The impact of dietary carbon sources on the blood characteristics of grass carp (*Ctenopharyngodon idella*) cultured in biofloc system

[1]Ayat M. Awad, [1]Ahmed E. A. Badrey, [2]Khaled Y. AbouelFadl, [1]Alaa G. M. Osman

[1] Department of Zoology, Faculty of Science, Al-Azhar University, 71524 Assiut, Egypt; [2]Faculty of Fish and Fisheries Technology, Aswan University, 81628 Aswan, Egypt. Corresponding author: A. E. A. Badrey, gmal_ahmed77@yahoo.com

Abstract. Biofloc technology degrades waste into useful resources exploiting microbes and can be used in zero-water exchange systems. To study the impact of different biofloc systems on blood characteristics of grass carp fingerlings, a 90-days experiment was conducted using three long lasting carbon sources. Two hundred and forty fingerlings having the mean weight of 1.8±0.2 g were stocked in each (20 fish/replicate). Four experimental groups were set in triplicate. T1 (control), T2 (molas), T3 (starch) T4 (flour) and all treatments (control, molas, starch and flour) include the same variables. In-situ biofloc was developed in 250 L fibre-reinforced plastic (FRP) tanks. In the current study, measures of the biofloc conditions included temperature, PH, DO, and ammonia. Water temperature and PH were not significantly different among groups while dissolved oxygen concentrations were significantly higher in biofloc groups compared to the control group. In the present study, results of fish blood biochemistry indicate that the feed supplemented with different carbon sources, especially flour and molas, can increase fish health. Accordingly, fish farming using biofloc system and feed supplemented with different carbon sources is very beneficial for fish farmers because it can reduce feed cost, toxicity, and improve the immunity of the fish. Additionally, no significant differences were observed for haematological and biochemical parameters, thus, indicating that the biofloc system in this study had no negative effect on this physical condition of the grass carp.
Key Words: Aquaculture, biofloc technology, grass carp, water quality.

Introduction. Aquaculture business plays a key role in producing aquatic products, eliminating hunger, solving world food shortages, reducing poverty, and promoting health (Bardach 1985; Perschbacher 2015; Zhifei et al 2019). However, aquaculture has to constantly deal with some problems, such as the environmental and economical limitations and the shortage of ingredients and their high prices (De Schryver et al 2008). Thus, strategies aimed to solve some of the above challenges are required. A relatively new alternative is the bioflocs technology (BFT) solving the previous limitations and problems and revolutionize aquaculture (Avnimelech 2006). BFT is considered as a new "blue revolution" based on minimal or zero water exchange and reducing the discharges of nutrient-rich effluents into the environment (Walker et al 2019). In this regard, aquaculture water quality parameters have been modified to promote the growth of some biotic communities in order to increase nutrient recirculation and to utilize the biomass of such biotic communities as a direct food source for the commercial organisms being cultivated (Martínez-Córdova et al 2015), reducing maintenance costs and provides an added value by improving the food consumption rate (Azim & Little 2008; Castro-Nieto et al. 2012). BFT was developed in the 70's at the French Research Institute for Exploitation of the Sea (IFREMER), based on the microbial communities that help avoid or minimize water exchange and, producing of microbial protein that can be used as food (Avnimelech 2009).

In biofloc aquaculture, a co-culture of heterotrophic bacteria and algae is grown in flocs with other kinds of particulate organic matter such as feces and uneaten feed under

controlled conditions within the culture pond (De Schryver et al 2008; Hargreaves 2006, 2013; Yassien et al 2021). Recently, BFT has been received considerable attention to maximize production yield, improve water quality and simultaneously recycle feed and protein production in the same culture unit (Avnimelech 2006; Crab et al 2007; Little et al 2008; Zaki et al 2020). To achieve low or zero-water exchange in BFT, the addition of organic carbon rich substrate to aquaculture is very important to promote production and nutrient retention (Crab et al 2007; Gao et al 2012). Thus, cheap or easily available waste carbon sources are required to further lower down the input cost and to increase the net return (Zaki et al 2020).

One way to know the health condition of fish in aquaculture is through the application of hematological and blood biochemical tools (Bañuelos-Vargas et al 2021). Blood characteristics have proven to be sensitive indices for monitoring physiological changes in fishes (Badrey et al 2019). Analysis of blood indices are an effective approach for the investigation of the health status of fish in aquaculture, providing reliable information on metabolic disorders (Osman et al 2019). Blood constituents could be affected by numerous dietary supplements (Animashahun et al 2006; Osman et al 2019). Hematological and blood biochemical variables are important for assessing the quality and suitability of feed ingredients for farm fish (Maxwell et al 1990; Ighwela et al 2012). They are usually sensitive to the dietary content, frequently used to evaluate the fish's health conditions (Solomon & Okomoda 2012; Abdel-Tawwab et al 2010; Habte-Tsion et al 2013; Kondera et al 2017)

Grass carp (*C. idella*) is an important aquaculture species and is one of the most important cultured fish worldwide. Grass carp have lots of characteristics such as strong adapt ability, a wide range of feed Sources, rapid growth and delicious meat. The literature regarding blood characteristics of fishes cultured in BFT is scarce, but it has been shown that the BFT system can have positive effects on fish health status, although some results are contradictory. According to the author knowledge's, little is known about the blood characteristics of grass carp in aquaculture especially those raised under different environmental and management conditions such as biofloc. Therefore, the present work aimed to investigate the effects of dietary carbon sources (molas, starch and flour) on the hematological and blood biochemical variables of grass carp (*C. idella*) reared in a biofloc system.

