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

FP 2018 79

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

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

March 2018

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

By

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.

ACKNOWLEDGEMENTS

All praises and adorations are due to Almighty Allah (SWT), for the journey so far. I sincerely and dutifully appreciate the support, guidance and encouragement of the chairman of my supervisory committee, Dr Nicholas Romano. My appreciation also goes to all the members of my supervisory committee; Professor Mohd Salleh Kamarudin, Dr Murni Marlina Abd Karim and Dr Natrah Fatin Mohd Ikhsan, for the guidance, attention and support received.

I am indebted to Federal Government of Nigeria for provision of scholarship for my study through her agency, Tertiary Education Trust Fund and to the management of Federal University, Dutsin-ma Katsina State, Nigeria, for my nomination. I appreciate every member of staff (teaching and non-teaching) and students of the Department of Aquaculture, Universiti Putra Malaysia who has contributed in one way or the other to ease my sojourn in Malaysia. Worthy of appreciation are members of Nigerian community in UPM, for the support, guidance and encouragement received.

Special, thanks to my pioneer Head of Department and Dean of Agriculture Federal University, Dutsin-Ma and now the Acting Vice Chancellor of the University, Professor Armayau Hamisu Bichi, for the tutelage and support all the time. To my family members, words cannot express my appreciation, especially to those who paid the price for my long-term absence from home, my darling wife Princess Temitope Hauwa Oladigbolu-Dauda and my Daughter Khadija Omotayo Dauda. I say a special thank you to my parents for the support and prayers and to all my siblings and my in-laws. I am highly indebted to the duo of Dr Ismail Olabisi Azeez and Mrs Morufat Bukola Wuraola-Tijani for their tireless support.

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:

Declaration by graduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been duly referenced;
- This thesis has not been submitted previously or concurrently for any other degree at any other institution;
- Intellectual property from the thesis and copyright of thesis are fully owned by Universiti Putra Malaysia (Research) Rules 2012;
- Written permission must be obtained from supervisor and the office of Deputy Vice-chancellor (Research and Innovation) before thesis published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules, or any other material as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/fabrication in the thesis, and Scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________________ Date: ________________________

Name and Matric No.: Dauda Akeem Babatunde GS43994

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision,
- 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.

REFERENCES

Abu Bakar, N.S., Mohd Nasir, N., Lananan, F., Abdul Hamid, S.H., Lam, S.S. & Jusoh, A. (2015). Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (*Clarias gariepinus*) utilizing Bioflocs Technology. *International Biodeterioration and Biodegradation* 102, 100-106.

Adewumi, A.A. & Olaleye, V.F. (2010). Catfish culture in Nigeria: Progress, prospects and problems. *African Journal of Agricultural Research* 6(6), 1281-1285.

Ahmad, I., Verma, A.K., Rani, A.M.B., Rathore, G., Saharan, N. & Gora, A.H. (2016). Growth, non-specific immunity and disease resistance of *Labeo rohita* against *Aeromonas hydrophila* in biofloc systems using different carbon sources. *Aquaculture* 457, 61-67.

Ahmad, I., Rani, A.M.B., Verma, .K. & Maqsood, M. (2017). Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture International* 25, 1215-1226.

Ajadi, A. Sabri, M.Y. Dauda, A.B. Ina-Salwany, M.Y. & Haslisa, A.H. (2016). Immunoprophylaxis: A Better alternative protective measure against shrimp vibriosis -A review, *Pertanika Journal of Scholarly Research Reviews* 2(2), 58–69.

Ajani, E.K., Akinwole, A.O., Ayodele, I.A. (2011). Fundamentals of Fish Farming in Nigeria. Walecrown publishers Ibadan, Nigeria.

Akinrotimi, O.A., Abu, O.M.G. & Aranyo, A.A. (2011). Environmental friendly aquaculture key to sustainable fish farming development in Nigeria. *Continental Journal of Fisheries and Aquatic Science* 5(2), 17-31.

Al-Harbi, A.H. & Uddin, N. (2005). Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture* 250, 566-572.

Anetekhai, M.A. (2013). Catfish Aquaculture Industry Assessment in Nigeria. Seisay M., and Nouala S.A edited. Inter-African Bureau for Animal Resources, African Union, Nairobi, Kenya.

AOAC. (2012). Official Methods of Analysis of the Association of Official Analytical Chemists, 20th Edition, Association of Official Analytical Chemists, Washington DC.

APHA (2012). Standard Methods for the Examination of Water and Waste Water. 22nd Edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC.

