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COMPARATIVE EFFECTS OF MOLASSES, SUGAR AND RICE BRAN AS CARBON SOURCES ON WATER QUALITY, GROWTH, AND IMMUNITY OF GIFT TILAPIA IN BIOFLOC SYSTEM

Saba Tahir[1*], Syed Waqqas Tahir[2], Ahmad Hassan[3], Sidra Saeed[4], Sadia Zahid[5], Zeeshan Ashraf[6], Nasir Ali[7], Nadeem Ahmad[8], Khadija Sana Ullah Cheema[9], Armeen Fatima Qamar[9]

[1*]*Department of Medical and Health, The Islamia University of Bahawalpur, Pakistan.*
[2]*Department of Zoology, University of Veterinary and Animal Sciences, Lahore, Pakistan*
[3]*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan.*
[4]*Department of Zoology, University of Okara, Okara, Pakistan.*
[5]*Department of Biotechnology, University of Okara, Okara, Pakistan.*
[6]*Department of Zoology, University of Education, Faisalabad Campus, Lahore, Pakistan.*
[7]*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan.*
[8]*Department of Molecular Biology, Üsküdar University, Istanbul, Turkey*
[9,10]*Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan.*

[1*]drsabatahir469@gmail.com

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Corresponding Author: *
Dr. Saba Tahir

Abstract

Biofloc technology (BFT) is a highly potential biofloating method to aquaculture that promotes nitrogen efficient usage, produces microbial biomass, and could improve fish growth and health. The choice of an appropriate carbon source, however, is crucial for optimizing water quality and production performance. The molasses, sugar and rice bran were compared for use as carbon sources in the culture of genetically improved farmed tilapia (GIFT) in biofloc culture. Four experimental treatments were tested, which comprised of the control without supplemental carbon, molasses, sugar and rice bran. The water quality, growth performance, feed conversion, hematological parameters, innate immune responses and antioxidant indicators were assessed. The results showed that for total ammonia nitrogen and nitrite-nitrogen, molasses supplementation resulted in the lowest levels and highest level was for rice bran, respectively, while for total suspended solids and settleable floc volume, the highest level was found in molasses and the lowest in rice bran, respectively. The fish that were fed molasses had the highest final body weight, weight gain, survival and biomass with the lowest FCR. The growth and physiological responses obtained after sugar supplementation were generally intermediate and moderate in rice bran although high floc development was observed. The comparative pattern of the hematological parameters (hemoglobin concentration, hematocrit), immune parameters (lysozyme activity, phagocytic activity and respiratory burst) was similar. The highest activities of antioxidant enzymes were observed in molasses treatment along with minimum malondialdehyde value. Based on the results of this study,

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it is hypothesized that carbon source treatment used in GIFT biofloc systems can affect the microbial community, nitrogen removal, growth efficiency and physiological status of the fish. Molasses seems to be a good candidate for further trials, however, the suitability is dependent on the cost, availability and the conditions of the system management.

INTRODUCTION

Aquaculture has become and is increasingly becoming an important source to satisfy the global demand for affordable animal protein; however, high nutrient density, environmental sustainability, water availability and production cost are limiting factors for intensive fish production. Biofloc technology (BFT) is an alternative method that encourages the growth of beneficial microorganisms that convert dissolved nitrogen back into microbial biomass which can enhance water quality and subsequently the availability of natural feed for cultured fish (Abakari et al., 2021; Khanjani et al., 2022). However, it requires careful control of the carbon to nitrogen (C:N) ratio, satisfactory aeration, stable water chemistry and balanced microbial activity (Silva et al., 2026). The supplementation of carbon is the main part of BFT as it encourages heterotrophic bacteria to convert inorganic nitrogen to organic nitrogen. Selection of the carbon source may affect microbial growth, biofloc composition, nutrient removal and the nutritional benefits provided to fish. Therefore, apart from nitrogenous waste reduction, the carbon sources must be assessed for their effects on the growth rate, feed utilization and physiological status of fish (Abakari et al., 2021; Khanjani et al., 2022). Recent studies also suggest that the response to carbon supplementation is influenced by the type of substrate and management strategies in the system, thereby making comparisons beneficial in identifying practical alternatives (Muthoka et al., 2026). Use of sugarcane molasses is common, as it contains fermentable carbohydrates that help the growth of microorganisms quickly. Biofloc systems using molasses have been reported to increase production performance and certain immune responses in Nile tilapia, but the results vary based on culture conditions and carbon-management strategies (Elayaraja et al., 2020; Rind et al., 2023). Simple sugar could also be a readily available carbon source, while rice bran could be a more complex substrate, which contains carbohydrates and other organic components. The suitability of fermented rice bran for biofloc production has been suggested to be influenced by the process of fermentation such as using beneficial microorganisms in genetically improved farmed tilapia (GIFT) fry (Mahadik et al., 2024). However, outcomes from one production system to another, using various substrates, various fish sizes and various experimental conditions should not be assumed to be the same. In addition to water-quality management, biofloc-related modifications may affect fish growth, haematological parameters, innate immunity and antioxidant status. Carbon source effects on production and physiological stability are important for assessing the potential of those sources to support physiological stability and production (Rind et al., 2023; Suloma et al., 2023). Hence, this study aims to compare the molasses, sugar and rice bran as carbon sources on the water quality parameters, growth performance, feed conversion, haematology, innate immunity and antioxidant status in GIFT tilapia biofloc system.