Material and Methods. The current experiment was carried out at the Aquaculture Lab, Department of Zoology, Faculty of Science, Al-Azhar University (Assiut Branch), Assiut, Egypt. The effects of dietary carbon sources on the blood characteristics of grass carp reared in a biofloc system were investigated.

Experimental design. A total of 240 healthy live grass carp (*C. idella*) with an average body weight of 1.8±0.2 g were obtained from the Ahywa hatchery-Sohag, Egypt. The fishes were kept in a recirculation system ensuring optimum water quality, and the system was thermostatically controlled at 23 ± 2°C. The study was conducted in twelve circular tanks (250 L each) filled with 200 L dechlorinated water. Fishes were acclimatized for six weeks at laboratory conditions before the start of the experiment, then they were divided into four equal groups, including control in three replicates each (20 fish/replicate) according to (Badrey et al 2019) with some modifications.

1 - The first group was considered as the control and fed the basal control diet containing 25% crude protein (CP).

2 - The second, third, fourth groups were fed on the basal diet containing 25% CP, which was supplied with molas, starch, flour, respectively, as sources of carbohydrates.

All fishes were fed with 5% of their body weight twice, daily, at 9:00 AM and 3:00 PM, six days a week. They were reared for 90 days, between 1 August to 29 October 2020.

Water quality. All water quality parameters (ammonia, pH, dissolved oxygen and temperature) were maintained in a suitable limit for grass carp rearing according to El-Sayed (2006).

Hematological analysis. Whole blood was assessed for hemoglobin concentration (Hb), hematocrit (Hct), and red blood cell (RBC) count by using an automated technical analyzer (Celltac α MEK- 6400J/K). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cell (WBC) count were calculated according to the methods of Dacie & Lewis (2002).

Biochemical analysis. Blood samples were collected through cardiac puncture as described by Osman et al (2011). No anesthetic was given to the fish, as anesthesia can affect blood parameters. The blood samples were then allowed to coagulate for 15–20 minutes at 4°C before centrifugation for 20 minutes at 3000 rpm to separate serum. The serum was stored at -20°C until use for biochemical. Serum total protein, cholesterol, triglyceride, glucose, urea, creatinine, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels were calculated according to the methods of Reitman and Frankel (1957), Henry (1964), Trinder (1969), Friedewald et al (1972) and Thomas (1992).

Statistical analysis. Data were presented as mean±SD (Standard Deviation). The results were subjected to a one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance (parameters presented in Tables 1, 2, and 3). Data were analyzed using SPSS (1997) program, Version 16. Differences between means were compared using Duncan's using Duncan's (1955) multiple range tests at $p < 0.01$ level.

Results

Water quality. The average values of the measured water quality variables (water temperature, pH, dissolved oxygen and total ammonia nitrogen) are presented in Table 1. Water temperature (23.3±4.2-23.9±5.1ºC) and pH (7.77±0.5-7.98±0.6) were not significantly different among groups (Table 1). Dissolved oxygen concentrations were significantly higher in biofloc groups (7.5±0.9-6.9±1.1 mg L^{-1}) compared to the control group (6.9±1.1mg L^{-1}). In contrast, the concentrations of ammonia were significantly lower in all the treated groups (molas 0.64±0.52, starch 0.77±0.72, and flour 0.8±0.75), compared to the control one (1.05±0.7). Non-significant differences were recorded among the biofloc groups in the values of dissolved oxygen (DO) and ammonia (Table 1).

Table 1

Water quality parameters in cultivation tanks of grass carp under different carbon sources during 90 days of experiment

Parameters	*Control*	*Molas*	*Starch*	*Flour*
Temperature ºC	23.9±5.1^{a}	23.6±4.9 ^{a}	23.5±5.0 ^{a}	23.3± 4.2^{a}
pH	7.77±0.5 ^{a}	7.87±0.6 ^{a}	7.95±0.6 ^{a}	7.98±0.6 ^{a}
DO (mg L^{-1})	6.9±1.1^{b}	7.6±0.95 ^{a}	7.5±0.9 ^{a}	7.5±0.98 ^{a}
Ammonia (mg L^{-1})	1.05±0.7 ^{b}	0.64±0.52 ^{a}	0.77±0.72 ^{a}	0.8±0.75^{a}

Means in the same row with different superscript letters are statistically significantly different (P<0.05).

