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Biofloc System and Stocking Density Effect on Water Quality, Feed Utilization, and Growth Performance of the Nile Tilapia (*Oreochromis niloticus*)

Ashraf M. Soliman[1], Anwer Abed Alazwari[2], Alam Eldeen Farouk[1]*

[1]Department of Limnology, Central Laboratory for Aquaculture Research Center, Agricultural Research Center

[2]Ministry of Environment, Water and Agriculture, KSA

[*]Corresponding Author: asolimanh@yahoo.com; alameldeenfarouk84@yahoo.com

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ABSTRACT

To assess the impact of varying stocking densities under the Biofloc system (BFS) on water quality, feed utilization, and growth performance of the Nile tilapia fingerlings, the current study was carried out over 90 days. It was implemented in 12 production rectangular concrete ponds (5.5×13×1.40m) with a water volume of about 100m^3 and different stocking densities (40, 60 or 80 fish/m^3) under Biofloc and Traditional system treatments. Overall, the Biofloc treatments outperformed the Traditional system treatments in terms of the water quality indicators. Regardless of stocking density, the water quality indicators were within the range advised for tilapia production. Biofloc system treatments showed elevated growth performance rates and reduced FCR, unlike Traditional system ponds. The total yield directly increased with stocking densities (40, 60, and 80 fish/m^3) in BFS. Generally, our results indicated that the higher fish densities relate to higher productivity but lower growth performance and survival rates. According to the feed consumption findings, the feed-saving rate under the Biofloc culture system improved significantly, reaching 29.83% at the stocking density of 40 fish/m^3 and 9.83% at the stocking density of 60 fish/m^3. The results also showed that, in contrast to the Traditional systems, the higher the fish stocking density, the more feed was consumed and the lower the saving rate. The findings also demonstrated that the Biofloc system had 0% daily water change rates in contrast to Traditional culture systems. Monthly water change rates were lower in the Biofloc treatments than in the Traditional systems, although it increased with the higher stocking density.

INTRODUCTION

One advantage of intensive aquaculture is the increased stocking density, which necessitates a rise in the quantity of high-quality artificial feed **(Avnimelech *et al*., 2008)**. However, increases in fish biomass raised in this system, along with the amount of feed used, lead to rapid water quality deterioration, resulting in impacts on productivity that should be avoided. There has been a trend toward constantly changing aquaculture water, which requires high operating energy at high cost **(Gutierrez-Wing & Malone, 2006)**. Therefore, scientists have turned their attention to recycling systems (RAS) for decades, but the high implementation and operating costs have prevented the extensive spread of this technology **(De Schryver *et al*., 2008)**. Biofloc technology was suggested as a substitute for conventional methods, such as extensive and semi-extensive aquaculture production systems **(Luo *et al*., 2014)**.

One of aquaculture's most inventive and promising sustainable methods is Biofloc Technology (BFT). With zero water exchange and enhanced water quality from beneficial bacterial biomass activity, this approach supports intensive aquaculture production. BFT also lowers production costs by serving as a nutrient-rich aquaculture feed **(Mugwanya *et al*., 2021)**. Nowadays, BFT is used to cultivate commercial species like shrimp and tilapia **(Emerenciano *et al*., 2021)**. Many researches have shown that the juvenile Nile tilapia is more effectively fed biofloc microorganisms than fattening ones **(Alves *et al*., 2017; Bossier & Ekasari, 2017; Sousa *et al*., 2019)**. Suspended biofloc is also considered an additional protein-rich diet for the Nile tilapia **(Hisano *et al*., 2019),** causing lower production costs **(Prabu *et al*., 2017)**.

In BFT, the stocking density of fish depends on manipulating the water quality's physicochemical parameters. Where, the first limiting factor for increasing culture density is dissolved oxygen while ammonia nitrogen is the second factor which comes from fish nutritional metabolism **(Ali *et al*., 2020; Eid *et al*., 2020)**.

The fundamental data which includes the appropriate stocking density during the Nile tilapia grow-out phase in BFT, is still lacking and is a deciding element for the tilapia production's economic viability in BFT, where the optimal density should be determined by a thorough assessment of fish ponds that takes into consideration growth rate data, water quality, fish health, production expenses, and profits. For this particular context, this experiment investigated how the water quality, growth, and feed consumption of the Nile tilapia (*Oreochromis niloticus*) fingerlings are impacted by stocking density when BFT is applied vs the Traditional aquaculture system

MATERIALS AND METHODS

Research location

The government fish farm served as the site of the study, on the desert highway near Bielbese City in El Sharkia Governorate, Egypt. The experiment period was 90 days, from the 1st of June until the 30th of August 2022.

Experimental ponds

Twelve production rectangular concrete ponds (5.5×13×1.40m)with a water volume of about 100m^3 were designed to allow the water current to turn around the 1.40m intermedial septum over 22h/day to preserve circumstance of water ponds in a suspension case for stabilizing the flock granules in a life state. All ponds were filled with pure well (ground) water (Fig. 1). Experimental ponds treated with a carbon source were cleaned and refilled with pure groundwater before being stocked with the Nile tilapia fingerlings. Feeding commenced after one day of acclimation, with continuous monitoring of key water quality parameters (unionized ammonia [NH_3], nitrite [NO_2^-], and nitrate [NO_3^-]) during the initial biofloc formation phase. Once biofloc granules developed, both inlet and outlet gates of all treated ponds were closed, establishing a zero-water-exchange system that was maintained throughout the experimental period. In contrast, control ponds (traditional system without carbon addition) underwent daily water exchange at rates of 10, 15, and 20% of total pond volume for treatments 1b, 2b, and 3b (stocking densities of 40, 60, and 80 fish/m³, respectively). This water exchange protocol was implemented to remove metabolic wastes (including feces) and to maintain optimal water quality conditions for tilapia culture.

Experimental design

Treatment description	Treatment synonym
Ponds stocked with 80 fish/m^3 treated with a carbon source	1a/ Biofloc system
Ponds stocked with 80 fish/m^3 without carbon source	1b/ Traditional aquaculture
Ponds stocked with 60 fish/m^3 treated with a carbon source	2a/ Biofloc system
Ponds stocked with 60 fish/m^3 without carbon source	2b/ Traditional aquaculture
Ponds stocked with 40 fish/m^3 treated with a carbon source	3a/ Biofloc system
Ponds stocked with 40 fish/m^3 without carbon source	3b/ Traditional aquaculture

