.7 million tonnes and 0.85 million tonnes per year (Food and Agriculture Organization Fisheries and Aquaculture Statistics, 2020). The increasing global population and the limiting global capture fisheries undeniably increase the demand of aquaculture product including tilapia. On the other hand, those will also bring about limitation to aquaculture expansion in particular of land and water utilization. Therefore, productivity enhancement in term of total production per input used becomes one of the major priority in the development of tilapia culture particularly and aquaculture in general (Brune *et al.*, 2003; Delgado *et al.*, 2003; Piedrahita, 2003; Avnimelech *et al.*, 2008).

Biofloc technology (BFT) is an aquaculture system which focused on a more efficient use of nutrient input with limited or zero water

exchange. The main principle of BFT is to recycle nutrient by maintaining a high carbon/ nitrogen (C/N) ratio in the water in order to stimulate heterotrophic bacterial growth that converts ammonia into microbial biomass (Avnimelech, 1999). The microbial biomass will further aggregate with other microorganisms and particles suspended in the water forming what has been called "biofloc", which eventually can be consumed in situ by the cultured animals or harvested and processed as a feed ingredient (Avnimelech, 1999; Avnimelech, 2007; Crab *et al.,* 2007; De Schryver*et al.,* 2008; Kuhn *et al.,* 2008; Kuhn *et al.,* 2009; Kuhn *et al.,* 2010).

Substrate (periphyton) based systems have traditionally been used in Africa (Hem and Avit, 1994) and Asia (Wahab and Kibira, 1994). Its application was recently expanded in Bangladesh and India, mainly in the polyculture of Indian carps, where introduction of the substrates had a positive effect on consequent periphyton development, production of the target species, and water quality (Beveridge *et al.,* 1998). Being an omnivore, tilapia has filter feeding, grazing and detritus feeding habits, making it an ideal candidate for substrate based aquaculture system, where periphyton ia a natural food source (Beveridge *et al.,* 1998; Azim, 2001a; Azim *et al.,* 2002b; Milstein and Omri, 2003; Cavalcante *et al.,* 2011). Periphyton based aquaculture is an appropriate technology, which can reduce costs and allow an economically viable organic *O.niloticus* production (Milstein and Omri, 2003).

Therefore, different substrate based Biofloc technology (BFT) is considered as a promising system for a sustainable and environmentally friendly aquaculture system, and has been applied both at laboratory and commercial scale for various aquaculture species such as tilapia (Avnimelech 2007; Azim and Little 2008; Crab *et al.,* 2009), shrimp (Burford *et al.,* 2004; Hari *et al.,* 2004; Taw, 2010), sturgeon and snook (Serfling, 2006). In relation to the former, biofloc technology could support the supply of good quality seeds by improving the reproductive performance of aquaculture animals and by enhancing the larvae immunity and robustness (Ekasari *et al.,* 2015; Ekasari *et al.,* 2016; Emerenciano *et al.,* 2013). The aim of the current study was to determine the effect of substrate based BFT application on water quality and hematological parameters of Nile tilapia *Oreochromis niloticus* cultured at various densities. Moreover, bioflocs primary nutritional parameters measurement was also performed in this study.

Materials and Methods

The experiment was carried out at Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Muthukur. Nile tilapia fingerlings *Oreochromis niloticus* (GIFT strain) were obtained from a commercial hatchery. The animals were transported in specific plastic bags and acclimated for one week until the beginning of the experiment.

Experimental design

The experimental group designed as three treatments and one control *viz*., T1 (only biofloc), T2 (biofloc + bamboo mat), T3 (biofloc + nylon mat) and C (control without biofloc and substrate) were designed and four replicates were maintained in separate tanks for each treatment and control. Biofloc inoculum was prepared in twelve glasses of one-liter capacity beakers using wheat flour as carbohydrate source.

The biofloc was maintained in all the treatment tanks throughout the experimental

period. Calculation for maintaining C/N ratio of 15:1 was done using Avinimelech (1999) method. In general, it is assumed that the carbohydrate source contains minimum 50% carbon.

Healthy and uniform size 240 number *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.

