OPEN ACCESS
Journal of Animal Health and Production

Research Article

Growth Performance, Biochemical, Antioxidant Status and Histopathology Changes of Clarias Gariepinus, Raised in a Biofloc Medium Utilizing Natural Carbon Sources

Ikele*, Chika Bright, Ujunwa Aghaji, Mgbenka, Benard Obialo

Department of Zoology and Environmental Biology, University of Nigeria, Nsukka.

Abstract | Integrated biofloc-centered aquaculture nutrient recycling offers a sustainable fish productivity with less environmental impact. A 28 days preliminary trial was conducted to evaluate the affect of biofloc technology application on *Clarias gariepinus* juveniles with emphasis on the growth performance and impact on physiological status. A total of two hundred and seventy juvenile *Clarias gariepinus* were randomly distributed into six plastic bioreactors groups (A-F) in triplicates with equal volume of inoculum, maintained in 30 L tank with constant aeration. Addition of organic carbon sources (CN 10:1, 20:1) were evaluated to stimulate rapid growth of microbial dynamics biomass whereas, no carbon sources were added in the control systems. The CN ratios (cassava and wheat) of 10:1 and 20:1 were; cassava (CA 10:1, 20:1) groups C, D, and wheat flour (WH10:1 and 20:1) groups E and F, respectively. Meanwhile, the normal controls, group A (inoculum, no carbon addition) and B (no inoculum, no carbon) had 50% water exchange and received only fish feed at 3% body weight. The physicochemical parameters maintained the tolerable limit for catfish farming. Significant weight gain, specific growth rate, LWRs, survival rate, and floc volumes were observed at the end of experiment. Observed changes in the catalase, superoxide dismutase, glutathione peroxidase, and malondialdehyde activities in the gill were between day 14 and 21. Significant levels ($p<0.05$) of floc volumes (67.33 to 72 ml) were obtained from the total suspended solids maintained between 111 to 324 mg/L. Total heterotrophic bacteria present in the biofloc media were higher in CA 20:1 with a significantly peak value of $390X10^4$ cfu/ml compared to other groups. The C:N at 10 and 20 utilizing cassava and wheat supported growth of *C. gariepinus* with a mean weight gain of 214.91±26.84g, specific growth rate 3.78±0.26 but did not enhance the condition factor >1 of *C. gariepinus* juveniles within 28-days. In conclusion, inclusion of C:N ratio of cassava and wheat flour in biofloc tank is beneficial for the growth and health parameters of *Clarias gariepinus*.

Keywords | Local carbon sources, C:N ratio, Biofloc, Growth performance, *Clarias gariepinus*

Received | January 22, 2022; **Accepted** | March 01, 2022; **Published** | June 01, 2022
***Correspondence** | Ikele, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka; **Email:** chika.ikele@unn.edu.ng
Citation | Ikele, Bright C, Aghaji U, Mgbenka, Obialo B (2022). Growth performance, biochemical, antioxidant status and histopathology changes of clarias gariepinus, raised in a biofloc medium utilizing natural carbon sources. J. Anim. Health Prod. 10(2): 168-182.
DOI | http://dx.doi.org/10.17582/journal.jahp/2022/10.2.168.182
ISSN | 2308-2801

INTRODUCTION

Aquaculture has persistently developed globally under best practices. A good health status from the function point of view is preponderant for both cultured and wild fish species in early development (Mota et al., 2019). Poor health precludes the reduced exponent of the fish to perform its physiological functions, conform to stressful conditions, and prevent disease (Caipang et al., 2015). Lofty intake of protein above 25% by both fin and shellfish is remarkably excreted as ammonia-nitrogen and influence the quality of water. When inorganic nitrogen, total suspended solids (TSS) are high in concentration in culture water, it leads to death of culture species, diseases, accumulation of

harmful residues in culture systems (Karunaarachchi et al., 2018), and obstruction of the growth of global aquaculture market (Caipang et al., 2015). Enforcement of agro-derived resources and aggrandize natural food sources towards intensifying aquaculture systems (Azim and Little, 2006) is preeminent. To promote efficient fish production, recycling aquaculture system though expensive to maintain, is most preferable. Therefore, it is apropos to utilize non-expendable ecological clues to resolve the problems associated with cost, poor fish production, etc. However, inappropriate husbandry conditions such as poor water quality, crowding leading to accentuated social interactions between similar schemes present in the culture strategies are a potential threat to animal health/welfare.

Therefore, biofloc technology (BFT) provides an alternate solution as a consequence of practicing zero-water exchange, waste nutrient recycling (Bossier and Ekasari, 2017) regulating water qualities by microbial communities, promoting fish growth (Hamidaghli et al., 2019; Mota et al., 2019; Lima et al., 2018; Zapata-Lovera et al., 2017, Yun et al., 2016, Avnimelech, 2007) with reduced environmental impact (Bossier and Ekasari, 2017) for sustainable aquaculture. It is a more beneficial system when heterotrophic bacteria, a nitrogen conversion agent is stimulated by adjusting the C:N ratio in the culture system by the addition of carbonaceous plant-based material or equivalent inorganic carbons for sustainable fish production to achieve sustainable development goals (Bossier and Ekasari, 2017). It has also offered practical solutions to effective water quality control (Panigrahi et al., 2017; Avnimelech, 2007) and improvement of fish growth in the healthy culture system (Lananan et al., 2014). In recent times, the technology is considered best back-up towards increased protein rich food. (Karunaarachchi et al., 2018). Proportionate carbon source agents utilized to intensify profitable microbial production as in aquaculture systems includes; yucca flour (Castro et al., 2018), dextrin (Hamidoghli et al., 2019, molasses (Bakhshi et al., 2018, Correia et al., 2010; Crab et al., 2010), glucose (Caipang et al., 2015; Ekasari et al., 2010), and plant-based, corn starch (Bakhshi et al., 2018), wheat flour (Caipang et al., 2015), sweet potato flour (Caipang et al., 2015), broken wheat and rice grain (Zaki et al., 2020), etc. There is a growing interest due to attributes associated to the choice of natural carbonaceous substrate for bioflocculation which includes; availability, low cost, solubility, and biodegradable as a replacement for protein utilized by fish. Organic and inorganic carbon sources are the key to the composition, formation, and nutriment values of bioflocs such as vitamin C, carotenoid, fatty acids, and trace minerals (Bossier and Ekasari, 2017). Considering the high stocking density, increased feed input utilized in rearing *C. gariepinus* in intensive aquaculture system, with a negative impact on water quality, it is imperative to adopt a better water quality control strategy in production systems (Popoola and Miracle, 2022). The bioflocculation is determined by the adjusted C:N ratio and qualities of substrate to correspond to fish species (Nguyen and Ly, 2018). Manipulation of the C:N ratio above 20 can promote the formation of microbial aggregates (protozoa, zooplankton, phytoplankton, autotrophic/heterotrophic bacteria), and yeast (Mato et al., 2019). Popoola and Miracle (2022) reported positive welfare of *C. gariepinus* raised in a biofloc for 72 days. CN 14:1 and CN 30:1 produced beneficial microbial floc for the growth of *Litopenaeus vannamei* (Silva et al., 2017). C:N ratio of 10 is beneficial for *Clarias gariepinus* farming, while C:N ratio below 10 (Liu et al., 2018) is essential in profit-making feeds but requires additional carbon for bioflocculation. There is need to develop more literatures on the effect of BFTs in rearing African Catfish considering its high values and possible acceptability among both fish farmers and consumers. However, the efficacy of different carbon sources (local cassava variety and commercial wheat flour) with constant availability on the growth performance, biochemicals, antioxidant activities and histological changes of African catfish, *Clarias gariepinus* raised in biofloc tank by steering different C/N ratios were evaluated.

MATERIALS AND METHODS

Experimental Fish

Two hundred and seventy healthy *C. gariepinus* fingerlings of mean weight 1.24±0.02g and length, 3.72±0.01 cm were procured from the catfish multiplication center, Aquafish Nigerian Limited, Awka, Nigeria. The fish were transported to the Department of Zoology and Environmental Biology, University of Nigeria Nsukka. The fish was acclimatized for two (2) weeks before the commencement of the study in 30 L tap water ((NO_3^- 0.91, Cl^- 7.4, SO_4^{2-} 15.2, Ca^{2+} 4.01, total phosphorus 0.03 and mg^{2+} 9.72, Na^+ 4.7, pH, 6.9, DO, 4.2) and was changed daily during acclimatization.

Procurement and Processing of Local Carbon Sources

5kg local variety cassava *Manihot esculenta* and 5kg wheat flour were both obtained from Ogige Market, Enugu State, Nigeria due to their biodegradable potential and availability to farmers. The cassava was peeled, washed, chopped (4cm long, 2mm thick), and dried in a Heraeus vacutherm oven at 40°C and ground into powder.

Experimental Design

A total of Two hundred and seventy juveniles *C. gariepinus* fingerlings were randomly distributed into 6 plastics tanks (54x54x67cm) (A-F) triplicated to 18 tanks at an initial density of 15 fish per tank. The experimental design

adopted a complete randomized design. The C:N ratios of the carbohydrates (cassava and wheat) sources in the culture system was adjusted (CN 10:1 and CN 20:1), respectively, and evaluated following the protocol of Avnimelech (2007) and Piedrahita (2003). Group A (CE) and B (NE) were maintained as controls with inoculum + no carbon addition, and no inoculum + no carbon addition, respectively with 50% water exchange every week. Two different natural carbon sources, were maintained at two different C:N ratio 10 and 20. Group C cassava (CA 10:1 + inoculum), group D cassava (CA 20:1, inoculum), Group E wheat flour (WH 10:1 + inoculum) and group F wheat flour (WH 20:1 + inoculums) while water was added when necessary to make up for loss caused by evaporation during aeration. The plastic tanks were sealed up using mosquito nets (0.024 inches) to prevent drosophila entrance and constantly aerated using 45L/min 200 W electromagnetic aquarium oxygen pump, model ACO-010, China) with air blowers distributed in all the tanks. Aeration was supported by using petroleum motor spirit (PMS) generator (Elemaq 3600) due to power failure. The plastic tank was maintained at 30 L volume of tap water (temperature 24.4° C to 25.1°C, pH 6.7-6.9). The experiment lasted for 28 days, but prior to the growth yield determination, the fish were starved overnight.

