UNIVERSITI PUTRA MALAYSIA

BIOFLOC TECHNOLOGY SYSTEM FOR IMPROVEMENT OF THE SURVIVAL, GROWTH, BIOCHEMICAL COMPOSITION AND PHYSIOLOGY OF AFRICAN CATFISH (Clarias gariepinus BURCHELL 1822) JUVENILES

DAUDA AKEEM BABATUNDE

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By

DAUDA AKEEM BABATUNDE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to Almighty Allah (SWT)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

BIOFLOC TECHNOLOGY SYSTEM FOR IMPROVEMENT OF THE SURVIVAL, GROWTH, BIOCHEMICAL COMPOSITION AND PHYSIOLOGY OF AFRICAN CATFISH (*Clarias gariepinus* BURCHELL 1822) JUVENILES

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DAUDA AKEEM BABATUNDE

March 2018

Chairman: **Nicholas Romano, PhD**
Faculty: **Agriculture**

Biofloc technology system (BFT) is an *in situ* low-cost water quality management technique. It works on the principle of stimulating heterotrophic bacteria to convert the toxic nitrogenous waste into potentially consumable biomass. This has been successfully applied in shrimp and tilapia aquaculture. However there is little information on its application on other commercially important species including African catfish, *Clarias gariepinus*. This study aimed at establishing the most appropriate conditions for the nursery culture of *C. gariepinus* in a biofloc technology system. The study was conducted in four phases. The first phase investigated the effects of three different carbon sources with different characteristics, sucrose, glycerol and rice bran, on the biofloc formation, water quality, growth and survival performance and physiological status of the African catfish juveniles. There was no difference in growth or feeding efficiencies parameters but survival, liver glycogen content and overall water quality parameters were significantly higher in glycerol than other treatments. The use of rice bran led to mass mortalities, likely due to stress associated with elevated nitrogenous waste and less soluble rice bran acting as an irritant. Glycerol was further investigated in the second phase at different carbon/nitrogen (C/N) ratios, 0, 10, 15 or 20. The growth performance and overall health status of the African catfish were examined and subsequently, the fish in the different treatments were challenged with *Aeromonas hydrophila* to investigate their resistance to pathogenic bacteria. C/N ratio of 15 appears to be the best in the management of ammonia-N. Although chymotrypsin activities and physiological parameters were higher in all the BFT treatments than the control but growth performance was not different among the treatments. Meanwhile, C/N ratios of 15 or 20 led to a significantly higher resistance to disease compared to C/N ratio of 10 or the control. The third phase investigated the effects of feeding habit of cultured fish on the growth performance in BFT. The previously established glycerol and C/N ratio of 15 was used in BFT for a more efficient filter-feeding fish, lemon fin barb hybrid (LFBH) and African catfish, an inefficient filter feeder. The control system was a recirculating aquaculture system (RAS). Fish feeding habit affected the nutritional value of biofloc but not the biofloc formation. There was no difference in water quality between RAS and BFT except for nitrate-N which was higher in RAS. BFT led to substantially improved

growth and feeding efficiencies performance in LFBH but the growth and feeding efficiencies in African catfish was only slightly higher in BFT than RAS. In the fourth phase, African catfish was cultured in BFT with differently processed rice bran as carbon sources. The rice bran was pretreated with a *Bacillus* sp. in either aerobic (cellular respiration; ResRB) or anaerobic condition (fermentation; FerRB) while raw rice bran (RRB) and RAS without carbon addition served as controls. The ammonia-N was significantly lower in RAS and FerRB in the first two weeks. However, the FerRB led to significantly improved growth and feeding efficiencies compared to the RAS or ResRB. The use of BFT with glycerol as the carbon source at C/N of 15 led to improved water quality management, and biochemical composition, physiological health status and diseases resistance of *C. gariepinus* in intensive nursery culture. In addition to efficient water quality management, FerRB led to improved growth and nutritional value of *C. gariepinus* juveniles in intensive nursery culture. The use of fermented rice bran is therefore recommended in BFT to culture African catfish. Meanwhile, there is need for further research on its optimum C/N ratio and the potentials to enhance the immunity and disease resistance of the fish culture in the system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