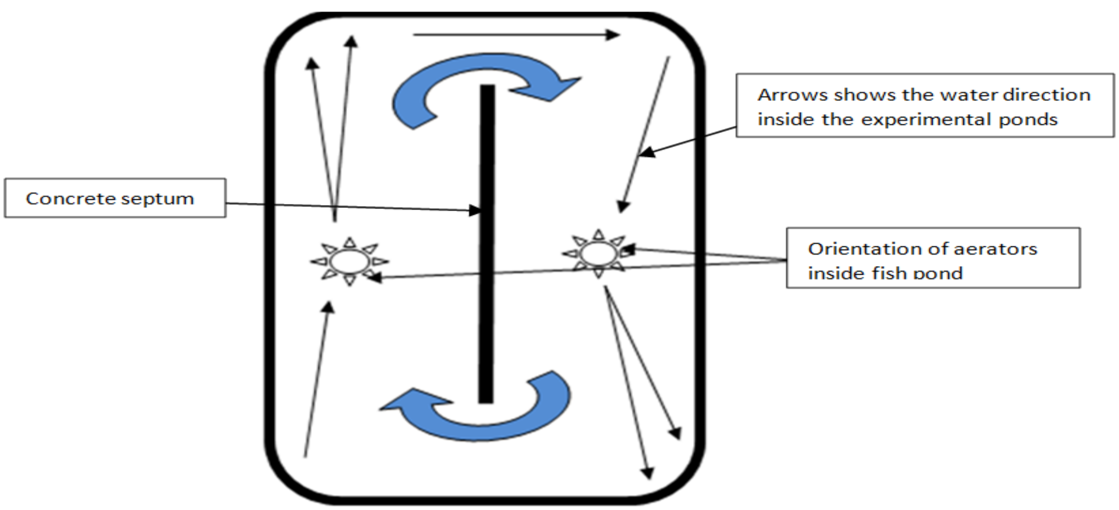

Fig. 1. Vertical view of the experimental ponds showing the orientation of aerators, concrete septum and the direction of water movement

Aeration and oxygenation

Aeration was continuously provided using two-air splashers 1.5 hp/pond. Both splashers were fixed in the middle of each pond on both sides of the concrete septum (Fig. 1) but in an opposite direction for pushing the water to circle movements around the central septum, so biofloc granules were still suspended for at least 22h/ day, in addition to maintaining the concentration of dissolved oxygen at levels of at least 5mg/ l.

Water quality monitoring

Salinity (ppt), pH, dissolved oxygen (mg/l), and water temperature were measured on-site using a YSI 556MPS meter (Yellow Spring Instrument Co., OH, USA). According to standard procedures **(APHA, 2000)**, unionized ammonia (NH_3 mg/l), nitrite (NO_2 mg/l), and nitrate (NO_3 mg/l) were measured every two weeks using a spectrophotometer (spectrophotometer 2000, CO, USA).

To obtain accurate measurements of total suspended solids (TSS), Whatman filter paper was used to filter 100 milliliters of each pond water. The gathered samples were subsequently dried for three hours at 105 degrees Celsius in an oven **(Khanjani *et al*., 2021)**. The volume of water-settable solids was measured weekly using an Imhoff cone to track the growth of Biofloc **(Avnimelech & Kochba, 2009)**.

Imhoff cone methods

The Imhoff sedimentation cone (graduated up to 1000ml) is clear plastic, therefore it is easy to see the level marks between the settled solids and the amount of the suspended solid that will settle out of liquids. When a liquid is allowed into the cone, the suspended solids settle to the bottom within 10 to 15 minutes. It refers to the volume of biofloc granules (masses).

Growth performance parameters

Fish from each pond were gathered and caught after 12 weeks, where the overall yield for each pond was evaluated. The following were the indexes of fish growth, productivity, and feed usage.

- Daily weight gain (DWG; gram/day) = WG (gram) / feeding period (day).

[Where, weight gain (WG; gram) = final fish weight (gram) – Initial fish weight (gram)].

- Survival rate (%) = 100 (fish final number at the end of the experiment / initial number).
- Feed conversion ratio (FCR) = feed added (kilogram/pond) / net yield (kilogram/pond).
- Condition factor (K Factor) = mean [body weight (gram) /body length^3 (Centimeter)] ×1000.
- Specific growth rate (SGR %/day) = 100 [Ln (final weight) – Ln (initial weight)] / experimental period.
- Total yield (Kilogram/pond) = (Average of individual final fish weight × no off survived fish.

Starting the biofloc inside the experimental ponds

Wheat flour (WF), which was acquired from a nearby wheat grinding company, was one of the suggested carbonaceous sources **(Soliman & Abdel-Tawwab, 2022)**. Table (1) displays the commercial diet's WF analysis (32% crude protein; CP).

Avnimelech (1999) used the following equation to determine how much carbohydrate supplement (ΔCH) was needed to lower the ammonium.

$$\Delta CH = \Delta C\ mic \times \%\ C\ X\ E$$

Where, ΔC mic symbolizes the amount of carbon as established by microorganisms. %C stands for the added carbohydrate's carbon content, which is typically about 50% for most substrates.

ΔN, Nitrogen quantity needed to cell material formation, is determined by the microbial biomass's C/N ratio, which is approximately 4:

$$\Delta N = \Delta\ C\ mic\ /\ [C/N]\ mic = \Delta CH\ X\ \%C\ X\ E\ /\ [C/N]\ mic$$

Considering the approximate values for [C/N] mic, % C, and E, we have 0.5, 0.4m, and 4, in that order:

$$\Delta\ CH = \Delta\ N/\ (0.5\ X\ 0.4/4) = \Delta\ N/0.05.$$

Biofloc was started in carbon-treated ponds after two weeks from stocking tilapia fingerlings, and TAN concentration came up to 2mg/ l and then WF was added after the commercial feed contenting of 32% crude protein, 7.1% total lipids, 4.2% crude fiber, and 6.6% total ash (Table 1).

To create biofloc biomass, carbohydrates were supplied to ponds treated with BFT while keeping in mind the 15:1 (C: N) ratio **(Avnimelech, 2009)**. Their proximate composition was used to compute the levels of WF. After three hours of the feeding schedule, the calculated source of carbohydrates was weighed, and put into 30-L plastic containers. The mixture was fully integrated with the growing ponds' waters and was then sprayed on the pond's water surface to promote biofloc growth. After being fed a commercial feed (32% CP) at a rate of 3% of their biomass, the Traditional system ponds fish and those treated with BFT were recalculated based on subsequent fish samples. The recommended feed amounts were introduced twice a day at 9 am and 15 am. Every two weeks, 50 fish samples from each pond were weighed after being gathered with a net and returned to the ponds. In light of this, feed amounts were changed every two weeks.

Table 1. The approximate chemical compositions of commercial fish diet and wheat flour (as a percentage of dry matter)

Contents (%)	Wheat flour	Commercial diet for fish
A dry substance	91.4	91.3
Unprocessed protein (as crude)	14.2	32
Total lipids	1.23	7.1
Carbohydrates	71.4	50.3
Crude fiber	2.31	4.2
Total ash	1.27	6.6

Calculated and consumed feed

Calculated feed was depended upon the total biomass and feeding rate, which was 3% daily of the total biomass and was readjusted again according to biweekly fish samples.