Inoculum Preparation, Biofloc, water quality and growth yield

Natural inoculum consisted of 200ml of *Chlorella lewinni* (cultivated in BG-11 medium in g/L of distilled water (pH 6.9) $NaNO_3$ 1.5, $K_3HPO_4.3H_20$ 0.04, $CaCL_2.H_20$ 0.036, $MgSO_4.7H_20$, Na_2CO_3 0.02, citric acid 0.006, $C_6H_80_7$ Fe_3, NH_3,0.006, Na_2 EDTA 0.001 and 1.0 ml trace metal solution (H_3BO_3 2.860, $ZnSO_4.7H_2O$ 0.222, $MnCl_2.4H_20$ 1.8, $CuSO_4.5H_2O$ 0.079, Na MOO4.$2H_20$ 0.390 and $C0(NO_3)2.6H_2O$ 0.0494) was prepared and supplemented with 30ml of filtered algae concentration obtained from pond water (pH, 5.5, TAN, 0.67, Temperature, 24.7°C, TDS 207 mgL^{-1}, dissolved oxygen 5.26 mgL^{-1}), put into each biofloc tank with 30 L of tap water.

Cassava and wheat flour were added together with coppens feed (45% crude protein) to support the build-up of the microbial community against the nitrogenous waste product. In consequence, CN 10:1 and CN 20:1 gave 1.08g and 2.16g, cassava, and wheat, respectively, and administered to induce heterotrophic medium for optimum biofloc production.

The floc volumes (ml) were obtained and measured weekly using improvised imhoff cone (seven observations) for 28 days. A 100 ml of heterogeneous aggregates were obtained at intervals, put in an improvised Imhoff cone, and flocculated for 10 minutes. The presence of floccules was observed and obtained by opening the central point of the improvised imhoff cone. Re-suspension of particles due to gas formation was avoided. The harvested flocs were measured in triplicates in a 100 ml measuring cylinder, to obtain the floc volume. The harvested flocs were examined and observed under an optika binocular microscope using X40 objectives.

Physicochemical parameters such as dissolved oxygen (mg/L), temperature (°C), pH, nitrite (mg/L), nitrate (mg/L), total suspended solids (mg/L), and total ammonia nitrogen (mg/L) were measured daily between 9:00 and 11:00 hours following the protocol of (APHA, 1995) throughout the experiment.

The growth parameters were determined throughout the experiment, by the measurement of both initial and final body weight (g) and length (cm) of fish. Meanwhile, the growth rate (%), length gain, percentage length gain (%), weight gain was determined. Other growth parameter includes;

WG=

Logarithmic transformation of length-weight relationship was determined

Log W=log a + b log L

Where W=weight of the fish (g)

L=length of the fish (g)

a=constant

b=exponent

Specific growth rate was determined in percentage;

Fulton Condition factor was expressed in percentage as K=

Survival rate was expressed in percentage (%) as;

X100

Heterotrophic Bacteria

Biofloc samples from each bioreactor were taken and approximately 1-2g flocs (net weight equivalent) were suspended in 10ml sterile saline solution (0.85% NaCl) and peptone water (Merck). After the preparation of ten-fold serial dilution (1ml of sample in 9 ml of diluent), 0.1 ml of the dilution was spread on the surface of sterile Petri dishes containing Tryptone Soy Agar (TSA) and De Mann-Rogosa and Sharpe Agar (MRS) in triplicate. Inoculated plates were incubated at 30°C for 48h aerobically and anaerobically for TSA and MRS cultures, respectively. Bacterial colonies formed on the incubated plates were counted and calculated against the cultured volume and dilution factor to get the colony forming units per ml (cfu/ml) thus;

$$C = \frac{n}{VD}$$

Where:

C = concentration in cfu/ml

n = number of colonies formed on culture plate

V = volume cultured

D = Dilution factor.

Total heterotrophic bacteria (THB count) expressed as colony-forming units (CFU) were observed within 21 days

Biochemicals

Aspartate aminotransferase and alanine aminotransferase Alkaline phosphatase, creatinine, Urea and total protein activities in the serum were assayed following the protocol of Reitman and Frankel, (1957) using randox kit.

Antioxidants

Antioxidants activities in the homogenate of fish gills (n=3) were used to assay for Glutathione peroxidase (GPx) (Lawrence and Buck, 1976) expressed as u/mg protein. Lipid peroxidation (TBARS) expressed as nanomoles of TBARS formed/mg protein (Sharma and Krishna-Murti, 1968). Catalase activity (CAT) assay (Aebi, 1984), and superoxide dismutase activity (SOD) in the gill tissue was measured based on the auto-oxidation of adrenalin due to the presence of superoxide anion (Misra and Fridovich, 1972).

Proximate composition of harvested flocs

The proximate composition of the flocs in triplicates was measured following the protocol of AOAC (1989).

Histopathology

The histological procedure of liver sections was carried out following the protocol of Bancroft and Cook (1994).

Statistical Analysis

Data were analyzed for significant differences ($p<0.05$) using analysis of variance (ANOVA). Also, All analyses were done using Statistical Package for Social Sciences windows version 20.0 (IBM Corporation, Armonk, USA).

RESULTS

Physicochemical

The simultaneous increase in temperature in CE and NE followed a clear-cut trend within 21 days. In all the setups, the temperature changes differed significantly at $p<0.05$ following the trend day 21>day 28>day14> day 7> baseline, respectively. The fluctuated pH varied between groups, especially in CA 10:1 and WH 20:1. The pH values significantly ($p<0.05$) increased in CA 10:1, WH 10:1, and WH 20:1 on day 14, followed by CE and CA 20:1. The dissolved oxygen levels maintained a static concentration throughout the study period, with a bit of variation in values in CA 10:1. Total suspended solids fluctuated among the groups, and 324.67mg/L peaked at WH 10:1. Inorganic nitrogen TAN concentration in the biofloc tanks depended on the components that produced the flocs duration-dependent. Generally, no significant difference was observed in the TAN in all BFTs groups. Irrespective of minor fluctuations in TAN, 0.31mg/L TAN was reached. Changes in nitrogen, nitrate, nitrite, and phosphate depended on the study duration and medium. No clear cut trend was observed in the variation of inorganic parameters (Table 1). The observed physicochemical parameters such as temperature, pH, and dissolved oxygen were still within the normal range for the rearing of *C. gariepinus*.

Growth Performance and Survival of *C. gariepinus* reared in Biofloc

The growth parameters of *C. gariepinus* to conditions of BFTs at the end of the experiment are presented in Table 2. The difference between weight gain, growth rate, specific growth rate, and the length-weight was insignificant ($p>0.05$). The growth performance normalized in the BFTs group at the end of the experiment with a significant condition factor of 0.88 observed in group B. At the end of the experiment, the length-weight relationship showed 'b' < 1 in all the groups (Table 3). The 'b' value of *C. gariepinus* ranged from 0.210 to 0.2597 throughout the study. The best relationship in the length-weight was observed in group C ($\log W=0.2101L^{0.7873}$, a, 6.128, b, 0.210, R=0.873, R^2= 0.762, $p<0.001$) (Table 3). The peak growth rate, the percentage weight gain (214±26.80), the specific growth rate (3.78±0.26) of *C. gariepinus* showed significantly higher values among the treatment ($p<0.05$) compared to CA and NE.

Survival was not 100% in any of the biofloc tanks. Mortality was recorded in groups that provided carbon sources compared to non-carbon sources (CE and NE). The survival percentage was most minuscule in NE and highest in WH 20:1. Similar magnitudes of survival were observed in WH 10:1 and CA 10:1 (Table 2). The survival differentials between groups ranged from 28.25-60.4% (CE) and 53.25-85.64 (NE). There was a significant difference in the survival of *C. gariepinus* in the BFTs groups ($F=14.982$, $p<0.001$).

Antioxidants

The antioxidant markers, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) activities in the gills of *C. gariepinus* were dependent on the biofloc medium and duration of the study. A significant variation in CAT, SOD, and GPx activities were noticed on day 14 between the groups, while MDA activities were substantial ($p<0.05$) on day 21. Also, CA 20:1 and WH 10:1 increased CAT activities ($P<0.05$). The activities of SOD were not significant in all the groups, but the GPx activities in the gill changed significantly in CA 10:1, CA 20:1, WH 10:1, and WH 20:1 throughout the study compared to the baseline. Lipid peroxidation activities observed in the fish exposed to WH 20:1 declined in concentration; the disparate was insignificant to CA

