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Research Article

Impact of Various Carbon Sources on the Growth Efficiency, Antioxidant Efficacy, and Immunity of Clariid Catfish *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The study examined the effects of various carbon sources on *Clarias gariepinus* (6.18±0.2g and 8±0.13 cm) growth efficiency, antioxidant efficacy, and immune function. The bioflocs system was developed using four carbon sources: tapioca, cassava flour, rice bran, and molasses. The fish were raised in concrete tanks for 10 weeks, and their water quality was monitored, after which they were challenged. The results demonstrated that the treatments significantly differed in terms of both survival rate and water quality parameters. Fish reared in biofloc systems exhibited significantly higher total cholesterol, total protein, superoxide dismutase (SOD), Lyzosome (LYZ), and Myeloperoxidase (MPO) activities compared with the control group. However, there was a reduction in the activities of ALT, AST, total glucose, and antiprotease in biofloc-treated C. gariepinus compared with the control. Expression of the IL-1 gene in the intestines was significantly elevated in fish raised in biofloc. Similarly, the transcription of GPX, GSR, IL-1, and IL-8 genes in the gut and liver of biofloc-treated fish was considerably enhanced. Applying biofloc to the rearing medium can enhance fish growth efficiency, immune system response, and the transcription of genes associated with immunity and antioxidant activities in *C. gariepinus*. The degree of immune system stimulation by the BFT system is impacted by the carbon source.

Keywords: Carbon sources, biofloc, African catfish, immunity, oxidative stress, gene expression

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INTRODUCTION

Food, fuel, shelter, clothes, and energy have been produced for human use by harvesting, farming, or cultivating the Earth's natural resources (Béné et al., 2016; Chan et al., 2019). However, these resources decline proportionately with population growth, leading to degradation of the environment, biodiversity loss, resource extinction, and climate change (Shah & Mraz, 2020). It is imperative to ensure that the supply of these resources occurs at a faster rate than consumption to guarantee sustainability. Aquaculture has been reported to provide more than 50% of fish production, and it is assumed that aquaculture production will expand to reach 202 million tons in 2030 aside from the production from algae (Ndondo, 2023)

Aquaculture systems result in the accumulation of inorganic nitrogen compounds, which are the primary excretory products of aquatic life. Ammonia, a product from the aquaculture system, has been reported to cause gill injury and predispose the cultured fish to disease stress, thereby affecting growth, reproductive patterns, oxygen expenditure, and death (Abdel Rahman et al., 2019; El-Sayed, 2020). Water exchange, bio-filtration systems, Recirculating Aquaculture Systems (RAS), cutting back on or stopping feeding, flushing ponds with fresh water, lowering stocking density, and aerating the pond are some of the techniques used to remove ammonia from aquaculture systems (Popoola & Miracle, 2022; Upadhyay et al., 2022). Unfortunately, the majority of these tech-

niques are costly, time-consuming, or harmful to the animal being raised.

Biofloc technology, a system that promotes the growth of microorganisms in aquaculture, has been explored as a potential feed source for ridding off ammonia in fish-rearing media (Abdel Rahman et al., 2019; El-Sayed, 2020). Biofloc can be cultivated using carbon sources such as molasses, wheat bran, maize bran, rice bran, and other organic substrates (Ekasari et al., 2014; Popoola & Miracle, 2022). The biofloc composition and carbon sources used could influence the fish's immunity and ability to withstand disease. Studies have demonstrated that biofloc, which contains vitamins, microbes, and other bioactive substances, can improve fish immune responses. In addition, its diets stimulate the fish's immune system, increasing disease resistance (Ahmad et al., 2017; Lumsangkul et al., 2021).

Assessing the expression of antioxidant enzymes (AOE) in response to environmental stressors, such as diseases and infections, is an extremely useful method for studying how aquatic species react to stress (Zhang et al., 2016).

To enhance comprehension of the immune system's impact on juvenile *Clarias gariepinus* raised in biofloc systems with varying carbon supplementation, this study examined the implications of four distinct carbohydrate sources as biofloculating agents, along with the growth performance and survival of *Clarias gariepinus*. Additionally, the study examined the transcription of immune- and antioxidant-associated genes in biofloc-reared *Clarias gariepinus* treated with *Aeromonas hydrophila*.

MATERIALS AND METHODS.

The Fisheries and Aquaculture Department of the Federal University of Technology, Akure (FUTA) in Nigeria provided the necessary resources for the research.

About 2,000 African catfish (*Clarias gariepinus*) juveniles (6.18±0.2g and 8±0.13 cm) were procured from a farm in Ondo Town and transferred to the Federal University of Technology Akure's teaching and research farm. The fish were maintained in 14-day mildly aerated rectangular concrete acclamation tanks with dimensions of 2m (length) x 1m (width) immediately after stocking. The fish were given commercially available feed with 35 % crude protein during acclimation at 08.00 and 17.00 h daily.

