Journal of Agriculture and Rural Development in the Tropics and Subtropics
Vol. 122 No. 1 (2021) 137–145

https://doi:10.17170/kobra-202105253967 ISSN: 2363-6033 (online); 1612-9830 (print) – website: www.jarts.info

Growth performance and immunological response of African Catfish (*Clarias gariepinus*) juveniles reared in biofloc system

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Abstract

This study was conducted to examine the growth and immune performance of catfish (*Clarias gariepinus*) juveniles cultured in a biofloc fish farming system. To achieve this, 900 *C. gariepinus* juveniles (9.0 ± 0.23 g) were cultured in an intensive zero water exchange biofloc system for 72 days in 9 separate tanks (2 m^3 each) aerated by an air blower, after which growth assessment of fish was conducted. The trial consisted of three treatments in triplicates, a control without carbon source addition, and two biofloc treatments with carbon source addition of rice bran or cassava flour. The two carbon sources added had a C/N rate of 15:1 to form the floc. The results showed no significant difference between dissolved oxygen (DO), pH, and temperature in all the treatments, however, conductivity, total dissolved solids, and salinity showed significant differences between the bioflocs and control treatments and the treatment group. Nevertheless, the obtained values for the water parameters were within the range required for culturing *C. gariepinus*. Survival rate of catfish was significantly higher in the biofloc culture with a cassava carbon source (98.3 %) compared with the control experiment (64.3 %). Weight gain of the fish was highest in rice bran based biofloc (44.9 ± 3.00 g) and lowest (37.0 ± 4.15 g) in the control treatment. The non-specific immune assay revealed that monocytes, serum lysozyme, and myeloperoxidase were higher in the biofloc treated groups compared to the control group. In contrast, neutrophils' percentage was lower in the treated groups than in the control group. Therefore, this study demonstrated the suitability of biofloc as an aquaculture wastewater purifier, growth-promoting, and immune-enhancing technology for the small-scale culture of *C. gariepinus*.

Keywords: Aquaculture, food production, immune system, lysozyme, myeloperoxidase

1 Introduction

Recently, aquaculture has rapidly expanded to overcome the dependence on fishery resources from the wild, and currently accounts for more than 50 % of total fisheries production (FAO, 2016). The aquaculture industry is a substantial global industry supplying a significant proportion of the aquatic food consumed and other aquatic products that are valuable sources of protein and essential nutrient components for global food security (FAO, 2018). To support the rapidly growing human population globally, food production industries such as aquaculture require an approach toward improving the output with minimal cost.

Biofloc technology is an emerging avenue in aquatic animal healthcare and nutrition not only horizontal but as well as vertical expansion (Daniel & Nageswari, 2017). The rapid growth of global aquaculture is faced with environmental and economic imitations. The intensification of aquaculture activities generates an immense amount of excess waste such as suspended solids, total nitrogen and total phosphorus (Twarowska *et al.*, 1997). With the high cost (price) of purchasing and installing Recirculatory Aquaculture Systems (RAS) a technology for farming fish by reusing the water in the production involving the use of mechanical and biological filters and the operational cost in terms of energy requirements, the small holders farming communities cannot afford to utilise RAS. It is, therefore, necessary to look for a low cost, sustainable, and environment-friendly technology with large-scale adoption. The use of biofloc has been adopted as a sustainable and eco-friendly system of aquaculture that controls water quality, together with the production of value-added microbial proteinaceous feed for the aquatic

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Published online: 8 June 2021 – Received: 4 December 2020 – Accepted: 15 May 2021

organisms (Ahmad *et al*., 2017). The use of Biofloc technology (BFT) systems in aquaculture has been extensively studied in shrimp culture (De Souza *et al*., 2014; Kumar *et al*., 2014) and also in Tilapia aquaculture (Menaga *et al*., 2019). This technology is cost-effective and environment-friendly and supports sustainable aquaculture (Naylor *et al*., 2000; Avnimelech & Kochba 2009).

