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Culture Performance of Nile tilapia (*Oreochromis niloticus*) raised in a biofloc-based intensive system

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ABSTRACT

In a 98-day experiment, young of the year Nile tilapia fingerlings (5.48±1.38 g) were raised in eight circular 500 l fiberglass tanks with a stocking density of 300 fish m^{-3}. Two treatments were evaluated and each was quadruplicated: (1) Culture in pure water i.e. conventional intensive system (four tanks kept as control) and (2) Culture in biofloc technology (BFT) system (four tanks). The juveniles Nile tilapia reared in the BFT showed a substantial improvement in the growth performance (in terms of final weight, weight gain, average daily gain, and specific growth rate) than the control ($P<0.05$). Meanwhile, the feed conversion ratio and the survival rates were comparable ($P>0.05$). The BFT system had significantly increased the total plasma protein and globulin. Whereas, the serum glucose and cortisol level of the control fish showed surprisingly increased levels compared to the biofloc group. What is more, a major difference was found within the antioxidant system in terms of, superoxide dismutase activity. Moreover, the NO levels were impressively decreased in the BFT tanks. The obtained results encourage the application of biofloc technology for intensive rearing of tilapia as implemented from the growth and immune response as well as the response to oxidative stress displayed by tilapia fish maintained at this system.

1. INTRODUCTION

Tilapia is a farmed species that is being produced intensively everywhere in the universe. In 2012, the world tilapia production reached 4.5 million MT (FAO, 2014) with eminent anticipations to extend more exponentially in the future. Tilapia intensification is a prime objective to deal with the increased demands and consumption of aquatic animal protein. Intensively cultured fish require higher levels of artificial feed. Aquatic feeds usually comprise more than 25 % protein. Whereas cultured fish solely retain 16-41 % of feed nitrogen (Avnimelech, 1999; Hari et al., 2004). Therefore, the intensive fish culture and the accompanied high levels of added feed load high levels of nitrogenous wastes in the aquatic ecosystem. Crab et al. (2007) described ammonia as the prime nitrogenous waste product of protein metabolism and fish feces decomposition as well as the uneaten feed. Due to its toxicity nature (i.e. ammonia), the extent of this product in an aquaculture medium should be sustained at 0.01 mg/L (Wedemeyer, 1996) by regular water replacement or by applying water management system.

On the other side, BFT is an aquaculture practice based on natural processes circulating in an aquatic ecosystem. In this system, the heterotrophic microbial biomass growth encouraged by the supplement of external organic carbon assimilates ammonia-nitrogen (Azim and Little, 2008). This microbial biomass may further gather with other organisms including phytoplankton and zooplankton to form the bioflocs (Hari et al., 2004). Bioflocs are a rich source of growth promoters and bioactive composites, which enhance both the digestive enzymes and the aquatic organism health status. Bioflocs (BF) also have 'natural probiotic' impact due to the presence of immunostimulants and antistress agents that helps to keep the fish healthy and resistant to diseases. On this context, tilapia farming, using biofloc technology (BFT), would have a distinguished priority over traditional fish farming in a low-to-zero exchange of water system that also supplies a complementary and inherently nutritious food for the commercial crop (Hargreaves, 2006; Avnimelech, 2007). Also, physiological functions such as immune and antioxidant systems are essential for tilapia in maintaining their health and growth performance, particularly beneath the stressful environmental, e.g. high-density culture (Shourbela et al., 2017) and thereby guarantee healthy tilapia culture. Otherwise, the decrease in the tilapia immune and antioxidant defense mechanism might simply result in disease outbreaks and non-profitable fish culture (Bachère, 2000). Rapid growth, good survival in high-density culture and disease tolerance make BFT a decent selection for intensive and/or bio-secure closed grow-out strategies. Therefore, the BFT system has received pronounced attention recently as it offers a practical solution to effectively manage water quality with minor water exchange and enhance tilapia growth performance, thus achieving a functional and healthy culture of tilapia. However, investigations regarding the impacts of BFT on the physiological health and overall performance of farmed fishes are scarce. Therefore, this study pursuit to estimate the impact of BFT on the growth performance and some non-specific immunological parameters as well as oxidative stress enzymes, of intensively reared Nile tilapia.

