



Effects of carbon sources on production performance, enzymatic efficiency and biochemical indices of Nile tilapia under biofloc culture system

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Abstract

Biofloc technology (BFT) is a sustainable aquaculture system based on the principle of nutrient recycling. This study evaluated the effects of starch, molasses and corn flour as carbon sources on water quality, growth performance, proximate body composition, hematological indices, immune responses and antioxidant status of Nile tilapia in biofloc system. A 12-week trial was conducted with juvenile Nile tilapia (25.3 ± 0.6 g), which were randomly assigned to four treatments. The treatments were BFT with corn flour (BFT+CF), molasses (BFT+M), starch (BFT+S) and a control group. Physicochemical parameters of water were affected by the carbon sources but remained within the optimal range for tilapia culture, with the highest biofloc volume (34.1 mL L^{-1}) observed in BFT+M. Growth performance was significantly increased in BFT+M, which yielded the superior weight gain (125 g), survival rate (99.03%) and the lowest feed conversion ratio (17.1). Proximate analysis revealed the highest crude protein levels in fish reared under BFT+M and BFT+S. Hematological parameters (hematocrit, hemoglobin, WBCs and RBCs) and non-specific immune responses (albumin, globulin, immunoglobulin and lysozyme activity) were significantly improved in BFT+M and BFT+S groups compared to the control. In conclusion, the supplementation of biofloc system with molasses and starch as carbon sources significantly improved growth performance, hematology, immunity and antioxidant capacity of Nile tilapia.

Keywords: antioxidant activity; biofloc technology; carbon source; fish growth; immune system

1 | INTRODUCTION

Aquaculture development is threatened by land and water scarcity, increasing feed cost and environmental concerns (Shourbela *et al.* 2021). The aquaculture sector has been exploring new environment-friendly production methods to use resources efficiently with less environmental impact. Biofloc Technology (BFT), which involves limited water exchange and the growth of dense communities of microorganisms known as "floc" (Ahmad and Abdel-Tawwab 2011), is one such technique that has gained popularity in recent times as it ensures sustainable feed management, yield high fish production with slight

water exchange (Rind *et al.* 2023).

Biofloc Technology, as a closed aquaculture system, offers several advantages over traditional methods, including improved management of water quality parameters such as temperature, dissolved oxygen (DO) and pH (Dauda 2020). Through continuous aeration and the addition of carbon sources, typically carbohydrates, to stimulate the growth of heterotrophic bacteria, the system maintains a high concentration of microbial flocs suspended in water column (Crab *et al.* 2012). The resultant microbial population is useful for two reasons: to serve as a bioreactor to uphold water quality and as a source of

protein for farmed fish (Pérez-Fuentes *et al.* 2016). El-Hawarry *et al.* (2021) recognised the impact of numerous carbon sources, including molasses, starch and glycerol, under low and high stocking densities, which shed light on each system's potential for tilapia culture. To fully understand how different carbon sources affect species hemato-immunological characteristics, growth and water quality, more research is needed.

Biofloc ability to regulate the C:N ratio is important to the system's overall productivity. Toxic inorganic nitrogen molecules could be immobilised into helpful bacterial cells using a certain C/N ratio (Xu *et al.* 2016). These cells then hide a single cell protein, which can nourish the cultivated organisms directly. Water quality is improved, and microbial biomass development is stimulated due to this changeover from autotrophic to heterotrophic systems (Khanjani *et al.* 2021a, 2021b). An appropriate carbon supply is vital for maintaining the correct C:N ratio and supporting the development of heterotrophic bacteria. The accessibility and composition of this carbon supply can heavily affect biofloc system. Silva *et al.* (2017) reported that the nutritional makeup of the microbial floc can be influenced by the choice of carbon source, which is commonly supplied through various carbohydrates in aquaculture.