Water quality parameters

Total ammonia nitrogen (TAN), nitrite, nitrate, pH, dissolved oxygen (DO) and temperature were measured at 10:00 am periodically. The tested water parameters were determined using following APHA (2012) methods.

Hematological parameters

Three fishes from each replicate, a total twelve fishes from each treatment were anaesthetized using 30 ppm MS 222 (Tricainemethane sulphonate). Blood was collected from the caudal vein by using No.24-gauge syringe, previously rinsed with 10% EDTA solution (Goldenfarb *et al.*, 1971). Blood collected was transferred immediately to a heparinized tube and shaken gently to prevent haemolysis of blood cells. 50 µl of collected blood sample from each treatment and control was immediately analyzed for Red Blood Cell (RBC) count, White Blood Cell (WBC) count, platelet count, hemoglobin content (Hb), hematocrit value, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). The hematological parameters were analyzed by using the hematology analyzer, (Pentagan Medical Instrument, PHA-23PLUS, China)

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 and Discussion

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.

Water quality parameters

The mean values of water quality parameters such as dissolved oxygen, temperature, pH, TAN, nitrite and nitrate were determined on weekly intervals in all the experimental tanks and the recorded values were represented in Table 1 to 6. All the obtained data in the selected experimental tanks were found in the approved range.

Dissolved Oxygen (DO)

The Dissolved Oxygen content in the control group was significantly higher ($p< 0.05$) than all the treatment groups and T2 recorded

lowest DO levels than T1 and T3 throughout the experimental period. Among the three treatments, T1 (only biofloc) has shown significantly higher DO values than T3 (biofloc + nylon mat substrate) and T2 (biofloc + bamboo mat substrate) during the experiment period. Between biofloc with substrate treatments DO content was significantly lower in T2 than T3 throughout the experiment. However, on day one there was no significant difference in DO content of treatments T2 and T3. The minimum and maximum DO levels observed in control and treatments T1, T2 and T3 ranged between 7.16±0.05 and 7.89±0.08, 6.29±0.12 and 7.62±0.04, 5.10±0.10 and 7.19±0.21 and 5.58±0.10 and 7.20±0.02 respectively. Overall Do content in all the experimental tanks was within the optimum range for growth and survival of *O. niloticus* (Table 1).

Temperature (^{0}C)

The experiment was conducted in indoor systems, uniform temperature conditions were maintained throughout the study period and no significant difference observed in both treatment and control tanks. The maximum and minimum water temperatures maintained in control, T1, T2 and T3 were, 30.30±0.11 and 27.62±0.16, 30.28±0.15 and 27.60±0.15, 30.25±0.12 and 27.65±0.13, 30.26±0.12 and 27.58±0.15 which were shown in Table 2 respectively.

pH

On day one average pH values in control and treatment groups have shown no significant difference. Significantly higher pH values were recorded in control than the treatment groups from 1st week to 8th week. The weekly average pH values have shown increasing trends in control and decreasing trends in treatments throughout the experiment. The average pH values on starting day and 8th week of experiment in control group were 8.22±0.07 and 8.68±0.09 respectively. Among the treatment groups, T1 has shown significantly ($p<0.05$) higher pH values than T3 and T2. During the experiment, though the weekly pH values in treatment T2 were lower than T3, significant difference was not observed between them. The pH values observed in all the experimental tanks is presented in Table 3.

Total Ammonia Nitrogen [TAN (mg/l)]

There was an increase in values of TAN in all the experimental groups from 1st week to 8th week. Throughout the experimental period, TAN values were significantly lower ($p<0.05$) in control than the treatments. Among the treatments, higher TAN values were recorded in T2 than T3 and T1, though they were not significantly different from each other (Table 4). The minimum and maximum TAN values in control and treatments T1, T2 and T3 recorded on 1st and 8th week were 0.2±0.05 and 0.54±0.08, 0.41±0.06 and 0.82±0.07, 0.43±0.02 and 0.84±0.06 and 0.39±0.02 and 0.88±0.03 respectively.