2. Materials and methods

2.1. Experimental fish

Young of the year juvenile Nile tilapia were sourced from a private fish farm at Kafr El-Sheikh Governorate, Egypt. Fish were transported in oxygenated well-inflated plastic bags to withstand the journey to the Laboratory of Fish Breeding and Production, located at the Faculty of Veterinary Medicine, Alexandria University. The fish were acclimatized in fiberglass tanks supplied with 500 l well water. Each tank supported with two air stone and subjected to a day after day complete water replacement. For a period of 2 weeks, a commercial pelleted diet (30% protein) was offered twice daily at a rate of 3% of fish biomass

2.2. Experimental design and fish rearing

Apparently, healthy juveniles Nile tilapia (5.48±1.38 g) were randomly allocated into eight circular 500 l fiberglass tanks with a stocking density of 300 fish m^{-3}. Two treatments were evaluated and each was quadruplicated: (1) Culture in pure water i.e. conventional intensive system (four tanks kept as control) and (2) Culture in biofloc technology (BFT) system (four tanks). The experiment extended for 98 days. The tanks were filled with a well water to maintain a water volume of 300 l. To meet the oxygen demand constant tank aeration were maintained by using an air blower 1.5 horse and porous rubber hoses and air stones for proper mixing of the floc. Feeding rates were adjusted to 2% of the total stocked biomass (Azim and Little, 2008). Daily ration was equally offered at 9 a.m. and 2 p.m. The amount of food was adjusted bi-weekly with regard to the actual body weight changes.

2.3. Biofloc Initiation

As an inoculum, all biofloc tanks were inoculated with10 L of fish pond water containing yeast and mud. The C: N ratio was maintained at 15: 1 by daily addition of 2.6 g molasses 53% as an organic source of carbon (Crab et al., 2012). For biofloc tanks, only a proper amount of fresh water was supplied to compensate water losses due to evaporation without any water exchange, whereas the control tanks were renewed twice weekly.

2.4. Growth performance

To assess the growth performance, 15 weighted fish were arbitrarily taken from each tank every 14 days using sensitive electronic balance. The growth performance was judged through the final body weight, average daily gain (ADG), and total weight gain, specific growth rate (SGR) and feed conversion ratio (FCR).

2.4.1. Weight gain (WG, g fish^{-1}):

WG = average final weight (g) – average initial weight (g)

2.4.2. Average daily gain (ADG, g fish^{-1} day^{-1}):

$$\text{ADG} = \frac{\text{Mean final weight - mean intial weight}}{\text{Days}}$$

2.4.3. Specific growth rate (SGR, % day^{-1}):

$$\text{SGR \%} = \frac{\text{Ln W}_1 - \text{Ln W}_0}{\text{T}} \times 100$$

Where W_1 = final mean weight (g) of fish, W_0 = initial mean weight (g) of fish at stocking time, t= time in days and Ln= natural log.

2.4.3. Feed conversion ratio (FCR):

It gives the weight of feed required to produce a unit weight gain of fish. It was calculated by the following equation:

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

2.5. Survival rate (SR %):

The survival rate (SR %) was determined using the following formulae;

$$\text{SR\%} = \frac{\text{Final number of live fish}}{\text{Initial number of fish}} \text{X } 100$$

2.6. Serum biochemical analyses:

The blood of five anesthetized juvenile fish (3 ml fish^{-1}) was collected from the fish caudal vein 24 hours after the last feeding. Randomly selected fish were anesthetized with MS222 (Sigma Aldrich, Steinheim, Germany) according to Smith et al., (2007). Blood was transferred in plastic biochemistry test tubes. Tubes with coagulated blood were centrifuged at 3500 rpm for 15 min for serum separation, which was stored below -20˚C according to Blaxhall and Daisley, (1973).

Biochemical indices in serum including total protein, albumin, globulin, and glucose were determined using analytical test kits (Biodiagnostic Industry, Co) and measured by a spectrophotometer (PG Instruments, UK).

Serum globulin was also calculated;

Globulin = total protein - albumin.

Also, A/G ratio was determined. The cortisol level was calorimetrically assayed via using commercial kit. The superoxide dismutase was also evaluated based on the prescript techniques and serum nitric oxide (NO) through colorimetric determination of nitrite using nitric oxide assay kit. The cortisol commercial kit (Cat., no. 500360), superoxide dismutase (SOD) assay kit and nitric oxide (NO) assay kit (Cat., no. 2533) were purchased from Cayman Co., USA., Sigma-Aldrich, St. Louis, USA and Biodiagnostic Co., (Cairo Egypt); respectively.