A number of important aquaculture metrics, including growth rates, feed conversion ratios and organismal health are affected by the carbon source used in this technology (Li *et al.* 2018). The quality of the microbial floc varies depending on the carbon source used to produce it, which in turn affects its viability as a natural food source for cultured species (Ahmad *et al.* 2017). Different carbon sources, such as molasses, starch and corn flour, provide variable C:N ratio that affects microbial growth dynamics and nutrient cycling (Bakhshi *et al.* 2018), thereby improving water quality and fish growth performance (Ahmad *et al.* 2019). While several studies have demonstrated the potential of carbon supplementation in enhancing growth and immune responses of cultured species (Emerenciano *et al.* 2013; Mansour and Esteban 2017; Khanjani *et al.* 2021a, 2021b), there is still comparative research on the specific effects of different locally available carbon sources on Nile tilapia culture in biofloc system. This gap highlights the need for systematic evaluation of starch, molasses and corn flour within biofloc systems to determine their effectiveness in improving water quality, growth performance, body composition, hematology and immune competency of Nile tilapia. Sustainable methods, better resource usage and healthier fish populations can all result from a better understanding of how different carbon sources influences growth, meat quality and immunity in aquaculture systems. As a result, the aquaculture sector may benefit from these discoveries since they may lead to methods for increasing productivity while decreasing negative effects on the en-

vironment (Mirzakhani *et al.* 2019; Zaki *et al.* 2020). Therefore, this study aimed to examine the impact of molasses, starch and corn flour, three distinct carbon sources, on the growth, meat quality and immune system of Nile tilapia under a biofloc system.

2 | METHODOLOGY

2.1 Experimental design

This research was conducted at the Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. The 12-week feeding trial was carried out from 1 July to 30 September 2024. Before the experiment, sex reversed juvenile Nile tilapia with an initial average weight of 25 g were taken from the Government Fish Hatchery, Faisalabad and acclimatised for one week in rectangular tanks. During the trial, fish were fed a basal feed containing 32% crude protein (CP) at 3% of body weight, twice daily. The experiment followed a completely randomised design comprising of four treatments, each with three replicates: a control group and three biofloc treatments supplemented with different carbon sources- corn flour (BFT+CF), molasses (BFT+M) and starch (BFT+S). Fish were reared in 12 rectangular tanks with 1000 L water capacity. Continuous aeration was provided using 3 cm, spherical air-stone connected to a 1.5 hp blower. The basal diet (32% CP) was provided to all groups, while different carbon sources were supplemented daily to biofloc treatments. Proximate analysis of the basal feed and carbon sources is presented in Table 1, and the daily addition of feed and carbon source is shown in Table 2.

TABLE 1 Proximate composition (mean $\pm$ standard deviation; $n = 3$) of basal feed and the carbon sources (corn flour, molasses and starch) supplemented in biofloc groups.

Composition (%)	Basal feed	Corn flour	Molasses	Starch
Dry matter	90.5 $\pm$ 1.1	88.1 $\pm$ 0.18	53.6 $\pm$ 0.39	91 $\pm$ 0.14
Crude protein	32 $\pm$ 1.0	9.1 $\pm$ 0.2	4.7 $\pm$ 0.17	0.4 $\pm$ 0.03
Crude lipid	10.5 $\pm$ 1.0	4.9 $\pm$ 0.08	0.2 $\pm$ 0.06	0.2 $\pm$ 0.03
Ash	11 $\pm$ 1.1	3.6 $\pm$ 0.9	8.3 $\pm$ 0.14	0.5 $\pm$ 0.05
Carbohydrate	30 $\pm$ 1.0	66.9 $\pm$ 0.1	39.3 $\pm$ 0.3	90 $\pm$ 0.14

TABLE 2 Amount of carbon sources and fish feed used in the experiment.

Organic materials	Daily added value (g)
Fish feed (32% CP, provided to all groups)	16.9
Corn flour	7.7
Molasses	10.1
Starch	4.5

2.2 Biofloc formation

One week before the experiment, biofloc inoculum was

developed by the method of Avnimelech (1999) by adding 20 g of pond soil, 10 mg L⁻¹ urea and 400 mg L⁻¹ of carbon sources in 1 L well-aerated water. Fresh water was then added to all the tanks and 100mL concentrated biofloc was added into each biofloc treatment group. During the experiment, immediately after feeding, the designated carbon sources were supplemented to the three BFT groups to promote biofloc development while maintaining a carbon-nitrogen ratio of 15:1.

2.3 Water quality measurements

Water quality parameters were measured daily at 10.00 am. Water temperature, pH and dissolved oxygen level (DO) were measured using pH meter and DO meter respectively. Total ammonia nitrogen (TAN), nitrate (NO₃) and nitrite (NO₂) were analysed using an API Freshwater Test Kit (model: API Master Test Kit, Mars Fishcare, USA).