Table 1: Physicochemical parameters of the culture system

Parameter	Groups	Baseline	Day 7	Day 14	Day 21	Day 28
Temperature (°C)	CE	25.30 ± 0.00^{a1}	25.80 ± 0.00^{a2}	26.00 ± 0.00^{b3}	27.07 ± 0.03^{b5}	26.70 ± 0.00^{a4}
	NE	25.30 ± 0.00^{a1}	25.70 ± 0.00^{a2}	26.07 ± 0.03^{ab3}	27.13 ± 0.035^{ab5}	26.70 ± 0.00^{a4}
	CA 10:1	25.30 ± 0.00^{a1}	25.77 ± 0.03^{a2}	26.07 ± 0.00^{ab3}	27.17 ± 0.03^{a5}	26.67 ± 0.17^{a4}
	CA 20:1	25.30 ± 0.00^{a1}	25.83 ± 0.09^{a2}	26.07 ± 0.03^{ab3}	27.20 ± 0.00^{a5}	26.50 ± 0.06^{a4}
	WH 10:1	25.30 ± 0.00^{a1}	25.77 ± 0.03^{a2}	26.10 ± 0.00^{a3}	27.17 ± 0.03^{a5}	26.63 ± 0.07^{a4}
	WH 20:1	25.30 ± 0.00^{a1}	25.80 ± 0.00^{a2}	26.07 ± 0.03^{ab3}	27.20 ± 0.00^{a5}	26.70 ± 0.00^{a4}
pH	CE	8.00 ± 0.90^{a1}	7.40 ± 0.10^{a1}	7.10 ± 0.06^{c1}	7.37 ± 0.03^{a1}	7.37 ± 0.03^{a1}
	NE	8.00 ± 0.90^{a1}	7.27 ± 0.09^{a1}	7.57 ± 0.03^{a1}	7.47 ± 0.12^{a1}	7.37 ± 0.03^{a1}
	CA 10:1	7.73 ± 0.09^{a2}	7.43 ± 0.09^{a1}	7.37 ± 0.03^{b1}	7.43 ± 0.07^{a1}	7.53 ± 0.03^{a12}
	CA 20:1	7.73 ± 0.09^{a2}	7.50 ± 0.10^{a12}	7.10 ± 0.06^{c1}	7.43 ± 0.09^{a12}	7.27 ± 0.07^{a1}
	WH 10:1	7.73 ± 0.09^{a2}	7.43 ± 0.07^{a1}	7.36 ± 0.03^{b1}	7.53 ± 0.19^{a1}	7.30 ± 0.10^{a1}
	WH 20:1	7.73 ± 0.09^{a2}	7.37 ± 0.03^{a1}	7.27 ± 0.06^{b1}	7.50 ± 0.21^{a1}	7.37 ± 0.18^{a1}
DO (mgL^{-1})	CE	5.45 ± 0.09^{a1}	5.37 ± 0.18^{a1}	5.57 ± 0.18^{a1}	4.93 ± 0.18^{b1}	5.43 ± 0.52^{a1}
	NE	5.45 ± 0.09^{a1}	5.73 ± 0.38^{a1}	5.33 ± 0.09^{a1}	4.97 ± 0.26^{ab1}	6.00 ± 0.37^{a2}
	CA 10:1	5.55 ± 0.09^{a12}	5.77 ± 0.15^{a2}	5.80 ± 0.31^{a2}	5.07 ± 0.03^{ab1}	5.87 ± 0.15^{a2}
	CA 20:1	5.55 ± 0.09^{a1}	5.53 ± 0.23^{a1}	5.40 ± 0.17^{a1}	5.30 ± 0.26^{ab1}	5.67 ± 0.15^{a1}
	WH 10:1	5.55 ± 0.09^{a1}	5.73 ± 0.49^{a1}	5.27 ± 0.20^{a1}	5.53 ± 0.32^{ab1}	5.13 ± 0.20^{a1}
	WH 20:1	5.55 ± 0.09^{a1}	5.83 ± 0.24^{a1}	5.63 ± 0.32^{a1}	5.73 ± 0.24^{a1}	5.43 ± 0.35^{a1}
TSS (mgL^{-1})	CE	311.50 ± 12.99^{a1}	222.00 ± 84.01^{ab1}	156.00 ± 103.71^{a1}	166.67 ± 38.44^{a1}	196.67 ± 39.23^{a1}
	NE	311.50 ± 12.99^{a1}	194.67 ± 96.45^{ab1}	294.67 ± 14.71^{a1}	220.67 ± 40.70^{a1}	154.70 ± 45.19^{a1}
	CA 10:1	270.50 ± 6.63^{b2}	144.67 ± 43.46^{ab1}	280.67 ± 40.83^{a2}	193.33 ± 23.22^{a12}	197.33 ± 24.34^{a12}
	CA 20:1	270.50 ± 6.63^{b2}	111.33 ± 21.46^{b1}	270.67 ± 46.34^{a2}	269.33 ± 38.68^{a2}	108.73 ± 81.83^{a1}
	WH 10:1	270.50 ± 6.63^{b2}	240.00 ± 23.86^{ab2}	167.43 ± 68.49^{a2}	346.67 ± 12.72^{b1}	188.67 ± 44.13^{a2}
	WH 20:1	270.50 ± 6.63^{b2}	324.67 ± 13.68^{a2}	226.00 ± 58.41^{a12}	177.33 ± 25.57^{a1}	197.67 ± 17.32^{a1}
TAN (mgL^{-1})	CE	0.22 ± 0.00^{a1}	0.23 ± 0.01^{a1}	0.23 ± 0.01^{b1}	0.24 ± 0.00^{a1}	0.24 ± 0.01^{ab1}
	NE	0.22 ± 0.00^{a1}	0.24 ± 0.00^{a12}	0.27 ± 0.02^{ab2}	0.26 ± 0.02^{a12}	0.24 ± 0.02^{ab12}
	CA 10:1	0.23 ± 0.00^{a1}	0.24 ± 0.02^{a2}	0.26 ± 0.01^{ab12}	0.24 ± 0.00^{a12}	0.24 ± 0.00^{ab12}
	CA 20:1	0.23 ± 0.00^{a1}	0.26 ± 0.02^{a2}	0.26 ± 0.03^{ab1}	0.26 ± 0.03^{a1}	0.23 ± 0.01^{b1}
	WH 10:1	0.23 ± 0.00^{a1}	0.26 ± 0.02^{a1}	0.31 ± 0.03^{a1}	0.23 ± 0.01^{a1}	0.26 ± 0.04^{ab1}
	WH 20:1	0.23 ± 0.00^{a1}	0.27 ± 0.02^{a12}	0.27 ± 0.01^{ab12}	0.25 ± 0.02^{a12}	0.30 ± 0.01^{a2}
Nitrite (mgL^{-1})	CE	1.83 ± 0.34^{a2}	1.40 ± 0.10^{b12}	1.21 ± 0.09^{c1}	1.39 ± 0.12^{a12}	1.28 ± 0.03^{a12}
	NE	1.83 ± 0.34^{a2}	1.23 ± 0.06^{b1}	1.41 ± 0.09^{c12}	1.36 ± 0.08^{a12}	1.31 ± 0.07^{a12}
	CA 10:1	1.19 ± 0.03^{a1}	1.78 ± 0.16^{a2}	1.45 ± 0.09^{c12}	1.38 ± 0.03^{a1}	1.50 ± 0.15^{a12}
	CA 20:1	1.19 ± 0.03^{a1}	1.52 ± 0.13^{ab1}	1.91 ± 0.23^{ab2}	1.33 ± 0.04^{a1}	1.30 ± 0.08^{a1}
	WH 10:1	1.19 ± 0.03^{a1}	1.27 ± 0.09^{b1}	1.55 ± 0.14^{bc1}	1.60 ± 0.21^{a1}	1.34 ± 0.13^{a1}
	WH 20:1	1.19 ± 0.03^{a1}	1.47 ± 0.08^{ab12}	2.27 ± 0.14^{a2}	1.45 ± 0.57^{a12}	1.52 ± 0.03^{a12}
Nitrate (mgL^{-1})	CE	0.15 ± 0.00^{a1}	0.15 ± 0.01^{a1}	0.14 ± 0.01^{c1}	0.20 ± 0.04^{a1}	0.13 ± 0.01^{a1}
	NE	0.15 ± 0.00^{a1}	0.14 ± 0.00^{a1}	0.14 ± 0.01^{c1}	0.16 ± 0.01^{a1}	0.13 ± 0.01^{a1}
	CA 10:1	0.14 ± 0.00^{b1}	0.18 ± 0.06^{a1}	0.16 ± 0.01^{c1}	0.12 ± 0.01^{a1}	0.17 ± 0.03^{a1}
	CA 20:1	0.14 ± 0.00^{b1}	0.20 ± 0.02^{a2}	0.20 ± 0.01^{ab2}	0.32 ± 0.17^{a12}	0.17 ± 0.02^{a12}
	WH 10:1	0.14 ± 0.00^{b1}	0.17 ± 0.01^{a1}	0.18 ± 0.03^{abc1}	0.15 ± 0.03^{a1}	0.15 ± 0.03^{a1}
	WH 20:1	0.14 ± 0.00^{b1}	0.21 ± 0.03^{a12}	0.23 ± 0.02^{a2}	0.15 ± 0.02^{a12}	0.20 ± 0.02^{a12}

Values as mean ± S.E. Values with different alphabet within a column were significantly different ($p < 0.05$). Values with different numeric superscript across a row were significantly different ($p < 0.05$). Group A (CE); Group B (NE), Group C (CA 10:1), Group D (CA 20:1) Group E (WH10:1), Group F (WH 20:1). DO, dissolved oxygen; TAN, total ammonia nitrogen; TSS, total suspended solid.

Table 2: Effects of different organic carbons on weight gain, growth rate, specific growth rate, condition factor and survival differences of *Clarias gariepinus* after 28 days growth trial under varied C:N ratios in a biofloc tank.

Parameters	CE	NE	CA 10:1	CA 20:1	WH 10:1	WH 20:1
Initial weight (g)	1.38 ± 0.03^{a}	1.38 ± 0.03^{a}	1.38 ± 0.03^{a}	1.38 ± 0.03^{a}	1.38 ± 0.03^{a}	1.38 ± 0.03^{a}
Initial length (cm)	5.78 ± 0.06^{a}	5.78 ± 0.06^{a}	5.78 ± 0.06^{a}	5.78 ± 0.06^{a}	5.78 ± 0.06^{a}	5.78 ± 0.06^{a}
Final Weight (g)	3.11 ± 0.25^{a}	3.24 ± 0.31^{a}	4.05 ± 0.30^{a}	3.46 ± 0.23^{a}	4.09 ± 0.32^{a}	4.19 ± 0.30^{a}
Weight gain (g)	1.80 ± 0.25^{a}	1.93 ± 0.24^{a}	2.69 ± 0.30^{a}	2.10 ± 0.24^{a}	2.73 ± 0.33^{a}	2.82 ± 0.31^{a}
Weight gain (%)	139.42 ± 20.30^{a}	146.77 ± 13.85^{a}	204.25 ± 24.32^{a}	162.18 ± 19.46^{a}	211.61 ± 29.75^{a}	214.91 ± 26.80^{a}
GR (%)	6.42 ± 0.90^{a}	6.88 ± 0.86^{a}	9.59 ± 1.09^{a}	7.50 ± 0.85^{a}	9.75 ± 1.18^{a}	10.06 ± 1.12^{a}
SGR (%)	2.99 ± 0.26^{a}	3.20 ± 0.21^{a}	3.74 ± 0.24^{a}	3.22 ± 0.24^{a}	3.73 ± 0.28^{a}	3.78 ± 0.26^{a}
Final Length (cm)	7.21 ± 0.17^{b}	7.16 ± 0.26^{b}	8.15 ± 0.11^{a}	7.70 ± 0.17^{ab}	8.15 ± 0.19^{a}	8.22 ± 0.18^{a}
LG(cm)	1.54 ± 0.18^{b}	1.56 ± 0.32^{b}	2.36 ± 0.15^{a}	1.92 ± 0.19^{ab}	2.36 ± 0.21^{a}	2.44 ± 0.19^{a}
LG (%)	27.40 ± 3.42^{b}	28.21 ± 5.82^{ab}	41.53 ± 2.91^{ab}	33.78 ± 3.49^{ab}	41.33 ± 3.95^{ab}	42.93 ± 3.54^{a}
CF (%)	0.82 ± 0.03^{ab}	0.88 ± 0.06^{a}	0.72 ± 0.03^{b}	0.75 ± 0.02^{b}	0.73 ± 0.02^{b}	0.73 ± 0.02^{b}
Survival (%)	38.89±7.35^{b}	13.89±7.35	77.78±7.35^{a}	75.10±12.73^{a}	77.78±2.78^{a}	88.89±2.78^{a}
Survival differences						
*CE	0	-25.00±10.64	38.89±10.64	36.11±10.64	38.89±10.64	50.00±10.64
*NE	25.00±10.64	0	63.89±10.64	61.11±10.64	63.89±10.64	75.00±10.64
Mean survival	4.67±-0.88^{b}	1.67±0.88^{c}	9.33±0.88^{a}	9.00±1.53^{a}	9.33±0.33^{a}	10.67±0.33^{a}

