Pakistan J. Zool., pp 1-8, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220414070405

Comparative Study of Growth, Hematology and Proximate Composition of Nile Tilapia (*Oreochromis niloticus*) in Biofloc Technology and Conventional Pond Culture System

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Article Information
Received 14 April 2022
Revised 20 April 2023
Accepted 06 May 2023
Available online 28 August 2023 (early access)

Authors' Contribution
YJ collected samples, analysed data and wrote the manuscript. MZA conceived idea, supervised the research, and revised the manuscript. ZM collected samples and revised the manuscript. SA revised the manuscript. MI did statistical analysis and guided manuscript writing.

Key words
Biofloc technology, Conventional pond, Nile tilapia, Growth, Hematology, Proximate composition

ABSTRACT

The current study was designed to evaluate the growth, hematology and proximate composition of Nile Tilapia (*Oreochromis niloticus*) cultured in super intensive Biofloc technology (BFT) and conventional pond culture systems (CPCS).Fish samples (n=5) of the same age (120 days) were collected and analyzed. Results showed significantly ($p>0.05$) higher growth performance in BFT as compared to CPCS in terms of daily weight gain (DWG), total weight gain (TWG), specific growth rate (SGR), productivity and feed conversion ratio (FCR). Various hematological parameters i.e., white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) were found significantly ($p \leq 0.05$) higher while the results of proximate analyesis i.e., crude protein (CP), crude fat (CF) and total ash (TA) were found significantly ($p \leq 0.05$) lowere in BFT as compared to CPCS. Moerover, the moisture content was observed significantly higher in BFT than CPCS. The survival rate was aproximatly 100% in both culture systems. Overall the current study showed improved growth performance and hematology in BFT while betterproximate composition of Nile tilapia reared in CPCS.

INTRODUCTION

Aquaculture is one of the fastest-growing food industries with an annual average growth rate of 5.3% during the period of 2001-2018. For the last five years, aquaculture has contributed 46% to world fish production and is estimated to grow from 82.1 million tonnes in 2018 to 109 million tonnes in 2050 (FAO, 2020). Due to the rapid growth in the world population which is expected to reach 9.5 billion in 2050 and the world food demand will increase about 70% (de Marsily and Abarca-del-Rio, 2016). Pakistan's human population increased with an annual growth rate of 2.4% from 132 million in 1998 to 208 million in 2017 and is estimated to rise to 250.2 million by 2050. Although this growth rate is lower than 2.7% of the previous intercensal period (1981-1998) but much higher than the anticipated growth rate of 1.9% (Goujon *et al*., 2020).Aquaculture and fisheries contribute a major role in the economy of a nation.Fisheries is a major source of nutrition, food, and occupation (Welcomme *et al*., 2010) supporting livelihoods of about 60 million people and supplying about 17% of global protein (FAO, 2018). Fish being an important source of various proteins, deal with the "hidden hunger" i.e. health-related issues due to micronutrients deficiency (Kennedy, 2002). To fulfill the increased food demand the aquaculture sector being an important source of nutrition, employment, and food needed to be well developed (Sharifinia *et al*., 2019). Intensification of the farming system is needed to meet the increasing food demand (Garlock *et al*., 2020).

Pakistan fish production mainly comes from capture fisheries and aquaculture practices. Initially, the aquaculture contribution was very low (3517 million tons/ year from 1950 to 1990) but with time it increases

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0030-9923/2023/0001-0001 $ 9.00/0

significantly (5460 million tons/ year from 1991 to 2017). The highest aquaculture production contributing to total capture fisheries production was recorded as 1,53,230 million tons in the year 2017 (Shah *et al*., 2018). Growth in Pakistan's fish production in the past two decades has come largely from aquaculture (FAO, 2018). The inland aquaculture in Pakistan is mainly relying on intensive, semi-intensive, and extensive culture systems. Although the growth rate of aquaculture production is increasing in Pakistan, but it is still far behind from the production level of other countries (Suplicy *et al*., 2017). To increase the production level within the available resources many new technologies are being developed worldwide e.g., Biofloc, recycling aquaculture system (RAS), Aquaponics, etc. Fish farmers in Pakistan are also adopting these technologies according to their environment, financial budget, and available resources.