Consumed feed refers to the actual amount of feed ingested by the stocked or total biomass of reared fish. In biofloc ponds, however, the fish may consume some of the biofloc granules as an alternative natural food source. As a result, some feed pellets may remain uneaten despite appearing to be consumed. This is due to the presence of natural food (biofloc) already existing in BFT-treated ponds. Therefore, a trial was conducted to address potential biases in feed intake estimation by calculating the amount of feed accurately consumed by the fish. The saved fish feed was then determined using the following formula:

- **Saved fish feed** = Calculated feed quantity – Actually consumed feed

Examining statistics

The statistical study employed maximum, minimum, and means ± standard error (SE) data. A two-way ANOVA was conducted to analyze water quality data, examining the effects of stocking density and farming system (Traditional system vs. BFS) as the two main components. Following a one-way ANOVA of the other data, Tukey's test was used as a post-hoc test to determine significant differences between treatments at the P<0.05 level. All statistical analysis was conducted using SPSS version 20, as described by **Dytham (2011)**.

RESULTS AND DISCUSSION

Water quality characteristics

Table (2) explains the changes in water quality monitored during the study period. All treatments showed significant differences (P<0.05) among the investigated water quality parameters, except for water temperature.

There was no discernible change in water temperature during the current experiment. Furthermore, the temperature recorded in this investigation falls within the range suitable for the development of biofloc and the production of tilapia **(Soliman & Abdel-Tawwab, 2022)**.

Aquaculture operations depend significantly on pH measurements, which might indicate how productive an aquatic ecosystem is **(Okbah *et al*., 2017; Farouk, 2018)**. Overall, the mean water pH values recorded in the present study ranged between 7.33 and 7.8 in the Biofloc treatments, with significant differences compared to Traditional system treatments (P< 0.05), which had higher water pH values, ranging from 8.37- 8.67. These results agree with those of **Ridha *et al*. (2020)** and **Soliman and Abdel-Tawwab (2022)**, who argued that Biofloc ponds had lower pH values than Traditional system ponds. This is explained by the significant growth of biofloc biomass, which releases CO_2 from fish respiration and microbes while consuming oxygen. The reduction of pH value in Biofloc-treated water may be due to the consumption of CO_3^{2-} and HCO_3^- ions by bacterial community, which reduces pH levels to become in the optimum values for Biofloc systems which ranges from 7 to 9 **(Avnimelech *et al*., 2012; Vicente *et al*., 2020; Soliman & Abdel-Tawwab, 2022; Elnady *et al*., 2023)**.

Fish stocking levels in experimental ponds are primarily limited by dissolved oxygen, followed by total ammonia nitrogen (TAN), which is impacted by pH **(Boyd & Tucker, 1998)**. For microbes in suspended flocks to breathe, dissolved oxygen levels must be kept between 5 and 8mg/ l **(Hargreaves, 2013)**.

Dissolved oxygen levels in the Biofloc and Traditional system ponds ranged from 5.3-5.6mg/ l to 5.1-5.5mg/ l, respectively (Table 2). The treatments of density (80 fish/m^3) exhibited the least dissolved oxygen content, likely because higher stocking densities increase aerobic metabolic activity **(De-Schryver *et al*., 2008; Hwihy *et al*., 2021)**.

Table 2. Water quality parameters (average ± SE; range) in ponds with the different Nile tilapia densities in BFS-based ponds compared to Traditional system ponds over 90 days

Stocking density (f/m^3)	Treatment	TC (°C)	pH	DO (mg/l)	NH_3 (mg/l)	NO_2^- (mg/l)	NO_3^- (mg/l)	TSS (mg/l)	IMMH OF CONE (ml/l)
40	Biofloc (3a)	25.97 ± 0.12	7.33 ± 0.03^d	5.60 ± 0.06^a	0.04 ± 0.01^b	0.09 ± 0.00^c	29.73 ± 0.03^a	339.47 ± 0.64^a	46.00 ± 1.53^a
	Traditional system (3b)	25.83 ± 0.09	8.37 ± 0.03^b	5.53 ± 0.09^a	0.11 ± 0.02^b	0.18 ± 0.03^b	2.53 ± 0.52^c	18.00 ± 1.78^d	5.33 ± 0.88^d
60	Biofloc (2a)	25.87 ± 0.12	7.67 ± 0.03^c	5.37 ± 0.13^{ab}	0.08 ± 0.01^b	0.10 ± 0.00^c	26.30 ± 0.46^b	299.80 ± 0.52^b	35.00 ± 1.15^b
	Traditional system (2b)	25.83 ± 0.09	8.60 ± 0.10^a	5.27 ± 0.15^{ab}	0.70 ± 0.11^a	0.25 ± 0.01^a	2.83 ± 0.88^c	18.00 ± 1.78^d	4.67 ± 0.88^d
80	Biofloc (1a)	26.03 ± 0.15	7.80 ± 0.06^c	5.30 ± 0.17^{ab}	0.09 ± 0.00^b	0.17 ± 0.03^b	26.03 ± 0.90^b	280.33 ± 5.66^c	31.00 ± 1.53^c
	Traditional system (1b)	25.83 ± 0.09	8.67 ± 0.12^a	5.07 ± 0.09^b	0.16 ± 0.01^b	0.26 ± 0.01^a	2.67 ± 0.74^c	18.00 ± 1.78d	4.33 ± 0.88^d
ANOVA in Two Ways		Value of *P*							
Treatment		0.201	0.0001	0.202	0.0001	0.0001	0.0001	0.0001	0.0001
Stocking density		0.755	0.001	0.024	0.0001	0.005	0.038	0.0001	0.0001
Treatment × Stocking density		0.755	0.526	0.771	0.0001	0.342	0.21	0.0001	0.0001

Means with different letters in the same column are substainally different at $P<0.05$ (Tukey's test).

It is noteworthy that there were no significant changes concerning dissolved oxygen concentrations between Traditional system and Biofloc ponds, which can be ascribed to the artificial aeration, matching those of **Soliman and Abdel-Tawwab (2022)** and **Elnady *et al.* (2023)**. The dissolved oxygen levels were consistently suitable for the growth of the Nile tilapia fingerlings during the experimental period, according to **El-Sayed (2019)** and **Soliman and Abdel-Tawwab (2022)**. Additionally, the BF ponds had appropriate levels of dissolved oxygen for BFT systems (> 4mg/ l) **(Avnimelech *et al*., 2012)**.

The obtained un-ionized ammonia (NH_3) concentrations along the study period were 0.04, 0.08, 0.09mg/ l in Biofloc treatments 1a, 2a, 3a, respectively, and 0.11, 0.7, 0.16mg/ l in Traditional system treatments 1b, 2b, 3b, respectively. While, nitrite (NO_2^-) concentrations fluctuated between 0.09 and 0.17mg/ l in Biofloc treatments 1a and 3a, respectively, and 0.18 and 0.26mg/ l in Traditional system treatments 1b and 3b, respectively. Nitrate concentrations fluctuated between 26.03 and 29.73mg/ l in Biofloc treatments 3a and 1a, respectively and 2.53 and 2.83mg/ l in the Traditional system treatments 1b and 2b, respectively.

