PHYSIOLOGY AND EXPRESSION OF GROWTH AND DISEASE RESISTANCE GENES OF *Clarias gariepinus* (Burchell, 1822) CULTURED IN STAGNANT RENEWAL AND SIMPILFIED-AQUAPONIC SYSTEMS

By

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CERTIFICATION

This is to certify that this project was carried out by SODIPE, MERCY AYOMIDE, with matriculation number 214614, under my supervision at the Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Oyo State, Nigeria.

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DEDICATION

This project is dedicated to the Triune God, in whom I live, move and have my being, for the privilege to carry out this study and to my beloved family and incredible friends, whose unwavering support and commitment have been instrumental in my journey.

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ABSTRACT

Production systems such as aquaponics, integrate fish and plant farming for improved production of diverse food components in a resource-use efficient and environmentally friendly manner. This system is being promoted for improved food production in cities. Cultural systems can influence the well-being of cultured fishes such as *Clarias gariepinus*. Knowledge of physiological and genetic responses of cultured fish to a production system provides information on the environmental suitability of the system, for management and policy interventions. Therefore, this study assessed the haematological, biochemical, and gene expression responses of *Clarias gariepinus* reared in a stagnant-renewal system and a simplified aquaponic system.

The study was conducted at the INCITIS-Food Living Lab, University of Ibadan, using blood samples from *Clarias gariepinus* collected over three weeks. Fish samples reared in aquaponic tanks (C) and the stagnant renewal /control tanks (A) were collected 2weeks intervals three times and subjected to standard procedures of physiology and gene expression studies. Blood and serum were collected for analysis of haematological using standard centrifugation methods, and biochemical analyses via spectrophotometry method. Subsamples of each collected blood were assessed for expression levels of IGF3 (growth), MHC (immunity), and Actin (cell integrity) through qPCR analysis. Statistical analysis was conducted using the *student's T-test*, with significance set at $p<0.05$.

The hematological and biochemical parameters were not significantly different across A and C. However, C had more stable haematological and biochemical parameters than A; PCV was slightly higher in C (37.50±9.19%) than A (34.50±12.02%) in the first week, both declined to 32.50±0.71% by week three. The RBC was higher in C (3.27±0.84 $\times 10^6/\mu L$) than A (2.56±0.72 $\times 10^6/\mu L$), while MCV was greater in A (112.00±24.04 fL) than C (64.50±6.36 fL), suggesting potential for compensatory oxygen transport mechanisms. Total protein levels fluctuated in A, peaking at 4.63±0.31 g/dL before dropping to 3.45±0.66 g/dL, while C increased steadily to 5.10±0.76 g/dL. The MHC and IGF genes were downregulated in C and A. However, higher MHC downregulation occurred in A (-0.71±0.92) compared to C (-2.87±1.15), indicating the signal for inhibitory immune action of MHC was less strong in A with significance of the expression of the gene at 0.0026. Conversely, IGF was -2.44±1.46 ΔCT in A and -1.90±1.16 ΔCT in C, reflecting the signal for the struggle to grow was strong in A.

The aquaponic system and the water-stagnant –renewal fish production systems had similar physiological characteristics, both displayed downregulation of growth and disease response genes as internal coping strategies with greater regulations from the aquaponic system.

Keywords: Haematology, Metabolism, Aquaculture, sustainability

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

The global demand for aquatic food products is expected to rise with population growth and economic development, with a potential to match or exceed the demand for other animal-based protein foods (Belton *et al.,* 2020; Costello *et al.,* 2020), creating the need for increased aquaculture production. The need for increased aquaculture is also conditioned by the stagnant nature and the risk of overexploitation in the capture industry.

Aquaculture uses water media for the production of organisms such as finfish, shellfish, and aquatic plants for human consumption and use (FAO,2020). Aquaculture has become the fastest-growing animal protein-producing sector worldwide (FAO,2011); its production has implications for global food security (Bush *et al.,* 2013; Bank *et al.,* 2020). Aquaculture is considered to have a strong ability to add resilience to the global food system in the face of climate change and increased demand for animal (Troell *et al.,* 2014). The continuous growth in aquaculture production has increased the average consumption of seafood at the global level(Food and Agriculture Organization, FAO, 2024).

Aquaculture is now a more essential and reliable source of seafood for human consumption(Handy, 2024). Fish consumption would increase in Asia, America, Africa, and European regions (Kobayashi *et al.,* 2015). Given the contribution of aquaculture to food security, the sustainable development of the aquaculture sector is a crucial requirement to meet the future needs of a world population of 9 billion by 2050 (FAO, 2016). Meanwhile, recent global trends point to a decline in landing from capture fisheries, an indicator that fish stocks have reached or even exceeded the point of maximum sustainable yield. Aquaculture therefore remains the only viable means for increasing for increasing fish production to meet the protein needs of the global population.

Aquaculture production depends on several factors and the interactions between them. These factors include fish seedlings, feed, farming area, climatic factors, production systems, management practices, and institutions. An increase in one or more of the aquaculture production input factors could influence output (Bostock *et al.,* 2010). Aquaculture resource abundance, farming practices, technology, and markets are critical factors that could contribute to aquaculture growth (Kumar and Engle, 2016). However, farming systems could influence the well-being, growth, reproduction, and vulnerability to diseases in cultured fish species.

Aquaculture in Nigeria is chiefly the story of catfish culture and the hope of fish supply would be dependent on its development and culture (Adewunmi and Olaleye, 2011). Catfishes of the family Clariidae comprise the most commonly cultivated fishes in Nigeria. The growth of aquaculture in Nigeria now is largely being boosted by a steady rise in catfish culture. Meanwhile, the African catfish is the most farmed fish in Nigeria (Nwachi and Toritseju, 2014). The catfish, *Clarias gariepinus* is famous among "point and kill" restaurants in Nigeria as the fish can be kept alive until it is ready to be prepared (Olagunju *et al.,* 2022).

Nigerian aquaculture dates back to the 1950s when the extensive production system of catfish, carp, and tilapia was introduced to various regions of the country (Anetekhai,2013). By the early 90s, an increased number of farmers had adopted the culture of fish species at a small-scale level. During the last decade, the farming of catfish has played a significant role in the growth of Nigerian aquaculture and accounts for over half of the total national aquaculture production (Olagunju *et al.,* 2022). The emergence of fish production in holding facilities such as ponds, fiberglass tanks, tarpaulin, plastic tanks, and concrete ponds in stagnant water-renewal production systems has increased opportunities for fish farming, especially, the culture of catfish in Nigeria.

There is an increasing trend of more sophisticated and environmentally friendly production systems such as the Recirculatory Aquaculture Systems (RAS) and the aquaponic systems. The

aquaponics system is relatively new and considered sophisticated in Nigeria's aquaculture sector. Aquaponics is the integration of a hydroponic plant production system on a nutrient solution or in some type of artificial media, the plants get the nutrients from the water instead of soil (Aires, 2018). Aquaponic is an efficient method for producing vegetables with minimal water and space (Asao ,2012). A recirculating aquaculture system (RAS) is most often a closed production system in which water quality is maintained through a filter system. In such systems, plants absorb nutrients permitting purified water to recirculate to fish tanks (Tyson *et al.,* 2011).

Aquaponics has been regarded as a sustainable and environmentally friendly method for producing plants and fish(Yildiz *et al.,* 2017). In addition , it supports the idea of circular economy as the wastes produced by the fish are turned into a resource for the plants(Faiqa *et al.,* 2022). Concerns about pollution have raised interest in aquaponic systems as a valid option to get rid of aquaculture wastes through the production of high-value vegetables (Roy *et al.,* 2013).

Various types of aquaponics systems can be classified based on the design and operational principles, ranging from the media-based aquaponics system to the Nutrient film technique (NFT) aquaponics (Padhiary, 2024). Aquaponics systems could operate on a single or multispecies fish or plant cultivation. In either case, the aquaaponic system's vitality and prosperity are based on fish, plant and bacterial welfare, of which the interrelatedness between these, is highly complex and in direct association with water quality (Yildiz *et al.,* 2017); such an approach could influence fish welfare, physiology, growth, and development. However, this has to be established for various aquaponic systems in the culture of different fish species.

Aquaponic systems is emerging, but the high cost of energy and the complexity of designs have been issues of concern. However, there are emerging low-cost and simple prototypes for

adoption. Such prototypes target the use of locally sourced low-cost materials and simplified designs. One of such is the INCITIS-Food simplified aquaponic prototype, available at the University of Ibadan, Nigeria. This aquaponic system is an advancement of the RAS, representing an integration of plant and fish production in an improved resource-efficient multi-plant species combination production approach. However, there is a need to generate baseline data on the suitability of this system and this has to be holistic, cutting across phenotypic including biochemical characteristics and genetic assessment on genes at play in reaction to the environmental condition posed by the rearing system.

Gene expression can be mobilized by prevailing environmental conditions. Whether a particular gene is expressed and the degree to which it is expressed depend strongly on the environmental conditions experienced by the organism (Kanellakis *et al.,* 2022). Moreover, vulnerability, and disease resistance can be strongly influenced by genetic endowment and environmental conditions during developmental stages (de Kloet *et al.,* 1998; Young *et al.,* 2013). There is the need to understand the different intrinsic genetic promptings of the cultured fish species in the culture environment of the aquaponic prototype.

1.2 STATEMENT OF PROBLEM

The physiological and immune health of the fish needs to be monitored as catfish farming is now the backbone of the aquaculture industry in Nigeria. There is also the concern on practicing aquaculture sustainably and the effects of aquaponics system on the overall health of the fish. The relationship between crucial biomarkers like haematological parameters, globulin levels, glucose and key genes involved in the overall health of the species remains unclear likewise in so many other species as well. These biomarkers are essential for assessing the immune competence, metabolic health, stress response in fish. It is expedient to investigate the interplay of gene expression and physiological parameters in cultured fish species in model aqauponic system.

1.3 JUSTIFICATION OF THE STUDY

This research work focuses on the characterisation of the gene expression of some selected genes and the physiological response of African catfish(*Clarias gariepinus*) , cultured in stagnant-water renewal and multispecies simplified aquaponics system. It is of extreme importance to compare the relationships as this will provide useful to every stakeholders for adoption and or rejection of the model system and assist in data-driven policy-making on the system. The results of this investigation are supposed to shed light on the extent of environmental influence on the health of the fish in the systems.

1.4 OBJECTIVES

Main Objectives

The main objective of this study is to understand whether the use of the incitis-food model simplified aquaponic system would influence change in physiological condition and gene expression of the cultured *Clarias gariepinus.*

Specific objectives

The specific objectives of the study are to:

1. Investigate the haematological and biochemical parameters of *Clarias gariepinus* cultured in water stagnant renewal and the incitis-food simplified aquaponic systems.
2. Evaluate the expression of reproduction (IGF3) gene in *Clarias gariepinus* cultured in water stagnant renewal and the incitis-food simplified aqauponics production systems.
3. Examine the disease resistance-related gene (MHC CLASS III) in *Clarias gariepinus* cultured in water stagnant renewal and the incitis-food simplified aquaponic production systems.

