



## Impact of Biofloc technology on growth performance and biochemical parameters of *Oreochromis niloticus*.

Hossam Hwihiy, Amr Zeina, Mohammad Abu Husien, Khaled El-Damhougy

Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

Corresponding author: [hossam.hwihiy@azhar.edu.eg](mailto:hossam.hwihiy@azhar.edu.eg)

### ARTICLE INFO

#### Article History:

Received: Jan. 27, 2021

Accepted: Feb. 16, 2021

Online: Feb. 19, 2021

#### Keywords:

Biofloc,  
Hepatosomatic index,  
Spleenosomatic index,  
Hematology,  
Biochemistry,  
*Oreochromis niloticus*

### ABSTRACT

In the present study, the experiments were conducted during the period from June to October 2019 (20-weeks) to evaluate the effect of Biofloc technology (BFT) on mono sex Nile tilapia, *Oreochromis niloticus* fingerlings. It was focused on water quality, growth parameters, hepatosomatic index (HIS), spleenosomatic index (SSI), Hematology, and biochemistry. Triplicated treatments were designed, two Biofloc treatments with two fish densities (12 & 16 fish/200 L tank) vs two control groups. All treatments were fed with 3% of body weight in two equally divided meals. Rice bran and Molasse (1:1) were added as a carbon source to Biofloc treatments twice daily after one hour of main meals. Results indicated that total ammonia-N and Nitrite-N were significantly decreased at Biofloc treatments. Biofloc treatments showed significantly high average final body weight, better feed conversion ratio (FCR), and one-ninth water consumption. Biofloc addition had generally no significant difference of hematology, biochemistry, and hepatosomatic index (HSI), while spleenosomatic index (SSI) was significantly increased at high density only. It could be concluded that Biofloc treatments improve water parameters stability and fish growth performance while it decreased water consumption. High density (16 fish/200 L) is a more yield productive treatment.

### INTRODUCTION

Aquaculture plays a key role in eliminating hunger, promoting health, reducing poverty, in the developing countries (FAO., 2014). High growth rate of aquaculture is needed to solve the problem of feeding's protein shortage (Subasinghe, 2005; Gutierrez-Wing and Malone, 2006 and Matos *et al.* 2006). However, environmental and economical limitations can hamper this growth. Intensive aquaculture coincides with the pollution of the culture water by an excess of organic materials and nutrients that are likely to cause acute toxic effects and long-term environmental risks (Piedrahita, 2003). High-density culture in intensive systems requires high amounts of feed to be added to the systems. This will cause water quality deterioration due to the high concentrations of organic compounds (Avnimelech, 2007). Elevated concentrations of ammonia affect growth, oxygen consumption and even can eventually cause mortality of fish. Increased nitrite concentration negatively affects the growth performance and survival of fish (Mallasen and Valenti, 2006).

Biofloc technology (BFT) system can be an environmentally friendly strategy to establish a near to zero water exchange culture system while providing potentially consumable biomass to the cultured animal (Avnimelech, 2014 and Bossier & Ekasari, 2017). This operates on the principle of increasing carbon to nitrogen ratios, through the addition of an exogenous carbon source that consequently stimulates natural heterotrophic bacterial growth in the system (Hargreaves, 2006 and De-Schryver *et al.* 2008). These bacteria will then convert otherwise toxic nitrogenous metabolites into microbial biomass known as Biofloc aggregates (Avnimelech, 2014 and Ebeling *et al.* 2006). Biofloc aggregates consist of various organic materials such as bacteria, microalgae, uneaten food and zooplankton which can serve as a constant supply of additional nutrients for aquatic animals that are capable of collecting and consuming these small particles (Emerenciano *et al.* 2013 and Bossier & Ekasari, 2017).

So, the aim of this work is to establish the effect of Biofloc technology (BFT) system on the fish density, water quality (total ammonia-N, Nitrite-N and Nitrate-N) and fish quality focused on growth parameters, haematology and biochemistry of produced fishes.

## MATERIALS AND METHODS

### Fish experiment:

The study was conducted in twelve 250-Liter (L) with used 200-L circular tanks. Monosex fingerlings of *Oreochromis niloticus* with  $30.0 \pm 2.1$  g weight and  $9.5 \pm 1.1$  cm length reared for 20 weeks extended from 11 June to 30 October 2019 at the Aquaculture Lab, Animal House, Faculty of Science, Al-Azhar University, Cairo.

Fishes were fed with 30% protein floating pellets. The proximate analysis of basic diet composed of 29.8% protein, 7.5% lipid, 12.4% ash and 6.9% water content. All fishes were fed with 3% of their body weight as two equal diets daily at 9:00AM and 4:00 PM, seven days a week. The feeding rate were calculated fortnightly by measuring not less than 30% of fishes of each group.

### Experimental design

Fishes were grouped into two stocking densities each of three replicates; low stocking density (T1) consists of 12 fish ( $60 \text{ fish/m}^3$ ) and high stocking density (T2) consists of 16 fish ( $80 \text{ fish/m}^3$ ). All previous groups had molasses and rice brane (1:1) as a carbohydrate additional diet to achieve C: N equal to 15:1 according to (Avnimelech, 1999). Carbohydrate was fed after one hour of main meals with only addition of evaporation loss of water every week. Another six tanks (C1&C2 each of three replicates) were used as control without any additional carbohydrate and 50-70 % water was replaced every week to maintain the nitrogenous wastes under tolerable conditions.