Challenges associated with the expansion and intensification of the aquaculture sector are aquatic animal disease (Stentiford *et al*., 2017), degradation of the ecosystem (Reverter *et al*., 2020), and water pollution due to high load of nitrogenous wastes (Crab *et al*., 2007). Biofloc technology is the best solution to the problems associated with the expansion of the aquaculture industry such as its impact on the environment and the limited water and land resources (Khanjani and Sharifinia, 2020).

Biofloc technology (BFT) is a cost-effective, sustainable, and environmental friendly (due to zero or minimum water exchange) way of aquaculture to improves water quality as well as in situ production of microbial protein that reduces the feed conversion ratio (FCR) (Sgnaulin *et al*., 2018). It is sustainable alternative for intensive aquaculture of Nile tilapia (*Oreochromis niloticus*), Pacific white shrimp (*Litopenaeus vannamei*) and polyculture of silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio* L) and bighead carp (*Aristichtys nobilis*) (Zhao *et al*., 2014). BFT might be the best solution for sustainable aquaculture production in culturing both detritivorous and filter-feeding species (Luo *et al*., 2014).

Tilapia (*Oreochromis niloticus)* is the most important aquaculture fish species (FAO, 2007). After carps, tilapia is the second widely cultured fish species (Ridha, 2006) boosting world aquaculture production and is the important source of animal proteins and income (Sosa *et al*., 2016). Tilapia is the best candidate for aquaculture due to its stress tolerance, widely accepted taste, fast growth, and easy breeding in captivity (Guerrero, 2019). Now a day'stilapia consumption has increased worldwide and is known as aquatic chicken (Fitzsimmons, 2005).

MATERIALS AND METHODS

Fish stocking

Monosex tilapia (*Oreochromis niloticus)* seed was obtained from Tawakul Tilapia Fish Hatchery Muradabad Muzaffargarh, Punjab. The fish was transported in Polythene bags with sufficient oxygen provided, to Smart Biofloc Fish Farm Peshawar and to Faisal Tilapia Fish Farm, chakri Rawalpindi for rearing under Biofloc technology and conventional pond culture system respectively. A total of 800 fish with an initial average weight of 25 (g) was stocked in BFT circular tank of 4×1.5-meter (Height X Diameter) with 10,000-liter water capacity. The CPCS pond was stocked at the rate of 3400 fingerling/acre.

Pond preparation and feeding

The conventional pond was prepared and fertilized pre-stocking by adding cow dung 250 kg/acre, Urea 2 kg/acre, and DAP 4 kg/acre while Biofloc tank was added with 100 g probiotics 15×10^9 CFU/gram (Napro company imported for China), 10 m^3 water, 2kg molasses, 100 g $CaCo_3$ and 10 kg raw salt.Fish was fed with commercially available 30% CP soya feed twice a day at the rate of 5% wet body weight in both BFT and CPCS.

Water management

Water parameters of the Biofloc tanks were maintained by adding fermented carbon organic (FCO). FCO was prepared by mixing probiotics (100 g) and molasses (5 kg) /200liter water and was provided with aeration for 24 h. FCO was added regularly 40% of the total feed used to maintain a C/N ratio of 10:1. The conventional pond were maintained by using AC-Pro aquaculture treatment chemical.

Growth performance

Growth performance of Nile Tilapia from both culture systems was obtained viz.,specific growth rate (SGR) and daily weight gain (DWG)using standard formulas (Hopkins, 1992).

$$\text{Specific growth rate (SGR)} = (\text{Ln}(W_f) - \text{Ln}(W_i))/t \text{ (days)}$$

$$\text{Total weight gain (TWG) (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Daily weight gain (DWG) (g/day)} = (W_t - W_i)/\text{days}$$

Feed conversion ratio (FCR) and Survival rate percentage was determined by using formula given by Khanjani *et al*. (2017).

$$\text{Feed conversion ratio (FCR)} = \text{total amount of dry feed consumed (g)/ wet weight gain of fish (g)}$$

$$\text{Survival rate (\%)} = \text{(final number of fish/ initial number of fish)} \times 100$$

Hematology

Neubauer haemacytometer was used to count the white blood cells (WBC), red blood cells (RBC) and platelets (PLT) in the blood of fish sampled randomly from both cuture systems. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were calculated (Wintrobe, 1933). The hemoglobin (Hb) and hematocrit (HCT) was measured by using Sahli's haemometer and micro haematocrit reader (NUVE, NF-048 Turkey), respectively.