Hematological parameters. The mean values of RBCs, Hb, Hct, MCV, MCH, MCHC, WBCs, Lymphocytes, Neutrophils, Monocytes, Eosinophils and Platelets are presented in Table 2. Statistical analysis of RBCs, Hb, Hct, MCV, MAHC and MCH, of the blood of groups supplemented with starch, molas and flour revealed non-significant differences compared to the control group after 45 and 90 days of exposure (Table 2). The values of MCHC in the blood of fish fed on starch and flour exhibited a significant reduction compared to the control one after 45 days of exposure, but the differences in the values of MCHC were non-significant for all treatments after 90 days of exposure (Table 2). Significant increase in the values of WBCs were recorded in the blood of fish fed on

molas, starch, and flour compared to the control. The highest level of WBCs was recorded in the blood of fish fed on flour and molas after 45 and 90 days of exposure, respectively (Table 2). The results indicated that no significant difference was observed in terms of Neutrophils, Monocytes and Eosinophils compared with the control group after 45 days of exposure (the excluded starch group which exhibited a significant difference). The same variables were significantly varied in nearly all the treatments compared to the control at the end of the experiment (Table 2). Significant increase in terms of platelets was observed in the blood of fish fed on the different carbon sources compared to the control. The highest platelets level was recorded in the blood of fish fed on flour after 45 and 90 days of exposure.

Table 2

Hematological parameters (mean ± Standard Deviation) of grass carp under different carbon sources for 45 and 90 days.

Treatment	*45 Days*				*90 Days*			
Items	*Control*	*Molas*	*Starch*	*Flour*	*Control*	*Molas*	*Starch*	*Flour*
RBCs ($\times 10^6$ µl)	3.4±0.2^a	3.03±0.15^a	3.5±1.3^a	3.5±0.5^a	3.4±0.2^a	3.5±0.2^a	4.0±0.1^a	3.6±0.2^a
Hb (g/dl)	10.03±0.3^a	10.5±0.5^a	9.6±1.4^a	9.7±0.5^a	10.6±0.2^a	9.4±0.2^a	10.4±0.4^a	9.5±0.4^a
Hct (%)	30.1±1.05^a	31.5±1.1^a	28.0±4.6^a	29.7±2.9^a	31.5±0.1^a	27.9±0.3^a	31.2±0.4^a	28.4±0.5^a
MCV (fL)	98.3±0.8^a	100±3.4^a	96.8±17.5^a	96.5±3.2^a	91.4±0.6^a	90.1±1.1^a	90.0±2.9^a	95.5±2.2^a
MCH (pg)	32.3±1.1^a	33.2±1.0^a	31.0±6.9^a	32.2±0.7^a	30.4±0.5^a	29.5±0.4^a	28.1±0.9^a	32.2±0.7^a
MCHC (g/dL)	35.5±2.2^b	33.5±1.8a^b	31.8±1.6^a	31.3±1.8^a	33.8±1.6^a	31.3±3.1^a	33.0±4.3^a	35.8±0.7^a
WBCs ($\times 10^3$ µl)	28.5±0.6^a	29.3±0.7^b	29.4±1.0^b	30.5±0.9^b	28.6±1.6^a	32.2±0.8^b	31.2±1.0^b	30.2±0.8^b
Lymphocytes (%)	81.3±1.1^{ab}	87.0±1.0^b	78.0±7.5^a	86.6±2.5^b	82.6±2.5^a	82.6±2.5^a	78.0±2.0^a	87.7±2.5^b
Neutrophils (%)	10.6±0.5^{ab}	7.3±3.0^a	17.3±9.2^b	7.3±1.5^a	13.6±1.5^{bc}	9.6±1.1^{ab}	15.3±4.1^c	8.6±1.5^a
Monocytes (%)	5.3±0.5^b	3.0±1.7^{ab}	2.3±0.5^a	4.3±1.5^{ab}	4.6±1.5^b	4.00±1.7^{ab}	2.6±0.5^{ab}	1.6±1.1^a
Eosinophils (%)	1.6±0.5^a	2.6±0.5^b	1.0±0.00^a	1.6±0.5^a	1.0±0.0^a	2.6±0.5^b	1.6±0.5^a	1.3±0.5^a
Platelets($\times 10^3$ µl)	163.3±6.4^a	205.0±7.0^b	175.3±17.9^{ab}	209.3±8.6^{bc}	184.6±8.5^a	194.0±6.0^b	210.3±10.5^b	257.0±6.0^c

Means in the same row with different superscript letters are significantly different ($P<0.05$).
Legend: Red blood cells (RBC); Hemoglobin concentration (Hb); Hematocrit (Hct); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC; white blood cells (WBC).