2.7. Water quality

Water quality parameters of each aquarium were measured bi-weekly at 15 cm water depth. Ammonia nitrogen (TAN), nitrite-nitrogen and nitrate-nitrogen using analytical kits (Lovebird®, Multidirect, co 210070 England. Dissolved oxygen was measured using a portable oxygen meter (DO-5510).

2.8. Statistical analysis

Data analysis was performed using SAS (2002), one-way analysis of variance. Duncan multiple ranges used to signify the differences between means.

3. RESULTS

3.1. Growth performance and survival rates:

Fish maintained in the BFT treatment attained the highest ($P < 0.05$) final weight, weight gain, weight gain per day, and the specific growth rate. Whereas the survival rate and FCR of juvenile tilapia kept at BFT treatment were compared with the control group (Table 1).

3.2. Serum biochemical indices:

The BFT system significantly improved the total protein and globulin content of juvenile tilapia serum than that of the control group. The tilapia fish cultured in the control non-biofloc treatment showed a significantly ($P < 0.05$) higher glucose and cortisol levels (139.8±13.97 mg dl^{-1} and 37.6±10.53 ng dl^{-1}) than the BFT based group (78±10.68 mg dl-1 and 17.52±2.05 ng dl^{-1}), respectively. Additionally, the BFT system had efficiently elevated ($P < 0.05$) the superoxide dismutase (SOD) activity of tilapia juveniles (39.8±3.70 U l^{-1}) over that of the control group (26±3.39 U l^{-1})

3.3. Water Quality

The monitored changes in the quality of tanks water are elucidated in Table 3. For all experimental tanks, the non-ionized ammonia was detected in levels that could be tolerated by juvenile tilapia (0.5 mg l^{-1}). Meanwhile, the BFT system had significantly reduced the ammonia, nitrites and nitrates level as compared to the control non-biofloc tanks (Table 3, Fig.1, Fig.2, and Fig. 3). Also, the BFT system had regularly reduced nitrites and nitrates levels over time. Still, all fish tanks showed comparable levels of dissolved oxygen (Table 3, Fig.4).

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Table (1): Effect of biofloc in the performance of Nile tilapia *Oreochromis niloticus* in comparison with a conventional intensive system (control) (mean±SD).

Parameter	Treatment	
	Control	Biofloc
Initial Weight (g $fish^{-1}$)	5.74±1.43	5.23±1.32
Final body Weight (g $fish^{-1}$)	35.52±3.94^b	40.25±2.56^a
weight gain (g $fish^{-1}$)	29.78±4.29^b	35.02±1.68^a
Average daily gain (AWG, g $fish^{-1}$)	0.30±0.04^b	0.36±0.02^a
Specific growth weight (SGR, % day^{-1})	1.88±0.31^b	2.11±0.20^a
Feed conversion ratio (FCR)	1.13±0.92	0.98±0.06
Survival rates (SR, %)	91.5±0.5	90.5±0.5

Different letters in the same row are significantly different at $P < 0.05$.

Table (2): Some biochemical indices and antioxidant enzymes of Nile tilapia *Oreochromis niloticus* cultured in a biofloc system and non-biofloc control tanks. (mean ± SD).

Parameter	Treatment	
	Control	Biofloc
Total protein (g dl^{-1})	3.82±0.74^{b}	4.91±0.81^{a}
Albumin (g dl^{-1})	1.19±0.30	1.47±0.07
Globulin (g dl^{-1})	2.63±0.78^{b}	3.45±0.77^{a}
A/G ratio	0.48±0.18	0.44±0.07
Glucose (mg dl^{-1})	139.8±13.97^{a}	78±10.68^{b}
Cortisol (ng dl^{-1})	37.6±10.53^{a}	17.52±2.05^{b}
Superoxide Dismutase (Ul^{-1})	26±3.39^{b}	39.8±3.70^{a}
Nitric oxide (Ul^{-1})	24.4±7.84^{a}	16.06±2.58^{b}

Different letters in the same row are significantly different at P <0.05.

