

Performance of Different Biomaterials as Carbon Sources on the Immunological Response and Oxidative Status of African Catfish *Clarias gariepinus* in Biofloc Systems

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Abstract

A trial was performed to investigate how carbon sources can affect the welfare status of African catfish (3.8±0.2g and 8±0.11 cm) juveniles in biofloc systems. Inocula was prepared in glass tanks (5L) by adding 20g of pond bottom soil in well aerated water (1L) containing 10mg L⁻¹ ammonium sulphate (NH)₄SO₄ and 400mg L⁻¹ of four different carbon sources (tapioca, wheat offal, brewery and cassava peel) for 24 hours. Each treatment group (carbon sources and control) were replicated and each tank contained 1000 *Clarias gariepinus* juvenile, fed with commercial feed (Crude protein 42%) at 5% of their body weight 72 days. The water quality showed that all water parameters remained at concentrations suitable for *Clarias gariepinus* culture in the studied systems. The enzymes activities were noticed to be different across the biofloc and the selected organs. There were significant differences in serological content in fish between the treatment's groups (P<0.05). The significant difference was found between the treatments in case of enzymes activity (P<0.05). The study shows that the welfare status with reference to digestive enzymes activity, oxidative status and extent of the immune system stimulation in BFT system is carbon source dependent.

Introduction

There are nearly seven billion people on the planet and the consumption of aqua food is rising, necessitating the growth and improvement of fish production. Fish farming, has a lot of potential for lifting people out of poverty, starvation, and malnutrition, as well as generating economic growth and ensuring better resource management (FAO, 2017). The FAO estimates that aquaculture production will increase from 40 million tonnes in 2008 to 82 million tonnes in 2050 (FAO, 2010). The increasing need of the world population has triggered the need to enhance aquaculture production. The ultimate focus of aquaculture development should be to generate more aquaculture products while using

fewer basic natural resources like water and land (Avnimelech, 2009). Also, production of environmentally friendly, long-term aquaculture systems (Naylor et al., 2000) and the develop systems that support economic and social sustainability by delivering an appropriate cost-benefit ratio (Avnimelech, 2009). Biofloc technology can provide all three of these requirements for long-term aquaculture development.

Biofloc technology (BFT) has been studied at several occasions and contributes to the maintenance of good water quality in the system and to the nutrition of the cultured animals (Avnimelech, 1999; Arnold et al., 2009; Megahed, 2010; Wasielesky et al., 2006; Crab et al., 2010; Buford et al., 2004; Hari et al., 2004, 2006). The

basic principle of the bioflocs system is to recycle waste nutrients, in particular nitrogen, into microbial biomass that can be used in situ by the cultured animals or be harvested and processed into feed ingredients. Heterotrophic microbial aggregates are stimulated to grow by steering the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source, so that the bacteria can assimilate the waste ammonia for new biomass production. A wide range of microorganisms and their cell components have been applied as probiotics or immunostimulants in order to improve the innate immunity, antioxidant status and disease resistance of aquatic organisms (Smith et al., 2003 and Vazquez et al., 2009). Bioflocs are rich in various bioactive compounds like carotenoids, chlorophylls, polysaccharides, phytosterols, taurine and fat-soluble vitamins (Jang et al., 2011). Intensive aquaculture system, especially in *Clarias gariepinus*, is characterized by high stocking density with a well formulated diet or feeds (Piedrahita, 2003), the high biomass and feed input negatively affect water quality, which in turn affect the health of the fish. It is therefore imperative to put in place an active water quality management like biofloc system to improve the water quality challenges in production system. However, little effort has been made to study the effect of bioflocs on physiological health and non-specific immunity of cultured fishes. Most studies on BFT focusses mainly on shrimps and it has shown to improve water quality in shrimp culture ((Megahed, 2010; Crab et al., 2010; Zhao et al., 2012), growth and digestive enzymes (Xu et al., 2012, Hargreaves, 2006; Becerra-Dorame et al., 2011). Few research has been carried out on the positive effect of bioflocs on fish has been carried out growth performance, immune response and digestive enzyme activity of fish, tilapia (Avnimelech, 2007; Azim and Little, 2008), in *Labeo rohita* (Verma et al., 2016). Studies have been carried out on effect of Bioflocs on *Clarias gariepinus* but this study is set out to examine the performance of different bio materials (cassava peel, tapioca, wheat offal and brewery waste) being readily available at affordable price with little effect on production cost, as carbon sources for Bioflocs on the water quality dynamics as well as the growth performance, biochemical composition, and digestive enzyme activities of African cat fish *Clarias gariepinus* with a few to recommend the best carbon source to *Clarias gariepinus* fish farmer.

Materials and Methods

Experimental Procedure

A total of 15000 African catfish (*Clarias gariepinus*) were obtained from a reputable farm and delivered to the Teaching and research farm, Federal University of Technology Akure. The fishes were stocked immediately into rectangular concrete acclimation tanks with

dimension of 2m x 1m x 1m for 14 days with gentle aeration. The fishes were fed with commercial feed of 42% crude protein. After the acclimation period, the fish were sorted into similar sizes (Initial mean weight \pm SE was 3.8 \pm 0.2g) and stocked in the experimental concrete tanks (2m x 1m x 1m).

During the period of acclimation, inoculum was prepared by adding 20g of pond bottom soil in well aerated water (1L) containing 10mg L⁻¹ ammonium sulphate (NH₄)₂SO₄ and 400mg L⁻¹ of different carbon sources (tapioca, wheat offal, brewery and cassava peel). The suspension was incubated for 24 h for development of microbial growth. The bioflocs tanks were inoculated following the method described by Xu and Pan (2012) with modifications. The inoculum was the added to each of the ten aquaria prior to stocking the catfish.

Experimental Design

A total of five treatments with different biomaterials as carbon sources (cassava peel, tapioca, wheat offal and brewery waste including control) to create a carbon nitrogen ratio of 20 based on the calculation of De-Schryver et al., (2008). The selection of carbon sources was due to their availability and cost friendliness to farmers. The experimental groups were duplicated with 100 fish (Initial mean weight) and each replicate was filled with 500l of fresh water. Prior to the stocking of the fish in the tank 200 mg L⁻¹ the prepared inocula were added to the tank and vigorous aeration was carried out using air blower installed at 15 lines (5 l/min per line).

Fish were fed at 3% of their body weight with the same commercial feed used during the acclimation period. The control received 70% water exchange every week. Water was added to the treatment to make up for the amount loss due to evaporation. The fishes were weighed bi-weekly to adjust the amount of food to daily add.

Water Quality Parameter

The temperature, pH, dissolved oxygen, total dissolved solids and conductivity were measured weekly using EXTECH instrument ExStik II according to manufacturer's instruction.