Blood biochemistry. The results of biochemical variables (total protein, glucose, cholesterol, triglycerides, creatinine, urea, ALT and AST) for control, molas, starch, and flour groups are presented in Table 3. Total protein was significantly varied in the blood of fish fed on different carbon sources compared to the control. The highest level of total protein was recorded in the blood of fish fed on flour compare to the other treatments (Table 3). Glucose was significantly higher in the blood of fish from the treated groups compared to the control. The highest level of glucose was recorded in the blood of fish fed on flour after 45 days of exposure and in the blood of fish fed on molas followed by flour at the end of the experiment (Table 3). Nearly in the same trend the cholesterol level significantly increased in the treated groups compared to the control one. The highest cholesterol level was observed in the blood of fish fed on flour after 45 and 90 days of exposure (Table 3). The present results indicated a remarkable increase in the level of triglyceride, recording the highest level in the blood of fish fed on flour at the end of the experiment. Higher levels of creatinine were recorded in the treated groups compared to the control. The highest creatinine value was recorded in the blood of fish fed on flour at the end of the experiment (Table 3). In term of urea, a significant increase was recorded in the blood of the treated groups compared to the control with the highest level in the blood of fish fed on flour after 45 and 90 days of exposure (Table 3). The

results of the present work indicated that remarkable increases were observed in terms of ALT and AST in the blood of fish fed on different carbon sources compared to the control. Such increase in ALT value was highly significant in the blood of fish fed on flour after 45 days and at the end of the experiment (Table 3). In case of AST level, the highest values were recorded in the blood of fish fed on flour after 45 days of exposure and the blood of fish fed on starch after 90 days of exposure (Table 3).

Table 3

Biochemical parameters (mean ± Standard Deviation) of grass carp under different carbon sources for 45 and 90 days

Treatment	*45 Days*				*90 Days*			
	Control	*Molas*	*Starch*	*Flour*	*Control*	*Molas*	*Starch*	*Flour*
TP (mg/dl)	5.16±0.5^{bc}	4.0±0.4^{ab}	2.8±0.5^{a}	6.5±1.3^{c}	4.7±1.1^{ab}	6.1±1.0^{bc}	3.9±0.3^{a}	7.3±1.2^{c}
GL(mg/dl)	1.15±2.5^{a}	1.16±6.1^{a}	1.61±11.0^{b}	1.87±17.0^{c}	1.6±7.0^{a}	3.2±11.1^{c}	1.6±25.8^{a}	2.2±5.5^{b}
Chlo (mg/dl)	1.78±3.2^{a}	1.47±16.1^{a}	2.28±20.9^{b}	2.31±30.5^{b}	1.79±3.6^{a}	2.4±23.4^{b}	2.7±11.6^{b}	3.0±9.5^{c}
Tg(mg/dl)	1.6±2.5^{a}	1.1±3.0^{a}	2.31±62.8^{b}	1.65±11.6^{a}	1.7±6.0^{a}	1.8±5.0^{a}	1.8±6.5^{a}	2.0±20.2^{b}
Crt (mg/dl)	0.98±0.3^{a}	0.83±0.25^{a}	1.56±0.66a^{b}	2.0±0.09^{b}	1.06±0.3^{a}	1.4±0.3^{a}	0.9±0.2^{a}	2.7±0.3^{b}
Ur (mg/dl)	24.6±3.0^{a}	31.0±2.6^{b}	21.0±1.00^{a}	39.0±3.6^{c}	31.0±2.6^{a}	39.3±3.0^{b}	49.6±1.5^{d}	44.0±2.00^{c}
ALT(U/L)	38.3±3.0^{a}	41.6±8.6^{a}	48.0±5.0^{a}	73.3±6.8^{b}	53.3±4.5^{a}	61.6±4.7^{ab}	61.6±4.07^{ab}	65.3±8.7^{a}
AST(U/L)	50.6±2.08^{ab}	45.3±4.0^{a}	53.0±3.0^{b}	60.0±3.6^{c}	51.3±3.2^{a}	64.6±8.7^{b}	66.3±3.5^{b}	63.3±8.2^{ab}

Means in the same row with different superscript letters are significantly different ($P<0.05$).
Legend: Total protein (TP); glucose (GL); cholesterol (Chol); triglyceride (Tg); creatinine (Crt); urea (Ur); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST).

Discussion

Water quality. Water quality parameters are very important for maintaining the health of aquatic species, acting as a limiting factor (Khanjani et al 2020, 2021). Biofloc technology improves water quality by the addition of extra carbon, encouraging nitrogen uptake by bacterial growth and consequently, decreasing the ammonium concentration to simpler compounds more rapidly than nitrification (Kumar et al 2019). This process improves the water quality and reduces the need to use a large volume of additional water in the pond. In this study, water physicochemical parameters including temperature, pH, DO, and Ammonia were in the suitable range. Temperature and PH were recorded to be non-significantly different in the treated groups compared to the control one. Both variables were within the appropriate range of floc formation and carp cultivation. Similar results were recorded for *Oreochromis niloticus* reared at different stocking densities compared to the control fish in biofloc systems, (28.5± 0.53, 7.8± 0.33) respectively (Hwihy et al 2021). The best temperature for biofloc was between 18 ºC to 20 ºC (De Schryver & Verstraete 2009). Therefore, the present study indicates that the water temperature values in all treatments were higher than the optimum level for biofloc, however, it still can be tolerated by the cultured carp. Dissolved oxygen is an important parameter for the identification of different water masses (Ibrahim & Ramzy 2013; Osman et al 2021). In the present work, DO was significantly higher in the three selected carbon sources compared to the control. This result was supported by Thilakan et al (2019), who recorded DO within the range of 6-7.8 mg L^{-1} during the experimental period. Hwihy et al (2021) reported a reduction in the level of DO in the Biofloc compared to the control.