BFT is an aquaculture system involving the efficient use of nutrient input with limited or zero water exchange. Its main principle is to recycle nutrients by maintaining a high carbon/nitrogen (C:N) ratio in the water to stimulate heterotrophic bacterial (group of microbes that use organic carbon as food) growth that converts ammonia into microbial biomass (Avnimelech, 1999). The microbial biomass further aggregates with other microorganisms and particles suspended in the water forming what has been called "biofloc", which eventually can be consumed *in situ* by the cultured animals or harvested and processed as a feed ingredient in fish feed (Avnimelech, 1999; Crab *et al*., 2007; De Schryver *et al*., 2008; Kuhn *et al*., 2009; 2010).

The carbon to nitrogen balance is enhanced through the addition of an extra carbon source to the system thus aiding the conversion of nitrogen supplied by uneaten food and faeces of the cultured organism, which together with the addition of organic carbon sources is converted to microbial protein (Crab *et al*., 2012). By adjusting the carbon-to-nitrogen ratio (C:N) in the culture water through carbohydrate supplementation, the ability of some heterotrophic bacteria to assimilate the inorganic nitrogen is promoted thus leading to an abundance of these bacteria.

African catfish, *Clarias gariepinus*, of the Clariid genus is known as the special freshwater fish in Nigeria and the West African sub-region of high economic value and delicacy. Because of its rapid growth rate, high production, and good disease resistance capability, *C. gariepinus* has become an important aquaculture species in Nigeria. There have been several investigations on the applications of biofloc technology to *C. gariepinus* culture, and these have mostly been positive (Ekasari *et al*., 2016; Dauda *et al*., 2018; Romano *et al*., 2018).

Romano *et al*. (2018) found that using rice bran led to lower growth of *C. gariepinus* juveniles, likely due to the rice bran acting as an irritant and having a low water solubility. However, fermenting rice bran substantially improved the solubility and, in turn, water quality and *C. gariepinus* growth (Romano *et al*., 2018).

The use of cassava starch as a biofloculating agent in the rearing of tilapia fish has been reported by Nootong, *et al*. (2011). However, there is a dearth of information on the use of cassava flour and rice bran as carbon sources when culturing *C. gariepinus* with biofloc technology. The present study was designed to evaluate the effect of rice bran and cassava flour induced biofloc on growth, survival and immune system of *C. gariepinus* juveniles.

2 Materials and Methods

2.1 Experimental setup

A single factor analysis (CRD) experimental design with three treatments in triplicate was used. The treatments consisted of biofloc systems using two easily and locally available carbohydrate sources viz, cassava flour (*Manihot esculenta*) and rice bran (*Oryza sativa*).

2.2 Biofloc preparation

Raw rice bran was obtained from a livestock feed ingredient store in Akure, Nigeria, then hammer milled to powdery form (425 μm), while cassava flour was procured from a cassava processing plant in Akure. Biofloc system was prepared by adding 2 mg of ammonium chloride (NH_4Cl) to 1 litre of water for 7 days as a source of nitrogen preceding the experiment. The rice bran and cassava flour were incubated with commercial *Bacillus megaterium* (15 L of water obtained from tilapia culture system) with aeration for pretreatments to initiate biofloc production.

2.3 Fish rearing

Nine units of outdoor rectangular concrete tanks (2 m^3 each) at the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure Nigeria, were assigned for this experiment. Before the experiment, tanks were prepared (cleaned, dried) and filled with fresh water to a volume of 1.10 m^3 (0.55 m water depth x 2 m length x 1 m breadth).