Final sampling

At the end of 72 days the remaining fish were weighed for their growth performance, feed efficiency and productive parameters were determined using the following equations:

$$SGR = \frac{\ln(\text{mean final weight}) - \ln(\text{mean initial weight})}{\text{Numbers of culture days}} \times 100$$

SGR: Specific Growth Rate

$$\text{Biomass gain} = \text{Harvested biomass} - \text{Stocked biomass}$$

$$\% \text{Biomass gain} = \frac{(\text{Harvested Biomass} - \text{Stocked Biomass}) \times 100}{\text{Stocked Biomass}}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Total feed intake}}{\text{Biomass gain}}$$

$$\text{Feed Efficiency Ratio} = \frac{1}{\text{Feed Conversion Ratio}}$$

Immunological and Oxidative Status

Fish was anaesthetized to collect blood sample from the caudal vein with the use of 27 GT needle attached to a 2 mm syringe. EDTA coated vials were used to collect the blood and to separate the serum, the blood was allowed to clot and centrifuged. The serum sample was analysed for glucose, albumin, total protein and globulin level using a kit (Beacon diagnostics Pvt. Ltd, India) with little modification to the manufacturer protocol. Blood glucose level was estimated by the method of Nelson (1944) and Somoyogi (1945). Blood was de-proteinized with zinc sulphate and barium hydroxide and filtered, and the supernatant was used for glucose estimation. The absorbance was recorded at 540 nm against the blank. Total protein was quantified colorimetrically by BCA method using Genei protein estimation kit (Cat. No. 105569). Albumin was quantified using bromocresol green binding method by Doumas et al., (1971). Globulin was quantified by subtracting albumin values from total plasma protein. Albumin-globulin ratio (A/G ratio) was determined through dividing albumin values by globulin values.

GPx activity in the sample was determined according to the method adopted by Rotruck et al., (1973). The reaction mixture containing 500 μ l phosphate buffer, 100 μ l of sodium azide, 200 μ l GSH, 100 μ l H₂O₂ were added to 500 μ l of the sample, after which 600 μ l of distilled water was added and mixed thoroughly. The whole reaction mixture was incubated at 37°C for 3 minutes after which 0.5 ml of TCA was added and thereafter centrifuged at 3000 rpm for 5 minutes. To 1 ml of each of the supernatants, 2 ml of K₂HPO₄ and 1 ml of DNTB was added and the absorbance was read at 412nm against a blank. In determining the SOD activity, a dilution of 1 ml of the sample was made with 9ml of distilled water to make a 1 in 10 dilutions. An aliquot of the diluted sample was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference curvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 secs for 150 secs. Catalase activity was determined according to the method described by Aebi, (1974). Sample (70 μ l) was mixed with 920 μ l Na-PO₄ buffer pH 7 containing 0.1 mM EDTA. The reaction started by adding 10 μ l of H₂O₂. The decrease in H₂O₂ concentration was

taken by reading the absorbance at 240 nm (10 seconds intervals) for 180 seconds.

Assay of Digestive Enzymes

Three fish was removed from each (replicate) with a total of fifteen fish from each treatment were randomly sampled, euthanized with clove powder (200 mg L⁻¹) and dissected to collect the liver, Stomach and intestine. They were homogenized in 100 mM Tris-HCl buffer with 0.1 mM EDTA and 0.1% triton X-100 at 9:1 ratio (pH 7.8) in an electric homogenizer. All these processes were performed on ice. The homogenate was centrifuged at 20000g for 20 min at 4°C, supernatant collected, and then stored at -20°C for further analysis. Total protease activity was assayed at 25°C using 2% (w/v) casein (Hammerstan casein, Merck, Germany) as a substrate in 0.2 M carbonnate buffer at pH 10.0 (Varley et al., 1995). Casein solution (0.5 ml) with an equal volume of homogenized samples (diluted enzyme solution) was incubated at 55°C. After 10 min, the reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid. The mixture was centrifuged and to the supernatant was added 5 ml of 0.44 M Na₂CO₃ and 1ml of two-fold diluted Folin Cioalteau reagent. After 30 min, the colour developed was read at 660 nm against a reagent blank prepared in the same manner. Tyrosin was used as a standard, and one unit of proteolytic activity and pepsin was defined as the amount of enzyme required for the formation of 1 mg of tyrosin per min. Amylase activity was determined by first preparing DNS reagent then the standard solution of maltose (8 μ moles/l) in test tubes. 1 ml of D.N.S. reagent was added in each tube and the mixture is agitated for a few seconds on vortex mixer. The samples were placed in a water bath (T=100°C) for 5 min and allowed to cool at room temperature. About 5 ml of deionized water was added in each sample, followed by agitation. The absorbance (A) of the samples was measured at λ =540 nm. A standard curve is being drawn, the absorbance of each one of the unknown samples is measured and the concentration of the converting sugars is determined, based on the standard curve.

Trypsin activity was assayed according to the method of Erlanger et al., (1961) and Chong et al., (2002) with little modification. The reaction was initiated by mixing 200 mL of the enzyme solution to 2.8 mL of the pre incubated reaction mixture containing 1 mM BAPNA as substrate in 50 mM Tris-HCl buffer (pH 7.5) with 20 mM CaCl₂. The mixture was incubated at 25°C and the release of p-nitroaniline was measured at 410 nm. Trypsin activity was then calculated using the formula described by Erlanger et al., (1961). Lipase activity was determined with p-nitrophenyl palmitate (pNPP) by the method reported by Licia et al., (2006). The substrate for this reaction was composed of solution A and solution B. Solution A contained 40 mg of pNPP dissolved in 12 ml isopropanol. Solution B contained 0.1 g of gum arabic and 0.4 ml of triton X-100 dissolved in 90 ml of water.

This was incubated at 40°C for 45 min. The enzyme activity was stopped by adding 0.2 ml of isopropanol. The absorbance was measured at 410 nm against substrate free blank. The standard graph was prepared by using para-nitro phenol (0.4 to 4 μmoles). One lipase unit (U) is defined as the amount of enzyme that liberated 1 μmol p nitrophenol per min under the assay conditions described (Maia et al., 1999).

Statistical Analysis

The data was tested for normality and heterogeneity of variance using Kolmogorov- Smirnov test and Levene’s respectively. All data were subjected to analysis of variance (ANOVA) at a level of significance of 0.05 (95% confidence) and results were presented as Mean ± SE. Duncan’s multiple range tests was used to examine significant differences among the treatments. All analysis was performed using IBM SPSS statistics version 22 for windows.

Results

Water Quality Parameter

The physio chemical parameters of the water observed during the trial revealed that salinity, total dissolved solid and conductivity show similar trends among the treatments. The salinity value was seen to be lower in the control and highest in with tapioca media. The TDS and conductivity value was highest in wheat offal and brewery waste respectively with control having the least value. (Figure 1). The values obtained for temperature, dissolved oxygen and pH in each of the

treatment show differences but the observed differences did not follow a regular pattern. The difference observed in pH, temperature, dissolved oxygen, total dissolved solid and conductivity between treatments (control and biofloc media) remained at concentrations suitable for *Clarias gariepinus* culture in the studied systems (Figure 1).