2.4 Growth performance

Growth performance of fish measured weekly and calculated according to the following formulas:

Weight Gain (g) = final weight (W_F) – initial weight (W_i)

Feed conversion ratio (FCR) = (Feed intake) / (Total weight gain)

Specific Growth Rate (SGR, % day⁻¹) = ((ln W_F - ln W_i) / (duration in days)) × 100

Survival Rate (%) = ((Final fish number) / (Initial stock number)) × 100

2.5 Proximate body composition

The proximate body composition was determined by using standard protocol. The crude protein level was determined through the Kjeldahl method to quantify nitrogen content. The lipid content was determined using a Soxhlet method, the dry matter was obtained via oven drying at 105°C and the ash content was measured by combustion at 600°C in a muffle furnace.

2.6 Haematological and immunological parameter

For haematological parameters, the fish was anaesthetised with clove oil. Blood samples were collected via a hepatic vein, and samples were kept in Eppendorf tubes with heparin-anticoagulants. Cyano-methemoglobin method was used to calculate the haemoglobin concentration (Hb). Hematocrit (HCT) was recorded by centrifugation method. Lysozyme activity was assessed using a turbidimetric assay following the standard protocol of Mörsky (1983). A suspension of *Micrococcus lysodeikticus* (0.15 mg mL⁻¹) was prepared in 66 mM phosphate buffer (pH 6.0). Serum samples (50 µL) were added to 1 mL of the bacterial suspension and reduction in absorbance at 450 nm was monitored with a spectrophotometer. Readings were recorded at three-minute intervals up to 4.5 minutes. Lysozyme activity was determined using the following formula: Lysozyme activity (U mL⁻¹) = ((Trans-

mittance (450 nm)) / (serum in mL used)) × 100

2.7 Data analysis

Shapiro-Wilk test used for the checking the normality of data. The experiment was carried out under a completely randomised design (CRD), and the data obtained was examined statistically using ANOVA. Tukey's pairwise was applied to compare the treatment means. Statistical software R (version 4.3.3) was used for correlation of water parameters with growth and haematological parameters. The data are presented as mean ± standard deviation (SD).

3 | RESULTS

3.1 Water Quality

Mean value of physicochemical parameters is presented in Table 1. The DO, temperature and pH were not different among the treatments and were maintained in optimum ranges (Figure 1). Meanwhile, ammonia (TAN), nitrite (NO₂) and nitrate (NO₃) differed significantly among treatment groups. Total ammonia ranged between 0.6 and 0.27 mg L⁻¹ in all treatments and remained below 1 mg L⁻¹. The control group showed a larger amount of TAN and a lesser amount of nitrite and nitrate than treatment groups.

3.2 Growth performance

The results of fish growth in biofloc tanks and control group with inclusion of different carbon sources are given in Table 4. The mean initial weight of fish was 25 ± 0.1 g, which increased significantly in all the treatments. Significantly increased weight gains and decreased FCR was recorded in the group with molasses (BFT+S). The lower FCR value was measured in biofloc groups BFT+M and BFT+S. Fish survival rates varied from 96 to 99% in all groups.

3.3 Proximate body composition

The fish muscle sample's proximate composition is shown in Table 5. The proximate composition significantly differed among the treatment group with the carbon source and control group. Fish in the treatment group with carbon sources molasses (BFT+M) and starch (BFT+S) had the highest protein content of 19.2 and 18.7 respectively. Crude fat content in the biofloc group ranged 1.1 to 1.19. Ash content in each group also differs significantly and the higher ash content recorded in the control.