SISTEM TEKNOLOGI BIOFLOK UNTUK PENINGKATAN KEMANDIRIAN, PERTUMBUHAN, KOMPOSISI BIOKIMIA DAN FISIOLOGI JUVENIL IKAN KELI AFRIKA (*Clarias gariepinus* BURCHELL 1822)

Oleh

DAUDA AKEEM BABATUNDE

Mac 2018

Pengerusi: Nicholas Romano, PhD
Fakulti: Pertanian

Sistem teknologi bioflok (BFT) adalah teknik pengurusan kualiti air kos rendah secara *in situ*. Ia berfungsi melalui prinsip stimulasi bakteria heterotrofik yang menukar sisa nitrogen toksik kepada biojisim yang boleh digunakan. Teknologi ini telah berjaya diaplikasikan pada kultur udang dan tilapia. Walaubagaimanapun hanya terdapat sedikit informasi mengenai aplikasinya pada spesies komersil yang lain termasuk ikan keli Afrika, *Clarias gariepinus*. Kajian ini bertujuan untuk mewujudkan keadaan yang paling sesuai untuk kultur asuhan *Clarias gariepinus* dalam sistem teknologi bioflok. Kajian ini dijalankan dalam empat fasa. Fasa pertama mengkaji kesan tiga jenis sumber karbon yang berbeza, sukrosa, gliserol dan dedak padi, pada pembentukan bioflok, kualiti air, pertumbuhan, kelestarian dan status fisiologi juvenil ikan keli Afrika. Tiada perbezaan antara pertumbuhan atau parameter kecekapan pemakanan walaupun kemandirian, kandungan glikogen hati dan parameter kualiti air keseluruhan adalah secara signifikan lebih tinggi dalam gliserol berbanding rawatan yang lain. Penggunaan dedak padi membawa kepada mortaliti yang tinggi, kemungkinan kerana tekanan yang disebabkan pertambahan keluaran buangan nitrogen dan kurang keterlarutan dedak padi yang menyebabkan iritasi. Gliserol telah dikaji dengan lebih lanjut dalam fasa kedua pada nisbah karbon/nitrogen (C/N) yang berbeza, 0,10,15,20. Kadar pertumbuhan dan status kesihatan ikan keli Afrika secara keseluruhan diperiksa dan seterusnya ikan dalam rawatan berbeza dicabar dengan *Aeromonas hydrophila* untuk mengkaji rintangan ikan tersebut pada bakteria patogenik. Nisbah C/N 15 didapati paling terbaik dalam pengurusan ammonia-N. Sementara itu, aktiviti kimotripsin dan parameter fisiologi adalah lebih tinggi di dalam rawatan BFT berbanding kawalan walaupun prestasi pertumbuhan tidak berbeza antara semua rawatan. Nisbah C/N 15 atau 20 membawa kepada kerintangan penyakit yang lebih tinggi berbanding C/N 10 atau kawalan. Fasa ketiga mengkaji kesan tabiat pemakanan terhadap pertumbuhan ikan yang dikultur dalam BFT. Gliserol dan nisbah C/N 15 digunakan dalam BFT pada ikan jenis pemakan menapis yang lebih efisien, kerai lampam (LFBH) dan ikan keli Afrika yang merupakan ikan pemakan menapis tidak efisien. Sistem kawalan adalah sistem akuakultur kitar semula (RAS). Tabiat pemakanan ikan mempengaruhi nilai nutrisi bioflok tetapi tidak pembentukan bioflok. Tidak terdapat perbezaan antara kualiti air RAS dan BFT kecuali nitrat-N yang lebih tinggi dalam RAS. BFT memberikan pertumbuhan yang lebih baik