4. Assess the cell signaling potentials (Actin expression) in *Clarias gariepinus* cultured in water stagnant renewal and the incitis-food simplified aquaponic production systems.

CHAPTER TWO

LITERATURE REVIEW

2.1 STUDY SPECIES

2.1.1 TAXONOMY

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Claridae

Genus: *Clarias*

Species: *gariepinus*

Binomial name: *Clarias gariepinus* (Burchell,1822)

2.1.2 DESCRIPTION

The African catfish or mudfish as they are usually named, have long bodies with dorsally flattened head enclosed by bony plates. They have huge terminal mouths and four pairs of barbels which look like the whiskers of a cat hence the name: catfish (Zakariah *et al.,*2017). The African catfish or *Clarias gariepinus* is a freshwater fish species in the family Clariidae and belongs to the order Siluriformes .Catfishes are a highly diverse and widely dispersed group of fish with over 3000 species around the world(Meri, 2016).African Catfish is known for its hardiness and high adaptability to adverse environmental conditions(Meri, 2016).

The African catfish(*Clarias gariepinus*) is widely found in Africa freshwater and the Middle East(Tesfahun,2017). In Ethiopia, it is widely distributed nearly in all water bodies such as in the rift valley, Abay, Awash, Tekeze and so on(Awoke, 2015). The African catfish is an

opportunistic and omnivorous feeder eating a wide variety of food items such as algae, macrophytes, zooplankton, insects, fish prey, detritus, amphibians and sand grains(Admasu *et al.,* 2015). However, in terms of prey importance, the foods of animal origins were consumed more by the fish in all of the water bodies(Tesfahun,2017).

The nostrils are far apart , with the anterior one tubular and the posterior ones equipped with a long tentacle. Both the dorsal and anal fins are without spines and are long almost reaching the caudal fin which is a single rounded lobe(Zakariah *et al.,* 2017). The pectoral fins all have spines and the air-bladder is bi-lobed. The numerous small teeth of this catfish are arranged in bands on the jaws and also on the roof of the mouth. Adult males can be differentiated from the females by the fact that they possess long, conical papilla, a projection from the vent containing the sexual opening, but this is always exposed(Zakariah *et al.,* 2016).

Catfishes have a suprabranchial air breathing organ which function as a lung enabling them to breath and survive in low oxygen environments and survive long periods out of water(Abidjan and Ivoire, 2014).The African Catfishes are air breathing fishes set apart by scale-less ,bony, elongated body, with long dorsal and anal fins and helmet like head. The colour varies from dark to light brown dorsally and cream to white colour in the underside. They can grow very large with maximum catch weight of over 290kg of the Mekong giant catfish recorded in the year 2005(Hogan, 2011).

The dorsal fin has 61-80 soft rays and the anal fin has 40-65 soft rays. They have strong pectoral fins with spines that are serrated on the outer side. Catfish are economically and ecologically essential fish species in Africa and in other tropical countries(Davies *et al.,* 2013).Spawning of catfish mostly takes place at night in shallow submerged areas of rivers and streams. Males show highly aggressive behavior during courtship. Mating and courtship takes place in the shallow waters between isolated pairs of both sexes. The male lies in the U-shaped curve with

the head of females held for a couple of seconds. A batch of milt and egg is released followed by a release of eggs by females over a wide area. There is no parental care to ensure survival of catfish offspring except for appropriate site selection(Meri, 2016). Egg and larval development is fleeting and the larva is able to swim within 48-72 hours after fertilization(FAO,2012).

Under favourable conditions, a matured of *Clarias gariepinus* can lay up to 60,000 eggs/kg of body weight. There is a direct relationship between fecundity and total length, total weight and gonad weight of fish. Usually, fecundity increases with increasing brood stock size, but the egg size may vary from one spawning season to another and the number of eggs contained in a particular volume may be also differ(Meri,2016).

2.1.3 ECONOMIC IMPORTANCE OF AFRICAN CATFISH

Global aquaculture production has increased by over 500% since the late 1980s (Yuan *et al.,* 2019). In 2018,global aquaculture fish production increased to about 82 million tonnes, accounting for 46% of global fish production(FAO,2020). Aquaculture is considered to have a strong ability to add resilience to the global food system in the face of climate change and increased demand for animal protein(Troell *et al.,* 2014). The dietary contribution of seafood is important in terms of protein and micronutrients. In 2013, world per capita fish consumption was over 19kg(FAO,2016). The continuous growth in aquaculture production has increased the average consumption of seafood at the global level .Aquaculture is now more essential than fisheries as a reliable source for seafood for human consumption. Fish consumption is estimated to increase further in countries in Asia, America, Africa and European regions during 2010 to 2030(Kobayashi *et al.,* 2015). Given the contribution by the global aquaculture sector to food security ,the sustainable development of the aquaculture sector is a crucial requirement to meet future needs from a world population of 9 billion by 2050(FAO,2016).

Generally, aquaculture production depends on several factors ,and the interactions between them, including fish seedlings ,feed ,farming area, climatic factors, farming systems, management practices, institutions. An increase in one or more input factor increases aquaculture output(Bostock *et al.,* 2010). However ,feed wastes, faeces, pathogens may have negative impacts .Marine resource abundance ,farming practices ,technology and markets have been discussed as critical factor that contributed to the growth experienced in recent decades(Kumar and Engle, 2016).

Nigerian aquaculture dates back to the 1950s when extensive culture of catfish,carp and tilapia was introduced to various regions of the country(Anetekhai,2013). Aquaculture growth was improved by the further promotion of the production of the African catfish in the early 1980s with the training of government extension agents on its artificial fertilization(Anetekhai,2013). By the early 90s,an increased number of farmers had adopted the culture of the culture of the species at a small-scale level. In 2003,African catfish farming took off owing to the increased availability of commercial protein-rich fish feeds(Anetekhai,2013). During the last decade,the farming of catfish has played a significant role in the growth of Nigerian aquaculture and accounts for over half of the total national aquaculture production(Olagunju *et al.,* 2022).

Fingerlings or juveniles are raised to table size catfish which takes place in different culture systems such as earthen ponds, concrete ponds, cages, fibre glass tanks and container that can stock the preferred number of fish. Sources of water can be through underground seepage ,rainfall or preferably borehole(Bassey *et al.,* 2013).

Earlier studies in Nigeria have reported good profitability of catfish farms, with 60 to 90% Return on Investment(ROI)(Tunde *et al.,* 2015).

One of Nigeria`s most essential aquaculture sectors is catfish farming, which is also widely referred as a profitable enterprise. In developing countries like Nigeria, which has an ever-

increasing population of close to 200 million people, catfish farming offers huge prospects for creating employment opportunities, generating revenue, minimizing poverty, and enhancing food security(Wuyep and Rampedi, 2018).

Due to its high protein content and the fact that it contains important nutrients for boosting the health of individuals with illnesses like cardiovascular diseases and others, fish is also a common food in every family in Nigeria. The average Nigerian consumes about 41% of their total protein from fish, which is less than the average global intake of about 13kg per person per year. This suggests that despite inefficient production, there is a significant demand for fish in the nation. This increasing demand for fish has demonstrated that the nation`s marketing and production industries have promising futures(Balami *et al.,* 2019;Ashley and Adelaja, 2022).

The world`s largest producer of African catfish, one of the most valuable freshwater species in Africa is Nigeria. The Federal Department of Fisheries(2013) estimates that the fishing sub-sector contributed around 5% of Nigeria`s Gross Domestic Product(GDP). In accordance with statistics from the Food and Agriculture Organization(2022), the nation`s aquaculture production increased from 22,000 tonnes in 1999 to more than 300,000 tonnes in 2017. The aquaculture sector is currently worth about 261.8 billion in the nation`s currency. Ninety percent of Nigerian farmers involved in the fish value chain produce catfish, which supports one million direct jobs across the chain(Daily Trust,2022).

According to Cowan(2021), economic analysis of catfish production is the assessment of the cost and benefits to determine the economic feasibility associated with catfish production in the state. Nigeria requires about 3.6 million metric tonnes(MMT) of fish yearly to meet the dietary needs of the ever growing population of over 180 million people in Nigeria. But, the total aggregate domestic fish supply from all sources both captured and farmed is about

1.2MMT with importation of frozen fish accounting for about 2.4 million metric tonnes per annum (Nnodium,2022).

This shows that Nigeria still depends on massive importation of fish to meet her fish needs as there still exists a deficit of 2.5 MMT as the current demand has skyrocketed to more than 3.6 million per annum. Between 2019 and 2020 importation of frozen fish had increased from 789.74 million dollars to1.27 billion dollars respectively, representing 38% increase. While between January and June in 2021 Nigeria imported the blue whittling species worth over 60 billion naira. While in 2022, Nigeria imported 2.14 billion dollar worth of fish into the country(Daily Trust,2022;Jaiyeola, 2022).

Nearly half of the fish consumed in Nigeria are produced in artificial ponds, making the catfish farming sub-sector one of the most profitable and rapidly expanding agricultural industries. *Clarias gariepinus*, popularly known as the African catfish, is the most widely cultured catfish species in Nigeria due to its tolerance of the local environment, resistance to diseases, and quality traits that minimizes loss for farmers(Idris-Adeniyi, 2018).

2.2 INDICATORS OF STRESS IN CATFISH SPECIES

Stress is a state of altered homeostasis which is rebuilt collectively by physiological and behavioural responses of an organism(Fazio,2019). It represents homeostasis disequilibrium raising both specific and non-specific responses which enable the animal to overcome agitation(Bouyoucos *et al.,* 2021).

Plasma glucose has been used as a biomarker for measuring stress levels and secondary metabolic responses in fish(Hafeez and Sherwani,2023). The level of plasma glucose in the blood depends on the metabolic production of glucose and the velocity at which it is removed from circulation. Animals obtain energy for cellular metabolism through glycolysis or the

breakdown of glucose-a pathway in the cytoplasm. Stressors cause alterations in glucose metabolism which can alter various tissues such as muscle, gill, and brain which usually leads glucose intolerance and insulin resistance. The liver is the seat of glucose production through the glycogenolysis and or gluconeogenesis pathways. It also acts as a reservoir of glucose until the animal needs it for metabolic activities. Closely related stress related hormones ,adrenaline(epinephrine) and cortisol can increase the plasma glucose levels in fish during stressful conditions(Hafeez and Sherwani, 2023).

Hyperglycemia can result from various environmental stressors and alter the development, health, and quality of fishes.(Sanches *et al.,* 2015).

Figure 1: An image showing the study species, *Clarias gariepinus*.

Source :Binay Chakraborty (2024).

2.3 HAEMATOLOGY INDICATIONS

Haematological characteristics are vital tools that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. They are very important and their changes depend on fish species, age,and diseases(Erhunmwunse and Ainerua, 2013).