Microorganisms associated with biofloc reduce nitrogen-containing compounds, especially toxic forms (ammonia), maintain water quality, and ingest foods (Suita et al. 2015; Wang et al. 2016). In the present study, the level of ammonia was significantly lower in all the treated groups compared to the control. The group fed on molas exhibited the lower level followed by starch and then flour group. The faster reduction of ammonia that recorded in the molas and starch groups as simple carbon sources is probably due to better absorption and degradation of carbon as a substrate for heterotrophic bacteria that

metabolize ammonia and thus improve water quality (Khanjani et al 2017; El- Shafiey et al 2018; Khanjani et al 2021). Still, all the selected carbohydrate sources were effective in bio flocculation resulting in a significant increase in total heterotrophic bacteria, which is consistent with the results of Khanjani et al (2021). The organic carbon source in the biofloc system reduces the total ammonia level and increases the diversity of microbial communities and ammonia-oxidizing bacteria as well (Deng et al 2018), which is consistent with this research. In aquaculture, blood characteristics are commonly used as an indicator of the nutritional status of fishes (Rijal et al 2020). Hematological parameters are sensitive and reliable indicators for monitoring the physiological status of fishes (Kim and Kang 2017). According to Blaxhall & Daisley (1973), the establishment of normal ranges of physiological parameters in healthy, diseased, and various stress conditions is important for some diagnoses, as these situations influence these parameters. Thus, a comparison of hematological results in the same experimental conditions is more appropriate. As well as, the literatures regarding the effect of bioflo aquaculture on the hematology of fish species are very scaring especially for carps. Complete blood cell count (CBC) is an important component of a minimum database, which can be used to assess the health status of fish in response to the water quality changes. Blood variables, such as hemoglobin and hematocrit, are critical parameters for assessing fish metabolism connected to the external environment via the circulatory system (Kim & Kang 2016). In this study, biofloc, had no apparent effect on RBC, Hb, Hct, MCV, MCH and MCHC, indicating that the biofloc system in this study had no negative effect on the physical conditions of the grass carp. The same result was recorded by Bakhsh et al 2018 for common carp fingerlings reared in biofloc system. Similarly, Azim & Little (2008) reported that blood Hct of Nile tilapia did not vary statistically between the biofloc groups and the control one. The same trend was also observed in other studies (e.g., Long et al 2015; Xu & Pan 2014). Furthermore, the total hemocyte count of shrimp did not vary significantly between biofloc-groups and control group (Xu & Pan 2014). In contrast, Xu & Pan (2013) found that the total hemocyte count in shrimp was significantly higher in the biofloc groups than in the control group. It seems that different experimental conditions and culture species may account for this phenomenon.

In the present study, white blood cells count (WBCs) and platelets values increase significantly in all the biofloc groups compared to the control. These results were partially in agreement with Azim & Little, (2008) & Long et al (2015). The highest level of WBCs was recorded in the blood of fish fed on flour and molas after 45 and 90 days of exposure, respectively. Such increase in the White blood cell count could be used as an indicator of an increase in the fish's immune system (Adel et al 2016). In the same trend, the highest platelets level was recorded in the blood of fish fed on flour.

Blood biochemistry indices are useful tools that aid in understanding the pathological process, the general state of fish health which can differ with water characteristics and nutritional state (Dawood et al 2015; El Basuini et al 2017). Overall, blood biochemical values recorded in the present study were within the acceptable limits of the grass carp. These results were supported by Ayyat et al (2017) and Mahmoud & El-Hais (2017). The results of biochemical variables (total protein, glucose, cholesterol, triglycerides, Creatinine, Urea, ALT and AST) exhibited significant increase in the blood of fish fed on different carbon sources compared to the control. Verma et al (2016) recorded a significant difference in the level of total proteins and albumin of *Labeo rohita* reared at Tapioca based biofloc system and a non-significant difference in the blood of *Labeo rohita* reared in wheat, corn, sugar bagasse based Biofloc system. In contrast, Bakhsh et al (2018); Long et al (2015) and Metwalli (2013) recorded non-significant differences in the biochemical variables between the treated biofloc groups and the courol one. According to the present results, the highest level of protein 7.3±1.2, glucose 2.2±5.5, cholesterol 3.0±9.5, triglycerides 2.0±20.2, creatinine 2.7±0.3, urea 44.0±2.00, ALT 65.3±8.7, and AST 63.3±8.2 were recorded in the blood of fish fed flour followed by molas. The present authors suggested that the differences between current results and Verma et al (2016) may be due to the different kinds of carbohydrate

sources, fish's species, and environmental conditions. The recorded increase in the level of total protein can increase non-specific immune responses.

Conclusions. The present study highlights the importance of different organic carbon sources in improving the biofloc systems, nitrogen dynamics, blood parameters, and water quality of grass carp. Sources of carbohydrates (molas-starch-flour) based biofloc had beneficial effects on haemato-biochemical responses in grass carp. Furthermore, according to the blood biochemistry results, feed supplemented with different carbon sources especially flour and molas can increase fish health. It can be concluded that fish farming using biofloc system and feed supplemented with different carbon sources is very beneficial for fish farmers because it can reduce feed cost, toxicity, and improve the immunity of the fish.