Aquacop. (1975). Maturation and spawning in captivity of penaeid shrimp: *Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives, *Metapenaeus ensis* de Haan and *Penaeus semisulcatus* de Haan. In: Avault W, Miller R, editors. Proceedings of the Sixth Annual Meeting World Mariculture Society, Louisiana State University, Baton Rouge, pp. 123–129.

Atanda, A.N. (2012). Fish species diversification in agriculture for the success of the agriculture transformation agenda: The role of tilapia production. Presented at Fisheries Society of Nigeria (FISON) Annual Public Lecture, July, Lagos, Nigeria.

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

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

Avnimelech, Y. (2009). Biofloc Technology-A Practical Guide Book. The World Aquaculture Society, Baton Rouge. LA, USA.

Avnimelech, Y. (2012). Biofloc Technology-A Practical Guide Book. 2nd ed. The World Aquaculture Society, Baton Rouge, LA, USA.

Avnimelech, Y., Mokady, S. & Schoroder, G.L. (1989). Circulated ponds as efficient bioreactors for single cell protein production. *Israeli Journal of Aquaculture-Bamidgeh* 41, 58–66.

Avnimelech, Y., Kochva, M. & Diab, S. (1994). Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. *Israeli Journal of Aquaculture-Bamidgeh* 46, 119–131.

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

Ballester, E.L.C., Abreu, P.C., Cavalli, R.O., Emerenciano, M., de Abreu, L. & Wasielesky, J.W. (2010). Effects of practical diets with different protein levels on the performance of *Farfantepeneau spaulensis* juveniles nursed in zero exchange suspended microbial flocs intensive system. *Aquaculture Nutrition* 16, 163–172.

Bano, Q., Ilyas, N., Bano, A., Zafar, N., Akram, A. & Hassan, F. (2013). Effect of *Azospirillum* inoculation on maize (*Zea mays* L.) under drought stress. *Pakistan Journal of Botany* 45 (SI), 13-20.

Bossier, P. & Ekasari, J. (2017). Biofloc technology application in aquaculture to support sustainable development goals. *Microbial Biotechnology* 10, 1012-1016

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, L. & Corner, R. (2010). Aquaculture: Global status and trends. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*. 365, 2897-2912.

Boyd, C.E. (2003). Guidelines for aquaculture effluent management at the farm-level. *Aquaculture* 226, 101-112.

Bradford, M.M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72*, 248–254.

Brune, D.E., Schwartz, G., Eversole, A.G., Collier, J.A. & Schwedler, T.E. (2003). Intensification of pond aquaculture and high rate photosynthetic systems. *Aquacultural Engineering* 28, 65–86.

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, 393–411.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. & Pearson, D.C. (2004). The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high intensity, zero-exchange system. *Aquaculture* 232, 525–537.

Cardona, E., Saulnier, D., Lorgeoux, B., Chim, L. & Gueguen, Y. (2015). Rearing effect of biofloc on antioxidant and antimicrobial response in *Litopenaeus stylirostris* shrimp facing an experimental sub-lethal hydrogen peroxide stress. *Fish & Shellfish Immunology.* 45, 933-939.

Cardona, E., Lorgeoux, B., Chim, L., Goguenheim, J., Le Delliou, H. & Cahu, C. (2016). Biofloc contribution to antioxidant defence status, lipid nutrition and performance of broodstock of the shrimp *Litopenaeus stylirostris*: consequences for the quality of eggs and larvae. *Aquaculture* 452, 252–262.

Chaignon, V., Lartiges, B.S., El Samrani, A. & Mustin, C. (2002). Evolution of size distribution and transfer of mineral particles between flocs in activated sludges: an insight into floc exchange dynamics. *Water Research* 36 (3), 676–684.

Chen, S., Ling, J. & Blancheton, J.P. (2006). Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* 34, 179–197.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P. & Verstraete, W. (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture* 270, 1–14.

Crab, R., Kochva, M., Verstraete,W. & Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering* 40, 105–112.

Crab, R., Chielens, B., Wille, M., Bossier, P. & Verstraete, W. (2010a). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research* 41, 559-567.

Crab, R., Lambert, A., Defoirdt, T., Bossier, P. & Verstraete, W. (2010b). The application of bioflocs technology to protect brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. *Journal of Applied Microbiology* 109, 1643–1649.

Crab, R., Defoirdt, T., Bossier, P. & Verstraete, W. (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* 356, 351-356.

Csavas, I. (1995). Status and perspectives of culturing catfishes in East and South East Asia. Presented at the International Workshop on the Biological Basis for Aquaculture of Siluriformes, May, Montpellier, France.