Fish lives favourably in aquatic environments, and their blood profile is a useful tool in the development of aquaculture system(Baraya and Abdulrazak,2022). Fish blood can inform quantifiable physiological changes faster than any other physiological assessment parameters(Baraya and Abdulrazak, 2022). Changes in the value of the component of a blood parameter in comparison with the reference values are used in the interpretation of the health status of living organisms(Etim *et al.,* 2014). Fish haematology has been used as an index to detect various stressful conditions such as diseases, exposure to pollutants, hypoxia and anaemia(Yanuhar *et al.,* 2021). Intensive system of fish culture has been characterised by high tendency of disease outbreaks. Diseases are among the limiting factors that diminishes fish production(Baraya and Abdulrazak,2022). Therefore, haematological evaluation is recommended in fish culture for early detection of diseases and environmental research(Yanuhar *et al.,* 2021).

Likewise, the examination of serum constituents has provided useful information for detection and diagnosis of diseases in fish(Baraya and Abdulrazak, 2022). In particular, a number of liver injury biomarkers have usually been used to detect unhealthy state in fish(Soulivongsa *et al.,* 2021)

2.4 HAEMATOLOGICAL PARAMETERS

Haematological parameters are vital indicators of fish health, providing insights into their physiological and pathological status. Haematological parameters ,red blood cell(RBC),haemoglobin(Hb), packed cell volume (PCV),white blood cell (WBC), and differential leucocyte counts ,namely lymphocytes, neutrophils, monocytes, eosinophils, basophils, and band cells (Baraya and Abdulrazak, 2022).

Low erythrocyte counts indicate anaemia, while high levels indicate polycythemia in fish(Ikun *et al.,* 2013). Anaemia has an impact on growth performance of fish due to the low RBC counts, and inevitably reduced food supply to cells ,tissues and organs (Baraya and Abdulrazak,2022). The WBC is the main component of the body`s defence system(Awolabi, 2011;Adedeji and Adegbile, 2011).

2.4.1 KEY HAEMATOLOGICAL PARAMETERS

i. Haemoglobin(Hb):is a metalloprotein found in red blood cells that binds and transports oxygen from the gills to tissues and organs. Its levels indicate the oxygen-carrying capacity of the blood, which is essential for metabolism. Low haemoglobin levels suggest anaemia or stress to low dissolved oxygen and high levels may indicate polycythemia, often a response to dehydration or stress(Baraya and Abdulrazak, 2022).

ii. Red Blood Cell(RBC) Count: RBCs are responsible for oxygen transport and are crucial for maintaining homeostasis. Their count reflects the fish`s ability to supply oxygen to tissues efficiently. A low RBC count can indicate anaemia, or exposure to pollutants, and an increased count may suggest dehydration or poor water quality(Adeyemo *et al.,* 2014).

iii. White Blood Cell(WBC) Count: WBCs are part of the immune system, defending against infections and diseases. The total WBC count and differentials(lymphocytes ,neutrophils ,monocytes) help assess immune status(Baraya and Abdulrazak, 2022).

iv. Packed Cell Volume(PCV)/ Hematocrit: is the proportion of blood volume occupied by RBCs ,measured as a percentage. It gives insights into blood viscosity ,oxygen transport efficiency, and hydration status. Low PCV may indicate anaemia, blood loss(Baraya and Abdulrazak, 2022).

v. Mean Corpuscular Volume(MCV): It measures the average size of RBCs, helping anaemia types. High MCV (macrocytosis) suggests regenerative anaemia and vitamin deficiencies(Okorie-Kanu and Unakalamba, 2015).

vi. Mean Corpuscular Haemoglobin(MCH): indicates the average amount of haemoglobin per RBC. High MCH suggests excessive haemoglobin production, often due to bone marrow disorders. Low MCH is typically linked to iron-deficiency anaemia(Okorie-Kanu and Unakalamba, 2015).

vii. Mean Corpuscular Haemoglobin(MCHC):represents the haemoglobin concentration within the RBCs. High MCHC suggests haemolysis or dehydration and low MCHC is commonly associated with iron-deficiency anaemia(Okorie-Kanu and Unakalamba, 2015).

viii. Platelets: are involved in blood clotting and immune responses. Low platelet counts indicate bleeding disorder or infections, high counts may signal stress or chronic inflammation(Ayotunde, 2021).

2.5 AQUAPONICS SYSTEMS

Aquaponics is fundamentally based on the concept of symbiosis,whereby fish and plants engage in mutual interactions within a closed-loop ecological system (Horn *et al.,* 2024). Fish emit nutrient rich excrement, which plant utilizes as fertilizer. Conversely, the plants filter and purify the water, making a balanced environment that promotes the development of both

aquatic animals and plants. The comprehensive methodology employed in aquaponics not only reduces water consumption but also obviates the necessity for inorganic fertilizers, rendering it an environmentally sustainable substitute for traditional agricultural methods. Aquaponics systems is well designed and made to provide a suitable environment for both plants and fish. Green leafy vegetables with low to medium nutrients requirements are well adapted to aquaponics system, including capsicum, tomatoes, lettuce, cabbage, basil, spinach, chives, herbs, and water cress. The fishes and plants grown in aquaponics are totally organic (Ravindrath, 2017)

2.5.1 MEDIA-BASED AQUAPONIC SYSTEMS

Aquaponics systems that rely on media involving cultivating plants in containers containing a medium, such as gravel, clay pellets. This medium promotes the growth of plants` roots and acts as a substrate for the expansion of beneficial bacteria . Nitrifying bacteria promotes the conversion of fish waste into nitrogenous compounds ,which are subsequently absorbed by the roots of plants as essential nutrients(Troell *et al.,* 2023).

The purified water is reinstituted into the fish tank, therefore concluding the cycle. Media based systems can be classified into two primary categories: flood-and-drain(or ebb-and –flow) systems , which involves frequently flooding and draining the grow beds to maintain oxygen levels in the root zone, and constant flow systems , which involve the continuous flow of water through the grow beds(Padhiary,2024).

2.5.2 RAFT(DEEP WATER CULTURE) AQUAPONIC SYSTEMS

Raft aquaponic systems include cultivating plants in buoyant rafts placed on the surface of a water tank abundant in nutrients. The fish tank situated underneath supplies water that is abundant in nutrients, which is consistently pumped into the raft beds(Padhiary,2024). The plants` roots suspend themselves in the water, immediately absorbing nutrients. The process of

nutrient extraction by plants contributes to the purification of the water, which is then recirculated back into the fish tank. Raft systems can be classified according to the configuration of the rafts and the water flow, including single-raft systems, multiple-raft systems(Padhiary,2024).

2.5.3 NUTRIENT FILM TECHNIQUE (NFT) AQUAPONIC SYSTEMS

A thin layer of water called nutrient film technology (NFT) is circulated through tubes or channels in aquaponic systems. The nutritional solution is mixed with plant roots. Beneficial bacteria filter and convert fish waste into soluble nutrients , which are then transferred into the NFT tubes (Jayaprakash *et al.,* 2024). The roots of the plants directly absorb nutrients from running water ,and afterwards, the purified water is reintroduced into the fish tank. NFT systems are commonly grouped according to the framework of the tubes, which might be horizontal ,vertical or inclined(Khaoula *et al.,* 2021).

2.5.4 VERTICAL AQUAPONIC SYSTEMS

Vertical aquaponic systems are designed to optimize the utilization of vertical space, rendering them well-suited for urban settings or areas with restricted land availability. These systems usually utilize water towers or stacked containers as a means of cultivating plants in a vertical manner(Al-Kodmany *et al.,* 2018).

The water coming from the fish tank is elevated to the topmost part of the system and thereafter distributed vertically through the plant beds, promoting the provision of vital nutrients. Subsequently, the cleansed water is collected at the base and reinstituted into the fish tank. The design and framework of vertical system can depict variability, encompassing tower systems, wall-mounted systems, and modular systems that possess the capability to be vertically enlarged as required(Ito and Lee,2024).

2.5.5 KEY COMPONENTS OF AN AQUAPONIC SYSTEM

The fish tank serves as the primary element within the aquaponic system ,promoting the cultivation of fish. The reservoir has aquatic fish species whose excrement functions as a vital supply of nutrients for the surrounding flora. Grow beds are enclosed structures that contain a growing medium, such as gravel ,clay pellet that is used for the growth of plants. The root systems of the plants protrude into the cultivation regions, absorbing essential nutrients from the water that has been surrounded with fish faeces. In order to promote the circulation of water between the fish tank and grow beds, a water pump is employed. It guarantees a non-stop supply of water that is rich in nutrients for the plants, enhancing their growth and well-being.

The effectuation of an aeration system is important in order to maintain the right amount of oxygen in the fish tank. The system usually consists of air pumps and diffusers that promote the release of oxygen into the aquatic environment, thereby facilitating the overall welfare of the fish. The effectuation of a filtration system serves to get rid of solid waste and excess nutrients from the water, thus upholding water quality and assuage the build-up of substances that may pose risks to aquatic organisms. The prevalent techniques incorporated for filtration encompass mechanical filters, biofilters, and settling tanks. In aquaponic systems, beneficial bacteria are important because they enable the conversion of fish waste, particularly ammonia, into nitrogenous compounds that plants can easily assimilate as essential nutrients(Gichana *et al.,* 2018).

The bacteria colonise in the grow beds and biofilter media, thereby facilitating the volatilisation of nitrogen(Khaoula *et al.,* 2021).

The monitoring of water pH and temperature is crucial in order to uphold optimal conditions for the well-being of fish and plants. It is advised that pH levels conform to an acceptable range, often between 6.5 to7.0. While ensuring that the water temperature is optimal, it is crucial to

have an alternative power source to guarantee uninterrupted functioning of critical components in the event of power failure(Farghali *et al.,* 2023).

2.5.6 WATER QUALITY MANAGEMENT IN AQUAPONICS SYSTEMS

Several critical water parameters must be monitored and managed in an aquaponics systems to ensure sustainability and productivity. Temperature , pH levels ,dissolved oxygen, and ammonia concentrations are the primary indicators of a healthy aquaponic environment. Maintaining a pH level between 6.5 to 7.5 is optimal for fish and plant health(Reidel,2023).

Nutrient availability is another important factor influenced by water quality. The nitrogen cycle plays a crucial role in converting fish waste into usable nutrients for plants. High ammonia and nitrite levels can be toxic to fish, making the presence of nitrifying bacteria essential for a balanced system. Regular testing ensures that toxic accumulation is prevented, leading to a stable ecosystem(Sallenave,2016).

Proper filtration and aeration are essential for maintaining optimal water quality. Additionally ,maintaining dissolved oxygen(DO) levels above 5 mg/L supports fish respiration and root oxygenation in plants. Oxygenation can be improved using air stones, diffusers, or water agitation techniques to ensure adequate gas exchange.(Go Green Aquaponics,2025)

Temperature fluctuations can negatively impact system stability. Different fish species have specific temperature requirements. Temperature inconsistencies may lead to reduced fish metabolism, affecting nutrient production for plants. Implementing heating or cooling systems, such as water heaters or shade nets, can help regulate temperature fluctuations and enhance system efficiency(Sallenave,2016).