Conflict of interests. The authors declare no conflict of interest.

Acknowledgement. We express our appreciation to Dr. Amr F. Zeina, Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt, for his help during the experimental design.

References

Abdel-Tawwab M., Ahmad M. H., Khattab, Y. A., Shalaby A. M., 2010 Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture 298(3-4):267-274.

Adel M., Yeganeh S., Dadar M., Sakai M., Dawood M. A., 2016 Effects of dietary Spirulina platensis on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso Linnaeus*, 1754). Fish & Shellfish Immunology 56:436-444.

Animashahun R. A., Omoikhoje S. O., Bamgbose A. M., 2006 Haematological and biochemical indices of weaner rabbits fed concentrates and Syndrella nodiflora forage supplement. Proc. 11th Annual Conference of Animal Science Association of Nigeria. Institute of Agricultural Research and training Nigeria, pp. 29-32.

Avnimelech Y., 2006 Bio-filters: the need for a new comprehensive approach. Aquacultural engineering 34(3):172-178.

Avnimelech Y., 2009 Biofloc technology: a practical guidebook. World Aquaculture Society.

Ayyat M. S., Mahmoud H. K., El-Hais A. E. A. M., Abd El-Latif K. M., 2017 The role of some feed additives in fish fed on diets contaminated with cadmium. Environmental Science and Pollution Research 24(30):23636-23645.

Azim M. E., Little D. C., 2008 The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283(1-4):29-35.

Badrey A. A., Osman A. G., Farrag, M.S., Toutou, M. M., Moustafa, A. M., 2019 Influences of diets supplemented with pomegranate peel on haematology, blood biochemistry and immune status in monosex Nile tilapia, *Oreochromis niloticus*. Egyptian Journal of Aquatic Biology and Fisheries 23(2):133-144.

Bakhsh E. M., Khan S. A., Marwani H. M., Danish E. Y., Asiri A. M., Khan S. B., 2018 Performance of cellulose acetate-ferric oxide nanocomposite supported metal catalysts toward the reduction of environmental pollutants. International journal of biological macromolecules 107:668-677.

Bañuelos-Vargas I., de Oca G. A. R. M., Martínez-Montaño E., Pérez-Jiménez A., Mendoza-Gamboa O. A., Estrada-Godínez J. A., Hernández C., 2021 Antioxidant and immune response of juvenile red tilapia (*Oreochromis sp*) cultured at different densities in sea water with biofloc plus probiotics. Aquaculture 544:737112.

Bardach J. E., 1985 The role of aquaculture in human nutrition. Geojournal 10(3):221-232.

Blaxhall P. C., Daisley K. W., 1973 Routine haematological methods for use with fish blood. Journal of fish biology 5(6):771-781.
Castro-Nieto L. M., Castro-Barrera T., De Lara-Andrade R., Castro-Mejía J., Castro-Mejía G., 2012 Biofloc systems: a technological breakthrough in aquaculture. El Hombre y su Ambiente 1(1):1-5.
Crab R., Avnimelech Y., Defoirdt T., Bossier P., Verstraete W., 2007 Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270(1-4):1-14.
Dacie S. J. V., Lewis S. M., 2002 Practical haematology 11th edition. UK, Churchill Livingstone.
Dawood M. A., Koshio S., Ishikawa M., Yokoyama S., 2015 Interaction effects of dietary supplementation of heat-killed Lactobacillus plantarum and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, Pagrus major. Fish & shellfish immunology 45(1):33-42.
De Schryver P., Crab R., Defoirdt T., Boon N., Verstraete W., 2008 The basics of bio-flocs technology:the added value for aquaculture. Aquaculture 277(3-4):125-137.
De Schryver P., Verstraete W., 2009 Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. Bioresource technology 100(3):1162-1167.
Deng M., Chen J., Gou J., Ho J., Li D., He X., 2018 The effect of different carbon sources on water quality, microbial community and structure of biofloc systems. Aquaculture 482:103-110.
El Basuini M. F., El-Hais A. M., Dawood M. A. O., Abou-Zeid A. S., El-Damrawy S. Z., Khalafalla M. S., Dossou S. J. A. N., 2017 Effects of dietary copper nanoparticles and vitamin C supplementations on growth performance, immune response and stress resistance of red sea bream, Pagrus major. Aquaculture Nutrition 23(6):1329-1340.
El-Sayed A. F. M., 2006 Tilapia Culture. CABI Publishing, CAB Internnational, Wallingford, Oxfordshire, UK, 277 pp.
El-Shafiey M. H. M., Mabroke R. S., Mola H. R. A., Hassaan M. S., Suloma A., 2018 Assessing the suitability of different carbon sources for Nile tilapia, *Oreochromis niloticus* culture in BFT system. AACL Bioflux 11(3):782-795.
Friedewald W. T., Levy R. I., Fredrickson D. S., 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18:499-502.
Gao L., Shan H.-W., Zhang T.-W., Bao W.-Y., Ma S., 2012 Effects of carbohydrate addition on Litopenaeus vannamei intensive culture in a zero-water exchange system. Aquaculture 342–343:89–96.
Habte-Tsion H. M., Liu B., Ge X., Xie J., Xu P., Ren M., Chen R., 2013 Effects of dietary protein level on growth performance, muscle composition, blood composition, and digestive enzyme activity of Wuchang bream (*Megalobrama amblycephala*) fry. The Israeli Journal of Aquaculture-Bamidgeh 65, 9p.
Hargreaves J. A., 2006 Photosynthetic suspended-growth systems in aquaculture. Aquacultural engineering 34(3):344-363.
Hargreaves J. A., 2013 Biofloc production systems for aquaculture, Southern Regional Aquaculture Center (SRAC). Publication no 4503, pp. 1-11. Stoneville.
Henry R. J., 1964 Clinical chemistry, New York and Row Publish.
Hwihy H., Zeina A., Abu Husien M., El-Damhougy K., 2021 Impact of Biofloc technology on growth performance and biochemical parameters of Oreochromis niloticus. Egyptian Journal of Aquatic Biology and Fisheries 25(1):761-774.
Ibrahim L. A., Ramzy E. M., 2013 Water quality and its impact on Tilapia zilli (case study) Qarun Lake-Egypt. International Water Technology Journal 3(4):170-191.
Ighwela K. A., Ahmad A. B., Abol-Munafi A. B., 2012 Haematological changes in nile tilapia (*Oreochromis niloticus*) fed with varying dietary maltose levels. World Journal of Fish and Marine Sciences 4(4):376-381.
Khanjani M. H., Alizadeh M., Sharifinia M., 2020 Rearing of the Pacific white shrimp, *Litopenaeus vannamei* in a biofloc system:The effects of different food sources and salinity levels. Aquaculture Nutrition 26(2):328-337.