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C. & Guillaume, J. (2004). Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. *Aquaculture* 235, 513–551.

Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W. & Bossier, P. (2008). Quorum sensing and quorum quenching in *Vibrio harveyi*: lessons learned from *in vivo* work. *ISME Journal* 2, 19–26.

Degani, G., Ben-Zvi, Y. & Levanon, D. (1989). The effect of different protein levels and temperatures on feed utilization, growth and body composition of *Clarias gariepinus* (Burchell 1822). *Aquaculture* 76, 293-301.

de Barros, H.P. & Valenti, W.C. (2003). Food intake of *Macrobrachium rosenbergii* during larval development. *Aquaculture* 216, 165–176.

de Lorenzo, M.A., Candia, E.F.S., Schleder, D.D., Rezende, P.C., Seiffert, W.Q. & Vieira, F.N. (2016). Intensive hatchery performance of Pacific white shrimp in the biofloc system under three different fertilization levels. *Aquacultural Engineering* 72-73, 40-44.

De Schryver, P. & Verstraete, W. (2009). Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. *Bioresource Technology* 100, 1162–1167.

De Schryver, P., Crab, R., Defoirdt, T., Boon,N. & Verstraete, W. (2008). The basics of bioflocs technology: the added value for aquaculture. *Aquaculture* 277, 125-137.

De Schryver, P., Boon, N., Verstraete, W. & Bossier, P. (2012). The biology and biotechnology behind bioflocs. In: Avnimelech Y, editor. Biofloc Technology - A Practical Guide Book, 2nd ed., The World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 217-230.

Divakaran, D., Chandran, A. & Pratap C. R. (2011). Comparative study on production of α-amylase from *Bacillus licheniformis* strains. *Brazilian Journal of Microbiology* 42, 1397-1404.

DOF. (2016). Annual Fisheries Statistics 2015. Department of Fisheries, Malaysia, Ministry of Agriculture and Agro-based Industries, Putrajaya, (www.dof.gov.my)

Dube, M.A., Tremblay, A.Y. & Liu, J. (2007). Biodiesel production using a membrane reactor. *Bioresource Technology* 98, 639-647.

Ebeling, J.M., Timmons, M.B., & Bisogni, J.J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture* 257, 346–358.

Ebrahimi, M., Raijon, M.A., Meng, G.Y. & Farjam, A.S. (2014). Omega-3 fatty acid enriched chevon (goat meat) lowers plasma cholesterol levels and alters gene expressions in rats. *BioMed Research International* 2014, 1-8

Ekasari, J. (2014). Biolfoc Technology as an Integral Approach to Enhance Production and Ecological Performance of Aquaculture. PhD thesis, Ghent University, Belgium

Ekasari, J., Crab, R. & Verstraete, W. (2010). Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *HAYATI Journal Biosciences* 17, 493 125-130.

Ekasari, J., Azhar, M.H., Surawidjaja, E.H., Nuryati, S., De Schryver, P. & Bossier, P. (2014a). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. *Fish & Shellfish Immunology* 41, 332-339.

Ekasari, J., Angela, D., Waluyo, S.H., Bachtiar, T., Surawidjaja, E.H., Bossier, P. & De Schryver, P. (2014b). The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture* 426-427, 105-111.

Ekasari, J., Rivandi, D.R., Firdausi, A.P., Surawidjaja, E.H., Zairin, M., Bossier, P. & De Schryver, P. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture* 441, 72–77.

Ekasari, J., Suprayudi, M.A., Wiyoto, W., Haganah, R.F., Tenggara, G.S., Sulistiani, R., Alkali, M. & Zairin, M. (2016). Biofloc technology application in African catfish fingerling production: The effects on the reproductive performance of broodstock and the quality of eggs and larvae. *Aquaculture* 464, 349-356.

El-Barbary, M.I. (2010). Some clinical, microbiological and molecular characteristics of *Aeromonas hydrophila* isolated from various naturally infected fishes. *Aquaculture International* 18, 943-954.

EL-Haroun, E.R. (2007). Improved growth rate and feed utilization in farmed African catfish *Clarias gariepinus* (Burchell 1822) through a growth promoter biogen supplementation. *Journal of Fisheries and Aquatic Science* 2, 319-327

Emerenciano, M., Cuzon, G., Goguenheim, J., Gaxiola, G., & Aquacop. (2012). Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. *Aquaculture Research* 44, 75-85.

