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Red tilapia reared in biofloc conditions: the impact of stocking density and dietary carbon sources on growth performance and hematological indices

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Biofloc technology, Carbon sources, stocking density, Growth performance, body chemical composition, hematological indices, red tilapia

Abstract:

The purpose of this experiment is to the effect of various carbon sources in the biofloc system on growth performance, feed utilization, hematological indices and body biochemical composition, of red tilapia under varying stocking densities. A set of 300 red tilapia fingerlings in good health, weighing 2.44:2.55±0.19 g on average, were cultivated for 14 weeks at a density of 100 and 150 fish/m^3 in fiberglass tanks (75 L). Eight treatments, representing four distinct carbon sources molasses, sugar, and rice bran as well as control (clear water) were used in the design of the experiment, with two stocking densities of 100 and 150 fish/m3. Three times a day, the fish were fed commercial feed with a protein content of 32%, up to 5% of their body weight. Growth, and feed utilization were all significantly impacted by the addition of carbon sources, according to the analysis of variance data. According to the data, molasses and rice bran produced the best growth performance, feed utilization and hematological indices for red tilapia at a stocking density of 100 fish/m^3.

Keywords: *Biofloc technology, Carbon sources, stocking density, Growth performance, body chemical composition, hematological indices, red tilapia*

INTRODUCTION

Due to their broad tolerance to salinity, red tilapia (*Oreochromis sp.*) are a fish species that may flourish in both freshwater and saltwater environments **(Yue *et al*., 2016; Ahmadi *et al*., 2016)**. Red tilapia is becoming more and more consumed, and it is now one of the fish exported **(FAO, 2018)**. Because tilapia are omnivorous fish with a wide range of environmental tolerances and the ability to live in large densities, biofloc technology has been utilized in the intensive production of tilapia.

A number of factors limit the amount of tilapia that can be produced to meet consumer demand, one of which is the reduced water quality caused by feed waste in the cultivation tank system. As inedible feed, feces, and fish metabolic waste products accumulate in aquaculture containers, this waste will break down and lower the water quality parameters **(Putra *et al*., 2017; Ombong and Indra, 2016)**. As a result, water quality in fish farming needs to be properly managed for the best possible living conditions **(Nurhatijah *et al*., 2016)**.

The foundation of Biofloc technology is the careful control of the carbon to nitrogen ratio and the input of carbonaceous organic materials to the cultivation system, which promotes the growth of heterotrophic bacteria **(Khanjani and Sharifinia, 2020)**. Carbohydrates have been identified by **Emerenciano *et al*. (2017)** and **Khanjani *et al*. (2017)** as an efficient means of mitigating the effects of nitrogenous wastes in aquaculture systems that have either very little or no water exchange.

When determining the amount of carbs required to maintain a high C:N ratio, it is important to account for the presence of proteins in complex carbohydrates, including wheat flour and barley flour **(Avnimelech, 2009; Walker *et al*., 2020)**. There are numerous other factors to take into account when choosing a carbon source, including accessibility, palatability and digestibility, bioavailability and bacterial uptake efficiency, water-dispersibility, and cost-effectiveness. According to **Khanjani *et al*. (2017)**, carbonaceous organic matter should be suspended in water to increase its accessibility to bacteria and should be liquid or finely powdered to decrease its settling rate.

The incorporation of carbonaceous organic materials into the cultivation system and meticulous regulation of the carbon to nitrogen ratio, which encourages the growth of heterotrophic bacteria, form the basis of Biofloc technology **(Khanjani and Sharifinia, 2020)**. In aquaculture systems with either very little or no water exchange, **Emerenciano *et al*. (2017) and Khanjani *et al*. (2017)** have found that carbohydrates are an effective way to mitigate the consequences of nitrogenous wastes.

Greater success is linked to the employment of the biofloc production system in the culture of species that have traits like feeding on detritus, tolerating moderate oxygen levels, and adapting to large densities **(Khanjani and Sharifinia, 2020)**. Because of its unique qualities, tilapia may be produced in the biofloc system under both intense and super intensive conditions **(Avnimelech, 2009; Samocha *et al*., 2017)**. According to **Durigon *et al*. (2019)**, this species can also eat bacteria that depend on biofloc. The management of the biofloc system is significantly influenced by the kind of carbon source that is employed in it **(Panigrahi *et al*., 2019)**.

The current study assessed the effects of three different carbon sources molasses, sugar, and rice bran as well as clear water in the biofloc system on the body biochemical compositions, growth performance, hematological indices, and water quality for red tilapia.

MATERIALS AND METHODS

The current study was carried out at the experimental fish unit, Department of Fish Production, Faculty of Agriculture, Azhar University, for a total of 14 weeks from 3 August to 10 November, 2021, in collaboration with the department of animal, poultry, and fish production, Faculty of Agriculture, Damietta University, Egypt. The purpose of this study was to determine how adding a carbohydrate source to different stocking densities in the biofloc system affected the red tilapia's (*Coptodon rendalli*) growth performance, feed consumption, body composition, blood components, and histological evaluation .