2.6 PHYSIOLOGICAL STRESS RESPONSE IN AFRICAN CATFISH

Albumins and globulin are essential blood proteins in fish, playing crucial roles in maintaining physiological balance and overall health. Albumin functions in the transport of different substances, including hormones ,vitamins and drugs throughout the bloodstream. Globulin on the other hand, are important for immune responses, helping in the defense against pathogens(Alalibo *et al.,* 2019).

In catfish such as *Clarias gariepinus* ,the measurement of these proteins through serum biochemical analyses provide valuable insights into their health status and physiological responses to environmental changes(Mahmoud *et al.,*2022).

2.7 GENE EXPRESSION IN FISH

2.7.1 IGF GENE

Reproduction and growth are the most basic attributes of living organisms;they are closely related but distinct from each other. Insulin-like-growth factors(IGFs) are important factors that regulate the growth and reproduction axis. IGFs consist of three ligands(IGF1, IGF2, IGF3), two receptors(IGF1R, IGF2R), and six IGF-binding proteins(IGFBPs). In fish, the basic function of Igf1 and Igf2 is to facilitate growth, but significant attention has been focused on their function in fish gonad development since Igf3 was discovered in fish ovary(Li *et al.,* 2011).

Igf3 has only been reported in zebrafish(*Danio rerio*)(Li *et al.,* 2011),tilapia(*Oreochromis niloticus*)(Li *et al.,* 2012), and orange-spotted grouper(*Epinephelus coiodes*)(Yang *et al.,* 2015). Two transcripts of the igf3 gene has been discovered in zebra fish, typically expressed in the ovary :transcript variant 1 is particularly expressed in the gonads ;transcript variant 2 is only expressed during early development(Li *et al.,* 2011).

In teleost fish,Igf1 and Igf2 are broadly expressed in various tissues;however, recent studies have shown that igf3 is particularly expressed in the gonads and brain tissue(Yang *et al.,* 2015),

beginning in the early stage of sex determination and differentiation and is related to gonad and reproductive development(Song *et al.,* 2016).

Current research suggests that Igf1 and Igf3 gradually increase in the follicle cells in zebra fish(Li *et al.,* 2011), and Igf2 expression is kept at a constant level in zebra fish(Song *et al.,* 2016). Igf3 expression in zebra fish is strongly regulates the gonadotropin analogue and human chorionic gonadotropin(HCG) and follicle-stimulating hormone(FSH) stimulates spermatogonial proliferation and differentiation in zebra fish by Igf3 (Nobrega *et al.,*2015).

2.7.2 MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) GENES

The MHC can be defined as a tightly link cluster of genes whose products play a crucial role in intercellular recognition and in discrimination between self and non-self. These genes code for antigens which involve determination of the compatibility of the transferred tissue(Cunningham,2024). The compatible tissues will be accepted by the immune system while the histo-incompatible ones are discarded. The rejection of foreign tissues leads to an immune feedback to cell surface molecules. The concept was first identified by Peter Gorer and George Snell. The main assignment of MHC molecules is to bring antigen to the cell surface for recognition by T cell(Aryal,2022).

The MHC is a genetic locus that encodes the glycoprotein molecules(transplantation antigens), which are responsible for tissue rejection of grafts between genetically dissimilar individuals. It is also the molecule that binds the peptide antigen processed by antigen-presenting cells and present them to the T-cells , therefore they are responsible for antigen recognition by the T-cell receptors(International journal on Advances in Life Sciences,2013).

Unlike the B-cell receptors that directly interact with the antigens, the T-cell receptors have an interwoven relationship with the MHC molecule, the T-cell receptors can only accept and bind processed antigens in form of peptides that are fixed to the MHC molecule, and so the T-cell

receptors are specific for MHC molecules. There are three common MHC molecules which are class I, class II, class III MHC proteins(Aryal,2022).

The MHC is also polymorphic, having a large number of alleles existing in the population for each of the genes. A large number of alleles exist in the population for each of the genes(Yamaguchi and Dijkstra, 2019).

Class III MHC molecule do not have any involvement in antigen presentation, the complement components such as C2,C4A, and factor B are the most essential compounds involved as class III MHC molecules(Aryal, 2022).

2.7.3 β ACTIN

Actin proteins are among the most important and abundant cytoskeletal elements of a cell. They play crucial roles in a wide array of cellular processes including cell division and the maintenance of gene expression. These functions are due to the ability of actin to form filaments that can quickly assemble and disassemble rendering to the needs of the cells.in vertebrates, six different but exceptionally conserved actin isoforms(Dominguez *et al.,* 2011).

The coding regions of these genes are highly conserved in different organisms. β
actin is a primary cytoplasmic actin isoform that is conserved and expressed in non-muscle eukaryotic cells as a housekeeping protein. It plays a vital role in regulation of the cell cytoskeleton, cell migration, cell division, and vesicle trafficking(Sangale *et al.,* 2020).
β actin is also involved in important nuclear processes such as transcription, mRNA processing, cell signaling and chromosome remodeling(Wang *et al.,* 2018). Studies also prove that the regulatory region the gene is highly efficient in regulating the expression of downstream foreign genes (Lee *et al.,* 2012), a good reason why it is widely used as internal marker or reference gene for studies quantifying gene expression(Reboucas *et al.,* 2013).

CHAPTER THREE

METHODOLOGY

3.1 DESCRIPTION OF THE STUDY AREA

This study was carried out at the living lab of the incites-food project located at the Department of Aquaculture and Fisheries Management, University of Ibadan, Oyo state, Nigeria. The living lab is a facility that combines fish culture and hydroponics to support a wide range of research and developmental activities on circular food systems for urban food production which involves hydroponics, aquaculture and aquaponics and water stagnant-renewal fish culture systems to create a closed-loop system that recycles nutrients and minimizes waste.

Plate 1: A picture taken of the study area at the University of Ibadan

3.2 EXPERIMENTAL FISH

The fish samples collected for this study were cultured from the juvenile stage to a table size in a stagnant and aquaponic systems of the living lab. Live samples of African Catfish(*Clarias gariepinus*) were collected from the living lab with permission from the authority in charge. A total of 12 fish were collected from the study sites for the study. Six (6) samples each were collected from the Static Renewal Aquaponics systems labeled as Tank A and the Multiple Plant Species Aquaponics systems labeled as Tank C. The collected fish had a mean weight of 445g for Treatment A and 452g for Treatment C. The mean standard length of the fish for Treatment A is 32.4cm and 31.8cm for Treatment C.

3.3 FISH PREPARATION AND BLOOD SAMPLING

The collected fish were immobilized by placing them on a clean laboratory table, and the face covered with a cloth.

Blood (1ml) was collected from the vertebral caudal blood vessel according to Schmit *et al.,* (1999),using disposable 5ml syringe and needle for biochemical analysis. The blood sample was emptied into a plain 5ml collection tube, each tube was slanted and left for 30 minutes for the blood to clot forming a clear portion of serum for the analysis. For the haematology analysis, the blood(1-2ml) was collected using method used for the biochemical analysis. The blood was emptied to a heparinized collection tube to prevent blood clotting, the same procedure was carried for the blood collection process for the qPCR (quantitative polymerase chain reaction)

For the hematology and biochemical tests, the blood from each fish was emptied into the heparinized bottle blood treated with Ethyl Diamine Tetracetic Acid (EDTA) and plain bottles respectively. Blood sample was centrifuge (1500 rpm for 7mins) to obtain the blood plasma and those for the biochemical analyses were collected into plain bottles. For the haematology analysis, the blood was mixed with EDTA and the analyses were carried out within 24 hours for the haematology tests.

3.4 HAEMATOLOGY TEST PROCEDURE FOR THE BLOOD SAMPLES

After the blood sample is emptied into the heparinized bottle, it was mixed gently with the anticoagulant to prevent clotting and ensure even distribution. Two blood smears on clean glass slides using a spreader to create a thin, even layer were prepared. The blood smears were allowed to air-dry completely and Giemsa's stain was used to stain. Micro-Haematocrit Centrifuge and Heamatocrit reader for measurement.

3.4.1 MICRO-HAEMATOCRIT CENTRIFUGE

A Micro-Haematocrit Centrifuge is a laboratory instrument used to separate blood components, specifically to measure the Packed Cell Volume(PCV) or Haematocrit(Hct)value. The centrifuge uses centrifugal force to separate the blood into its components ,with the heavier red blood cells settling at the bottom of the tube. The centrifuge consists of a rotor, tubes, and a control panel.

3.4.2 HAEMATOCRIT READER

A Haematocrit Reader is a device used to measure the Packed Cell Volume (PCV) or Haematocrit (Hct) value from a blood sample after centrifugation. The reader measures the height of the red blood cell column in the centrifuged blood sample, which corresponds to the PCV or Hct value. The reader consists of a calibrated scale , a viewing window, and sometimes a digital display.

3.5 BIOCHEMICAL TESTS

The biomolecules that were analyzed for this project include globulin, total protein, albumin and glucose levels.

For globulin analysis, the serum was diluted with a buffer solution and a precipitating agent was added to the sample to precipitate globulins. The sample was centrifuged to separate the precipitated globulins. The absorbance of the supernatant was measured at 450nm using a spectrophotometre.

For the total protein ,the serum sample was diluted with a buffer solution. A protein- binding dye(Coomassie Brilliant Blue) was added to the sample and the sample was then incubated for 5 to 10 minutes to allow the dye to bind to the proteins. The absorbance of the sample was measured at 595nm using a spectrophotometre.

For the glucose analysis, the serum was diluted with a buffer solution and glucose oxidase-peroxidase was added to the sample. The sample was incubated for 10 to 30 minutes to allow the enzyme to react with glucose. The absorbance of the sample was measured at 505nm using a spectrophotometre. The concentration was then calculated.

For the albumin analysis, the serum sample was diluted with a buffer solution. A bromocresol green dye was added to the sample. The sample was incubated for 30 seconds to 5 minutes to allow the dye to bind to albumin. The absorbance of the sample was measured at 628nm using a spectrophotometre. The concentration was then calculated.

3.6 SPECTROPHOTOMETRE

A spectrophotometer is a laboratory instrument used to measure the interaction between light and molecules. In biochemical testing, spectrophotometres are employed to quantify the concentration of specific biomolecules. In this case, globulin, total protein, glucose and albumin in the blood samples.

Spectrophotometry relies on the principles that molecules absorb, transmit or reflect light at specific wavelengths. When a sample is exposed to light, the molecules within the sample absorb certain wavelengths and transmit or reflect others. By measuring the amount of light absorbed or transmitted by a sample, the concentration of the molecule of interest can be determined.

3.7 QUANTITATIVE POLYMERASE CHAIN REACTION

The qPCR is a laboratory technique used to amplify and quantify specific DNA sequences, such as genes. It is a powerful tool for gene expression analysis, genetic diagnosis, and

research. It is based on the principle of Polymerase Chain Reaction(PCR),which involves the amplification of a specific DNA sequence using thermal cycling.

