EFFECT OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS A FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*)

An Undergraduate Thesis Presented to the
Faculty of the Fisheries Department
Bicol University Tabaco
Tabaco City, Albay

In Partial Fulfillment
of the Requirements for the Degree
Bachelor of Science in Fisheries

by

JOAN B. BUEN
VINCE C. BUENVENIDA

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ABSTRACT

EFFECTS OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*), March 2025

Fisheries Department, Bicol University Tabaco, Tabaco City, Albay, Philippines

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Adviser: BENYL JOHN A. AREVALO, M.Sc.

Feed additives are essential substances infused in the diet of aquatic organisms to enhance the growth, health, and overall productivity of aquaculture species. In this study, the pili pulp extract, known for its rich antioxidant capacity, was infused into commercial feeds as a feed additive. The study focuses on the effect of chitosan microparticles loaded with pili pulp extract as a feed additive on the growth performance and innate immune response of African catfish. The African catfish was cultured for 28 days to evaluate its innate immune response, and another setup was done to investigate its growth performance for 60 days. The effect of PE-CMPs on the growth performance was analyzed using ANOVA. The treatment had a significant effect on both weight ($p < 0.001$) and length ($p < 0.001$), with a notable interaction between treatment and culture period ($p < 0.001$). It was determined that the WBC and RBC have a significant value ($p<0.05$) in the culture period but none in the treatment and culture period treatment, during the 28-day and 60-day feeding trials. During the 28-day feeding trial, there was a significant difference ($p<0.05$) observed in the lymphocytes and monocytes in terms of culture period and treatments, but none in their interactions, while the neutrophil had a significant difference ($p<0.05$) in treatments. Basophils and eosinophils have no significant difference. In addition, all the different leukocytes are normally distributed except eosinophils. It was determined that the 500 mg/kg of PE-CMPs increases white blood cells and red blood cells, compared to the control, and has a significant difference in the culture period. It was also determined that longer exposure to PE-CMPs feed additive increases the presence of lymphocytes, while lowering the neutrophils, monocytes, eosinophils, and basophils, indicating a shift toward adaptive immunity. The results indicated that PE-CMPs are effective in enhancing the growth performance and innate immune response of African catfish, demonstrating their promise as a feed additive for aquaculture applications.

Key words: Growth; Innate Immune; Leukocytes; Red Blood Cells

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RECOMMENDATION FOR ORAL EXAMINATION

This thesis entitled "**EFFECTS OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS A FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*),"** prepared and submitted by **JOAN B. BUEN and VINVE C. BUENVENIDA**, in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN FISHERIES,** is hereby recommended to the thesis committee for consideration and approval.

March 11, 2025

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EDITOR'S CERTIFICATION

This thesis entitled "**EFFECTS OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS A FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*),"** prepared and submitted by **JOAN B. BUEN and VINVE C. BUENVENIDA**, in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN FISHERIES,** has been edited by the undersigned.

May 23, 2025

JULIE ANNE BORJAL PEÑA, MAEd
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APPROVAL SHEET

This thesis entitled "**EFFECTS OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS A FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*)**," prepared and submitted by **JOAN B. BUEN and VINVE C. BUENVENIDA**, in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN FISHERIES** is hereby recommended to the thesis committee for consideration and approval.

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STUDENTS' CERTIFICATION

This is to certify that the ideas and information contained in this undergraduate thesis entitled "**EFFECTS OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS A FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*)**" are truly of the researchers and borrowed information are appropriately acknowledged.

March 13, 2025

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RESULT OF ORAL EXAMINATION

Result of oral examination for **JOAN B. BUEN** and **VINCE C. BUENVENIDA**, candidate for the degree Bachelor of Science in Fisheries.

Thesis: **EFFECTS OF CHITOSAN MICROPARTICLES-LOADED WITH PILI PULP EXTRACT AS A FEED ADDITIVE ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus*)**

Place: Bicol University Tabaco
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Date: March 13, 2025

PANEL OF EXAMINERS	**ACTION**
MARIA CORAZON R. COLLANTES, MSc. Chairman	_____
ALDRIN MEL B. MACALE, MSc. Member	_____
MARVY CLAIRE N. MORTEGA, MSc. Member	_____

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J.B.B
V.C.B

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CHAPTER 1

THE PROBLEM

Introduction

In the Philippines, aquaculture contributes significantly in meeting global seafood demands contributing significantly in the fisheries sector. Even with high production volumes in recent years, challenges like diseases on fish, environmental impacts on farming, and rise in feed costs are inevitable (TahiLuddin & Terzi, 2010). Addressing this concern is important for the industry's development and stability, which can further contribute to economic growth and global food security (FAO, 2020). Due to expansion of aquaculture activities, the cost of commercial aquatic feeds continues to increase making efficient use of feed essential not only for growth, but also for economic viability, as inefficient feeding directly affects production costs (Naorbe, 2022; Mihelakakis *et al.*, 2007). Therefore, the sectors current challenges require improved feed management to ensure economic sustainability while supporting global food security and development.

The African catfish or *Clarias gariepinus* is a known freshwater species for its robustness and adaptability, making it a crucial player in the aquaculture industry. This species originates from the river basins of Africa and the Middle East, renowned for its high growth potential, can tolerates most of the environmental stress, and is not very sensitive to extreme environment such as low dissolved oxygen concentration thus reducing the possibility of mass mortality in diverse environmental conditions (Adewolu, *et al.*, 2008). The introduced species (*C. gariepinus*) is more abundant and can grow bigger than the native catfish (*Clarias macrocephalus*), making it ideal for various farming conditions, which aids its rapid adoption by local farmers (Catanos, 2000). However, the

present-day problems in African catfish nutrition are complicated which includes matters of feed quality, cost, and accessibility to other conditions that requires formulation of right diet in order to improve the growth rate and the health of the fish.

Feed additives are essential in enhancing the growth, health, and overall productivity of aquaculture species. These additives such as vitamins, minerals, prebiotics, probiotic, enzymes, and plant extracts, are added to the fish's diet to improve feeding efficiency and utilize the nutrients (Gatlin *et al.*, 2007). According to the NRC, (2011), feed additives subsidize sustainable aquaculture practices by limiting toxic wastes while highlighting faster growth. Adewolu *et al*. (2008) and Ng *et al*. (2012) stated that feed additives have the capacity to enhance feed conversion ratio and growth performance of African catfish which led to the increase in profitability and efficiency.

Chitosan, a promising antimicrobial agent, from the waste of crustacean shell, is known for its reactive amino and hydroxyl groups, allowing modifications to enhance its properties (Rinaudo, 2006; Kumar *et al*., 2004). It is characterized as biodegradable, non-toxic, and biocompatible which makes it reliable in in biochemical applications such as drug delivery and tissue engineering (Sinha *et al*., 2004). In the field of aquaculture, chitosan's antimicrobial properties are significantly valuable in maintaining water quality and boost fish resistance to infections (Rinaudo, 2006; Saleh *et al*., 2022). Moreover, its biodegradability property contributes to sustainable aquaculture practices (Gong *et al*., 2022).

Infusing natural extracts with complementary benefits enhances the potential of chitosan and its applications. For example, the pili tree's pulp, provide potential health benefits and applications in food and medicine due to its rich in bioactive compounds,

(Dumandan *et al*., 2022; Rosario, 2008). It is significant for natural therapeutic treatments due to its antioxidants and anti-inflammatory properties (Dumandan *et al*., 2022). Researchers can create a novel, natural feed additive that not only enhances the growth and immune response of fish, such as African catfish, but also enhance sustainability and reduces the dependance on synthetic enhancers by combining pili pulp extract with chitosan microparticles (Brul and Canapit, 2024). This combined approach aims to enhance aquaculture productivity while supporting eco-friendly practices.

Objectives of the Study

This study aims to determine the effects of chitosan microparticles loaded with pili-pulp extracts (PE-CMPs) on the growth performance and innate immune response of African catfish (*C. gariepinus*). Specifically, it aims to:

1. Determine the growth performance of African catfish fingerlings fed with PE-CMPs infused commercial feeds in terms of length, weight, survival rate, and assess its condition factor.
2. Evaluate the innate immune response of juvenile African catfish fed with PE-CMPs particularly on white blood cells, red blood cells, and differential leukocytes.
3. Determine the water quality parameters during the culture period such as Dissolved Oxygen (DO), pH, Total Dissolved Solids (TDS), Oxidation-Reduction Potential (ORP), and temperature.

Significance of the Study

The purpose of this research is to provide significant insights and knowledge regarding the possible outcome of synthesized chitosan microparticles loaded with pili-pulp extract as a feed additive to African catfish. Important findings from this study could be highly significant and beneficial specifically to the following:

Fisheries Industry and Catfish Farmers. The results of this study provide sufficient information on the effects of chitosan microparticles loaded with pili-pulp extract to the growth and innate immune response of African catfish as a feed additive. It will improve the feed formulations and production of African catfish in the Philippines.

Fisheries Students. The findings of this study will help the fisheries students to learn the advantages and disadvantages of using chitosan microparticles loaded with pili-pulp extracts on the growth performance and innate response of African catfish. Furthermore, this will help them expand their knowledge on the topic.

The Researchers. The results of this study will help them widen their knowledge, abilities, and approach in conducting research.

Future Researchers. The findings of this study will provide information to those who would undergo similar research for further research and development as well as additional literature in their study. The results of the study would serve as a tool for future researchers to become more knowledgeable about the effectiveness of chitosan microparticles loaded with pili-pulp extract when applied as a feed additive to the African catfish to its growth parameters and innate immune response.

To Science. The findings of this study will contribute to biotechnology, aquaculture, and sustainable agriculture by understanding the natural product extraction, optimization techniques, and practical applications of chitosan-based delivery systems in aquaculture.