The inorganic nitrogen compounds results indicated that the decreased concentrations of NH_3 and NO_2^- paired with the increased NO_3^- concentrations in all experimental treatments, which can be traced back to the artificial aeration in all ponds (BFT and CT) increasing the levels of dissolved oxygen in ponds water **(Soliman & Abdel-Tawwab, 2022)**, which consequently increase nitrification process by nitrifying bacteria in water that can oxidize NH_3 to NO_2^- and then NO_3^-, which may be re-incorporated into plant or microbial proteins **(Maciel *et al*., 2018; Robles-Porchas *et al*., 2020)**. As a result of these factors, the ultimate product of nitrification, NO_3^-, is typically far more concentrated than NH_3 and NO_2^- **(Camargo *et al*., 2005; Hamlin, 2006)**.

The lower concentrations of NH_3 and NO_2^- in the Biofloc (BF) system compared to the Traditional system, along with a significant increase in NO_3^- levels in the BFT system, indicate a higher abundance of biofloc microorganisms, including nitrifying bacteria such as ammonia-oxidizing bacteria. These bacteria convert NH_3 to NO_2^- and subsequently to NO_3^- **(Correia *et al.*, 2014; Soliman & Abdel-Tawwab, 2022)**. This process is further supported by the presence of phytoplankton, which helps remove ammonia, promotes bacterial uptake, and facilitates nitrification—from NH_3 to NO_2^-, followed by its oxidation to NO_3^-.

Biofloc consists of suspended organic matter and microbial biomass in the water column, containing living microbes that are essential to the functioning of the Biofloc System (BFS) **(Ebeling *et al.*, 2006; Hargreaves, 2006)**. These biological mechanisms play a crucial role in reducing ammonia and nitrite levels, maintaining water quality at non-toxic levels that support healthy fish growth **(Ahmed *et al.*, 2019)**.

Various microorganisms collected from the BF system have been shown in numerous studies to play essential roles in removing nitrogenous pollutants while contributing to sustenance, diet and general health **(Ray *et al.*, 2010; Emerenciano *et al.*, 2013a; Cardona *et al.*, 2016; Ahmad *et al.*, 2017; Daniel & Nageswari, 2017)**.

A significant part of the total suspended solids (TSS) in the BF system is particulate organic carbon, including bacteria, algae, zooplankton, uneaten feed, excrement, and detritus **(Burford *et al.*, 2004; Ray *et al.*, 2010)**.

The current experiment revealed that the total suspended solids (TSS) results in ponds treated with the Biofloc Technology system were significantly higher than those in the Traditional system ponds. This finding aligns with the research conducted by **Khanjani *et al*. (2021a)** and **Soliman and Abdel-Tawwab (2022)**. Additionally, the obtained results indicated that, according to **Emerenciano *et al*. (2017)** and **Soliman and Abdel-Tawwab (2022)**, the TSS in the BFT system was at an ideal level (less than 500 mg/l) for biofloc formation. Furthermore, **Avnimelech (2006)**, **Hargreaves (2013)** and **Long *et al*. (2015)** suggested that this concentration is optimal (less than 1000mg/ l) for promoting fish growth.

Biofloc biomass abundance in culture ponds was estimated by Imhoff Cone volumes to explain the materials of Biofloc abundance in terms of its effect on the nutrition of fish **(Elnady *et al*., 2023)**.

The Imhoff cone volume results reflected a similar trend to total suspended solids, indicating that these were greater levels in the BFT system vs the Traditional system. Increased feed supply and organic carbon supplements are to blame for this, the same conclusion has previously been reported **(Liu *et al*., 2017; Adineh *et al*., 2019; Elnady *et al*., 2023)**. Imhoff cone values fall within the permissible range for the Nile tilapia culture (25-50ml/ l), as advised in several studies on Biofloc water **(Ray *et al*., 2010; Hargreaves, 2013; Green *et al*., 2014; Emerenciano *et al*., 2017; Lima *et al*., 2018; Ridha *et al*., 2020; Elnady *et al*., 2023)**. **Dos Santos *et al.* (2021)** proposed tolerance limits of up to 50ml/ l for SS levels in Biofloc water.

It is possible to say that all investigated water quality parameters during the present study were constantly within acceptable limits for tilapia aquaculture, irrespective of fish stocking density. This guarantees a favorable atmosphere for the fish's optimum development and well-being **(Monsees *et al*., 2017; Alvarenga *et al*., 2018; El-Sayed, 2019; Manduca, *et al*., 2021)**.

Growth performance variables

The growth performance variables of the Nile tilapia in Biofloc vs Traditional systems at different fish stocking densities are indicated in Table (3). Significant differences ($P < 0.05$) were observed for all investigated variables among all treatments except K factor and SGR, which did not have significant differences ($P > 0.05$) among BFS. Additionally, no notable variations ($P > 0.05$) were recorded across ponds using

Traditional systems. While, their interaction had no notable variations (P>0.05) for all investigated growth performance parameters, except FCR.

Additionally, obtained results revealed that stocking density of 40 (fish/m^3) had the maximum average of final weight, daily weight gain, and survival rate with values of 255.83g/ fish, 2.04g/ fish/ day, and 98%, respectively. In contrast, their minimum values were 155.37g/ fish, 0.91g/ fish/ day, and 79.33%, respectively, at 80 fish/m^3 stocking density. It is obtainable that BFT ponds had higher values of these parameters than CT ponds. Additionally, the lowest density of fish recorded the highest values, as shown in Fig. (2).

This may be clarified through that the Biofloc system is a nutrient-rich environment containing beneficial microorganisms like protozoa, bacteria, and phytoplankton. These provide high-protein food for the Nile tilapia, such as rotifers and ciliates, which enhances fish survival and growth in this system **(Rono *et al*., 2018)**.
The results above confirm those previously revealed by **Li *et al*. (2012)** and **Eid *et al*. (2020)**, who assumed that increased fish stocking density leads to reduced feed consumption. According to **Wendelaar Bonga (1997)**, chronic stress brought on by the high density was thought to raise the fish's overall energy requirement, which would otherwise be used for growth. Additionally, fish density can influence how well feed is used. In addition, the stock density of fish affects their survival rate. Other investigations related to the Biofloc system showed an adverse connection between survival rate and stocking density **(Widanarni *et al*., 2012)** that can be attributed to greater concentration of NH_4^+ at the higher fish density contributed to growth performance **(Vicente *et al*., 2020)**. Overall, the content of N-metabolites in the system affected productive performance, which has an opposite relationship with the density of fish stocks **(Widanarni *et al*., 2012)**.

Condition factor and specific growth rate had the same value among different stocking densities in biofloc treatments (3.43 and 1.27%/ day, respectively) and Traditional system ponds (2.33 and 0.91%/ day, respectively). (Table 2 & Fig. 3). The higher survival rate in the current trial is linked to the Biofloc system's superior water quality, which is supported by **Wasielesky *et al*. (2013)** and **Elnady *et al*. (2023)**. This demonstrates that the amount of biofloc material consumed by the Nile tilapia during the experiment provided them with essential nutrients necessary for survival. According to **Adineh *et al.* (2019)**, fish growth performance in biofloc systems is significantly influenced by the consistently improved water quality, where fish benefit from consuming suspended biofloc material rich in high-quality protein.