Proximate analysis

Forproximate composition the fish were dissected andvisera was removed along with body scales. Fish meat was oven dried at 60 °C till constant weight and grinded. Moisture content, crude protein, crude fat and total ash were determined by following the standard procedure of AOAC (2005).

Statistical analysis

The data is expressed in terms of Mean±SD. All hematological and meat data were incorporated into SPSS software version 21 for desktop. T-test (two-tailed) was used to calculate the difference between the proximate composition of fish samples from the BFT and CPCS. Significance was set at ($p < 0.05$).

RESULTS

Growth performance

Growth performance, survival and productivity of Nile Tilapia (*O. niloticus*) in BFT and CPCS at the end of 120 days is presented in Figure 1. Survival rate was 100% in both culture systems while SGR, DWG, TWG, and productiovity were significantly higher ($p \leq 0.05$) in BFT as compared to CPCS. FCR in CPCS was significantly higher ($p \leq 0.05$) than BFT.

Hematology

Hematological parameters of Nile Tilapia (*O. niloticus*) reared in biofloc technology and conventioanal pond culture systes (CPCS) are shown in Table I. The various hematological parameters were found significantly higher ($p \leq 0.05$) in BFT.

Proximate analysis

The proximate analysis of Nile Tilapia (*O. niloticus*) cultured in a conventional pond was significantly higher($p \leq 0.05$) in terms of crude protein (CP), crude fat (CF), and total ash (TA) than fish cultured in a biofloc system. The moisture content was recorded higher ($p > 0.05$) in biofloc cultured fish (Fig. 2).

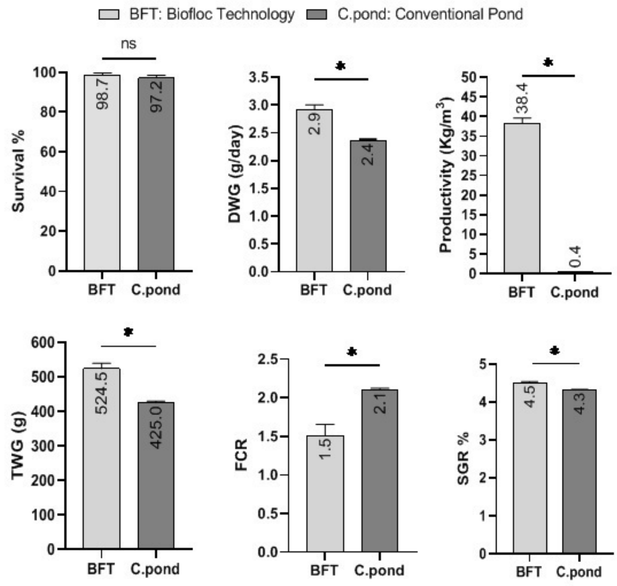

Fig. 1. Growth performance of nile tilapia (*O. niloticus*) reared in biofloc technology and conventional pond culture system. DWG (g/day) = daily weight gain; TWG(g) = Total weight gain; FCR = feed conversion ratio; SGR%, specific growth rate; ns, not different significantly; *, significantly different.

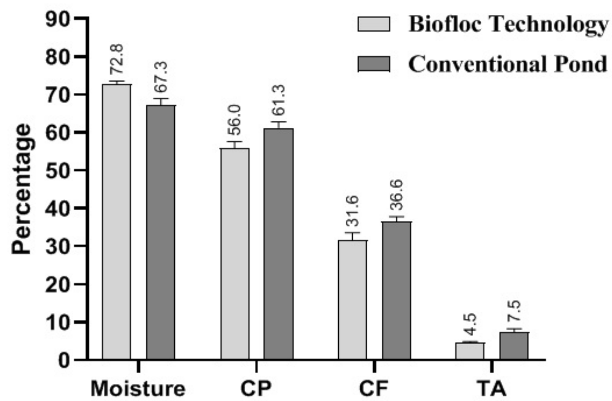

Fig. 2. Comparative proximate composition of nile tilapia (*O. niloticus*) reared in biofloc technology and conventional pond culture system. CP, crude protein; CF, crude fat; TA, total ash.

