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Biofloc effects on body composition, plasma protein, lipid profile, zooplankton community, and economics of Nile Tilapia fingerlings reared under different stocking densities

Mohamed M. Said[1], Esraa M. Taha[2]
1. Faculty of Fish Resources, Suez University, Egypt.
2. Faculty of Agriculture, Suez Canal University, Egypt.
*Corresponding author: msaid226@yahoo.com

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ABSTRACT

This study aimed to investigate the effects of biofloc on body composition, plasma protein, lipid profile, zooplankton community, and economics of Nile tilapia fingerlings in different stocking densities. Three stocking densities (200, 300 and 400 fish/m^3) under biofloc system (BS) and clear system (CS) were applied. Nile tilapia with an initial body weight of 20.35 ± 0.35 grams were stocked in 18 cylindrical fiberglass tanks with a water volume of 50 liters for 75 days. Starch was added on Biofloc system treatments as a carbon source to set the C / N ratio at 15/1. Body proximate composition showed that the highest protein and ash content were recorded in the highest stocking density (400/m^3). The highest fat content was noticed in the lowest density of 200/m^3. For system conditions, the biofloc system showed significantly higher dry matter, fat, and ash content, compared to the clear water system. Biofloc sample analysis showed an increase in protein, fat, and ash contents with increasing stocking density. The highest stocking density of 400/ m^3 showed the highest significant (P<0.05) total protein, albumin, and globulin. Plasma total cholesterol, triglyceride, HDL, and LDL were all significantly higher in the lowest density (200/m^3) than the other treatments. Biofloc system results recorded significantly higher total protein, albumin, globulin albumin/globulin ratio than the clear system. In addition, cholesterol triglyceride, and LDL were significantly increased with BFT treatments. All biofloc treatments showed higher zooplankton count, when compared with the clear system. Under biofloc conditions, increasing stocking density led to an elevation in zooplankton count as the highest was recorded for a density of 400 fish/m^3. Three groups of organisms were identified, Rotifera, Protozoa, and Copepoda. Economical benefits in terms of feed costs and relative feed costs per kg of fish were recorded in the treatments of (BS) with superiority of stocking density of 200 fish/ m^3 under these experimental conditions.

INTRODUCTION

In the last decades, aquaculture has become the best option for providing sustainable seafood. Its high rate of return on investment has attracted farmers and investors to the intensification systems with the application of modern technologies in order to increase profits **(Towers, 2015; Dawood *et al.*, 2016).** Intensive farming depends on high fish densities with the use of large quantities of high-protein levels diets (25–55%) aiming for increasing productivity in closed or semi closed systems to overcome the limit of water and lands resources **(Delong *et al.*, 2009; Dawood, 2016).**

High stocking densities combined with highly nitrogenous diets in intensive fish culture negatively affect the water quality, especially the accumulation of inorganic nitrogen forms (NH_3 and NO_2) **(Hargreaves and Tucker, 2004).** The common approaches to maintain water from deterioration and avoid nitrogen increases are through water exchange, the use of nitrifying biofilters and the microorganisms that grow while using a carbon sources **(Avnimelech and Kochba, 2009).** For decades, re-circulating system (RAS) has been considered as the main application for intensive rearing of several species. However, operating and implementation costs of all structure considered highfor tilapia culture, biofloc technology (BFT) was more effective in terms of cost–benefit than RAS **(Luo *et al.*, 2014).**

The BFT provides the intensive aquaculture with no or minimum water renew, and reduces its environmental impact **(Poli *et al.*, 2019).** In this system, the management of the microbial community is determinanted to keep the water quality, especially the growth of heterotrophic bacteria; through the complementary carbon source, which stimulates its growth and improves the process of removing inorganic nitrogen from water, besides allowing its transformation into bacterial biomass **(Robinson *et al.*, 2019).**

In order to determine the optimum density under the biofloc system, some criteria is needed to be examined to determine the effects of stress on fish. Chemical composition, plasma protein and lipid profile of Nile tilapia (*Oreochromis niloticus*) fingerlings are useful tools that indicate the general statof fish health, which can differ with water quality and nutritional status **(El Basuini *et al.*, 2017).** In this context, the objective of this experiment was to study the effect of different fish stocking densities under BFT application on the total zooplankton count, chemical composition, plasma protein and lipid profile of Nile tilapia (*Oreochromis niloticus*) fingerlings.