The Nile tilapia showed improved survival rates in BFS with carbon supplementation vs the Traditional system. These results may be attributed to the system with carbon supplementation maintaining better water quality parameters compared to the traditional system. The biofloc system offers a sustainable approach to maintaining water quality within a range that is beneficial for the Nile tilapia **(Ekasari *et al*., 2015; Rono *et al*., 2018)**.

Feed conversion ratio indicates more nutrients are efficiently converted into flesh compared to Traditional feed, suggesting an effective food utilization **(Bhijkajee & Gobin, 1997)**. In BFT, the Nile tilapia feed conversion ratio (FCR) was at its best values (1.07, 1.28, and 1.49, respectively) for stocking densities (40, 60, and 80 fish/m^3) compared to 1.97, 2.32, and 2.65 for Traditional system treatments (Table 2 & Fig. 3). Whereas, the lowest (best) values of FCR were recorded at the lowest fish density (40 fish/m^3).

Feed conversion ratios in the BFS treatments were improved compared to those in the Traditional system, demonstrating the benefits of using biofloc material as a nutritional source for the Nile tilapia during the fattening phase. This improvement can be attributed to the protein being consumed by tilapia in two forms: first through the formulated feed, and second through microbial protein present in the biofloc **(Ogello *et al*., 2014)**. In this situation, microbial biomass is considered an additional food source **(Hisano *et al*., 2021)**.

The feed conversion ratio can be affected by changes in fish size and age, stocking density, environmental conditions, cleanliness methods, and other unknown variables **(Watanabe *et al.*, 1990)**.

Table 3. Nile tilapia growth performance (average ± SE; range) in BFS-based ponds vs the Traditional system ponds over 90 days with varying stocking densities

Stocking density (f/m^3)	Treatment	Initial weight (g/fish)	Final weight (g/fish)	DWG (g/fish)	Survival rate (%)	FCR	K Factor	SGR (%/day)	Total Yield (Kg/ pond)
40	Biofloc (3a)	72.33 ± 0.60	255.83 ± 6.71^a	2.04 ± 0.08^a	98.00 ±1.00^a	1.07 ± 0.02^f	3.43 ± 0.07^a	1.27 ± 0.02^a	1003.17 ± 33.21^c
	Traditional system (3b)	73.00 ± 0.58	185.30 ± 3.03^c	1.25 ± 0.03^c	87.33 ± 1.45^c	1.97 ± 0.03^c	2.33 ± 0.15^b	0.91 ± 0.02^b	647.30 ± 15.08^e
60	Biofloc (2a)	72.17 ± 0.17	232.23 ± 1.41^b	1.78 ± 0.01^b	93.67 ± 0.88^b	1.28 ±0.02^e	3.43 ± 0.07^a	1.27 ± 0.02^a	1305.20 ±16.82^b
	Traditional system (2b)	73.00 ± 0.58	161.67 ± 1.11^d	0.98 ± 0.02^d	84.67 ± 1.45^c	2.32 ± 0.04^b	2.33 ± 0.15^b	0.91 ± 0.02^b	821.20 ±13.36^d
80	Biofloc (1a)	72.33 ± 0.60	227.13 ± 2.77^b	1.72 ± 0.03^b	91.67 ± 0.88^b	1.49 ± 0.01^d	3.43 ± 0.07^a	1.27 ± 0.02^a	1665.93 ± 33.01^a
	Traditional system (1b)	73.00 ± 0.58	155.37 ± 4.33^d	0.91 ± 0.05^d	79.33 ± 1.20^d	2.65 ± 0.04^a	2.33 ± 0.15^b	0.91 ± 0.02^b	985.23 ± 13.53^c
ANOVA in Two Ways		Value of *P*							
Treatment		0.127	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.127
Stocking density		0.984	0.0001	0.0001	0.0001	0.0001	1.0	1.0	0.984
Treatment × Stocking density		0.984	0.983	0.977	0.392	0.002	1.0	1.0	0.984

Means with different letters in the same column are substainally different at P<0.05 (Tukey's test).