Values as mean ± S.E. Values with different alphabet superscript across a row were significantly different ($p < 0.05$). GR, growth rate; SGR, specific growth rate; LG, length gain, CF, condition factor. Group A (CE); Group B (NE), Group C (CA 10:1), Group D (CA 20:1) Group E (WH10:1), Group F (WH 20:1)

Table 3: Length-weight relationship of *C. gariepinus* raised in a biofloc tank after 28 days growth trial

	Groups	Logarithmic equation	A	b	R	R^2	P
Baseline		LogW = 1.319Log L – 0.8699	0.134927	1.319	0.540	0.292	0.000
Day 7	CE	LogW = 1.3244LogL – 0.7775	0.16799	1.324	0.579	0.335	0.001
	NE	LogW = 0.9622LogL-0.5371	0.290335	0.962	0.477	0.228	0.008
	CA 10:1	LogW = 2.5973LogL - 1.8175	0.015223	2.597	0.925	0.852	0.000
	CA 20:1	LogW = 1.7867LogL-1.1834	0.065554	1.787	0.747	0.558	0.000
	WH 10:1	LogW = 1.4296LogL-0.8647	0.136553	1.430	0.608	0.370	0.000
	WH 20:1	LogW = 2.1463LogL-1.4276	0.037359	2.146	0.813	0.661	0.000
Day 14	CE	LogW = 1.7162LogL-1.0684	0.085428	1.716	0.740	0.548	0.000
	NE	LogW = 1.859Log-1.2188	0.060423	1.859	0.734	0.539	0.001
	CA 10:1	LogW = 1.5605LogL-0.9289	0.117788	1.561	0.696	0.485	0.000
	CA 20:1	LogW = 1.985LogL-1.301	0.050003	1.986	0.731	0.534	0.000
	WH 10:1	LogW = 1.7075LogL-1.0433	0.090511	1.708	0.707	0.500	0.000
	WH 20:1	LogW = 2.2317LogL-1.4872	0.031827	2.232	0.796	0.634	0.000
Day 21	CE	LogW = 1.8218LogL-1.1097	0.077678	1.822	0.821	0.675	0.000
	NE	LogW =1.2646LogL-0.7051	0.197197	1.265	0.655	0.429	0.021
	CA 10:1	LogW = 2.5071LogL-1.7029	0.01982	2.507	0.953	0.908	0.000
	CA 20:1	LogW = 1.9171LogL-1.2097	0.061702	1.917	0.741	0.549	0.000
	WH 10:1	LogW = 2.5697LogL-1.7731	0.016862	2.570	0.929	0.863	0.000
	WH 20:1	LogW = 2.0387LogL-1.275	0.053088	2.039	0.888	0.788	0.000
Day 28	CE	LogW = 0.2727LogL+0.7258	5.318633	0.273	0.826	0.682	0.000
	NE	LogW = 0.3426Logl+0.6813	4.80065	0.343	0.830	0.689	0.082
	CA 10:1	LogW = 0.2101LogL+0.7873	6.127735	0.210	0.873	0.762	0.000
	CA 20:1	LogW = 0.356LogL+0.6988	4.998043	0.356	0.911	0.830	0.000

WH 10:1	LogW = 0.3226LogL+0.7193	5.239623	0.323	0.917	0.840	0.000
WH 20:1	LogW = 0.3024LogL+0.7325	5.401321	0.302	0.889	0.790	0.000

Group A (CE); Group B (NE), Group C (CA 10:1), Group D (CA 20:1) Group E (WH10:1), Group F (WH 20:1).W, weight; L, length

Table 4: Responses of oxidative stress biomarkers in *Clarias gariepinus* reared in a different BFT conditions

Parameters	Groups	Baseline	Day 7	Day 14	Day 21
CAT (U/mg protein)	CE	1.13 ± 0.02^{a12}	1.17 ± 0.04^{a2}	1.16 ± 0.04^{ab2}	1.06 ± 0.01^{b1}
	NE	1.23 ± 0.08^{a1}	1.25 ± 0.03^{a1}	1.18± 0.05^{ab1}	1.08 ± 0.00^{b1}
	CA 10:1	1.08 ± 0.11^{a1}	1.25 ± 0.03^{a1}	1.20 ± 0.07^{ab1}	1.10 ± 0.01^{ab1}
	CA 20:1	1.30 ± 0.21^{a1}	1.19 ± 0.04^{a1}	1.25 ± 0.03^{ab1}	1.14 ± 0.00^{a1}
	WH 10:1	1.14 ± 0.14^{a1}	1.23 ± 0.03^{a1}	1.13 ± 0.00^{b1}	1.14 ± 0.03^{a1}
	WH 20:1	1.17 ± 0.01^{a12}	1.17 ± 0.06^{a12}	1.38 ± 0.14^{a2}	1.09 ± 0.01^{ab1}
SOD (U/mg protein)	CE	10.50 ± 0.26^{a1}	10.92 ± 0.03^{a12}	11.32 ± 0.20^{a2}	11.22 ± 0.03^{b2}
	NE	10.66 ± 0.12^{a1}	10.89 ±0.14^{a1}	10.88 ± 0.20^{b1}	11.52 ± 0.16^{a2}
	CA 10:1	10.83 ± 0.12^{a1}	10.98 ± 0.09^{a12}	11.04 ± 0.03^{ab12}	11.24 ± 0.03^{b2}
	CA 20:1	10.77 ± 0.28^{a1}	10.94 ± 0.08^{a12}	11.05 ± 0.05^{ab12}	11.22 ± 0.07^{b2}
	WH 10:1	10.95 ± 0.06^{a1}	10.93 ± 0.06^{a1}	11.01 ± 0.06^{ab1}	11.37 ± 0.03^{ab2}
	WH 20:1	10.97 ± 0.10^{a12}	10.87 ± 0.10^{a1}	10.91 ± 0.02^{ab1}	11.22 ± 0.07^{b2}
GPx (ng/mg protein)	CE	11.09 ± 0.21^{a2}	10.06 ± 0.29^{a1}	9.91 ± 0.25^{b1}	9.91 ± 0.25^{b1}
	NE	11.58 ± 0.15^{a3}	11.34 ± 0.39^{a23}	10.35 ± 0.50^{ab12}	9.91 ± 0.25^{b1}
	CA 10:1	11.78± 0.21^{a2}	10.92 ± 0.29^{a12}	10.78 ± 0.25^{ab12}	10.35 ± 0.50^{b1}
	CA 20:1	11.30 ± 0.48^{a1}	10.63 ± 0.29^{a1}	10.78 ± 0.25^{ab1}	11.64 ± 0.25^{a1}
	WH 10:1	11.82 ± 0.39^{a2}	10.34 ± 0.50^{a1}	11.21 ± 0.50^{a12}	10.34 ± 0.00^{b1}
	WH 20:1	11.04 ± 0.22^{a1}	10.06 ± 0.58^{a1}	10.77 ± 0.25^{ab1}	9.91 ± 0.25^{b1}
MDA (U/mg protein)	CE	1.16 ± 0.02^{a1}	1.22 ± 0.04^{a1}	1.27 ± 0.12^{a1}	1.29 ± 0.12^{ab1}
	NE	1.17 ± 0.07^{a1}	1.16 ± 0.07^{a1}	1.50 ± 0.07^{a2}	1.58 ± 0.07^{a2}
	CA 10:1	1.20 ± 0.05^{a1}	1.22 ± 0.16^{a1}	1.44 ± 0.08^{a1}	1.25 ± 0.06^{ab1}
	CA 20:1	1.17 ± 0.04^{a1}	1.21 ± 0.08^{a1}	1.41 ± 0.01^{a2}	1.14 ± 0.08^{ab1}
	WH 10:1	1.16 ± 0.16^{a1}	1.23 ± 0.07^{a1}	1.29 ± 0.05^{a1}	1.16 ± 0.03^{ab1}
	WH 20:1	1.27 ± 0.08^{a1}	1.13 ± 0.06^{a1}	1.22 ± 0.12^{a1}	1.05 ± 0.02^{c1}

Values as mean ± S.E. Values with different alphabet superscript within a column were significantly different ($p < 0.05$). Values with different numeric superscript across a row were significantly different ($p < 0.05$). CE; NE, CA 10:1 carbon (cassava) 10:1; CA 20:1 carbon (cassava) 20:1; WH10:1, carbon (wheat) 10:1; WH20:1, carbon (wheat) 20:1. CAT, catalase, SOD, superoxide dismutase; GPx, glutathione peroxidase, MDA, malondialdehyde

Table 5: Changes in the liver biomarkers of *C. gariepinus* raised in biofloc tank.

Parameters	Groups	Baseline	Day 7	Day 14	Day 21
ALT (IU/L)	CE	8.03± 0.34^{a1}	7.24 ± 0.51^{b1}	8.53 ± 0.61^{b1}	8.45 ± 0.52^{b1}
	NE	8.39± 0.10^{a1}	8.99 ± 0.31^{a12}	10.37 ± 1.08^{a2}	8.78 ± 0.14^{ab12}
	CA 10:1	8.43± 0.41^{a1}	8.12 ± 0.85^{b1}	12.05 ± 0.28^{a2}	9.44 ± 0.11^{a1}
	CA 20:1	8.16± 0.21^{a1}	9.11 ± 0.25^{a2}	11.82 ± 0.38^{a3}	8.94 ± 0.23^{ab12}
	WH 10:1	8.26± 0.41^{a1}	9.38 ± 0.42^{a1}	11.90 ± 0.29^{a2}	8.96 ± 0.13^{ab1}
	WH 20:1	8.25± 0.22^{a1}	9.03 ± 0.36^{a1}	10.89 ± 0.41^{a2}	8.86 ± 0.19^{ab1}