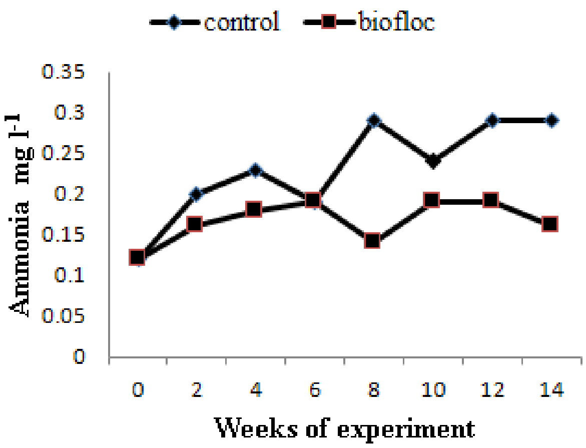


Fig. 1. Level of un-ionized ammonia (NH3–N) in intensive Nile tilapia Oreochromis niloticus cultured in a biofloc system and non-biofloc control tanks

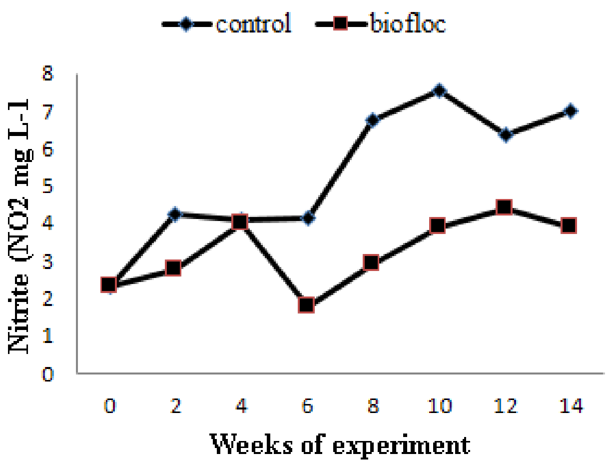
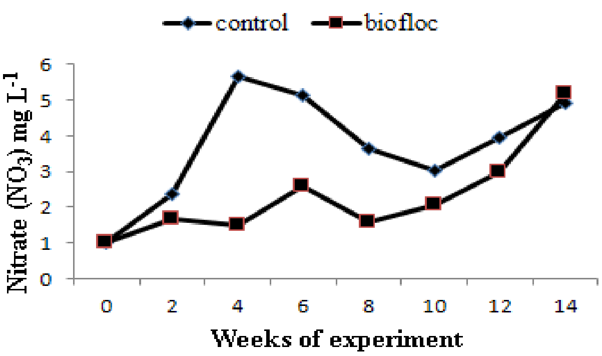
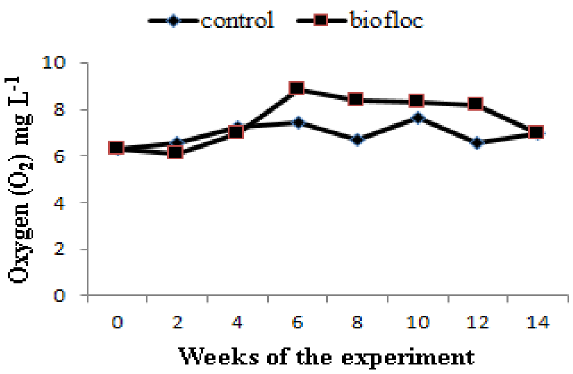


Fig. 2. Level of Nitrite (NO2 mg L-1) in intensive Nile tilapia Oreochromis niloticus culture in a biofloc system and non-biofloc control tanks.





(DO) in
niloticus
n-biofloc

4. DISCUSSION

The BFT treatment had substantially improved the growth performance of intensively reared juveniles Nile tilapia (in terms of final weight, WG, ADG, and SGR). This finding confirmed the results of different researchers who reported that the BFT system improved Tilapia growth (Azim and Little 2008; Avnimelech and Kochba 2009). Physiological adaptations allow shrimp and tilapia to consume biofloc and digest microbial protein (Azim and Little, 2008; Hargreaves, 2013). The increased growth performance in the BFT treatment might be in part due to the maintenance of optimum quality tank water and to the constant availability of nutritious floc (the richest source of many essential fatty acids (Ekasari et al., 2010; Toledo et al., 2016). Ju et al. (2008) evaluated many bioactive compounds in biofloc such as carotenoids, chlorophylls, and phytosteroids, which are expected to contribute better growth of cultured organisms in the biofloc system. Likewise, Avnimelech (2007) quoted that biofloc contribute to 50% of the feed protein requirement of tilapia. Additionally, Avnimelech and Kochba (2009) evaluated nitrogen uptake and excretion by tilapia in biofloc tanks using 15N tracing, they concluded that net uptake of microbial protein from a biofloc suspension contributes about 25% of the classical protein ration. Hence, higher growth observed in the BFT treatment indicated efficient biofloc utilization assimilation into body mass. In contrast, growth impairment of fish was noted in indoor and outdoor BFT systems as compared to the ordinary recirculating culture system. The biological turbidity provoked by biofloc may represent a direct cause of such growth impairment as it reduces the water visibility and thus the pelleted feed intake. Also, fish might have subjected to chronic stress arisen from deteriorated and fluctuated water quality (Little et al., 2008).