2. Materials and Methods

2.1. Experimental Design and Culture Conditions

The experiment aimed to assess the water quality, growth performance, hematological parameters, innate immunity, and antioxidant status of genetically improved tilapia (GIFT; Oreochromis niloticus) in biofloc culture conditions in the presence of various carbon sources. Four treatments were used with four replicate tanks for each number of experimental unit (16). The treatments consisted of control (T1), molasses (T2), refined sugar (T3) and rice bran (T4). They were conducted using circular tanks with a

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working water volume of 500 L. The husbandry conditions in all tanks were similar, such as stock density, feeding schedule, aeration, and photoperiod. A continuous aeration was ensured for maintaining dissolved oxygen level and to keep the biofloc particles in suspension. To minimise environmental variation due to location, the positions of tanks were randomised where possible. Dissolved oxygen (DO), water temperature and pH were measured during the entire experiment.

2.2. Experimental Fish and Acclimatization

Fingerlings of relatively uniform size were obtained from a reliable hatchery and were healthy GIFT tilapia. The fish were checked for any obvious scuffles, uncoordinated swimming and disease symptoms prior to stocking. They had been acclimatized to the experimental conditions for 10–14 days before being fed a commercial tilapia diet. After acclimatization, the fish were weighed to get their initial body weight and randomly assigned to the experimental tanks at a stocking density of [number of fish per tank]. The number and the biomass of the fish were measured at the beginning of the experiment in each tank. Fish handling and sampling procedures were designed to minimize stress to the fish and comply with pertinent animal welfare and institutional requirements.

2.3. Biofloc Establishment and Carbon-Source Treatments

All biofloc systems were set up prior to the experimental period for microbial community to grow. Culture water and microbial inoculum (when used) were provided to all tanks alike. Biofloc establishment and the whole trial period were provided continuous aeration. Four treatments were considered – a control with no carbon (T1), molasses (T2), refined sugar (T3) and rice bran (T4). Carbon supplementation was carried out based on a pre-established carbon-to-nitrogen (C) strategy. The desired C ratio was [specify ratio] and the amount of carbon added was determined from the carbon content of each substrate and from the assumed amount of nitrogen added through feed and other inputs. Each tank had recorded Carbon additions and frequency and quantity of supplementation were varied as per the management plan. The moisture content and proximate composition of rice bran (crude protein, crude lipid and ash) were analysed according to standard methods of analysis. Product specifications or laboratory analysis were used to determine the composition of molasses and refined sugar. The amount of carbon available and extra nutrients provided with the different substrates were taken into account in determining supplementation rates.

2.4. Feeding Management and Experimental Duration

The experimental feeding test was 8 weeks (56 days) in length. Commercial tilapia feed with 32% crude protein and 6% crude lipid was used for feeding fish. An availability of 5% feed according to the estimated biomass per day was provided in 3 meals at regular times. The feeding ration was adjusted based on the biomass measurements taken at regular intervals and actual feed intake. The quantity of feed offered to each tank was recorded each day. The percentage of feed not consumed was observed when it was possible to recover. The feed and husbandry were standardized among the treatments while the carbon supplementation was applied according to the treatment protocol. Data were collected for mortality, fish removal, water exchange, sludge removal, and management interventions for each tank.

2.5. Water Quality and Biofloc Characterization

Daily measurements of physical parameters of water temperature, dissolved oxygen, and pH were made by calibrated digital instruments. Total ammonia nitrogen (TAN), nitrite-nitrogen, nitrate-nitrogen and total alkalinity were measured twice weekly by colorimetric methods and with appropriate commercial water-analysis kits, according to the manufacturers' instructions. Water samples were taken at different locations in each tank at the same time. The total suspended solids (TSS) were determined by filtering a known

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volume of culture water on to pre-weighed filters and gravimetrically measuring the solids. Filters were allowed to dry to constant weight, and TSS was determined by the weight of the filters compared to the volume of water filtered. After 15 minutes of settling, the volume of the bioflocs that settled was measured in the Imhoff cone and reported as mL/L. The calibration of the instruments, sample collection and analytical procedures were standardized among treatments. Other corrective actions such as water exchange or increases or decreases in alkalinity were documented where they would have a potential impact on water quality and treatment comparisons.