Red tilapia (*Coptodon rendalli*) fingerlings were obtained from fish hatchery Kilo 21, Lakes and Fish Resources Protection and Development Authority, Egypt. The average body weight and length of red tilapia are 2.7 g and 6.8 cm, respectively. A total of 300 fingerlings of two distinct stocking densities (100 and 150 fish /m3) were placed in 24 tanks (66×47×44 cm - 75 L) (triplicated for each) using a (2×4) factorial design with two stocking density 100 and 150 fish/m3 and four external carbon sources (clear water - control), molasses, sugar and rice bran, respectively). The experimental design was summarized by the following groups:

T1: Clear water(control) 100 fish /m3 (D1+ CS)
T2: Clear water(control) 150 fish /m3 (D2+ CS)
T3: Molasses 100 fish / m3 (D1+ MO)
T4: Molasses 150 fish / m3 (D2+ MO)
T5: Sugar 100 fish / m3 (D1+ SU)
T6: Sugar 150 fish / m3 (D2+ SU)
T7: Rice bran 100 fish / m3 (D1 + RB)
T8: Rice bran 150 fish / m3 (D2 + RB)

Feeding was done by hand disseminating equal amounts of 5% biomass per day at 9:00, 13:00, and 16:00 hours. To help the fish's culturing environment, the feeding was done 6 days weekly with one day off **(Price and Morris, 2013)**. The addition of carbon is done every 8 days.

Table (1) Chemical composition of the experimental diets (DM%).

Chemical composition	DM%
Dry mater	90.1
Crude protein	30.3
Fat	6.1
Ash	4.95
Crude fiber	4.8
NFE1	53.85
Organic carbon	37.45
GE Kcal/100g2	450.70

1-Nitrogen Free Extract= 100- (protein + fat + ash +fiber)
2- GE = gross energy was calculated as 5.65,9.45 and 4.12 Kcal /100gram of protein, lipid, and carbohydrate, respectively after **(NRS, 2011)**

Every two weeks, ten fish individuals from each tank were randomly picked. A digital weighing scale was used to measure the individual's body length (BL) and body weight (BW), respectively. After sampling, each fish was put back into its tank, and the feeding schedule was modified based on the weight change.

Water quality parameter:

The experiment involved monitoring several water quality measures, including temperature, pH, O2 and total ammonia, in order to track the impact of the biofloc system and compare it to the clear water (control).

Water temperature and dissolved oxygen were measured using a portable oxygen meter (Oxy Guard meter) daily at 13.00 h. pH values were recorded daily using a portable pH meter (Oxy Guard meter). Total ammonia nitrogen (TAN) was measured biweekly using the spectrophotometer model. Soluble reactive phosphorus (SRP), sludge volume index (SVI), and TSS values were measured twice during the experimental TSS period.

Biofloc volume:

Weekly measurements of the biofloc volume (water settleable solids) were made with an Imhoff cone. Imhoff or settling cones are an easy approach to index the concentration of suspended solids in order to track the growth of biofloc. The volume of sediments that settle in one liter of system water can be measured using the graduations marked on the outside of the cones. after 15 to 20 minutes of sedimentation, measuring the amount of biofloc in 1000 milliliters of tank water **(Avnimelech & Kochba, 2009)**. Tilapia biofloc systems will perform well if the settleable solids concentration is kept between 25 and 50 mL/L. **(Hargreaves, 2013)**.

Growth Performance and Feed utilization:

Weight gain (WG) = Average Final body weight (g) – Average Initial body weight (g). **(Carlos, 1988).**

Weight gain/day =Average Weight gain / day of experiment

According **to De-Silva and Anderson, (1995).**

Specific growth rate % (SGR) = [(Ln Average Final body weight - Ln Average Initial body weight (g)/ day of experiment] ×100.

As reported by **El-Sayed and Jamal, (2004).**

Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g)

According to **El-Sayed and Jamal, (2004).**

Feed conversion efficiency (FCE) = Weight gain (g)/ Feed intake (g)

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)

Chemical analysis of fish body:

Precipitated flocs were taken from various treatments to determine the proximate composition of the fish, and a random pooled sample of fish was sampled from each tank of different treatments at the end of the experiment. Standard procedures were followed for the chemical analysis of biofloc and whole-body DM%, CP%, EE%, and ash content % **(AOAC, 1995)**. Fish and samples were grounded and kept at -20 ˚C for further examination after being dried in an oven at 70 ˚C for 24 hours until they attained a constant weight. By burning for two hours at 600 degrees Celsius, the amount of ash was found. The Kjeltech (Model 1030, Tecator, Höganäs, Sweden) auto analyzer was utilized to determine crude protein using the micro-Kjeldhal technique, which yielded %N × 6.25. Using sechelt extraction and diethyl ether at 40–60 °C, the crude fat content of several samples was calculated.

Blood sampling protocol:

Fish were put to sleep with 100 µg mL[1] MS222 (Tricaine methane-sulfonate, Sigma-Aldrich Co. LLC) before blood was taken, as stated by **Cicia *et al*. (2012)**. Blood samples were randomly taken from each of the two fish in each tank (ten fish for each treatment). Using sterile 2.5-mL syringes, blood samples were extracted from the caudal vein and divided into equal portions. The first portion was kept for use in heparinized tubes for hematological assays, while the second part was chilled for three hours at 4 °C after coagulating at room temperature for thirty minutes. Serum was then extracted from the clotted samples by centrifuging them for ten minutes at 4 °C and 3000 rpm. Following that, the serum was stored at -20 °C until further immunological, biochemical, and antioxidant investigations could be conducted.