Following a completely randomized design, fifteen concrete tanks measuring 1 x 2 x 1 m were divided into five experimental groups based on the carbon sources (rice bran, molasses, tapioca, and cassava peel flour) and in triplicate. A week before the trials, concrete tanks were prepared, and two days before stocking, carbon sources were added. Daily additions of organic carbon sources were made following the anticipated 20:1 carbon-to-nitrogen ratio in the rearing medium and the approximate amount of feed nitrogen supplied to the culture tank (De-Schryver et al., 2008). Each tank held 120 pieces of *C. gariepinus*, vigorously aerated for 10 weeks using an air blower at 5 L/min per line (15 lines). During this period, conventional fish feed was provided to the fish (35% Crude Protein) twice daily, between 08:00 and 10:00 hours in the morning and 15:00 and 17:00 hours in the evening at 5% body weight.

The water parameters like temperature, dissolved oxygen (DO), biological oxygen demand (BOD), pH, salinity, alkalinity, and TDS were measured on a weekly basis using a multi-sensor EXTECH instrument ExStik II following the manufacturer's procedure.

After 10 weeks of stocking, surviving fish were counted and various growth parameters were determined.

The total weight gain was obtained as;

TWG (g/fish) is equal to (W_F–W_I), $\mathrm{TWG}\left(\frac{\mathrm{g}}{\mathrm{fish}}\right) = \mathrm{WF} - \mathrm{WI}$

where W_F is the fish's weight in grams at the end of the trial (Final) and W_I is its initial weight in grams.

The survival rate (SR) was calculated as

$\text{Specific Growth Rate (SGR)}\ \mathrm{SGR}\left(\frac{\%}{\mathrm{day}}\right) = \frac{\ln \mathrm{WF} - \ln \mathrm{WI}}{\text{trial}}\ \text{duration in days} * 100$

where ln is the natural logarithm and W_F, W_I represents the final and initial weight.

The feed conversion ratio (FCR) is obtained as;

Feed conversion ratio (FCR)=Weight Gain (g)/Feed Intake (g) x 100

Survival rate (SR)=total fish harvested /total quantity of fish stocked x100

The Animal Care Laboratory, Ogere, identified the pathogenic strain of *A. hydrophilia* (MPSTR 2143) and used it to feed the control fish and those in various biofloc systems. The bacteria were cultured in a 100-mL conical flask containing 30-mL autoclaved tryptic soy broth (TSB; Merck) as it progressed to a log phase. To obtain bacterial pellets, the culture was centrifuged for 20 min at 3500 x g and 4°C. The bacterial pellets were then cleaned using a sterile 0.15 M phosphate-buffered saline (PBS) with a pH of 7.2. Using the findings of the lethal dosage 50% (LD_{50}) trial, the pellets were redissolved in PBS, and the concentration was adjusted to 1.5 × 108 $cfuml^{-1}$ (Popoola et al., 2023). The 50 fishes (10 fish/treatment) were challenged via intraperitoneal injection, and mortality and survival were monitored and documented for further analysis.

Following confirmation of *A. hydrophila* as a source of mortality via challenge, dead and sick fish were collected. The challenging strain was then aseptically isolated and identified from samples collected from the kidneys, liver, intestines, and gills.

With the aid of a 2 ml heparinized syringe, blood was drawn from the caudal vein after 50 µl of clove oil was used to anesthetize the fish (Malick et al., 2020) and poured into a citrate-treated anticoagulant-coated test tube. Serum was isolated from blood and collected without the use of an anticoagulant. After centrifuging the blood for 15 min at 3,500 rpm and 4°C, the straw-colored serum was recovered and stored for later use at 20°C.

Total counts for leucocytes and erythrocytes were obtained using the Schaperclaus et al. (1991) method involving combining 20 µL of the blood sample with 3,980 µl of WBC and RBC diluting solution in a sterile vial, respectively. Using a Neubauer hemocytom-

eter (Rohem, India), the diluted fluids were examined, and the number of cells was recorded. The packed cell volume was determined using capillary action to pull non-dotted blood into the microhaematocrit tubes. A microhaematocrit reader was used to measure the PCV, which was then reported as a percentage (%). Using Drabkin's Fluid (Qualigens, India), the cyanomethemoglobin technique was used to determine blood hemoglobin levels. This involved mixing 5 mL of Drabkin's working solution and 20 µ blood and the spectrophotometer (Thermo Electron, Merck) was used to detect the absorbance at 540 nm. The hematological characteristics of the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formula provided by Haney et al. (1992).

MCV (fl) = Hct x 10/RBC, MCH (pg/cell) = Hgb (gm/dL) x 10/RBC, MCHC (g / dL) = Hgb (g/dL) / Hct.