MATERIALS AND METHODS

1. Location and duration

The experiment was conducted at the experimental Fish Lab, Department of Animal and Fish Resources, Faculty of Agriculture, Suez Canal University, Egypt (at latitude 30° 37’ 08” North, longitude 32° 16’ 19” East) during the period from July 2019 to October 2019.

2. Experimental Fish

Monosex Nile tilapia, *O. niloticus* fingerlings were obtained from private fish Hatchery that located in Tall El Kebir, Ismailia Governorate (at latitude 30° 18’ 45” North, longitude 31° 53’ 02” East). Fish were transported in oxygenated containers and experimental fish were acclimatized to laboratory conditions for two weeks and fed twice daily with commercial diet (38 % CP) prior to start the experiment.

3. Experimental design

All experimental tanks were stocked with monosex (all male) Nile tilapia fingerlings with an initial average body weight of 20.35 ± 0.35 grams and average body length 10 ± 0.25 cm at three different stocking densities. Completely randomized designed experiment with three different stocking densities (200, 300 and 400 Fish/m^3) under Biofloc system and Clear system representing six experimental treatments (three densities X two systems) in triplicate. Experiment was carried out in 18 cylindrical fiberglass tanks with water volume 50 litre.

4. Experimental management

All experimental units were supplied with aerated water. Aeration was continuously provided using an air blower (220 Watt add the power and the flow of the blower). Fish were held under natural light (12:12 h, light: dark schedule). In the tanks representing the control treatments (clear system); water was exchanged daily, while for experimental BFT tanks, no water exchange was done (zero water exchange) except the evaporation compensate. Starch was used in biofloc treatments as an external carbon source, it was added at the same amount of feeding ration to maintain the optimal C: N ratio for activate heterotrophic bacteria growth (>15) **(Avnimelech, 1999).** Starch was completely mixed in a glass beaker with tank water sample and spread to the tank surfaces at the afternoon time **(Azim and Little, 2008).**

The experiment lasted 75 days. Fish in all experimental groups were fed six days per week$^-$ with commercial floating pellet containing 30% crude protein and its diameter is 3 mm from Skretting Egypt for animal feed Company **(Table 1).** The daily ration was 3% of the total stocked biomass, divided into two equal amounts and offered two times a day (9.00 and 14.00). Fish in each tank were weighed every 15 days and the amount of the daily feed allwance was accordingly adjusted**.**

Table (1). The approximate composition (% of experimental diets)

Chemical Composition	**%**
Dry mater	90.1
Crude Protein	30.3
Fat	6.1
Ash	4.95
Carbohydrate	53.85
Crude fiber	4.8
Organic carbon	37.45
Gross Energy **Kcal/ 100g**	450.70

1. Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to **(NRC, 2011).**

5. Chemical analysis of fish and biofloc

At the end of the experimental period, a random pooled sample of fish was collected and precipitated flocs from each tank were collected from different treatments for determination of proximate composition. chemical analysis of biofloc and whole-body dry mater %, crud protein %, fat % and ash content % were performed according to standard **(AOAC, 1995)** methods. Fish and biofloc samples were dried in an oven at 80°C till constant weight, then were grounded and stored at – 20°C for subsequent analysis. By incineration at 550ºC for 6 h, ash content was detected. Crude protein was determined by micro-Kjeldhal method, % Nitrogen × 6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden). Soxhlet extraction with diethyl ether (40-60ºC) was used to estimate crude fat content of different samples.

6. Blood plasma estimates

At the end of the trial, fish were systematically captured (per treatment replicates, tank after tank) for blood sample collection; Five fish from each replicate tank in the respective treatments were obtained. Prior to blood sample collection, fish were captured, transferred and retrieved unconscious from a bucket of water containing tricaine methane sulfonate (2% MS-222), to avoid changes in measurement parameters that could be caused by fighting due to handling stress. Blood samples were centrifuged at 4000 rpm for 10 min. for separating plasma, stored at - 20ºC and used later for biochemical determination.

Total protein by the Biuret method according to **Gornal *et al.* (1949).** Albumin concentration was determined by the method of **Doumas *et al.* (1977).** Globulin was calculated as the difference between total protein and albumin. Albumin Globulin Ratio (A/G) the ratio of albumin to globulin in plasma.