Growth Performance

No Significant difference was observed in the growth performance of fish in all the treatment groups (P<0.05), the FCR were not significantly different showing that the fish effectively utilized the feed. Tapioca based treatment registered lowest FCR (0.64±0.03) compared to 0.66±0.05 obtained in control. Specific growth rate did not vary significantly among different treatments (Table 1)

Digestive Enzymes

The digestive enzymes activities in *C. gariepinus* were noticed to be different across the biofloc and selected organs as presented in Figure 2-5. The amylase activity was seen to be highest in the control across the selected organs and decrease across the biofloc (Cassava peel > Tapioca > Wheat offal > Brewery waste). The activity of amylase was observed to be highest in the liver, intermediate in the stomach, and lowest in the intestine. The Specific activity of protease was observed to produce no significant difference across the treatments (biofloc and control) and the selected organs. Lipase activity observed in the stomach and liver of the reared fish was high for cassava peel biofloc,

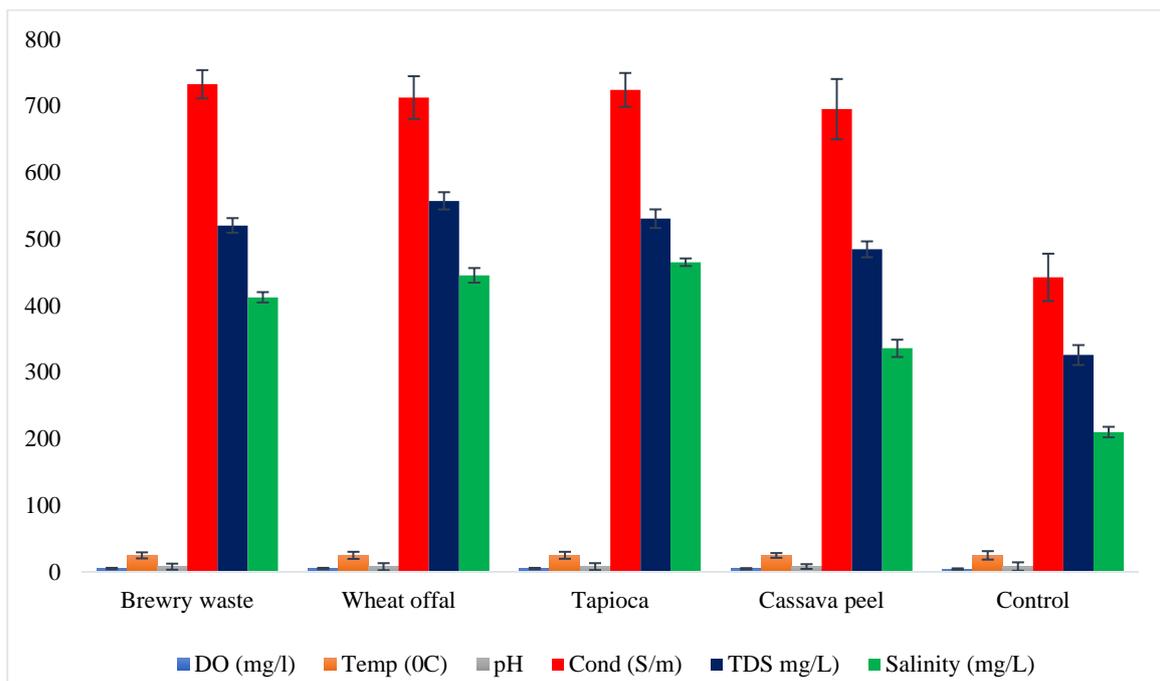


Figure 1. Physicochemical parameters of the biofloc systems in raising *C. gariepinus* (mean±SEM, n=3) for 72 days. Significant difference (P<0.05) from the control is indicated by asterisks.

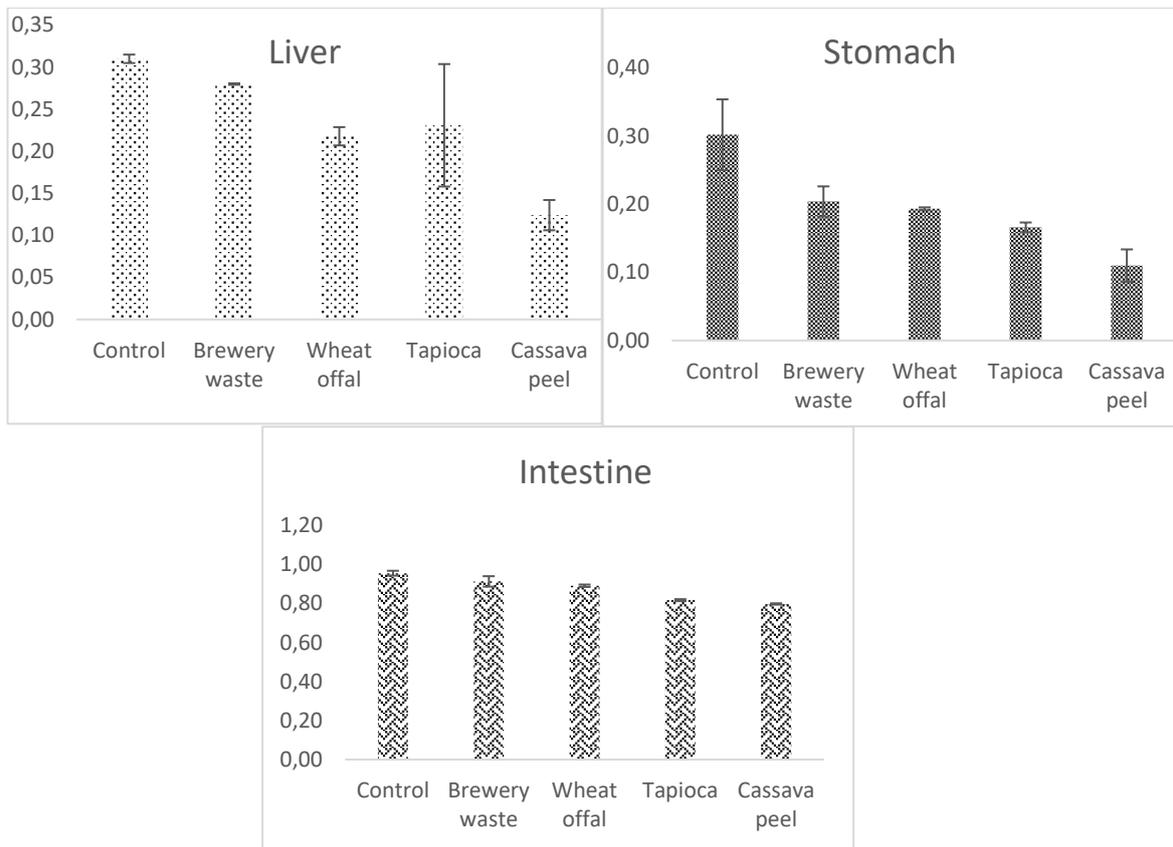


Figure 2. Specific activity of amylase (mg min⁻¹mg⁻¹protein) in *Clarias gariepinus* juveniles reared in different biofloc systems