3.7.1 QUANTIFITATION OF GENE EXPRESSIONS

RNA extraction and amplification

Total RNA was extracted from tissue samples of specimens from Treatment A and Treatment C were collected biweekly at three times using a modified CTAB extraction protocol. Briefly, approximately 100mg tissue was grinded in 1ml CTAB extraction buffer (2% CTAB (Cetyltrimethyl ammonium bromide) powder (w/v), 100mM Tris-HCl, 20mM EDTA, 1.4M NaCl and 0.2% β-Mercaptoethanol (v/v) (Add just before use)) in a sterile mortal and pistil. sap was poured into new sterile tube and vortex briefly followed by an incubation at 600C for 10mins. Immediately, tubes were placed on ice and add equal volume of Phenol, Chloroform and Iso-amyl alcohol at 25:24:1 was added followed by vortex and centrifuge at 12000g for 10mins. 450µl of the supernatant was collected into a new sterile tube to which 300µl of cold Isopropanol was then added and Mixed gently and incubate for 1hr at -200C. Samples were then Centrifuge at 12000g for 10mins. to sediment the nucleic acid followed by decanting the supernatant gently and ensured the pellets were not disturbed. pellets were then washed by adding 500µl of 70% ethanol and centrifuge at 12000g for 5mins. This was repeated twice. Ethanol was then decanted and the RNA air dried at room temp. Pellets were then suspended in 50µl TE buffer or sterile water treated with EDPC till further use/storage.

PRIMER DESIGN

The relative expression of Major histocompatibility complex (MHC) and Insulin Growth factor (IGF3) genes was investigated in the presence of beta-actin as a housekeeping gene used as a reference gene. To design specific qPCR primers for these genes specific to *clarias gariepinus*, mRNA reference sequences of individual genes were obtained from the genebank database on

NCBI website (National Center for Biotechnology Information). https://www.idtdna.com/PrimerQuest/Home/Index site was then accessed and sequence pasted in the sequence entry box and multiple intercalating dye PCR primers were generated. It is very necessary to ensure that the primers will have a perfect match, this will enhance primer annealing during PCR. To do this, primers must anneal to regions where the sequences are conserved. Each primer pair was then checked for specificity to be sensitive to only the genes of interest to which it was designed to detect and also ability to cut across all aligned genes then the best primer was selected and synthesized at Inqaba in South Africa.

TABLE 1:THE PRIMER CHARACTERISTICS OF THE SEQUENCED GENES

Gene	Forward primer sequence	Reverse primer sequence
CG_IGFF	CAAGCGTGTTTCTTGGTTCAG	GTGTGCTATCATTGGGACCAT
MHC	AAATGATGTTCTGCCTAATGGT	AGCTGATCTTCTGTGTCCTC
Fish actin	TGATGAAATCGCCGCACT	CCACAATGGATGGGAAGACA

RNA treatment

20 ng total RNA was treated with NEB DNase 1 **(M0303) t**o eliminate extracted DNA. Briefly, a mixture of 2µl of 10ng/ µl RNA, 10 µl DNase I Reaction Buffer (10X), 1 µl DNAse I (RNase-free) and up to 100 µl with Nuclease-free H_2O. The mixture was then incubated at 37°C for 10 minutes followed by addition of 1 µl of 0.5 M EDTA to a final concentration of 5 mM. This was then heat inactivated at 75°C for 10 minutes and stored at -20ºC till use.

Gene quantification

20 µl reactions (following manufacturer's instructions) using Luna® Universal qPCR Master Mix Protocol (M3003) was used to detect the presence of the selected genes in the extracted RNA. Expression of Actin gene was used as an internal control. Briefly, A mix of 10 µl Luna Universal qPCR Master Mix, 0.5 µl Forward primer (10 µM) 0.5 µl Reverse primer (10 µM) and 0.06 Reverse Transcriptase (Promega) was made up to 18 µl with Nuclease-free water to which 2 µl of the treated RNA Template was added. This was then run with the profile: Initial Denaturation 95°C for 60 seconds followed by 40-45 of Denaturation 95°C 15 seconds Extension and plate reading at 60°C for 30 seconds followed by a termination at 72oC for 10 minutes. Amplification was conducted using the cfx96tm real-time system from bio-rad following manufacturer manual.

3.8 STATISTICAL ANALYSIS

All data were statistically analysed by using analysis of variance (ANOVA) to identify specific difference between means and the statistical significance level was established at $p<0.05$ using SPSS version 17 computer statistical software package.

CHAPTER FOUR

RESULTS

4.1 Heamatology and Biochemical Parameters of the C. gariepinus produced from Stagnant renewal and aquaponic Production Systems

Table 2 shows the mean concentration and standard deviation of the analysis of the haematology parameters in African catfish in Treatments A and C during the study for the first weekly sampling. The PCV and Hb indicate the oxygen-carrying capacity of the blood. Treatment A had a slightly higher PCV (34.50±12.02) compared to Treatment C (37.50±9.19). Similarly, Hb values were marginally higher in Treatment C(12.05±3.05) than in Treatment A (10.95±3.89). The WBC count, which reflect immune response, was relatively similar in both Treatments. However, lymphocyte values showed slight differences(Treatment A : 38.50±0.71, Treatment C:37.50±0.71), while heterophil counts were higher in Tank C (62.80±0.71) than in Tank A (61.00±1.41) . Platelet counts were similar between the tanks with Treatment A having 10.50±2.12 and Treatment C 11:00±1.41. Treatment C exhibited significantly higher MCV values (112.00±24.04) compared to Treatment A (64.50±6.36), indicating larger blood cells in Treatment C. However, MCH and MCHC remained similar across the groups, suggesting a consistent haemoglobin concentration within erythrocytes.

Table 3 shows the results of the analysis of the study in December 30, 2024. PCV and Hb levels showed a decrease in both tanks compared to the first weekly sampling. Treatment A had a PCV of 31.50±2.12, while Treatment C had 35.50±9.19. Similarly, Hb levels dropped in both tanks, with 10.30±0.57. The RBC counts showed a significant compared to the first table. Treatment A had 3.43±0.16, while Treatment C had 4.13±1.45. WBC counts increased slightly in Tank A (4.20±0.85) but remained the same in Tank C (3.20±1.13). Lymphocytes remained stable between the two points, with Treatment A at 39.50±2.12 and Treatment C at

40.00±0.00. Heterophils showed no significant variation, maintaining a similar ratio in both Treatments (59.50±0.71). Monocytes increased in both Treatments but remained with a low range (1.00±1.41 for Treatment A and 0.50±0.71 for Treatment C). Platelets levels remained relatively stable, with Treatment A at 10.00±0.00 and Treatment C at 10.50±2.12. MCV increased in both Treatments compared to the first weekly sampling. Treatment A had 91.50±10.61, and Treatment C had 87.00±8.49. MCH also increased slightly, with Treatment A at 29.00±2.58 and Treatment C at 28.00±2.83, indicating slightly more haemoglobin per red blood cell. MCHC remained stable at 32.50±0.71 for both tanks, suggesting that while RBC size increased, haemoglobin concentration remained constant.

Table 2: Mean value and Standard deviation of haematology parameters of *Clarias gariepinus* for the first weekly sampling.

Haematology parameters	Treatment A	Treatment C
PVC (%)	34.50 ± 12.02	37.50 ± 9.19
Hb (g/dl)	10.95 ± 3.89	12.05 ± 3.04
RBC (10^6/µL)	5.45 ± 2.40	3.33 ± 0.11
WBC (10^3/µL)	3.80 ± 0.28	3.20 ± 0.00
Platelets (10^3/µL)	10.50 ± 2.12	11.00 ± 1.41
MCV (fl)	64.50 ± 6.36	112.00 ± 24.04
MCH (pg)	20.00 ± 1.4	35.50 ± 7.78
MCHC (g/dL)	32.00 ± 0.00	32.00 ± 0.00
Lymphocytes (%)	38.50± 0.71	37.50 ± 0.71
Heterophils (%)	61.00 ± 1.41	62.80 ± 0.71
Monocytes (%)	0.50 ± 0.71	0.00 ± 0.00

values in each row without superscript letter are not significantly different at P<0.05

Table 3:Mean value and Standard deviation of haematology parameters of *Clarias gariepinus* for the second weekly sampling.

Haemoglobin Parameters	Treatment A	Treatment C
PCV (%)	31.50 ± 2.12	35.50 ± 9.19
Hb (g/dL)	10.30 ± 0.57	11.65 ± 3.18
RBC (10^6/µL)	3.43 ± 0.16	4.13 ± 1.45
WBC (10^3/µL)	4.20 ± 0.85	3.20 ± 1.13
Platelets(10^3/µL)	10.00 ± 0.00	10.50 ± 2.12
MCV (fL)	91.50 ±10.61	87.00 ± 8.49
MCH (pg)	29.00 ± 2.58	28.00 ± 2.83
MCHC (g/dL)	32.50 ± 0.71	32.50 ± 0.71
Lymphocytes (%)	39.50 ± 2.12	40.00 ± 0.00
Heterophils (%)	59.50 ± 0.71	59.50 ± 0.71
Monocytes (%)	1.00 ± 1.41	0.50 ± 0.71

Values in each row without superscript letter are not significantly different at $P<0.05$

Table 4 shows the mean concentration and standard deviation for the study for third weekly sampling. PCV values remained stable between the two tanks (32.50±0.71 for both). Hb levels were also similar, with 10.50±0.14 in Treatment A and 10.60±0.42 in Treatment C. This suggests that oxygen-carrying capacity was maintained across both groups with no significant deviations from previous values. RBC count in Treatment A was 4.04±0.28, slightly higher than in Treatment C (3.82±0.25).

WBC count was 3.10±0.14 in Treatment A and 3.70±0.42 in Treatment C. Lymphocytes remained stable, with 38.50±2.12 in Treatment A and 39.50±0.71 in Treatment C. Heterophils showed minimal variation, with 61.00±2.83 in Treatment A and 59.50±0.71 in Treatment C, indicating consistent immune function. Monocytes were slightly higher in Treatment C (1.00±1.41) than in Treatment A (0.50±0.71), the values remained low, suggesting no significant inflammatory response. Platelets counts were consistent across both Treatments (10.00±0.00), similar to previous values. MCV was 80.50±3.54 in Treatment A and 84.50±3.54 in Treatment C. Compared to the last results, MCV slightly decreased, suggesting a return to normal RBC size after the previous increase. MCH was 26.00±1.14 in Treatment A and 27.50±0.71 in Treatment C. MHCH remained stable at 32.50±0.71 for both Treatments, suggesting that RBCs maintained their normal haemoglobin saturation.

Table 5, 6, and 7 shows the mean values and standard deviations of biochemical parameters (Total Protein, Albumin, Globulin, and Glucose) in *Clarias gariepinus* from Treatments A and

C over three C over three weekly sampling periods. The increase in total protein in Treatment C the third weekly sampling suggests improved protein metabolism, the fluctuation in Treatment A, with a peak in the first and a decline in the last weekly sampling, may imply environmental stress and metabolic adjustments.