### Water quality:

Temperature ( $^{\circ}\text{C}$ ) and pH were checked every day by portable pH meter Adwa. Floc volume were measured by Imhoff cone within 20 minutes of precipitation according to (Avnimelech, 1999). Dissolved oxygen (mg/L), were measured two times weekly by

Winkler titration method while total ammonia nitrogen (TAN), nitrite nitrogen (NO<sub>2</sub>-N) and nitrate nitrogen (NO<sub>3</sub>-N) were measured spectrophotometrically every week according to (APHA, 1995)

### **Measurements of growth performance**

Growth in weight (%), total weight gain (g/fish), average daily weight gain (mg/fish/day), specific growth rate (%/day), feed conversion ratio, Protein efficiency ratio (%) and feed efficiency of *Oreochromis niloticus* were determined according to (Recker., 1975 and Castell & Tiews., 1980) and using the following equations:

- Total weight gain (g/fish) = final fish weight (W<sub>F</sub>) - initial fish weight (W<sub>I</sub>)
- Average daily weight gain (mg /fish/day) = total weight gain (mg)/ duration period (days)
- Growth in weight (%) = Total weight gain (g) - Initial fish weight (g)
- Specific growth rate (SGR, % / day) = (Ln W<sub>F</sub>- Ln W<sub>I</sub>) \* 100/ duration period
- Food conversion ratio (FCR)= feed intake (g)/ total weight gain (g) (Tacon, 1987)
- Protein efficiency ratio (PER)= total gain (g)/ protein intake (g) (Davies & Morries, 1997)
- Protein intake (PI) = feed intake (g) × Protein% in the diet/100
- Feed efficiency (FE) = Weight gain (g) / Feed intake (g)

### **Hepatosomatic (H.S.I) & Splenosomatic (S.S.I) indices:**

Whole fish, liver and spleen were wet weighted to the nearest 0.01 g. ; these indices were calculated according to the following equations:

$$\text{Hepatosomatic index} = \frac{\text{wet weight of Liver (g)}}{\text{wet weight of fish (g)}} \times 100$$

$$\text{Spleenosomatic index} = \frac{\text{wet weight of Spleen (g)}}{\text{wet weight of fish (g)}} \times 100$$

### **Haematological and biochemical analysis:**

Total red blood cells (RBCs, 10<sup>6</sup> /ml), total white blood cells (WBCs, 10<sup>3</sup> /ml) and blood platelets (10<sup>3</sup> /ml) were counted according to (Dacie & Lewis, 2010). Hemoglobin concentration (HGB, g/dl) was determined spectrophotometrically based on cyanomethemoglobin method (blood was diluted in a Drabkin solution) then the concentration determined using standard curve (Noga, 2010).

Total protein was analyzed according to (Reinhold, 1953) and Albumin was analyzed by (Dumas *et al.* 1971) using a kit of Vitro Scient Company. Alanine amino transferase and Aspartate amino transferase (ALT and AST) were analyzed spectrophotometrically according to (White *et al.* 1970) using a Bio-Edwic kits.

### **Statistical analysis:**

The Shapiro-Wilk normality test and Bartlett's homoscedasticity test were employed at 5% significance. Three-way analysis of variance (ANOVA) was applied to the growth parameters and nutritional values using (SAS, 2003)

## RESULTS

### 1. Water quality

The data in **Table 1**, showed water quality parameters of tanks that contain *Oreochromis niloticus* reared at different densities of Biofloc versus control groups for 20 weeks. They were at an acceptable range. Water temperature values fluctuated between  $25.8 \pm 2.7$  at control low density group and  $25.6 \pm 2.7$  C° at control high density group. pH values fluctuated between  $7.6 \pm 0.15$  for control low density group and  $7.0 \pm 0.36$  for Biofloc high density group.