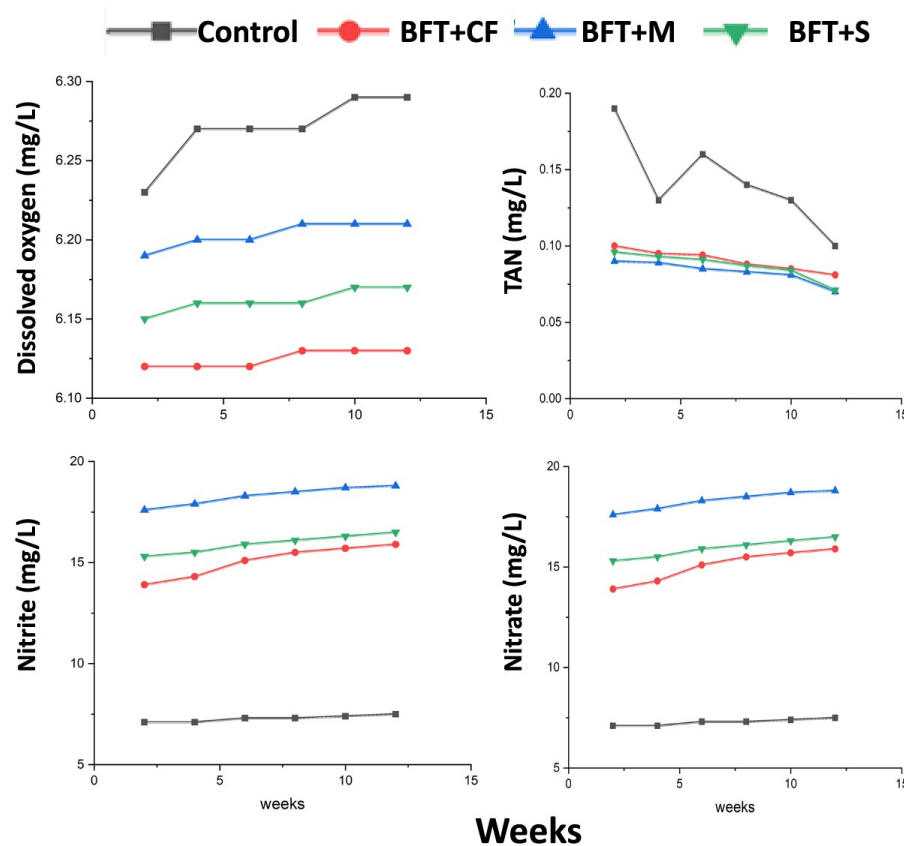
3.4 Haematological parameters

The addition of different carbon sources significantly influenced WBCs, RBCs, haemoglobin (Hb) and hematocrit (HCT) values (Table 6). Fish reared in biofloc treatments with corn flour, molasses and starch showed significantly higher RBCs Hb and HCT levels compared to the control group (*p* < 0.05).

TABLE 3 Water quality parameters in different treatments.

Treatments	Temperature (°C)	DO (mg L ⁻¹)	pH	TAN (mg L ⁻¹)	Nitrite (mg L ⁻¹)	Nitrate (mg L ⁻¹)
Control	27.03±0.5	5.9±0.5	7.48±0.8	0.6±0.4	0.21±0.3	7.5±0.01
BFT+CF	27.2±0.44	5.61±0.4	7.3±0.3	0.27±0.8	0.38±0.2	15.9±0.14
BFT+M	27.6±0.49	5.83±0.7	7.39±0.9	0.14±0.6	0.47±0.5	18.8±0.09
BFT+S	27.2±0.6	5.8±0.3	7.33±0.5	0.18±0.1	0.4±0.8	16.5±0.1

Values are presented as mean ± SD (*n* = 3). DO = Dissolved oxygen; TAN = Total ammonia nitrogen; BFT = Biofloc technology; CF = corn flour; M = Molasses; S = Starch

**FIGURE 1** Variability in Water quality parameters in different treatments (mean ± SD, *n* = 3). BFT = Biofloc technology; CF = corn flour; M = Molasses; S = Starch.**TABLE 4** Growth response of Nile Tilapia reared in biofloc culture system with different carbon sources.

Treatments	Initial weight (g)	Final weight (g)	Weight gain	FCR	SGR	Survival (%)
Control	25.2±0.3	127.4±1.3	102.2±1.3 ^c	2.2±0.9 ^a	1.09±0.89 ^a	96.8±1.29 ^a
BFT+CF	25.3±0.29	138.3±1.1	113±0.8 ^b	1.98±0.7 ^b	1.39±0.73 ^c	97.01±1.43 ^b
BFT+M	25.1±0.23	150.6±0.9	125.5±1.1 ^a	1.71±1.3 ^c	1.65±0.9 ^b	99.03±1.51 ^c
BFT+S	25.6±0.19	143.9±0.89	118.3±0.8 ^b	1.85±0.5 ^{bc}	1.51±0.54 ^{bc}	97.9±1.2 ^b

Values are presented as mean ± SD (*n* = 3). Means within the same column with different superscript letters differ significantly (*p* < 0.05). FCR= Feed conversion ratio; SGR= Specific growth rate; BFT+CF = corn flour; BFT+M = Molasses; BFT+S = Starch

TABLE 5 Proximate body composition of Nile tilapia reared in biofloc culture system with different carbon source.