Emerenciano, M., Gaxiola, G. & Cuzon, G. (2013). Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry', In: Biomass Now – Cultivation and Utilization, INTECH, pp 1-28. London, UK. http://dx.doi.org/10.5772/53902

FAO. (2014a). The State of World Fisheries and Aquaculture- Opportunities and challenges. Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations Rome.

FAO. (2014b). FAO Yearbook of Fishery and Aquaculture Statistics, Food and Agriculture Organization of the United Nations, Rome.

FAO. (2014c). FAO and Rice Market Monitor, Trade and Markets Division Food and Agriculture Organization of the United Nations, vol.17, no. 4, 2014

FAO. (2016a). The State of World Fisheries and Aquaculture- Contributing to food security and nutrition for all. Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome.

FAO. (2016b). Cultured Aquatic Species Information Programme: *Clarias gariepinus*. Fisheries and Aquaculture Department, Food and Agricultural Organization of the United Nations, Rome. Accessed at 1325 hours, 22nd April 2017 http://www.fao.org/fishery/culturedspecies/Clarias_gariepinus/en.

FAO. (2017). Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (FishstatJ). In: FAO Fisheries and Aquaculture Department. Accessed at 0944 hours, 3rd May 2017 www.fao.org/fishery/statistics/software/fishstatj/en.

Faturoti, E.O., Balogun, A.M. & Ugwu, L.C. (1986). Nutrient utilization and growth responses of *Clarias* fed different protein levels. *Nigerian Journal of Appied Fisheries and Hydrobiology* 1, 31-49.

FDF. (2008). Fisheries Statistics of Nigeria, Projected Human Population; Fish Demand and Supply in Nigeria, 2000 – 2015. Federal Department of Fisheries, Nigeria.

Ferreira, G.S., Bolívar, N.C., Pereira, S.A., Guertler, C., F.N., Vieira, Mouriño, J.L.P. & Seiffert, W.Q. (2015). Microbial biofloc as source of probiotic bacteria for the culture of *Litopenaeus vannamei*. *Aquaculture* 448, 273-279.

Fuentes, J., Garbayo, I., Cuaresma, M., Montero, Z., González-del-Valle, M. & Vílchez, C. (2016). Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. *Marine Drugs* 14, 100. doi:10.3390/md14050100.

Furtado, P.S., Poersch, L.H. & Wasielesky Jr.,W. (2012). Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. *Aquaculture* 321, 130–135.

Green, B.W. (2015). Performance of a temperate-zone channel catfish in biofloc technology production system during winter. *Aquacultural Engineering* 64, 60-67.

Hagopian, D.S & Riley, J.G. (1998). A closer look at the bacteriology of nitrification. *Aquacultural Engineering* 18, 223–244.

Hapsari, F. (2016). The effect of fermented and non-fermented biofloc inoculated with bacterium *Bacillus cereus* for catfish (*Clarias gariepinus*) juveniles. *AACL Bioflux* 9, 334-339.

Hancock, L., Goff, L. & Lane, C. (2010). Red algae lose key mitochondrial genes in response to becoming parasitic. *Genome Biology and Evolution* 2, 897–910.

Hargreaves, J.A. (1997). A simulation model of ammonia dynamics in commercial catfish ponds in the southeastern United States. *Aquacultural Engineering* 16, 27–43.

Hargreaves, J.A. (2006). Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering* 34, 344–363.

Hart J.P., Lovis W.A., Schulenberg J.K. & Urquhart G.R. (2007). Paleodietary implications from stable carbon isotope analysis of experimental cooking residues. *Journal of Archaeological Science* 34, 804–813.

Hecht, T., Uys, W. & Britz, P.J. (Editors). (1988). Culture of Sharp Tooth Catfish, *Clarias gariepinus*, in Southern Africa. National Scientific Programmes Unit: CSIR, SANSP Report 153.

Hepher, B. (1985). Intensification under land and water limitations. *Geojournal*, 10(3), 253-259.

Hepher, B. (1988). Nutrition of Pond Fish. Cambridge University Press, Cambridge, UK.

Irfan M., Asghar, U., Nadeem M., Nelofer R. & Syed, Q. (2016). Optimization of process parameters by *Bacillus* sp. in submerged fermentation. *Journal of Radiation Research and Applied Sciences* 9, 139-147.

Irigoyen, J.J., Emerich, D.W. & Sanchez-Diaz, M. (1992). Water stress induced changes in the concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum* 8, 455-460.

Ismail, S., Kamarudin, M.S. & Saad, C.R. (2016). Dietary lipid requirement of lemon fin barb hybrid. *Journal Environmental Biology* 37 (SI) ,765-774.

Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., Kuo, W.C. & Horgen, F.D. (2008). Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. *Aquaculture Research* 39, 118–133.

Karaki, N., Aljawish, A., Humeau, C., Muniglia, L. & Jasniewski, J. (2016). Enzymatic modification of polysaccharides: Mechanisms, properties, and potential applications: A review. *Enzyme and Microbial Technology* 90, 1-18

Karami, A., Romano, N., Hamzah, H., Simpson, S.L. & Yap, C.K. (2016). Acute phenanthrene toxicity to juvenile diploid and triploid African catfish (*Clarias gariepinus*): Molecular, biochemical, and histopathological alterations. *Environmental Pollution* 212, 155-165.

Kechik, I. A. (1995). Aquaculture in Malaysia. In T. U. Bagarinao & E. E. C.Flores (Eds.), Towards Sustainable Aquaculture in Southeast Asia and Japan: Proceedings of the Seminar-Workshop on Aquaculture Development in Southeast Asia, Iloilo City, Philippines, 26- 28 July 1994 pp. 125-135. Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

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

Kim, S.K., Pang, Z., Seo, H.C., Cho, Y.R., Samocha, T. & Jan, I.K. (2014). Effect of bioflocs on growth and immune activity of Pacific white shrimp, *Litopenaeus vannamei* postlarvae. *Aquaculture Research* 45, 362-371.

Kreutz, L.C., Barcellos, L.J.G. Valle, S.D.F., Silva, T.D.O., Anziliero, D., Santos, E.D.D., Pivato, M. & Zanatta, R. (2011). Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish and Shellfish Immunology. 3*0, 51-57.

Krishna, C. & Van Loosdrecht, M.C.M. (1999). Effect of temperature on storage polymers and settleability of activated sludge. *Water Research* 33 (10), 2374–2382.

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, 1168-1182.

Kusdarwati, R., Kismiyati, Sudarno, Kurniawan, H. & Prayog Y.T. (2017). Isolation and identification of *Aeromonas hydrophila* and *Saprolegnia* sp. on catfish (*Clarias gariepinus*) in floating cages in Bozem Moro Krembangan Surabaya. IOP Conf. Ser.: Earth Environ. Sci. 55, 012038, http://iopscience.iop.org/17551315/55/1/012038.

Liong, P.C., Hanafi, H.B., Merican, Z.O. & Nagaraj, G. (1988). Aquaculture development in Malaysia. In: J.V. Juario & L.V. Benitez (Eds.) Perspectives in Aquaculture Development in Southeast Asia and Japan: Contributions of the SEAFDEC Aquaculture Department. Proceedings of the Seminar on Aquaculture Development in Southeast Asia, 8-12 September 1987, Iloilo City, Philippines. pp. 73-90. Tigbauan, Iloilo, Philippines: SEAFDEC, Aquaculture Department.

Liu, C.H. & Chen, J.C. (2004). Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish & Shellfish Immunology* 16, 321-334.

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.

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L. & Tan, H. (2014). Growth, digestive activity, welfare and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) in recirculating aquaculture system and an indoor biofloc systems. *Aquaculture* 422-423, 1-7.

Lynch, S.M. & Frei, B. (1993). Mechanisms of copper-and iron dependent oxidative modification of human low-density lipoprotein. *Journal of Lipid Research* 34, 1745–1751.

Ma, H. (2004). Cholesterol and human health. *Nature and Science* 2(4), 17-21.

Maisonnier-Grenier, S., Clavurier, K., Saulnier, L., Bonnin, E. & Geraert, P. (2006). Biochemical characteristics of wheat and their relation with apparent metabolisable energy value in broilers with or without non-starch polysaccharide enzyme. *Journal of the Science of Food and Agriculture* 86, 1714-1721

Malone, R.F. & Pfeiffer, T.J. (2006). Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquacultural Engineering* 34, 389–402.

Mannan, H., Moh, J.H.Z., Kasan., Suratman, S., Ikhwanuddin, M. (2017) Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp,*Penaeus vannamei*, culture in closed hatchery system. *Applied Water Science* 7, 2437–2446.

Martins, A.M.P., Heijnen, J.J. & van Loosdrecht, M.C.M. (2003). Effect of dissolved oxygen concentration on sludge settleability. *Applied Microbioliology and Biotechnology* 62 (5–6), 586–593.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d'Orbcastel, E. & Verreth, J.A.J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering* 43(3), 83–93.