dan kecekapan pemakanan yang signifikan di LFBH tetapi BFT hanya memberikan sedikit sahaja peningkatan pertumbuhan dan kecekakapan makanan berbanding RAS. Dalam fasa keempat, ikan keli Afrika dikultur dalam BFT dengan dedak padi yang diproses secara berbeza sebagai sumber karbon. Dedak padi tersebut telah dipra-rawat dengan *Bacillus* sp. samada secara aerobik (respirasi selular; ResRB) atau kondisi anaerobik (fermentasi; FerRB) manakala dedak padi mentah (RRB) dan RAS tanpa penambahan karbon bertindak sebagai kawalan. Kandungan ammonia-N dalam RAS dan FerRB adalah rendah secara signifikan pada dua minggu pertama. Walaubagaimanapun, FerRB telah membawa kepada pertumbuhan dan kecekapan makanan yang ketara lebih baik berbanding kawalan ResRB. Penggunaan BFT menggunakan gliserol sebagai sumber karbon pada nisbah C/N 15 telah meningkatkan pengurusan kualiti air, dan komposisi biokimia, status kesihatan fisiologi dan kerintangan penyakit dalam kultur nurseri intensif *C. gariepinus.* Penggunaan FerRB bukan sahaja meningkatkan pengurusan kualiti air yang efisien tetapi juga pertumbuhan dan nilai nutrisi yang lebih baik pada juvenil *C. gariepinus* dalam kultur intensif asuhan. Dengan itu, penggunaan dedak padi yang telah difermentasi adalah dicadangkan dalam sistem BFT bagi ikan keli Afrika. Penyelidikan lanjut diperlukan untuk menentukan nisbah C/N yang optimum dan keupayaan dedak padi yang difermentasi dalam mempertingkat imuniti dan ketahanan penyakit ikan yang dikultur dalam sistem tersebut.

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I certify that a Thesis Examination Committee has met on 9 March 2018 to conduct the final examination of Dauda Akeem Babatunde on his thesis entitled "Biofloc Technology System for Improvement of the Survival, Growth, Biochemical Composition and Physiology of African Catfish (*Clarias gariepinus* Burchell 1822) Juveniles" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Fatimah binti Md Yusoff, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

S. M. Nurul Amin, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Aziz bin Arshad, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

A. Jesu Arockia Raj, PhD
Professor
SRM University
India
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 June 2018

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Nicholas Romano, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mohd Salleh Kamarudin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Murni Marlina Abd Karim, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Natrah Fatin Mohd Ikhsan, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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Name and Matric No.: Dauda Akeem Babatunde GS43994

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This is to confirm that:

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- Supervision responsibilities as slated in Rule 41 in Rules 2003(Revision 2012-2013) were adhered to.

Signature: ______________________
Name of
Chairman
of supervisory
committee: Dr Nicholas Romano

Signature: ______________________
Name of
Member
of supervisory
committee**:** Professor Dr Mohd Salleh Kamarudin

Signature: ______________________
Name of
Member
of supervisory
committee: Dr Murni Marlina Abd Karim

Signature: ______________________
Name of
Member
of supervisory
committee: Dr Natrah Fatin Mohd Ikhsan