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; 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.

Increased levels of serum cortisol and glucose were detected in the control non-biofloc treated fish group. The serum cortisol and glucose levels were reported to increase in stressed Nile tilapia (Barreto and Volpato, 2006; 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.

The oxidative stress is highly determined by the animal antioxidant defense system (Zahran and Risha, 2014). SOD plays a decisive role against radioactive oxygen species ROS (Kumaravelu et al., 1995). In addition, Tao et al. (2013) conveyed that the superoxide anions are catalyzed by SOD to produce hydrogen peroxide which, in turn, decomposed by CAT to water and oxygen, hence preventing lipid peroxidation. The present study revealed the potentiality of the BFT system to improve the Nile tilapia antioxidant defense system as deduced from the increased fish welfare and reduced oxidative stress. The SOD activity of fish cultured in the BFT treatment showed expressively higher values than the control one. Mansour and Esteban (2017) and Luo (2014) also reported this increased trend of SOD activity. Early investigations concluded that the biofloc community obtained from the water of BFT tanks containing tilapia could potentially improve the antioxidant defense system of fish. Xu and Pan (2013) attributed this effect to the numerous bioactive products (phytosterols, taurine and natural antioxidants such as polyunsaturated fatty acids (PUFA), different vitamins (C, E), phenolic compounds, carotenoids, and minerals) that are existed in the biofloc microbial community. Additionally, in the present investigation, the BFT system substantially decreased the activity of NO as compared to the control. This result plus the recorded reduced glucose and cortisol levels give further support to the effect of BFT as a stress relieving effect in intensively cultured fish. On the other hand the reduced NO activity detected in the BFT could be due to the generous source of many bioactive compounds (Ju et al., 2008) and antibacterial compounds (Crab et al., 2010) of the biofloc which helps to reduce the density-related stress and positively influences the robustness of the fish immune system. Similar results were conveyed by El-Hawarry et al. (2018) where they recorded increased NO activity in juvenile tilapia maintained in the conventional intensive system.

Concerning the water quality criteria recorded in this study; a remarkable reduction in the ammonia and nitrite levels (i.e. improved water quality) was noticed in BFT treatment. These reductions are due to the uptake of inorganic nitrogen by heterotrophic and nitrifying bacteria existed in the biofloc community of culture water (Aizm and Little, 2008; Long et al., 2015). This explanation was further supported by the notable increase in the total heterotroph bacteria count of the BFT treatment. Accordingly, the addition of molasses had potentially increased the C/N ratios in the BFT treatment tanks which in turn enhance the growth of heterotroph bacteria to create a unique ecosystem of biofloc along with algae, protozoa and organic particles (Emerenciano et al., 2012; Gao et al., 2012). Similarly, several authors recognized accepted limits of water quality in BFT system based tanks (e.g. Gaona et al., 2011; Long et al., 2015; Ekasari et al., 2015).

However, the reduction in NO2 --N and NO3 --N in biofloc treatment from the 4th-6th week to the end of the experiment likely occurred due to immobilization by heterotrophic bacteria, which inhibited the nitrification process providing that, denitrification may also have arisen during this period of the experiment (Azim and Little, 2008; Luo et al., 2013).

5. CONCLUSION

The results of this study demonstrated the possibility of rearing Nile tilapia juveniles in a zero-water exchange biofloc-based intensive culture tanks without affecting its survival and growth. This BFT system would boost the water quality and along with its nutritious rich compounds, better fish growth will be augmented. Hence, the BFT system would provide a sustainable approach for profitable and ecological fish culture. In addition, the BFT system had enhanced the fish physiological status concerning the non-specific immune response and the antioxidant capability. This is, of course, would guarantee a proper health status of intensively cultured tilapia within this system. Therefore, the BFT system could efficiently contribute to the physiological health of cultured tilapia.

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