Scope and Delimitation

The study focused only on the effects of the synthesized chitosan microparticles loaded with Pili-pulp extracts (PE-CMPs) infused commercial feeds on the growth performance in terms of length, weight, and condition factor and innate immune response of African catfish (*C. gariepinus*) particularly on white blood cells, red blood cells and differential leukocytes and assess the effects on the key water parameters including the Dissolved Oxygen (DO), pH, Total Dissolved Solids (TDS), Oxidation-reduction potential (ORP), and temperature. The findings of this study will serve as a reference for future recommendations to enhance the growth and development of African catfish.

Primary methods of data collection and information were from electronic references and utilized resources available at the Bicol University Tabaco Library for relevant published literature. Specifically, the procedure for the optimized synthesis of chitosan microparticles loaded with pili pulp extract was adopted from the study of Brul & Canapit (2024). Moreover, the starting size of the African catfish for the growth performance was 10.43 cm in length and for the innate immune response was 16.31 cm and was cultured for 60 days and 28 days respectively at the wet laboratory in Bicol University Tabaco. Possible effects of PE-CMPs such as resistance to specific diseases and bacteria were not included in this study.

Definition of Terms

The following are the conceptual and operational definitions of the terminology used in this study.

African Catfish. The African Catfish, *C. gariepinus*, is a hardy, air-breathing catfish species native to African freshwater systems. Distinguished by its long, cylindrical body, broad, flat head, and long barbels around the mouth, this species is well-adapted to diverse environmental conditions, including low oxygen levels. Burchell's description highlights its adaptability, rapid growth rate, and significant role in both aquaculture and wild fisheries. As an adaptable and resilient species, it serves local community food security across its native (Burchell, 1822). In this study, the African catfish was used as an experimental subject to investigate the impact of PE-CMPs to the growth parameters and innate immune response to the said species.

Basophils. Basophils is a typer of white blood cells that belongs to the granulocyte group and is the rarest type. It has a crucial part in innate immune response to parasitic infections and allergic reactions by releasing histamine (Robinson, 2024). In this study, basophils are one of the types of white blood cells that was counted and observed during differential leukocyte.

Chitosan. Chitosan is a biopolymer made from chitin from exoskeletons of crustaceans. It is used in diverse applications in different fields such as in biomedicine, agriculture, and water treatment because of its microbial activities and ability to form films and gels and its biodegradable, biocompatible, and non-toxic characteristics (Kumar, 2000). In this study,

chitosan was used as a polymer for encapsulation of pili pulp extract to avert the deterioration of the antioxidant properties of pili extract.

Chitosan Microparticles (CMPs). Chitosan microparticles are small spherical particles derived from chitosan; a biopolymer made from the deacetylation of chitin which is found in the exoskeletons of crustaceans. This is known for its ability to encapsulate active ingredients, control release rates, enhance the stability of encapsulated substances, high surface area, biocompatibility, and biodegradability (Agnihotri *et al*., 2004). In this study, chitosan microparticles was used to encapsulate the pili pulp extract as a feed additive for the African catfish as well as to improve its effectiveness as an antioxidant.

Eosinophils. Eosinophils is a type of white blood cells having a crucial role in destroying viruses, parasites and also in modulating allergic reactions by releasing toxic granules proteins and enzymes (Underwood, 2024). In this study, eosinophils is one of the types of white blood cells that was observed and counted during differential leukocytes.

Fulton's Condition Factor. Fulton's Condition Factor (K), is an indicator representing the relationship between biotic and abiotic factors in the bodily condition of fish (Lizama and Ambrosio, 2002). It is derived based on the relation between fish's weight and length to illustrate its well-being (Froese, 2006). In this study, Fulton's Condition Factor was used as an indicator of overall the fish's condition during the experiment.

Growth performance. Growth performance is the measurement and assessment of the growth rate and overall development of an organism over a specified period (Bureau *et al*., 2003). In this study, the growth performance was one of the variables that was measured to assess the impacts of PE-CMPs as a feed additive.

Innate Immune Response. The innate immune response of fish refers to the immediate, non-specific defense mechanisms that are activated upon encountering pathogens. This system plays the vital role in the survival of fish in pathogen-rich aquatic environments, enabling rapid responses to infections, parasites, and diseases and maintaining overall health and homeostasis (Kordon *et al*., 2018). In this study, the innate immune response was one of the variables that was assessed to determine the effects of PE-CMPs as a feed additive. Specifically, the red blood cells, white blood cells, and differential leukocytes was counted and analyzed.

Lymphocytes. Lymphocytes are a type of white blood cell that represent the primary cells of immune system, responsible in regulating immune response, production of anti-bodies, and direct cell-mediated killing of cells infected with virus (Morris, 2018). It can turn into a memory cell and respond to antigens once it recognizes it again (Professional, 2025). In this study, lymphocytes are a type of white blood cells that was observed and counted as a part of differential leukocytes.

Microencapsulation. Microencapsulation is a process in which small or microparticles are coated with containing effective properties to serve lightweight capsules with diameters ranging from micrometers to millimeters and is used to encapsulate active ingredients (Sris & Prabha, n.d.). In this study, microencapsulation was used to lengthen the bioavailability of pili pulp extract with the help of chitosan microparticles.

Monocytes. Monocytes is a type of white blood cells known for playing a central role in both innate and adaptive immunity, contributing to the pathogenesis of many chronic inflammatory conditions and degenerative diseases. It also response against viral, bacterial,

fungal, or parasitic infections (Sapkota, 2022). In this study, monocytes is one of the types of white blood cells that was observed and counted during differential leukocytes.

Neubauer Hemocytometer. The Neubauer hemocytometer is a precision instrument used for counting cells in a specified volume of fluid under a microscope. It consists of a thick glass microscope slide with a grid of precisely defined dimensions, allowing for accurate quantification of cells in biological and medical research (Holmskov, 2023). In this study, the Neubauer Hemocytometer was used as a counting chamber for white blood cells and red blood cells of African catfish.

Neutrophils. Neutrophils are a type of white blood cell that serves as the first line of defense against diseases, responsible for responding against infections, pathogens, and releasing antimicrobial substances (Madayas *et. al*., 2013). In this study, neutrophils are one of the types of white blood cells that were observed and counted during differential leukocyte counts.

PE-CMPs. Chitosan Microparticles loaded with Pili-pulp extract. In this study, commercial feeds were infused with PE-CMPs as a feed additive to enhance the growth performance and innate immune response of African catfish.

Pili Pulp. Pili pulp, derived from the fruit of the Pili tree, is known for having rich, essential nutrients, including vitamins, minerals, and dietary fiber. It is a valuable ingredient in functional foods and traditional medicine known for its antioxidant properties and potential health benefits (Coronel, 1996). In this study, pili pulp was used as a raw material as a source of antioxidants and as a potential feed additive.

Pili Pulp Extract (PE). Pili Pulp extract is a macerated form of pili pulp. In this study, pili pulp extract was macerated in ethanol for it to be encapsulated with chitosan microparticles.

Tripolyphosphate (TPP). Tripolyphosphate (TPP) is an inorganic compound commonly used as a sequestrant, preservative, and texturizer in food processing. It is used in different industrial applications, including water treatment and as a cross-linking agent in the production of chitosan microparticles (Xiong *et al*., 2023). In this study, the TPP was added to the chitosan solution to create stable microparticles, which are desirable for the application as a microcapsule.

CHAPTER 2

REVIEW OF RELATED LITERATURE AND STUDIES

This chapter provides an extensive analysis of the previous studies, theories, and findings conducted by previous researchers relevant to this study. The insights and knowledge acquired from these previous studies serve as valuable information that guided the progress of the present study.

Related Literature

African catfish, or *C. gariepinus*, are known for their cylindrical body having scaleless skin, flattened bony heads, small eyes, spineless dorsal fins, and four pairs of barbels around a broad mouth. The body is usually greyish-black on the underside of the head and the body, creamy white color (Ng, 2022). It originated from the river basins of Africa and the Middle East, has a high growth potential, is known to tolerate most of the environmental stress, and is not very sensitive to low dissolved oxygen concentration, hence reducing the risk of mass mortality in diverse environmental conditions (Adewolu *et al*., 2008). Being one of the major freshwater aquaculture species in the world, it has been reported to have an annual production of about 370,000 tons per year. catfish's aquaculture contributes for 17.5 % of the overall production of freshwater fish aquaculture (Cacot & Hung, 2011). The species is known for its hardiness and adaptability, making it suitable for various farming conditions, which aids in its rapid adoption by local farmers (Castaños, 2000). However, despite these advantages, challenges such as disease management, accessibility of high-quality feed, water quality, and the need for improved farming practices remain prevalent.

The nutrient requirements of African catfish (*C. gariepinus*) are crucial for enhancing their health and growth. Studies suggested that 35% of dietary protein is the optimal in maximizing nutrient utilization and growth making protein a crucial component of diet (Keremah & Beregha, 2014). To improve the overall health and physiological functions of a fish, additional support from essential amino acids, minerals, and lipids are required. Proper balance in nutrients is essential in optimizing dietary formulations to aid the specific needs of catfish, leading to sustainable aquaculture practices and economic viability for farmers. In essence, feed additives play a crucial part in aquaculture in general. Feed additives are edible substances that are added or infused to animal feeds diet in small quantities to improve the feed quality in order to boost growth performance, reduce mortality (Dada, 2015), enhance immune responses, and disease resistance in fish (Onomu & Okuthe, 2024). Common additives like vitamins, minerals, enzymes, probiotics, and other bioactive compounds can increase feed efficiency and enhance immune function in fish. Recently, using sustainable components and replacing chemical additives with natural or organically produced components has been a trend. Moreover, innovations such as plant-based ingredients, algae, and microbial-derived additives have gained popularity due to their prospects in reducing environmental impact and improving the nutritional profile of aquafeeds (Naylor *et al*., 2021). This establishes the need for eco-friendly and health-oriented products in the market to drive this shift.