Nitrite (mg/l)

The nitrite levels gradually increased from 1st week to 8th week in all the treatments and as well in control. Throughout the experimental period the nitrite levels of control were significantly low ($p<0.05$) in comparison with all the treatments. The average weekly values of nitrite observed in all the experimental tanks are presented in the Table 5. Among the treatments groups, there was no significant difference in the values of nitrite, though higher levels were recorded in treatment T2. The minimum and maximum nitrite levels were observed on 1st and 8th week in all the experimental groups. 1st and 8th week nitrite values in control and treatments T1, T2 and T3 were 0.03±0.00 and 0.40±0.06, 0.07±0.02

and 0.62±0.05, 0.10±0.02 and 0.68±0.11 and 0.08±0.01 and 0.65±0.04 respectively.

Nitrate (mg/l)

Both control and treatment group have shown gradual increase in the nitrate values from 1st week to 8th week. Throughout the study period significantly lower nitrate levels were observed in control than treatments (Table 6). Among the treatment groups T2 followed by T3 have recorded with significantly higher nitrate levels in comparison with T1. The minimum and maximum average nitrate levels were observed on 1st and 8th week in both control (0.62±0.05 and 3.65±0.05) and treatments T1 (0.90±0.09 and 16.26±0.82), T2 (1.02±0.02 and 22.52±0.15) and T3 (1.00±0.02 and 21.68±0.21).

Hematological parameters

Hematological Parameters *viz*., total red blood cell count, total white blood cell count, platelet count, hemoglobin content, hematocrit value and erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were analyzed at the end of the experiment and the results obtained were compared for significant difference ($p<0.05$).

Total RBC Count

In control, the RBC count was observed as lower (1.28±0.10) as compared to the treatments significantly. Among the treatments T2 (2.52±0.44) showed significantly higher difference than the treatments T1 (1.83±0.35) and T3 (1.79±0.19). There was no significance in RBC count between the treatments T1 and T3. The recorded values of total erythrocyte count in *O. niloticus* from control and all the three treatments are presented in Table 7.

Total WBC count

There was a highly significant difference between the treatment groups and control. The total WBC count showed significantly higher value in T2 (81.55±3.10) and T3 (77.30±0.62) when compared to the treatment T1 (70.03±3.58). As compared to the treatment groups, the WBC count was significantly lower in control (54.2± 5.68) (Table 7).

Platelet count

Significantly lower platelet count was observed in control (4.25±1.71) in comparison with the treatments. Significantly higher platelet count was observed in the treatment T2 (12.25±0.96) when compared to T1 and T3. The treatments T1 (8.00±3.74) and T3 (7.25±0.96) were not significantly different for platelet count which were shown in Table 7.

Hemoglobin content

The mean Hemoglobin value in all the experimental groups is presented in Table 7. When compared to the treatments, significantly low Hb content was observed in control group (3.50±0.18). Between the treatments, T2 (6.65±0.58) and T3 (6.13 ± 0.61) showed the higher significant Hb values than T1 (5.33± 0.97).

Hematocrit value [HCT (%)]

The hematocrit value was observed at the end of experiment and the result was presented in Table 7 and it shown significantly higher difference in HCT value was observed between the treatment groups and control (19.45±3.72). Between the treatments T2 (32.10±3.27) and T3 (31.35±1.75) showed the higher significant difference compared to T1 (26.10±4.49).

Erythrocyte indices

All the three erythrocyte indices showed significantly lower values in control as compared to the treatments. Among the treatments, T2 recorded significantly higher values for MCV (154.93±3.86), MCH (32.23±1.37) and MCHC (24.23±0.62) than T3 and T1. The treatments T3 and T1 were not significantly different with each other for MCV, MCH, and MCHC (Table 7) respectively.

The water quality parameters remained within recommended levels for the species (El-Sayed, 2006), and similar to those found by Azim and Little (2008) with *O. niloticus* in BFT system. As expected no high oscillation over the six weeks in the ammonia levels was observed and demonstrates the advantage of the biofloc system since the microorganisms present in the water can control this toxic compound (Kishida *et al.*, 2008). In addition, the inoculation of mature biofloc water before the experiment began guaranteed low levels of nitrite (one of the most harmful compounds presented in closed systems) as observed by Krummenauer *et al.*, (2014).