2.6. Growth Performance and Feed Utilization

Growth performance was measured based on initial and final body weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), survival rate and final tank biomass. The initial and final weight of the fish were measured using a calibrated digital balance while other biomass measurements were conducted every two weeks to adjust feeding quantities. The measurement of the tank level was used for the statistical analysis. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were obtained from the following equations:

[WG;(\mathrm{g})=W_f-W_i]
[SGR;(%!/\mathrm{day})=
\frac{\ln W_f-\ln W_i}{t}\times100]
[FCR=\frac{\text{Total dry feed supplied (g)}}{\text{Net biomass gain (g)}}]
[Survival;(%)=\frac{N_f}{N_i}\times100]

where (W_i) and (W_f) are the mean body weights at the start and end of the experiment, respectively, (t) is the duration of the experiment (days), and (N_i) and (N_f) are the numbers of fish at the start and end of the experiment, respectively. Where applicable, biomass losses due to mortality and fish removals were compensated for to calculate consistently net biomass gain.

2.7. Hematological and Biochemical Analyses

Three fish were randomly selected from each replicate tank at the end of the 56 days for blood collection and 12 fish per treatment were used for blood collection. Fish were starved for about 12 hours prior to sampling to minimise variation due to recent feeding. Blood was drawn from the caudal vein via sterile syringes under the proper fish handling and anesthesia protocol. Samples for hematological analysis were put into tubes containing the anticoagulant, EDTA. The number of erythrocytes (RBC) and leukocytes (WBC) were counted by hemocytometric method which was validated. Hemoglobin concentration (Hb) was measured by colorimetric method validated by the manufacturer of the instrument and hematocrit (Hct) was determined by microhematocrit method. Blood was drawn into tubes without anticoagulant and allowed to clot before being spun in a centrifuge to get serum. Valuable colorimetric assays were used to measure serum glucose, total protein and albumin. If both measurements were on compatible units of measurement, then globulin concentration was calculated as total protein minus albumin.

2.8. Innate Immune and Antioxidant Analyses

The activity of lysozyme, phagocytic and respiratory burst were used as indices for innate immune responses. The turbidimetric method of lysozyme activity was determined by measuring the decrease in turbidity of a suspension of Micrococcus lysodeikticus. A particle-uptake assay on prepared blood leukocytes was used to assess phagocytic activity and the percentage of phagocytic cells determined from the number of blood leukocytes examined. Nitroblue tetrazolium (NBT) reduction assay was used to evaluate the respiratory burst activity. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were used to assess antioxidant status. Lipid peroxidation was determined by measuring malondialdehyde (MDA). The values of these parameters were measured in serum by validated laboratory assays or assay kits, according to the protocol. Enzyme activity and MDA concentrations were adjusted

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where necessary to the total protein concentration. Analytical controls and technical replicates were conducted where necessary as per each assay protocol. Technical replicate measurements were averaged prior to statistical analysis, and fish-level observations were considered to be subsamples within replicate tanks.

2.9. Statistical Analysis

One-way analysis of variance (ANOVA) was performed followed by post hoc Tukey test for treatment comparisons. Linear mixed-effects models were used to analyze repeated water-quality measurements where treatment and sampling time were both fixed effects, and tank was a random effect. The data were presented as mean ± standard error (SE); $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Water Quality and Biofloc Characteristics

The four carbon-source treatments had different water quality parameters (Table 1). There were no treatment differences in water temperature (27.7–27.9°C). The pH level of the water samples ranged from (7.22–7.42) and the total alkalinity of the water samples ranged from (112–126) mg/L as $CaCO_3$, which shows that the water samples had relatively stable acid–base conditions throughout the experimental period. The molasses treatment (T2; 6.12 ± 0.18 mg/L) had significantly higher levels of dissolved oxygen than the control (T1; 5.72 ± 0.21 mg/L) and rice bran treatment (T4; 5.65 ± 0.24 mg/L), while the sugar treatment (T3; 5.86 ± 0.20 mg/L) exhibited intermediate response. Total ammonia nitrogen (TAN) varied significantly between treatments with the highest concentration in treatment T1 (0.88 ± 0.09 mg/L), and the lowest in treatment T2 (0.42 ± 0.05 mg/L). The sugar treatment (0.49 ± 0.06 mg/L) also exhibited lower TAN than the control whereas rice bran exhibited intermediate concentration (0.61 ± 0.07 mg/L). The trend of nitrite-nitrogen was similar, dropping from 0.48 ± 0.06 mg/L in T1 to 0.24 ± 0.03 mg/L in T2. The concentration of 0.29 ± 0.04 mg/L and 0.35 ± 0.05 mg/L was recorded in the sugar and rice bran treatments, respectively. In contrast, nitrate-nitrogen was highest in T4 (9.35 ± 0.83 mg/L), compared with 8.42 ± 0.71 mg/L in T1 and 7.18 ± 0.62 mg/L in T2. There were also differences in biofloc characteristics between the treatments. Total suspended solids were highest in T4 (318 ± 22 mg/L), followed by T2 (248 ± 18 mg/L), T3 (231 ± 16 mg/L), and T1 (185 ± 15 mg/L). Likewise, the average volume of floc that settled was highest for T4 (24.6 ± 2.1 mL/L), and lowest for the control (12.4 ± 1.5 mL/L). The results showed the difference of nitrogenous excrement and biofloc development for the carbon sources tested (Figure 1A–D).