Blood Hematological assessments:

A hemocytometer and Natt-Herrik solution were used to quantify the erythrocyte and leukocyte counts according to the **Stoskopf (1993)** technique. However, the hemoglobin level was ascertained using the cyanomethemoglobin technique, which was endorsed by **Balasubramaniam and Malathi (1992)**. Moreover, the PCV%, MCV, MCH, and MCHC were computed using the microhematocrit method **(Dacie and Lewis, 1991)**. Blood smear slides were prepared according to the protocol described by **Blaxhall and Daisley (1973)**, allowed to air dry, fixed with methanol for three to five minutes, stained with gimsa stain for eight to ten minutes, rinsed with distilled water, and then allowed to dry at room temperature in order to calculate the differential leukocytic count.

Statistical analysis:

The data homogeneity and normalcy were assessed using the Shapiro-Wilk test. Additionally, two-way ANOVA statistical analysis approach was used to all calculated and estimated data, and Tucky's range test was used to confirm any discrepancies between the means. $P < 0.05$ served as the tested threshold of significance. The findings are shown as mean± pooled SE values **(SAS, 2012)** was used for all statistical analyses.

RESULTS AND DISCUSSION

Water quality red tilapia:

The measured values of the water quality variables (temperature, pH, dissolved oxygen and total ammonia nitrogen) are shown in Table 2 but have not clear trend. The pH ranged from 6.90±0.17 to 7.50±0.05, while the average water temperature was between 27.70±0.11 and 28.5±0.05ºC. The averages of ammonia concentration were ranged from 0.039±0.001 to 0.087±0.001, respectively. However, there were no notable variations between the treatments in terms of total ammonia. Dissolved oxygen concentration averages ranged from 4.80±0.11 to 6.30±0.17, with significant differences among treatments.

Table 2: Water quality parameters for red tilapia reared in cultivation tanks under different carbon sources during the experimental period.

Items	Measured parameters			
	°C	PH	O_2	NH_3
D1+ CS (T1)	27.80±0.11^{bc}	7.10±0.11^{bc}	5.10 ±0.05^{c}	0.064±0.002^{b}
D2+ CS (T2)	28.40±0.05^{ab}	7.10±0.05^{bc}	4.90 ±0.11^{d}	0.087±0.002^{a}
D1+ MO (T3)	28.20±0.11^{b}	7.30 ±0.11^{ab}	6.30±0.17^{a}	0.039±0.001^{d}
D2+ MO (T4)	27.90±0.17^{bc}	6.90 ±0.17^{c}	5.80 ±0.11^{b}	0.040 ±0.002^{cd}
D1+ SU (T5)	28.50±0.05^{a}	7.50 ±0.05^{a}	6.20±0.05^{a}	0.041 ±0.002^{c}
D2+ SU (T6)	28.10±0.17^{b}	7.40 ±0.17^{a}	6.10 ±0.17^{a}	0.042 ±0.002^{c}
D1 + RB (T7)	27.70±0.11^{c}	7.00±0.11^{c}	6.00 ±0.12^{ab}	0.040 ±0.001^{cd}
D2+ RB (T8)	27.90±0.12^{bc}	7.20±0.12^{b}	5.90 ±0.11^{ab}	0.041 ±0.002^{c}
One Way ANOVA (*P* Value)				
P-Value	0.003	0.043	0.0143	0.0398

CNT, fish group stocked at clean water;
MO, fish group reared under biofloc system supplemented with molasses;
SU, fish group reared under biofloc system supplemented with sugar;
RB, fish group reared under biofloc system supplemented with rice bran;
Data were presented as the mean ± standard error mean (SEM).

The PH and temperature ranges were suitable for tilapia farming and floc production. Comparing *Oreochromis niloticus* raised at varying stocking densities to the control fish in biofloc systems, comparable outcomes were observed (28.5± 0.53, 7.8 ± 0.33), respectively **(Hwihy *et al*., 2021)**. 18 to 20 °C was the ideal temperature range for biofloc **(De Schryver and Verstraete 2009)**. Thus, the current investigation shows that, although the water temperature values in all treatments were greater than the ideal range for biofloc, the cultivated red tilapia were still able to withstand them. One crucial metric for distinguishing between various water masses is dissolved oxygen **(Ibrahim and Ramzy, 2013; Osman *et al*., 2021)**. In the current study, the three carbon sources that were chosen had considerably more DO than the control. **Thilakan *et al*. (2019)**, who measured DO between 6-7.8 mg L^{-1} throughout the experimental period, corroborated this conclusion.