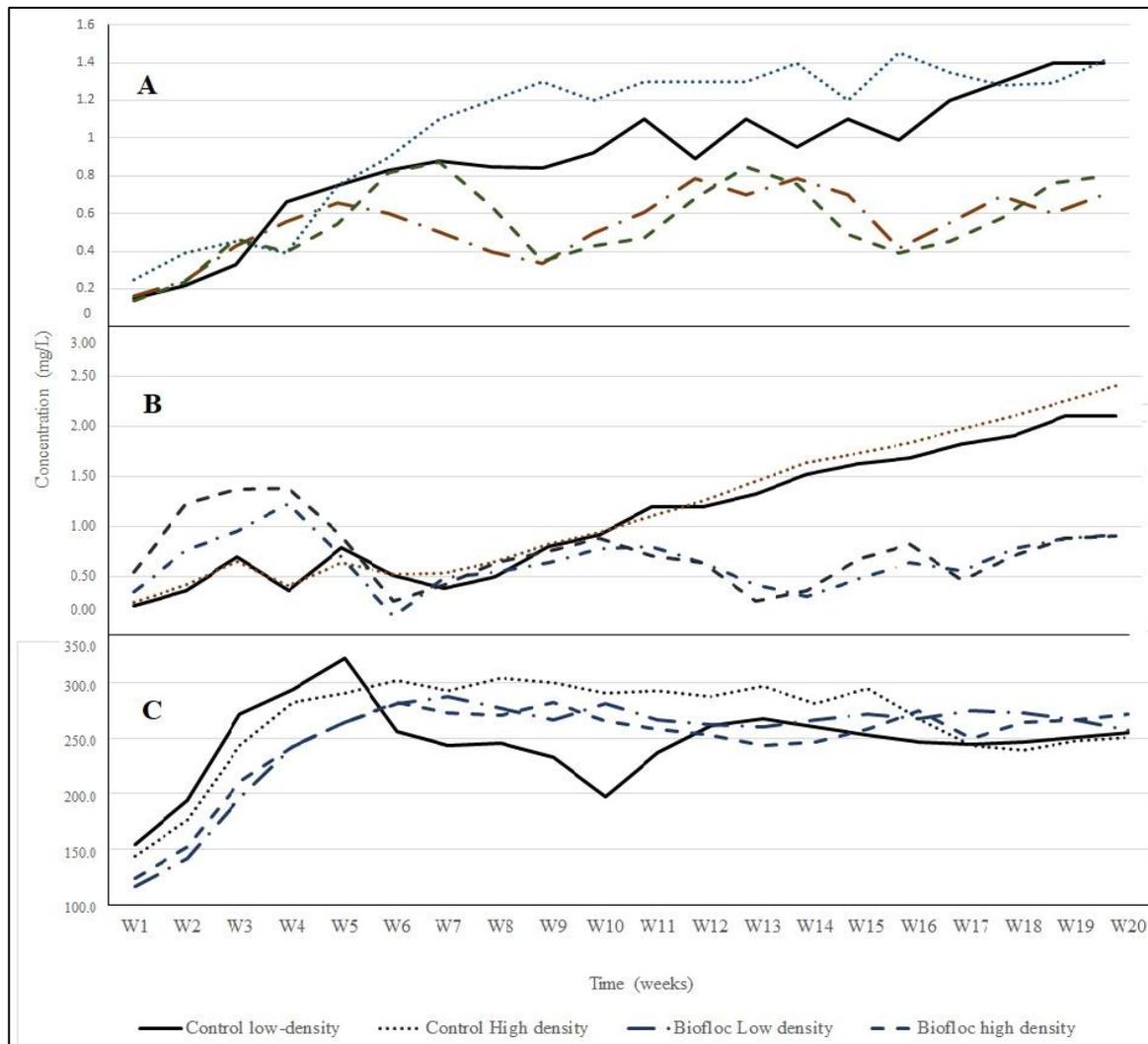
**Table 1. Water quality parameters (average  $\pm$  SD) of experimental groups**

Parameter	Density	Treatment		p-value
		Control	Biofloc	
Temp (C°)	Low density	$25.8 \pm 2.7$	$25.6 \pm 2.7$	0.063
	High density	$25.7 \pm 2.8$	$25.7 \pm 2.6$	0.055
pH	Low density	$7.6 \pm 0.15$	$7.4 \pm 0.16$	0.655
	High density	$7.1 \pm 0.37$	$7.0 \pm 0.36$	0.773
DO (mg/L)	Low density	$5.8 \pm 0.57$	$5.7 \pm 0.65$	0.420
	High density	$5.8 \pm 0.54$	$5.4 \pm 0.69$	0.093
TAN (mg/L)	Low density	$0.89 \pm 0.34$	$0.55 \pm 0.17^*$	> 0.001
	High density	$1.06 \pm 0.38$	$0.55 - 0.20^*$	> 0.001
Nitrite-N (mg/L)	Low density	$1.10 \pm 0.61$	$0.65 \pm 0.25^*$	> 0.001
	High density	$1.18 \pm 0.67$	$0.74 \pm 0.32^*$	> 0.001
Nitrate-N (mg/L)	Low density	$246.8 \pm 34.4$	$251.3 \pm 44.8$	0.730
	High density	$266.6 \pm 41.8$	$247.8 \pm 40.3$	0.166
FV ml/L	Low density	$1.7 \pm 0.5$	$19.0 \pm 4.7^*$	> 0.001
	High density	$2.6 \pm 0.6$	$21.7 \pm 5.0^*$	> 0.001

DO= Dissolved oxygen, TAN= total ammonia Nitrogen and FV= Floc volume. \* significant at ( $p < 0.05$ ).

Dissolved oxygen concentration ranged between  $5.8 \pm 0.57$  and  $5.4 \pm 0.69$  mg/L for low density control group and high density Biofloc group respectively. Total ammonia nitrogen (TAN) concentrations ranged between  $1.06 \pm 0.38$  and  $0.55 \pm 0.17$  mg/L for control high density and Biofloc low density groups, respectively. Nitrite-N concentrations fluctuated between  $1.18 \pm 0.67$  and  $0.65 \pm 0.25$  mg/L for control high density and Biofloc low density groups, respectively. Nitrate-N concentrations fluctuated between  $266.6 \pm 41.8$  and  $246.8 \pm 34.4$  mg/L for control high-density and low-density groups, respectively. Whereas the Floc volume measured were fluctuated between  $21.7 \pm 5.0$  and  $1.7 \pm 0.5$  ml/L for low density control and high density Biofloc groups, respectively (Table 1 and Figure 1).

Statistical analysis revealed that, no significance in temperature, pH, DO and Nitrate-N between Biofloc and control groups while total ammonia, Nitrite-N and Floc volume revealed a significant difference at all cases ( $p < 0.05$ ).



**Figure 1.** Nitrogen waste compounds, TAN (A), Nitrite-N (B) and Nitrate-N (C) of tanks of *Oreochromis niloticus* reared at different experimental groups for 20 weeks.