The organic carbon source in the biofloc system was added daily at a dose depending on the estimated total feed nitrogen added to the culture tank on the previous day and the expected C/N ratio in the water as described by Avnimelech (1999) and De Schryver *et al*. (2008). A pre-weighed carbon source was mixed in a glass beaker with the water collected from the corresponding culture tanks and was added and poured directly into the water column after first feeding (Avnimelech, 1999). Ten thousand individuals of *C. gariepinus* (9.0 ± 0.23 g), purchased from a local hatchery in Akure, Nigeria, acclimated to laboratory condition for 14 days before experimentation, were placed in each tank. The fishes were fed twice daily at 5 % body weight (08.00 and 17.00 hours) with a locally available commercial fish feed (40 % crude protein). Aeration was supplied by an air

blower installed at 9 lines (5 l/min per line). Clarias gariepinus juveniles were randomly and equally distributed into 9 culture tanks (1000 juveniles/tank) (2 m^3 each) in three experimental groups: control (without carbon addition), biofloc (with rice bran as carbon source) and biofloc (with cassava flour as carbon source) systems in triplicate following a completely randomized design. The carbon-nitrogen ratio in the rearing media was determined and calculated as follows: C = weight of the cassava flour for biofloculation × % dry matter of the cassava flour × 70 % of waste that remains in water)/2 (carbon content of the feed on dry matter) N = weight of cassava flour for biofloculation × % dry matter of the cassava flour × 70 % of waste that remains in water) × % crude protein content of feed)/6.25 (constant) The C:N ratio was adjusted by multiplying the C and N in the biofloculating agents to get a 15:1 C:N ratio. This also was done for rice bran This experiment was conducted for 72 days between 13th June and 24th August 2018.

2.4 Water quality maintenance

To maintain water quality, in the control experiment, a consistent water exchange at 30 % of the total volume was done every 7 days, however, in both biofloc systems, water was only supplemented to replace loss due to brick absorption or evaporation. Temperature, pH, and dissolved oxygen (DO) concentration were measured daily by using thermometer, pH meter, and DO meter respectively. Specific conductivity, salinity, and total dissolved solids (TDS) were measured using the EXTECH instrument (ExStik ii model) according to the standard method for the examination of water and wastewater. However, total ammonia nitrogen (TAN) was measured weekly in line with the standard procedure described in the standard for water and wastewater quality analyses (APHA, 1998). Analyses of the nitrite (NO_2-N) and nitrate (NO_3-N) concentrations were performed every 5 days as described by Strickland & Parsons (1972).

2.5 Zootechnical parameters

After 72 days of rearing, the fish were harvested by draining the tanks and were anesthetized using clove oil at 50 μl per litre of water for weighing and counting purposes (Popoola, 2016). Fishes were counted and measured to determine the final body length and various growth parameters. The effects of biofloc on fish growth were determined by evaluating final weight, weight gain, specific growth rate, and percent survival. The calculated indices for the growth were as follows:

$$WG = FW - IW$$

$$SGR = \left[\frac{(Ln\ FW\ -\ Ln\ IW)}{culture\ days}\right] \times 100$$

$$SR = \left[\frac{\text{Final number of fish}}{\text{Initial number of fish}}\right] \times 100$$

where:
WG = weight gain (g);
IW = initial weight (g);
FW = final weight (g);
SGR = specific growth rate expressed as % body weight gain per day;
SR = survival rate.

2.6 Immunological analyses

Blood and serum samples were collected for immunological assays from the fish (10 fishes per treatment) using a 1.0 ml hypodermal syringe and 24-gauge needles and rinsed with 2.7 % Ethylene Diamine tetra acetic acid (EDTA) solution before use. The collected blood was immediately transferred to the test tube coated with a thin layer of EDTA (as an anticoagulant) and shaken properly to prevent clotting. Serum collection was done without the use of anticoagulant and separated from blood by keeping the tubes in slanting position for about 2 hours and centrifuged at 1370 g for 15 min at 4 °C, followed by the collection of straw-coloured serum with a micropipette and stored at 20 °C for further analysis. Total myeloperoxidase content present in serum was measured according to Quade & Roth (1997). Serum lysozyme activity was measured using spectrophotometric assay by (Anderson and Siwicki, 1995). Other Haematological values were measured by following standard methods as described by Terry *et al*. (2000).