Table 1. Effects of different carbon sources on water quality and biofloc characteristics of GIFT tilapia cultured in a biofloc system

Parameter	T1: Control	T2: Molasses	T3: Sugar	T4: Rice bran
Temperature (°C)	27.8 ± 0.4	27.9 ± 0.3	27.8 ± 0.4	27.7 ± 0.3
Dissolved oxygen (mg/L)	5.72 ± 0.21^{b}	6.12 ± 0.18^{a}	5.86 ± 0.20^{ab}	5.65 ± 0.24^{b}
pH	7.31 ± 0.12	7.42 ± 0.10	7.36 ± 0.11	7.22 ± 0.14
TAN (mg/L)	0.88 ± 0.09^{a}	0.42 ± 0.05^{c}	0.49 ± 0.06^{c}	0.61 ± 0.07^{b}
Nitrite-N (mg/L)	0.48 ± 0.06^{a}	0.24 ± 0.03^{c}	0.29 ± 0.04^{bc}	0.35 ± 0.05^{b}
Nitrate-N (mg/L)	8.42 ± 0.71^{b}	7.18 ± 0.62^{b}	7.64 ± 0.68^{b}	9.35 ± 0.83^{a}
Alkalinity (mg/L $CaCO_3$)	112 ± 8	126 ± 9	120 ± 7	116 ± 8
Total suspended solids (mg/L)	185 ± 15^{c}	248 ± 18^{b}	231 ± 16^{bc}	318 ± 22^{a}
Settleable floc volume (mL/L)	12.4 ± 1.5^{c}	18.7 ± 1.8^{b}	16.9 ± 1.6^{b}	24.6 ± 2.1^{a}

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Values are expressed as mean ± SE (n = 4 replicate tanks per treatment). Different superscript letters within a row indicate significant differences at $P < 0.05$. Rows without superscripts represent parameters for which significance has not been established.