AST (IU/L)	CE	11.24± 0.78^{a1}	10.90 ± 0.37^{ab1}	11.82 ± 0.14^{b1}	11.07 ± 0.06^{b1}
	NE	11.10± 0.35^{a1}	12.33 ±0.22^{a2}	12.27 ± 0.21^{a2}	11.87 ± 0.37^{a12}
	CA 10:1	11.53± 1.39^{a1}	11.60 ± 0.23^{a1}	11.79 ± 0.12^{b1}	11.99 ± 0.28^{a1}
	CA 20:1	10.83± 0.58^{a1}	12.29 ± 0.15^{a2}	11.00 ± 0.08^{c1}	11.38 ± 0.03^{ab12}
	WH 10:1	11.11± 0.80^{a1}	11.19 ± 0.19^{ab1}	11.75 ± 0.09^{b1}	11.52 ± 0.05^{ab1}
	WH 20:1	10.66± 0.40^{a1}	10.59 ± 0.89^{b1}	11.42 ± 0.05^{b1}	11.20 ± 0.08^{b1}
ALP (IU/L)	CE	52.49± 4.04^{a1}	45.47 ± 2.32^{a1}	46.30 ± 1.31^{bc1}	42.42 ± 6.57^{b1}
	NE	54.07± 0.66^{a2}	46.60 ± 2.84^{a1}	49.90 ± 1.67^{b12}	52.90 ± 2.48^{a12}
	CA 10:1	49.42± 2.00^{a1}	51.73 ± 5.33^{a1}	56.70 ± 1.10^{a1}	55.10 ± 1.67^{a1}
	CA 20:1	47.42± 3.04^{a1}	47.00 ± 4.08^{a1}	45.80 ± 2.19^{bc1}	50.00 ± 0.46^{ab1}
	WH 10:1	49.90± 1.82^{a12}	51.93 ±1.88^{a2}	45.20 ± 0.58^{c1}	50.80 ± 1.27^{ab2}
	WH 20:1	51.36± 2.82^{a2}	46.00 ± 3.36^{a12}	41.80 ± 0.69^{c1}	46.30 ± 0.29^{ab12}

Values as mean ± S.E. Values with different alphabet superscript within a column were significantly different ($p < 0.05$). Values with different numeric superscript across a row were significantly different ($p < 0.05$). CE (normal control); NE,; CA 10:1 carbon (cassava) 10:1; CA 20:1 carbon (cassava) 20:1; WH10:1, carbon (wheat) 10:1; WH20:1, carbon (wheat) 20:1. ALT, alanine aminotransferase, AST, aspartate aminotransferase; ALP, alkaline phosphatase

10:1, CA 20:1, and WH 10:1. The MDA level in the fish reared in NE was significantly different ($p<0.05$) (Table 4).

Biochemicals

Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) indicated variations dependent on the media harbouring the fish and the duration of the experimental setup. AST only showed a duration-dependent variation in concentration. In the cassava carbon source (CA 10:1 and CA 20:1) and wheat carbon source (WH 10:1 and WH 20:1) groups, ALT on day 14 was significantly higher than day 14 and baseline concentrations ($p < 0.05$) (Table 5). The ALT level in these groups declined on day 21 to day 7 level. ALT in NE appeared to follow the same trend as the groups that provided carbon sources. No significant difference occurred in ALT in CE between baseline and day 21 ($p > 0.05$). AST concentration in groups provided carbon sources were within the same range except WH 20:1 with reduced AST level compared to CA 20:1 ($p < 0.05$). The AST level was significantly lower in CA 20:1 than all other groups, while NE was considerably higher ($p < 0.05$) on day 14.

Fluctuations in the concentration of ALP for the duration of the study only occurred in groups provided wheat carbon source (WH 10:1and WH 20:1). The significant variations did not follow a clear cut pattern in both groups. The marked difference in ALP activities between the groups occurred on days 14 and 21. On day 14, ALP was significantly higher in CA 10:1 group compared to the other groups ($p < 0.05$). But ALP in CA 10:1 was within the same limits as other groups except for CE on day 21 (Table 5).

The total protein level on days 7, 14 and 21 were within the baseline range. Complete protein within any group was not significantly different between days 7, 14 and 21 ($p > 0.05$). On day 21, marked differences occurred in total protein level between the groups (Figure 1); WH 10:1 and WH 20:1 total protein concentration was significantly higher than NE ($p < 0.05$). Creatinine concentration in all the groups dropped from day 7, through day 14 to day 21, except CA 10:1 group (Figure 2). On day 21, creatinine concentration in CA 10:1 was significantly higher.

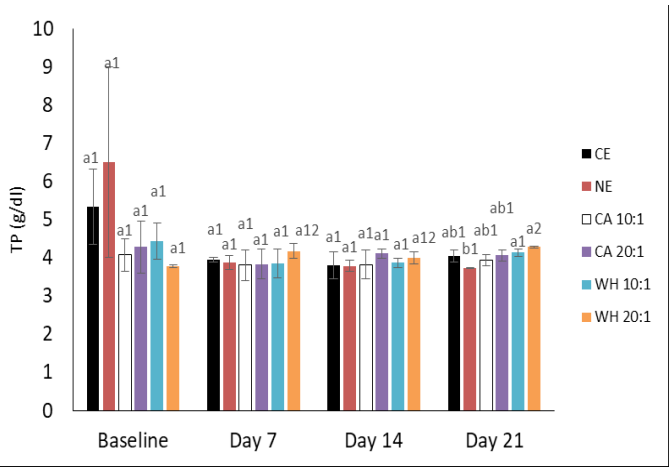

Figure 1: Changes in total protein under different BFT conditions.
Bars with different alphabet label were significantly different between the groups on days 7, 14 and 21($p < 0.05$). Bars with different numeric superscript were not significantly different based on duration ($p < 0.05$).

Variations in urea concentration were dependent on biofloc media used and the setup duration. The level of urea in *C. gariepinus* in CE, NE, CA 10:1 and CA 20:1 were in the same range as the baseline level through the experiment (Figure 3). From day 7 to day 21, urea concentration in *C.*

Table 6: Floc volume (ml) of different carbon sources (n=7, observations)

Groups	Duration (Days)			
	7	14	21	28
A (CA 10:1)	17.33 ± 1.33^{c1}	37.33 ± 2.67^{b2}	17.33 ± 1.33^{b1}	29.33 ± 7.06^{c12}
B (CA 20:1)	56.00 ± 4.62^{a1}	69.33 ± 5.33^{a1}	52.00 ± 6.23^{a1}	50.67 ± 7.06^{ab1}
C (WH 10:1)	32.00 ± 2.31^{b1}	60.00 ± 6.93^{a2}	37.33 ± 10.67^{ab1}	36.00 ± 4.00^{bc1}
D (WH 20:1)	50.67 ± 2.67^{a1}	72.00 ± 8.00^{a1}	65.33 ± 13.92^{a1}	55.33 ± 2.91^{a1}

Values as mean ± S.E. Values with different alphabet within a column were significantly different ($p < 0.05$). Values with different numeric superscript across a row were significantly different ($p < 0.05$). Group A (CE); Group B (NE), Group C (CA 10:1), Group D (CA 20:1) Group E (WH10:1), Group F (WH 20:1).

Table 7: Nutritional composition of flocs produced by local cassava variety and wheat flour

Composition	CA 10:1	CA 20:1	WH 10:1	WH 20:1
Moisture	65.55 ± 3.94^{b}	69.40 ± 1.46^{a}	69.64 ± 1.06^{a}	63.31 ± 0.47^{a}
Ash	0.47 ± 0.05^{a}	0.38 ± 0.02^{ab}	0.29 ± 0.03^{c}	0.31 ± 0.01^{c}
Fats	0.05 ± 0.02^{a}	0.06 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}
Protein	20.95 ± 1.79^{a}	18.65 ± 1.31^{ab}	20.46 ± 1.52^{ab}	21.17 ± 1.62^{b}
Fibre	0.58 ± 0.06^{a}	0.55 ± 0.02^{a}	0.55 ± 0.04^{a}	0.64 ± 0.05^{a}
Carbohydrate	12.40 ± 5.64^{a}	10.96 ± 0.13^{a}	9.02 ± 0.48^{a}	14.52 ± 1.39^{a}
Vitamin A	0.51±0.01^{a}	0.47±0.01^{a}	0.51±0.01^{a}	0.46±0.01^{a}
Vitamin E	0.24±0.02^{a}	0.25±0.02^{a}	0.21±0.02^{a}	0.30±0.04^{a}
Potassium	0.32±0.02^{a}	0.27±0.01^{a}	0.34±0.01^{a}	0.28±0.02^{a}
Calcium	0.47±0.01^{a}	0.39±0.02^{a}	0.42±0.01^{a}	0.43±0.02^{a}
Sodium	0.28±0.02^{a}	0.24±0.02^{a}	0.22±0.01^{a}	0.19±0.01^{b}

Values as mean ± S.E. Values with different alphabet superscript across a row were significantly different ($p < 0.05$)

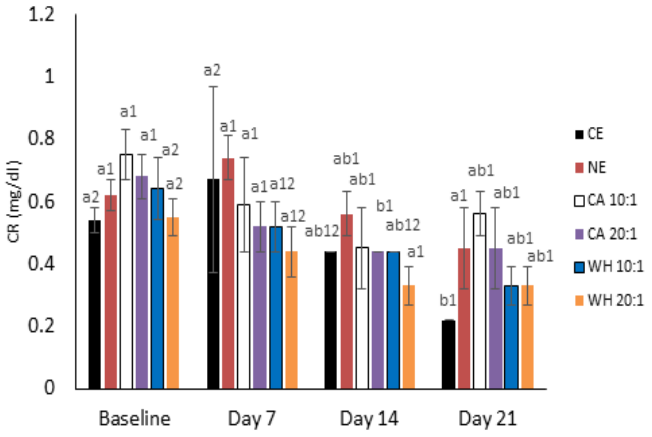

Figure 2: Changes in creatinine under different BFT conditions. Bars with different alphabet label were significantly different between the groups on days 7, 14 and 21($p < 0.05$). Bars with different numeric superscript were not significantly different based on duration ($p < 0.05$).

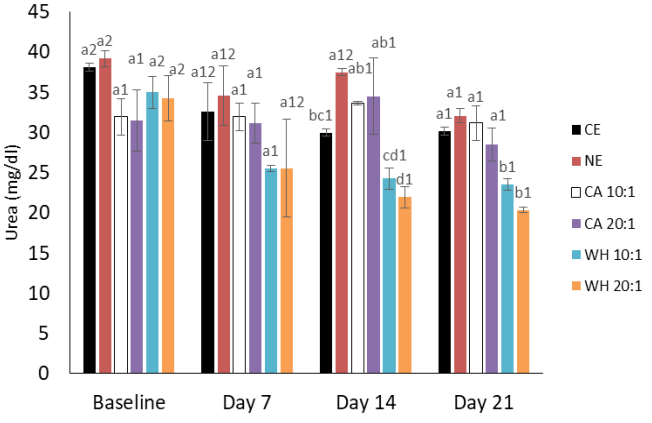

Figure 3: Changes in urea under different BFT conditions. Bars with different alphabet label were significantly different between the groups on days 7, 14 and 21($p < 0.05$). Bars with different numeric superscript were not significantly different based on duration ($p < 0.05$).

gariepinus from WH 10:1, and WH 20:1 did not change significantly ($p > 0.05$). There was no significant difference in urea concentration between the groups on day 7.