Table.1 Weekly variation of DO (Mean±SD in mg/l) in the treatment tanks and control

Treatments / Days	C	T1	T2	T3
1	7.89^{c}±0.08	7.62^{b}±0.04	7.19^{a}±0.21	7.20^{a}±0.02
7	7.88^{d}±0.04	7.53^{c}±0.05	6.43^{a}±0.41	6.64^{b}±0.08
14	7.43^{d}±0.04	6.81^{c}±0.21	5.82^{a}±0.18	6.25^{b}±0.14
21	7.54^{d}±0.05	6.33^{c}±0.06	5.57^{a}±0.06	6.05^{b}±0.27
28	7.65^{d}±0.08	7.24^{c}±0.06	5.95^{a}±0.24	6.63^{b}±0.25
35	7.83^{d}±0.05	7.20^{c}±0.26	6.00^{a}±0.48	6.44^{b}±0.43
42	7.16^{d}±0.05	6.83^{c}±0.08	6.29^{a}±0.38	6.51^{b}±0.04
49	7.76^{d}±0.10	6.56^{c}±0.04	5.58^{a}±0.16	6.35^{b}±0.05
56	7.42^{d}±0.05	6.29^{c}±0.12	5.10^{a}±0.10	5.58^{b}±0.10

The significant difference was observed at $p<0.05$

Table.2 Weekly variation of temperature (Mean±SD in ^{0}C) in the treatment tanks and control

Treatments / Days	C	T1	T2	T3
1	27.62^{a}±0.16	27.60^{a}±0.15	27.65^{a}±0.13	27.58^{a}±0.15
7	28.45^{a}±0.13	28.48^{a}±0.11	28.50^{a}±0.13	28.46^{a}±0.14
14	28.72^{a}±0.10	28.70^{a}±0.15	28.67^{a}±0.12	28.65^{a}±0.11
21	28.70^{a}±0.15	28.78^{a}±0.10	28.70^{a}±0.12	28.75^{a}±0.14
28	27.75^{a}±0.13	27.80^{a}±0.13	27.76^{a}±0.15	27.72^{a}±0.11
35	29.22^{a}±0.13	29.17^{a}±0.10	29.21^{a}±0.12	29.20^{a}±0.10
42	29.86^{a}±0.14	29.80^{a}±0.14	29.85^{a}±0.10	29.82^{a}±0.15
49	30.30^{a}±0.11	30.28^{a}±0.15	30.25^{a}±0.12	30.26^{a}±0.12
56	30.15^{a}±0.20	29.95^{a}±0.21	30.10^{a}±0.18	30.05^{a}±0.15

The significant difference was observed at $p<0.05$

Table.3 Weekly variation of pH (Mean±SD) in the treatment tanks and control

Treatments Days	C	T1	T2	T3
1	8.22^{a}±0.07	8.18^{a}±0.15	8.10^{a}±0.05	8.15^{a}±0.18
7	8.20^{a}±0.05	8.12^{a}±0.67	8.05^{a}±0.12	8.10^{a}±0.11
14	8.25^{c}±0.08	8.08^{b}±0.06	8.00^{a}±0.20	8.05^{a}±0.15
21	8.40^{c}±0.13	8.05^{b}±0.12	7.90^{a}±0.12	7.94^{a}±0.16
28	8.52^{c}±0.05	7.95^{b}±0.08	7.85^{a}±0.11	7.80^{a}±0.13
35	8.58^{c}±0.09	7.90^{b}±0.09	7.70^{a}±0.07	7.75^{a}±0.16
42	8.60^{c}±0.10	7.85^{b}±0.07	7.62^{a}±0.08	7.68^{a}±0.12
49	8.60^{c}±0.15	7.80^{b}±0.14	7.58^{a}±0.11	7.62^{a}±0.07
56	8.68^{c}±0.09	7.62^{b}±0.06	7.46^{a}±0.08	7.50^{a}±0.17

The significant difference was observed at $p<0.05$

Table.4 Weekly variation of total ammonia nitrogen (Mean±SD in mg/l) in the treatment tanks and control