Pili Pulp as a Potential Feed Additive

Pili tree is usually easy to grow especially in tropical countries and is native to the Philippines. Pili belongs to the *Burceraceae* family, has four genera including the

Canarium genera, and has about 40 species in the Philippines. Some authorities believe that pili, described as a fairly large tree, reaching a height of about 20 meters or more and has a trunk diameter of about 40 cm, is the main nut-producing species in the country.

The pili tree contributes to the livelihoods of local communities and plays a significant role in culinary practices and traditional medicine, exhibiting its great cultural and economic importance in its native regions. Due to its beneficial nutritional value and potential contribution to the economy, pili has been recognized as a valued tree species with distinct functions in the food industry and sustainable agriculture (Dumandan *et. al*., 2022). In 2019, Philippine pili nut production reached up to 7,158 tons, with an estimated value of PHP 385.67 million. The largest producers are located in the provinces in the Bicol region, specifically Sorsogon, ranking first, and Albay, in second. The region is the largest core producer and supplies the country with around 78% (BAS, 2012; PSA, 2020; Millena *et al*., 2022).

The pili tree is recognized for its fruits as which range from 4 to 6 cm in length and come in different shapes such as elliptical, oblong, oval, or obovate. The fruit is made up of three parts, namely: the nut, pulp, and skin. As the fruit ripens, its exocarp eventually turns black, and the pulp of the fruit is made up of fibrous mesocarp (Millena *et al*., 2022).

According to Millena & Sagum (2018), the main product of the tree is the pili nut, which is surrounded by a hard shell. It is stated to have a unique taste, buttery texture, and valuable nutritional composition, which consists of substantial concentrations of unsaturated fatty acids, amino acids, vitamins, and minerals. Pili Tree has diverse applications due to its nutritional value and its economic importance in the economy,

especially in the food industry. It has gained recognition as a sustainable agriculture as a tree species (Dumandan *et al*., 2022).

The pili pulp is an edible fibrous mesocarp that is locally consumed. Its abundant bioactive compounds and essential nutrients are beneficial to health. Pili pulp contains a large amount of essential fatty acids, proteins, and high carbohydrates that serve as a readily available source of energy to animals. Additionally, the pili pulp is a valuable protein source because of the presence of amino acids. Locally, the oil from the pulp is used for the food and cosmetic industry as it is rich in fiber and essential nutrients that have a positive effect on the health and growth of the animals. Moreover, Millena *et al*., (2021) stated in the study that pili pulp is effective in improving intestinal health and nutrient absorption in animals, due to its excellent fermentable fiber. The fibers present in pili pulp strengthen digestive health and also serve as a prebiotic and promoting beneficial intestinal flora. In addition, a study by Briñas (2022) highlighted and assessed the potential of pili pulp pomace in tilapia feeds and its high content of carbohydrates and essential fatty acids, which are vital for the growth and overall development of fish. The study found that the inclusion of pili pulp in the diet of tilapia improved its growth performance and overall health status, implicating its effectiveness as a feed.

Pili pulp is renowned for its rich polyphenols, flavonoids, and other bioactive compounds. Pili pulp extract is known for its potent antioxidant activity that is necessary in protecting cells from oxidative stress by combating harmful free radicals within biological systems (Gupta, 2015). In addition to its antioxidant properties, pili pulp extract also demonstrates antimicrobial and anti-inflammatory effects, which makes it essential in managing inflammatory conditions and enhancing overall health (Dumandan *et al*., 2022).

A study by Arenas and Trinidad (2017) found that polyphenol-rich pili pomace contains notable antioxidant properties that help in boosting the immune response in animals, which further leads to increasing its resilience to diseases. Moreover, the antimicrobial potential of pili pulp oil was highlighted by Ramirez (2021), by lessening the microbial load, which further results in a healthier gut environment and prevents infections. Furthermore, the fatty acids in pili pulp oil are another crucial aspect as it consists of linoleic and oleic acids that is vital in maintaining the cell membrane integrity (Millena & Sagum, 2023). Pili pulp is also known having a notable concentration of vital minerals such as calcium, potassium, magnesium, and phosphorus that are important for the development of bones, muscles, and enzyme activity of animals.

The antioxidant properties present in pili pulp makes it an auspicious candidate as a feed additive in aquaculture, offering significant benefits such as promoting normal physiological functions and enhancement of immune system, growth, and reproduction in fish. (Alemayehu *et al*., 2018; Dumandan *et al*., 2022).

Chitosan Microparticles

Chitin is available in many organisms, mostly found on the exoskeleton of crustaceans, mollusks, insects, algae, fungi, and cell walls of fungi (Pellis *et al*., 2022). In general, the proportion of chitin in these organism ranges from 30–40% in shrimp cuticles which is the highest, 2–44% fungi cell walls (Elsoud & Kady, 2019, Ahmad *et al*., 2020), while 20–30% crustacean exoskeletons (Yeul & Rayalu, 2012) and 5–25% in insect cuticles (Abidin *et al*., 2020). Chitin is not soluble in common solvents because of its high

crystallinity, acetamido, and the strong hydrogen bonding between hydroxyl and carbonyl groups (Rinaudo, 2006).

According to Kou *et al*. (2021), chitosan is the outcome when chitin, a polymer of N-acetyl-D-glucosamine, is subjected to deacetylation and the repeating units in the polymer are predominantly without the acetyl functional group, such as β-1,4-D-glucosamine. Nowadays, the chitosan derived mainly from crustaceans, especially crab, prawns, and shrimp shells, where their exoskeletons are treated as waste from the food processing industry, is used for industrial applications (Kumar, 2000). By converting waste into a commercially valuable product, these industries can improve their profitability and contribute to the development of a sustainable bioeconomy. The chitosan market is growing due to its wide range of applications in agriculture, pharmaceuticals, water treatment, and food industries, making the extraction of chitosan from crab shell waste a lucrative venture (Shahidi & Abuzaytoun, 2005). This leads up to the reduction of costs related to the waste disposal and management for seafood processing facilities, which contributes economically (Rinaudo, 2006).

Extraction of chitosan from crab shells includes several steps such as deproteinization, demineralization, and deacetylation. This step provides an effective way for creating an economically practical product and waste management by transforming waste material into a valuable biopolymer. Recent studies enhanced the extraction processes of making crab shell waste into optimized chitosan yield and purity, increasing its potential for industrial applications (Kumari & Rath, 2014). This chitosan production lessens the environmental footprint due to the production of synthetic polymers and lowers the reliance on non-renewable resources (Muanprasat & Chatsudthipong, 2017).

The degree of deacetylation (DD) suggests the amount of deacetylated units, which substantially affects the characterization of chitosan, such as solubility, reactivity, and biological effects. A study by Cheng *et al*. (2023) indicated that the partial deacetylation and ultrasonic treatment show a higher degree of deacetylation, enhancing the yield and mechanical properties of chitin nanofibrils. Generally, to dissolve in acidic aqueous solutions, chitosan needs a DD greater than 50 %. Higher DD chitosan improves the reaction extent, antioxidant, and antimicrobial properties of chitosan-mannose derivatives (Yu *et al*., 2023). To adapt and enhance its properties and functionalities, chitosan contains reactive amino and hydroxyl groups in its chemical structure (Rinaudo, 2006). According to Kumar *et al*. (2005), these groups allow the development of derivatives with enhanced solubility, antimicrobial activity, and conjugation with bioactive molecules. Chitosan's ability to be dissolved in weak acidic solutions makes it convenient in applications in biomedicine such as hydrogels, films, and nanoparticles that are used in drug delivery, wound healing, and tissue engineering (Sinha *et al*., 2004).

Molecular weight (MW) of chitosan influences its biodegradability, affecting its structural integrity, water absorption, and its properties in microbial degradation. In terms of microbial degradation, low MW degenerates more rapidly and extensively (Manatsittipan *et al.,* 2018). Relatively, low MW chitosan from delayed degradations is more easily biodegraded (Hussain *et al*., 2019). In comparison to low MW, chitosan with higher MW relatively has higher tensile strength, retains more, which can affect its biodegradation rate, making it less prone to fast degradation (Gomes *et al.*, 2021; Mutasher and Al-Lami, 2022; Supernak *et al*., 2023).

A substantial amount of research has been conducted towards developing safe and effective chitosan-based particulate drug delivery systems. Due to its adaptable applications in drug delivery, food industry, and cosmetics, microencapsulation of chitosan microparticles has become a widely renowned topic in studies.

Microencapsulation using Chitosan

Microencapsulation, ranging from a few micrometers to a few millimeters in size, involves the enclosing of active ingredients or core materials within a coating or shell to form microparticles. To protect sensitive substances, improve the stability and bioavailability of materials, and oversee the release of active reagents, it is widely used and accepted in different industries such as pharmaceuticals, food, and cosmetics (Desai & Park, 2005). When applied to chitosan, microencapsulation influences its properties such as biocompatibility, biodegradability, and non-toxicity. Due to the unique chemical structure of chitosan, including its protonatable amino groups, it can recognize and flexibly interact with different cross-linking agents and encapsulated materials, thus optimizing its functions in diverse applications (Ahmed & Aljaeid, 2016).