Treatments	Crude protein (%)	Crude lipid (%)	Moisture (%)	Ash (%)
Control	17.6±0.53 ^c	1.4±0.03 ^a	78.9±0.5 ^a	2.2±0.8 ^a
BFT+CF	18.5±0.4 ^b	1.19±0.1 ^b	78.1±0.3 ^{ab}	1.8±0.6 ^b
BFT+M	19.2±0.3 ^a	1.05±0.3 ^b	77.5±0.8 ^{bc}	1.07±0.3 ^c
BFT+S	18.7±0.5 ^b	1.1±0.09 ^b	77.9±0.4 ^b	1.3±0.9 ^c

Values are presented as mean ± SD (*n* = 3). Means within the same column with different superscript letters differ significantly (*p* < 0.05).

TABLE 6 Hemato-biochemical indices of Nile tilapia reared in biofloc culture system with different carbon sources.

Treatments	RBCs ($10^6 \mu\text{L}^{-1}$)	WBCs ($10^3 \mu\text{L}^{-1}$)	Haemoglobin (mg dL^{-1})	Haematocrit (%)
Control	1.9 ± 0.5^a	27.3 ± 0.9^c	8.79 ± 0.1^c	28.7 ± 0.5^b
BFT+CF	2.7 ± 0.8^c	30.1 ± 0.1^a	10.1 ± 0.3^b	33.5 ± 0.4^a
BFT+M	3.94 ± 0.1^b	33.3 ± 0.6^b	11.8 ± 0.5^a	35.3 ± 0.7^b
BFT+S	3.58 ± 0.5^b	32.9 ± 0.4^{ab}	11.01 ± 0.19^{ab}	34.6 ± 0.9^{ab}

Values are presented as mean $\pm$ SD ($n = 3$). Means within the same column with different superscript letters differ significantly ($p < 0.05$). RBCs= red blood cells; WBCs= white blood cells.

3.5 Serum biochemical and immunological analysis

The addition of carbon sources significantly affected serum biochemical analysis of Nile tilapia cultured in biofloc system (Figure 2). Immunoglobulin, albumin, globulin and lysozyme activity positively improved in the experimental group reared with molasses (BFT+M) and starch (BFT+S)

as a carbon source. The treatment with carbon sources resulted in a considerable rise in fish serum levels of the antioxidant enzymes and catalase. Fish reared in the molasses-based biofloc group presented higher values of SOD (56.3 U L^{-1}) and CAT (35.3 U L^{-1}) than other groups.