Khanjani M. H., Alizadeh M., Sharifinia M., 2021 Effects of different carbon sources on water quality, biofloc quality, and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings in a heterotrophic culture system. Aquaculture International 29(1):307-321.

Khanjani M.H., Sajjadi M.M., Alizadeh M., Sourinejad I., 2017 Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system:the effect of adding different carbon sources. Aquaculture Research 48:1491-1501. DOI:10.1111/are.12985.

Kim J.H., Kang J. C., 2016 The toxic effects on the stress and immune responses in juvenile rockfish, Sebastes schlegelii exposed to hexavalent chromium. environmental toxicology and pharmacology 43:128e133.

Kim J.H., Kang J.C., 2017 Toxic effects on bioaccumulation and hematological parameters of juvenile rockfish Sebastes schlegelii exposed to dietary lead (Pb) and ascorbic acid. Chemosphere 176:131e140.

Kondera E., Kościuszko A., Dmowska A., Witeska M., 2017 Haematological and haematopoietic effects of feeding different diets and starvation in common carp *Cyprinus carpio* L. Journal of Applied Animal Research 45(1):623-628.

Kumar A., Reddy A., Rani A., Rathore G., Lakra W., Jayant M., 2019 Water quality and nutrient dynamics of biofloc with different C/N ratios in inland saline water. Indian journal of animal research Oct 15;9(5):783–791. 10.30954/2277-940X.05.2019.23

Little D.C., Murray J.F., Azim M.E., Leschen W., Grady K., Young J., Watterson A., 2008 Warm-water fish production in the UK:limits to green growth? Trends in Food Science and Technology 19:255–264.

Long L., Yang J., Li Y., Guan C., Wu F., 2015 Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture 448:135-141.

Mahmoud H. K., El-Hais A., 2017 Effect of dietary garlic (allium sativum) supplementation on Nile tilapia *Oreochromis niloticus* juveniles' performance under two stocking denisties. Egyptian Journal of Nutrition and Feeds 20(1):115-124.

Martínez-Córdova L. R., Emerenciano M., Miranda-Baeza A., Martínez-Porchas M. 2015 Microbial-based systems for aquaculture of fish and shrimp: an updated review. Reviews in Aquaculture 7(2):131-148.

Maxwell M. H., Robertson G. W., Spence S., McCorquodale C. C., 1990 Comparison of haematological values in restricted-and ad libitum-fed domestic fowls:White blood cells and thrombocytes. British Poultry Science 31(2):399-405.

Metwalli A., 2013 Effects of partial and total substitution of fish meal with corn gluten meal on growth performance, nutrients utilization and some blood constituents of the Nile tilapia *Oreochromis niloticus*. Egyptian Journal of Aquatic Biology and Fisheries 17(1):91-100.

Osman A. G. M., Abd El Reheema A.-E.-B. M., Moustafa M. A., Mahmoud U. M., 2011 In situ evaluation of the genotoxic potential of the river Nile:I. Micronucleus and nuclear lesion tests of erythrocytes of Oreochromis niloticus niloticus (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). Toxicological and Environmental Chemistry 93:1002-1017.