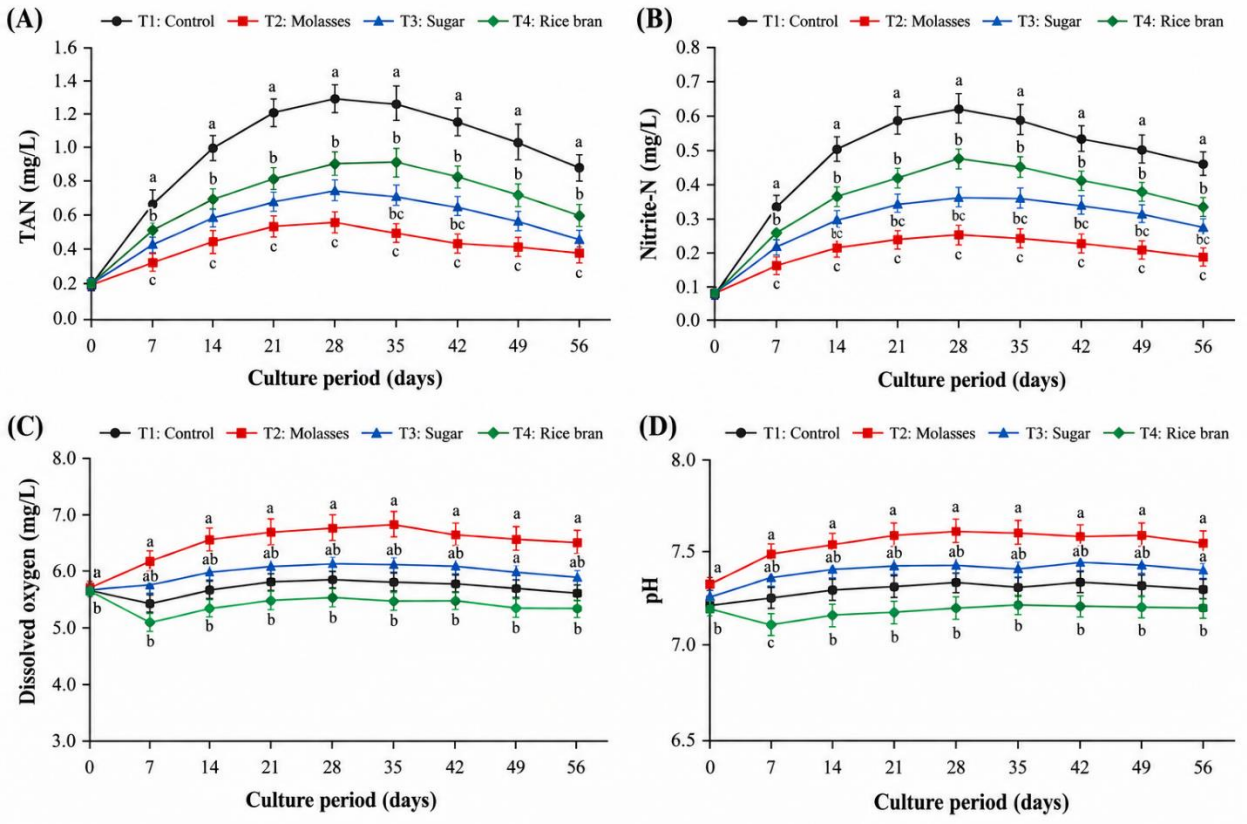


Figure 1. Temporal variation in water quality parameters of GIFT tilapia cultured under different carbon-source treatments in a biofloc system. (A) Total ammonia nitrogen (TAN), (B) nitrite-nitrogen, (C) dissolved oxygen, and (D) pH. Treatments comprised T1, control without supplemental carbon; T2, molasses; T3, refined sugar; and T4, rice bran. Values should be presented as treatment means ± SE based on independent replicate tanks at each sampling time.

3.2 Growth Performance and Feed Utilization

The treatments in the carbon-source test had different growth performance (Table 2). All initial body weights were similar with treatments ranging from 10.2 to 10.3 g. The fish fed with molasses showed the maximum final body weight (82.6 ± 3.4 g), which was followed by fish fed sugar (79.4 ± 3.2 g), fish fed rice bran (75.8 ± 2.9 g) and fish fed control (68.2 ± 3.1 g) at the end of experimental period. The difference in final body weight between T2 and T1 and T4 was significant with T3 having an intermediate response. Weight gain followed a similar pattern, with the highest value in T2 (72.3 ± 3.3 g/fish), compared with 69.2 ± 3.1 g/fish in T3, 65.5 ± 2.8 g/fish in T4, and 58.0 ± 3.0 g/fish in T1. Specific growth rate (SGR) was also highest in T2 (2.98 ± 0.07%/day), followed by T3 (2.92 ± 0.06%/day), T4 (2.85 ± 0.07%/day), and T1 (2.70 ± 0.08%/day). There was not much difference between the treatments in their feed intake, which ranged from 93.4 to 95.4 g/fish. The feed conversion ratio (FCR) was, however, found to vary greatly. The molasses treatment had the lowest optimum FCR (1.32 ± 0.06) followed closely by the sugar treatment (1.36 ± 0.07) while the rice bran treatment had an FCR

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of 1.43 ± 0.06. The FCR of the control was the highest (1.61 ± 0.08). Survival ranged from 90.0 ± 2.9% in T1 to 97.5 ± 1.4% in T2. The sugar and rice bran treatments showed the survival rate of 95.0 ± 2.0% and 93.8 ± 2.4%, respectively. Final biomass yield was highest in T2 (1.61 ± 0.09 kg/tank), followed by T3 (1.51 ± 0.08 kg/tank), T4 (1.42 ± 0.07 kg/tank), and T1 (1.23 ± 0.08 kg/tank). Generally the pattern of growth and feed utilization indicated by the molasses treatment was the most desirable of the four treatments tested (Figure 2A, B).

Table 2. Growth performance, feed utilization, and survival of GIFT tilapia under different carbon-source treatments

Parameter	T1: Control	T2: Molasses	T3: Sugar	T4: Rice bran
Initial body weight (g)	10.2 ± 0.3	10.3 ± 0.3	10.2 ± 0.2	10.3 ± 0.3
Final body weight (g)	68.2 ± 3.1^{c}	82.6 ± 3.4^{a}	79.4 ± 3.2^{ab}	75.8 ± 2.9^{b}
Weight gain (g/fish)	58.0 ± 3.0^{c}	72.3 ± 3.3^{a}	69.2 ± 3.1^{ab}	65.5 ± 2.8^{b}
SGR (%/day)	2.70 ± 0.08^{c}	2.98 ± 0.07^{a}	2.92 ± 0.06^{ab}	2.85 ± 0.07^{b}
Feed intake (g/fish)	93.4 ± 4.2	95.4 ± 4.0	94.0 ± 3.8	93.8 ± 4.1
FCR	1.61 ± 0.08^{a}	1.32 ± 0.06^{c}	1.36 ± 0.07^{c}	1.43 ± 0.06^{b}
Survival (%)	90.0 ± 2.9^{b}	97.5 ± 1.4^{a}	95.0 ± 2.0^{ab}	93.8 ± 2.4^{ab}
Final biomass (kg/tank)	1.23 ± 0.08^{c}	1.61 ± 0.09^{a}	1.51 ± 0.08^{ab}	1.42 ± 0.07^{b}

Values are expressed as mean ± SE (n = 4 replicate tanks per treatment). Different superscript letters within a row indicate significant differences at $P < 0.05$. SGR = specific growth rate; FCR = feed conversion ratio.