According to **Hwihy *et al*. (2021),** the biofloc's DO level was lower than that of the control. According to **Suita *et al*. (2015)** and **Wang *et al*. (2016)**, microorganisms linked to biofloc absorb food, maintain water quality, and reduce nitrogen-containing chemicals, particularly ammonia. In the current investigation, all treated groups' ammonia levels were considerably lower than those of the control. The group that was fed molasses showed the lowest amount, which was then followed by rice bran and sugar, as well as a lower stocking density. The increased absorption and degradation of carbon as a substrate for heterotrophic bacteria that metabolize ammonia and thereby improve water quality is likely the cause of the faster ammonia reduction observed in the molas, sugar, and rice bran groups as simple carbon sources **(Khanjani *et al*., 2017; El-Shafiey *et al*., 2018; Khanjani *et al*., 2021)**. Yet, all of the chosen carbohydrate sources demonstrated efficacy in bio flocculation, leading to a noteworthy rise in the overall number of heterotrophic bacteria. This is in line with the findings of **Khanjani *et al*. (2021)**.

Growth performance red tilapia:

Table 3 illustrates that the ultimate body weight (BW) of red tilapia grew significantly ($P<0.05$) as the levels of stocking density decreased. Consequently, fish with a stocking density of 100 fish/m3 (D1) exhibited the highest BW in comparison to the other group of 150 fish/m3 (D2). The final BW of the fish rose with all biofloc groups as compared to the control, according to the results in this table. When compared to other carbon sources and control groups, the dietary carbon source molasses (Mo) released the highest BW by a significant margin ($P<0.05$). Fish in T3 (D1+ MO), which had a stocking density of 100 fish/m^3 and molasses as a carbon source, had the highest final BW, while fish in T2, which had a control stocking density of 150 fish/m3 (D2+ CS), had the lowest final BW. This indicates that the interaction between stocking density and different carbon sources affects final BW.

Table 3: Effect of stocking density levels and different carbon sources on body weight (BW), wight gain (WG), Average daily gain (ADG) of red tilapia.

Items	Measured parameters			
	Initial weight	**Final weight**	**WG/g**	**ADG g/d**
Effect of stocking density				
Stocking density 100 fish/m^3 (D1)	2.52±0.06	37.68±0.17a	35.16±0.17a	0.36±0.01a
Stocking density 150 fish/m^3 (D2)	2.49±0.06	36.04±0.14b	33.56±0.14b	0.34±0.01b
P Value	0.895	0.0389	0.380	0.380
Effect of carbon resources				
Clear water (CS)	2.51±0.07	29.00±0.22	26.49±0.22	0.27±0.002
Molasses (MO)	2.52±0.07	41.03±0.22	38.51±0.22	0.39±0.002
Sugar (SU)	2.49±0.07	38.83±0.22	36.34±0.22	0.37±0.002
Rice bran (RB)	2.47±0.07	38.57±0.22	36.10±0.22	0.36±0.002
P-Value	0.985	>0.001	>0.001	>0.001
Effect of stocking density and carbon resources				
D1+ CS (T1)	2.48±0.09	29.60±0.29	27.11±0.30	0.28±0.003
D2+ CS (T2)	2.53±0.09	28.40±0.29	25.87±0.30	0.26±0.003
D1+ MO (T3)	2.53±0.09	42.54±0.29	40.01±0.30	0.41±0.003
D2+ MO (T4)	2.51±0.09	39.53±0.29	37.02±0.30	0.38±0.003
D1+ SU (T5)	2.55±0.09	39.29±0.29	36.74±0.30	0.37±0.003
D2+ SU (T6)	2.44±0.09	38.38±0.29	35.94±0.30	0.37±0.003
D1 + RB (T7)	2.51±0.09	39.30±0.29	36.79±0.30	0.38±0.003
D2+ RB (T8)	2.43±0.09	37.84±0.29	35.41±0.30	0.36±0.003
P-Value	0.983	0.114	0.109	0.109

CNT, fish group stocked at clean water;
MO, fish group reared under biofloc system supplemented with molasses;
SU, fish group reared under biofloc system supplemented with sugar;
RB, fish group reared under biofloc system supplemented with rice bran;
Data were presented as the mean ± standard error mean (SEM).

The weight gain (WG) averages as influenced by stocking density were 35.16 and 33.56 g for two stocking densities of 100 and 150 fish/m^3, respectively, according to the results shown in Table 3, and the differences between means were significant ($p<0.005$). The average daily gain (ADG), which was 0.36 and 0.33 g on average for the two stocking densities, showed the similar pattern. The weight gain (WG) influenced by the four carbon sources (CNT, MO, SU, and RB) was 26.49, 38.51, 36.34, and 36.10 g, as indicated in this table. The same trend was also seen in the average daily gain (ADG), with the averages for the four groups being 0.27, 0.39, 0.37, and 0.36 g.

Table 3 displays the effects of stocking density and various carbon sources together on red tilapia weight growth (WG) and average daily gain (ADG). red tilapia in T3 (W1+ S3) recorded the highest WG and the best ADG, according to the table's data. For all carbon sources employed in this experiment, a drop in stocking density was associated with an almost substantial rise in average WG and ADG.

With a zero-water exchange system, the current study verified the positive impacts of each of the stocking density and crbon sources enhanced bioflocs on growth performance in red tilapia fingerlings. These findings suggest that adding a carbohydrate, such as molasses, sugar, or rice bran, to the commercial diet in the BFT system encourages the establishment of heterotrophic bacterial populations.