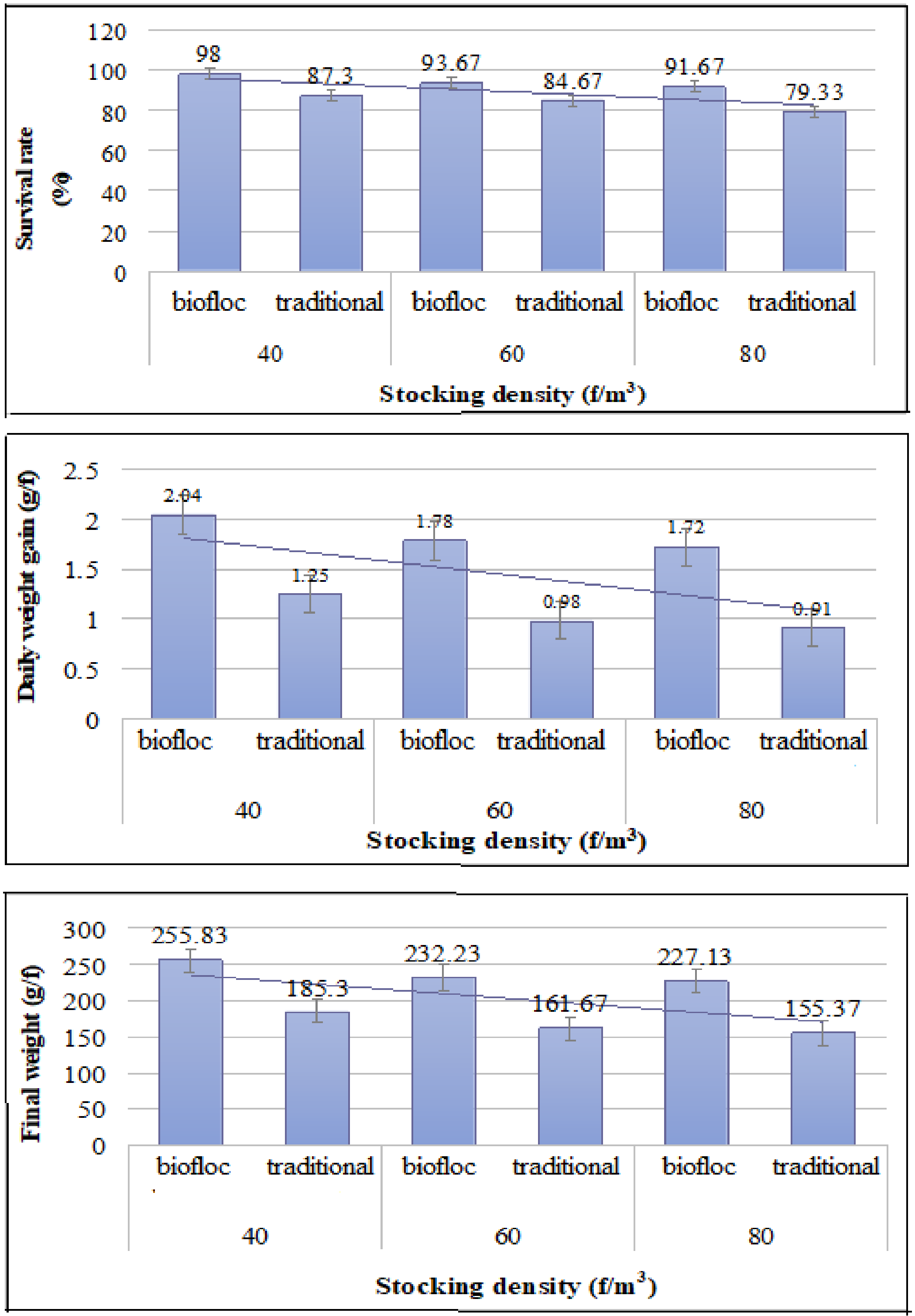

Fig. 2. Changes in the Nile tilapia survival rate (%), daily weight gain (g/f/day), and final weight (g/f) between BFS ponds vs the Traditional system with different stocking levels

In this experiment, reduced stocking density led to a correspondingly enhanced FCR compared to high stocking density. These findings are in line with the findings of **Bolivar *et al.* (2006)** and **Eid *et al.* (2020)**, who noted that the FCR deteriorated as the density of fish stocks increased. This can be related to elevated energy requirements linked to stress, which negatively affects FCR **(Moniruzzaman *et al*., 2015)**.

The beneficial effect of microbial flocs on improved weight gain, SGR, and FCR in cultured tilapia was established by **Nguyen *et al*. (2021)**. Numerous research studies support these findings, including **Luo *et al*. (2014)**, **Long *et al*. (2015)** and **Mansour and Esteban (2017)**.

The Biofloc system (BFS) treatments resulted in lower FCR and higher growth performance rates compared to Traditional system ponds. In these treatments, most of the feed is converted to body mass (meat), indicating that fish culture in the carbon-supplemented BFS enhances protein utilization. Biofloc can enhance the growth, consumption, and digestion of aquatic animals and artificial feeds, according to a similar finding by **Emerenciano (2013)**.

Biofloc technology provides continuous additional food sources for tilapia 24 hours a day in the form of microorganisms and microbial communities, enabling a reduction in FCR **(Emerenciano *et al*., 2017; Luo *et al*., 2017)**. Using less pelletized feed inputs in Biofloc tanks improved FCR lowers production costs **(Correa *et al*., 2020)**. In addition, fish productivity and feed efficiency are also increased by ingesting Biofloc, according to **Luo *et al*. (2014)** and **Long *et al*. (2015)**.

Results demonstrate that microbial flocs enhance nutrient digestion in the stomach by promoting the development and activation of digestive enzymes. This likely contributes to a better performance of microbial floc treatments regarding growth and feed utilization. The Nile tilapias' ability to readily adjust to novel feeding conditions was also demonstrated **(Xu & Pan, 2012; AbouelFadl *et al*., 2022)**.

The BFS treatments of this experiment achieved the highest total yield of fish, which were 1003.17, 1305.20 and 1665.93kg/ pond compared to the Traditional system treatments (647.30, 821.20 and 985.23 kg/ pond) for the stocking densities of 40, 60 and 80 fish/pond, respectively. Although the increase in total yield corresponded with higher stocking densities, as shown in Fig. (3), it was also accompanied by a significant rise in costs due to sharply increasing feed prices. Feed costs represented the largest portion of total expenses; therefore, as stocking density increased, so did the overall production cost **(Moniruzzaman *et al.*, 2015; Manduca *et al.*, 2021)**.

Following the data gathered during this research, higher fish densities correlate to higher productivity but lower growth and survival rates. This finding agrees with **Gall and Bakar (1999)**, **Azim and Little (2008)**, **Avnimelech and Kochba (2009)**, **Widanarni *et al*. (2012)**, **Long *et al*. (2015)** and **Vicente *et al*. (2020)**.