Treatments Days	C	T1	T2	T3
7	0.20^{a}±0.05	0.41^{b}±0.06	0.43^{b}±0.02	0.39^{b}±0.02
14	0.27^{a}±0.08	0.45^{b}±0.67	0.48^{b}±0.07	0.42^{b}±0.12
21	0.34^{a}±0.06	0.49^{b}±0.09	0.56^{b}±0.06	0.52^{b}±0.05
28	0.38^{a}±0.03	0.56^{b}±0.08	0.61^{b}±0.03	0.59^{b}±0.03
35	0.42^{a}±0.02	0.60^{b}±0.00	0.67^{b}±0.07	0.63^{b}±0.05
42	0.46^{a}±0.04	0.67^{b}±0.03	0.71^{b}±0.04	0.68^{b}±0.06
49	0.50^{a}±0.02	0.70^{b}±0.04	0.79^{b}±0.06	0.75^{b}±0.08
56	0.54^{a}±0.08	0.82^{b}±0.07	0.84^{b}±0.06	0.88^{b}±0.03

The significant difference was observed at $p<0.05$

Table.5 Weekly variation of nitrite (Mean±SD in mg/l) in the treatment tanks and control

Treatments Days	C	T1	T2	T3
7	0.03^{a}±0.00	0.07^{b}±0.02	0.10^{b}±0.02	0.08^{b}±0.01
14	0.06^{a}±0.01	0.10^{b}±0.03	0.14^{b}±0.03	0.11^{b}±0.02
21	0.08^{a}±0.01	0.14^{b}±0.03	0.19^{b}±0.03	0.16^{b}±0.02
28	0.12^{a}±0.03	0.20^{b}±0.04	0.26^{b}±0.02	0.22^{b}±0.05
35	0.20^{a}±0.04	0.26^{b}±0.02	0.32^{b}±0.03	0.29^{b}±0.03
42	0.28^{a}±0.03	0.36^{b}±0.05	0.42^{b}±0.05	0.38^{b}±0.05
49	0.33^{a}±0.04	0.46^{b}±0.06	0.52^{b}±0.06	0.48^{b}±0.07
56	0.40^{a}±0.06	0.62^{b}±0.05	0.68^{b}±0.11	0.65^{b}±0.04

The significant difference was observed at $p<0.05$

Table.6 Weekly variation of nitrate (Mean±SD in mg/l) in the treatment tanks and control

Treatments / Days	C	T1	T2	T3
7	0.62^{a}±0.05	0.90^{b}±0.09	1.02^{c}±0.02	1.00^{c}±0.02
14	0.70^{a}±0.07	1.03^{b}±0.09	1.15^{c}±0.05	1.05^{c}±0.04
21	0.77^{a}±0.06	1.16^{b}±0.02	1.44^{c}±0.06	1.36^{c}±0.07
28	1.09^{a}±0.09	4.40^{b}±0.27	4.83^{c}±0.05	4.67^{c}±0.04
35	1.76^{a}±0.05	7.03^{b}±0.05	9.64^{c}±0.93	8.84^{c}±0.08
42	2.35^{a}±0.08	11.94^{b}±0.09	14.12^{c}±0.46	12.43^{c}±1.98
49	2.90^{a}±0.04	14.41^{b}±0.14	17.88^{c}±0.19	16.95^{c}±0.57
56	3.65^{a}±0.05	16.26^{b}±0.82	22.52^{c}±0.15	21.68^{c}±0.21

The significant difference was observed at $p<0.05$

Table.7 Hematological parameters (Mean±SD) observed in *O. niloticus* at the end of experiment

Treatments / Parameters	C	T1	T2	T3
RBC ($\times10^{6}$ cells/µL)	1.28^{a}±0.10	1.83^{b}±0.35	2.52^{c}±0.44	1.79^{b}±0.19
WBC ($\times10^{3}$ cells/µL)	54.20^{a}±5.68	70.03^{b}±3.579	81.55^{c}±3.10	77.30^{c}±0.62
Platelet count ($\times10^{3}$ cells/µL)	4.25^{a}±1.71	8.00^{b}±3.74	12.25^{c}±0.96	7.25^{b}±0.96
Hb (g/dL)	3.50^{a}±0.18	5.33^{b}±0.97	6.65^{c}±0.58	6.13^{c}±0.61
Hct (%)	19.45^{a}±3.72	26.10^{b} ±4.49	32.10^{c}±3.27	31.35^{c}±1.75
MCV(fL)	130.40^{a}±5.76	148.80^{b}±7.32	154.93^{c}±3.86	150.80^{b}±16.27
MCH(pg)	24.83^{a}±2.00	27.80^{b}±0.57	32.23^{c}±0.62	28.68^{b}±1.29
MCHC(g/dL)	18.85^{a}±0.76	22.02^{b}±0.57	24.23^{c}±0.62	21.73^{b}±1.24