Plasma concentrations of total lipids, cholesterol and triglycerides (TG) were determined according to the methods of **Zollner and Kirsch (1962), Allain *et al.* (1974), Fossati and Principe (1982)**, respectively. High-density lipoprotein-cholesterol (HDL-c) was determined according to the methods of **Grove (1979).** Low-density lipoprotein-cholesterol (LDL-c) was determined by the calculation (LDL-c = (cholesterol-(HDL+VLDL)) according to **Warnick *et al.* (1983).** Very low-density lipoprotein-cholesterol (VLDL-c) was calculated by dividing the values of TG by factor of 5 according to **Warnick *et al.* (1983).**

7. Zooplankton assessment

Zooplankton was collected from experimental tanks on the last week of culture period; 5 liters of every water sample were filtered through plankton net 55 µ mesh size, 25 cm diameter and 80 cm length. Each collected sample was transferred to a labelled clean bottle and immediately fixed with 4 % formaldehyde. In the laboratory, three subsamples (one ml for each) of the homogenized plankton samples were transferred into a counting cell and zooplankton species were identified. The subsamples were examined under a binocular research microscope with magnification varied from 100X to 400X. Zooplankton population density was then calculated as the number of individuals per cubic meter from the equation conducted by **(APHA,1995):** No. X m^{-3} = (c X v′) / (v″ x v‴) x 1000

Where: - c= number of organisms counted.
v′= volume of concentrated sample, ml.
v″ = volume counted, ml.
v″′= volume of the grab sample, liters.

Zooplankton species were identified according to the following references: **Edmondson (1963); Ruttner-Klisko (1971); Pennek (1978); Pontin (1978); Wallace and Snell (1991) and Foissner and Berger (1996).**

8. Economic evaluation

Economic evaluation the cost of feed required to produce a unit of fish biomass was estimated using the given formula below (simple economic analysis):

1- Feed conversion ratio = Feed intake / Weight gain
2- Feed cost /1Kg weight gain (LE) = FCR (Feed intake /Weight gain) × Feed cost of 1 kg diets.
3- Relative % of feed cost of Kg weight gain = (Respective figures for step 2/ highest figure in this step) × 100.

*Reduction % of feed cost of Kg gain was calculated as a percentage from the highest value.

*Feed cost of 1 kg diets used were 8.40 L.E. Skretting Egypt diets (Price at end of 2020).

9. Statistical analysis

The data were statisticaly analyzed by Two-way with interaction ANOVA using Statistical Analysis System (SAS) version 9.0.0 (2004) program at *P<0.05* level to test the effects of stocking densities (200, 300, and 400 Fish/m^3) and system condition (clear and biofloc system) supplementation, as well as their interactions The ANOVA was followed by **Duncan test (1955)** at P<0.05 level of significant when needed.

RESULTS and DISCUSSION

1. Proximate body and biofloc composition

The proximate composition of the fish whole body was shown in **Table (2).** The proximate composition of the whole fish body may changed as a reflection of a number of factors, including water quality, stress factors, availability of nutrients, feed intake and utilization (**Dawood *et al.,* 2016 and El Basuini *et al.,* 2017**). Regarding stocking density, the highest protein and ash content were recorded in the highest stocking density (400/m^3). Highest fat was noticed on the lowest density. As for system condition biofloc system showed significantly higher dry matter, ether extract, and ash content as compared to clear water system. Interaction results showed that higher crude protein and ash content was recorded for fish density 400 fish/m^3 under either clear or biofloc system. While, the highest dry matter and ether extract content were noticed for fish 200 fish/m^3 under biofloc system.

In contrast with our results, **(Azim and Little, 2008)** revealed that no significant difference between clear and biofloc system in chemical composition of Nile tilapia were recognized. In the same context, no significant difference between biofloc and clear system in shrimp dry matter and crud protein content but biofloc showed superiority for ether extract and ash content **(Xu and Pan, 2012).** It was hypothesized that under biofloc system shrimp (*L. vannamei)* have better nutrient assimilation when compared to those fed only the formulated feed, because of the greater amount of essential amino acids, fatty acids (PUFA and HUFA) and other nutritional elements supplied by the bioflocs (**Tacon *et al*., 2002 and Ju *et al*., 2008**). This finding support our results as increase in biofloc protein and lipid content recorded with the elevation of dietary protein. **Tacon *et al*. (2002)** reported that the increased whole body ash content of the shrimp might be explained by continuous availability of abundant minerals and trace elements from the bioflocs as indicated by high ash content.