## **2. Growth parameters.**

Data in **Table 2.** revealed that final body weight values fluctuated between  $192.9 \pm 20.0$  and  $168.3 \pm 17.5$  g/fish for Biofloc high density and control high density groups, respectively. Total weight gain was fluctuated between  $164.9 \pm 17.2$  g/fish for Biofloc high density and  $139.5 \pm 14.8$  g/fish for control high density group. Average daily weight gain values ranged between  $1175.9 \pm 123.1$  and  $996.4 \pm 106$  mg/fish/day for Biofloc high density and control high density groups, respectively. Specific growth rate values ranged between  $1.4 \pm 0.1$  and  $1.3 \pm 0.04$  %/day for Biofloc high density and control high density groups, respectively.

Feeding conversion ratio fluctuated between  $1.9 \pm 0.2$  and  $1.4 \pm 0.2$  for high density control group and low density Biofloc groups, respectively. Protein efficiency ratios ranged between  $2.3 \pm 0.3$  and  $1.8 \pm 0.2$  for Biofloc low density and control high density groups, respectively. Feed efficiency fluctuated between  $0.7 \pm 0.1$  and  $0.5 \pm 0.1$  for Biofloc high density and control low density groups, respectively (**Table 2**).

Hepatosomatic index values fluctuated between  $3.35 \pm 0.69$  % and  $1.8 \pm 0.52$  % for high density Biofloc and control groups respectively. Splenosomatic index values fluctuated between  $0.28 \pm 0.09$  % and  $0.07 \pm 0.04$  % for high density Biofloc and control groups respectively (**Table 2**).

All growth parameters revealed significant difference between Biofloc and control groups at high density while at low density only final body weight, Specific growth rate (SGR) and FCR were significant.

PER and FE were significantly different between Biofloc and control groups at all cases. Hepatosomatic index was non significant between Biofloc and control groups, while Splenosomatic index was non significant only at low density.

**Table 2.** Growth parameters and protein efficiency of *Oreochromis niloticus* monosex condensed with different densities in Biofloc vs control groups for 20 weeks.

Parameter	Density	Treatment		p-value	
		Control	Biofloc		
Growth parameters	Final body weight (g)	Low	$171.5 \pm 15.3$	$187.9 \pm 20.7^*$	0.045
		High	$168.3 \pm 17.5$	$192.9 \pm 20.0^*$	>0.001
	Total weight gain (g/fish)	Low	$142.6 \pm 14.6$	$155.2 \pm 17.4$	0.079
		High	$139.5 \pm 14.8$	$164.6 \pm 17.2^*$	>0.001
	ADWG (mg /fish/day)	Low	$1018.3 \pm 104.2$	$1108.3 \pm 124.3$	0.573
		High	$996.4 \pm 106$	$1175.9 \pm 123.1^*$	>0.001
	SGR (%/day)	Low	$1.3 \pm 0.1$	$1.3 \pm 0.1$	0.548
		High	$1.3 \pm 0.04$	$1.4 \pm 0.1^*$	>0.001
	FCR	Low	$1.8 \pm 0.2$	$1.4 \pm 0.2^*$	0.001
		High	$1.9 \pm 0.2$	$1.6 \pm 0.2^*$	0.001
Protein efficacy ratio (PER)	Low	$1.9 \pm 0.2$	$2.3 \pm 0.3^*$	>0.001	
	High	$1.8 \pm 0.2$	$2.0 \pm 0.2^*$	>0.001	
Feed efficacy (FE)	Low	$0.6 \pm 0.1$	$0.7 \pm 0.1^*$	>0.001	
	High	$0.5 \pm 0.1$	$0.6 \pm 0.1^*$	>0.001	
Indices	Hepatosomatic	Low	$2.39 \pm 0.58$	$2.44 \pm 0.27$	0.914
		High	$1.80 \pm 0.52$	$3.35 \pm 0.69$	0.065
	Spleenosomatic	Low	$0.09 \pm 0.03$	$0.16 \pm 0.11$	0.398
		High	$0.07 \pm 0.04$	$0.28 \pm 0.09^*$	0.043

ADWG=Average daily weight gain, SGR=Specific growth rate, FCR=Food conversion ratio, PER=Protein efficiency ratio and FE=Feed efficiency.

### **3. Haematology and biochemistry**

Haematological parameters of *Oreochromis niloticus* monosex fingerlings reared at BFT vs control groups were given in **Table 3**, and it revealed that: White blood cells (WBC's) count fluctuated between  $54.7 \pm 2.0$  and  $40.8 \pm 1.6$  cell $\times 10^3$  /ml for Biofloc low density and control low density groups respectively. Red blood corpuscles (RBC's) count fluctuated between  $2.0 \pm 0.6$  and  $1.4 \pm 0.4$  cell $\times 10^6$  /ml for control low density and control high density groups respectively. Hemoglobin (HGB) concentrations ranged between  $9.7 \pm 2.7$  and  $7.3 \pm 2.6$  g/dl for control high density and control low density

groups respectively. Platelets count fluctuated between  $210.0 \pm 88.6$  and  $93.8 \pm 11.1$  cell $\times 10^3$  /ml for control high density and Biofloc low density groups, respectively. All hematological parameters revealed non-significant difference between Biofloc and control groups except WBC's at low density groups.