Martins, A.C.D., Flores, J.A., Porto C. Wasielesky Jr, W. & Monserrat, J.M. (2015). Antioxidant and oxidative damage responses in different organs of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) reared in a biofloc technology system. *Marine and Freshwater Behaviour Physiology* 48, 279-288.

Martins, G. B., Tarouco, F., Rosa, C. E., & Robaldo, R. B. (2017). The utilization of sodium bicarbonate, calcium carbonate or hydroxide in biofloc system: water quality, growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus*). *Aquacultur*e 468, 10-17.

Mohanta, K.N., Mohanty, S.N., Jena, J.K. & Sahu, N.P. (2008). Protein requirement of Silver barb, *Puntius gonionotus* fingerlings. *Aquaculture Nutrition* 14, 143-152.

Moss, S.M., Moss, D.R., Arce, S.M., Lightner, D.V. & Lotz, J.M. (2012). The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture. *Journal of Invertebrate Pathology* 110, 247–250.

Nadeem, M., Qazi, J.I. & Baig, S. (2009). Effect of aeration and agitation on alkaline production by *Bacillus licheniformis* UV-9 mutant. *Turkish Journal of Biochemistry* 34, 89-96

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature* 405, 1017–1024.

Ng, W.K. (2009). The current status and future prospects for the aquaculture industry in Malaysia. World Aquaculture (Magazine) 40 (3), September, 26-30.

Oduguwa, O.O., Edema, M.O. & Ayeni, A.O. (2008). Physico-chemical and microbiological analyses of fermented corn cob, rice bran and cowpea husk for use in composite rabbit. *Bioresource Technology* 99, 1816–1820.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Magaji, U., Hussin, G., Ramli, A. & Miah, G. (2016). Fermentation quality and addittives: A case of rice straw silage. *BioMed Research International* article ID 7985167, 14pages.

Oloyede, O.O., James, S., Ochenne, O.B., Chinma, C.E. & Akpa, V.E. (2015) Effects of fermentation on the functional and pasting properties of defatted *Moringa oleifera* seed flour. *Food Science & Nutrition* 4, 89-95

Osman, M.A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences* 10, 1–6.

Pérez-Fuentes, J.A., Hernández-Vergara, M.P., Pérez-Rostro, C.I. & Fogel, I. (2016). C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc systems under high density cultivation. *Aquaculture* 452, 247-251.

Piedrahita, R.H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* 226, 35-44.

Rakocy, J.E., Bailey, D.S., Thoman, E.S. & Shultz, R.C. (2004). Intensive tank culture of tilapia with a suspended, bacterial based treatment process: new dimensions in farmed tilapia. In: Bolivar R, Mair G, Fitzsimmons K, editors. Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, 584–596.

Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V. & Purushothaman, C.S. (2016). Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research* 47, 3432-3444.

Ray, A.J. & Lotz, J.M. (2017). Shrimp (*Litopenaeus vannamei*) production and stable isotope dynamics in clear-water recirculating aquaculture systems versus biofloc systems. *Aquaculture Research*. 48, 4390-4398.

Ray, J.A., Lewis, B.L., Browdy, C.L. & Leffler, J.W. (2010). Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture* 299, 89–98.

Rick, W. (1974a). Trypsin. In Methods of Enzymatic Analysis. 2nd ed. Editor, H.U. Bergmeyer, Academic Press. New York and London, pp 1013-1024.

Rick, W. (1974b). Chymotrypsin. In Methods of Enzymatic Analysis. 2nd edition. Editor, H.U. Bergmeyer, Academic Press. New York and London, pp 1006-1012.

Romano, N. (2017). Aquamimicry: A revolutionary concept for shrimp farming. Global Aquaculture Advocate. February 10.

Romano, N. & Zeng, C. (2013). Toxic effects of ammonia, nitrite, and nitrate to decapod crustaceans: a review on factors influencing their toxicity, physiological consequences, and coping mechanisms. *Reviews in Fisheries Science* 21(1), 1-21.

Romano, N., Simon, W., Ebrahimi, M., Fadel, A. H., Chong, C. M., Kamarudin, M. S. (2016). Dietary sodium citrate improved oxidative stability in red hybrid tilapia (*Oreochromis* sp.) but reduced growth, health status, intestinal short chain fatty acids and induced liver damage. *Aquaculture* 458, 170-176.

Romano, N., Kanmani, N., Ebrahimi, M., Chong, C.M., Teh, J.C., Hoseinifar, S.H., Amin, S.M.N., Kamarudin, M.S. & Kumar, V. (2018). Combination of dietary pre-gelatinized starch and isomaltooligosaccharides improved pellet characteristics, subsequent feeding efficiencies and physiological status in African catfish, *Clarias gariepinus*, juveniles. *Aquaculture* 484, 293-302.