Table (2). Chemical composition of Nile tilapia fingerlings reared in different stocking densities under clear (CS) and biofloc system (BS).

Items		Dry mater %	Protein %	Fat %	Ash %
Stocking density	**200**	25.80[a]	58.67[c]	20.05[a]	17.36[b]
	300	24.46[b]	60.84[b]	19.13[a]	18.16[ba]
	400	23.44[c]	63.36[a]	18.86[b]	18.37[a]
SE		0.16	0.46	0.12	0.12
System Condition	**Clear system (CS)**	24.09[b]	61.54[a]	19.33[b]	16.05[b]
	Biofloc system (BS)	25.05[a]	60.37[b]	20.11[a]	19.88[a]
SE		0.13	0.37	0.09	0.26
Interaction	**CS200**	24.40[cb]	61.30[bc]	19.33[c]	15.39[d]
	BS200	26.57[a]	57.70[d]	21.22[a]	19.33[b]
	CS300	24.07[c]	63.04[ba]	18.92[dc]	16.27[c]
	BS300	25.03[b]	59.64[c]	20.20[b]	20.04[a]
	CS400	22.82[d]	63.68[a]	18.77[d]	16.49[c]
	BS400	24.51[cb]	60.38[c]	19.90[b]	20.26[a]
SE		0.21	0.64	0.16	0.46

* Data are presented as means ± standard error (SE).
** Means in the same column having the same superscript letter are not significantly different ($P<0.05$).

Biofloc chemical composition was summarized in **Fig. (1).** Significant differences ($P<0.05$) in biofloc composition in terms of protein and ash content were noted between different stocking densities. Meanwhile, chemical analysis of biofloc samples showed increase in protein, fat and ash contends with increasing stocking density. Same results were suggested by (**Azim *et al*., 2007)** they observed significant difference in biofloc composition in terms of protein and ash content between different stocking densities. **Azim *et al.* (2007)** reported that there were significant differences in protein and fat composition of biofloc with the highest stocking density, but the oboist were recorded for ash and fiber composition.

Some results suggest that biofloc conditions stimulate accumulation of both fat and ash in tilapia carcass especially with the elevation of stocking density. Biofloc chemical analysis confirmed this hypothesis as with increase of dietary protein biofloc content of lipid and ash elevated. The nutritional value for the protein of the evaluated floc was within the range obtained in other studies carried out in BFT **(Liu *et al.*, 2018**), as well as the ash concentrations (**Widanarni and Maryam, 2012**). The development of autotrophic bacteria, which use a greater amount of alkalizing compounds, incorporating a greater number of ions in the biofloc, resulting in higher ash content. **Martins *et al.* (2017)** reported that the greater use of biofloc by fish probably contributed to the maintenance of a lower organic load in the water, resulting in greater ash residue in the system.

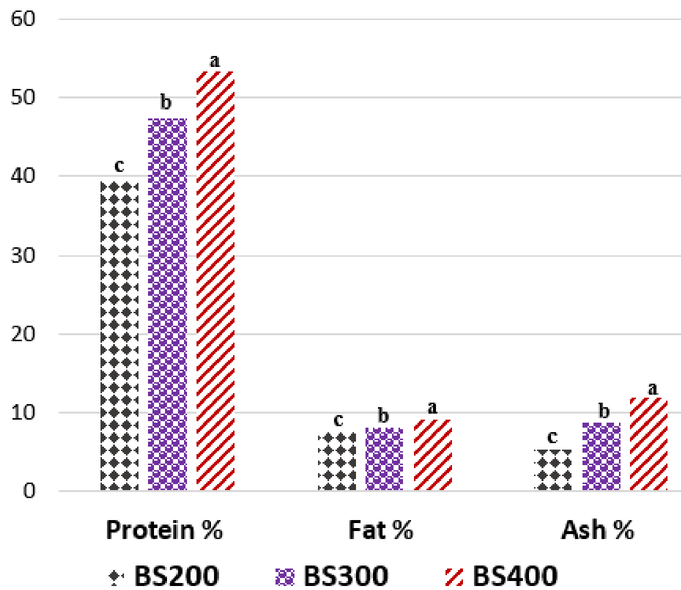

Fig.1: Chemical composition of biofloc under different stocking densities of Nile tilapia.