DISCUSSION

Growth performance

Results of the present study revealed better growth performance of Nile Tilapia reared in biofloc technology as compared to conventional pond culture system.

Table I. Hematology of Nile Tilapia (*Oreochromis niloticus*) reared in biofloc technology (BFT) and conventional pond culture system (CPCS).

Parameters	Culture system	
	BFT	CPCS
WBC (10^3/µL)	185.00 ± 5.2[b]	151.60 ± 6.10[a]
RBC (10^6/µl)	2.44 ± 0.08[b]	2.32 ± 0.14[a]
Hb (g/dL)	13.80 ± 0.83[b]	11.94 ± 0.48[a]
HCT (%)	52.44 ± 1.18[b]	41.75 ± 2.27[a]
MCV (fL)	189.60 ± 7.70[b]	174.20 ± 3.96[a]
MCH (pg)	49.56 ± 1.69[b]	46.52 ± 1.47[a]
MCHC (g/dL)	31.82 ± 1.12[b]	28.78 ± 0.95[a]
PLT (10^3/µL)	287.20 ± 7.19[b]	272.80 ± 4.08[a]

Means ± SD with different alphabets are significantly different ($p \leq 0.05$). WBC (10^3/µL), white blood cells, RBC (10^6/µl), red blood cells; HGB (g/dL), hemoglobin; HCT(%), hematocrit; MCV(fL), mean corpuscular volume; MCH (pg), mean corpuscular hemoglobin, MCHC (g/dL), mean corpuscular hemoglobin concentration, PLT (10^3/µL), platelets.

These results are in accordance withvarious studies that reported increased growth performance of cultured animals by the presence of biofloc in culture systems (Panigrahi *et al*., 2019; Adineh *et al*., 2019). In support to our studyAzim and Little (2008) also observed higher individual fish weight in biofloc treatment as compared to the control using a recirculating system. BFT treatments in the experiment also contributed 44-46% greater individual weight gain and net fish production than those in controls. Food conversion ratio (FCR) value was also significantly higher in the control as compared to the BFT treatment tanks.In biofloc probiotics, essential nutrients and several organic compounds are present (Najdegerami *et al*., 2016). The presence of biofloc and the supplementary feed provides a complete diet to the cultured aquatic animals (Khanjani and Sharifinia, 2020). 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). Many bioactive compounds were evaluated in biofloc such as carotenoids, chlorophylls, and phytosteroids, which are expected to contribute to better growth of cultured organisms in the biofloc system (Ju *et al*., 2008). In contrast to our study Little *et al.* (2008) reported better growth performance in ordinary recirculating culture systemas compared to that in indoor and outdoor BFT systems. Such results might be due to the biological turbidity provoked by biofloc and/or the chronic stress arising from deteriorated and fluctuated water quality.

Hematology

Hematological parameters are important to monitor health of cultured animals and for detecting abnormalities and disease etc. (Harikrishnan *et al*., 2012).In the presentstudy hematological parameters including WBC, RBC, Hb, HCT, MCV, MCH, MCHC and PLT were significantly higher ($p \leq 0.05$) in BFT than in the conventional pond. These results were in accordance with Zafar *et al*. (2021) who also reported increased hematological parameters of stinging catfish *(Heteropneustes fossilis)* in BFT as compared to control. Higher values of hematological perameters in *O. niloticus* reported by Gayed *et al*. (2021) with probiotics supplemented diet support the results of present stsudy. The improved hematological parameters in BFT as compared to conventional pond may be due to the application of probiotics (Salam *et al*., 2021; Saravanan *et al*., 2021; Iwashita *et al*., 2015). To the best of our knowledge data regarding the effect of BFT on hematological parameters is insufficient and contradictory. Azim and Little (2008) observed non significant difference in HCT% between control and BFT groups in Nile Tilapia. In contrast to our study Hisano *et al*. (2021) found non significant difference in hematological parameters of *O. niloticus* cultured in BFT and RAS and in anonther study conducted by Long *et al*. (2015) also non significant hematological parameters were found between indoor BFT with no water exchange and control with water exchange.