The significant difference was observed at $p<0.05$

In the present study, the water quality variables were within the appropriate levels for the culture of Nile tilapia, *O. niloticus* (Azim and Little, 2008). At higher C/N ratio in biofloc systems, immobilization of inorganic nitrogen by hetertrophic bacteria will decrease the ammonia levels in biofloc systems (Lancelot and Billen, 1985; Avnimelech *et al.*, 1989). Similarly, in substrate based ponds, nitrifying bacteria develop on the substrate, which enhances nitrification keeping ammonia levels low. Thus, both biofloc and substrate based biofloc systems demands for more DO than the conventional culture systems (Hargreves, 2006). In the present study, the DO levels in biofloc and substrate based biofloc treatments were significantly lower than the control. Among the treatments, the DO levels in the both T2 and T3 were significantly lower than biofloc treatment (T1) indicating more nitrification process by bioflocs as well by periphyton in T2 and T3. The results on the levels of nitrogen species corroborate the results of the other studies (Azim and Little, 2008; Schveitzer *et al.*, 2013; Luo *et al.*, 2014; Ahmad *et al.*, 2016).

Hematological parameters are used as an index of health status in a number of fish species (Harikrishnan *et al.*, 2011), to detect physiological changes following different stress conditions (Agrawal and mahajan, 1980). The results of the current study of total RBC count were in agreement with the findings of Gupta *et al.*, (2008) in *L. rohita* fingerlings fed an immune-stimulant (Levan) and Ahmad (2016) in *L.rohita* fingerlings reared in biofloc culture systems. Similar results of Total WBC Count were also obtained by Sahoo and Mukherjee (1999) and Gupta *et al.*, (2008) in *L.rohita* fingerlings treated with immune-stimulants such as levamisole, ascorbic acid and Levan in biofloc systems using different carbon sources (Ahmad, 2016) reported increased number of WBC in *Catla* fish fed with yeast RNA,Ω-3 fatty acids and β- carotene.

Increased number of platelets is related to enhanced nonspecific immune system, as these cells are involved in defense system further to blood clotting (Martins *et al.*, 2008). Whereas, Ahmad (2016) observed increased hemoglobin levels in *L.rohita* reared in biofloc based systems in comparison to clear water control group. Mohan and Senthilkumar (2015) stated that, fish hematocrit values usually range between 20 – 35% and attain greater than 50% (Clark *et al.*, 1976). In the present study, the Hematocrit values obtained were within the above range and no anemic conditions were recorded in *O.niloticus.* Erythrocytes indices MCV, MCH and MCHC are used for the diagnosis of different forms of anemia. They are also used in the identification of RBC activities. In the present study, *O.niloticus* reared in biofloc + bamboo mat substrate has shown significantly higher values for MCV, MCH and MCHC and lowest values recorded in control group.

In conclusion the present study provides baseline data for validation of biofloc mediated enhancement of growth of Nile tilapia *Oreochromis niloticus*. Based on the experimental data we concluded that it is evident from the data that biofloc formation showed no deteriorating effect on water quality. Water quality parameters during the experimental period were normal for growth and health status of the fish. Toxic nitrogen species (TAN, NO2-N and NO3-N) were under tolerable limits of fish, under minimal water exchange. Increased levels of hematological parameters viz., total RBC, WBC, Platelet count, Hb, HCT, MCV, MCH and MCHC were observed in Nile tilapia fingerlings reared in biofloc cum substrate based and biofloc treatments than that of control. Among treatments, biofloc+bamboo mat substrate treatment has shown better hematological response. The present study revealed the suitability of both biofloc and biofloc cum substrate based systems in enhancing the production and non-specific immunity of Nile tilapia.

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