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABA	antibacterial activity
ALT	alanine aminotransferase
Ammonia-N	ammonia-nitrogen
ANOVA	analysis of variance
AST	aspartate aminotransferase
BFT	biofloc technology system
BLA	bacteriolytic activity
CAT	catalase activity
CHO	carbohydrate
C/N	carbon to nitrogen ratio
DO	dissolved oxygen
FerRB	pretreated rice bran without aeration (fermentation)
GPX	serum glutathione peroxidase
GSH	reduced glutathione
GSSG	oxidized glutathione
GSSG/GSH	oxidized glutathione/reduced glutathione ratio
HMT	hematology parameters
HSI	hepatosomatic index
LC-PUFA	long-chain polyunsaturated fatty acids
LFBH	lemon fin barb hybrid
LPD	myeloperoxidase
LSZ	serum lysosyme activities
MDA	malondialdehyde
Min	minutes
MUFA	monounsaturated fatty acids
ND	not determined
Nitrate-N	nitrate-nitrogen
Nitrite-N	nitrite-nitrogen
PAS	periodic-acid Schiff
PCY	phagocytic activity
PHA	polyhydroxyalkanoates
PHB	poly-β-hydroxybutyrate
PO	phenoloxidase
PUFA	polyunsaturated fatty acids
RAS	recirculating aquaculture system
ResRB	pretreated rice bran with aeration (Cellular respiration)
RB	respiratory burst
RBC	red blood cells
RRB	raw rice bran
SE	standard error
SFA	saturated fatty acids
SOD	superoxide dismutase
SP	serum total protein
TDS	total dissolved solid
TAOC	total antioxidant
THC	total haemocyte count
TIG	total immunoglobulin
TSS	total suspended solid

v/v	volume for volume
VSI	viscerosomatic index
WAI	water absorption index
WBC	white blood cells
WSI	water solubility index

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background to the study

Global fish production has been increasing steadily in the last six decades, with food fish supply growing at an average annual rate of 3.2%, which is now outpacing world population growth at 1.6%. The increment in the last three decades was due to a great expansion of world food fish aquaculture production with an average annual rate of 6.2% in the period of 2000–2012 (as against 9.5% in 1990–2000) and from 32.4 million to 66.6 million tonnes. The latest aquaculture production figure stood at 73.8 million tonnes (FAO, 2016a). The world per capita fish consumption is also on the increase and has grown from an average of 9.9 kg in the 1960s to 19.2 kg in 2012, 19.7 kg in 2013 and 20.1 kg in 2014, although with wide variation across countries and regions of the world (FAO, 2014a; FAO, 2016a). The needs to meet-up with increasing fish demand may necessitate increasing expansion of aquaculture production.

One of the limiting factors to the continued aquaculture expansion is access to both suitable land and water resources (Widanarni *et al*., 2012) as well as sustainability issues associated with pollution from waste products, especially those from intensive aquaculture systems (Crab *et al*., 2012). One of the major water quality problems in intensive aquaculture systems is the accumulation of toxic inorganic nitrogenous substances (NH_3 and NO_2^-) (Avnimelech, 1999; Avnimelech, 2012), which results from metabolic by-products of protein catabolism (Romano and Zeng, 2013). Aquatic animals excrete ammonium that can accumulate to high levels in closed culture systems due to the high input of feeds with high protein content (Helpher 1988; Crab *et al*., 2007). This can subsequently necessitate water exchanges to the surrounding environment to prevent adverse consequences to the farmed animals, which may include, reduced feed intake, poor growth, reduced feeding efficiencies, increased susceptibility to bacterial infection and diseases, gill and tissue damage, extreme lethargy and death (Timmons *et al*., 2002; Boyd, 2003; Schneider *et al*., 2005; Martins *et al*., 2010).

However, there are increasing regulations regarding the amount of permissible water exchanges due to concerns over nutrient rich water leading to eutrophication as well as the potential introduction of pathogens and/or alien species into the open water bodies (Piedrahita, 2003). Even in areas that have fewer laws regarding water exchanges, frequent use of this practice can increase pumping costs to the farmer. One approach in closed aquaculture systems includes encouraging bacterial nitrification process whereby highly toxic ammonium and nitrites are converted to less toxic nitrate. This is primarily used in recirculating systems where biofilters are employed as immobile surfaces to serve as substrates to nitrifying bacteria (Timmons *et al*., 2002). A large surface area is typically provided for the nitrifying microorganisms in order to enhance the nitrification process (Avnimelech, 1999). However, the main disadvantages includes a requirement to remove solids in the water (i.e. mechanical filtration), which can increase installation and maintenance costs, along with the accumulation of nitrate to levels that may potentially harm the cultured animal (Schneider *et al*., 2006; Verdegem *et al*. 2006; Martins *et al*., 2010).