Total proteins concentrations ranged between  $3.83 \pm 0.20$  and  $3.57 \pm 0.14$  g/dl for control high density and Biofloc low density groups, respectively. Albumin concentrations ranged between  $1.20 \pm 0.22$  and  $0.97 \pm 0.14$  g/dl for control low density and Biofloc high density groups, respectively. Alanine aminotransferase (ALT) activity fluctuated between  $87.5 \pm 15.1$  and  $69.1 \pm 13.9$  IU/ml for control high density and control low density groups respectively. Aspartate aminotransferase (AST) activity fluctuated between  $97.3 \pm 13.5$  and  $75.7 \pm 15.6$  IU/ml for Biofloc low density and Biofloc low density groups, respectively. All biochemical investigations revealed non-significant difference between Biofloc and control groups (**Table 3**).

**Table 3.** Hematological and biochemical parameters of *Oreochromis niloticus* monosex fingerlings reared at BFT vs Control groups for 20 weeks.

Parameter	Density	Treatment		p-value	
		Control	Biofloc		
Haematology	WBCs ( $10^3$ cells/ml)	Low	$40.8 \pm 1.6$	$54.7 \pm 2.0$ *	0.001
		High	$44.4 \pm 2.7$	$45.3 \pm 3.2$	0.752
	RBCs ( $10^6$ cells/ml)	Low	$1.4 \pm 0.4$	$1.8 \pm 0.6$	0.734
		High	$2.0 \pm 0.6$	$1.6 \pm 0.7$	0.524
	HGB (g/dl)	Low	$7.3 \pm 2.6$	$8.8 \pm 3.0$	0.947
		High	$9.7 \pm 2.7$	$7.8 \pm 2.8$	0.502
Platelets ( $10^3$ cells/ml)	Low	$120.3 \pm 47.2$	$93.8 \pm 11.1$	0.546	
	High	$210.0 \pm 88.6$	$124.3 \pm 33.1$	0.303	
Biochemistry	Total proteins	Low	$3.73 \pm 0.20$	$3.57 \pm 0.14$	0.124
		High	$3.83 \pm 0.20$	$3.72 \pm 0.16$	0.232
	Albumin	Low	$1.20 \pm 0.22$	$0.98 \pm 0.11$	0.066
		High	$1.07 \pm 0.12$	$0.97 \pm 0.14$	0.218
	AIT	Low	$69.1 \pm 13.9$	$81.5 \pm 11.9$	0.128
		High	$87.5 \pm 15.1$	$78.3 \pm 14.5$	0.28
	AsT	Low	$85.1 \pm 13.3$	$97.3 \pm 13.5$	0.144
		High	$88.3 \pm 14.4$	$75.7 \pm 15.6$	0.169

WBC's = White blood cells, RBC's= Red blood corpuscles and HGB= Haemoglobin.

\*= Significant difference ( $P < 0.05$ ).

## DISCUSSION

The present study revealed that, Water quality parameters; DO, temperature, pH, TAN, Nitrite-N and Nitrate-N; were within the normal limits of fish culture. This results agreed with the finding of **Stone & Thomforde, (2004)** and **Emerenciano et al. (2013)**. Temperature, pH, DO and Nitrate-N didn't show significant difference between Biofloc and control treatments that agreed with **El-Shafiey et al. (2018)**, while **Azim & Little, (2008)** observed significant decrease at pH due to respiration and the processes of

degradation, assimilation and nitrification carried out by microorganisms, including autotrophic bacteria. The present work mentioned low of DO at Biofloc high density (T2) to 4.3 mg/dl that may be due to an increase in the metabolic activity of the aerobic bacteria present in the culture environment because of addition of Carbohydrate organic load that agreed with **De-Schryver *et al.* (2008)**.

The present study revealed that Nitrate-N gradually increased at first four weeks as a result of nitrification, then showed slight fluctuation that may be due to Denitrification that can convert nitrate to nitrogen gas by microbial process that agreed with **Ray *et al.* (2010)**.

Generally, TAN, Nitrite-N and Floc volume revealed a significant difference at all cases. At first 6 weeks there were no differences between behavior of TAN and NO<sub>2</sub>-N. Biofloc treatments slightly fluctuated and didn't increase after the 6<sup>th</sup> week. Because of carbohydrate addition that either assimilate nitrogen wastes by heterotrophic bacteria to bacterial biomass or convert nitrite to nitrate. This result agrees with **Avnimelech (2014)**, where the NO<sub>3</sub> produced can also be reduced to NO<sub>2</sub> and NH<sub>3</sub> by the process of denitrification (**Luo *et al.* 2014**).

Growth performance of *Oreochromis niloticus* fingerlings at the current study showed significance difference in all the treatments ( $P < 0.05$ ) at high density while at low density only FCR, PER and FE showed significant difference. This could be due to the Biofloc produced a medium rich in organic matter made of bacteria, phytoplankton, protozoa, filamentous bacteria, nematodes, ciliates, flagellates and rotifers which serve as natural food and contains a high protein level ( $15 \pm 2$  % protein, unpublished data) for *O. niloticus* and thus improving growth performance and survival for the cultured fish in the system. The treatments resulted in increased growth rate and lower FCR compared to control tanks. A non-significant difference between different carbon sources on both tilapia and shrimps was found (**Serra *et al.* 2015; Da-Silva *et al.* 2017; Khanjani *et al.* 2017 and El-Shafie *et al.* 2018**). On the other hand, growth performance of *Peneus monodon* was higher with addition of jaggery in Biofloc system than other carbon sources like cane sugar and molasses (**Sakkaravarthi & Sankar, 2015**). Meanwhile, mixture of different carbon sources (60% molasses + 20% corn flour + 20% wheat bran) positively affected the growth of *Litopenaeus vannamei* in comparison with molasses as a single carbon source (**Wang *et al.* 2016**).