With the aid of a BioVision USA SOD activity assay kit, the inhibition rate of superoxide anion in serum obtained from *A. hydrophila*-dosed *Clarias gariepinus* fish (control and biofloc raised) was calculated to determine the amount of superoxide dismutase (SOD). The Assay Kit for Lysozyme (USA: Sigma-Aldrich) was utilized to detect lysozyme activity by the instructions provided by the manufacturer. The serum concentration resulting in a 0.001-min decrease in absorbency at 450 mM was considered a single lysozyme activity unit. The techniques of (Quade & Roth, 1997) were used to measure the serum level of myeloperoxidase function (MPO) at 450 mM absorbance. The trypsin inhibition rate was used to indicate antiprotease activity according to the technique of Heo et al. (2013). The following formulas were used to determine the trypsin inhibition rate: (Control OD−Sample OD)/Control OD × 100, with PBS serving as the control. Serum levels of total glucose, cholesterol, and protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a clinical chemistry analyzer, Fuji DRI-CHEM 3500i (Fuji Film, Japan),

Observing the guidelines provided by the manufacturer for the Assay kit TRIZOL (Invitrogen, Carlsbad, CA, USA), with a few adjustments, 1 mL of TRIZOL reagent was used to extract total RNA from organs like the liver, muscle, and gills from five distinct juvenile C. gariepinus individuals. Total RNA was evaluated using a Nano-photometer (Implen®, CA, USA), and an optimal purity threshold of 1.8 and 2.0 was selected. With adherence to the manufacturer's instructions, upon diluting a sample of the total RNA to 300 ng µL−1, DNAse I (1 U/µL, Sigma-Aldrich, Missouri, US) was added to transcribe the cDNA. Reverse transcriptase (Promega, WI, USA) with oligo (dT20) primers was used according to the manufacturer's recommendations to synthesize cDNA from 1.0 µg of RNA (10 µL). Before qPCR analysis, the cDNA was kept at − 70 °C after dilution 10 times with ddH2O. For each qRT–PCR reaction, 2.5 L of this cDNA dilution served as the template. With SYBR Green PCR Reagents from Applied Biosystems and β-action as a housekeeping gene, real-time PCR was performed to evaluate the transcription of the selected genes (Table 1). The primer synthesis was designed based on the reference's

Table 1. Primers and sequences used for gene transcription.

Gene of Interest		Target sequence	Optimum Tm	References
GSR	F	GACTGGTTCCCAACGTGTCA	60.2	XM_034139725
	R	CGCCACCTATCACCAGGAAG		
GPX	F	ACTGCACACTCATGGGAACA	60.2	DQ355022
	R	TTAAAAGCCAGCGGATTGAC		
IL-8	F	CTGTTCGCCACCTGTGAAGG	61.2	NM_001279704
	R	ATGTGGCGGCCAATAGGTTT		
IL-1	F	GTCTGTCAAGGATAAGCGC	59.3	XM_019365844
	R	ACTCTGGAGCTGGATGTT		
β-actin	F	CTACGAGGGTTATGCCCTGC	62.0	XM_003443127.5
	R	ATGTCACGCACGATTTCCCT		

GPX, glutathione peroxidase; GSR, glutathione-disulfide reductase; IL-8, interleukin 8; IL-1, interleukin 1.

Table 2. Water quality parameters of the biofloc systems rearing *Clarias gariepinus*.

	Control	Molasses	Tapioca	cassava flour	Rice bran
Temperature (°C)	27.23±0.14	28.94±0.19	28.37±0.11	28.11±0.41	29.01±0.10
DO (mg/ L)	5.30±1.02	4.70±0.23	4.81±2.17	4.91±1.76	4.60±0.92
BOD (mg /L)	2.52±1.05	2.87±1.02	2.81±1.14	2.91±0.82	2.83±0.82
pH	6.80± 0.98	6.71±1.45	6.74±0.94	6.84±1.23	6.80±0.39
Salinity (g/L)	31.00±1.04	32.02±0.97	31.02±1.23	31.64±1.43	30.85±2.10
Alkalinity (mg/ L)	121.00± 3.23	114.17±4.22	117.10±1.33	119.01±2.35	114.21±3.12
TDS (mg/ L)	93.23±4.23	180.14±5.13	155.19±3.17	160.24±3.12	184.19±2.19

The growth characteristics of *Clarias gariepinus* cultured in systems using biofloc technology with various carbon sources were measured, and mean values ± standard deviation were recorded for each of the six individuals. Values for the same variable show significant variations ($P < 0.05$), denoted by an alternate superscript letter.

sequences obtained from GenBank sequences. According to (Livak & Schmittgen, 2001), the transcription levels of the indicated transcripts were evaluated for changes using standard curves and the 2−ΔΔCt approach.

Minitab version 14 was used to evaluate the study data following any necessary log transformation. To determine whether or not there was a difference of statistical significance at P<0.05, The New Duncan Multiple Range Test (NDMRT) was employed to differentiate the mean differences between the treatment groups. A descriptive statistic was used for gene expression analysis.

RESULT AND DISCUSSION

Water quality parameters

Salinity, BOD, and total dissolved solids were found to be significantly lower in the control group than in the biofloc group, according to the physicochemical parameters of the trial media. It was observed that the molasses medium had the highest salinity value, whereas the control group had the lowest. The control group had greater values for alkalinity and dissolved oxygen than the biofloc groups. However, there were variations in each treatment's pH, and these variations did not follow a consistent pattern. The study exhibited sustained differences in pH, dissolved oxygen, total dissolved solids, and salinity between the control and biofloc media treatments, all of which were suitable for the growing of *Clarias gariepinus*. (Table 1).