Rosenberry, B. (2010). Controlling pH in biofloc ponds. The shrimp news international. Accessed at 1100 hours, 24th May, 2017. http://www.shrimpnews.com/FreeReportsFolder/phContronBioflocPonds.html

Rudel, L.L. & Morris, M.D. (1973). Determination of cholesterol using o-phthalaldehyde. *Journal of Lipid Research* 14, 364–366.

Saavedra, M.J., Guedes-Novais, S., Alves, A., Rema, P., Tacao, M.,Correia, A. & Martinez-Murcia, A. (2004). Resistance to B-Lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (*Oncorhynchus mykiss*). *International Microbiology* 7, 201-211.

Sakkaravarthi, K. & Sankar, G. (2015). Identification of effective organic carbon for biofloc shrimp culture system. *Journal of Biological Sciences* 15(3), 144-149.

Samocha, T.M., Patnaik, S., Speed, M., Ali, A.M., Burger, J.M., Almeida, R.V., Ayub, Z., Harisanto, M., Horowitz, A. & Brock, D.L. (2007). Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering* 36, 184–191.

Schneider, O., Sereti, V., Eding, E.H. & Verreth, J.A.J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquacultural Engineering* 32, 379–401.

Schneider, O., Blancheton, J.P., Varadi, L., Eding, E.H. & Verreth, J.A.J. (2006). Cost Price and Production Strategies in European Recirculation Systems. Linking Tradition & Technology Highest Quality for the Consumer, Firenze, Italy, WAS.

Serfling, S.A. (2006). Microbiol flocs: natural treatment method supports freshwater, marine species in recirculating systems. Global Aquaculture Advocate 9, 34–36.

Serra, F.P., Gaona, C.A.P., Furtado, P.S., Poersch, L.H. & Wasielesky, W. (2015). Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquaculture International* 23, 1325-1339.

Sinha, A.K., Baruah, K. & Bossier, P. (2008). Horizon Scanning: the potential use of biofloc as an anti-infective strategy in aquaculture – an overview. Aquaculture Health International 13, 8-10.

Sohier, L. (1986). Microbiologie Appliquée à l'Aquaculture Marine Intensive. Thèse Doctorat d'Etat, Université Aix-Marseille II Marseille, France.

Souza, D.M.D., Suita, S.M., Romano. L.A., Wasielesky Jr, W., & Ballester, E.L.C., (2014). Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille,1817) in a Biofloc technology system. *Aquaculture Research* 45, 270–277.

Stehr, C.M., Johnson, L.L. & Myers, M.S. (1998). Hydropic vacuolation in the liver of three species of fish fro the U.S. West Coast: lesion description and risk assessment associated with contaminant exposure. *Diseases of Aquatic Organisms* 32, 119-135.

Su, Y., Zhang, Y., Zhou, X. & Jiang M. (2013). Effect of nitrate concentration on filamentous bulking under low level of dissolved oxygen in an airlift inner circular anoxic-aerobic incorporate reactor. *Journal Environmental Sciences* 25(9), 1736-1744.

Subasinghe, R.P. (2005). Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive Veterinary Medicine* 67, 117–124.

Suharmili, R., Kamarudin, M.S., Saad, C.R., Ina-Salwany, M.Y., Ramezani-Fard E. & Mahmud M.H. (2015). Effects of varying dietary protein level on the growth, feed efficiency and body composition of lemon fin barb hybrid fingerlings. *Iranian Journal of Fisheries Sciences* 14(2), 425-435.

Summerfelt, S.T., Sharrer, M.J., Tsukuda, S.M. & Gearheart, M. (2009). Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. *Aquacultural Engineering* 40, 17-27.

Supriyati, Haryati, T., Susanti, T. & Susana, I.W.R. (2015). Nutritional value of rice bran fermented by *Bacillus amyloliquefaciens* and humic substances and its utilization as a feed ingredient for broiler chickens. *Asian-Australasian Journal of Animal Science* 28, 231-238

Suzuki, Y., Maruyama, T., Numata, H., Sato, H. & Asakawa, M. (2003). Performance of a closed recirculating system with foam separation, nitrification and denitrification units for intensive culture of eel: Towards zero emission. *Aquacultural Engineering* 29(3), 165-182.