The present results revealed that Biofloc system increases utilization of protein and converts most of the diet to the body mass in these treatments. Similar finding conquers with **Emerenciano *et al.* (2013)** result which demonstrated that Biofloc can enhance the digestion and utilization of artificial feeds as well as improving the growth performance of aquatic animals. Molasses containing sucrose, a disaccharide, which is more effective compared to other carbohydrate sources that contain starch, which is a polysaccharide. **Ballester *et al.* (2010)** showed that Biofloc is a good food, low cost strategy which is better than traditional culture system because the formed flocs have high protein, lipids, carbohydrates and ashes content and can be used as food in aquaculture industry. It is possible that Biofloc stimulates digestive enzyme activity (**Avnimelech, 1999 and Kuhn *et al.* 2010**). This could contribute to increased growth of Nile tilapia at Biofloc against control treatments. As well as some other studies demonstrated that, Bioflocs improved the growth performance and proteins utilization as

they produce exogenous microbial enzymes such as proteases (Arnold *et al.* 2009; Xu & Pan, 2012 and Zhang *et al.* 2016) and also induce the generation of endogenous digestive enzymes (Xu & Pan, 2014 and Najdegerami *et al.* 2016) that facilitating the digestion and absorption of feed nutrients. Water consumption in the present work at Biofloc treatments was 9 times lower than control once. This result agrees with De-Lima *et al.* (2018) who found decrease of water consumption with 11.5 times compared with control treatments.

Hepatosomatic index (HSI) and transaminases ALT and AST were not significantly different between Biofloc and control treatments. The same results were obtained by many authors (El-Mohammady *et al.* 2015 and Romano *et al.* 2018). Adineh *et al.* (2019) noticed that HSI was significantly lower in the clear water group than all Biofloc groups. An increased mobilization of liver reserves caused by higher energy demands under high density conditions can be responsible for lower HSI in clear water group (Trenzado *et al.* 2007).

At the present work, Splenosomatic index (SSI) showed no significant difference between Biofloc and control treatments at low density but at high density there were significant difference that may be due to condensed bacterial biomass which may stimulate lymph organ (spleen) to be enlarged. This splenomegaly slightly effected on hematological parameters. Hemre *et al.* (2005) and El-Mohammady *et al.* (2015) mentioned that soya bean meal might cause early release of immature erythrocytes which may be responsible for increased spleen size enlargement of *Oreochromis niloticus* whose feed on plant origin protein.

Blood biochemistry indices are useful tools that aid in indicating the general state of fish health which can differ with water characteristic and nutritional state (Dawood *et al.* 2015 and El Basuini *et al.* 2017). Overall, blood parameter values recorded in the present study are within the acceptable limits of the Nile tilapia, the same results were mentioned by Ayyat *et al.* (2017) and Mahmoud & El-Hais, (2017). The present work revealed non significant difference between Biofloc and control groups at all hematological and biochemical parameters except WBC's at low density. However, there were slightly decrease at hemoglobin and platelets that may be due to splenomegaly. These results were partially in agreement with Azim & Little, (2008) and Long *et al.* (2015) where their results revealed no significant effect on all hematological parameters between Biofloc and control groups of *Oreochromis niloticus*. On the other hand, significantly decreased hematological indices (RBC's, WBC's, HGB and blood platelets) were reported by increasing stocking density of Nile tilapia (Mehrim, 2009; Kpundeh *et al.* 2013 and Zaki *et al.* 2020). On the other side, Xu & Pan, (2013) found that the total hemocyte count in shrimp was significantly higher in Biofloc treatments than in the control group due to different experimental conditions.

Total protein and albumin biochemical parameters appeared non significant difference among Biofloc and control groups at the present work. The same results were mentioned by Long *et al.* (2015) while Verma *et al.* (2016) mentioned significantly difference of total proteins and albumin of *Labeo rohita* reared at Tapioca based Biofloc system while wheat, corn, sugar bagasse based Biofloc system were non significant.

## CONCLUSION

The present study concluded that Biofloc aquaculture technology improves water parameters stability and fish growth performance with the same fish quality. As well as, the high stocking density (80 fish/m<sup>3</sup>) is more yield productive treatment.