2. Plasma protein and lipid profile

Plasma protein and lipid profile of different investigated groups showed that, fish reared in biofloc system revealed higher significantly plasma total protein, albumin and globulin than clear system **(Table 3).** As for stocking density, highest stocking density 400/ m^3 showed the significantly higher total protein, albumin and globulin. Meanwhile, plasma total cholesterol, triglyceride, HDL, and LDL were all significantly higher in the lowest density (200/m^3). Biofloc system results showed significantly higher total protein, albumin, globulin albumin/globulin ratio than clear system. Also, cholesterol triglyceride, and LDL were significantly increased with BFT treatments **(Table 4).**

Total Protein, Albumin and Globulin levels increased with increasing stocking density and under biofloc system (P<0.05); the highest levels were recorded in fish reared at 400 fish/m^3, whiles the lowest was recorded in in fish reared at 200 fish/m^3 **(Table 3)**. However, increased levels of cholesterol, triglycerides and LDL were noted within the biofloc system with decreased stocking density (**Table 4).**

Table (3). Plasma protein profile of Nile tilapia reared in different stocking densities under clear and biofloc system.

Items		Albumin (g/dl)	Globulin (mg/dl)	Albumin Globulin Ratio (A/G)	T.Protein (mg/dl)
Stocking density	200	1.810^b	2.760^b	0.656^c	4.57^c
	300	1.855^b	2.805^b	0.661^b	4.66^b
	400	2.018^a	2.842^a	0.710^a	4.86^a
SE		0.054	0.089	0.017	0.090
System Condition	Clear system (CS)	1.827^b	2.742^b	0.676^b	4.613^b
	Biofloc system (BS)	1.962^a	2.817^a	0.700^a	4.780^a
SE		0.044	0.073	0.073	0.073
Interaction	CS200	1.59^f	2.53^f	0.73^{cb}	4.42^f
	BS200	1.99^c	2.78^c	0.54^e	4.71^c
	CS300	1.84^e	2.55^e	0.74^b	4.51^e
	BS300	2.03^b	2.92^b	0.63^d	4.72^b
	CS400	1.87^d	2.72^d	0.80^a	4.61^d
	BS400	2.05^a	3.18^a	0.69^c	5.21^a
SE		0.565	0.561	0.176	1.062

* Data are presented as means ± standard error (SE).
** Means in the same column having the same superscript letter are not significantly different (P<0.05).

Stress has a wide range of negative impacts on production characteristics of fish **(Øverli *et al.*, 2015 and Trenzado *et al.*, 2003)**. Higher stocking densities and poor water quality are chronic stressors commonly encountered by fish **(De Oliveira *et al.*, 2012)** High density groups of fish may have suffered from weakened immunity that resulted to their death. **Mehrim (2009)** did similar study correlating stocking density and dietary probiotic, and observed that; at optimal density, the probiotic improved fish immunity, meanwhile, when stocking density went beyond the optimal, the effect of probiotic was suppressed and led to reduced levels of hematological parameters. The sustainability of any aquaculture system is largely limited by disease incidence and management. Meanwhile, the fish health and disease prophylaxis are directly related to the non-specific immune response of fish. In the current study, the BFT system significantly improved the non-specific immunity as deduced from the levels of total protein, albumin, and globulin measured in tilapia serum. These results were in agreement with the numerous earlier studies e.g. **(Haridas *et al.*, 2017; Emerenciano *et al.*, 2013 and Kim *et al.*, 2014).** The enhancement effect of biofloc on immunity could be explained by the phenomenon of 'natural probiotic' effect that found in BFT. These results are supported by the findings of **Azim and Little (2008)**, they reported that welfare indicators in terms of gill histology, blood haematocrit and plasma cortisol levels, which did not changed with BFT.

Table (4). Lipid profile of Nile tilapia at different stocking density under clear and biofloc system.