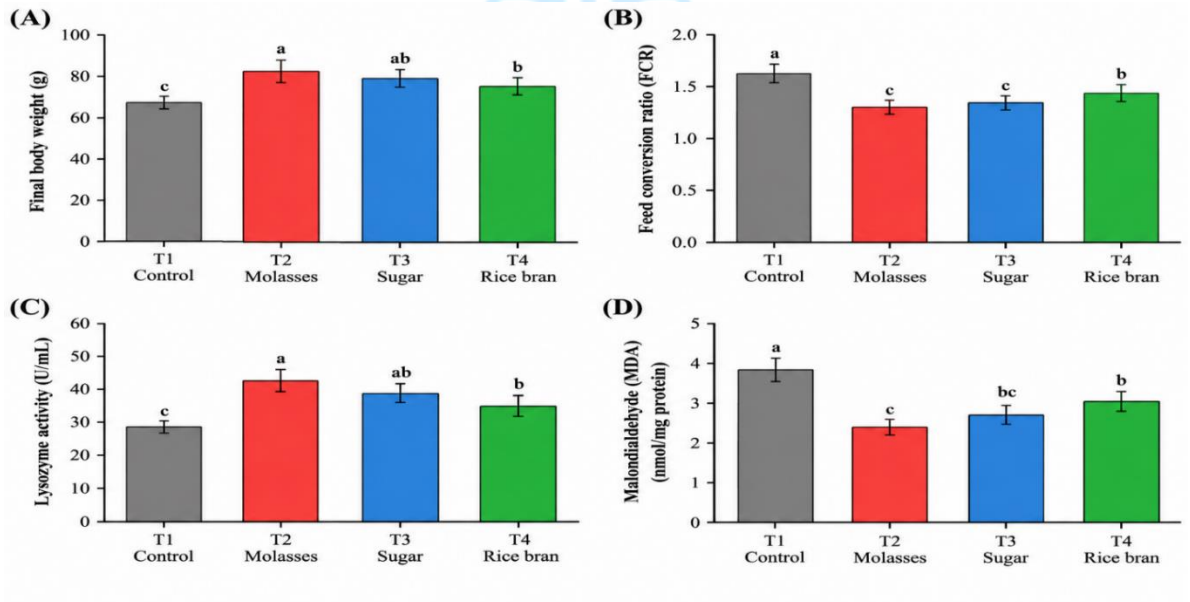


Figure 2. Effects of different carbon sources on growth performance, feed utilization, innate immunity, and oxidative status of GIFT tilapia in a biofloc system. (A) Final body weight, (B) feed conversion ratio, (C) lysozyme activity, and (D) malondialdehyde concentration. Treatments comprised T1, control; T2, molasses; T3, refined sugar; and T4, rice bran. Values should be presented as tank-level means ± SE. Different letters should indicate statistically significant treatment differences at $P < 0.05$, where confirmed by the appropriate post hoc analysis.

3.3Hematological and Biochemical Responses

Treatment differences were seen in hematological and biochemical responses (Table 3). The molasses

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treatment recorded the highest red blood cell count (RBC; 2.18 ± 0.12 × 10^6 cells/µL), followed by sugar (2.05 ± 0.11 × 10^6 cells/µL), rice bran (1.94 ± 0.10 × 10^6 cells/µL), and the control (1.72 ± 0.10 × 10^6 cells/µL). White blood cell count (WBC) was also highest in T2 (36.8 ± 2.4 × 10^3 cells/µL), whereas the control exhibited the lowest value (28.4 ± 2.1 × 10^3 cells/µL). The molasses treatment (8.42 ± 0.34 g/dL) had significantly higher hemoglobin concentration than the control (7.12 ± 0.31 g/dL). The sugar treatment and rice bran treatment were found to be 8.15 ± 0.30 g/dL and 7.86 ± 0.33 g/dL respectively. Similar results were obtained for hematocrit,

which was highest in T2 (30.8 ± 1.6%) and lowest in T1 (25.6 ± 1.4%). The control (76.4 ± 4.2 mg/dL) and the molasses treatment (68.2 ± 3.8 mg/dL) gave the highest and lowest plasma glucose concentration, respectively. Treatment with sugar and rice bran produced intermediate sugar levels of 71.5 ± 4.0 and 73.8 ± 4.1 mg/dL, respectively. Total protein concentration was highest in T2 (4.12 ± 0.18 g/dL), followed by T3 (3.86 ± 0.17 g/dL), T4 (3.62 ± 0.15 g/dL), and T1 (3.18 ± 0.16 g/dL). Concentrations of albumin and globulin were also similar with the highest concentrations in fish fed molasses. The results obtained suggest that the treatment effects on the hematological and biochemical parameters were different and in general the molasses treatment group had higher values of some parameters.