3 Results

3.1 Zootechnical studies

The growth performance result of *C. gariepinus* raised in biofloc and control tanks showed that there were no significant variations ($p > 0.05$) in the initial weight of stocked fishes, hence fishes stocked were of similar weight (Table 1). Statistical evaluation of growth data obtained from this experiment showed that there was a significant difference in the growth parameters obtained. This study consistently recorded higher weight gain, specific growth rate and percentage weight gain in *C. gariepinus* raised in biofloc culture with rice bran as carbon source. However, *C. gariepinus* juveniles raised in biofloc with both carbon sources had higher growth indices than those raised in control tanks. The survival rate was highest (98.30 %) in the biofloc culture with

Table 1: *Growth indices for Clarias gariepinus raised in biofloc systems.*

Parameters	*BC*	*BR*	*C*
IW (g)	9.25 ± 1.008^a	9.23 ± 0.935^a	9.19 ± 0.278^a
FW (g)	52.56 ± 7.411^a	54.12 ± 3.323^a	45.18 ± 5.943^b
WG (g)	43.31 ± 7.137^a	44.89 ± 3.001^a	36.98 ± 4.152^b
PWI (%)	470.65 ± 80.438^a	489.08 ± 51.193^a	472.70 ± 123.831^a
SGR (%/day)	0.77 ± 0.120^b	0.80 ± 0.053^a	0.66 ± 0.073^c
Survival (%)	98.30 ± 1.518^a	91.67 ± 10.014^b	64.33 ± 22.028^c

Values are means ± standard error (SE) of three replicate groups (n = 3), Data on the same row with different superscripts are statistically significant ($p < 0.05$). IW = initial weight, FW = final weight, WG = weight gain, PWI = percentage weight increase, SGR = specific growth rate, BC = biofloc cassava, BR = biofloc rice bran, C = control

added cassava flour. Biofloc with rice bran had the highest growth indices except for survival rate.

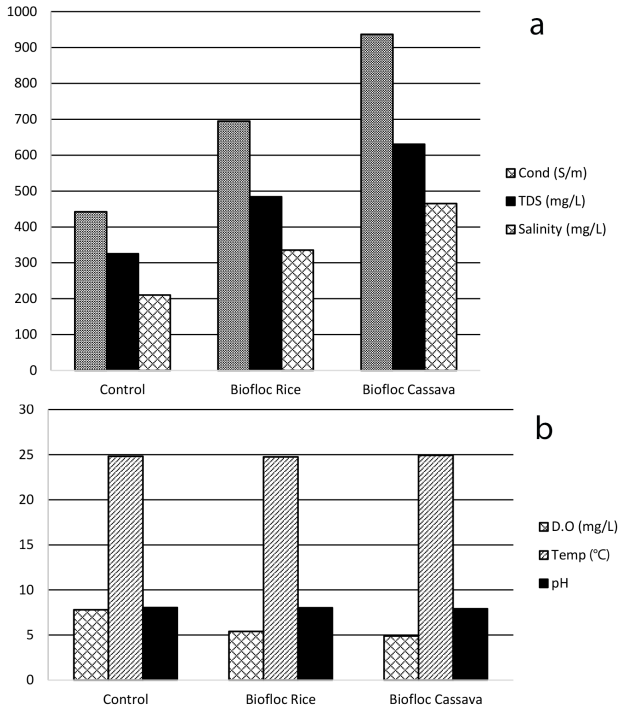

Fig. 1: *Water quality conditions during cultivation of Clarias gariepinus in Biofloc systems (a) Conductivity (Cond.), Total Dissolved Solids (TDS), and Salinity; (b) Dissolved Oxygen (DO), Temperature (Temp.), and pH.*