Items		Cholesterol (mg/dl))	Triglycerides (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)
Stocking density	200	185.5[a]	157.67[a]	112.50[a]	48.6[a]
	300	177.0[b]	155.33[a]	99.50[b]	46[ab]
	400	172.5[b]	132.50[b]	92.33[c]	44.5[b]
SE		7.675	5.299	3.659	3.136
System Condition	Clear system (CS)	173[b]	143.78[b]	102.67[a]	40[b]
	Biofloc system (BS)	183.67[a]	153.22[a]	100.22[a]	52.733[a]
SE		6.267	4.326	2.987	2.560
Interaction	CS200	170[d]	135[d]	115[a]	36[d]
	BS200	204[a]	179[a]	101[c]	63[a]
	CS300	150[e]	131[e]	114[ab]	34[e]
	BS300	201[b]	170[b]	85[d]	59[b]
	CS400	145[f]	130[ef]	110[b]	30[f]
	BS400	200[c]	145[c]	84[de]	56[c]
SE		120.849	77.092	50.062	59.814

* Data are presented as means ± standard error (SE).
** Means in the same column having the same superscript letter are not significantly different ($P<0.05$).

Effect of stressors on fish has been correlated with reduced body lipid content **(Svobodova *et al.*, 2006).** In this trial, triglyceride and cholesterol levels were found to decrease with increasing stocking density **(Table 4).** Their levels in blood serum have been associated with stress management **(Lupatsch *et al.*, 2010 and Pérez *et al.*, 2008). Vijayan *et al.* (1990)** reported a reduction in triglyceride level when brook charr (*Salvelinus fontinalis*) was exposed to a stressful situation that triggered higher energy demand; and was further supported by **Da Rocha *et al.* (2004)**, who also reported significant change in the above parameter in matrinxã (*Brycon cephalus*) after handling and acute crowding stress. The animals could have utilized substantial amount of metabolizable energy in their response to the stressful condition. The decreased trends of triglyceride and cholesterol in our trial are similar to what was observed in Senegalese sole; *Solea senegalensis* **(Costas *et al.*, 2011).**

Table (5). Zooplankton count and species under different stocking density levels and culture conditions (biofloc and clear water systems).

Groups	Types of zooplankton	200CS	300CS	400CS	200BS	300BS	400BS
Rotifera	*Lepadellaovalis(O.F. Muller)*	10000	30000	310000	950000	1500000	4380000
	Monostyllaclosterocerca (Schmarda)	120000	120000	300000	600000	6200000	3200000
	Philodena sp.	60000	40000	200000	20000	600000	80000
	Trichocerca sp.	0	0	10000	300000	0	0
	Colurellaadriatica (Ehrenberg)	0	50000	40000	0	300000	250000
	Colurellaobtusa (Gosse)	0	0	10000	0	0	0
	Trichocerca sp.	20000	0	0	40000	0	100000
	Cephalodella sp.	20000	10000	0	40000	0	0
	Euchlanis sp.	0	0	0	80000	80000	0
Protozoa	*Paramecium sp.*	0	0	10000	0	0	0
	Tokophyraquadripartita (Goodrich &Jahn)	0	0	20000	0	0	10000
	Vorticella campanula(Ehrenberg)	310000	240000	60000	1170000	190000	2830000
	Centropyxisoculeatea(Stein.)	0	0	0	0	30000	240000
	Arcella vulgaris (Ehrenberg)	0	0	0	30000	0	10000
	Difflugia corona (Bovee)	0	0	0	0	0	240000
	Didinium sp.	0	0	0	10000	0	0
Copepoda	*Copepotidae*	20000	130000	50000	420000	60000	0
Total count zooplankton		560000	620000	1010000	3660000	8960000	11340000

* Data are presented as means ± standard error (SE).
** Means in the same column having the same superscript letter are not significantly different (P<0.05).

Cholesterol, triglycerides and HDL levels were reported to increase in stressed Nile tilapia **(Barreto and Volpato, 2006 and EL-Khaldi, 2010)**, which might bring us to the conclusion that the BFT system does induce stress-related risks in intensive culture despite the zero-water renewal rate. Similar results were denoted by **Azim and Little (2008)** and **Haridas *et al.* (2017).** Whereas, **Bakhshi *et al.* (2018)** reported comparable glucose levels in common carp maintained in biofloc and non-biofloc based system.

3. Zooplankton assessment

Zooplankton count and species in different experimental treatments are presented in **Table (5).** All biofloc treatments showed higher zooplankton count when compared with the clear system. As described in this table; the highest total count of zooplankton was recorded for (400 fish/ m^3).