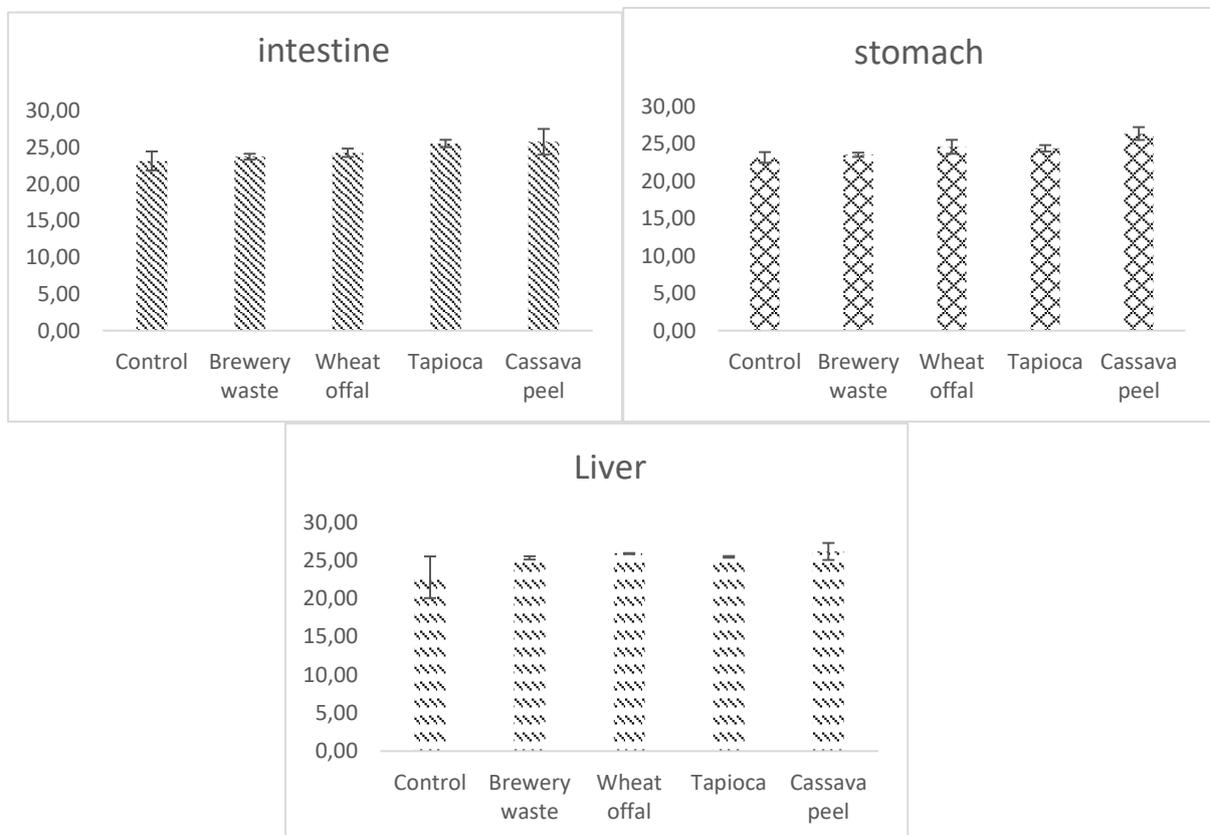


Figure 3. Specific activity of protease (µg min⁻¹mg⁻¹protein) in *Clarias gariepinus* juveniles reared in different biofloc systems

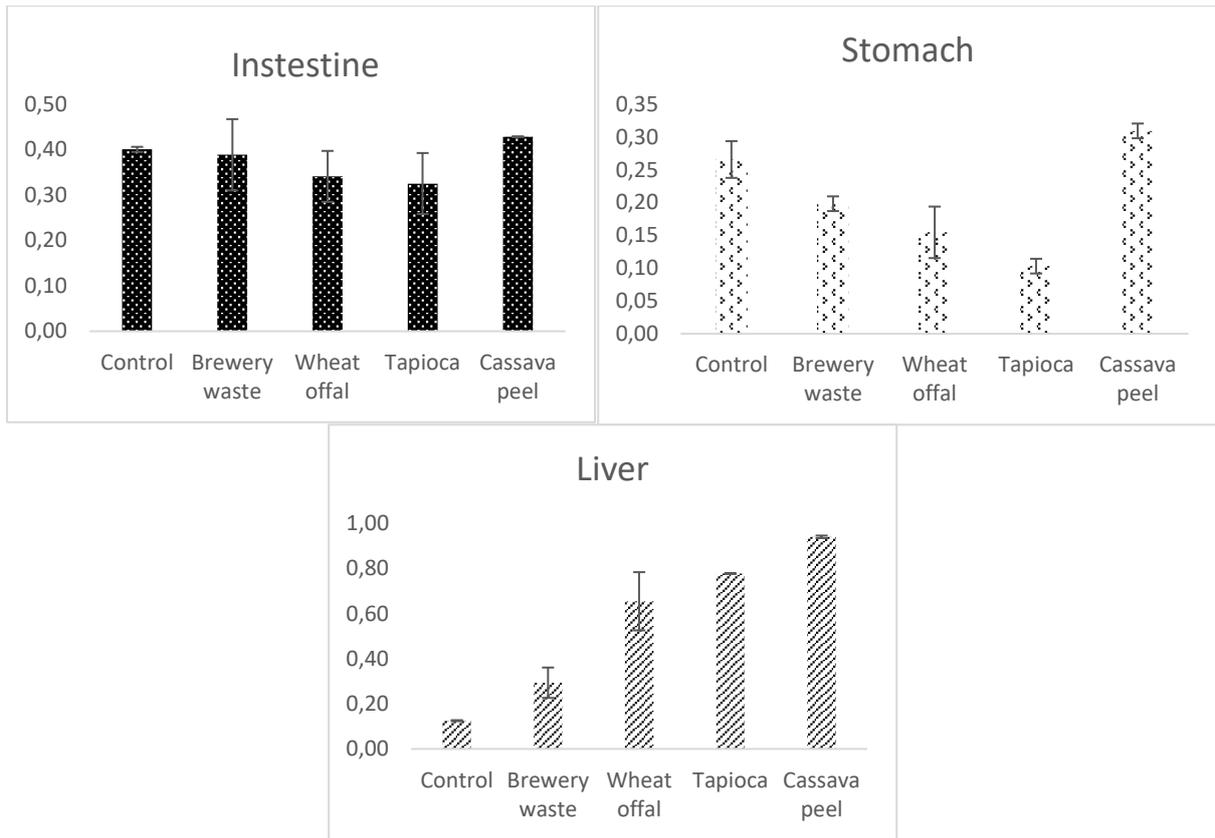


Figure 4. Specific activity of lipase (mg min⁻¹·mg⁻¹protein) in *Clarias gariepinus* juveniles reared in different biofloc systems