3.2 Water quality parameters

The water temperature of the different treatments ranged from 24.2 °C to 25.6 °C, however, no significant difference existed between the treatments (Fig. 1b). Water quality parameters at the end of the experimental period revealed considerable variation. The values for dissolved oxygen ranged between 7.74–8.37 mg L^{-1} at the experimental period. Dissolved oxygen showed a significant difference ($p < 0.05$) among experimental groups. The control (7.8 mg L^{-1}) had significantly higher DO than BC (5.40 mg L^{-1}) and BR (4.90 mg L^{-1}) (Fig. 1b). The mean pH ranged from 7.74 to 8.37 (Fig. 1b).

The total dissolved solids (TDS) fluctuated from 283.57 to 871.57 mg L^{-1} in the experimental groups. There is a significant difference between the BC and BR treatments compared to the control. The TDS in rice bran biofloc and cassava flour biofloc were significantly higher than the control (Fig. 1a). The concentration of total ammonia nitrogen (TAN) within the experimental groups varied significantly ($p < 0.05$) with the highest mean values of 0.32 mg L^{-1} in the control, 0.17 mg L^{-1} in BR, and the lowest mean value of 0.11 mg L^{-1} in BC, respectively (Table 2). A similar trend was observed in nitrite values among the Treatment groups. The control group had the highest value of 0.39 mg L^{-1} followed by BR (0.23 mg L^{-1}) and the lowest in BC (0.29 mg L^{-1}). The values recorded for nitrate among the treatments showed that BR had the highest (25.2 mg L^{-1}) and the least (16.18 mg L^{-1}) in the control. The alkalinity values also show the same trend with BR having the highest (125.9 m L^{-1}) and control having the least value (113.7 mg L^{-1}).

3.3 Immunological studies

Data obtained from various immunological parameters are shown in Table 3. Most non-specific immune parameters showed significant differences across treatment groups. Serum lysozyme activity (U/min) was found to be significantly different across treatment groups, with the control having the lowest value of 0.61 U/min. Myeloperoxidase (MPO) activity (OD at 450 nm) showed significant difference across treatments, MPO showed higher value for control compared with biofloc culture. Neutrophils percentage showed a significant difference between biofloc culture and also between biofloc and control. Lymphocytes and neutrophils percentage shows no significant difference across treat-

Table 2: *The average values of total ammonia nitrogen, nitrite, nitrate, and alkalinity concentrations in the respective treatments (biofloc and control).*

	TAN (mg L^{-1})	*Nitrite (mg L^{-1})*	*Nitrate (mg L^{-1})*	*Alkalinity (mg L^{-1})*
Control	0.32 ± 0.01^a	0.39 ± 0.02^a	16.18 ± 1.31^a	113.7 ± 1.62^a
Biofloc Cassava	0.11 ± 0.02^b	0.23 ± 0.02^b	23.03 ± 0.71^b	118.1 ± 2.22^a
Biofloc Rice Bran	0.17 ± 0.01^b	0.29 ± 0.01^b	25.2 ± 1.03^b	125.9 ± 2.03^b

Means ± SD on the same row with different superscripts are statistically significant ($p < 0.05$). TAN: total ammonia nitrogen

Table 3: *Non-specific immune parameters for Clarias gariepinus raised in biofloc systems*

Parameters	*BC*	*BR*	*C*
Neutrophils (%)	55.00 ± 1.73^b	54.56 ± 4.16^a	61.67 ± 1.45^c
Lymphocytes (%)	38.37 ± 2.60^c	39.33 ± 4.81^a	35.33 ± 1.45^b
Serum Lysozyme (U/min)	0.89 ± 0.02^b	0.96 ± 0.01^c	0.61 ± 0.01^a
Myeloperoxidase activity (OD at 450 nm)	0.91 ± 0.01^c	0.83 ± 0.05^b	0.61 ± 0.01^a
Monocytes	1.66 ± 0.57^a	1.50 ± 1.00^a	0.66 ± 0.57^a

Values are means ± SE of three replicate groups (n = 3). Data on the same row with different superscripts are statistically ($p < 0.05$) significant. BC = Biofloc Cassava, BR = Biofloc Rice Bran, C = Control.

ment groups. However, both parameters have higher percentages across both biofloc cultures.