According to Gallardo-Collí et al. (2019), BFT is dubbed the "new blue revolution" since it uses microorganisms (bacteria, fungus, microalgae, and zooplankton) to proliferate and preserve water quality. The recommended values for *C. gariepinus* were met by the water quality indicators used in this investigation.

Growth and survival of experimental fish

Fish grown in biofloc systems showed considerably higher WG and survival values relative to the group under control (p<0.05) (Fig. 1a). In the meantime, there were no discernible differences in survival between fish receiving biofloc treatments for survival (p>0.05). Notable differences were observed among the biofloc treatments, with the control group exhibiting a significantly lower SGR than the other groups (p<0.05). Although tapioca and cassava flour exhibited no appreciable change, the FCR in the systems (tapioca and cassava flour) was higher than that of the other treatments, including the control. However, there was no statistical difference (p>0.05) between the control, molasses, and rice bran, but the FCR was the lowest in the control. Growth indicators and the survival rate of *C. gariepinus* after a 10-week feeding period showed significant differences (p<0.05) across all treatments (Fig. 1b).

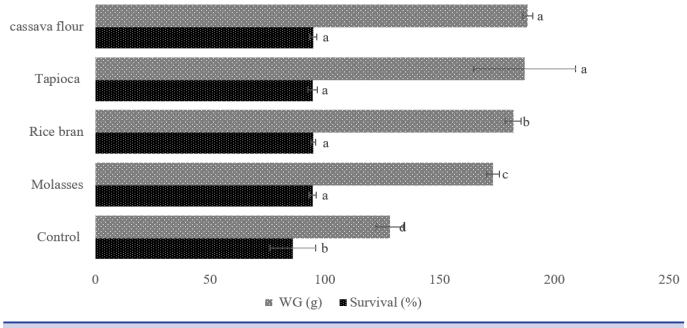

Figure 1a. Survival and weight gain in *Clarias gariepinus* raised under different carbon biofloc system.

Within the same row, mean values (Means, n =5) with different letters exhibit significant differences (P < 0.05).

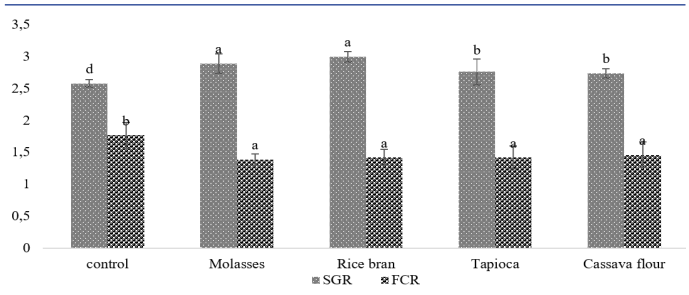

Figure 1b. Growth parameters of *Clarias gariepinus* reared in different carbon bioflocs system.
SGR-Specific Growth Rate; FCR- Feed Conversion Ratio

In the present study, fish reared within the biofloc performed better than fish kept in normal water devoid of carbon. This was revealed in the FCR of the BF groups, which was lower than that of the control groups. This implies that *Clarias gariepinus* could be produced more intensively using BF with less feed. Several factors could account for the beneficial features of biofloc technology, particularly its growth efficiency and feed utilization of *Clarias gariepinus*. According to several studies (Crab et al., 2012, Wang et al., 2015), biofloc improves water quality and stability, and fish can consistently obtain Flocs as a rich nutrient source (Fauji et al., 2018; Bakhshi et al., 2018). In addition, fish resilience to stress is increased by the environment in which they live and by consuming biofloc. (Fauji et al., 2018, Liu et al., 2018). The probability that the raised fish in this study consumed biofloc could be linked with the decreased values of FCR in the BFT groups. According to Yi et al. (2018), the FCR of biofloc groups was considerably lower than that of the control group. Further research has revealed that the biofloc improves fish's feed utilization while both the control and BF groups showed improved percentage survival rates, whereas the BF groups showed greater percentage survival rates; this finding may be related to the biofloc's immunostimulatory effects against stress (Keiko et al. 2015). Improvements in the aquatic environment that decreased stress and supplementation of nutritional components, essential amino acids, and fatty acids available in biofloc may have contributed to the dramatic increase in survival rate observed in this study.

Mortality-Disease resistance

At the end of the challenge experiment, the number of fish that died was deducted from the treated fish to determine whether the survival of fish reared in biofloc media with various carbon sources was significantly higher (P < 0.05) than that of fish kept in regular water. (Fig. 2).