Lower growth was observed in the groups raised at a high stocking level (150 fish per m^3), which is in line with the findings of **Bakeer *et al*. (2007)** and **Sorphea *et al*. (2010)**. Although tilapia can withstand high densities, they are an aggressive and territorial fish, and the ongoing stress from overcrowding and competition for territory can be the reason for the negative effect of density on growth **(Zhang *et al*., 2016)**. Numerous studies **(Ju *et al*., 2008; Ray *et al*., 2010b; Emerenciano *et al*., 2013a, 2013b, 2013c; Cardona *et al*., 2016; Ahmad *et al*., 2017; Becerril-Cortés *et al*., 2017; Daniel and Nageswari, 2017)** have shown that a variety of microorganisms that are assembled in biofloc systems play a crucial role in removing nitrogenous waste and supplying food, nutrition, and general health. Furthermore, research by **Kamilya *et al*. (2017)** and **Khatoon *et al*. (2016)** suggests that the presence of microbial floc and the system's ability to maintain water quality may be responsible for the improved growth performance in a biofloc system. According to **Ekasari (2014)**, when compared to non-biofloc systems like traditional and recirculating aquaculture systems, biofloc systems can increase net productivity by 8:43%. More crucially, biofloc a waste rich in nutrients—can be used as a feed in BFT to reduce environmental problems in the aquaculture business.

In comparison to the control group, the study's final weight, weight gain, specific growth rate, and relative growth rate all increased significantly ($P > 0.05$). The present study's findings are consistent with a number of studies on the following species: *Carassius auratus* **(Wang *et al*., 2015)**, *Litopenaeus vannamei* **(Burford *et al*., 2004; Xu *et al*., 2013)**, *Marsupenaeus japonicas* **(Zhao *et al*., 2012)**, *Oreochromis mossambicus* **(Avnimelech 1999, 2007)**, and *Macrobrachium rosenbergii* **(Asaduzzaman *et al*., 2008)**.

Feed utilization of red tilapia:

Table 4 displays feed utilization information for red tilapia fingerlings. Because of the treatment effect on feed intake (FI), the average FI values as influenced by stocking density of 100 and 150 fish/m^3 increased from 46.59 to 46.24 g, respectively, with significant differences ($P<0.05$). (Table, 4). Regarding the impact of various carbon sources independent of stock density levels, Table 4's data revealed that the FI values for each of the carbon sources (CNT, MO, SU, and RB) were 42.11, 48.01, 48.12, and 47.34 g, respectively. The differences in feed intake resulting from treatment effects are statistically significant.

Regarding the impact of the interaction between stocking density and various carbon sources on FI values, Table 5 data illustrated that, at the end of the experiment, (D1+ MO) T3 recorded the highest average FI value (48.15 g) at a stocking density of 100 fish/m^3 and with molasses as a carbon source. The disparities between FI values are large, while (D2+ CNT) T2 recorded the lowest value (41.65 g) at a stocking density of 100 fish/m^3 and a clear system.

The FCR values for red tilapia at two stocking densities of 100 and 150 fish/m^3 were displayed in Table 4's results. At the conclusion of the experiment, the FCR averages were 1.35 and 1.40, with large variations between these means (Table, 4). The data collected showed that fish supplied at a density of 50 fish/m^3 had the highest FCR. In the red tilapia biofloc, as shown in Table 4, the various carbon sources had a substantial impact on FCR.

Table 4: Effect of stocking density levels and different carbon sources on feed utilization of red tilapia.

Items	Measured parameters		
	Fi	**FCR**	**PER**
Effect of stocking density levels			
Stocking density 100 fish/m3 (D1)	46.59±0.18a	1.35±0.01b	2.51±0.02a
Stocking density 150fish/m3 (D2)	46.24±0.14b	1.40±0.01a	2.41±0.02b
P Value	0.755	0.652	0.737
Effect of carbon resources			
Clear water (CNT)	42.11±0.23c	1.59±0.01	2.01±0.2
Molasses (MO)	48.01±0.22a	1.25±0.01	2.68±0.2
Sugar (SU)	48.12±0.22a	1.33±0.01	2.52±0.2
Rice bran (RB)	47.34±0.22b	1.32±0.01	2.54±0.2
P-Value	>0.001	>0.001	< 0.001
Effect of Stocking size and stocking density levels			
D1+ CNT (T1)	42.57±0.35	1.57±0.02	2.12±0.03
D2+ CNT (T2)	41.65±0.35	1.61±0.02	2.07±0.03
D1+ MO (T3)	48.15±0.35	1.21±0.02	2.77±0.03
D2+ MO (T4)	47.87±0.35	1.30±0.02	2.58±0.03
D1+ SU (T5)	48.31±0.28	1.32±0.02	2.54±0.02
D2+ SU (T6)	47.94±0.28	1.34±0.02	2.50±0.02
D1 + RB (T7)	47.35±0.28	1.29±0.02	2.59±0.02
D2+ RB (T8)	47.52±0.28	1.36±0.02	2.49±0.02
P-Value	0.043	0.867	0.901

CNT, fish group stocked at clean water;
MO, fish group reared under biofloc system supplemented with molasses;
SU, fish group reared under biofloc system supplemented with sugar;
RB, fish group reared under biofloc system supplemented with rice bran;
Data were presented as the mean ± standard error mean (SEM).