4 Discussion

The improvement in growth afforded by BFT in the present study might be due to the abundance of active heterotrophic bacteria, which can assimilate the waste nitrogen from the uneaten feed and faeces of the reared fish and produce a new cellular protein of a proper amino acid profile for fish consumption (Avnimelech, 2009; Crab *et al*., 2012; Wang *et al*., 2015). The improved growth performance of *C. gariepinus* reared in different biofloc treatments has been observed also by Dauda *et al*. (2018) in Malaysia and Romano *et al*. (2018) in Indonesia. The reason for the improved growth could be due to the synergistic effects of the improved water quality, and higher bacterial and zooplankton densities as suggested by Dauda *et al*., (2018). Studies have indicated that carbohydrate addiction can result in the production and accumulation of biofloc (Avnimelech 2007), which could serve as an important food source for the zooplankton and thus could increase the growth of the *C. gariepinus*.

Overall, the growth performance of African catfish production in a biofloc system was higher than that of the control. This may be an indication that biofloc systems could be applied to enhance the production of African catfish. Also, the nutritive nature of biofloc offered the cultured organisms (*C. gariepinus*) additional feed in the rearing media as compared with the control group. Also, the reduction of ammonium and nitrite level in the biofloc system could probably aid to the improvement of growth performance in *C. gariepinus*. The potential benefits of BFT may be multilayered and can include maintaining water quality as well, which would minimize stress as well as increase biosecurity and potentially reducing the prevalence of diseases. Another factor may be the consumption of the bioactive compounds in the biofloc that leads to an enhanced nutritional status and / or immunity of the fish against diseases (Xu & Pan 2013, Ekasari*et al*., 2014). The survival rate of fish in the biofloc treatments was higher ($p < 0.05$) than for the control. Tseng *et al*. (2009) and Verschuere *et al*. (2000) also noted that biofloc culture was able to increase growth performance and survival rate of *C. gariepinus* fish.

Balancing the concentration of ammonium in the biofloc culture system by adding a carbon source is possible because the heterotrophic bacteria in biofloc can absorb ammonia 40 times faster than nitrifying bacteria found in non-biofloc system (Ebeling *et al*., 2006). The relationship between adding carbon via carbohydrates in biofloc, reducing ammonium, and producing microbial proteins has been reported already by Avnimelech (1999). This relationship depends on the microbial conversion coefficient, the C/N ratio in the microbial biomass, and the carbon content of the added material. Avnimelech (2009) demonstrated that the addition of carbohydrate lessens the need for dietary protein concentration and also decreases the TAN level in the system. The average concentration of TAN observed was higher in the control group (Table 2). However, the mean value of a lower

concentration of NO_3-N was observed in the Biofloc treated group. This low level probably relates to NO_3-N uptake by microbes in the treatments (Hargreaves, 1998). In general, the TAN concentrations found in this study were still in optimum ranges for *C. gariepinus* production (Taslihan *et al*., 2003).

Rostika (2014) recommended total dissolved solids (TDS) levels up to 1000 mg L^{-1} to be appropriate for the culture of *C. gariepinus*, but beyond this level, it may lead to stress. The values recorded in this study in the biofloc and control groups were less than the threshold for *C. gariepinus* culture. The lower pH values in the biofloc tanks may be appropriated to high respiration rates by the large quantities of microorganisms, which might consequently increase CO_2 concentrations. A similar trend was observed by Wasielesky *et al*. (2006). Also, the decrease in pH during the chemolithotrophic process as reported by Chen *et al*. (2006) leads to the release of CO_2 and H^+ into the culture medium. Dissolved oxygen observed during the experimental period was within the range required for culturing *C. gariepinus* production, which is an indication of the positive effect on plankton nutritional quality (Azim *et al*. 2003).