A diverse approach has been developed to synthesize chitosan microparticles for their specific advantages and applications. A notable method is spray drying, which is described by its scalability and ability to produce uniformly sized particles, making it suitable for various industrial applications (Estevinho *et al*., 2013). In addition, ionic gelation is a common method that does not need harsh chemicals or solvents where ionic interactions between chitosan a polyanions such as sodium tripolyphosphate (TPP) form microparticles, thus simplifying the production process (Ko *et al*., 2002). Another method

is emulsion crosslinking. A chitosan emulsion is created in an oil phase and then crosslinked with active ingredients such as glutaraldehyde, which is ideal for controlled drug delivery due to the stable microparticles (Ahmed & Aljaeid, 2016).

Concentration of chitosan and cross-linking agents, and various factors such as pH and temperature, affect the synthesis and properties of chitosan microparticles. The pH value and temperature during synthesis affect the solubility, reactivity of chitosan, and the size and stability of microparticles. For example, optimal pH conditions are crucial for efficient ionic interactions during the synthesis process, while fluctuating temperatures can modify the viscosity and reactivity of chitosan solutions (Sinha *et al*., 2004). Likewise, higher amount of chitosan and cross-linking agents determines the density and size of the resulting microparticles, generally resulting in larger and denser particles (Ko *et al*., 2002). Each method requires specific conditions to optimize particle formation and stability; thus, preparation method plays a significant role. For instance, spray drying requires definite control of atomization and drying parameters to provide uniform-sized particles (Desai & Park, 2005).

Chitosan microparticles have a wide range of uses, especially in drug delivery systems. A wide range of medications is encapsulated in chitosan microparticles to prolong their bioavailability and contribute to the sustained release profiles, which are both advantageous for therapeutic adequacy (Mitra and Dey, 2011). It is also used in the food industry to stabilize flavors and nutrients by enhancing the release profiles of bioactive compounds (Estevinho *et al*., 2013). In addition, chitosan microparticles are used to stabilize the efficacy of the formulations by encapsulating active ingredients (Casanova *et al*., 2016). Furthermore, chitosan microparticles demonstrated significant potential in

aquaculture, specifically in applications in drug delivery, vaccine encapsulation, and as nutritional supplements to improve the health and growth of aquatic life. It provides a promising solution for different applications due to its crucial physicochemical properties, adaptable synthesis methods, stability enhancement, and effectiveness in controlled release.

Chitosan microparticles enhance the effectiveness of the delivery of vaccines in fish immunization (Rivas-Aravena *et al*., 2013). It shows that disease can be prevented using chitosan microparticles in encapsulating the vaccines, which helps to enhance the immune response of fish (Rivas-Aravena *et al*., 2013). Similarly, González-Chavarría *et al*. (2023) determined that the chitosan microparticles can effectively boost the immune system of *Oncorhynchus mykiss* (rainbow trout), aiding in the treatment of infectious diseases in aquaculture. Another vital use of chitosan microparticles is nutrient delivery in aquaculture, is the study by Anas *et al.* (2008). The study found that chitosan-coated microcapsules are efficient in delivering nutrients and drugs, causing the enhanced growth and survival rates of giant prawn (*Macrobrachium rosenbergii*) larvae. Moreover, Behera and Swain (2013) demonstrated that oral immunization with chitosan microspheres loaded with antigens in fish is effective in preventing infections and enhancing the overall immune response in fish. Chitosan microparticles offer various applications in aquaculture, from improving disease control through vaccination strategies to providing effective nutrient and drug delivery systems. Its unique properties and capabilities make it an invaluable tool in promoting the growth and health of aquatic species.

Related Studies

Fish nutrition must be carefully investigated and discussed as there is a need to search for innovative feed additives or supplements that aid in maximum digestibility with less side effects, a high feed conversion ratio, and low feed cost (Kumar *et al*., 2016). Various literature examined demonstrate that the application of effective feed additives in aquaculture lessens stress, aids digestion, enhances growth, water quality, reduces the footprint of aquaculture on the environment and parasitic infestation, improving the chances of survival of aquatic animals after exposure to infections (Onomu, 2024). According to Ogunkalu (2019), the improvement of the growth performance of fish is one of the vital goals in aquaculture. Growth performance index is an essential component of aquaculture as it demonstrates production yield and is affected by genetic, environmental, and dietary factors. Plant-based feed additives demonstrated improvements in growth performance and development in antioxidant activity that enhances the innate immune response of cultured species. Various plant extracts are recorded to boost appetite and improve weight gain when it is given to cultured fish (Reverter *et al.*, 2014). For instance, Shalaby *et al*. (2006) and Diab *et al*. (2002) stated that when Nile Tilapia (*O. niloticus*) is fed with a diet containing garlic powder, there is a notable development in weight gain, feed efficiency, and specific growth rate observed. A study conducted by Turan and Yiğitarslan (2016) shows that rosemary extract can induce effective technical and economic growth in catfish reared when used as a dietary additive.

The innate immune system is the first line of defense against invading pathogens. It has major components such as macrophages, granulocytes, monocytes, and humoral elements, which include complement systems or lysozymes (Harikrishnan *et al*., 2009). An

immunostimulant is a substance that improves the defense mechanisms or specific and nonspecific immune response, thus providing resistance to diseases and environmental issues to animals (Anderson, 1992; Reverter *et al*., 2014). Plant extracts have gained popularity in the last decade as fish immunostimulants. Reverter *et al.* (2014) further stated that their use could be more advantageous to the environment as they are more biodegradable and reduce treatment costs compared to synthetic molecules and decrease drug resistance in parasites because of the high diversity of plant extract molecules (Reverter *et al*., 2014). Some of the research analyzes the effect of natural immune-stimulant additives on fish feed. African Catfish (*C. gariepinus*) fed with 0.5% garlic-supplemented diets had significantly higher White Blood Cells (WBC) counts, Red Blood Cells (RBC) counts, plasma protein, hemoglobin, and packed cell volumes compared to those without (Onomu, 2018). A study done by Purbomartono *et al*. (2021) shows that the dietary inclusion of turmeric and ginger increases the weight growth (WG) and enhances the feed conversion ratio (FCR), leucocyte differential count of lymphocyte (%), and hematocrit (%) of African Catfish.

Although there are no specific studies on Pili pulp extract loaded with chitosan microparticles, research on chitosan microparticles and their applications in various fields is abundant and offers valuable insights. Shakib *et al*. (2023) evaluated the properties of anti-biofilm of Mentha piperita essential oils encapsulated within chitosan nanoparticles, demonstrating significant antimicrobial effects against *Acinetobacter baumannii* on catheter surfaces, highlighting the possibility of chitosan nanoparticles as carriers for bioactive compounds which could be applied to pili pulp extract in future research (Shakib *et al*., 2023). Similarly, the study of Yang *et al*. (2020) emphasizes the effectiveness of

chitosan microparticles in the establishment of stability and flavor retention of encapsulated substance, implying that a similar method could be favorable for pili pulp extracts to improve their stability and bioavailability.

The antimicrobial and antioxidant activities of cornsilk extract were investigated by Mady (2021), enclosed in polysaccharide nanoparticles, including chitosan. This study showed that bioactive properties of the extract can be enhanced using chitosan as an encapsulating tool, supporting the ability of chitosan as a versatile courier for various bioactive extracts, including pili pulp.

In addition, Noremylia *et al*. (2022) investigated recent improvements in isolation and processing of nanocellulose, including its extraction from pili pulp. The study highlighted the promising application of nanocellulose due to its excellent mechanical properties and biocompatibility in different industries like cosmetics and food packaging. This review provides an underlying recognition of the extraction techniques and applications that could be applied to further studies on pili pulp extract encapsulated with chitosan microparticles.

These studies generally highlighted the versatility and efficacy of chitosan microparticles in encapsulating numerous bioactive compounds, implicating probable avenues for future research on pili pulp extract in related encapsulation methods. These studies also demonstrate that plant extracts have a potential application in boosting the growth performance of African catfish as well as enhancing the innate immune response.

Synthesis of the Art

This section focuses on the theories and studies collected about the have valuable information and results of the utilization of pili pulp extract as a feed additive from the investigations from both published and unpublished sources. Furthermore, there are related studies that investigate the use of chitosan microparticles and their microencapsulation process.

The pili pulp extract, known for providing antibacterial and antioxidant activity due to its abundant bioactive compounds such as phytosterols and triterpenoids, has gained popularity in the field of aquaculture as a feed additive (Dumandan *et al*., 2022). Different cultured aquatic species have been proven by several studies to enhance their growth performance and immune system by the use of plant-based additives. For instance, a study by Lim *et al*. (2001) indicated that increasing levels of dietary palm oil fed to African catfish enhances the growth performance, protein retention, and fillet vitamin E concentration of the fish. Briñas (2022) states that fatty acids from pili pulp are a source of potential alternative fatty acid feed ingredient for tilapia. Briñas (2022) further stated that pili pulp has explicit potential for nutraceuticals industries due to its fatty acids characteristics and minor components such as carotenoids, tocopherols. Tocopherols also known as “vitamin E” also gained attention in clinical and nutritional applications for their usefulness as antioxidants (Catelo & Jimenez, 2016). A recent study by Pham (2015) indicates that pili pulp encompasses significant number of carotenoids, particularly astaxanthin, have been found to enhance the antioxidative state and immune system of farmed fish, minimizes cytotoxicity and side effects, resulting to increase in resistance in diseases, growth performance, survival, and egg quality (Nakano & Wiegertjes, 2020; Brul

& Canapit, 2024). Additionally, pili pulp possesses tannins and different organic compounds that supports in conditioning of the culture water (Pham & Dumandan, 2020). Furthermore, Brul & Canapit (2024) have reported that PE-CMPs does not have negative impact on the water quality parameters of betta fish (*Betta splendens*) culture.