There are three primary goals of sustainable aquaculture development. First is to expand aquaculture production in order to create more products without a major increase in the use of basic natural resources such as water and land (Avnimelech, 2009). The second is to develop sustainable aquaculture systems in an environmentally friendly manner (Naylor *et al*., 2000). The third involved building up of culture systems that will provide a good cost/benefit ratio in order to ensure both economic and social sustainability (Avnimelech, 2009). Biofloc technology (BFT) has the potential to meet all these goals (Crab *et al*., 2012).

Biofloc technology is an *in situ* water treatment method that promotes suspended floc growth by aggregating both living and dead particulate organic matter, such as phytoplankton, bacteria, and grazers of the bacteria (Hargreaves, 2006). If the carbon to nitrogen (C/N) ratio is well elevated in a solution, then potentially toxic nitrogenous wastes generated by the cultivated organisms will be converted into bacterial biomass that may be consumed by the fish or crustacean (Schneider *et al*., 2005; Bossier and Ekasari, 2017). To elevate the C/N ratio, carbohydrates are often added to the water, which stimulates heterotrophic bacterial growth that recycles dissolved nitrogen into microbial protein (Avnimelech, 1999; Crab *et al*., 2010a). This subsequently decreases the dissolved nitrogen levels within a few hours while nitrification in conventionally used biofilters is a much slower process, particularly when the biofilter is new and there is little nitrifying bacteria present in the water or filters (Hargreaves, 2006; Ebeling *et al*., 2006). For instance, heterotrophic bacteria produced per substrate is 40 times greater than that of nitrifying bacteria (Hargreaves, 2006). Typically it takes around 1 – 2 weeks for a biofilter to become fully established and during this time ammonia and nitrite might spike to potentially dangerous levels until nitrifying bacteria grow to sufficient numbers (Martins *et al*., 2010). In contrast, establishing a biofloc-based system may be achieved within 3 – 5 days, depending on the type and quantity of the inoculant used, the carbon type and C/N ratio (Crab *et al*., 2012; Rajkumar *et al*., 2016, Perez-Fuentes *et al*., 2016; Xu *et al*., 2016). In addition to being an inexpensive system without any need to invest in an external water treatment system (Crab *et al*., 2007), BFT has also been described as a 'neutral cost' in terms of feeding, because it converts carbohydrate to protein.

Different organic carbon sources can be used to establish a biofloc-based system, and some of these include acetate, sucrose, glucose, glycerol, molasses and rice bran. However, it is desirable that the carbon source is low-value and readily available. For example, glycerol is a by-product of biodiesel production, which is readily available at a cheap cost in most countries of the world (Dube *et al*., 2007; De Schryver *et al*., 2008). Meanwhile, rice bran is another cheap carbon source and is an agricultural by-product that is readily available in most regions of the world (Oladosu *et al*., 2016).
To date, most of the research since the early 1980's on BFT has focused on shrimp (Serfling, 2006). The general consensus by many researchers is that BFT can improve water quality maintenance, and if properly managed, can be a zero-exchange system (Ray *et al*., 2010; Xu and Pan, 2012; Zhao *et al*., 2014) and thus increase biosecurity in shrimp culture systems (Crab *et al*., 2010b; Kumar *et al*., 2017). There are some additional benefits that BFT can provide such as enhancing shrimp growth that has been

attributed to an additional supply of natural food that may also stimulate digestive enzyme activities (Xu and Pan, 2012) as well as improving the antioxidant and immune defense of shrimp (Xu and Pan, 2013; Kim *et al*., 2014; Souza *et al*., 2014).