## REFERENCES

- Adineh, H.; Naderi, M.; Hamidi, M. K. and Harsij, M. (2019).** Biofloc technology improves growth, innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. *Fish & Shellfish Immunology*, **95**: 440-448.
- APHA (1995).** Standard Methods for Examination of Water and Wastewater. American Public Health Association, 18: 769. Washington D.C., USA.
- Arnold, S. J.; Coman, F. E; Jackson, C. J. and Groves, S. A. (2009).** High- intensity, zero water exchange production of juvenile tiger shrimp, *Penaeus monodon*: An evaluation of artificial substrates and stocking density. *Aquaculture*, **293**(1, 2): 42-48.
- Avnimelech, Y. (1999).** Carbon/Nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, **176**: 227–235.
- Avnimelech, Y. (2007).** Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. *Aquaculture*, **264**: 140–147.
- Avnimelech, Y. (2014).** Biofloc Technology-A Practical Guidebook, 3<sup>rd</sup> ed. The World Aquaculture Society, Baton Rouge, United States:285.
- Ayyat, M.S.; Mahmoud, H.K.; El-Hais, A.E. and Abd El-Latif, K. (2017).** The role of some feed additives in fish fed on diets contaminated with Cadmium. *Environmental Science and Pollution*, **24**: 23636–23645.
- Azim, M. E. 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**(2): 29-35.
- Ballester, E.; Abreu, P.; Cavalli, R.; Emerenciano, M.; Abreu, L. and Wasielesky, 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. *Aquaculture Nutrition*, **16**(1): 163–172,
- Bossier, P. and Ekasari, J. (2017).** Biofloc technology application in aquaculture to support sustainable development goals. *Microbial biotechnology*, **10**(5): 1012-1016.
- Castell, J.D. and Tiews, K. (1980).** Report of the EIFAC, IUNS and ICES Working Group on the Standardization of Methodology in Fish Research. Hamburg, FRG, Germany, IFAC Tech., **3**: 24.

- Dacie, J. V. and Lewis, S. M. (2010).** Preparation and Staining Methods for Blood and Bone-Marrow Films. In: Dacie JV, Lewis SM, eds. Practical Haematology. 8th ed. Churchill Livingstone. Pp: 83-96.
- Da-Silva, M.; De-Alvarenga, É.; Alves, G.; Manduca, L.; Turra, E.; De-Brito, T. and Teixeira, E. (2018).** Crude protein levels in diets for two growth stages of Nile tilapia (*Oreochromis niloticus*) in a Biofloc system. *Aquaculture Research*, **49**(8): 2693-2703.
- Davies, S. J. and Morris, P. C. (1997).** Influence of multiple amino acid supplementation on the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed soya based diets. *Aquaculture Research*, **28**(1): 65-74.
- Dawood, M.; Koshio, S.; Ishikawa, M. and Yokoyama, S. (2015).** Effects of heat killed *Lactobacillus plantarum* (LP20) supplemental diets on growth performance, stress resistance and immune response of Red Sea bream, *Pagrus major*. *Aquaculture*, **442**: 29-36.
- De-Lima, E.; De-Souza, R.; Girao, P.; Braga, I. and Correia, E. (2018).** Culture of Nile tilapia in a Biofloc system with different sources of Carbon. *Revista Ciência Agronômica*, **49**(3): 458-466.
- De-Schryver, P.; Crab, R.; Defoirdt, T.; Boon, N. and Verstraete, W. (2008).** The basics of bioflocs technology: the added value for aquaculture. *Aquaculture*, **277**(3-4): 125-137.
- Doumas, B. T.; Watson, W. A. and Biggs, H. G. (1971).** Albumin standards and the measurement of serum albumin with Bromocresol green. *Clinica Chimica Acta*, **31**(1): 87-96.
- Ebeling, J.; Timmons, M. B. and Bisogni, J. J. (2006).** Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, **257**(1-4): 346-358.
- El Basuini, M.; El-Hais, A.; Dawood, M.; Abou-Zeid, A.; EL-Damrawy, S.; Khalafalla, M.; Koshio, S., Ishikawa, M. and Dossou, S. (2017).** Effects of dietary copper nanoparticles and vitamin C supplementations on growth performance, immune response and stress resistance of red sea bream, *Pagrus major*. *Aquaculture Nutrition*, **23**: 1329–1340.
- El-Mohammady, E. Y.; Goda, A. S.; Mohamed, A.; Mohamed, R. A. and Mohamed, M. S. (2015).** Partial and total substitution of dietary fishmeal by conventional or genetically modified soybean meal with or without formic acid supplementation for *Oreochromis niloticus* fingerlings. *Journal of Scientific Research in Science*, **32**(2): 337-359.
- El-Shafiey, M. H. M.; Mabroke, R. S.; Mola, H. R. A.; Hassaan, M. S. and Suloma, A. (2018).** Assessing the suitability of different carbon sources for Nile tilapia, *Oreochromis niloticus* culture in BFT system. *AAACL Bioflux*, **11**(3): 782-795.
- Emerenciano, M.; Gaxiola, G. and Cuzon, G. (2013).** Biofloc Technology (BFT): A review for aquaculture application and animal food industry. In: *Biomass Now - Cultivation and Utilization*, 301–328.