Recent advancements in the synthesis of chitosan microparticles have shown auspicious utilization in aquaculture, specifically in enhancing the delivery of bioactive compounds and improving fish health. Researchers are focusing on different synthesis methods, along with green synthesis techniques that apply natural extracts as cross-linkers, enhancing the biocompatibility and promoting eco-friendliness. For example, Rahman *et al*. (2024) achieve the optimal conditions for maximum yield and stability of nanoparticles using green synthesis of chitosan nanoparticles using citrus lemon extract. Furthermore, comparative studies feature different synthesis methodologies that make them suitable for various aquaculture applications, such as ionic gelation, which produces chitosan nanoparticles with varying sizes and toxicity profiles. This development indicates the potential of chitosan microparticles as effective carriers for drugs and nutrients, contributing to sustainable practices in aquaculture.

Several studies researched African catfish's (*C. gariepinus*) growth performance and innate immune response fed with different plant-based feed additives. However, these published data have not yet investigated the effects of chitosan microparticles loaded with pili-pulp as a feed additive on the growth performance and innate immune response of African catfish culture.

With the expanding demand for African catfish in aquaculture, providing artificial and reliable alternative feed additives at a feasible cost to improve fisheries and acquire

maximum production is vital. All the related studies concluded that pili pulp extract as a feed additive enhances the growth performance and innate immune response of African catfish.

Gap Bridged by the Study

The study aimed to fill the gap in the research studies that can bring added value and potential increase in the field of aquaculture, specifically in African catfish culture. This study focused on the effects of the microencapsulation of chitosan microparticles loaded with pili pulp extract as a feed additive for African catfish (*C. gariepinus*). Thus, this study analyzes the possible effects of PE-CMPs on the growth parameters, innate immune response, and assesses the water quality on the African catfish culture, and the microencapsulation method.

Previous studies were done in synthesizing and optimizing the PE-CMPs and applied them as a feed additive for the enhancement of color pigmentation of *B. splendens*, and determined their effects on water quality parameters. The researchers used the same methods of synthesizing PE-CMPs. However, no previous studies have focused on the growth parameters enhancement, and the innate immune response specifically in African Catfish (*C. gariepinus*) using pili pulp extract as a feed additive. The researchers concluded that this study, entitled “Effect of Chitosan Microparticles-Loaded with Pili Pulp Extract as Feed Additive on the Growth Performance and Innate Immune Response of African Catfish (*Clarias gariepinus*),” is a unique contribution and significant in addressing the gap.

Theoretical Framework

This section exhibits the connection of multiple theories from different literature sources and their relationship to the problem statement and study objectives. It demonstrates hypotheses of the utilization of pili pulp extract as a natural source of antioxidants, enhancing growth performance and innate immune response, the significance of microencapsulation, and the inclusion of chitosan microparticles as a feed additive.

Pili pulp possesses phytosterols and triterpenoids with antioxidant and antibacterial properties that are known bioactive compounds (Dumandan *et al*., 2022), making it valuable for African catfish for improving the immune response, promoting growth, and overall physiological conditions. Incorporating chitosan microparticles loaded with pili-pulp extracts into catfish feed presents a low-stress alternative to traditional immunostimulants. However, factors like heat, light, pH, oxidation, and chemical structure, along with processing methods and individual biological factors, can impact the bioavailability of these natural antioxidants (Parisi, 2014; Abourashed, 2013).

Chitosan microparticles contain bioactive properties such as immunogenic activities, antimicrobial, antiviral, hemostatic, tumor inhibition, anti-inflammatory, tissue regeneration, and wound healing, which enhances the effectiveness of antioxidants (Arevalo, 2022). Relatively, encapsulating antioxidants with chitosan microparticles has a promising potential as it increases the effectiveness and bioactivity of these substances, while also protecting their antioxidant properties (Parisi, 2014).

Bioactive compounds contain beneficial properties, such as antioxidants, anti-inflammatory agents, or anticancer properties, which aid in disease prevention or delay

(Mascheroni, 2013). Antioxidants affect by neutralizing free radicals and reactive oxygen species that cause oxidative damage to cells and can lead to various serious diseases (Tiwari & Jatawa, 2011). Antioxidants, whether naturally occurring or added, can be degraded by various factors during food processing and storage, such as exposure to heat, light, oxygen, and pH changes which can lead to different biological effects and can no longer effectively neutralize free radicals therefore increasing the risk of oxidative stress and associated health problems like chronic diseases (Poljsak *et al*., 2021). Encapsulation emerges as a potential solution to this challenge by enhancing the bioavailability and stability of bioactive compounds (Mascheroni, 2013). Microencapsulation has also been employed in the pharmaceutical industry to improve the stability and bioavailability of drugs (Vila *et al*., 2015).

Fish are significantly affected by stress from environmental challenges such as water quality issues and hypoxia, along with health problems such as parasites and infectious diseases, which negatively impact their well-being, performance, and economic value (Oliva-Teles, 2012). To alleviate these issues, it is important to equip fish with a well-balanced diet that sustains their specific nutrient requirements based on their developmental stages, thereby enhancing growth, feed efficiency, stress tolerance, overall health, and disease resistance (Oliva-Teles, 2012). Moreover, dietary supplementation is vital to achieve weight gain, feed efficiency, disease resistance, and stress tolerance that can further enhance growth parameters and innate immune response of African catfish. The inclusion of bioactive compounds further supports fish health by activating antioxidants that improve their overall well-being.

A nutraceutical is a bioactive compound or substance that is believed to contribute health benefits apart from basic nutritional value and is made out of natural resources, such as plants, animals, or marine organisms (Pandey *et al*., 2024). They are utilized as growth promoters and immunity boosters to prevent and control diseases in farmed aquaculture species. It also has antioxidants that help counteract oxidative stress caused by factors like poor water quality and handling stress, and can lead to improved fish health and quality. Chitosan is an immunostimulant that enhances the immune system's response to pathogens and improves disease resistance in fish. Microencapsulation can be mixed into feeds and is used to encapsulate microcapsules, providing controlled release and protection against deterioration. In conclusion, nanoparticle nutraceuticals can be applied in the field of aquaculture, such as for water quality management. They improve water quality and create a healthier environment for aquatic organisms due to their capacity to absorb heavy metals, organic compounds, and pathogens (Samanta *et al*., 2022). Most importantly, nanoparticles can help improve the immune system and also enhance growth and development. They can increase metabolic processes that result in better growth rates and improved feed conversion.

Figure 1 shows the relation of this hypothesis and theory that are significantly valuable in terms of applications for the synthesis and evaluation of microencapsulated PE-CMPs and their ability to determine the effects on the growth parameters and innate immune response of *C. gariepinus* culture.

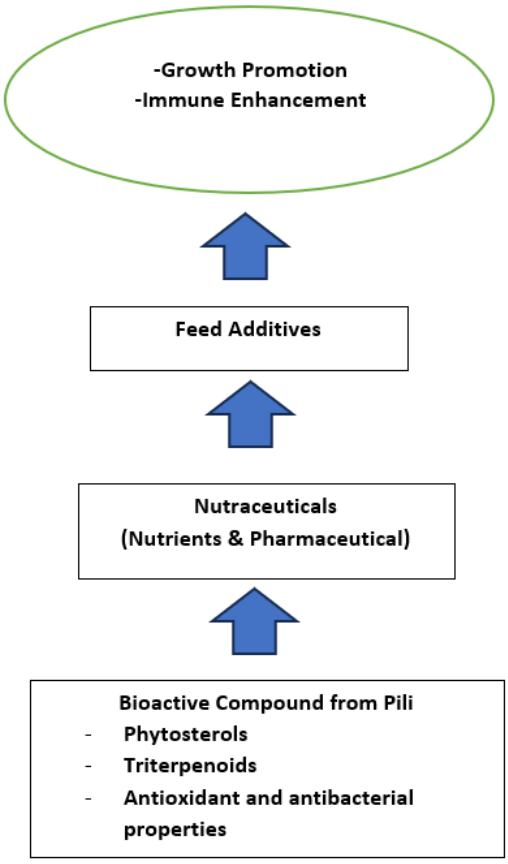

Figure 1. Theoretical Paradigm of the Study

Conceptual Framework

This study included the main concepts and variables on the preparation of chitosan microparticles, the Pili pulp extraction, and the integration of these two to create a feed additive. To assess the feed additive's efficacy, the variables of interest include the growth performance of African catfish, innate immune response, and various water quality parameters.

The study's foundations are grounded in several key theories. The theory of bioavailability and encapsulation suggests that encapsulation significantly enhances the

stability and bioavailability of bioactive compounds (Mascheroni, 2013). Additionally, the theory of antioxidant mechanisms and oxidative stress suggests that antioxidants neutralize free radicals, which helps in reducing oxidative stress and promotes cellular health (Tiwari & Jatawa, 2011). Furthermore, the hypothesis on growth variation proposes that the genetic, environmental, and social factors influence the growth rate in fish, and that appropriate feeding strategies can optimize growth by improving feed intake and efficiency (Oliva-Teles, 2012). The theory on immune modulatory indicated that the innate immune response can be enhanced using certain feed additives, improving disease resistance and overall growth of an organism. Therefore, these theories on economic and environmental sustainability highlighted the benefits of using natural feed additives for enhancing economic viability and environmental sustainability in aquaculture.

Several hypotheses are formulated for this study based on these theoretical foundations. The first hypothesis is that chitosan microparticles loaded with Pili pulp extract will significantly enhance the growth performance of African catfish fingerlings compared to the control group. It is expected in the second hypothesis that this feed additive will improve the innate immune response of the African catfish. Furthermore, the third hypothesis is anticipated to be economically viable and environmentally sustainable in incorporating this feed additive into fish feed.

A feedback loop is included in this conceptual framework as illustrated in the visual diagram, demonstrating the dynamic nature of the research process. The findings from the study that were used to refine and optimize the synthesis and encapsulation processes, adjust feeding protocols, and implement improvements in water quality management are indicated in this feedback loop.