The results showed that FCR rose when more carbon sources were added. When compared to other carbon sources and clear systems, molasses was shown to have the highest FCR. Significant differences were seen between the treatments ($P<0.001$). Additionally, Table 5 showed that the range of FCR values affected by the combination of carbon sources and stocking density was 1.21 to 1.57. Fish of treatment T3, stocked at 100 fish/m3, had the highest FCR with molasses serving as a carbon source. Treatment differences were statistically significant ($P<0.001$).

Table 4 shows that a decrease in stocking density was associated with a significant ($P<0.05$) increase in the average protein efficiency ratio (PER). The addition of carbon sources to the biofloc system enhanced the PER values, as Table 4 demonstrates, and there were notable variances between the three carbon sources and clear water. Additionally, Table 5's data demonstrated that fish stocked at 100 and 150 fish/m^3 using molasses as a carbon source had increased PER values.

The development and production of microbial flocs recycles wastes and leftover feeds, which helps fish recycle and repurpose feed nutrients and ultimately improves overall feed assimilation, particularly in situations where there is no water exchange **(Avnimelech, 2006; Avnimelech *et al.*, 2008; Xu and Pan, 2012)**. Regarding the beneficial impacts of BFT on growth performance, these findings showed that red tilapia fingerlings are adaptable to novel nutritional environments and that microbial flocs induced the production and/or activity of digestive enzymes, leading to better nutrient digestion in the gut. This enhanced feed utilization and growth performance may be explained by these findings **(Moss *et al*., 2001; Xu *et al*., 2012a, b; Xu and Pan, 2012)**. The FCR (1.25) for red tilapia in the molasses group was comparable to those of **Sagar *et al*. (2009)** and **Hari and Kurup (2003)**. After 98 days, red tilapia cultivated in a sugar biofloc system showed an FCR of 1.33, according to **Ballester *et al*. (2017)**. However, L. vannamei grown in the biofloc system showed improved FCR (0.89–1.76) **(Serra *et al*., 2015; Khanjani *et al*., 2017; Peixoto *et al*., 2018)**.

In general, the growth of red tilapia fingerlings cultured in BFT was influenced by the supply of carbon. In similar study, *L. vannamei* post larvae have grown in the presence of molas, starch, and wheat flour groups **(Khanjani *et al*., 2017)**. For *L. vannamei* in a grow-out stage, **Ekasari *et al*. (2014)** also showed comparable growth performance utilizing molasses, tapioca, tapioca by-product, and rice bran. Similar growth performance was also shown by **Lobato *et al*. (2019)** and **Vilani *et al*. (2016)** while producing white leg shrimp using molasses and cassava flour and molasses and rice bran.

Hematological parameters of red tilapia:

Blood index analyses are a useful method for examining the health of fish kept in aquaculture since they yield trustworthy data on metabolic illnesses **(Osman *et al*., 2019)**. As well as, various dietary supplements may have an impact on blood components **(Animashahun *et al*., 2006; Osman *et al*., 2019)**. Assessing the quality and suitability of feed ingredients for farm fish requires consideration of hematological and blood biochemical factors **(Maxwell *et al*., 1990; Ighwela *et al*., 2012)**. As a result of their typical sensitivity to food composition, they are widely employed in the assessment of fish health **(Abdel-Tawwab *et al*., 2010; Solomon and Okomoda, 2012; Habte-Tsion *et al*., 2013; Kondera *et al*., 2017)**.

As shown in Table (5), two stocking densities of 100 and 150 fish/m^3 did not affect hemoglobin (Hb), red blood cell count (RBCs), or mean cell volume (MCV) of red tilapia, and the differences between the two groups were non-significant. While mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and white blood cell (WBC) were affected by two stocking densities of 100 and 150 fish/m^3, while D1 (50 fish/m^3) had the highest averages and D2 (150 fish/m^3) had the lowest averages, the differences between the two groups were significant.

The results in Table 5 showed that adding carbon sources to biofloc of red tilapia enhanced their means of hemoglobin (Hb), mean cell hemoglobin concentration (MCHC), and white blood cell (WBC). When compared to other carbon sources and pure water, the fish groups in the system using molasses as a source of carbon emitted the most significantly ($P<0.05$). while sources of carbon had no effect on the increasing values of RBCS, MCV, or MCH.

Hb, RBCS, MCV, MCH, and MCHC of red tilapia were not affected by the interaction between stocking density and different carbon sources (Table, 5). while WBCs of red tilapia were affected by interaction between stocking density and different carbon sources, where the highest value of WBCs was in T3 (D1+ MO) and the lowest value was in T2 (D2+ CNT).

The results of this study suggest that the biofloc system, which is a combination of stocking density and carbon source, did not affect the physical conditions of the red tilapia. Specifically, there was no discernible influence on RBC, Hb, MCV, MCH, or MCHC. **Bakhsh *et al*. (2018)** observed the same outcome for common carp fingerlings raised in the biofloc method. In a similar vein, **Azim and Little (2008)** found no statistically significant differences in the blood Hct of Nile tilapia between the biofloc groups and the control group. A similar pattern was noted in additional research **(Xu and Pan, 2014; Long *et al*., 2015)**.