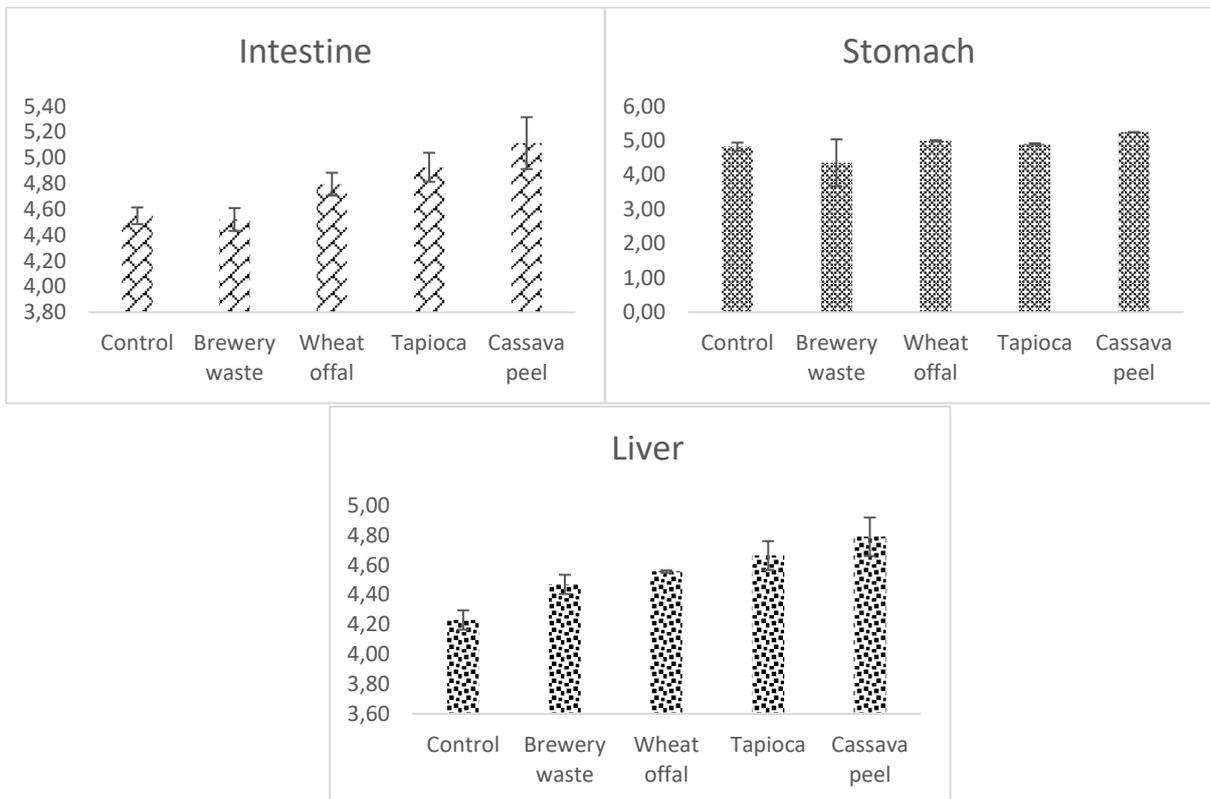


Figure 5. Specific activity of trypsin (µg min⁻¹·mg⁻¹protein) in *Clarias gariepinus* juveniles reared in different biofloc systems

tapioca produce least in the stomach while control has least in the liver. The trypsin activity was also observed to produce significant difference across the biofloc and increase across the treatments in the intestine and liver. A general enhancement in protease and trypsin activities of the *C. gariepinus* in bioflocs treatments was observed. Serum Biochemistry

The serological parameters observed in *C. gariepinus* juveniles are shown in Table 2. Results showed that the biochemical parameters of the different carbon sources varied significantly in terms of albumin, glucose, protein, and globulin in treatments ($P<0.05$). But the highest and lowest values for albumin glucose and protein were seen in wheat offal and control but a deviation from trend was observed in globulin in which the highest was the control and lowest wheat offal.

Immunological Parameters

The results of immunological parameters are shown in Table 3. The results showed that significant difference was observed the lowest value and highest value observed were control and wheat offal respectively ($P<0.05$). With regard to SOD, there was no significant difference found between tapioca and wheat offal treatments while other treatment varied

significantly ($P<0.05$). GPx activity was significantly different among treatment with no significant difference between tapioca and cassava peel BFT treatments. Each of the biofloculating agents also demonstrated the effect on the reared fish thus, given room for different values.

Discussion

The growth and composition of bioflocs in a system varies and its dependent on the type of carbon source used (brewery waste, tapioca, wheat offal, rice bran). The biofloc growth of each of the various system was adequate in maintaining the overall water quality parameters in a normal range for catfish culture. Higher levels of salinity were found in biofloc treatments compare with control due to the presence of dominant heterotrophic bacterial groups which are responsible for nitrogen uptake due to carbon supplementation. This was in agreement with the study of Wendelaar. (1997) who reported that as alkalinity concentration alters the buffering capacity of the water it was found that the effect of low alkalinity leads to lower pH levels. The TDS accumulation was higher in the biofloc treatments than in the control treatment. This difference may have been due to a greater abundance of microalgae in the bioflocs system, resulting from the nutrients derived from the

Table 1. Growth performance of *Clarias gariepinus* juveniles reared in different biofloc systems

Treatment	Initial (g)	Final (g)	WG (g)	SGR	FCR	FER	PER	Survival (%)
Control	8.13±0.48	88.45±5.45	80.31±5.93	2.98±0.15	0.66±0.05	1.53±0.11	1.78±0.13	50.00±10.0a
BW	8.07±0.56	81.30±2.20	73.23±2.76	2.89±0.12	0.72±0.03	1.39±0.05	1.63±0.06	72.00±1.50 bc
WO	8.18±0.25	90.35±3.25	82.16±3.50	3.00±0.08	0.64±0.03	1.56±0.07	1.83±0.08	57.00±1.00ab
Tapioca	8.61±0.01	81.70±22.16	73.08±22.09	2.76±0.35	0.79±0.24	1.39±0.42	1.62±0.49	76.00±2.00c
CP	8.62±0.07	77.10±2.20	68.47±2.14	2.74±0.03	0.77±0.02	1.30±0.04	1.52±0.05	76.00±1.50 c

The mean values (mean±SEM, n=3) with different superscript within the same row are significantly different ($P<0.05$).

SGR-Specific growth rate, PER-Protein Efficiency Ratio, FER- Feed Efficiency Ratio, FCR-Feed Conversion Ratio, WG- Weight Gain, BW-Brewery Waste, CP- Cassava Peel, WO- Wheat Offal. Values are expressed as mean ± SE.

Table 2. Serological characteristics of *C. gariepinus* juveniles raised in biofloc systems for 72 days

	Albumin (mg/dl)	Glucose (mg/dl)	Protein (mg/dl)	Globulin mg/dl
Brewery Waste	3.601±0.09 ^b	105.91±3.21 ^a	4.44±0.05 ^a	0.83±0.04 ^a
Cassava peel	3.76±0.05 ^b	141.36±7.07 ^b	4.63± 0.02 ^b	0.87±0.07 ^a
Tapioca	4.05±0.06 ^b	169.09±7.71 ^c	4.80±0.20 ^b	0.75±0.14 ^a
Wheat Offal	4.66±0.60 ^c	192.73±10.29 ^d	5.16±0.15 ^c	0.50±0.46 ^a
Control	2.99±0.21 ^a	93.64±1.29 ^a	4.30±0.16 ^a	1.30±0.37 ^b

The mean values (mean±SEM, n=3) with different superscript within the same row are significantly different ($P<0.05$).