Despite the fact that among aquatic animals finfish constitute the major (66.3%) aquaculture production (FAO, 2014a), only a few studies have been conducted on the feasibility and application of BFT to finfish and, moreover, most of these were on tilapia. The use of BFT has been established to lead to improved water quality, enhanced growth, digestive enzymes activities and immune responses of *Tilapia species.* This improved performance of the fish was suggested to likely due to the ability of tilapia to collect and consume the small biofloc particles (Long *et al*., 2015). Similarly in the recent time, various cyprinids which include rohu *(Labeo rohita)*, bighead carp (*Aristichtys nobilis),* silver carp (*Hypophthalmichthys molitrix*) and bottom feeding carp (*Cyprinus carpio*) were experimented in BFT and improved water quality performance was observed. However, the less efficient filter feeding common carp (*Cyprinus carpio*) did not have an improved growth perfomance, while the bighead carp and silver carp that were cultured with it in a polyculture system had improved growth benefits (Zhao *et al*., 2014). In addition to improve growth in *Labeo rohita*, further experimentaton showed an improved immune response and resistance to pathogenic bacteria when cultured with BFT (Ahmad *et al*., 2016).

African catfish (*Clarias gariepinus*) has also received some research attention in BFT and these include optimal C/N ratios for nutrient removal (Abu Bakar *et al*., 2015), growth performance of *C. gariepinus* juveniles in a BFT system (Yusuf *et al*., 2015) as well as the reproductive performance and subsequent larval productivity (Ekasari *et al*., 2016). Most of these studies relied on optimal conditions for tilapia or shrimp culture. Early researchers assumed that BFT might only be suitable for filter-feeding fish such as shrimps and tilapia and that more carnivorous fish species, such as *C. gariepinus*, may not be a good candidate for BFT (Avnimelech, 2012; Emerenciano *et al*., 2013). However, rather than relying on assumption, there is need to test the feasibility of BFT to this commercially important species as not just a water quality management strategy but to examine whether BFT can confer additional advantages. This may be accomplished through comprehensive research to establish basic design and operational parameters for culture of *C. gariepinus* in BFT.

1.2 Justification for the study

The development of BFT has been established to be a more sustainable strategy to manage water quality in aquaculture compared to other systems. Some of these include flow through systems that continually release nitrogenous wastes and phosphorus into the environment. Moreover, recirculating aquaculture systems (RAS) employ expensive filtration methods that generally cannot manage nitrate removal. Despite numerous research and adoption of BFT in farms, there has been little focus on finfish, possibly due to assumption that BFT is not applicable to some species in this animal group. However, this may not necessarily be the case and should be explored. This is because African catfish are a highly important commercial species that is farmed throughout Asia, Africa, some parts of Europe and South America as well as requiring high protein feeds. Therefore, any method that improves their cost-effective production more sustainably should greatly improve the prospects of their culture. Some of the lacking information regarding the optimal operating protocols to the implementation of BFT to finfish, including African catfish, includes the optimal carbon source and C/N ratio as well as any influence these have to the nutritional value and production of biofloc and any subsequent changes to the physiological health status and resistance to diseases. In a series of experiments, the influence of BFT to the growth, survival, nutritional value, biochemical composition and physiological health status of African catfish (*Clarias gariepinus*) were investigated.

1.3 Research objectives

This study primarily seeks to establish the most appropriate conditions for the sustainable nursery production of *C. gariepinus* in biofloc-based systems. The specific objectives were:

1. To identify the most appropriate carbon sources (sucrose, glycerol or rice bran) for the intensive culture of *C. gariepinus* juveniles.
2. To determine the optimal carbon to nitrogen (C/N) ratio for nursery culture of *C. gariepinus* in a biofloc technology system (BFT).
3. To elucidate whether BFT can improve the immunity of *C. gariepinus* juveniles and aid in their resistance to bacterial challenge (*Aeromonas hydrophila*).
4. To examine the effect of feeding habit on the performance of fish culture in BFT.
5. To investigate the effects of pretreatments on the performance of complex carbohydrate (rice bran) as carbon source in BFT and the subsequent effect on the performance of *C. gariepinus* juveniles reared in the system.

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