Albumin increased in Treatment A significantly in the second weekly sampling but then declined in third weekly sampling, which might suggest changes in protein synthesis or metabolic stress. Treatment C exhibited a gradual increase over time, indicating stable metabolic function and improved protein synthesis. Globulin levels increased over time in both Treatments, with Treatment C showing a significant increase by the third weekly sampling. This suggests an enhanced immune response or improved health status.

Table 4: Mean value and Standard deviation of haematology parameters of *Clarias gariepinus* for the sample period, third weekly sampling.

Haematology parameters	Treatment A	Treatment C
PCV (%)	32.50± 0.71	32.50 ± 0.71
Hb (g/dL)	10.50 ± 0.14	10.60 ± 0.42
RBC (10^6/µL)	4.04 ± 0.28	3.82 ± 0.25
WBC (10^3/µL)	3.10 ± 0.14	3.70 ± 0.42
Platelets (10^3/µL)	10.00 ± 0.00	10.00 ± 0.00
MCV (fL)	80.50 ± 3.54	84.50 ± 3.54
MCH (pg)	26.00 ± 1.14	27.50 ± 0.71
MCHC (g/dL)	32.50 ± 0.71	32.50 ± 0.71
Lymphocytes (%)	38.50 ± 2.12	39.50 ± 0.71
Heterophils (%)	61.00 ± 2.83	59.50 ± 0.71
Monocytes (%)	0.50 ± 0.71	1.00 ± 1.41

Values in each row without superscript letter are not significantly different at $P<0.05$

Table 5: Mean value and Standard deviation of biochemical parameters of *Clarias gariepinus* for the first weekly sampling.

Parameters	Treatment A	Treatment C
Total protein (g/dL)	3.42± 0.31	3.52 ± 0.31
Albumin (g/dL)	2.85± 0.02	2.25 ± 1.15
Globulin (g/dL)	0.57 ± 0.33	1.28 ± 0.42
Glucose (mg/dL)	65.50 ± 19.09	59.50 ± 9.19

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 6: Mean value and Standard deviation of biochemical parameters of *Clarias gariepinus* for the second weekly sampling.

Parameters	Treatment A	Treatment C
Total protein (g/dL)	4.63 ± 0.31	3.55 ± 0.21
Albumin (g/dL)	3.96 ± 1.13	2.44 ± 0.25
Globulin (g/dL)	0.67 ± 0.49	1.11 ± 0.04
Glucose (mg/dL)	58.50 ± 4.94	64.00 ± 1.41

Values in each row marked by the same superscript letter are not significantly different at $P<0.05$

Table 7: Mean value and Standard deviation of biochemical parameters of *Clarias gariepinus* for the third weekly sampling.

Parameters	Treatment A	Treatment C
Total protein (g/dL)	3.45 ± 0.66	5.10 ± 0.76
Albumin (g/dL)	2.16 ± 0.74	3.06 ± 0.33
Globulin (g/dL)	1.29 ± 0.85	2.04 ± 0.44
Glucose (mg/dL)	57.00 ± 2.83	64.00 ± 4.24

Values in each row without superscript letter are not significantly different at $P<0.05$

Table 8 shows the expression of MHC I using ACTIN as a reference gene in two treatments (A and C). MHC I expression was significantly lower in Treatment C (28.61 ± 0.04^{a}) compared to Treatment A (26.84 ± 0.24^{b}), while ACTIN remained stable across treatments. The ΔCT value was higher in Treatment A (0.33 ± 0.014^{a}) than in Treatment C (-1.56 ± 0.21^{b}), indicating higher relative expression of MHC I in Treatment C. The negative ΔΔCT further confirms this up regulation.

Table 9 shows the expression of MHC I using ACTIN as a reference gene in two Treatment types (A and C). MHC I expression was significantly lower in Treatment A (27.05 ± 0.04^{a}) compared to Treatment C (25.25 ± 0.08^{b}), while ACTIN remained stable across treatments (28.47 ± 0.07 vs. 28.57).The ΔCT value was higher in Treatment A (-1.41 ± 0.04^{a}) than in Treatment C (-3.32 ± 0.07^{b}), indicating higher relative expression of MHC I in Treatment C. The negative ΔΔCT (-1.76) in Treatment C further confirms MHC I up regulation compared to Treatment A.

Table 10 shows MHC I gene expression in *Clarias gariepinus* cultured in Aquaponic (C) and Stagnant-Renewal (A) Fish Production Systems, using ACTIN as a reference gene. MHC I expression was significantly higher in Stagnant-Renewal (27.57 ± 0.05^{a}) compared to Aquaponic (25.10 ± 0.14^{b}).ACTIN expression was slightly higher in Aquaponics (28.83 ± 0.18^{b}) than in Stagnant-Renewal (28.62 ± 0.00^{a}).ΔCT was lower in Aquaponics (-3.73 ± 0.035^{a}) compared to Stagnant-Renewal (-1.05 ± 0.05^{a}), indicating a higher relative expression of MHC I in the Stagnant-Renewal system. The negative ΔΔCT (-2.175) in Aquaponics further confirms MHC I regulation.

Table 8: Expression of MHC and Actin Genes in *C. gariepinus* cultured in Aquaponic and Stagnant renewal Fish Production Systems in the First Weekly Sampling

Specimen type	MHC 1	ACTIN	ΔCT	ΔΔCT
A1.1	28.58	28.24	0.34	
A2.1	28.63	28.31	0.32	
Mean±SD	28.61±0.04[a]	28.27±0.05[a]	0.33±0.014[a]	
C1.1	26.66	28.37	-1.71	
C2.1	27.01	28.42	-1.41	
Mean±SD	26.84±0.24[b]	28.39±0.04[a]	-1.56±0.21[b]	

**the more the negative the more expressed*

Multiple plant species aquaponic systems labeled Treatment C

A1.1= Stagnant Renewal systems Tank A1 collected in 1st Weekly sampling

A2.1= Stagnant Renewal systems Tank A2 collected in 1st Weekly sampling

C1.1= Aquaponic system Tank C1 collected in 1st Weekly sampling

C2.1= Aquaponic system Tank C2 collected in 1st Weekly sampling

A1.2= Stagnant Renewal systems Tank A1 collected in 2nd Weekly sampling

A2.2= Stagnant Renewal systems Tank A2 collected in 2nd Weekly sampling

C1.2= Aquaponic system Tank C1 collected in 2nd Weekly sampling

C2.2= Aquaponic system Tank C2 collected in 2nd Weekly sampling

A1.3= Stagnant Renewal systems Tank A1 collected in 3rd Weekly sampling

A2.3= Stagnant Renewal systems Tank A2 collected in 3rd Weekly sampling

C1.3= Aquaponic system Tank C1 collected in 3rd Weekly sampling

Table 9: Expression of MHC and Actin Genes in *C. gariepinus* cultured in Aquaponic and Stagnant renewal Fish Production Systems in the Second Weekly Sampling

Specimen type	MHC 1	ACTIN	ΔCT	ΔΔCT
A1.2	27.03	28.42	-1.39	-1.415
A2.2	27.08	28.52	-1.44	
Mean±SD	27.05±0.04[a]	28.47±0.07[a]	-1.41±0.04[a]	
C1.2	25.19	28.56	-3.37	-1.76
C2.2	25.31	28.58	-3.27	
Mean±SD	25.25±0.08[b]	28.57±0.01[a]	-3.32±0.07[b]	

**the more the negative the more expressed*

Stagnant Renewal systems labeled Treatment A

Multiple plant species aquaponic systems labeled Treatment C

A1.1= Stagnant Renewal systems Treatment A1 collected in 1st Weekly sampling

A2.1= Stagnant Renewal systems Treatment A2 collected in 1st Weekly sampling

C1.1= Aquaponic system Treatment C1 collected in 1st Weekly sampling

C2.1= Aquaponic system Treatment C2 collected in 1st Weekly sampling

A1.2= Stagnant Renewal systems Treatment A1 collected in 2nd Weekly sampling

A2.2= Stagnant Renewal systems Treatment A2 collected in 2nd Weekly sampling

C1.2= Aquaponic system Treatment C1 collected in 2nd Weekly sampling

C2.2= Aquaponic system Treatment C2 collected in 2nd Weekly sampling

A1.3= Stagnant Renewal systems Treatment A1 collected in 3rd Weekly sampling

A2.3= Stagnant Renewal systems Treatment A2 collected in 3rd Weekly sampling

C1.3= Aquaponic system Treatment C1 collected in 3rd Weekly sampling

C2.3= Aquaponic system Treatment C2 collected in 3rd Weekly sampling

Table 10: Expression of MHC and Actin Genes in *C. gariepinus* cultured in Aquaponic and stagnant-Renewal Fish Production Systems in the Third Weekly Sampling

Specimen type	MHC 1	ACTIN	ΔCT	ΔΔCT
A1.3	27.53	28.62	-1.09	-1.385
A2.3	27.60	28.62	-1.02	
Mean±SD	27.57±0.05[a]	28.62±0.00[a]	-1.05±0.05[a]	
C1.3	25.00	28.71	-3.71	-2.175
C2.3	25.20	28.96	-3.76	
Mean±SD	25.10±0.14[b]	28.83±0.18[b]	-3.73±0.035[a]	

**the more the negative the more expressed*

Stagnant Renewal systems labeled Treatment A

Multiple plant species aquaponic systems labeled Treatment C

A1.1= Stagnant Renewal systems Treatment A1 collected in 1st Weekly sampling

A2.1= Stagnant Renewal systems Treatment A2 collected in 1st Weekly sampling

C1.1= Aquaponic system Treatment C1 collected in 1st Weekly sampling

C2.1= Aquaponic system Treatment C2 collected in 1st Weekly sampling

A1.2= Stagnant Renewal systems Treatment A1 collected in 2nd Weekly sampling

A2.2= Stagnant Renewal systems Treatment A2 collected in 2nd Weekly sampling

C1.2= Aquaponic system Treatment C1 collected in 2nd Weekly sampling

C2.2= Aquaponic system Treatment C2 collected in 2nd Weekly sampling

A1.3= Stagnant Renewal systems Treatment A1 collected in 3rd Weekly sampling

A2.3= Stagnant Renewal systems Treatment A2 collected in 3rd Weekly sampling

C1.3= Aquaponic system Treatment C1 collected in 3rd Weekly sampling

C2.3= Aquaponic system Treatment C2 collected in 3rd Weekly sampling

Table 11 analyses the expression of Insulin-like Growth Factor (IGF) in two treatment (A and C), using ACTIN as a reference gene. IGF expression was significantly lower in Treatment A (27.52 ± 0.31^{a}) compared to Treatment C (27.84 ± 0.08^{b}).ACTIN expression remained stable across treatments (28.28 ± 0.05^{a} vs. 28.40 ± 0.04^{a}), validating it as a reference gene. ΔCT was lower in Treatment C (-0.56 ± 0.05^{b}) than in Treatment A (-0.75 ± 0.26^{a}).