This conceptual framework served as a guide to the research methodology by informing the processes in synthesis and encapsulation of feed additives, structuring the feeding trials, monitoring protocols, guiding the proper data collection and analysis, and providing a basis for the evaluation of the economic and environmental impacts of the feed additive. Additionally, understanding this framework is essential for the flow of activities and the iterative nature of the research, where findings continuously inform and improve the study.

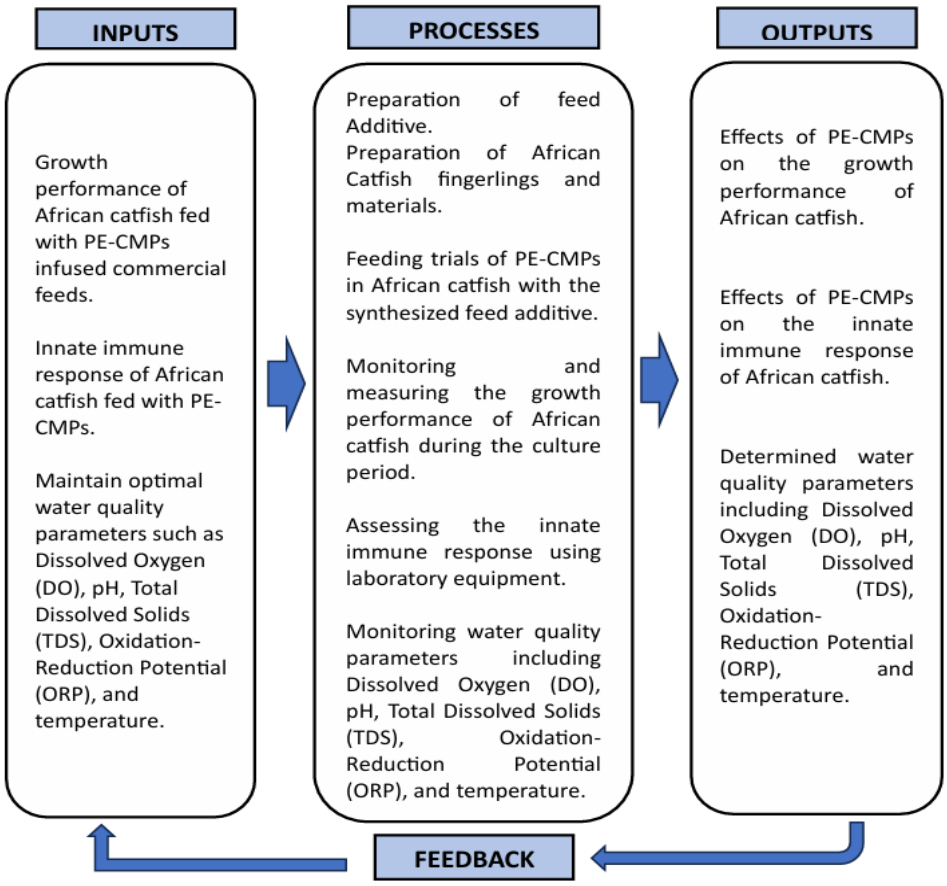

Figure 2. Conceptual Paradigm of the Study

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

This chapter describes the research design and methodologies used in the synthesis of chitosan microparticles loaded with pili pulp extract (PE-CMPs) as a feed additive and its effect on the growth parameters and innate immune response of the African catfish. It also focuses on determining the water quality during the culture period. Furthermore, this chapter presents the study area, materials, as well as the resources, the methodology, including data gathering, data collection, data processing, data analysis, and evaluation.

Research Design

This section outlines the research design and methodologies employed in the study. The research is purely experimental, manipulating both independent and dependent variables to assess their effects. The primary focus is on the preparation of chitosan microparticles loaded with pili pulp extract (PE-CMPs), incorporating them into fish feeds, and evaluating their impact on the growth performance, innate immune response, and water quality of African catfish. Furthermore, to determine the effects of PE-CMPs on the growth performance of African catfish, three feeding groups were established. The Complete Randomized Design (CRD) was applied to ensure unbiased assignment of treatments.

Place and Duration of the Study

The research study was conducted through experimental activities, which were undertaken at the Multi-Purpose Laboratory Building located at Bicol University Tabaco in Tayhi, Tabaco City for 2 months. All laboratory analyses were conducted at the said location.

The data results were collected as soon as the researchers obtained the outcomes. The UV-Vis Spectrophotometry technique was used to determine the optimal parameters for using chitosan microparticles in microencapsulating pili pulp extracts, resulting in the synthesis of PE-CMPs.

Experimental Design and Layout

A. Growth Performance

To determine the effects of PE-CMPs on the growth performance of African catfish fingerlings, the researchers established three feeding groups. Three feeding treatments, including the control, namely: T0- Control, T1- 100 mg/kg, T2- 500 mg/kg, which are shown in Table 1, were used in the experiment. The treatments were modified from the previous study of Brul & Canapit (2024). This was adapted to the type of feeds used and to the species used in the experiment. To guarantee unbiasedness during the experiment, the Complete Randomized Design (CRD), illustrated in Table 2, was applied as a foundation for assigning treatments. The researchers measured the growth metrics, specifically the initial and final weight, length measurements, weight gain, and length gain. The data collected was analyzed using ANOVA.

Table 1. Experimental Groups for the Evaluation of PE-CMPs as a Feed Additive on the Growth Performance and Innate Immune Response of African Catfish Fingerlings.

TREATMENT	FEED GROUP
0	Control
1	100 mg/kg of PE-CMPs
2	500 mg/kg of PE-CMPs

Fish and Culture Conditions

In this study, a total of 90 African catfish with a mean length of 10.43 cm and a mean weight of 8.42 grams were used for a 60-day feeding trial to determine the effect of the optimized PE-CMPs on the growth performance of *C. gariepinus.* African catfish that was cultured was bought from a trusted supplier located within Tabaco City. The fish underwent acclimatization for them to adjust to the environment for about 15 days in their crates. The fish was reared in a rectangular wooden crate with a dimensions of 15 x 17 x 22.5 inches covered with polyethylene plastic. There were 10 fish per crate with 45.97 liters of water. The water was sourced from the Tabaco Water District (TAWAD). Experimental fish were fed twice a day with 5% of their body weight. Samples were measured for growth performance during each sampling time (0 day, 30 days, 60 days) and were collected randomly.

Daily Feeding Rate

Table 2 presents the Standardized Daily Feeding Rate (DFR) at month 1 and month 2 across all treatments on the overall average body weight (BW)of fish. The average BW was recorded at the start (Day 0) and after one month (Day 30), and the feeding rate was adjusted accordingly. The same amount of feed was given to all replicates throughout the experimental period. The feeding rate was set at 5% of BW and multiplied by the number of fish per replicate (10 fish) to determine the exact amount of feed, calculated using the formula:

$$DFR = (BW \times 0.05) \times N \qquad \textit{(Eq. 1)}$$

Where:

DFR= Daily Feeding Rate (g/day)

BW= Average Body Weight of fish (g)

Feeding rate (5% of BW)= 0.05

N= Number of fish er replicate

Table 2. Standardized Daily Feeding Rate for African Catfish grow-out set-up in 60 day feeding trials

SAMPLING TIME	Average Body Weight (g)	Daily Feeding Rate (g/day per replicate)
Day 0	8.42	4.21
Day 30	45.57	22.79

Table 3. Experimental Layout

TREATMENT		
T_0R_1	T_1R_1	T_2R_1
T_2R_2	T_0R_2	T_1R_2
T_1R_3	T_2R_3	T_0R_3

B. Innate Immune Response

A total of 90 African catfish (*C. gariepinus*) with a mean body weight of 26.84 grams and a mean length of 16.31 cm were used for a 28-day feeding trial to determine the effect of PE-CMPs on the innate immune response of African catfish. It was reared at the Bicol University Tabaco wet laboratory, separated from the growth performance set up. However, it was reared using the same dimensions of wooden crates and the same source of water. To evaluate the effects of PE-CMPs on the innate immune response of African

catfish, three feeding groups and three feeding treatments (refer to Table 1) were established and used in the experiment. Complete Randomized Design (CRD) was applied in assigning treatments (refer to Table 2). Experimental fish were fed twice a day with 5% of their body weight. Experimental fish were fed twice a day with 5% of their body weight; a total of 13.42 grams/day per replicate of feeds was given (refer to the formula of DFR for the computation). Blood samples were collected during each sampling time (0 day, 7 days, 14 days, 21 days, and 28 days) and were collected randomly in triplicate.

Figure 3. Actual Experimental Set-up of 28 days and 60 days feeding trial on African Catfish (*C. gariepinus*)

Preparation of Feed Additives

A. Collection, Processing, and Preparation of Pili Pulp Extract

The preparation of Pili pulp followed the methodology by Abion *et al*. (2022) with slight modification. Pili pulp was collected from San Isidro Iraya, Malilipot, Albay. After acquiring the pili pulp, it underwent air-drying for 2 hours and dehydration at 80°C for 8 hours. The dried pulp was then ground into powder and stored in a refrigerator.

For Pili pulp extraction, powdered pulp was macerated in 95% ethanol at a 1:3 (w/v) ratio for 48 hours. The mixture was filtered using Whatman filter paper and a PES syringe filter (0.22 μm pore size), followed by solvent removal via rotary evaporation or water bath heating.