Tal, Y., Schreier, H. J., Sowers, K. R., Stubblefield, J. D., Place, A. R. & Zohar, Y. (2009). Environmentally sustainable land-based marine aquaculture. *Aquaculture* 286, 28-35.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. & Vinci, B.J. (2002). Recirculating Aquaculture Systems, 2nd Edition. Cayuga Aqua Ventures, New York. 769 pgs.

van Rijn, J., Tal, Y. & Schreier, H. J. (2006). Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering* 34 (3), 364–376.

Ventola, C.L. (2015). The antibiotic resistance crisis, Part 1: Causes and threats. *Pharmacy & Therapeutics* 40, 277-283.

Verdegem, M.C.J., Bosma, R.H. & Verreth, J.A.J. (2006). Reducing water use for animal production through aquaculture. *International Journal Water Resources Development* 22, 101-113.

Verschuere, L., Rombaut, G., Sorgeloos, P. & Verstraete, W. (2000). Probiotic bacteria as biocontrol agents in aquaculture. *Microbiology and Molecular Biology Reviews* 64, 655–671.

Vilani, F.G., Schveitzer, R., Arnates, R.F., Vieira, F.N., Santo, C.M.E. & Seiffert, W.Q. (2016). Strategies for water preparation in a biofloc system. Effects of carbon source and fertilization dose on water quality and shrimp performance. *Aquacultural Engineering* 74, 70-75.

Vinatea, L., Malpartidaa, J., Carbób, R., Andreeb, K.B., Gisbertb, E. & Estévezb A. (2018). A comparison of recirculation aquaculture systems versus biofloc technology culture system for on-growing of fry of *Tinca tinca* (Cyprinidae) and fry of grey *Mugil cephalus* (Mugilidae). *Aquaculture* 482, 155–161.

Wang, X., Li, Z., Su, J., Tian, Y., Ning, X., Hong, H. & Zheng, T. (2010). Lysis of a red-tide causing alga, *Alexandrium tamarense*, caused by bacteria from its phycosphere. *Biological Control* 52, 123–130.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L. & Zheng., Z. (2015). Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus. Aquaculture* 443, 98-104.

Wasielesky, W., Atwood, H., Stokes, A. & 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, 396-403.

Wei, Y.F., Liao, S.A. & Wang, A.L. (2016). The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. *Aquaculture* 465, 88–93.

Wetzel, R. G., & Likens, G. E. (2000). Limnological Analyses. New York: Springer

Widanarni, Ekasari, J. & Maryam, S. (2012). Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp. cultured at different stocking densities. *HAYATI Journal Biosciences* 19(2), 73-80.

Wilen, B.M. & Balmer, P. (1999). The effect of dissolved oxygen concentration on the structure, size and size distribution of activated sludge flocs. *Water Research* 33(2), 391–400.

Wilen, B.M., Jin, B. & Lant, P. (2003). The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water Research* 37(9), 2127–2139.

Xu, W.J. & Pan, L.Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture* 356–357, 147–152.

Xu, W.J. & Pan, L.Q. (2013). Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture* 412–413, 117–124.

Xu, W.J. & Pan, L.Q. (2014). Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. *Aquaculture* 426-427, 181-188.

Xu, W.J., Pan, L.Q., Sun, X. & Huang, J. (2013). Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. *Aquaculture Research* 44, 1093–1102.

Xu, W.J., Morris, T.C. & Samocha, T.M. (2016). Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. *Aquaculture* 453, 169–175.

Yusuf, M.W., Utomo, N.B.P., Yuhana, M. & Widanarni (2015). Growth performance of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp. *Journal of Fisheries and Aquatic Science* 10, 523-532.

Zhang, K., Pan, L., Chen, W. & Wang, C. (2017). Effect of using sodium bicarbonate to adjust the pH to different levels on water quality, the growth and the immune response of shrimp *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. *Aquaculture Research* 48, 1194-1208

Zhao, Z., Xu, Q., Luo, L., Wang, C., Li, J. & Wang, L. (2014). Effect of feed C/N ratio promoted bioflocs on water quality and production performance of bottom and filter feeder carp in minimum-water exchanged pond polyculture system. *Aquaculture* 434, 442-448.

Zhao, D., Pan, L., Huang, F., Wang, C. & Xu, W. (2016). Effects of different carbon sources on bioactive compound production of biofloc, immune response, antioxidant level, and growth performance of *Litopenaeus vannamei* in zero-water exchange culture tanks. *Journal of the World Aquaculture Society Soc*. 47, 566- 576.

Zmyslowska, I., Korzekwa, K. Szarek, J. (2009). *Aeromonas hydrophila* in fish aquaculture. *Journal of Comparative Pathology* 141, 313.