Table 3. Immunological parameters for *C. gariepinus* juveniles reared in different carbon sources in BFT system

	Catalase activity (KU)	GPx (U/ml)	SOD (U/ml)
Brewery waste	42.08±2.17 ^b	153.27±3.01 ^b	34.50±0.71 ^{ab}
Cassava peel	41.64±0.48 ^b	185.84±0.60 ^c	41.50±7.78 ^b
Tapioca	46.16±2.24 ^c	196.37±0.15 ^{cd}	55.00±00 ^c
Wheat Offal	51.01±0.91 ^c	209.67±15.04 ^d	64.50±3.53 ^c
Control	36.67±2.82 ^a	132.75±1.65 ^a	24.00±4.24 ^a

The mean values (mean±SEM, n=3) with different superscript within the same row are significantly different ($P<0.05$).

mineralization processes carried out by bacteria and protozoa present in BFT systems (Avnimelech, 2009). A decrease in water pH was recorded among the treatments culture. This pH reduction in the water was accentuated in the biofloc treatments, which suggests greater pH stability resulted from the use of reuse water within the biofloc treatments. This observation is congruent with the report of Avnimelech. (2009) that minimal pH variation in the water occur in BFT cultures. Emerenciano et al., (2017) indicated that values below 7.0 are considered normal in BFT systems, although they can affect nitrification processes.

Previous studies have suggested that in biofloc cultures and the increase in microbial biomass reduce the concentration of dissolved oxygen in the water (Hargreaves, 2013). This is in contrast with the results of the present study whereby, the DO concentration in the cultured media increased when compared with the control and also among bioflocs treatments which was attributed to the continuous increase in the concentration of TDS. Also, the C:N ratio is another factor that could affect the dissolved oxygen concentration for instance, values in the range of 15:1–20:1 cause a dramatic reduction in the DO concentration immediately after the carbon source enters the water (Pe´rez-Fuentes et al., 2016).

Under biofloc system conditions, growth performance indices did not clearly represent the effect of different carbon sources compared with control. Although, Kumar et al., (2017) found that rice flour, a complex carbon source, had a better effect on shellfish growth than simple carbon source. Meaning that the nature of carbon has effects on the fish growth. Many writers claimed that there were no changes in organic carbon sources as a control element in the biofloc system (Prajith & Madhusoodana 2011; Khanjani et al., 2017) as observed in this study. Then, choosing less expensive carbon sources, proved to be more cost-effective and environmentally friendly. The fish yield performance showed that the Bioflocs contributed to the individual weight of the fish than those of the control thereby showing that Bioflocs utilization as food by fish. Ekasari et al., (2016) mentioned that the increasing growth rate of aquatic organisms provided with probiotic in their feed was related to higher enzymatic digestion activity together with vitamin synthesis, therefore, digestibility and organism weight were also increased. The survival rate was significantly higher in the Bioflocs based treatment group compared to the control ($P < 0.05$). Higher rate of survival in the BFT was observed compared to the control making BFT an effective technology for rearing/ raising *Clarias gariepinus* fish species. The profile of digestive enzymes plays a pivotal role in the hydrolysis of protein, carbohydrate, and lipid to form small absorbable units which then easily transported into the tissues by the circulatory system and transformed into energy for growth and development (Furne´ et al., 2005). Therefore, for better understanding of the digestive

physiology, quantification of protease, amylase, and lipase activities is very essential. During physiological condition under biofloc-rearing condition in the laboratory, the activities of the digestive enzymes namely amylase, lipase trypsin and protease were not significantly increased in stomach, intestine and liver of the test fish, indicating that biofloc offers no serious tissue damage due to the decreased synthesis of the enzymes in the tissues. Digestion and absorption occurs mainly in the stomach and intestine respectively. This is reflected by the levels of enzyme activity in the three digestive tissues in this present study, an enhancement of protease and trypsin compared to the control. It could be said that this enzyme is enhanced as a result of the increase protein intake of the fish in the Bioflocs systems. Lipase activity in the liver was higher than the control group. It is well known that stress condition resulted in less intake of food, therefore, to overcome this situation the activity of digestive enzymes will be to enhance the break down the protein, carbohydrate, and lipid molecules to fulfill the additional energy requirement. In the present study, the level of production of these enzymes in the biofloc-reared fish compared with control is an indication that the system posed no stress to *C. gariepinus*.

Oxidative stress in animals is a consequence of the imbalance between reactive oxygen species (ROS) production and antioxidant capacity (Ahmadpoor et al., 2009). The ROS change structural and functional molecules, resulting in tissue and organ dysfunction (Zalba et al., 2006). Bacterial and viral infections, dietary limitations such as antioxidative vitamins (E, C), physical and chemical stress are the main causes of oxidative stress in animals (Ahmadpoor et al., 2009; Castex et al., 2010; Lutgendorff et al., 2008). Three classes of antioxidative enzymes (SOD, catalase, GPx) play crucial roles against ROS (Kumaravelu et al., 1995), and natural antioxidants such as polyunsaturated fatty acids (PUFA), different vitamins (C, E), phenolic compounds, carotenoids and minerals (Se, Zn) improve antioxidant system efficiency (Podsednik, 2013). Previous findings indicated that microbial flocs derived from fish and shrimp water are rich in various bioactive products that contain fat-soluble vitamins, carotenoids, phytosterols and taurine (Xu and Pan, 2013). In reference to later findings, we expected BFT system improved antioxidant defense system in juveniles. In the present study, experimental treatments did change SOD activity in juveniles, which is in disagreement with the results obtained in Long et al., (2015) on genetically improved farmed tilapia (*Oreochromis niloticus*), however BFT significantly increased the serum Gpx and CAT activities of fish $P < 0.05$ but in agreement with the result of Luo et al., (2014) who stated that biofloc increased the SOD activity in *Oreochromis niloticus* reared in bioflocs compared with those reared in recirculatory aquaculture system.

Microbial flocs formed in BFT system are considered to be a good source of many organic

compounds such as carotenoids, chlorophylls, bromophenols, amino sugars, phytosterols and antibacterial compounds which have positive influence on immune parameters (Crab et al., 2010b; Ju et al., 2008). In this study, different carbon sources affected non-specific immune parameters (Albumin, Glucose, total serum protein and total immunoglobulin) and the highest values were observed in wheat offal based biofloc treatment. Based on the literature, production of short-chain fatty acids (SCFAs) such as lactic acid by starch microbial degradation has been reported by several studies (Anuradha et al., 1999; McBurney et al., 1990). The SCFAs improves gut health and support the immune system by decreasing epithelial permeability and modulating cytokines in the intestine (Van-Nuenen, 2005). In contrast to our results, Verma et al., (2016) reported that the tapioca-based biofloc improves nonspecific parameters (total serum protein) in Rohu fingerlings (Verma et al., 2016). It seems that the extent of the immune system stimulation in BFT system is carbon source dependent.

Conclusively, the study had shown that, BFT has beneficial effects on the maintenance of good water quality, growth performance compared to control group. The study also, revealed that BFT improved the digestive enzymes of the fish, oxidative status which also enhanced feed utilization and growth performance. In addition, BFT produced a stimulatory effect on the immune response in some ways.

Ethical Statement

The experiments were performed according to the guidelines for animal welfare and ethics as prescribed by the Federal University of Technology, Akure Nigeria Animal Ethics Committee. (No. SAAT/FAT/MTECH/2018)

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Author Contribution

Conceptualization: POM, Data Curation: OAM, Formal Analysis: POM, OAM, Funding Acquisition: POM, OAM, Investigation: OAM, Methodology: POM, Project Administration: POM, Resources: POM, OAM, Supervision: POM, Writing -original draft: OAM, Writing -review and editing: POM, OAM.