Three groups of organisms were identified, Rotifera, Protozoa and Copepoda. The first group (Rotifera) included eight genera namely *Lepadellaovalis* (O.F. Muller*), Monostylla closterocerca (Schmarda), Philodena* sp*.*, *Collotheca* sp., *Anuraeopsisfissa (Gosse), Anuraeopsisfissa (Gosse), Colurellaadriatica* (Ehrenberg), *Euchlanis* sp. The second group (Protozoa) included three genera namely *Tokophyraqua dripartita* (Goodrich &Jahn), *Vorticella campanula* (Ehrenberg**)** and *Arcella vulgaris* (Ehrenberg). Only *copepotidae* was found in the group Copepoda.

Increased stocking density results on higher total count of zooplankton. Elevated fish output (feces) and in the presence of starch established a suitable environment for microorganisms (biofloc) and zooplankton growth. Meanwhile, active growth of biofloc under these conditions exceeds the fish ability of consuming. **Crab *et al.* (2007)** conclude that the biological flocs can be considered as a kind of fast growing microbial mixed culture, in which the 'waste'-nitrogen is recycled to young cells, which subsequently are grazed by the fish. Adding carbohydrate activated the growth of zooplankton. **Gao *et al*. (2012) ; Avnimelech. (2007) and Emerenciano *et al*. (2011)** reported similar results. Regarding the species **Azim and little (2008)** showed that under biofloc system, three groups of organisms were identified: Protozoa, Rotifera and Oligochaeta. Among protozoans, three genera, namely, *Paramecium*, *Tetrahymena* and *Petalomonas*d ominated. Four genera of rotifers were identified, namely, *Lecane, Trichocerca, Polyarthra*and *Asplanchna*. Only *Tubifex*was found in the group Oligochaeta.

4. Economic Evaluation

Calculations of economical efficiency of stocking densities under Biofloc system and Clear system based on the cost of feed, costs of one Kg fish weight gain and its ratio with the highest treatment 400 fish/m^3 under clear system are shown in **Table (6).** The results of the current study demonstrated that the biofloc system is more economical in aquaculture in terms of water consumption needed to produce fish under limited water availability and high pumping costs. When fingerlings of Nile tilapia were reared in the biofloc tanks, the amount of daily feed inputs can be reduced without affecting the production costs, indicating that biofloc could contribute to the nutrition and physiological health of Nile tilapia. Feed costs for producing one kilogram of Nile tilapia were better when applying the biofloc system, it reduced feed costs compared to the clear system. Feed costs required to produce one kilogram of fish were higher in 400, 300, 200 fish/m^3 under clear system, respectively treatment.

It is well known that feeding cost in fish production is about 60% and more of the total production costs. Under the present experimental condition, all other costs are constant; therefore, the feeding cost to produce one Kilogram of fresh body weight could be used as a measure to compare between different stocking densities under Biofloc system and Clear system. The main factors affecting the growth and development of the aquaculture industry are environmental protection and feed cost (**Avnimelech, 2009).** Reducing production costs and more profitability are considered as important goals in the aquaculture industry. Growth rate and feed conversion ratio play an important role in aquaculture costs, which are improved in biofloc system compared to conventional system and profitability is also better in the biofloc treatments (**Khanjani, 2015).** Better feed recycling, improved feed conversion ratio, increased specific growth rate zero water exchange) are key components of aquaculture management costs.

Table (6). Economical evaluation (Mean ± SE) of Nile tilapia under biofloc and clear system throughout the experimental period (75 days).

Item	Stocking density (fish/m^3)						SE
	200		300		400		
	CS	BS	CS	BS	CS	BS	
Feed intake (g)	48.793^c	55.260^a	45.339^e	50.373^b	43.995^f	47.93^d	2.680
Weight gain (g)	26.52^d	39.19^a	24.21^e	30.34^b	20.36^f	27.37^c	7.489
Feed conversion ratio (FCR)	1.840^b	1.410^e	1.872^b	1.661^d	2.164^a	1.752^c	0.256
Feed cost /kg weight gain L.E	15.456^{bc}	11.844^e	15.725^b	13.952^c	18.178^a	14.717^d	1.05
Relative % of feed cost /kg weight gain	85.03^{bc}	65.16^e	86.51^b	76.75^d	100.00^a	80.96^c	4.65

* Data are represented as means ± standard error (SE).
** Means in the same row having the same superscript letter are not significantly different ($P<0.05$).
***Reduction % of feed cost of Kg gain was calculated as a percentage from the highest value) 400 fish/m3 under clear system).
****Feed cost of 1 kg diets used were 8.40 L.E. Skretting Egypt diets Price at end of 2020.