Proximate analysis

Proximate composition of fish meat i.e., Crude protein, crude fat, ash and moisture are the important factors to evaluate the muscle quality of edible organisms (Lie, 2001; Yang *et al*., 2018a). Different environmental conditions lead to alteration in the proximate components (Yang *et al*., 2018). Data regarding comparative analysis of the proximate composition of *O. niloticus* is insufficient in BFT. Numerous studies have been conducted to evaluate the proximate composition of tilapia and various other species in BFT, RAS, Floating cages. Comparative studies of the proximate composition of wild and pond-reared fish have also been conducted (Hisano *et al*., 2021; Long *et al*., 2015). In our study, significantly higher values of crude protein, crude fat, and total ash of *O.niloticus* were found in the conventional pond culture system as compared to fish reared in biofloc technology while moisture content was significantly low in the conventional pond. Significantly higher moisture content in BFT (72.82%) was recorded as compared to the conventional pond (67.26%). Azim and Little (2008) also found higher moisture content (71.25%) for *O. niloticus* fed with 35% crude protein along with biofloc and that of 67.83%in fish fed with 24% crude protein feed with addition of biofloc. Zafar *et al*. (2021) reported the moisture content of 75.74% and 75.62% in BFT and control for Asian stinging catfish (*Heteropneustes fossilis*). Another study conducted by Desta *et al*. (2019) on the proximate composition of *O. niloticus* reported higher moisture content (73.62%) in semi-intensive pondsas compared to wild fish (66.77%) of Lake Hawassa.

The crude protein content in the present study was (56.00 ± 1.58) and (61.26 ± 1.58) for BFT and conventional pond, respectively. These results were in accordance to Azim and Little (2008) who reported the crude protein values of 53.41%, 55.28%, and 49.55% for *O. niloticus* cultured in indoor tanks and fed with different experimental diets i.e. 35% CP with biofloc, 35% CP without biofloc and 24% CP with biofloc respectively. Khanjani *et al*. (2021) also reported similar values of crude protein 59.23% for *O. niloticus* in Biofloc systems provided with Barley flour as a carbon source. The improved proximate parameters in pond cultured fish may be due to the foraging movement which leads to the breakdown of lipids and accumulation of protein content in the fish muscles (Yang *et al*., 2018; Xu *et al*., 2006, 2015). Chowdhury *et al*. (2000) and Uddin *et al*. (2001) also reported higher protein content in paddy culture systems as compared to conventional pond due to the availability of more diverse microbes and other supplementary nutrients.

Crude fate content in the current study calculated as 31.60±1.95 and 36.58±1.22 for BFT and conventional pond, respectively was in accordance with Khanjani *et al*. (2021) and Azim and Little (2008) who reported 25.69% and 27.83% respectively for *O. niloticus* in biofloc cultured systems.

Total ash content found in present study (7.46%) for the conventional pond was significantly higher as compared to BFT i.e., 4.52%. These results were in contrast to Desta *et al*. (2019) who reported low ash content in pond reared (1.51%) as compared to wild *O. niloticus* (1.89%).

Overall the present study showed that BFT is one of the best option to adopt for intensive fish culture to fulfill the increasing world food demand in a sustainable way. Beter Growth and hematological parameters of Nile Tilapia indicating healthy environment in BFT as compared to CPCS. However beter proximate composition of fish meal was recorded in conventional pond culture system but it requires more land as well as water. Further improvements in BFT is needed to to produce quality fish with beter proximate composition. Hybrid culture system of BFT and conventional pond may also fill the gap to atain beter quality fish meat.

ACKNOWLEDGEMENT

The provision of research facility at Smart Biofloc Fish Farm in Peshawer, KP by Mr. Salim Nawaz and the conventional fish pond at Rawalpindi, Punjab by Mr. Muhammad Faisal is greatly acknowledged.

Funding

It was a self funded research work, no funds were acquired from any organization for this study.

IRB approval

Not applicable.

Ethics statement

All the rules and regulations approved by ethical committee of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi were followed.

Statement of conflict of interest

The authors have declared no conflict of interest.

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