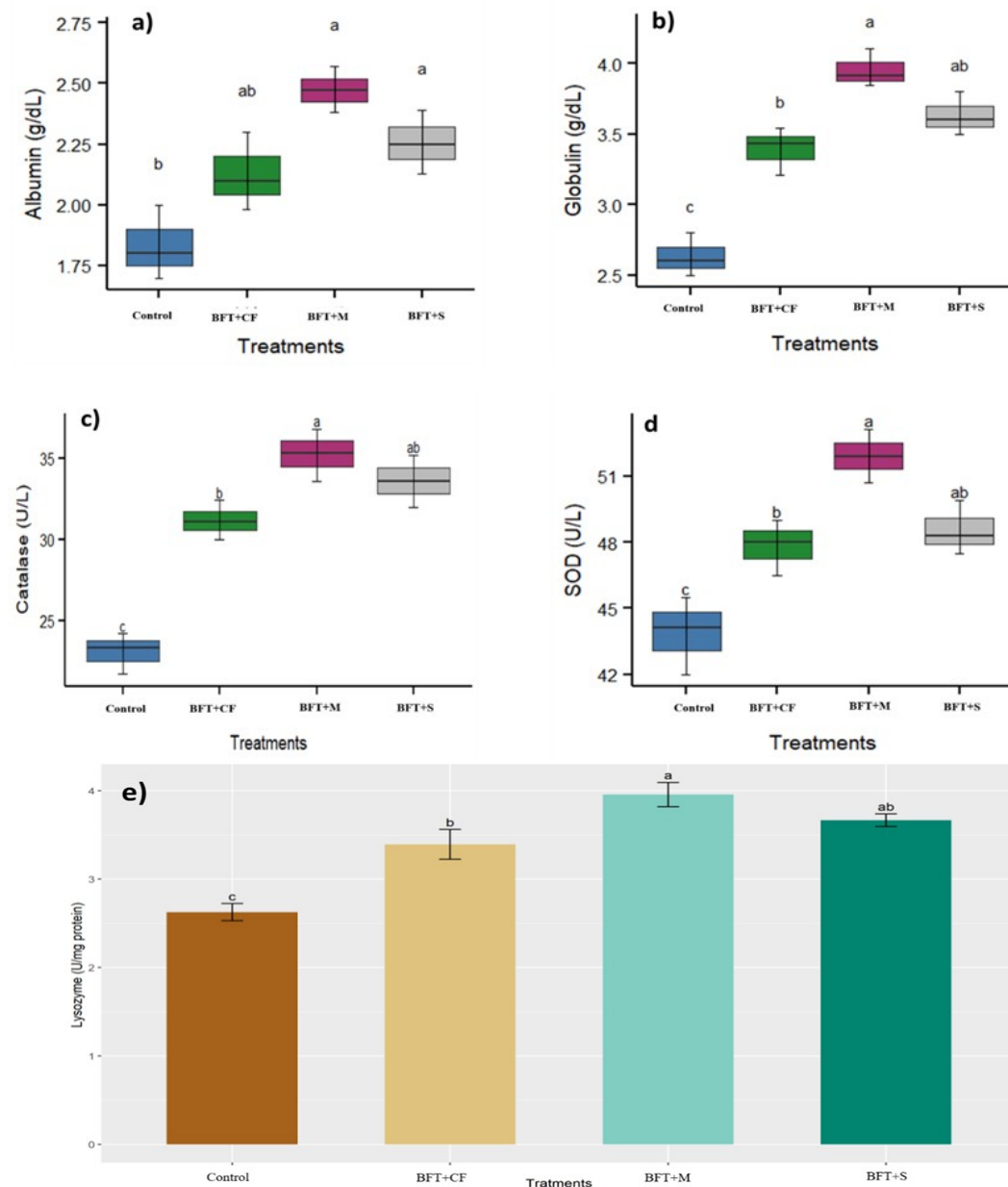


FIGURE 2 Non-specific immune parameters of Nile Tilapia reared in biofloc culture system with different carbon sources (mean $\pm$ SD; $n = 3$).

4 | DISCUSSION

With the intensification in human population, the demand for food to fulfil the nutritional requirements of almost seven billion people on earth has also increased (Dauda *et al.* 2019). Therefore, expansion in a sustainable culture system is indispensable without affecting the environment and support social and economic sustainability (Bhutia and Bhutia 2021). In this regard, biofloc technology is an ecologically intensive culture system and a new blue revolution in aquaculture. It ensures sustainable production of aquatic food organisms with minimum or zero water exchange and provides greater yield by retaining the carbon-nitrogen ratio. The current research, therefore, evaluated the impact of various carbon sources on production performance, proximate body composition, haematological parameters and immune status of Nile tilapia reared in a biofloc culture system.

4.1 Water quality

The ability of the biofloc system to maintain the C:N proportion is essential to the overall system productivity (Xu *et al.* 2016). Addition of carbon sources into biofloc tanks enhances the microbial species present in culture system, which then act as valuable feed source for cultivated species thus contributing to the improved culture output and lower production costs (Rind *et al.* 2023). Aquatic organisms predominantly depend on water to maintain their growth and health; therefore, maintaining water parameters for healthy and high production is paramount. In current study, the level of biofloc and physicochemical parameters including pH, DO, TAN, nitrates and nitrites were within the optimal range for fish growth. The findings of our research showed that, DO level and pH reduced in biofloc groups as compared to control group, likely due to increased microbial respiration (Rind *et al.* 2023). Microorganisms in the biofloc system have an imperative role as it helps in sustaining the quality of culture water (Khanjani and Sharifinia 2020) by recycling undigested food, reducing ammonia and nitrite and converting them to microbial floc protein (Wang *et al.* 2016). In present research, the reduction in the ammonia level of the group grown on different carbon sources could have resulted from the addition of carbon (Luo *et al.* 2014; Bakhshi *et al.* 2018) which accelerates the ammonia assimilation by the action of heterotrophic bacteria in biofloc culture (Deng *et al.* 2018). Concentration of ammonia in all biofloc groups rose during the first three weeks, and steadily decreased over time, possibly due to the suppression of nitrification activity by the heterotrophic microbial flora in the biofloc system.