An important measure of the wellness of fish is their survival after a pathogenic bacteria challenge test (Ringø et al., 2010). Qiao et al., (2018) reported that biofloc provides reliable fish immunity to diseases. According to the current investigation, the different carbon sources offered different immune capabilities to *C. gariepinus* and increased survival after challenge testing with *A. hydrophila* and *C. gariepinus*. Liu et al. (2018) observed comparable outcomes, noting that O. niloticus infected with *V. harveyi* and cultivated with biofloc exhibited a greater survival rate compared with the untreated group, suggesting that stimulation of immunity is caused by biofloc coupled with different materials used as flocculating agents. Kishawy et al. (2020) reported that *O. niloticus* infected with *A. hydrophila* had a higher chance of survival in groups using biofloc as the carbon source. Furthermore, Kishawy et al. (2020) argued that by increasing fish immunity and infection tolerance through the use of mannan oligosaccharides as a source of carbon in biofloc, the percentage of fish that survive is increased. Haridas et al. (2017) also discovered that *O. niloticus* raised with biofloc had a good survival rate when infected with *A. hydrophila*, supporting the positive effects on infection resistance. According to Fauji et al. (2018), *C. gariepinus* grown in biofloc cultures exhibited a much higher survival rate following *A. hydrophila* infection. According to Verma et al. (2016), *L. rohita* raised with biofloc had a greater survival probability for *A. hydrophila* infection than the control group. A high survival rate of *C. carpio* reared in biofloc from *A. hydrophila* infection *L.* was also noted by Bakhshi et al. (2018). Kim et al. (2020) found that *P. olivaceus* with an *E. tarda* infection that was farmed in biofloc had a significantly higher chance of survival. This was attributed to the biofloc's capacity to boost immunological function, thereby enhancing disease resistance.

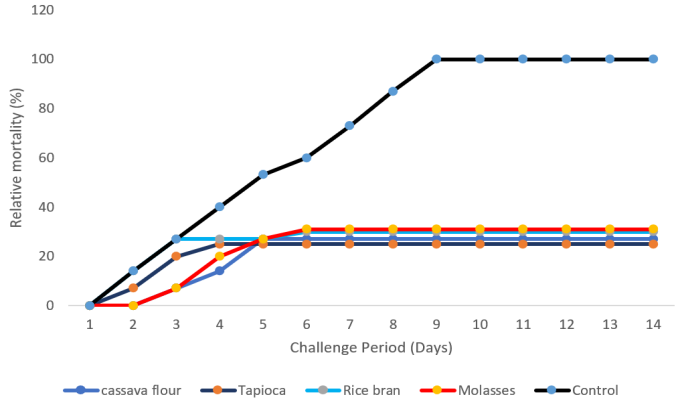

Figure 2. Relative mortality of *C. gariepinus* in different carbon biofloc systems against *A. hydrophila* compared with the control treatment.

Table 3 presents the changes in various Serum non-specific immune factors in *C. gariepinus* raised under different carbon biofloc systems for 10 weeks. Significant differences ($P < 0.05$) were observed for every parameter and treatment group (biofloc and control). For SOD, lysosome, and MPO, compared with other treatments, the control group's levels were noticeably lower (biofloc) unlike the antiprotease that was highest in levels in control when compared with other treatments.

Immune -antioxidant response

Superoxide dismutase (SOD) (% superoxide inhibition). Lysozyme is the Activity of serum lysozyme. Myeloperoxidase activity (MPO) was measured at 450 nm. An antiprotease is a percentage of trypsin suppression.

In contrast to AST, total glucose, and total cholesterol, which exhibit substantial differences between the carbon sources (biofloc) and control (Table 4), serum biochemical parameters, including ALT and total protein, were not significantly affected by biofloc treatment ($P > 0.05$).

Table 3. Serum non-specific immunological markers of *C. gariepinus* cultured in various carbon biofloc systems.

	SOD (UL^{-1})	Lysosome (mg ml^{-1})	MPO (UL^{-1})	Antiprotease UmL^{-1}
Control	35.95 ± 2.21^{c}	0.51 ± 0.03^{a}	0.78 ± 0.03^{e}	50.17 ± 3.14^{a}
Molasses	55.64 ± 0.50^{ab}	0.62 ± 0.00^{ab}	0.82 ± 0.03^{d}	43.18 ± 2.27^{b}
Tapioca	56.14 ± 0.54^{a}	0.65 ± 0.01^{b}	0.89 ± 0.05^{c}	41.73 ± 1.28^{bc}
cassava flour	43.64 ± 0.36^{b}	0.69 ± 0.02^{b}	1.06 ± 0.01^{a}	41.27 ± 2.01^{c}
Rice bran	55.76 ± 0.05^{a}	0.75 ± 0.11^{c}	0.90 ± 0.02^{b}	42.56 ± 1.38^{b}

The results show the average ± standard deviation of three duplicate observations. Values for the same variable show significant variations ($P < 0.05$), denoted by an alternate superscript letter.

Table 4. Biochemical parameters of *C. gariepinus* grown under different carbon biofloc systems.