Bacteria Counts in different BFT Media

The total heterotrophic bacteria count (THBC) in the baseline was similar. On day 14, there was no significant difference between the groups for THBC ($p < 0.05$). The apparent discrepancies between the THBC among the groups occurred on day 21 (Figure 4). Meanwhile, THBC was significantly higher in CA 20:1, while WH 10:1 produced the highest ($p<0.05$) THBC.

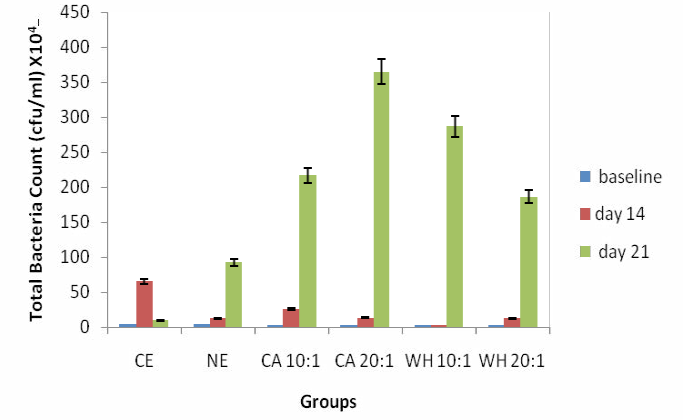

Figure 4: Total heterotrophic bacteria count (cfu/ml) in different biofloc tank

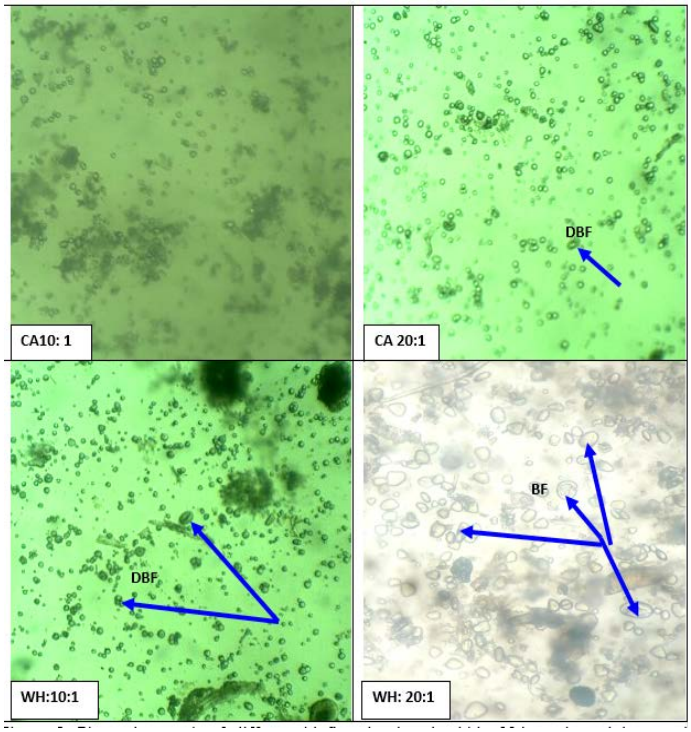

Figure 5: Photomicrograph of different biofloc developed within 28days showed increased macroaggregates biofloc size (**BF**) (blue arrow). Small developed bioflocs (**DBF**). Mag. X100.

Floc Volume under Different Carbon Sources

After 28 days of growth trial (Table 6), the volume of floccules harvested after flocculation was higher in WH 20:1 than WH 10:1 on days 7 to 28. The most minor floc volumes were recorded with a cassava carbon source (CA 10:1) compared to other harvested floc volumes from other groups ($p < 0.05$) on day14. Meanwhile, the floc volume of CA 20:1 and WH 20:1 was within the same range but significantly higher than CA 10:1 and WH 10:1 ($p < 0.05$) on day 7 only.

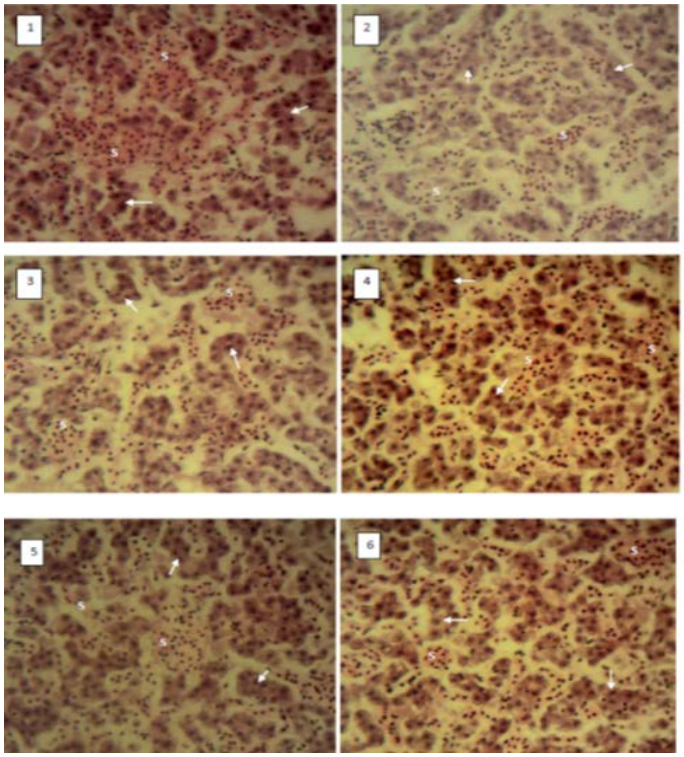

Figure 6: Photomicrograph of liver of *C. gariepinus* showed normal liver histology with presence of Sinusoid (S); Hepatic cord (arrow) in all the experimental groups before raised in biofloc; Groups 1 (CE), 2 (NE), 3 (CA 10:1), 4 (CA 20:1), 5 (WH 10:1) and 6 (WH 20:1). H&E x400.

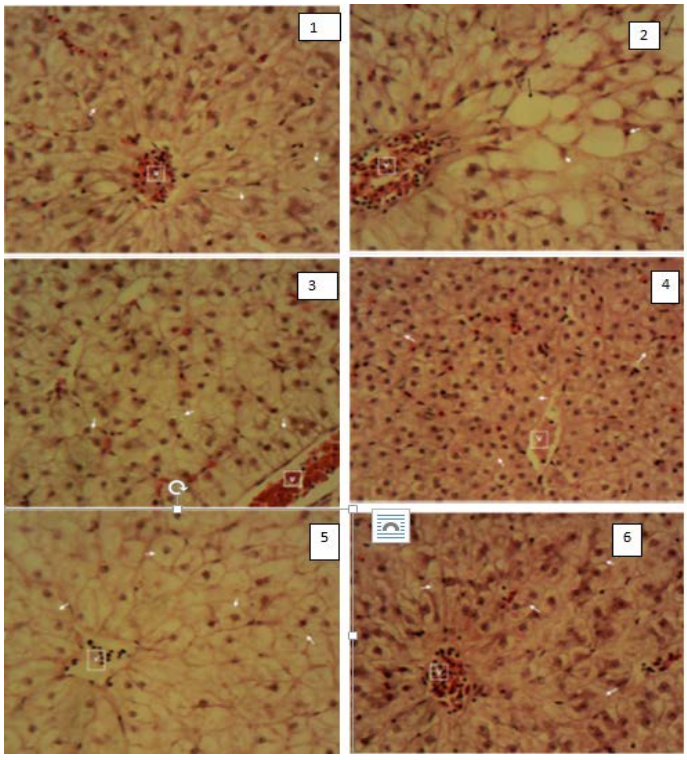

Figure 7: Photomicrograph of liver showed normal liver histology with presence of central vein (Cv) in all the experimental groups; Groups 1 (CE), 2 (NE), 3 (CA 10:1), 4 (CA 20:1), 5 (WH 10:1) and 6 (WH 20:1). H&E x400.

Proximate composition of harvested flocs

Significant changes in the proximate and mineral composition of the floc produced by different carbon sources at the end of the experiment were observed. The fats, fibre, carbohydrate, and ash composition were similar between carbon sources (Table 7).

Microscopic view of biofloc developed by local cassava variety and wheat flour at different C:N ratios

Biofloc developed by other C:N ratios of carbon sources (cassava and wheat flour) within 28 days showed increased macroaggregates biofloc size mostly in the WH 20:1. Small developed bioflocs were observed in the CA 10:1 (Figure 5) followed by CA 20: 1.

Histopathology

The hepatocytes, polyhedral in shape, were arranged in two cell thick laminas/hepatic chords separated by engorged sinusoid containing nucleated red blood cells. Hepatic parenchyma is not organized into distinct lobules with indistinguishable interlobular connective tissues. At the end of the experiment, the liver sections in the groups showed similar indistinct cords of hepatocytes composed of branching anastomosing, two cell thick laminae of hepatocytes radially arranged around the central veins non-obvious portal triad. Cytoplasmic vacuolation with a consequent eccentric displacement of the nucleus was observed in the NE group (Figure 6-7).

DISCUSSION

Physicochemical, growth performance and Biofloc Media

Biofloc technology system supported the regulation of physicochemical parameters of culture medium preventing the accumulation of toxicants and monitoring the pollution status of the medium. From the preceding, the temperature range obtained from the present study ranged between 25 ºC and 27 ºC, which is within the optimal degree of catfish fingerling production. The observed temperature in the experimental group supported the optimal temperature for juvenile *Clarias gariepinus* is 25 -35 ºC. The pH range 7-8 was optimal for the production and survival of *Clarias gariepinus*, which suggested pH stability within the biofloc treatment (Popoola and Miracle, 2022). Uzoka et al. (2015) reported pH 7.8 acceptable for fish production (FAO, 1996). The observed dissolved oxygen concentration from the present study suggested that increased microbial biomass may affect the DO in biofloc culture (Popoola and Miracle, 2022). The continuous stability of DO concentration was attributed to the C:N ratio variation. The TSS was within the range of 21.95-338.35 mg/L. TSS level of 400-600 mg/L was preferred for *Litopeneaus vannamei* production. More so, Ekasari et al. (2016) reported a much higher range of TSS for biofloc (188-1250 mg/L) and non-biofloc (160-1150 mg/L). TAN was generally within the acceptable limits. The initial increase was probably returned to baseline levels in groups provided carbon sources by activities of heterotrophic bacteria that assimilate nitrogen (Hargreaves, 2013). From the present study, TAN was between the ranges of 0.22-0.30mg/L. The TAN level was below 0.35-22.8 mg/L, usually considered toxic for freshwater organisms. The initial increase in TAN between 7 and 14 days was due to metabolic activities, decompositions of leftover feed, and initial lag in establishing heterotrophic and nitrification bacteria (Hagreaves, 2013). The nitrite concentration in the present study was high (1.12-2.41 mg/L) compared to recommended levels for biofloc media, 0.07-0.42 mg/L (Ekasari et al., 2016; Roques et al., 2015) in *Clarias gariepinus* fingerlings culture. The impact of nitrate concentration in the present study is uncertain, especially as the nitrate concentration in control and biofloc media were similar. The activities of the biofloc media did not facilitate the nitrification processes, which would have lowered the nitrite concentration by the production of nitrates. This is the cause of the observed drop of nitrate concentration >0.35 mg/L in all the groups.