4.2 Growth performance and feed utilisation

Due to the diverse nutritional value, variety of amino acids and minerals with the microbial protein (Khanjani *et al.* 2021a, 2021b), biofloc system acts as a complete meal

for cultured fish, which beneficially affects fish growth performance and health (Wei *et al.* 2016). Therefore, the results of current study exhibited a significant increase in growth parameters of tilapia in all biofloc groups. This study revealed a clear difference in growth and survival of fish, with increased survival and growth rate in the biofloc group grown on carbon sources molasses and starch. In this research, the control group had the highest FCR, while the molasses-supplemented biofloc group showed the lowest, which are in accordance with the results of Mansour and Esteban (2017). Recent studies have proved that aquatic species cultured in biofloc systems showed improved growth rates than in traditional culture systems (Mirzakhani *et al.* 2019). Feed cost and efficiency are the major factors controlling the farmer's economy as feed represent almost 60% of production cost. Thus, improving nutritional efficiency is key for developing a sustainable aquaculture industry and in this regard, applying biofloc technology for intensive fish culture can effectively reduce production costs (Durigon *et al.* 2020).

4.3 Proximate body composition

Nutritional value of biofloc can positively influence the proximate body composition of fish (Long *et al.* 2015) and in present study, crude protein and lipid content were significantly affected by addition of carbon sources. The highest crude protein and lipid content were in BFT+M, BFT+S and BFT+CF treatment groups. The crude protein content was significantly greater in BFT+M than in other groups while increased ash and moisture content were measured in the control group (Akinwande *et al.* 2016). This increase in the protein content may have been connected to the nutritional composition and microbial populations in the biofloc culture system, which gives essential nutrients to the cultured species (Crab *et al.* 2012).

4.4 Haematological response

Evaluation of haematological indices is crucial as they serve as an effective tool to observe fish health and physiological status. A slight change in these constraints indicates abnormalities in fish health (Akinwande *et al.* 2016). The findings of this research showed that inclusion of different carbon sources significantly affected WBCs, Hb, HCT and RBCs among treatments and the BFT+M group had higher levels of each of these blood parameters than the other groups. Rind *et al.* (2023) reported a substantial improvement in haematocrit value of tilapia in the biofloc group grown on carbon sources. Mansour and Esteban (2017) observed the same trend in WBC's count and HB value in tilapia and reported improved Hb, RBC and HCT content in the biofloc group grown on carbon sources.

4.5 Immune and antioxidant response

The non-specific immune system in fish plays an im-

portant part in maintaining fish health and disease resistance (Najdegerami *et al.* 2016). The immunostimulatory role of the biofloc culture medium has been reported in many studies. In this study, inclusion of carbon sources influence innate immune parameters and the group recorded a considerable increase in albumin, globulin, immunoglobulin and lysozyme activity levels. The results of our research are in accordance with the findings of Bakhshi *et al.* (2018), who reported that biofloc systems grown on carbon sources significantly improved the immune competency of fish in those systems. Mansour and Esteban (2017) and Rind *et al.* (2023) also showed similar results and concluded that fish reared in biofloc culture systems had significantly greater levels of immunoglobulin along with lysozyme in addition to increased plasma protein. The antioxidant enzymes are involved in the body's defence mechanism against the oxidative stress produced mainly during the redox reaction and in response to an increase in ROS. Earlier studies have revealed that microbial flocs are a great source of bioactive compounds such as carotenoids, natural antioxidants, and different types of vitamins that help improve the antioxidant defence system of cultured species (Khanjani and Sharifinia 2020). In this study, fish raised in BFT with molasses as a carbon source had greater levels of the antioxidant enzymes CAT and SOD and these outcomes were similar to the results of Rind *et al.* (2023), in which increased SOD and CAT level were observed in biofloc groups with addition of carbon source.

5 | CONCLUSIONS

This study demonstrates that biofloc culture systems supplemented with specific carbon sources significantly enhance the growth performance, body composition, water quality, antioxidant status, haematological and immunological responses of Nile tilapia. Among the tested carbon sources, molasses and starch were particularly effective in improving water quality and promoting superior growth performance. These findings highlight the potential of carbon source based biofloc systems as a sustainable aquaculture strategy to reduce environmental impact while supporting healthy fish production.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

AM, NA and KZ designed the study; NA performed the research experiment; AM devised ideas and supervised the experiment; KZ, AA, DR and SR helped in experimental work and data analysis; NA, AM, KZ and DR provided the critical revision and final approval of the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are avail-

able on a reasonable request from the corresponding author.

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