Table 12 shows the expression of Insulin-like Growth Factor (IGF) in two treatments (A and C), using ACTIN as a reference gene. IGF expression was significantly lower in Treatment A (25.25 ± 0.5^{a}) compared to Treatment C (26.03 ± 0.01^{b}). ACTIN expression remained stable across treatments (28.47 ± 0.07^{a} versus 28.57 ± 0.01^{a}), ΔCT was lower in Treatment C (-2.54 ± 0.00^{b}) than in Treatment A (-3.23 ± 0.02^{a}).

Table 13 shows the expression of Insulin-like Growth Factor (IGF) in two treatments (A and C), using ACTIN as a reference gene. IGF expression was significantly lower in Treatment A (25.28 ± 0.42^{a}) compared to Treatment C (26.25 ± 0.47^{b}).ACTIN expression remained stable across treatments (28.62 ± 0.00^{a} vs. 28.84 ± 0.18^{a}), confirming its suitability as a reference gene. ΔCT was lower in Treatment A (-3.34 ± 0.04^{a}) than in Treatment C (-2.59 ± 0.65^{b}), indicating higher relative IGF.

Figure 2 and 3 both show the expression of the genes in the two Treatments highlighting the difference in the expression of the genes in both Treatments.

Table 14 and 15 shows the significance of the gene expression of both IGF and MHC for the total three weekly sampling periods. In which there is a significant difference in the expression of the MHC gene between the two treatments over the three weekly periods.

TABLE 11. Expression of IGF and Actin Genes in *C. gariepinus* cultured in Aquaponic and Stagnant renewal Fish Production Systems in the First Weekly Sampling

Specimen type	IGF	ACTIN	ΔCT	ΔΔCT
A1.1	27.30	28.24	-0.94	
A2.1	27.74	28.31	-0.57	
Mean±SD	27.52±0.31[a]	28.28±0.05[a]	-0.75±0.26[a]	
C1.1	27.78	28.37	-0.59	
C2.1	27.90	28.42	-0.52	
Mean±SD	27.84±0.08[b]	28.40±0.04[b]	-0.56±0.05[b]	

**the more the negative the more expressed*

Multiple plant species aquaponic systems labeled Treatment C

A1.1= Stagnant Renewal systems Treatment A1 collected in 1st Weekly sampling

A2.1= Stagnant Renewal systems Treatment A2 collected in 1st Weekly sampling

C1.1= Aquaponic system Treatment C1 collected in 1st Weekly sampling

C2.1= Aquaponic system Treatment C2 collected in 1st Weekly sampling

A1.2= Stagnant Renewal systems Treatment A1 collected in 2nd Weekly sampling

A2.2= Stagnant Renewal systems Treatment A2 collected in 2nd Weekly sampling

C1.2= Aquaponic system Treatment C1 collected in 2nd Weekly sampling

C2.2= Aquaponic system Treatment C2 collected in 2nd Weekly sampling

A1.3= Stagnant Renewal systems Treatment A1 collected in 3rd Weekly sampling

A2.3= Stagnant Renewal systems Treatment A2 collected in 3rd Weekly sampling

C1.3= Aquaponic system Treatment C1 collected in 3rd Weekly sampling

C2.3= Aquaponic system Treatment C2 collected in 3rd Weekly sampling

Table 12: Expression of IGF and Actin Genes in *C. gariepinus* cultured in Aquaponic and Stagnant renewal Fish Production Systems in the Second Weekly Sampling

Specimen type	IGF	ACTIN	ΔCT	ΔΔCT
A1.2	25.21	28.42	-3.21	

A2.2	25.28	28.52	-3.24	
Mean±SD	25.25±0.05[a]	28.47±0.07[a]	-3.23±0.02[a]	
C1.2	26.02	28.56	-2.54	-1.985
C2.2	26.04	28.58	-2.54	
Mean±SD	26.03±0.01[b]	28.57±0.01[a]	-2.54±0.00[b]	

**the more the negative the more expressed*

A1.1= Stagnant Renewal systems Treatment A1 collected in 1st Weekly sampling

A2.1= Stagnant Renewal systems Treatment A2 collected in 1st Weekly sampling

C1.1= Aquaponic system Treatment C1 collected in 1st Weekly sampling

C2.1= Aquaponic system Treatment C2 collected in 1st Weekly sampling

A1.2= Stagnant Renewal systems Treatment A1 collected in 2nd Weekly sampling

A2.2= Stagnant Renewal systems Treatment A2 collected in 2nd Weekly sampling

C1.2= Aquaponic system Treatment C1 collected in 2nd Weekly sampling

C2.2= Aquaponic system Treatment C2 collected in 2nd Weekly sampling

A1.3= Stagnant Renewal systems Treatment A1 collected in 3rd Weekly sampling

A2.3= Stagnant Renewal systems Treatment A2 collected in 3rd Weekly sampling

C1.3= Aquaponic system Treatment C1 collected in 3rd Weekly sampling

C2.3= Aquaponic system Treatment C2 collected in 3rd Weekly sampling

Table 13: Expression of IGF and Actin Genes in *C. gariepinus* cultured in Aquaponic and Stagnant renewal Fish Production Systems in the Third Weekly Sampling

Specimen type	IGF	ACTIN	ΔCT	ΔΔCT
A1.3	25.25	28.62	-3.37	-2.585
A2.3	25.31	28.62	-3.31	
Mean±SD	25.28±0.42[a]	28.62±0.00[a]	-3.34±0.04[a]	
C1.3	26.58	28.71	-2.13	-2.035

C2.3	25.91	28.96	-3.05
Mean±SD	26.25±0.47[b]	28.84±0.18[a]	-2.59±0.65[b]

**the more the negative the more expressed*

A1.1= Stagnant Renewal systems Treatment A1 collected in 1st Weekly sampling

A2.1= Stagnant Renewal systems Treatment A2 collected in 1st Weekly sampling

C1.1= Aquaponic system Treatment C1 collected in 1st Weekly sampling

C2.1= Aquaponic system Treatment C2 collected in 1st Weekly sampling

A1.2= Stagnant Renewal systems Treatment A1 collected in 2nd Weekly sampling

A2.2= Stagnant Renewal systems Treatment A2 collected in 2nd Weekly sampling

C1.2= Aquaponic system Treatment C1 collected in 2nd Weekly sampling

C2.2= Aquaponic system Treatment C2 collected in 2nd Weekly sampling

A1.3= Stagnant Renewal systems Treatment A1 collected in 3rd Weekly sampling

A2.3= Stagnant Renewal systems Treatment A2 collected in 3rd Weekly sampling

C1.3= Aquaponic system Treatment C1 collected in 3rd Weekly sampling

C2.3= Aquaponic system Treatment C2 collected in 3rd Weekly sampling

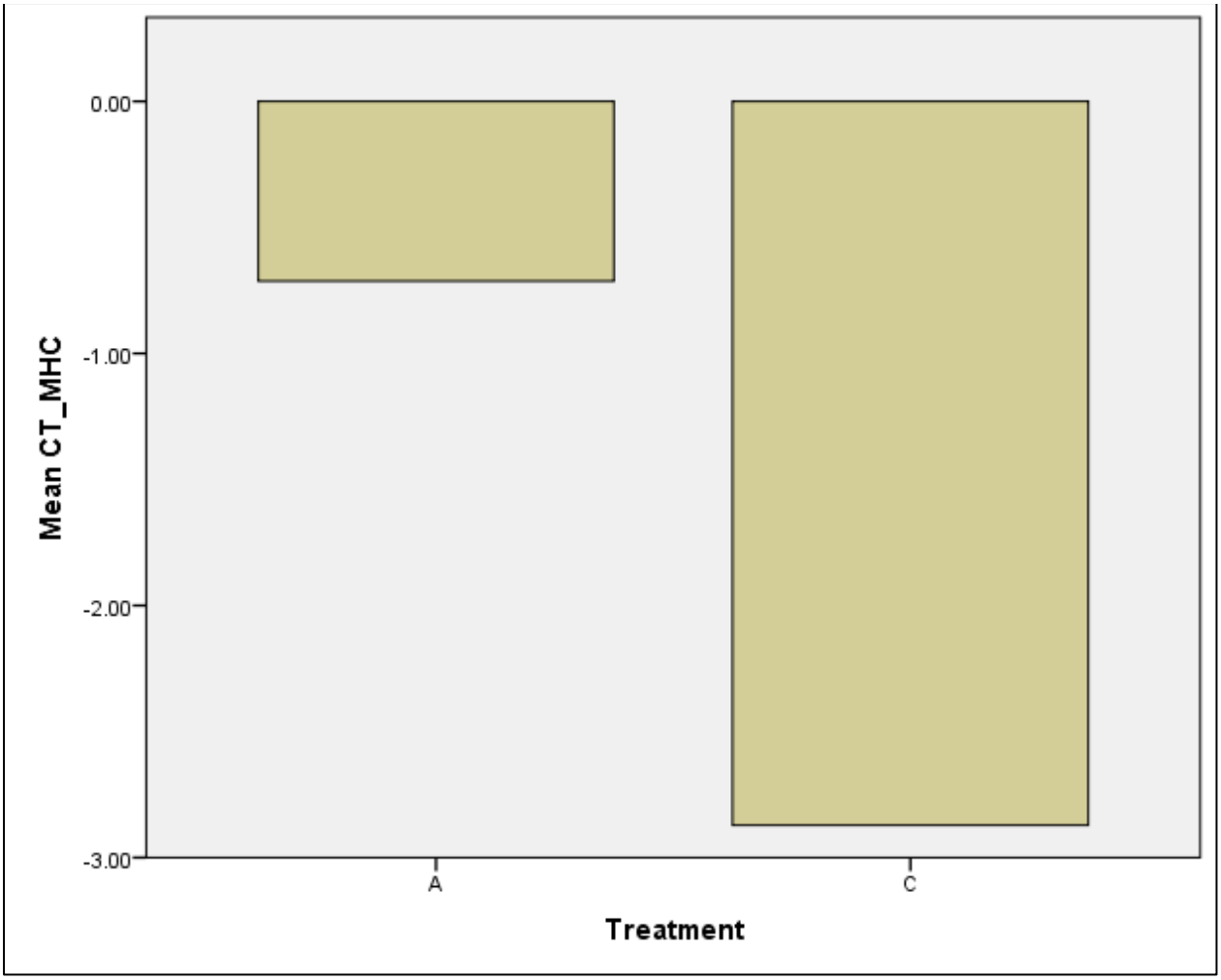

Figure 2: showing the mean ΔCT for both Treatments with MHC as a response.