Fish cultured in biofloc media did not clearly show a better growth performance than the standard control. Several studies have reported better growth performances in fish (Kuhn et al.,. 2009; Ekasari et al., 2016) and shrimps (Hussain et al., 2015; Sakkaravarthi and Sankar, 2015) cultured using BFT or fed with biofloc formulated feeds compared to those cultured in non-biofloc media or fed commercial feeds. The length-weight relationship of *C. gariepinus* for the duration of the study and in all the treatment groups showed a negative allometric growth pattern. Factors possibly responsible for this observation were physicochemical properties of the water (Nehemia et al., 2012), insufficient feed, lack of suitable feed types, poor feeding (Khatoon et al, 2016), pathogenic stress (da Silva et al, 2016), including deficiencies in experimental setups (Goana et al., 2011). The quantity of microbial biomass generated in proportion to the number of fish per group was insufficient to support growth. Provision of additional carbon sources may serve as feed sources even though inadequate to correct the deficiency. The suitability of microbial biomass generated as feed for the *C. gariepinus* fingerlings is yet another factor that may have affected growth performance and the Length-weight relationship of the fish. The microbial biomass is unsuitable for the fish, so they may have avoided it or fed less on it. Despite choosing cost-effective biomaterials and using bioflocs as food by *C. gariepinus*, the contribution to the growth was comparable to the control.

Floc volume and TSS are valid indicators of biofloc formation (Avnimelech, 2007). In the present study, the increased

high floc volume observed in CN 20:1 was in agreement with the report of El-Husseiny et al. (2018), who recorded the highest floc volume with wheat bran. The floc volume was a little above the safe range of 5-50 ml/L, according to Avnimelech (2011). Meanwhile, the flocs produced may depend on the carbon sources utilized (Zhou et al., 2002). This indicated that the nature of carbohydrates affects the quantum of biofloc produced by the application of simple sugars.

Biochemicals

The biochemical liver markers such as AST, ALT and ALP did not change significantly in all the groups, indicating non-hepatoxic conditions. The levels of these enzymes increased in the NE, which may indicate possible exposure to pollution stress than other experimental groups. Provision of aeration and sometimes additional carbon sources by BFT caters to water pollution and the need for water exchange (Ekasari et al., 2016; Crab et al. 2007); in NE, the problem of water pollution was obvious. The observed increase in creatinine and urea in NE compared to other groups may indicate higher absorption of nitrogen-related toxicants (e.g. ammonia and nitrite).

Production of reactive oxygen species (ROS) and antioxidant capacity was due to oxidative stress in *C. gariepinus* caused by chemical or physical stressors. Under normal conditions, animal cells produced ROS such as H_2O_2, OH^- and O^-, damaging cellular components leading to cell death. From the present study, the catalase activity played a crucial role in the antioxidant defence of cells by reducing H_2O_2 and O_2 was critical for scavenging free radicals (Bello et al., 2000). The BFT increased the CAT activities of fish which supported the report of Popoola and Miracle (2022), who stated that BFT increased the CAT and GPx activities of *Clarias gariepinus*. Meanwhile, SOD activities catalyzed the destruction of superoxide radicals, and their activity indicated the tissues coping with oxidative stress (Misra, 1972). The increased SOD level in the BFTs group was attributed to lower oxidative stress caused by the bioflocs. These findings supported the report of Long et al. (2015), Popoola and Miracle (2022). Glutathione peroxidase catalyzes the reduction of hydrogen peroxides by glutathione and protects the body from oxidation and damaging effect of endogenously formed hydrogen peroxides. The observed changes in the GPx activities were due to the sequestering potentials of GPx in eliminating and scavenging free radicals from the body of the fish raised in the Biofloc tank. Lipid peroxidation occurred when fatty acids came in contact with ROS produced a series of reactive aldehyde, including MDA. The changes in the MDA levels of the fish raised in biofloc may be due to a reduction in the level of radicals produced.

Nutritional composition

Floc biomass provided a complete source of nutrition and various bioactive compounds (Akiyama et al., 1992). Different factors such as carbohydrate source, microbial community, floc density etc., affect the nutritional value of bioflocs (Hargreaves, 2006). Meanwhile, the type of carbon source also influences the palatability and digestibility of the cultured organisms (Crab et al., 2009; Crab, 2010a). The protein content of biofloc in the present study is in the same range with findings of Soares (2004), who reported a crude protein level of 16-20%, while Hende et al. (2014) wrote 15.8-27% crude protein. A reduced level of biofloc crude protein was reported by Magondu (2012) and Megahed (2014). Bakhshi et al. (2018) said crude protein of 21-25% in biofloc is utilized for carp farming. Hence, the nutritional composition of the floc obtained in the present study (18.65%-21.17% protein, 9.02-14.52% carbohydrate, and 0.04-0.06% fat) was appropriate for fingerlings, despite the reduced lipid content observed. The reduced lipid content was because biofloc is a poor source of lipid (Azim et al., 2008). The variations in the nutrient composition of harvested flocs from biofloc tanks were due to the cultured species' different carbon sources and feeding preferences. These findings agreed with the reports of Khanjani et al. (2016), who opined that wheat flour resulted in high protein levels as observed in the biofloc produced by WH 20:1.

Histopathology

The liver is an essential organ in nutrient metabolism, and tissue changes are considered a reliable indicator in the evaluation of toxic conditions (Bakhshi et al., 2015). No histological changes were observed in the control (CE) and biofloc media in the present study. This result supported the assertion of Bakhshi et al. (2016), who confirmed no changes in proteolytic enzymes, liver histology in common carp raised in biofloc with zero water exchange. He further reported no change in the liver tissues. Meanwhile, in the NE, slight changes in the liver tissues characterized by cytoplasmic vacuolation indicated hepatoxicity, supported by the reports of Stefan et al. (2008).

CONCLUSION

The adjustable C:N ratio of cassava and wheat flour in flocculation is essential, efficient, and indicated a positive reduction of inorganic nitrogen and noxious metabolites produced in the culture system of African catfish, *Clarias gariepinus*. It is pertinent to observe the peculiar attributes of natural carbohydrates sources such as locally available, not harmful to the cultured fin and shellfish, and cheap. Fish culture using BFT is more beneficial for better growth performances with a reduced pathophysiology changes. CA 10: 1 and WH 20:1 is better utilized in *Clarias gariepi-*

nus culture compared to CA 20:1 and WH 10: 1. There is a need to investigate other cheaper sources of carbohydrates such as white yam tuber (*Dioscorea rotundata*), water yam (*Dioscorea alata*), cocoyam (*Xanthosoma sagittifolia, Colocasia esculenta*) and relevant agricultural by-product as natural carbon sources in culture of finfish in a biofloc media.

AUTHORS CONTRIBUTIONS

Chika Bright Ikele designed the experiment, proof read the manuscript and provided technical information of the research. Uju, Aghaji, conducted the experiment by routinely monitoring the experiment. Mgbenka, Obialo proof read the manuscript and analyzed the data.

ETHICAL APPROVAL

The fish were handled following the approved regulatory guidelines of the University of Nigeria, Nsukka Senate Committee on Medical and Research Ethics (UNN-ACC, Protocol No. 0764/2015) and does not contain clinical or patient data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

Research Data are not shared.

REFERENCES

A.O.A.C (Association of Official Analytical Chemists). (1989). Official Methods of Analysis, 17th Edition, Washington, D.C.; U.S.A.

Aebi H (1984). Catalase in vitro. Methods in Enzymol. 105: 121-126

Akiyama D, Doming W.G., Lawrence A.L. (1992) Penaeid shrimp nutrition. In: Fast AW, Lester LJ (eds) Marine shrimp culture: principles and practices. Elsevier, Amsterdam, pp 535–568

APHA (1999) Standard Methods for the Examination of Water and Waste Water (19th ed.). American Public Health Association, Washington, DC. 1082 pp.

Avnimelech Y (2011). Tilapia production using biofloc technology, saving water, waste recycling improves economics. Global aquaculture Advocate,. 66-68.

Avnimelech, Y (1999). Carbon and nitrogen ratio as a control element in aquaculture systems. Aquaculture., 176: 227–235.

Avnimelech, Y (2007). Feeding with microbial bioflocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture., 264: 140–147.

Azim ME, Little DC (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture, Amsterdam., 283(1-4): 29-35.

Azim ME, Little DC (2006). Intensifying aquaculture production through new approaches to manipulating natural food. CAB Reviews: Perspectives in Agriculture, Vet. Sci. Nutrit. Nat. Resou. 062, 23 pp

Bakhshi F, Najdegerami EH, Manaffar R, Tukmechi A, Farah KR (2018). Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture, 2018; 48(4): 259-267. http//doi.org/10.1016/j.aquaculture.2017.11.036.

Bancroft JD, Cook HC (1994). Manual of Histological Techniques and Their Diagnostic Application. Churchill Livingstone, 457

Bello ARR, Fortes E, Bello-Khlein AA (2000). Lipid peroxidation by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhandia quelen*. Dis. Aquat. Organism. 42: 233-238.

Bossier P, Ekasari J (2017). Biofloc technology application in aquaculture to support sustainable development goals. Microb. Biotechnol. 10(5): 1012-1016.

Caipang C.M.A., Choo H.X., Bai Z., Hunag H, Lay-yag C.M. (2015). Viability of sweet potato flour as carbon source for the production of biofloc in freshwater culture of tilapia, Oreochromis sp. Int. Aquat. Res. 7(4): 329-336.

Castro M.G., Castro J.M., Ramírez J.N., Castro A.E.C (2018). Zooplankton growth in a biofloc system with different carbon sources in a *Cyprinus carpio* culture. International J. Fisher. Aquat. Stud. 6(6): 253-258

Correia E.S., Wilkenfeld J.S., Morris T.C., Wei L., Prangnell D.I., Samocha T.M (2014). Intensive nursery production of the Pacific white shrimp, *Litopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system. Aquaculture Engineering, 2014; 59, 48–54. https://doi.org/10.1016/j.aquae ng.2014.02.002

Crab R., Kochva M., Verstraete W., Avnimelech Y. (2009). Bioflocs technology application in over-wintering of tilapia. Aqua. Enginr. 40: 105-112. https://doi.org/10.1016/j.aquaeng.2008.12.004.