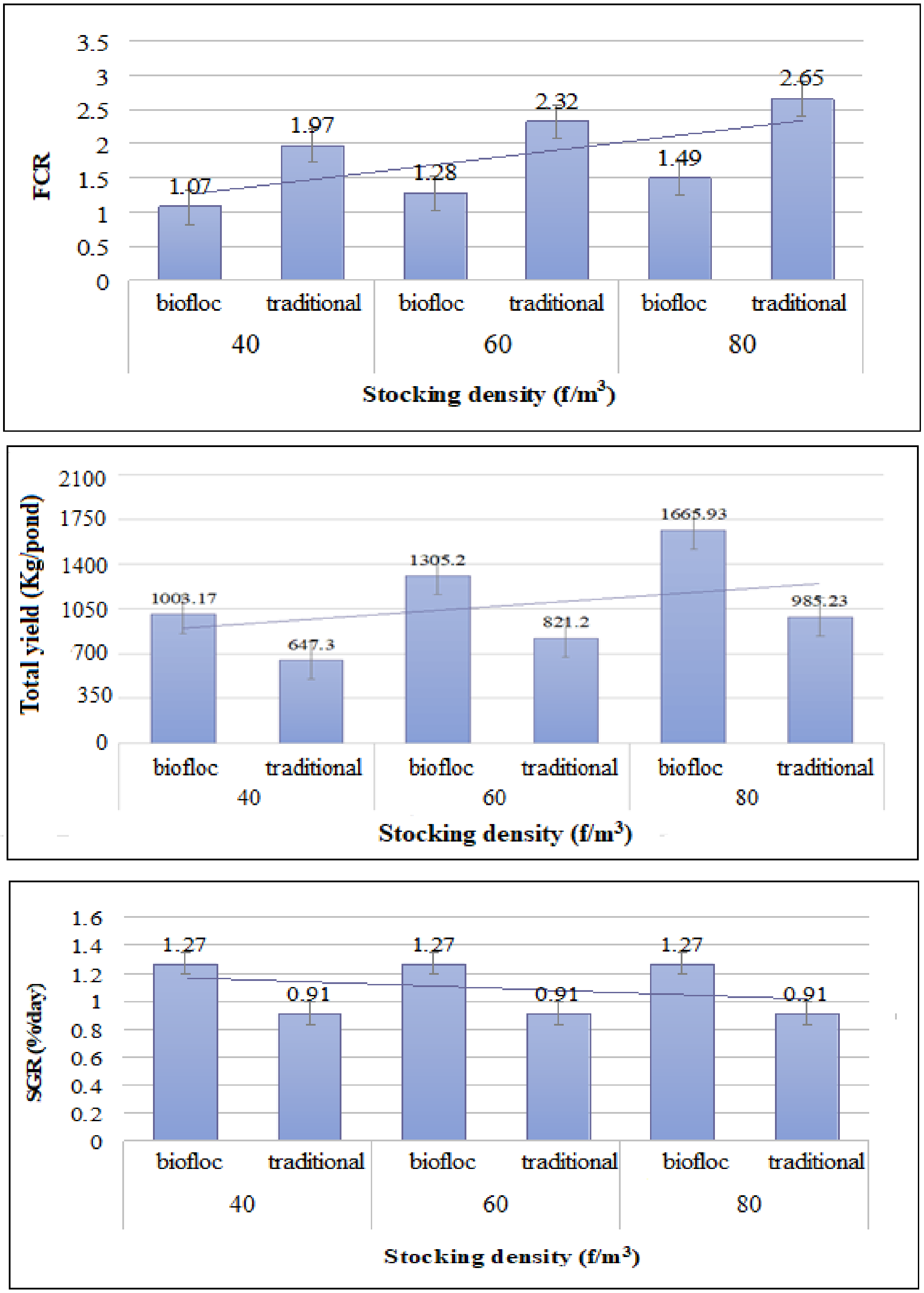

Fig. 3. Changes in the Nile tilapia feed conversion ratio (FCR), total yield (Kg/pond), and specific growth rate (SGR, %/day) between BFS ponds vs the Traditional system with different stocking levels

It was suggested that greater death rates are caused by larger stocking densities of the Nile tilapia (larval and juvenile) **(Ridha, 2006; Garcia *et al*., 2013; Ferdous *et al*., 2014; Lima *et al*., 2018; Wu *et al*., 2018)**. Fish stocking density, growth stage, production system, species, and nutritional status can all affect these results. The probiotic impact of Biofloc may also be linked to improved fish immune system **(Haridas *et al*., 2017; Liu *et al*., 2018; Menaga *et al*., 2019)**.

BFT's flocs have a high content of protein, fat, carbohydrate, and ash, which makes them a food suitable for the aquaculture system. This system is an inexpensive, high-quality food technology that outperforms the Traditional culture system **(Ballester *et al*., 2010)**. In addition, Biofloc may increase the activity of digestive enzymes **(Avnimelech, 1999; Kuhn *et al*., 2010)**. This could affect the Nile tilapia's growth at Biofloc vs the Traditional system treatments, previously approved in the study of **Hwihy *et al*. (2021)**.

Feed utilization and water consumption

Feed utilization (including calculated feed, actually consumed feed, the amount saved, and the saving ratio), daily water exchange rate, and monthly water consumption during the experimental treatments in both the Biofloc and traditional systems are presented in Table (4). The data revealed that stocking densities, treatment types, and their interactions had a significant effect on all measured parameters ($P< 0.05$).

The feeding results (Table 4 & Fig. 4) showed a significant difference between the calculated and actually consumed feed by farmed tilapia, highlighting the benefits of the Biofloc system. This was further supported by the calculated saved feed and feed-saving ratios, where the Biofloc system achieved feed-saving ratios of 29.88, 20.87, and 9.83% at stocking densities of 40, 60, and 80 fish/m³, respectively—compared to 0.00% in all treatments of the traditional system.

These findings confirm that the continuous availability of biofloc particles as a supplemental food source throughout the day enhanced feed efficiency. As a result, the Nile tilapia raised in the Biofloc system consumed less formulated feed **(Ekasari & Maryam, 2012; Haridas *et al*., 2017)**. This reduction in daily feed intake with biofloc supplementation has been reported in the studies of **Perez-Fuentes *et al*. (2016)** and **Elnady *et al.* (2023)**.

By decreasing water exchanges and lowering the discharge of nutrient-rich wastewater, the Biofloc system technology represents a new "blue revolution" **(Awad *et al*., 2021)**. An increasingly popular long-term and sustainable substitute for no-water exchange culture systems is biofloc technology **(De Schryver *et al*., 2008; Crab *et al*., 2009)**. Aquaculture water quality parameters have been altered in this way to promote nutrient circulation and to use the biomass of specific biotic communities as a direct food source for the commercial organisms being farmed **(Martinez-Córdova *et al*., 2015)**.

The investigated technique increases the rate at which value is added while lowering maintenance costs **(AbouelFadl *et al*., 2022)**. In comparison with traditional systems, Biofloc techniques enable a zero-water replacement, which reduces 30% from water treatment costing, shortens the aquaculture period, and boosts aquatic species' survival and growth rates **(Sontakke *et al*., 2018; Holanda *et al*., 2020)**. Therefore, they are economically feasible for the cultivation of aquaculture.

Regarding water conservation, as shown in Table (4), the daily water exchange rates for the traditional system treatment ponds, which had stocking densities of 40, 60, and 80 fish/m³, were 10, 15, and 10m³, respectively, throughout the trial. In contrast, the biofloc treatment ponds had a 0% water exchange rate. However, the monthly water consumption rates for the same stocking densities in the biofloc treatment ponds were significantly lower, at 4.67, 10.67, and 19m³, respectively. These amounts were much less than the water consumption in the Traditional system treatment ponds, which were 400, 550, and 646m³, as indicated in Table (4) and Fig. (5).

Table 4. Changes in feed utilization and water exchange (daily and monthly) (average ± SE; range) in BFT-based ponds versus Traditional system ponds over 90 days with varying stocking densities

Stocking density (f/m^3)	Treatment	Calculated Feed (kg)	Consumed Feed (kg)	Saved Feed (kg)	Saving Ratio (%)	Daily Water Exchange (cm)	Monthly Water Consumed (m^3)
40	Biofloc (3a)	1083.40 ± 35.89^c	759.67 ± 19.80^d	323.73 ± 17.06^a	29.83 ± 0.67^a	0.00 ± 0.00^c	4.67 ± 0.88^f
	Traditional system (3b)	699.10 ± 16.29^e	699.07 ± 16.27^d	0.03 ± 0.03^c	0.00 ± 0.00^d	10.00 ± 0.00^b	400 ± 0.00^c
60	Biofloc (2a)	1409.63 ± 18.15^b	1115.33 ± 6.06^b	294.30 ± 13.92^a	20.87 ± 0.72^b	0.00 ± 0.00^c	10.67 ± 0.88^e
	Traditional system (2b)	886.90 ± 14.42^d	886.87 ± 14.45^c	0.03 ± 0.03^c	0.00 ± 0.00^d	15.00 ± 0.00^a	550 ± 0.00^b
80	Biofloc (1a)	1799.17 ± 35.65^a	1622.33 ± 36.36^a	176.83 ± 8.91^b	9.83 ± 0.52^c	0.00 ± 0.00^c	19.00 ± 1.53^d
	Traditional system (1b)	1064.10 ± 14.59^c	1064.03 ± 14.61^b	0.07 ± 0.03^c	0.00 ± 0.00^d	10.00 ± 0.00^b	646 ± 3.33^a
ANOVA in Two Ways		Value of *P*					
Treatment		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Stocking density		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Treatment × Stocking density		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Means with different letters in the same column are substainally different at $P<0.05$ (Tukey's test).