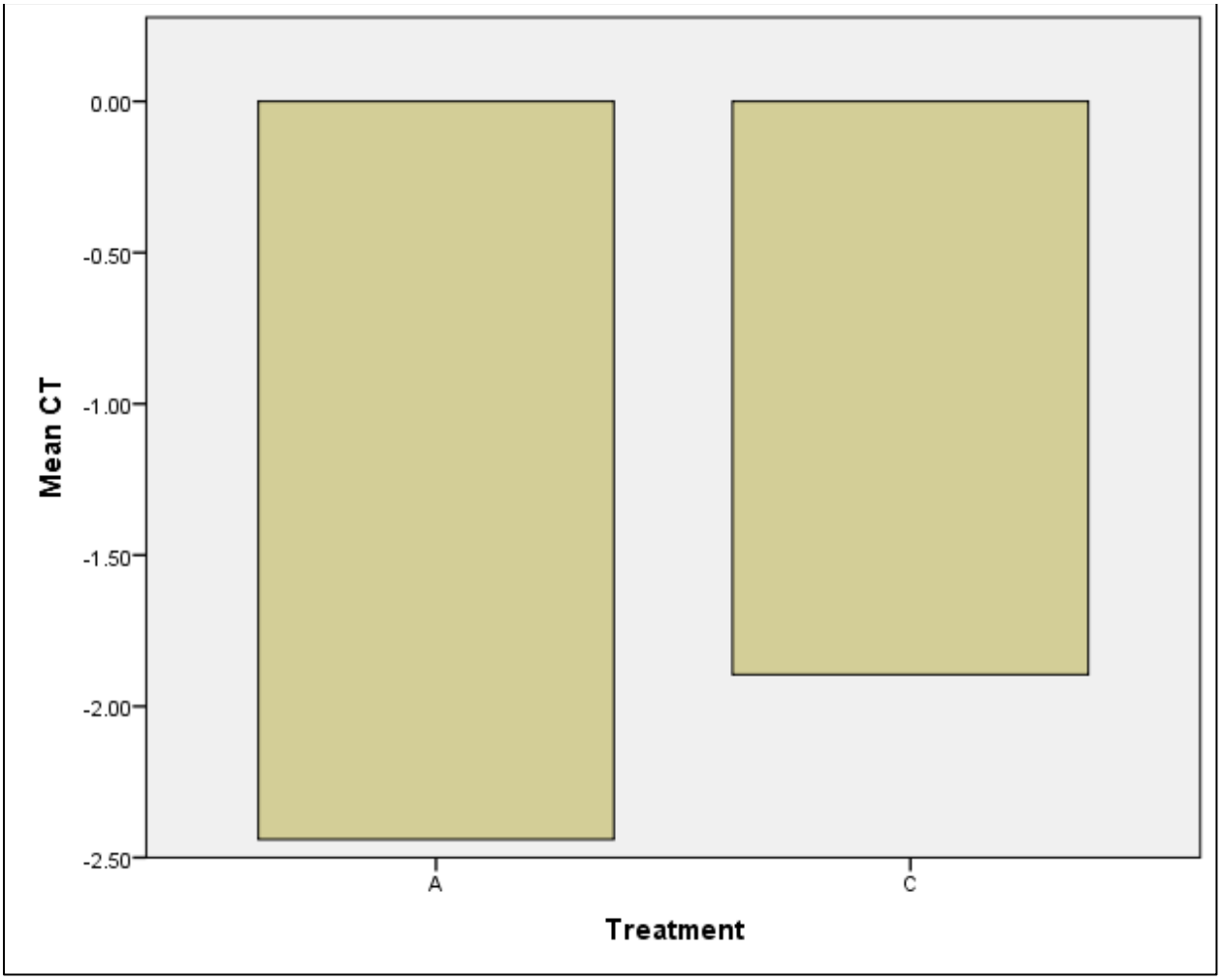

Figure 3: Showing the mean ΔCT for both Treatments with IGF as a response.

TABLE 14: IGF Mean, Standard deviation and P-value for the total three weekly sampling periods

PARAMETER	TREATMENT A	TREATMENT C	P-value
IGF	-26.02±1.30	26.71±0.99	0.2819
ACTIN	28.46±0.17	28.60±0.22	0.9252
Δ CT	-2.44±1.46	-1.90±1.16	0.4499

Values with P>0.05 are significant

TABLE 15: MHC Mean, Standard deviation and P-value of the treatment for the total three weekly sampling periods

PARAMETER	TREATMENT A	TREATMENT C	P-value
MHC	27.74±0.79	25.73±0.96	0.0013
ACTIN	28.45±0.18	28.60±0.22	0.2449
Δ CT	-0.71±0.92	-2.87±1.15	0.0026

Values with P>0.05 are significant

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The results presented in the tables above provide the haematological, biochemical, and gene expression responses of Clarias gariepinus reared in stagnant-renewal (Treatment A) and mutiplant species aquaponic (Treatment C) systems over three weekly sampling periods.

The haematological parameters, packed cell volume (PCV) and haemoglobin (Hb) serve as critical indicators of oxygen transport efficiency and fish health. In the first week, Treatment A exhibited higher PCV and Hb levels than Treatment C, suggesting a greater initial oxygen-carrying capacity. However, by the second week, both parameters declined in both systems, likely reflecting metabolic adjustments to environmental conditions (Gabriel *et al.,* 2015). By the third week, PCV and Hb levels converged between the two treatments, indicating an eventual physiological adaptation, regardless of the production system. The reduction in these parameters over time may be attributed to stress, variations in water quality, or nutritional factors affecting erythropoiesis (Akinrotimi et *al.,* 2010).

The red blood cell (RBC) count was consistently higher in Treatment C, whereas the mean corpuscular volume (MCV) was greater in Treatment A. This suggests that fish in the aquaponic system had more, but smaller RBCs, while those in the stagnant-renewal system had fewer, but larger RBCs, possibly as a compensatory mechanism for oxygen fluctuations (Olaniyi & Salau, 2013).

White blood cell (WBC) counts remained relatively stable across both treatments. However, a transient increase in WBCs was observed in Treatment C during the second week, which may indicate an immune response to environmental stressors such as fluctuations in water quality.

Lymphocyte levels remained stable across the study, showing no significant differences between treatments. However, heterophil counts were consistently higher in Treatment A, suggesting a more pronounced innate immune activation, possibly in response to environmental stress or pathogenic exposure (Fagbenro *et al*., 2013). Additionally, monocyte counts increased slightly in Treatment A by the third week, indicating a sustained immune response, potentially due to variations in pathogen load or environmental conditions.

Platelet counts remained stable in both production systems, indicating that coagulation processes were not significantly affected. MCV was significantly higher in Treatment A, suggesting that fish in the stagnant-renewal system produced larger RBCs as a compensatory mechanism for oxygen availability variations (Fernandes and Moron, 2014). Mean corpuscular haemoglobin (MCH) increased slightly in both systems over time, reflecting a gradual increase in haemoglobin content per RBC, while mean corpuscular haemoglobin concentration (MCHC) remained stable, indicating consistent haemoglobin regulation.

Total protein levels increased in Treatment A over time, suggesting more stable protein metabolism and better nutrient assimilation. In contrast, fluctuations in total protein were observed in Treatment C, with a peak in the first week followed by a decline, possibly due to metabolic stress or environmental adjustments (Kumar *et al.,* 2018).

Albumin levels spiked in Treatment C during the second week but later declined, whereas a gradual increase was observed in Treatment A, suggesting more stable protein synthesis regulation in the stagnant-renewal system . Globulin, an important immune function indicator, increased in both systems but was significantly higher in Treatment C, indicating a stronger immune response .

Glucose levels were initially higher in Treatment C but declined over time, suggesting that fish in the aquaponic system may have experienced early metabolic stress before stabilizing. Meanwhile, glucose levels in Treatment A remained relatively stable, indicating a better-regulated metabolic state (Barcellos *et al.,* 2010).

Expression of the Major Histocompatibility Complex (MHC) gene was significantly higher in Treatment A, aligning with the increased heterophil and globulin levels observed in the haematological and biochemical analyses. This suggests that fish in the stagnant-renewal system experienced heightened immune activation, potentially due to environmental stress or increased microbial exposure (Puvanendran *et al.,* 2019).

Actin gene expression remained stable across both treatments, indicating that cellular structural integrity was not significantly affected by differences in production systems.

Insulin-like Growth Factor (IGF) expression was initially higher in Treatment C, indicating better growth potential in the aquaponic system. However, by the third week, IGF expression declined slightly in Treatment C, suggesting metabolic adjustments. Conversely, Treatment A exhibited a more stable IGF expression pattern, indicating consistent nutrient assimilation and metabolic balance (De Santis *et al.,* 2011).

The multiplant species aquaponic system (Treatment C) supported higher RBC counts and stable IGF expression, suggesting better oxygen transport and initial growth advantages. However, it exhibited fluctuations in protein metabolism and glucose levels, possibly due to initial stress or metabolic adjustments.

The stagnant-renewal system (Treatment A) promoted larger RBCs, stronger immune activation (higher heterophils, MHC expression, and globulin levels), and more stable protein metabolism, suggesting a better long-term immune response but a higher initial physiological stress burden.

5.2 CONCLUSION

The haematological, biochemical, and gene expression responses of Clarias gariepinus reared in stagnant-renewal and multiplant aquaponic systems over three weekly periods. The results showed that the aquaponic system supported higher RBC counts and better initial oxygen transport, while the stagnant-renewal system led to larger RBCs, likely as a compensatory mechanism for oxygen fluctuations. White blood cell counts remained stable, though a transient increase in Treatment C suggested an immune response to environmental stress.

Biochemical analysis revealed that protein metabolism was more stable in the stagnant-renewal system, while the aquaponic system experienced fluctuations, possibly due to initial metabolic stress. Glucose levels were initially higher in the aquaponic system but declined over time, whereas they remained steady in the stagnant-renewal system, suggesting better metabolic regulation.

Gene expression analysis showed that the stagnant-renewal system triggered a stronger immune response, with increased MHC expression , heterophil counts, and globulin levels, indicating heightened immune activation due to environmental stress. Meanwhile, the aquaponic system initially had higher IGF expression, suggesting early growth advantages, but this declined over time, indicating metabolic adjustments.

Overall, the aquaponic system supported early growth and oxygen efficiency but required adaptation to metabolic stress. The stagnant-renewal system induced a stronger immune response and stable protein metabolism, despite higher initial physiological stress. These results emphasize the role of production systems in shaping fish health, metabolic balance, and immune function, which are crucial for optimizing aquaculture practices.

5.3 RECOMMENDATIONS

Aeration in the stagnant-renewal system should be improved to support oxygen transport and reduce physiological stress, while protein-rich diets should be optimized to stabilize metabolism and enhance immune function in the aquaponic system. Regular monitoring of haematological parameters, including MHC expression and heterophil counts, is necessary to assess immune responses and mitigate stress-related effects. Controlled feeding strategies should be employed to regulate IGF expression and sustain growth performance. Water quality parameters must be consistently monitored and adjusted to minimize metabolic fluctuations. Long-term studies on haematological, biochemical, and gene expression responses should be conducted to assess adaptation and system efficiency. Lastly, an Aeration in the stagnant-renewal system should be improved to support oxygen transport and reduce physiological stress, while protein-rich diets should be optimized to stabilize metabolism and enhance immune function in the aquaponic system.

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