Moreover, there was no discernible difference in the total hemocyte count of shrimp between the biofloc groups and the control group **(Xu and Pan, 2014)**. Conversely, **Xu and Pan (2013)** discovered that there was a substantial difference in the total hemocyte count between the shrimp in the biofloc groups and the control group. It appears that this phenomenon could be explained by several cultural species and experimental settings. White blood cell count (WBC) levels in the current study increased significantly across all biofloc groups as compared to the control. These findings somewhat concurred with the findings of **Long *et al.* (2015)** and **Azim and Little (2008)**. Fish fed rice bran and molasses had the highest concentration of white blood cells (WBCs) in their blood. The blood biochemical values obtained in this study were generally within the Nile tilapia's permissible ranges. **Mahmoud and El-Hais (2017) and Ayyat *et al*. (2017)** both endorsed these findings.

Table 5: Effect of stocking density levels and different carbon sources on hematological parameters of red tilapia.

Items	Measured parameters					
	Hb	RBCS	MCV	MCH	MCHC	WBCs
Effect of stocking density levels						
Stocking density 100 fish/m3 (D1)	11.66±0.04	4.05±0.25	98.14±0.08a	30.77±0.08	32.20±0.14a	27.18±0.44a
Stocking density 150 fish/m3 (D2)	11.71±0.04	4.07±0.25	97.47±0.08b	30.82±0.08	31.44±0.14b	24.74±0.44b
P Value	0.395	0.995	<0.001	0.66	0.001	0.001
Effect of carbon resources						
Clear water (CNT)	10.98±0.06d	3.61±0.32	97.96±0.11	30.54±0.11	31.45±0.20b	19.27±0.62c
Molasses (MO)	12.20±0.06a	3.73±0.32	97.65±0.11	31.24±0.11	32.40±0.20a	30.71±0.62a
Sugar (SU)	11.95±0.06b	3.88±0.32	97.96±0.11	30.88±0.11	31.92±0.20ab	27.72±0.62b
Rice bran (RB)	11.63±0.06c	4.00±0.32	97.64±0.11	30.53±0.11	31.86±0.20ab	26.14±0.62b
P-Value	<0.001	0.148	0.076	0.001	0.010	<0.001
Effect of Stocking size and stocking density levels						
D1+ CNT (T1)	10.96±0.80	3.60±0.51	98.20±0.15	30.51±0.16	31.14±0.27	20.49±0.87e
D2+ CNT (T2)	10.61±0.80	3.72±0.51	98.08±0.15	31.21±0.16	32.02±0.27	18.05±0.87f
D1+ MO (T3)	12.22±0.80	4.01±0.51	98.20±0.15	30.86±0.16	31.54±0.27	31.93±0.87a
D2+ MO (T4)	12.27±0.80	3.99±0.51	98.08±0.15	30.50±0.16	31.07±0.27	29.49±0.87b
D1+ SU (T5)	11.01±0.80	3.62±0.51	97.72±0.15	30.56±0.16	31.90±0.27	26.50±0.87cd
D2+ SU (T6)	11.66±0.80	3.74±0.51	97.22±0.15	31.26±0.16	32.78±0.27	24.92±0.87d
D1 + RB (T7)	11.97±0.80	3.99±0.51	97.72±0.15	30.91±0.16	32.30±0.27	27.36±0.87c
D2+ RB (T8)	11.92±0.80	3.87±051	97.22±0.15	30.55±0.16	31.83±0.27	28.94±0.87b
P-Value	1.00	1.00	0.429	1.00	1.00	0.001

CNT, fish group stocked at clean water;
MO, fish group reared under biofloc system supplemented with molasses;
SU, fish group reared under biofloc system supplemented with sugar;
RB, fish group reared under biofloc system supplemented with rice bran;
Data were presented as the mean ± standard error mean (SEM).

Proximate analysis of red tilapia:

Proximate analysis of red tilapia as affected by stocking density is presented in Table 6. It is clear that the stocking density D1 (100 fish/m^3) significantly increased crude protein content (P<0.05), while the stocking density D2 (150 fish/m^3) significantly increased lipid and ash contents (P<0.001), but moisture and dry matter content were not significantly affected (Table, 6).

Results of the proximate analysis Table 9 showed that the addition of sources of carbon increased dry matter (P<0.05) as well as protein, lipid, and ash contents (P<0.001), while dry matte content was not significantly affected.

The interaction between stocking density and source of carbon (Table, 6) showed that proximate analyses of whole fish showed no significant differences among treatments for moisture contents. According to Table 9, T8 (D2 + RB) was found to have the highest value of crude protein contents (52.39%), while T2 (D2 + CNT) was found to have the lowest value (51.49%).

As found in this table, the highest value of crude fat was (11.27%) in the T2 (D2+ CNT) group that received clear water (control) at a density of 150 fish/m^3, whereas the T7 (D1 + RB) group that received rice bran at a density of 100 fish/m^3 had the lowest value of crude fat contents (9.15%). The results in Table 6 indicated that T2 (D2 + CNT) recorded the highest value of ash contents (17.96%), and the lowest value (14.97%) was in T7 (D1 + RB). The differences among treatments were significant (<0.05, 0.001, and <0.001) for crude protein, crude fat, and ash contents, respectively.

Table 6: Effect of stocking density levels and different carbon sources on measurements of chemical composition of red tilapia.