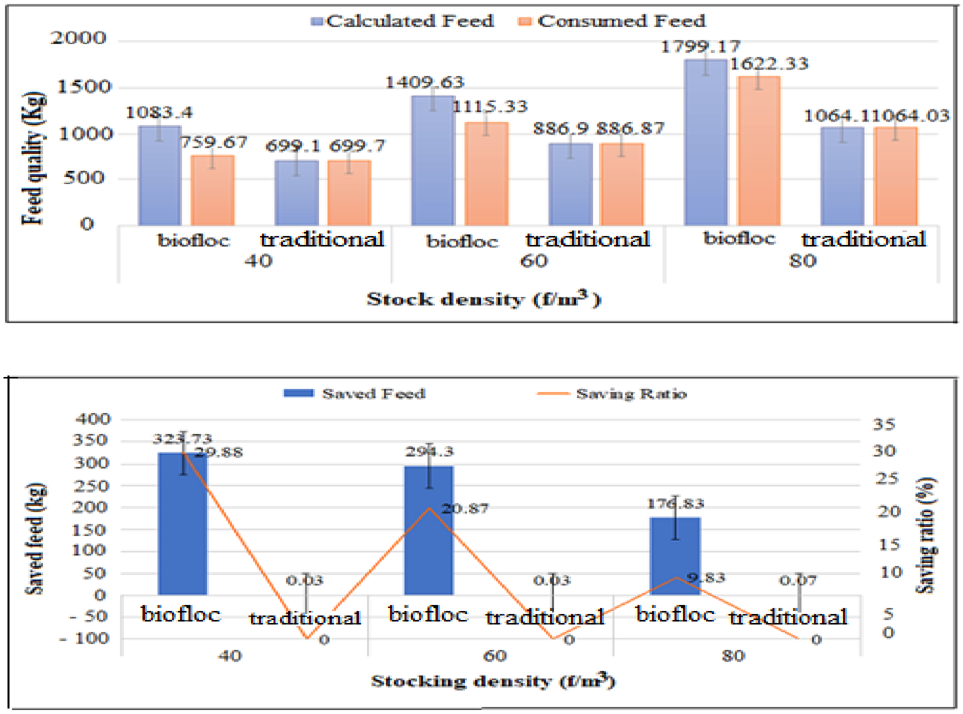

Fig. 4. Changes of calculated feed, consumed feed, saved feed, and feed saving ratio in BFT-based ponds versus Traditional system ponds over 90 days with varying stocking densities

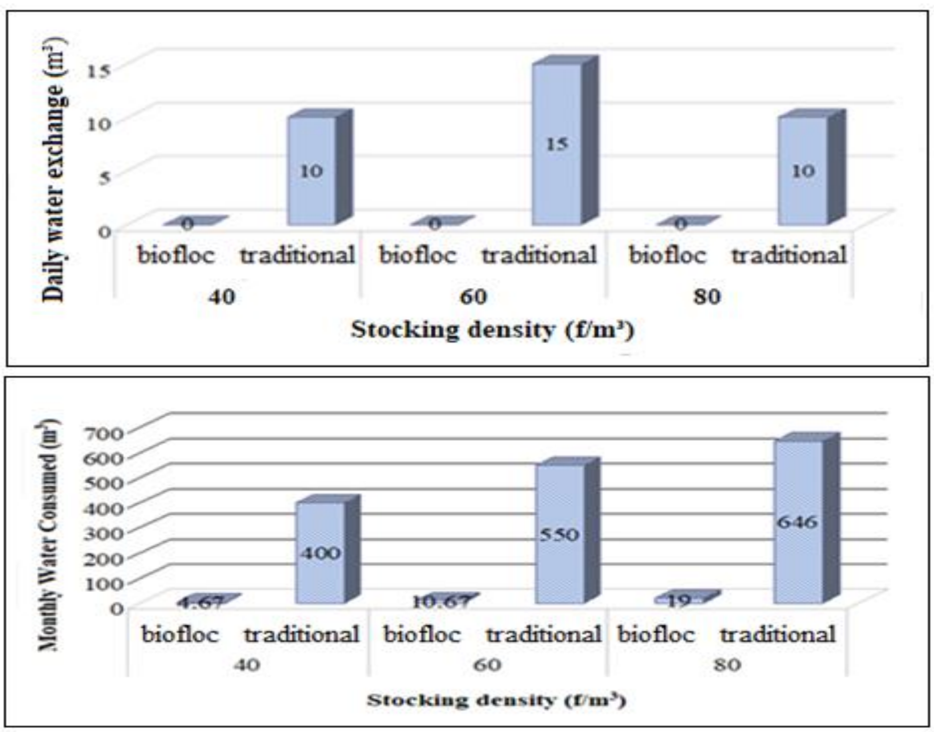

Fig. 5. Water exchange rates (daily and monthly) in BFT-based ponds versus Traditional system ponds over 90 days with varying stocking densities

The findings are in line with the outcomes of **De-Lima *et al*. (2018)**, who elucidated that Biofloc Technology (BFT) utilized 11.5 times less water than traditional aquaculture systems. Similarly, **Hwihy *et al.* (2021)** reported that Biofloc treatments reduced water usage by nine times compared to Traditional system treatments. BFT is an environmentally sensitive technique that allows for a culture system with minimal water exchange while supplying aquaculture fish with possibly edible biomass **(Avnimelech, 2014; Bossier & Ekasari, 2017)**. This can be achieved by adding an external carbon supplier to aquaculture pond, which raises the (C:N) ratio and promotes the growth of naturally occurring heterotrophic bacteria **(Hargreaves, 2006; De-Schryver *et al*., 2008)**.

These beneficial microbes will subsequently transform harmful nitrogenous compounds into Biofloc agglomerates, a biomass produced by bacteria **(Ebeling *et al*., 2006)**. The components of BF agglomerates comprise diverse organic substances that provide a stable source of nutrients for aquatic species. These organisms can collect and digest microscopic particles like microalgae, bacteria, plankton, and leftover food **(Emerenciano *et al*., 2013; Bossier & Ekasari, 2017)**.

Biofloc technology has been suggested by many researchers and producers of sustainable aquaculture **(Emerenciano *et al*., 2014; Fauzi *et al*., 2017; Kaya *et al*., 2019; Gallardo-Collí *et al*., 2020; Costa *et al*., 2021)** as one of the most promising substitutes for traditional food production. This is because (1) water exchange prevents the release of nutrient-rich wastewater into the environment and enables efficient use of finite water resources, and (2) production costs are lower because it uses less artificial feed input.

CONCLUSION

The outcomes of the current study have proven the viability of Biofloc technology as a sustainable and efficient alternative to traditional tilapia culture. The Biofloc system provides an effective solution to the issues of high-density aquaculture by significantly enhancing water quality, feed utilization, and growth performance. Furthermore, its ability to reduce feed costs and to eliminate the need for daily water replacement makes it an environmentally friendly and economically viable strategy. These findings support the expanded use of Biofloc technology in modern aquaculture, especially in light of limited resources and the need for sustainability.

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