B. Preparation of Chitosan and Tripolyphosphate (TPP) Stock Solutions

Chitosan was dissolved in 1% (v/v) acetic acid to achieve a final concentration of 5 mg/mL (w/v). The dissolution process involved overnight stirring on a magnetic stirrer at 400 rpm, followed by filtration through a PES membrane syringe filter with a pore size of 0.22 μm (Arevalo, 2022). On the other hand, TPP was dissolved in distilled water to a final volume of 5 mg/mL (w/v) and also filtered through a PES membrane syringe filter (Arevalo, 2022). The chitosan microparticles (5 mg/mL) were used in this study.

C. Synthesis of Chitosan Microparticles Loaded with Pili Pulp Extracts (PE-CMPs)

The synthesis of chitosan Microparticles Loaded with Pili Pulp Extracts (PE-CMPs) involves several key steps. Initially, the optimal formulation factors were identified from the study of Brul & Canapit (2024), which adopted the ionic gelation technique by Agnihotri *et al*. (2004), with modifications, involving electrostatic interactions between chitosan's amino groups and TPP to form cross-linked structures encapsulating bioactive compounds. To enhance encapsulation efficiency, a 1% polysorbate solution was added to the formulation.

For the synthesis process of CMPs, 9 mL of TPP solution (5 mg/mL) was gradually added to 18 mL of chitosan solution (5 mg/mL) under mild agitation (400 rpm) at room temperature for 30 minutes. The resulting chitosan microparticles were centrifuged at

5,000xg for 10 minutes, with the supernatant discarded. The pellet was rinsed with distilled water, re-dispersed in acetone, filtered, and dried at room temperature in a desiccator. Additionally, 1 mL of Pili pulp extract was incubated before cross-linking under mild agitation at room temperature for 30 minutes.

D. Preparation of PE-CMPs Infused Commercial Feeds

The standard procedure for the mass production of PE-CMPs feed additive at the highest encapsulation efficiency was derived from the study conducted by Brul & Canapit (2024). The researchers used 270 mL of chitosan solution (5mg/mL), placing it in a magnetic stirrer for gentle agitation (400 rpm) at room temperature for 10 minutes. Then, 15 mL of pili-pulp extract was added, and after another 10 minutes of mild agitation, 1 % polysorbate was added and stirred for an additional 10 minutes. Subsequently, 135 mL of TPP solution (5mg/mL) was slowly added to the solution. Mild agitation of 400 rpm is maintained at room temperature for 30 minutes. The PE-CMPs undergo centrifugation at 5000xg for 10 minutes, followed by the removal of supernatant. The pellet is washed with distilled water. Sequentially, the wet weight for the T1 and T2 with the values of 100mg/kg and 500 mg/kg was measured and recorded, respectively. After weighing, it was diluted in 20 mL of distilled water and sprayed on the commercial feeds.

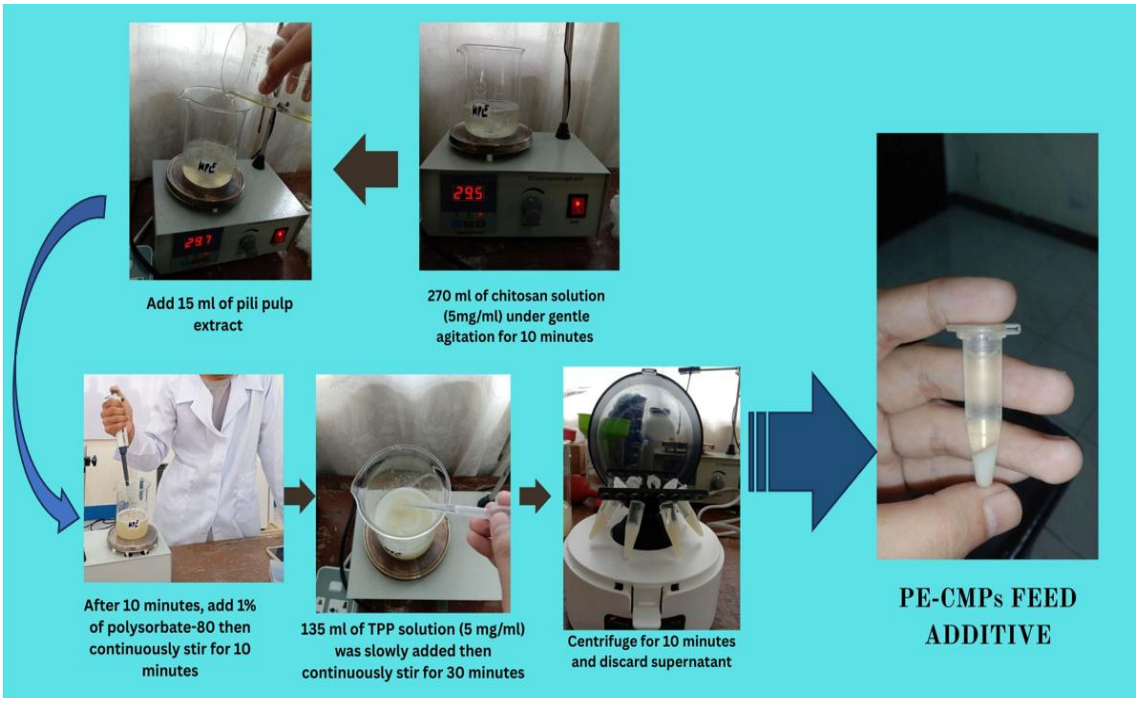

Figure 4. Synthesis Process of PE-CMPs

Data Collection

A. Growth Performance

To determine the effects of PE-CMPs on the growth performance of African catfish, fish sampling was done every 30 days. The method of Awad *et al.* (2015) was adopted, where the fish were deprived of food for 24 hours before weighing and sampling. The samples were measured using a digital weighing scale and a tape measure. The analysis of the growth performance of African catfish fingerlings (*C. gariepinus*) was done by measuring and recording the growth parameters, specifically the weight gain, Feed Conversion Ratio (FCR), Feed Efficiency Ratio (FER), and Condition Factor. Survival rate was also determined. After measuring and recording, the growth parameters were calculated using the following formula.

$$Weight\ Gain = FinalWeight - InitialWeight \quad \textit{(Eq. 2)}$$

$$K = 100 \times \frac{W}{L^3} \quad \textit{(Eq. 3)}$$

$$FCR = \frac{TotalFeedConsumed}{WeightGain} \quad \textit{(Eq. 4)}$$

$$FER = \frac{Weightgain}{TotalFeedConsumed} \quad \textit{(Eq. 5)}$$

$$SR(\%) = (Total\ fish\ stocked - Mortality) \times 100 \quad \textit{(Eq. 6)}$$

B. Innate Immune Response

Collection of data was done weekly by getting 0.5 ml of blood from 3 treatments and replicates through the dorsal part of the fish. Blood samples were gathered from 45 mixed sex sample species of African catfish, with a total average body weight of 26.84±SD. Blood samples were diluted using Hayem's Solution and WBC diluting fluid for RBC and WBC, respectively. The volume of white cell diluting fluid and red blood cell diluting fluid is 4.975ml and diluted to a 25µl blood sample. The diluted blood sample was placed in a hemocytometer for microscopy analysis and counted in ImageJ. The total WBC counted was multiplied by 50, then the product was multiplied by 1000. While the total RBC counted was multiplied by 10000 then the product is then multiplied by 1000.

Water Quality Parameters

To evaluate the water quality parameters, specifically Dissolved Oxygen (DO), pH, Total Dissolved Solids (TDS), Oxidation-Reduction Potential (ORP), and temperature, the researchers used a multi-parameter tester tool. The water quality parameters were measured weekly as part of the research protocol.

The cultured media was siphoned every day while letting new water flow to the crates to replace the water lost from siphoning. This was done to reduce the risk of ammonia and nitrogen build-up.

The data collected on the water quality parameters with the use of a multi-parameter tester tool were statistically analyzed using one-way ANOVA.

Statistical Analysis

All the data that were collected in this study were presented as means ± SD and were statistically analyzed using Jamovi software version 2.5.6. Specifically, the data that were collected for the growth performance, such as weight gain, FCR, FER, condition factor, and mortality rate, were analyzed using ANOVA then further analyzed using a normality test. The data obtained on the blood samples for the innate immune response of the African catfish fed with PE-CMPs were analyzed using a non-parametric test, which is the Kruskal-Wallis test. The data on the water quality parameters were statistically analyzed using ANOVA as well.

CHAPTER 4

EFFECT OF CHITOSAN MICROPARTICLES LOADED WITH PILI PULP EXTRACT (PE-CMPs) ON THE GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF AFRICAN CATFISH (*Clarias gariepinus)*

This chapter presents the data, analysis, and discussion of the results of the study of the effect of PE-CMPs on the growth performance and the innate immune response of African catfish.

Growth Performance of African catfish

A. Weight Gain

Table 4 displays the average weight gain of African catfish (*Clarias gariepinus*) fingerlings fed commercial feeds at a feeding rate of 5% of their body weight. The diet included varying levels of Pili Pulp Extract Loaded with Chitosan Microparticles (PE-CMPs). The fish were reared in 15x17x22.5-inch wooden frame tanks with polyethylene plastic, and their weights were measured using a digital weighing scale.

Table 4. Mean Weight Gain of the African catfish (*Clarias gariepinus*)

Treatment	Initial Weight (g)	Weight on Day 30 (g)	Final Weight on Day 60 (g)	Mean Weight gain (g)
T0 (Control)	8.49	38.49	74.02	65.53
T1 PE-CMPs (100mg/kg)	8.03	42.89	77.23	69.20
T2 PE-CMPs (500mg/kg)	8.75	55.33	87.97	79.23

Results in Table 4 showed better weight gain in all treatments. The initial weights of the fish were relatively similar across treatments, with slight variations. However, as the culture period progressed, differences in weight gain were observed among treatments. By day 30, fish fed with 500 mg/kg PE-CMPs (T2) exhibited the highest weight, followed by the 100 mg/kg PE-CMPs group (T1), while the control group (T0) had the lowest weight.