Items	Measured parameters				
	Moisture	**Dry matter**	**Crude protein**	**Crude Fat**	**Ash**
Effect of stocking density levels					
Stocking density 100 fish/m3 (D1)	72.21±0.19	27.79±1.39	52.58±0.07a	10.24±0.08a	15.64±0.05b
Stocking density 150 fish/m3 (D2)	71.91±0.09	26.31±1.39	52.19±0.07b	9.64±0.08b	16.45±0.05a
P Value	0.021	0.364	0.047	<0.001	<0.001
Effect of carbon sources					
Clear water (CNT)	73.14±0.12a	26.86±1.79	51.60±0.10c	10.71±0.11a	17.14±0.06a
Molasses (MO)	72.34±0.12b	27.66±1.79	52.61±0.10b	10.35±0.11b	16.72±0.06b
Sugar (SU)	71.55±0.12c	28.90±1.79	52.93±0.10a	9.39±0.11c	15.26±0.06c
Rice bran (RB)	71.22±0.12d	28.78±1.79	52.80±0.10a	9.30±0.11c	15.06±0.06d
P-Value	<0.001	0.469	<0.001	<0.001	<0.001
Effect of Stocking size and stocking density levels					
D1+ CNT (T1)	73.366±0.17	26.646±6.77	51.65±0.13	10.16±0.15	16.32±0.09
D2+ CNT (T2)	72.926±0.17	27.086±6.77	51.49±0.13	11.27±0.15	17.96±0.09
D1+ MO (T3)	72.56±0.17	27.446±6.77	52.65±0.13	9.79±0.15	15.90±0.09
D2+ MO (T4)	72.12±0.17	27.88±6.77	52.56±0.13	10.90±0.15	17.54±0.09
D1+ SU (T5)	71.800±0.17	28.20±6.77	52.80±0.13	9.44±0.15	15.35±0.09
D2+ SU (T6)	71.29±0.17	28.71±6.77	53.07±0.13	9.33±0.15	15.17±0.09
D1 + RB (T7)	71.13±0.17	28.87±6.77	53.22±0.13	9.15±0.15	14.97±0.09
D2+ RB (T8)	71.30±0.17	28.70±6.77	52.39±0.13	9.45±0.15	15.14±0.09
P-Value	0.183	0.431	0.005	0.001	<0.001

CNT, fish group stocked at clean water;
MO, fish group reared under biofloc system supplemented with molasses;
SU, fish group reared under biofloc system supplemented with sugar;
RB, fish group reared under biofloc system supplemented with rice bran;
Data were presented as the mean ± standard error mean (SEM).

Table 6's results demonstrated that, when compared to the control group, the different sources of carbon (molasses, sugar and rice bran) obtained the greatest significance in terms of crude protein and dry matter and the lowest in terms of fat and ash content. However, **Azim and Little (2008)** found that the chemical makeup of the Nile tilapia (*O. niloticus*) fish in the tanks under biofloc and control did not significantly differ from one another. Same observations were documented by **Wasielesky *et al*. (2006)**, who found that while the crude lipid and ash content tended to increase in the biofloc treatments, there were no significant differences (P > 0.05) in the chemical composition of the shrimp whole body between the biofloc treatments and the control group in the moisture and protein content.

Conclusion:

In this study, demonstrated the effective application of molasses, sugar and rice bran as carbon supplement for biofloc. The addition of carbon sources particularly, molasses at stocking density 100 fish per m^3 of red tilapia fingerlings, promotes growth performance, feed utilization and anti-stress markers of red tilapia cultured in biofloc system as an effective environmentally friendly aquaculture technique.

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الملخص العربي

أسماك البلطي الأحمر المرباة في ظروف البيوفلوك: تأثير كثافة التخزين ومصادر الكربون الغذائي على أداء النمو ومؤشرات الدم

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الغرض من هذه التجربة هو دراسة تأثير مصادر الكربون المختلفة في نظام البيوفلوك على أداء النمو، واستخدام الأعلاف، ومؤشرات الدم، والتركيب الكيميائي الحيوي لجسم أسماك البلطي الأحمر تحت كثافات تخزين متفاوتة. تم استزراع300 إصبعيات أسماك البلطي الأحمر بحالة صحية جيدة، بوزن 2.44: 2.55 ± 0.19 جرام في المتوسط، لمدة 14 أسبوعًا بكثافة 100 و 150 سمكة / م^3 في خزانات من الألياف الزجاجية (75 لترًا). وتم إجراء ثماني معاملات، تمثل أربعة مصادر كربون مميزة هي الدبس والسكر ونخالة الأرز بالإضافة إلى التحكم (المياه النقية) في تصميم التجربة، بكثافات تخزين 100 و 150 سمكة / م3. تم تغذية الأسماك ثلاث مرات في اليوم على علف تجاري يحتوي على نسبة بروتين 32٪، بمعدل 5٪ من وزن الجسم. وقد تأثر النمو واستخدام الأعلاف بشكل كبير بإضافة مصادر الكربون. ووفقًا للبيانات، فقد حقق الدبس ونخالة الأرز أفضل أداء للنمو واستخدام الأعلاف ومؤشرات الدم لأسماك البلطي الأحمر تحت كثافة تخزين تبلغ 100 سمكة/م^3.