Conflict of Interest

The author(s) declared that they have no known competing conflicts be it financial or non-financial, professional, or personal that could have appeared to influence the work reported in this paper.

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References

- Ahmadpoor, P., Eftekhari, E., Nourooz-Zadeh, J., Servat, H., Makhdoomi, K. & Ghafari, A. (2009). Glutathione, glutathione-related enzymes, and total antioxidant capacity in patients on maintenance dialysis. *Iranian Journal of Kidney Dialysis*, 3, 22–27. PMID: 19377255.
- Asaduzzaman, M.; Wahab, M.A.; Verdegem, M.C.J.; Huque, S.; Salam, M.A. & Azim, M.E., (2008). C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*, 280(1), 117-123. <https://doi.org/10.1016/j.aquaculture.2008.04.019>
- Avnimelech, Y. (1999). Carbon and nitrogen ratio as a control element in Aquaculture systems. *Aquaculture*, 176, 227–235. [https://doi.org/10.1016/S0044-8486\(99\)00085-X](https://doi.org/10.1016/S0044-8486(99)00085-X)
- Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*, 264(1), 140-147. <http://dx.doi.org/10.1016/j.aquaculture.2006.11.025>
- Avnimelech, Y. (2009). Biofloc Technology. A Practical Guide Book. The World Aquaculture Society, Baton Rouge, Louisiana, United States,
- Azim, M. E.; Little, D.C. & Bron, J.E. (2008). Microbial protein production in activated suspension tanks manipulating C: N ratio in feed and the implications for fish culture. *Bioresource Technology*, 99(9), 3590-3599. <https://doi.org/10.1016/j.biortech.2007.07.063>
- Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. & Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary Parasitology*, 132(3), 249-272. <https://doi.org/10.1016/j.vetpar.2005.07.005>
- Browdy, C., Hargreaves, J., Hoang, T. & Avnimelech, Y. (2013). Biofloc Technology and Shrimp Disease Workshop.
- Browdy, C.L. (2006). Effect of natural production in a zero-exchange suspended microbial floc based super-intensive culture system for white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 258(1), 396-403. <http://dx.doi.org/10.1016/j.aquaculture.2006.04.030>
- Burford, M., Thompson, P., McIntosh, R., Bauman, R., & Pearson, D. (2004). The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*, 232, 525-537
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. & Pearson, D.C. (2003). Nutrient and microbial dynamics in high intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, 219(1), 393-411. [https://doi.org/10.1016/S0044-8486\(02\)00575-6](https://doi.org/10.1016/S0044-8486(02)00575-6)
- Castex, M., Lemaire, P., Wabete, N. & Chim, L. (2010). Effect of probiotic *Pediococcus acidilactici* on antioxidant defenses and oxidative stress of *Litopenaeus stylirostris* under *Vibrio nigripulchritudo* challenge. *Fish & Shellfish Immunology*, 28, 622–631.

- <https://doi.org/10.1016/j.fsi.2009.12.024>
- 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. *Aquaculture Research*, 41(4), 559-567.
<http://dx.doi.org/10.1111/j.1365-2109.2009.02353.x>
- Crab, R., Defoirdt, T., Bossier, P. & Verstraete, W. (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*, 356, 351-356
<https://doi.org/10.1016/j.aquaculture.2012.04.046>
- Crab, R., Lambert, A., Defoirdt, T., Bossier, P. & Verstraete, W. (2010). The application of bioflocs technology to protect brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. *Journal of Applied Microbiology*, 109(5): 1643-1649.
<https://doi.org/10.1111/j.1365-2672.2010.04791.x>
- Daniel, N.; Amit K.; Mohamed F.M. & Nageswari, P. (2016). Investigation of Vitamin C [Ascorbic Acid] in Nutrition and Health of Fish. *Aquaculture*, 2(1), 20-24. PMID: 29763052.
- Dawood, M.A, O.; El-Dakar, A.; Mohsen, M.; Abdelraouf, E.; Koshio, S. Ishikawa, M. & Yokoyama, S. (2014). Effects of using exogenous digestive enzymes or natural enhancer mixture on growth, feed utilization, and body composition of Rabbitfish, *Siganus rivulatus*. *Journal of Agricultural Science & Technology*, B.4(3B), 4 (3): 180-187
- De Schryver, P., Sinha, A.K., Kunwar, P.S., Baruah, K., Verstraete, W., Boon, N., De Boeck, G. & Bossier, P. (2010). Poly- β -hydroxybutyrate (PHB) increases growth performance and intestinal bacterial rangeweighted richness in juvenile European sea bass, *Dicentrarchus labrax*. *Applied Microbiology & Biotechnology*, 86(5): 1535 - 1541. <https://doi.org/10.1007/s00253-009-2414-9>
- Decamp, O., Moriarty, D.J. & Lavens, P. (2008). Probiotics for shrimp larviculture: review of field data from Asia and Latin America. *Aquaculture Research*, 39(4): 334- 338.
<https://doi.org/10.1111/j.1365-2109.2007.01664.x>
- Doumas, B.T., Watson, W. & Biggs, H.G. (1971). Albumin standards and measurement of serum albumin with bromocresol green, *Clinica Chimica Acta*, 31,87-96.
[https://doi.org/10.1016/0009-8981\(71\)90365-2](https://doi.org/10.1016/0009-8981(71)90365-2)
- Emerenciano, M.; Gaxiola, G. & Cuzon, G. (2013). Biofloc technology (BFT): a review for aquaculture application and animal food industry. *Biomass Now: Cultivation and Utilization*. Rijeka, Croatia: In Tech, Rijeka, Croatia: 301-328. <http://dx.doi.org/10.5772/53902>
- Food and Agriculture Organization, 2010 (FAO). The State of World Fisheries and Aquaculture 2010. Rome.
- Food and Agriculture Organization, 2017 (FAO). FAO and the SDGs. Indicators: Measuring up to the 2030 Agenda for Sustainable Development.
- Francis-Floyd, R., Watson, C., Petty, D. & Pouder, D.B. (2009). Ammonia in aquatic systems. University of Florida IFAS Extension Publication, FA-16.
- Ghosh, S., Sinha, A. & Sahu, C. (2007). Effect of probiotic on reproductive performance in female livebearing ornamental fish. *Aquaculture Research*, 38(5), 518-526.
<http://dx.doi.org/10.1111/j.1365-2109.2007.01696.x>
- Halet, D., Defoirdt, T., Van Damme, P., Vervaeren, H., Forrez, I., Van de Wiele, T., Boon, N., et al. (2007). Poly- β -hydroxybutyrate-accumulating bacteria protect gnotobiotic *Artemia franciscana* from pathogenic *Vibrio campbellii*. *FEMS Microbiology Ecology*, 60(3), 363-369.
<https://doi.org/10.1111/j.1574-6941.2007.00305.x>
- Hargreaves, J.A. (2006). Photosynthetic suspended growth systems in aquaculture. *Aquaculture Engineering*, 34(3), 344-363.
<http://dx.doi.org/10.1016/j.aquaeng.2005.08.009>
- Hari, B., Kurup, B.M., Varghese, J.T.; Schrama, J.W. & Verdegem, M.C.J. (2004). Effects of carbohydrate addition on production in extensive shrimp culture systems. *Aquaculture*, 241(1), 179-194.
<http://dx.doi.org/10.1016/j.aquaculture.2004.07.002>
- Hari, B.; Kurup, B. M.; Varghese, J. T.; Schrama, J. W. & Verdegem, M. C. J. (2006). The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. *Aquaculture*, 252(2), 248-263.
<https://doi.org/10.1016/j.aquaculture.2005.06.044>
- Huys, G., Bartie, K., Cnockaert, M., Oanh, D.T.H., Phuong, N.T., Somsiri, T., Chinabut, S., Yusoff, F.M., Shariff, M., Giacomini, M. & Teale, A. (2007). Biodiversity of chloramphenicol-resistant mesophilic heterotrophs from Southeast Asian aquaculture environments. *Research in Microbiology*, 158(3), 228-235.
<https://doi.org/10.1016/j.resmic.2006.12.011>
- Khanjani, M.H., Sajjadi, M.M., Alizadeh, M. & Sourinejad, I. (2017). Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. *Aquaculture Research*, 48(4),1491-1501.
- Kumar, S., Anand, P.S.S., De, D., Deo, A.D., Ghoshal, T.K., Sundaray, J.K., Ponniah, A.G., Jithendran, K.P., Raja, R.A., Biswas, G. & Lalitha N. (2017). Effects of biofloc under different carbon sources and protein levels on water quality, growth performance and immune responses in black tiger shrimp *Penaeus monodon* (Fabricius, 1978). *Aquaculture Research*, 48(3), 1168-1182.
- Liu, G., Ye, Z., Liu, D., Zhao, J., Sivaramasamy, E., Deng, Y. & Zhu, S. (2018). Influence of stocking density on growth, digestive enzyme activities, immune responses, antioxidant of *Oreochromis niloticus* fingerlings in biofloc systems. *Fish and Shellfish Immunology*, 81,416-22.
<https://doi.org/10.1016/j.fsi.2018.07.047>
- Long, L., Yang, J., Li, Y., Guan, C. & Wu, F. (2015). Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*, 448, 135-141.
<http://dx.doi.org/10.1111/are.13420>
- Luo, G.Z., Gao, Q., Wang, C.H., Liu, W.C., Sun, D.C., Li, L. & Tan, H.X. (2014). Growth, digestive activity, welfare and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture*, 422-423, 1-7.
<https://doi.org/10.1016/j.aquaculture.2013.11.023>
- Lutgendorff, F.; Trulsson, L.M.; Van-Minnen, L.P.; Rijkers, G.T.; Timmerman, H.M.; Franzen, L.E. & Sandstrom, P.A. (2008). Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. *American Physiology, Gastrointestinal & Liver Physiology*, 295, 1111-1121.
<https://doi.org/10.1152/ajpgi.00603.2007>
- MacIntosh, R.P. (2000). Changing paradigms in shrimp farming: IV: low protein feeds and feeding strategies. *Global Aquaculture Advocate*, 2, 40-47.