Azim & Little (2006) stated that the presence of optimum concentration of microbial cells in biofloc was able to increase fish health status. Bacterial cells in the biofloc accumulate the poly-β-hydroxybutyrate (PHB) which has an alleged role in microbial pathogens inhibition of fish culture. The higher PHB content in the biofloc tanks made that the fish was able to increase its immune system in order to be more resistant to environmental interference during the study period (De-Schryver *et al*., 2008). This might likely be the reason for improved survival observed in the biofloc treatments.

Lysozyme activity functions as the primary defence of nonspecific humoral immunity, its capability to disrupt cell walls of pathogens make it a natural antagonist to harmful organisms which include bacteria and viruses (Machado *et al*., 2014). Neutrophils are considered the source of lysozyme; lysozyme has been found predominantly in fish serum and mucus (Ellis *et al*., 2011). An increased level of lysozyme is a natural protective mechanism (Ingram, 1980, Basha *et al*., 2013) and it helps destroy gram-positive bacteria. In this study, the serum lysozyme showed a significant difference in biofloc culture with cassava flour as a carbon source having the highest value. It can thus be inferred that *C. gariepinus* juveniles' serum lysosome activity was enhanced in biofloc culture.

Myeloperoxidase (MPO) being the most abundant pro-inflammatory enzyme that is deposited in the azurophilic granules of neutrophilic granulocytes in fish, catalyses the production of hypochlorous acid from hydrogen peroxide as well as produces reactive oxygen species, which help in the killing of bacteria (Heinecke, 1999; Benelli, 2016). In this study, the production of a higher level of ammonia and nitrite in the control treatment showed that the cultured fish were under stress. During stress, the oxygen consumption of cells increases leading to the production of reactive oxygen species through the NADPH-oxidase system. MPO acts as the enzymatic defender of reactive oxygen species, removing excess reactive oxygen species, thus helps in cell detoxification (Wang *et al*., 2008). Neutrophils are an important component of host defence against many bacterial, viral, and fungal infections, it produces cytokines to recruit immune cells to infected areas of the fish. The evaluation of neutrophil function is valuable for assessment of the health status, in this study, neutrophils percentage was found to show a significant difference between the biofloc culture and also between the nonbiofloc culture. *Monocytes* are capable of differentiating between pathogenic and non-pathogenic mycobacteria, it produces cytokines the primary cells involved in phagocytosis. Monocytes showed no significant difference; however, it was higher in both biofloc cultures compared with the nonbiofloc culture (control) an indication that a normal immune response to rearing conditions (El-Etr *et al*., 2001) as observed in experimental fish (*C. gariepinus*). Lymphocytes percentage also shows no significant difference.

Cassava flour and rice bran utilisation as carbohydrate sources to biofloc development for the rearing of *C. gariepinus* was proved to be beneficial. Both carbohydrate sources utilised for biofloc treatments indicated better growth performance, improved innate immunity as well as maintained good water quality for the cultured *C. gariepinus* than the control treatment. The result of this study could be adopted by (catfish) farmers as it support over production which consequently improve food availability. In places where water is scarce or land is expensive, biofloc forms of aquaculture could be practiced for cost-effective production.

Acknowledgements

The authors thank Mr Ojuola M and Ali A of the Department of Fisheries and Aquaculture Technology, Federal University of Technology Akure, Nigeria, for their assistance in water exchange and blood collection.

Funding

This work was supported by the Tertiary Education Trust Fund (TETFUND) [grant numbers VCPU/TETFund/155C.

Conflict of interest

Authors have declared that no competing interests exist.

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