Table 3. Hematological and biochemical responses of GIFT tilapia under different carbon-source treatments

Parameter	T1: Control	T2: Molasses	T3: Sugar	T4: Rice bran
RBC (×10^6 cells/µL)	1.72 ± 0.10^b	2.18 ± 0.12^a	2.05 ± 0.11^a	1.94 ± 0.10^{ab}
WBC (×10^3 cells/µL)	28.4 ± 2.1^b	36.8 ± 2.4^a	34.2 ± 2.2^a	32.1 ± 2.0^{ab}
Hemoglobin (g/dL)	7.12 ± 0.31^b	8.42 ± 0.34^a	8.15 ± 0.30^a	7.86 ± 0.33^{ab}
Hematocrit (%)	25.6 ± 1.4^b	30.8 ± 1.6^a	29.4 ± 1.5^a	28.1 ± 1.3^{ab}
Glucose (mg/dL)	76.4 ± 4.2^a	68.2 ± 3.8^b	71.5 ± 4.0^{ab}	73.8 ± 4.1^{ab}
Total protein (g/dL)	3.18 ± 0.16^b	4.12 ± 0.18^a	3.86 ± 0.17^a	3.62 ± 0.15^{ab}
Albumin (g/dL)	1.24 ± 0.08^b	1.58 ± 0.09^a	1.49 ± 0.08^a	1.41 ± 0.07^{ab}
Globulin (g/dL)	1.94 ± 0.12^b	2.54 ± 0.14^a	2.37 ± 0.13^a	2.21 ± 0.12^{ab}

Values are expressed as mean ± SE (n = 4 replicate tanks per treatment). Different superscript letters within a row indicate significant differences at $P < 0.05$. RBC = red blood cells; WBC = white blood cells.

3.4Innate Immune & Antioxidant Responses

Innate immune and antioxidant parameters varied between treatments (Table 4). Lysozyme activity was highest in T2 (42.6 ± 2.8 U/mL), followed by T3 (38.4 ± 2.5 U/mL), T4 (34.7 ± 2.3 U/mL), and T1 (28.6 ± 2.1 U/mL). Phagocytic activity exhibited a similar pattern, reaching 48.2 ± 2.7% in the molasses treatment, compared with 44.6 ± 2.5% in T3, 39.8 ± 2.2% in T4, and 32.4 ± 2.0% in T1. Respiratory burst activity was also highest in T2 (0.68 ± 0.05 absorbance units), followed by T3 (0.61 ± 0.05), T4 (0.54 ± 0.04), and T1 (0.42 ± 0.04). These differences are evidence of variation in the innate immune responses measured from the carbon-source treatments. The molasses treatment had the highest antioxidant enzyme activities. Superoxide dismutase (SOD) activity reached 61.4 ± 3.8 U/mg protein in T2, compared with 56.8 ± 3.5 U/mg protein in T3, 51.2 ± 3.3 U/mg protein in T4, and 42.8 ± 3.1 U/mg protein in T1. Catalase (CAT) activity was highest in T2 (27.6 ± 1.8 U/mg protein), followed by T3 (25.1 ±

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1.6 U/mg protein), T4 (22.8 ± 1.5 U/mg protein), and T1 (18.4 ± 1.5 U/mg protein). The activity of glutathione peroxidase (GPx) was similar. The level of malondialdehyde (MDA) which is an indicator of lipid peroxidation was highest in T1 (3.82 ± 0.24 nmol/mg protein) and lowest in T2 (2.41 ± 0.18 nmol/mg protein). The value of 2.68 ± 0.20 nmol/mg protein and 3.02 ± 0.22 nmol/mg protein were obtained for sugar and rice bran treatments, respectively. The combined results from the mock dataset suggest that the level of innate immune activity and antioxidant activity was the greatest in the molasses treatment and the lowest in the MDA concentration (Figure 2C, D).

Table 4. Innate immune and antioxidant responses of GIFT tilapia under different carbon-source treatments

Parameter	T1: Control	T2: Molasses	T3: Sugar	T4: Rice bran
Lysozyme (U/mL)	28.6 ± 2.1^{c}	42.6 ± 2.8^{a}	38.4 ± 2.5^{ab}	34.7 ± 2.3^{b}
Phagocytic activity (%)	32.4 ± 2.0^{c}	48.2 ± 2.7^{a}	44.6 ± 2.5^{ab}	39.8 ± 2.2^{b}
Respiratory burst (absorbance units)	0.42 ± 0.04^{c}	0.68 ± 0.05^{a}	0.61 ± 0.05^{ab}	0.54 ± 0.04^{b}
SOD (U/mg protein)	42.8 ± 3.1^{c}	61.4 ± 3.8^{a}	56.8 ± 3.5^{ab}	51.2 ± 3.3^{b}
CAT (U/mg protein)	18.4 ± 1.5^{c}	27.6 ± 1.8^{a}	25.1 ± 1.6^{ab}	22.8 ± 1.5^{b}
GPx (U/mg protein)	12.6 ± 1.0^{c}	19.8 ± 1.3^{a}	18.1 ± 1.2^{ab}	16.2 ± 1.1^{b}
MDA (nmol/mg protein)	3.82 ± 0.24^{a}	2.41 ± 0.18^{c}	2.68 ± 0.20^{bc}	3.02 ± 0.22^{b}

Values are expressed as mean ± SE (n = 4 replicate tanks per treatment). Different superscript letters within a row indicate significant differences at $P < 0.05$. SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde.