Crab R., Chielens B., Wille M., Bossier P., Verstraete W (2010). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquacult. Res. 41: 559–567. https://doi.org/10.1111/j.1365-2109.2009.02353.x

Da Silva Campos, C.V.F., da Silva Farias R. da Silva S. M.B.C. et al (2020). Production of *Daphnia similis* Claus, 1876 using waste water from tilapia cultivation in biofloc system. Aquaculture international, 2020; 28:403-419.

Ekasari J., Zairin M., Putri D.U., Sari N.P., Surawidjaja E.H., Bossier P (2016). Biofloc based reproductive performance of Nile tilapia, *Oreochromis niloticus* L., broodstock. Aquacult. Res. 46(2): 509-512.

El-Husseiny O.M., Soliman A.Z.M, Elsherif H.M.R (2018). Influence of Dietary Methionine, Folic Acid and Cyanocobalamin and their Interactions on the Performance of Broiler Breeder. Int. J. Poult. Sci., 17: 189-196. https://doi.org/10.3923/ijps.2018.189.196

El-Sherif M. Mohamed A., El-Feky I. (2009). Performance of Nile Tilapia (Oreochromis niloticus) Fingerlings. I. Effect of pH. Int. J. Agric. Bio. 11

FAO (1996). World food summit. Rome, Italy.

Gaona C.A.P., Poersch L.H., Krummenauer D., Foes G.K., Wasielesky W.J. (2011). The Effect of Solids Removal on Water Quality, Growth and Survival of Litopenaeus vannamei in a Biofloc Technology Culture System. Int. J. Recircul Aqua. 12: 54-73.

Hamidoghli A., Won S., Aya F.A., Yun H., Bae J., Jang I., Bai S.C (2019). Dietary lipid requirement of white leg shrimp *Litopenaeus vannamei* juveniles cultured in biofloc system. Aquaculture Nutrition, 2019; 00:1–10. https://doi.org/10.1111/anu.13021

Hargreaves JA (2013). Biofloc production systems for aquaculture. Southern regional Aquaculture Center, United States Department of Agriculture, National Institu. Food Agricult. n. 4503, 12 p.

Hussain A.S., Mohammad D.A., Ali E.M., Sallam W.S (2015). Growth Performance of the Green Tiger Shrimp *Penaeus semisulcatus* Raised in Biofloc Systems. J. Aquacult. Marine Biol. 2(5): 1-10.

Karunaarchchi K.A.C.M., Kumari M.A.A.P., Adikari A.M.J.B., Nayananjalie W.A.D (2018). Effect of biofloc on growth of genetically improved farmed tilapia juveniles in indoor condition. Int. J. Fisher. Aquat. Sci. 6(4): 295-299.

Khanjani M.H., Sajjadi M.A., Mortez S.I (2016). Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: The effect of adding different carbon sources. Aquaculture Research, 2016; 48. https://doi.org/10.1111/are.12985.

Khatoon H., Banerjee S., Yuan G.T.G., Haris N.,Ikhwanuddin M., Ambak M.A., Endut A (2016). Biofloc as a potential natural feed for shrimp postlarvae. Int. Biodeteriorat. Biodegrad. **113**: 304 - 309.

Kuhn D.D., Boardman G.D., Lawrence A.L., Marsh L., Flick G.J. Jr (2009). Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture, 2009; 296, 51–57. https://doi.org/10.1016/j.aquaculture.2009.07.025

Lananan F., Abdul Hamid S.H., Din W.N.S., Ali N.A., Khatoon H., Jusoh A., Endut A (2014). Symbiotic bioremediation of aquaculture wastewater in reducing ammonia and phosphorus utilizing Effective Microorganism (EM-1) and microalgae (Chlorella sp.). Int. Biodeteriorat. Biodegrad. 95: 127–134. https://doi.org/10. 1016/j.ibiod.2014.06.013

Lawrence R.A., Burk R.F (1976). Gluthathione peroxidase activity in selenium-deficient rat liver. Biochemical and Biophysical Research Communication, 1976; 71: 952-958.

Lima P.C., Abreu J.L., Silva A.E., Severi W., Galvez A.O., Brito L.O (2018). Nile tilapia fingerling cultivated in a low-salinity biofloc system at different stocking densities. Spanish J. Agric. Res. 16(4): 0612. https://doi.org/10.5424/sjar/2018164-13222

Liu G., Ye Z.Y., Liu D.Z., Sivaramasamy E., Deng Y., Zhu S.M (2018). Influence of stocking density on growth, digestive enzyme activities, immune responses, antioxidant of, *Oreochromis niloticus,* fingerlings in biofloc systems. Fish Shellfish Immunol., 81: 416–422. https://doi.org/10.1016/j.fsi.2018.07.047.

Long L.N., Yang J., Li Y., Guan C.G., 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. https:// doi.org/10.1016/j.aquaculture.2015.05.017.

Magondu E., Charo-Karisa H., Verdegem M. (2013) Effect of C/N ratio levels and stocking density of Labeo victorianus on pond environmental quality using maize flour as a carbon source. Aquaculture; 410–411: 157–163.

Megahed M., Mohamed K (2014). Sustainable Growth of Shrimp Aquaculture through Biofloc Production as Alternative to Fishmeal in Shrimp Feeds. J. Agric. Sci. 6. 10.5539/jas.v6n6p176.

Misra A., Fridrovich I (1972). The role of superoxide anion in the autooxidation of epinephrine and a simply assay for superoxide dismuatase. J. Biol. Chem., 247: 3170 – 3175.

Mota G.C.P., Figueiredo C.V., Campos D., Silva de Moraes L.B., Bruzaca D.N.A., Brito L.O., Gálvez A.O. (2019). Effect of the c:n ratio on *Daphnia magna* (Straus, 1820Bol. Inst. Pesca 2019, 45(3): e463. https://doi.org/10.20950/1678-2305.2019.45.3.463

Nehemia A, Maganira J., Rumisha C (2012). Length-Weight relationship and condition factor of tilapia species grown in marine and fresh water ponds. Agric. Bio. J. North Am. 3: 117-124. https://doi.org/10.5251/abjna.2012.3.3.117.124.

Nguyen T.D, Ly V.K. (2018). Effect of molasses addition at different C:N ratios on growth and survival rate of spotted scat (*Scatophagus argus*) fingerlings in biofloc system. Int. J. Scient. Res. Publicat. 8(5): 452-459. https://doi.org/10.29322/USRP.8.5.2018.P7759

Panigrahi A., Sundaram M., Chakrapan S., Rajasekar S., Dayal J.M., Chavali G (2017). Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. Aquacult. Res., 50:29–41. https://doi.org/10.1111/are.13857

Piedrahita RH (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture., 226(1): 35-44. https://doi.org/10.1016/S0044-8486(03)00465-4

Popoola OM, Miracle OA (2022). Peformance of different biomaterials as carbon sources on the immunological response and oxidative status of African catfish, Clarias gariepinus in biofloc system. Aquacult. Stud., 22(2): AQUAST800. http://doi.org/10.4194/AQUAST800.

Pormohammad A., Mohammadabadi T., Chaji M., Mirzadeh K., Marammazi J (2018). Study of biofloc production from fish breeding system and using as supplement in ruminant diet. Basrah J. Vet. Res., https://doi.org/10.33762/bvetr.2018.143556

Prabu E., Rajagopalsamy C. B. T., Ahilan B., Jegan M.A.J., Renuhadevi M. (2018). Effect of dietary supplementation of biofloc meal on growth and survival of GIFT tilapia. Indian J. Fish, 65(1): 65-70. https://doi.org/10.21077/ijf.2018.65.1.72074-11.

Reitman S, Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic transaminases. American J. Clin. Pathol. 28: 56-63.

Roques J.A.C., Tom S., Tirsa V.S., Wout A., Jeroen B., Pepijn V., Hans van de Vis GF (2015). The impact of elevated water nitrite concentration on physiology, growth and feed intake of African catfish Clarias gariepinus (Burchell 1822). Aqua. Res., 46(6): 1384-1395. https://doi.org/10.1111/are.12292.

Sakkaravar K., Sankar G. (2015). Identification of Effective Organic Carbon for Biofloc Shrimp Culture System. J. Biol. Sci. 15. 144-149. https://doi.org/10.3923/jbs.2015.144.149.

Schveitzer R., Arantes R., Costódio P., Fóes S., do Espírito S, Carlos M (2013). "Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquacult. Eng., 56: 59-70.

Sharma S.K., Krishna-Murti C.R (1968). Production of lipid peroxides by brain. J. Neurochem. 15: 147-149.

Silva U.L., Falcon D.R., Pessôa M.N.D.C., Correia E.D.S. (2017). Carbon sources and C:N ratios on water quality for

Nile tilapia farming in biofloc system. Revista Caatinga,. 30(4): 1017–1027. http://dx.doi.org/10.1590/1983-21252017v30n423rc

Stefan L., Johannes, H., Stephan K., Christian F., Peter R.G., Hans P.D., Christoph S., Ansgar W.L. (2008). Serologic markers compared with liver biopsy for monitoring disease activity in autoimmune hepatitis. J. Clin Gastroenterol. 42(8):926-30

Ukwe, I.O., Abu, O.M. Physico-chemical parameters of water in holding tanks of *Clarias gariepinus* Induced with ovaprim and ovulin hormones. International Journal of Innovative Studies in Aquatic Biology and Fisheries, 2016; 2 (4), 12-19.

Yun, H., Shahkar, E., Katya, K., Jang, I.K., Kim, S.K., Bai, S.C (2016). Effects of bioflocs on dietary protein requirement in juvenile whiteleg shrimp, *Litopenaeus vannamei*. Aquacult. Res. 47, 3203–3214.

Zaki M.A.A., Alabssawy A.N., Nour A.M., El Basuini M.F., Dawood M.A.O., 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. Aquacult. Rep., 16: 1-8. https://doi.org/10.1016/j.aqrep.2020.100282

Zapata-Lovera K.P., Brito L.O., Lima P.C.M., Vinatea L.A.A., Gálvez A.O., Cárdenas J.M.V. (2017). Cultivo de alevines de tilapia en sistema biofloc bajo diferentes relaciones carbono/nitrógeno. Boletim do Instituto de Pesca., 43(3): 399-407. https://doi.org/10.20950/16782305.2017v43n3p399

Zhou Z., Robards K., Helliwell S., Blanchard C (2002). Composition and functional properties of rice. International J. Food Sci. Technol. 37: 849–868.