CONCLUSION

It could be concluded that biofloc system and stocking density of 200 fish/m^3 have many advantages over the clear water system and the higher densities (300 and 400 fish / m^3) in terms of body proximate chemical composition, Plasma protein and lipid profile of Nile tilapia (*Oreochromis niloticus*) fingerlings. Zooplankton community improved dramatically with following biofloc system and increasing stocking density. Applying biofloc system with the lowest density (200/ m^3) results on economical advantages especially feed costs over clear water and higher densities conditions. These rearing conditions can play a key role in developing a sustainable Nile tilapia fingerlings production via better body composition, improved biochemical parameters, better biological community, higher production, and decreased feeding costs.

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تأثيرالبيوفلوك على تكوين الجسم، بروتين البلازما، تركيب الدهون، العوالق الحيوانية واقتصاديات تربية إصبعيات البلطي النيلي تحت كثافات تخزين مختلفة

محمد محمد سعيد سيد [١] اسراء محمد ابراهيم طه[2]

[١] كليه الثروة السمكيه، جامعه السويس
[٢] كليه الزراعه ، جامعه قناه السويس

تهدف هذه الدراسة إلى معرفة تأثير نظام البيوفلوك على التركيب الكيميائي للجسم ، وبروتين البلازما ، خصائص الدهون ، مجتمع العوالق الحيوانية وكذلك اقتصاديات تربية إصبعيات البلطي النيلي بكثافات مختلفة. تم استخدام ثلاث كثافات تربية (200 ، 300 ، 400 سمكة / م ^{3}) في نظام بيوفلوك ونظام المياه الصافية (بدون إضافة الكربون الخارجي). تم وضع البلطي النيلي بوزن أولي قدره 20.35 ± 0.35 جرام في 18 تانك أسطواني من الفيبر جلاس بحجم ماء 50 لتر لمدة 75 يومًا. تم تغذية الأسماك على علف تجاري يحتوي على 30٪ بروتين وأضيف النشا كمصدر للكربون للوصول إلى نسبة C / N عند 1/15. أظهر التركيب الكيميائى للجسم أن أعلى محتوى من البروتين والرماد كان عند أعلى كثافة تخزين (400 / م ^{3}). أعلى نسبة دهون لوحظت عند أقل كثافة تخزين 200 / م^3. أظهر نظام البيوفلوك محتوى أعلى من الماده الجافة والدهون والرماد مقارنة بنظام المياه الصافية. أظهر تحليل عينات البيوفلوك زيادة في البروتين والدهون والرماد مع زيادة كثافة التخزين. أظهرت أعلى كثافة تخزين 400 / م ^{3} زياده معنويه احصائيا فى إجمالى بروتين الألبيومين والجلوبيولين. الكوليسترول الكلي في البلازما والدهون الثلاثية و HDL و LDL كانوا جميعا أعلى معنويا في أقل كثافة (200 / م ^{3}). نظام البيوفلوك كان أعلى معنويا فى البروتين الكلي ، الألبومين ، الجلوبيولين / الجلوبيولين مقارنة بنظام المياه الصافية. ارتفع كلا من الكوليسترول والدهون الثلاثية ، و LDL بشكل ملحوظ فى كافة معاملات البيوفلك. أظهرت كافة وحدات البيوفلوك ارتفاع فى عدد العوالق الحيوانية مقارنة بنظام المياه الصافية. أدت زيادة كثافة التخزين إلى ارتفاع عدد العوالق الحيوانية حيث سجلت أعلى كثافة عند 400 سمكة/ م^3 ، وقد تم تحديد ثلاث مجموعات من الكائنات الحية ، Rotifera، Protozoa و Copepoda. وبشكل عام ، تم تحسين تكوين الجسم ، وبروتين البلازما ، والدهون ، ومجتمع العوالق الحيوانية باتباع نظام البيوفلوك. تم تسجيل فوائد اقتصادية من حيث تكاليف الأعلاف وتكاليف الأعلاف النسبية لكل كجم من الأسماك في معاملات البيوفلوك مع أفضلية كثافة التخزين 200 سمكة / م^3.