	ALT (UL^{-1})	AST (UL^{-1})	Total glucose level (mg dl^{-1})	Total cholesterol level (mg dl^{-1})	Total protein level (mg ml^{-1})
Control	13.30 ± 1.01^{a}	17.03 ± 0.20^{a}	45.73 ± 2.06^{a}	119 ± 5.81^{d}	6.71 ± 0.31^{c}
Molasses	12.15 ± 1.13^{b}	15.65 ± 1.32^{b}	42.66 ± 2.17^{d}	129 ± 7.41^{c}	7.21 ± 0.14^{b}
Tapioca	11.35 ± 1.52^{c}	15.87 ± 2.11^{b}	44.90 ± 3.17^{b}	138 ± 4.21^{a}	7.46 ± 0.43^{a}
Cassava flour	11.16 ± 1.11^{c}	15.15 ± 1.02^{c}	43.67 ± 3.31^{c}	128 ± 2.44^{c}	7.23 ± 0.13^{b}
Rice bran	12.13 ± 1.22^{b}	15.21 ± 2.11^{c}	44.75 ± 2.17^{b}	133 ± 3.18^{b}	7.28 ± 0.26^{b}

Three replicates' mean ± standard deviation of three replicates is represented by the values. Values for the same variable show significant variations ($P < 0.05$), denoted by an alternate superscript letter.

The main antioxidant enzyme found in fish, superoxide dismutase (SOD), functions as a primary defense against oxidative damage by converting superoxide anion (O_2^-) into hydrogen peroxide, shielding fish from damage caused by reactive oxygen compounds, and maintaining the metabolic equilibrium of ROS (Kim et al., 2019). When *C. gariepinus* was cultivated in biofloc, it was discovered that the SOD and CAT values were amplified. This conclusion aligns with research by Mansour & Esteban, (2017) and Shourbela et al. (2021), who observed a significant increase in the serum SOD and CAT activity of *O. niloticus* grown in biofloc. Furthermore, *O. niloticus* cultivated with biofloc exhibited increased serum SOD and CAT activity. The reduction in oxidative stress, consequentially leading to fish health improvement recorded in this study was earlier observed by Shourbela et al. (2021). According to Menaga et al., (2019), biofloc may increase fish SOD and CAT activity levels, and low levels of these enzymes may cause high concentrations of free radical accumulation in cells, leading to cell damage. The explanation of the increased antioxidant capacity is believed to be an increase in the antioxidant enzyme that inhibits lipid peroxidation, as reported by Youse et al. (2020), who also observed a discernible increase in the blood concentrations of SOD and CAT in *C. carpio* cultured in biofloc. According to Popoola & Miracle, (2022), *C. gariepinus* cultured in biofloc medium with high stocking density exhibited better growth performance because of higher levels of SOD and CAT. Yu et al. (2020) found that fish grown with biofloc exhibited increased levels of SOD and CAT activity. Lipid peroxidation levels decreased as a result, and the body's ability to combat free radicals was enhanced. *P. hypophthalmus* cultivated in a biofloc environment exhibited a significant increase in SOD and CAT activities, according to (Nabi, 2021). The implication of this is that the biofloc environment provides fish with a high level of resistance to oxidative stress and acts as an effective antioxidant. However, Haridas et al. (2017) found that *O. niloticus* cultivated with biofloc had lower liver tissue SOD and CAT activity. They speculated that this could be because the bioactive chemicals decreased the production of SOD and CAT. This implies that antioxidant enzymes are not stimulated by reduced levels of oxidative stress. Yu et al. (2020) reported that fish health, environmental stress, and exposure to toxins can affect the activity of lysozyme, which acts as the first line of defense against various diseases, including bacteria, viruses, and parasites. Leukocytes in the blood produce an enzyme called lysozyme, which breaks down bacterial cell walls and promotes the formation of phagocytes to fight infections and other harmful diseases (Hwihy et al., 2021). Biofloc showed noticeably more LYZ activity than the control. According to Ali et al. (2018), consumption of the floc may have led to increased LYZ levels, which are indicative of *C. gariepinus* supplementation. Innate immunity in fish is measured by analyzing LYZ activity (Skouras et al., 2003). The outcome showed that elevated serum LYZ activity in C. *gariepinus* fed biofloc would have strengthened the fish's immune system. This finding is similar to the findings by Mansour & Esteban, (2017), who found that *O. niloticus* cultivated with biofloc exhibited a substantial increase in lysozyme levels, indicating improved immunity. Several researchers have observed this increase, and it has been suggested that the fish's defense mechanism is stimulated by bioactive chemicals found in biofloc, especially carotenoids, endogenous microbes, and fat-soluble vitamins (Long et al., 2015; Hwihy et al. (2021). Verma et al. (2016) reported that when *L. rohita* was grown on biofloc, lysozyme activity increased considerably, indicating that non-specific immunity can be improved by raising fish within a biofloc-based system. According to Yu et al. (2020), diverse species of fish, including *O. fopingensis, C. auratus*, and *C. argus*, exhibit increased lysozyme activity when reared with biofloc. Accordingly, fish may obtain protein from microbial flocs to support their immune system.

According to Borgia et al. (2018), serum antiprotease is essential for preventing bacterial infection in host cells from invading and multiplying. Therefore, a notable increase in the serum antiprotease activity of *C. gariepinus* cultured in biofloc prompted the prevention of *A. hydrophila* proliferation.