- FAO (2014).** The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations. Opportunities and Challenges. 223.
- Gutierrez-Wing, M. T. & Malone, R. F. (2006).** Biological filters in aquaculture: trends and research directions for freshwater and marine applications. *Aquaculture Engineering*, **34**(3): 163-171.
- Hargreaves, J. A. (2006).** Photosynthetic suspended-growth systems in aquaculture. *Aquaculture Engineering*, **34**: 344–363 .
- Hemre, G.I.; Sanden, M.; Bakke-Mckellep, A.M.; Sagstad, A. and Krogdahl, A. (2005).** Growth, feed utilization and health of Atlantic salmon *Salmo salar L.* fed genetically modified compared to non-modified commercial hybrid soybeans. *Aquaculture Nutrition*, **11**: 157-167.
- Khanjani M. H.; Sajjadi M. M.; Alizadeh M. and Sourinejad I. (2017).** Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a Biofloc system: the effect of adding different carbon sources. *Aquaculture Research*, **48**(4): 1491-1501.
- Kpundeh, M. D.; Xu, P.; Yang, H.; Qiang, J. and He, J. (2013).** Stocking densities and chronic zero culture water exchange stress' effects on biological performances, hematological and serum biochemical indices of gift tilapia juveniles (*Oreochromis niloticus*). *J Aquac Res Development*, **4**(189): 2.
- Kuhn, D. D.; Lawrence, A. L.; Boardman, G. D.; Patnaik, S.; Marsh, L. and 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.
- 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. *Aquaculture*, **422**: 1–7.
- Mahmoud, H. and El-Hais, A.M.A. (2017).** Effect of dietary garlic (*Allium sativum*) supplementation on Nile tilapia *Oreochromis niloticus* juveniles performance under two stocking densities. *Egypt J. Nutr. Feed.*, **20**: 115–124.
- Mallasen, M. and Valenti, W. C. (2006).** Effect of nitrite on larval development of the giant river prawn, *Macrobrachium rosenbergii*. *Aquaculture*, **261**(4): 1292-1298.
- Matos, J.; Costa, S.; Rodrigues, A.; Pereira, R. and Pinto, I. S. (2006).** Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. *Aquaculture*, **252**(1): 31-42.
- Mehrim, A.I. (2009).** Effect of Dietary Supplementation of Biogen® (Commercial Probiotic) on Mono-Sex Nile tilapia *Oreochromis niloticus* under Different Stocking Densities. *J. Fish. Aquat. Sci.*, **4**: 261–273.

- 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.
- Noga, E. J. (2010).** *Fish Disease Diagnosis and Treatment*, 2<sup>nd</sup> ed. Wiley-Blackwell, Ames, Pp: 519.
- Piedrahita, R.H. (2003).** Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, **226**(1): 35–44.
- Ray, A.; Venero, J.; Browdy, C. and Leffler, J. (2010).** Simple settling chambers aid solids management in Biofloc system. *Global Aquaculture advocate*, 28-30.
- Recker, W.E. (1975).** Computation and interpretation of biological statistics of fish populations. *Fish. Res. Board Can. Bull.*, **191**: 1-382.
- Reinhold, J.G. (1953).** Manual Determination of Serum Total Protein, Albumin and Globulin Fractions by Biuret Method. In: Reiner, M. (Ed.), *Standard Method of Clinical Chemistry*. Academic Press, New York. Pp:88.
- Romano, N.; Dauda, A. B.; Ikhsan, N.; Karim, M. and Kamarudin, M. S. (2018).** Fermenting rice bran as a carbon source for Biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles. *Aquaculture Research*, **49**(12): 3691-3701.
- Sakkaravarthi, K. and Sankar, G. (2015).** Identification of effective organic carbon for Biofloc shrimp culture system. *Journal of Biological Sciences*, **15**:144-149.
- SAS, (2003).** Campus Drive, SAS Institute Inc, Cary, NC USA: 27513:2414.
- Serra, F.P.; Gaona, C.A.P.; Furtado, P.S.; Poersch, L.H. and Wasielesky, W. (2015).** Use of different carbon sources for the Biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquaculture International*, **23**: 1325–1339.
- Stone, N. M. and Thomforde, H. K. (2004).** *Understanding Your Fish Pond Water Analysis Report*. Cooperative Extension Program, University of Arkansas at Pine Bluff, US Department of Agriculture and Country governments Cooperating. Pp:1-4.
- Subasinghe, R. (2005).** Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive Veterinary Medicine*, **67**(2, 3): 117-124.
- Tacon, A. (1987).** The essential nutrients. In: *The Nutrition and Feeding of Farmed Fish and Shrimp A Training Manual*, FAO, **61**: 117-130.
- Trenzado, C. E.; Higuera, M. and Morales, A. E. (2007).** Influence of dietary vitamins E and C and HUFA on rainbow trout (*Oncorhynchus mykiss*) performance under crowding conditions. *Aquaculture*, **263**: 249-258.
- Verma, A. K.; 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.

- Wang, C.; Pan, L.; Zhang, K.; Xu, W.; Zhao, D. and Mei, L. (2016).** Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. *Aquaculture Research*, **47**(10): 3307-3318.
- White, B. A.; Erickson, M. M. and Steven, S. C. (1970).** Chemistry for Medical Technologists, 3<sup>rd</sup> Ed C. V., Mosby Company Saint Louis, USA. Pp: 293 – 296.
- Xu, W. J. and Pan, L. Q. (2012).** Effect 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*. **356**: 147-152.
- Xu, W.J. and Pan, L.Q. (2013).** Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in Biofloc-based culture tanks manipulating high C/Nratio of feed input. *Aquaculture* **412**: 117–124.
- Xu, W. J. and Pan, L. Q. (2014).** Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange Biofloc-based culture tanks. *Aquaculture*, **426**: 181–188.
- Zaki, M. A.; Alabssawy, A. N.; Nour, A. E. A. M.; El Basuini, M. F.; Dawood, M. A.; Alkahtani, S. and Abdel-Daim, M. M. (2020).** The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under Biofloc conditions. *Aquaculture Reports*, **16**: 100282.
- Zhang, N.; Luo, G.; Tan, H.; Liu, W. and Hou, Z. (2016).** Growth, digestive enzyme activity and welfare of tilapia (*Oreochromis niloticus*) reared in a Biofloc-based system with poly- $\beta$ - hydroxybutyric as a carbon source. *Aquaculture*, **464**: 710–717.