Osman A. G. M., Farrag M. M. S., Badrey A. E. A., Khedr Z. M. A., Kloas W., 2021 Water quality and health status of monosex Nile Tilapia, *Oreochromis niloticus* cultured in aquaponics system (ASTAF-PRO) Egyptian Journal of Aquatic Biology & Fisheries 25(2):785–802.

Osman A. G. M., Gadel-Rab A. G. Mahmoud F. A., Hamed H. S., Elshehaby M. M., Ali A. E., Kloas W., 2019 Blood Characteristics and Tissue Histology of Nile Tilapia (*Oreochromis Niloticus Niloticus*) Fed A Diet Containing Cheese Skipper (*Piophila Casei*) Larvae. Scholarly Journal of Food and Nutrition 25(3):219-228.

Perschbacher P. W., 2015 The future of fish as food. Journal of Fisheries & Livestock Production 3 doi:10.4172/2332-2608.1000143. Article 3.

Reitman S., 1957 Colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvate transaminases. Journal clinical pathology 28:56-63.
Rijal R., Cadena L. A., Smith M. R., Carr J. F., Gomer R. H., 2020 Polyphosphate is an extracellular signal that can facilitate bacterial survival in eukaryotic cells. Proceedings of the National Academy of Sciences 117(50):31923-31934.
Solomon S. G., Okomoda V. T., 2012 Growth performance of *Oreochromis niloticus* fed duckweed (*Lemna minor*) based diets in outdoor hapas. International Journal of Research in Fisheries and Aquaculture 2 (4):61-65.
SPSS. (1997). Statistical package for the social sciences, Versions16, SPSS in Ch, Chi-USA.
Suita S. M., Ballester E. L., Abreu P. C., Wasielesky Jr. W., 2015 Dextrose as carbon source in the culture of *Litopenaeus vannamei* (Boone, 1931) in a zero-exchange system. Latin American Journal of Aquatic Research 43(3):526-533.
Thilakan A. P., Kumar K., Shukla S. P., Kumar S., Saharan N., Karmakar S., 2019 Occurrences of triclosan in Versova creek of Mumbai, India and its toxicity on selected aquatic organisms. Journal of Experimental Zoology, India 22:737-742.
Thomas K. W., 1992 Conflict and conflict management:Reflections and update. Journal of Organizational Behavior 13:265-274.
Trinder P., 1969 Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Clinical and biochemistry 6:24-27.
Verma V., Ravindran P., Kumar P. P., 2016 Plant hormone-mediated regulation of stress responses. BMC plant biology 16(1):1-10.
Walker T. R., Adebambo O., Del Aguila Feijoo M. C., Elhaimer E., Hossain T., Edwards S. J., Morrison C. E., Romo J., Sharma N., Taylor S., Zomorodi S., 2019 Chapter 27:Environmental effects of marine transportation. In:Sheppard, C. (Ed.), World Seas:An environmental evaluation, 2nd edn. Academic Press, pp. 505-530.
Wang C., Pan L., Zhang K., Xu W., Zhao D., Mei L., 2016 Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. Aquaculture research 47 (10):3307-3318.
Xu W. J., Pan L. Q., 2013 Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture 412:117-124.
Xu W. J., Pan L. Q., 2014 Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. Aquaculture 426:181-188.
Yassien M. H., Ashry O. A., Mohamed M. A., 2021 Effect of bioflocs on growth performance and survival of the white-leg shrimp *Litopenaeus vannamei* raised in zero-water exchange culture tanks. Egyptian Journal of Aquatic Biology & Fisheries 25(2):645-657.
Zaki M. A., Alabssawy A. N., Nour A. E., El Basuini M. F., Dawood M. A., Alkahtani S., Abdel-Daim M. M., 2020 The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. Aquaculture Reports 16:100282.
Zhifei Li., Guangjun W., Ermeng Yu., Kai Z., Deguang Y., Wangbao G., Jun Xie., 2019 Artificial substrata increase pond farming density of grass carp (*Ctenopharyngodon idella*) by increasing the bacteria that participate in nitrogen and phosphorus cycles in pond water. Peer j DOI 10.7717/peerj.7906.

Received: 09 October 2021. Accepted: 01 November 2021. Published online: 04 November 2021.
Authors:
Ayat M. Awad, Post graduate from the Faculty of Science, Al-Azhar University (Assiut), Assiut, Egypt, e-mail: Ayatmoubark37@gmail.com
Ahmed E. A. Badrey, Department of Zoology, Faculty of Science, Al-Azhar University, 71524 Assiut, Egypt, e-mail: gmal_ahmed77@yahoo.com
Alaa G. M. Osman, Department of Zoology, Faculty of Science, Al-Azhar University, 71524 Assiut, Egypt, e-mail: agosman2@yahoo.com.
Khaled Y. AbouelFadl, Faculty of Fish and Fisheries Technology, Aswan University, e-mail: Khaled_almasry@yahoo.com

How to cite this article:
Awad A. M., Badrey A. E. A., AbouelFadl K. Y., Osman A. G. M., 2021 The impact of dietary carbon sources on the blood characteristics of grass carp (*Ctenopharyngodon idella*) cultured in biofloc system. AACL Bioflux 14(6):3178-3188.