One of the peroxidases that is most frequently expressed in neutrophil granulocytes is myeloperoxidase (MPO). This ferrous lysosomal polypeptide is found in myeloid cells and is composed of monocytes and neutrophils. MPO causes hydrogen peroxide (H_2O_2) and chloride anions (Cl^-) to release hypochlorous acid (HOCl) during neutrophil respiratory bursts. Sontakke et al. (2018) reported that MPO is mostly found in basic neutrophil granules, where it combines with phagocyte packets to expedite the killing of pathogens and then transports these materials to invasive pathogens. To provide a potent antibacterial response, HOCl is generated (Kumar et al., 2005). Menaga et al. (2019) reported a large increase in MPO in *O. niloticus* cultivated in biofloc cultures, which they attributed to the fish's immune system being stimulated or triggered. Verma et al. (2016) reported that *L. rohita* cultivated in biofloc exhibited increased MPO, although the level of stimulation differed depending on the carbon source used to create the floc. Some carbon sources produced biofloc with a significant decrease in MPO, whereas biofloc made from additional carbon sources demonstrated a notable increase in MPO. This suggests that carbon sources have strong antimicrobial effects because they produce hypochlorous acid during respiratory bursts (Verma et al. 2016).

The AST and ALT enzymes were observed to be significantly low in all carbon-containing BFT treatments, whereas ALP showed no significant difference between BFT and control. These results demonstrate the stress-releasing effect of BFT in cultured *C. gariepinus*. Similar observation was reported by Abduljabbar et al. (2015) on Tilapia reared in a biofloc system. From another perspective, it could be mentioned that BFT systems in zero water exchange systems are suitable for Catfish culture and comparable with common intensive culturing methods.

A prior study found that when crucian carp were fed a supplemented diet, the levels of glucose were lower and the levels of total protein were much higher (Tan et al., 2018). Analogously, in our investigation, the biofloc system worked regardless of the carbon source employed to raise total protein and glucose. Serum components, including total cholesterol, have been widely established to be associated with immunological function and overall health (Zhou et al., 2012). The current study found that fish produced in biofloc had higher total cholesterol levels than those raised in a control group, and the total cholesterol levels of fish raised in different carbon inclusion environments varied.

Gene Expression Profiling

The significant effects of carbon sources on transcription of the GPX, GSR, IL1, and IL8 genes in *C. gariepinus* livers are illustrated in Fig. 3. Both IL-1 and IL-8 expression levels were significantly increased in the biofloc system, and there was a significant ($p < 0.05$) variance in their expression. There is upregulation of IL-1 and IL-8 in fish reared in all biofloc systems (molasses, tapioca, rice bran, and cassava flour), with the highest levels observed in tapioca, which is included in biofloc. Comparing fish in biofloc to those in other treatments and the control, we observed that the fish with molasses included in biofloc had significantly greater expression levels of GSR genes ($p < 0.05$).

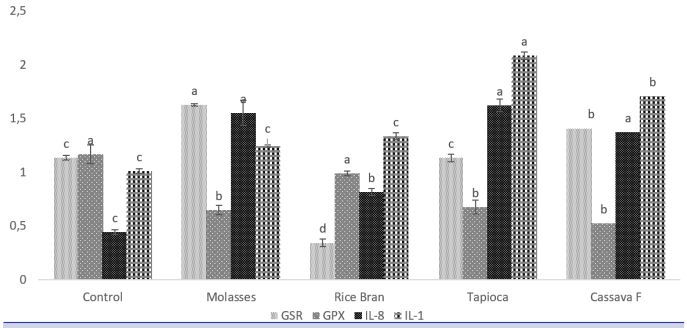

Figure 3. The liver of *Clarias gariepinus*, grown in various carbon-induced biofloc systems, expresses genes associated with immunity (interleukin 1, IL1; interleukin 8), as well as antioxidants (glutathione-disulfide reductase, GSR; glutathione peroxidase, GPX). (n = 5). Significant variations ($p<0.05$) are designated by superscripts in identical columns.

There were notable variations existing in GPX and GSR expression in fish raised in biofloc and in control *C. gariepinus* guts (Fig. 4). The transcription levels of the selected genes in the intestines of *C. gariepinus* (IL1, IL8, GPX, and GSR) displayed significant changes ($p < 0.05$) between the biofloc-treated and untreated groups (Fig. 4). Nevertheless, there was no discernible variation in GSR transcription levels among the fish raised in the Molasses, Tapioca, and control groups, respectively. In addition, the expression of IL-8 was not significant in rice bran, tapioca, and cassava flour, however, IL-1 was clearly upregulated among the biofloc groups.

Wang and Secombes (2013) asserted that cytokines; a byproduct of white blood cells, are essential for controlling and establishing connections between specific and non-specific immune systems. The current investigation showed that when *C. gariepinus* was challenged with *A. hydrophila* and raised in a biofloc system, there was a markedly elevated IL-1 and IL-8 levels. These important cytokines are expressed in fish in response to pathogenic pathogens, as reported by Sakai et al. (2021) in aquatic species.