- Megahed, M.E. (2010). The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) fed with different crude protein levels. *Journal of Arabian Aquaculture Society*, 5(2), 119-142.
- Moriarty, D.J. (1997). The role of microorganisms in aquaculture ponds. *Aquaculture*, 151(1), 333-349. [https://doi.org/10.1016/S0044-8486\(96\)01487-1](https://doi.org/10.1016/S0044-8486(96)01487-1)
- Moriarty, D.J.W. (1998). Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, 164(1), 351-358. [http://doi.org/10.1016/S0044-8486\(98\)00199-9](http://doi.org/10.1016/S0044-8486(98)00199-9)
- Moss, S.M., LeaMaster, B.R. & Sweeney, J.N. (2000). Relative Abundance and Species Composition of Gram Negative, Aerobic Bacteria Associated with the Gut of Juvenile White Shrimp *Litopenaeus vannamei* Reared in Oligotrophic Well Water and Eutrophic Pond Water. *Journal World Aquaculture Society*, 31(2), 255-263 <https://doi.org/10.1111/j.1749-7345.2000.tb00361.x>
- Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay J et al. (2000). Effect of aquaculture on world fish supplies. *Nature*. 405:1017-1024.
- Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, 153, 375-80.
- Panigrahi, A., Kiron, V., Satoh, S., Hirono, I., Kobayashi, T., Sugita, H., Puangkaew, J. & Aoki, T. (2007). Immune modulation and expression of cytokine genes in rainbow trout *Oncorhynchus mykiss* upon probiotic feeding. *Developmental & Comparative Immunology*, 31(4), 372-382. <https://doi.org/10.1016/j.dci.2006.07.004>
- Piedrahita RH. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*. 2003; 226:35-44.
- Prajith, K.K., & Madhusoodana K.B. (2011). Application of Biofloc Technology (BFT) in the nursery rearing and farming of giant freshwater prawn, *Macrobrachium rosenbergii* (deMan) (Doctoral dissertation, Cochin University of Science and Technology)
- Pulkkinen, K., Suomalainen, L.R., Read, A.F., Ebert, D., Rintamäki, P. & Valtonen, E.T (2010). Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681), 593-600. <https://doi.org/10.1098/rspb.2009.1659>
- Pybus, V., Loutit, M.W., Lamont, I. L. & Tagg, J.R. (1994). Growth inhibition of the salmon pathogen *Vibrio ordalii* by a siderophore produced by *Vibrio anguillarum* strain VL4355. *Journal of Fish Diseases*, 17(4), 311-324. <https://doi.org/10.1080/08997659.2014.1001534>
- Schmidt, A.S., Bruun, M.S., Dalsgaard, I., Pedersen, K. & Larsen, J.L. (2000). Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. *Applied Environmental Microbiology*, 66(11), 4908-4915. <https://doi.org/10.1128/aem.66.11.4908-4915.2000>
- Schneider, O., Sereti, V., Eding, E.H., & Verreth, J.A.J. (2006). Molasses as C source for heterotrophic bacteria production on solid fish waste. *Aquaculture*, 261(4), 1239-1248. <https://doi.org/10.1016/j.aquaculture.2006.08.053>
- Somogyi M.A. (1945). New reagent for the determination of sugars. *Journal of Biological Chemistry*, 160: 61-8.
- Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P. & Decamp, O.E. (2002). Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition*, 8(2), 121-137. <https://doi.org/10.1046/j.1365-2095.2002.00199.x>
- Taw, N. (2010). Biofloc technology expanding at white shrimp farms. *Global aquaculture advocate*. P. 1-5.
- Verschuere, L., Rombaut, G., Sorgeloos, P. & Verstraete, W., (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, 64(4), 655-671. <https://dx.doi.org/10.1128%2Fmbr.64.4.655-671.2000>
- Wendelaar B.S.E. (1997). The stress response in fish. *Physiological Reviews*, 7:591-625. <https://doi.org/10.1152/physrev.1997.77.3.591>
- Zalba, G.; Fortuño, A. & Diez, J. (2006). Oxidative stress and atherosclerosis in early chronic kidney disease. *Nephrology Dialysis Transplantation*, 21, 2686-2690. <https://doi.org/10.1093/ndt/gfl398n>