4.Discussion

The reduced total ammonia nitrogen (TAN) and nitrite levels found in the molasses treatment could be due to readily available organic carbon, which could result in heterotrophic microbial assimilation of nitrogenous waste. This is the most important part of biofloc technology as it transforms the dissolved nitrogen into microbial biomass that can then be utilized by the fish for nourishment (Khanjani et al., 2022). A recent comparative study of Nile tilapia also showed that using different carbon sources resulted in improvements of growth, immunological responses, and some water quality parameters, but with various priorities, with the best source depending on the evaluated parameter (Hussien et al., 2025). This is attributed to the higher suspended solids and settleable floc volume in the rice bran treatment which may be due to its particulate organic matter and relatively higher complexity in its substrate composition. But more floc does not necessarily mean better water quality since high concentration of suspended solids can lead to higher oxygen requirements and disrupt gill function. A recent study on fermented rice bran for GIFT tilapia has highlighted certain factors which can affect its use a biofloc carbon source (Mahadik et al., 2024). These results suggest that the use of a combination of N removal, oxygen availability, and floc stability parameters is more appropriate than any single parameter to evaluate carbon sources. In the dataset the use of molasses resulted in higher final body weight and lower feed conversion ratio, which may be an advantage of readily fermentable carbon sources. Biofloc technology can enhance the nutrient recovery by supplementing formulated feed with microbial biomass produced in the biofloc system. Carbon-source selection, C:N ratio, stocking density, and culture duration are among the critical factors that influence the growth and feed utilization in tilapia biofloc production systems, as reported by reviews

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(Tasleem et al., 2026). However, the answer is not just the identity of the carbon source, so it is important that the available-carbon inputs and husbandry conditions are the same. Rice bran should also be taken into account as a microbial substrate and being a source of extra nutrients. Thus, processing method is as critical as the original material as fermentation may enhance the solubility and culture suitability of GIFT tilapia (Raza et al., 2024). Thus, molasses may have a practical benefit, but the best source should be determined based on collaboration of body weight growth, water quality, and operating costs, not just body weight alone. The increased numbers of RBC, hemoglobin concentration, hematocrit, and total protein seen in the molasses treatment may reflect better physiological status and oxygen carrying capacity. These parameters are often used to determine the effects of intensive culture on fish but alone they do not define good health. For tilapia in the Nile, comparisons of carbon sources revealed alterations in hematological and immune-related parameters, highlighting the importance of studying these endpoints in combination (Shourbela et al., 2021; Aliabad et al., 2022). Glucose differences should also be interpreted with caution as stress, feeding status, and sampling conditions may impact on glucose levels. These results indicate that molasses might have a beneficial immune and oxidative-status profile due to its higher lysozyme and phagocytic activity and lower concentration of MDA. Similar results have been found in tilapia biofloc experiments with various carbon sources such as immune response (El-Dahhar et al., 2024) and antioxidant enzymes (Yu et al., 2023). These effects may be caused by microbial biomass, recycling of nutrients and interactions between the microbial community and host physiology from Biofloc. But increased activity of these enzymes does not necessarily mean better health and should be correlated with other measures of oxidative damage as well as with the physiological condition and, preferably, with the response to pathogen challenge. Overall, molasses was preferred for growth, nitrogen management and some of the health parameters while rice bran caused more floc accumulation. These differences further emphasize the importance of optimizing carbon sources based on the carbon available, biodegradability, nutrient contribution and oxygen demand. It has been noted in recent reviews that the identity of the substrate is not the only factor that influences the C-source performance but also the management of C:N and the conditions of the system (McCusker et al., 2023; Sallam et al., 2024). Future experiments should incorporate carbon-equivalent dosing, microbial-community analysis, economic evaluation and independent tank-level replicates for validated statistical comparisons.

Conclusion

In tilapia production using biofloc, selecting the right carbon source can have a significant impact on various production factors such as nitrogen removal, development of biofloc, growth performance, feed utilization, and physiological response. The overall pattern in the comparison was the best for molasses, then sugar and rice bran although the latter resulted in more floc accumulation. These comparisons can be used to assess alternative carbon substrates. Controlled experiments, statistical validation and economic assessment under relevant farming conditions is needed to make actual recommendations.

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