De Schryver et al. (2010) and Ray et al. (2010) speculated that the significantly improved immunity shown by *C. gariepinus* in the present study may be related to the biologically active ingredients (taurine, carotenoids, phytosterols, and polysaccharides) available in the flocs, which contain a significant amount of pos-

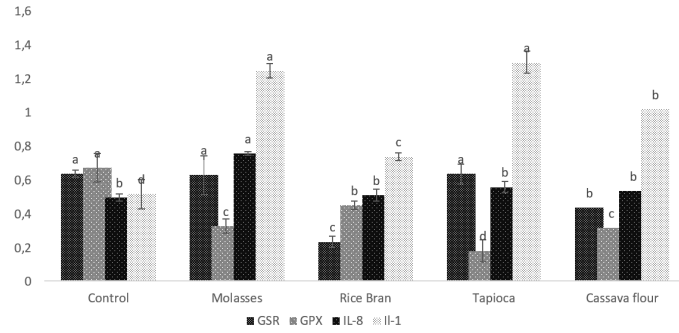

Figure 4. In the gut of *Clarias gariepinus* cultured in various carbon-induced biofloc systems, the expression of genes linked to immunity (interleukin 1 and IL1; interleukin 8) and antioxidants (Glutathione peroxidase, GPX, and glutathione-disulfide reductase, GSR). Significant variations ($p<0.05$) indicated by superscripts in identical columns.

sible prebiotics. Popoola and Miracle (2022) reported that biofloc carotenoids perform bioactive physiological tasks, strengthen fish immunity, and supply vital nutrients.

Bioactive compounds with specific antagonistic uses against pathogens have been reported to be present in biofloc, which can control disease occurrence in addition to boosting the immunity of farmed fish (Yu et al., 2023). The probiotic Bacillus, which is the main bacterium in the biofloc and increases fish immunity and resistance to infections, is beneficial to fish farming. Together, GPx and GSR remove hydrogen peroxide (H_2O_2) through a glutathione protection mechanism. Through the oxidation of glutathione (GSH) to glutathione disulfide (GSSG), GPx converts H_2O_2 into water. GSR uses the oxidizing reduction of NADPH to restore GSH after it has been oxidized (Imai and Nakagawa, 2003). Phase II xenobiotic metabolic catalyst glutathione S-transferase (GST) builds larger endogenic compounds through phase I reactions and is easily discharged through the kidney or bile (Diamond, 1993). Based on this study, it is possible that *C. gariepinus* raised in biofloc were fed supplemental diets from rearing medium that significantly increased GSR and GPX transcription in their livers.

Compared with controls, fish in the biofloc group had a greater capacity for defense against infection. These findings may be attributed to the antioxidant activity of the experimental fish. In a related study, Kheti et al. (2017) fed the Rohu microbial floc to their diets and found that this boosted the survival rate of the animals after contracting *Edwardsiella tarda*.

Additionally, it has been determined that the floc components of biofloc contribute to its immune-boosting and antioxidant qualities by scavenging its oxidative activity and enhancing fish immunity (Ahmad et al., 2016: Van Doan et al., 2018; Popoola et al., 2023).

It is interesting to note that in fish raised on biofloc, the expression of the GSR, IL-1, and IL-8 genes was higher in the liver than in the intestine. (Lumsangkul et al., 2021) additionally observed

that fish livers exhibit markedly higher relative immunological and antioxidant gene transcription. but not even a discernible variation was observed in *C. gariepinus* intestines other than IL-1. Differences in the quantity of immune cells in each tissue (Lumsangkul et al., 2021) as well as the types of carbon sources that support floc development (Popoola and Oyelade 2021) may explain the variation in relative immune gene expression. Comparable results were noted for antioxidant gene expression in common carp, with increased expression of these genes in the liver rather than the gut. This phenomenon could be explained by the expression of antioxidant genes differently in different tissues under oxidative stress (Lumsangkul et al., 2021). According to Hermesz and Ferencz (2009), oxidative stress enhances the expression of antioxidant genes in the liver of carp and lowers their transcription in other organs.

CONCLUSION

Because fish produced in biofloc exhibit better growth and survival rates, they can help increase output in the aquaculture sector. In comparison with the control, fish species grown in biofloc exhibited improved physiological indicators and reduced stress in biochemical measures, such as total protein, glucose, cholesterol, aspartate transaminase, and alanine transaminase. Fish grown on biofloc exhibited increased antioxidant responses, such as SOD and CAT, indicating a greater capacity to eliminate reactive oxygen species (ROS) resulting from environmental stress. Significant immune responses, lysozyme activity, and MPO were activated in a biofloc-reared fish species. In studies of *A. hydrophila* disease resistance, fish reared in biofloc showed a greater level of resistance. In conclusion, various carbon sources, particularly Molasses, Tapioca, cassava flour, and rice bran, could be successfully used to induce flocs in a rearing medium, and any of them could be used to improve fish immunity against pathogenic organisms while at the same time lessening production stress.

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Ethics Committee Approval: The ethical requirements of the Experimental Animal Welfare Ethics Committee of the Federal University of Technology, Akure, Nigeria were strictly adhered to during the conduct of this study. On the other hand, every attempt was made to reduce the experimental fish's pain and anguish.

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