To assess differences in weight (grams) among groups, ANOVA was used for a parametric test, and Kruskal-Wallis was used for a Kruskal-Wallis non-parametric test was conducted. Since the Shapiro-Wilk test ($p < 0.001$) indicated a violation of normality, the Kruskal-Wallis test was used as a more reliable alternative. The test result ($p = 0.189$) suggests no significant differences in weight among the groups. Since the p-value is greater than 0.05, there is insufficient evidence to reject the null hypothesis. This indicates that there is no strong statistical evidence to support weight differences across groups.

The trend remained consistent by the end of the experiment (Day 60). This observation in the mean weight gain was further supported over the entire period as T2 achieved the highest mean gain, followed by T1, while the lowest gain is in control group. The observed enhanced growth performance in African catfish fed with PE-CMPs-

enriched diets may be attributed to the potential bioactive compounds present in the Pili pulp extract, which could have contributed to improved nutrient absorption, metabolism, and overall growth efficiency (Abdel-Tawwab *et al*., 2019). Furthermore, the chitosan microparticles have potentially played a role in enhancing the feed utilization and immune response of African catfish while promoting better growth (Abdel-Ghany & Salem, 2020).

The results in this study indicated that the supplementation of PE-CMPs at both 100 mg/kg and 500 mg/kg concentrations had a positive effect on the weight gain of African catfish, with the higher dosage (500 mg/kg) yielding the most significant gain in weight. These results suggest that the integration of PE-CMPs into fish diets is an effective strategy to enhance the growth performance of fish in aquaculture settings.

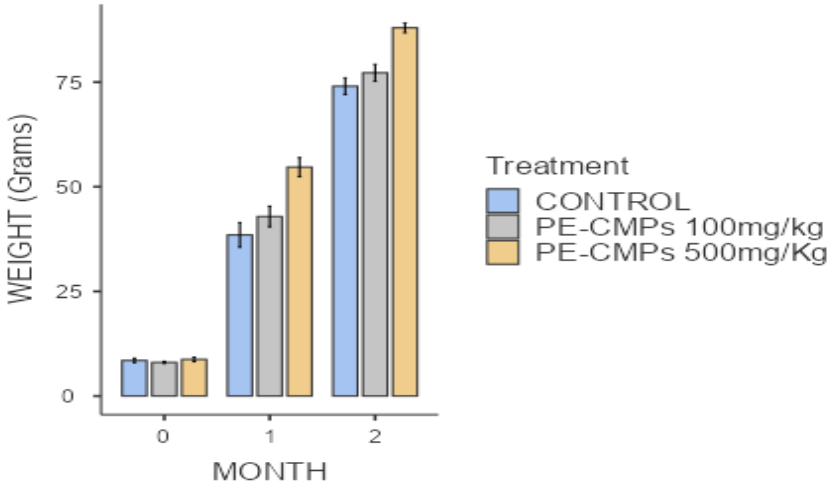

Figure 5. Changes in Body Weight of African catfish (*Clarias gariepinus*) over a 60-day feeding trial in response to PE-CMPs supplementation at different concentrations (p-value <0.05).

In Figure 5, the results of the test in ANOVA showed significant effects of both MONTH ($p < .001$) and Treatment ($p < .001$), demonstrating that both time and treatment significantly influenced the weight changes of African catfish. Additionally, the MONTH

× Treatment interaction ($p < .001$) implicates that the impact of treatment varies over time. The residual variance remains relatively low, reinforcing the robustness of the observed effects.

The post hoc comparisons further clarify these relationships, demonstrating no significant differences between groups at Month 0, confirming comparable baseline weights across all conditions. However, as time progressed, significant differences emerged. Both treatment groups exhibited weight increase compared to the control, with PE-CMPs 500 mg/kg showing the highest weight gain ($p < .001$ for all comparisons). The dose-dependent response suggests that higher concentrations of PE-CMPs exert a more substantial impact on weight progression over time.

The graphical representation in Figure 5 aligns with these statistical findings, illustrating a progressive increase in weight across months for all groups, with a particularly marked separation of the PE-CMPs 500 mg/kg group by Month 2. Zaki *et al.* (2015) reported similar positive effects of chitosan supplementation in sea bass (*Dicentrarchus labrax*), where fish fed with chitosan-enhanced diets exhibited higher weight gain and improved feed conversion efficiency. The study attributed these benefits to chitosan's role in improving nutrient absorption and digestive efficiency. These findings support the results of the present study, where PE-CMPs appeared to enhance the growth performance of African catfish over time, particularly in the higher dosage group.

B. Length gain

The results shown in Figure 6 from the length gain analysis of African catfish (*Clarias gariepinus*) from this study indicate a significant difference between the treatments and time on fish growth. The ANOVA results revealed that the type of treatment had a statistically significant impact on fish length ($p < 0.001$), suggesting that feed infused with PE-CMPs promoted higher length gain compared to the control group. Additionally, the effect of time was highly significant ($p < 0.001$), confirming that length increased progressively over the experimental period. However, the interaction between treatment and time was not statistically significant ($p = 0.064$), indicating that the effects of different treatments on the length gain of fish remained relatively consistent over time.

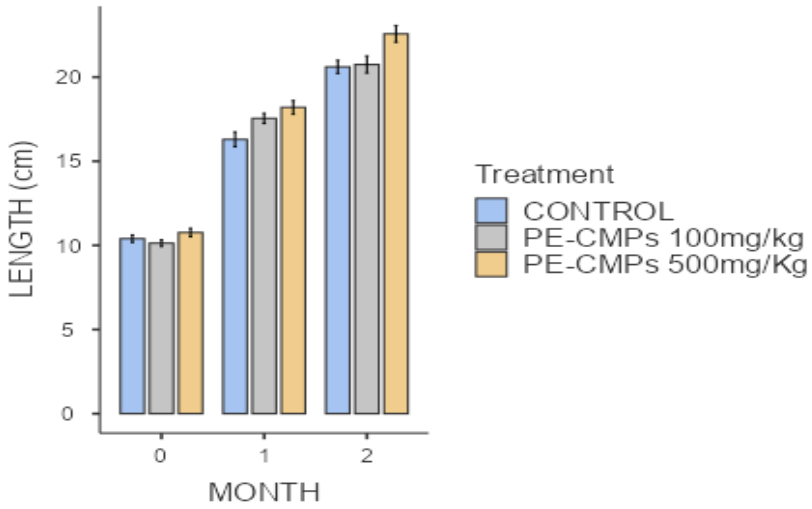

Figure 6. Changes in Body Length of African catfish (*Clarias gariepinus*) over 60-day feeding trial in response to PE-CMPs supplementation at different concentration (p-value <0.05).

The data presented statistical analyses, including the Shapiro-Wilk normality test, ANOVA, and post hoc comparisons as shown in Figure 6. The Shapiro-Wilk normality test result ($p = 0.066$) indicates that the data do not significantly deviate from normality, as

the p-value is greater than 0.05. Since the p-value is greater than 0.05, there is insufficient evidence to reject the null hypothesis. This indicates that there is no strong statistical evidence to support length differences across groups.

These findings were further supported by the post hoc test results, which demonstrated that African catfish that received PE-CMPs-infused feeds, particularly at the 500 mg/kg dosage, exhibited significantly greater length gain as compared to the control group. The observed increase in the length of African catfish in this study aligns with the previous study on dietary chitosan-based supplementation, which has reported enhanced growth performance in aquaculture species. For example, the study of Yang *et al.* (2020) found that dietary chitosan nanoparticles improved the growth rates and feed efficiency of Nile tilapia (*Oreochromis niloticus*), potentially due to the better nutrient utilization and immune enhancement. Additionally, the study by Abdel-Tawwab *et al*. (2018) demonstrated that the bioactive compounds in plant extracts could stimulate growth in *C. gariepinus*, likely through antioxidant and antimicrobial properties that reduce physiological stress.

The interaction effect between treatment and time was not significant, which suggests that while the PE-CMPs treatments consistently promoted growth, there is no significant variation in the rate of increase across the study period. This stability may indicate that the effects of PE-CMPs on length are sustained over time without decreasing the treatment's efficacy. These findings suggest that PE-CMPs supplementation can serve as a functional and effective dietary additive to enhance the fish growth performance.

C. Feed Conversion and Efficiency

In aquaculture, feed utilization efficiency is an essential factor, as it directly affects the production costs and overall growth performance. The FCR (Feed Conversion Ratio) and FER (Feed Efficiency Ratio) are commonly used to determine the validity of feed utilization in fish culture. The FCR is used to measure the requirement of feed to gain a unit of weight; a lower value indicates better feed efficiency. On the other hand, the FER determines the efficiency of feed conversion into body mass, where the lower the value of FER, the better. It means that less feed is required to produce one unit of fish weight.

Table 5. Feed Conversion Ratio (FCR) and Feed Efficiency Ratio (FER) of African Catfish in a 60-day culture period.

Treatment	Total Feed Consumed	Total Weight Gain (g)	FCR	FER/FCE
T0 (Control)	2,327.85	1,965.9	1.18	0.84
T1 PE-CMPs (100mg/kg)	2,327.85	2,076.0	1.12	0.89
T2 PE-CMPs (500mg/kg)	2,327.85	2,376.9	0.98	1.02

The results shown in Table 5 indicate that PE-CMPs 500 mg/kg (T2) exhibited the most efficient feed utilization, with the lowest FCR (0.98) and highest FER (1.02). These results suggest that incorporating a higher concentration of PE-CMPs in the diet of African catfish significantly improved the feed conversion and efficiency. In this study, the control group (T0) obtained the highest FCR (1.18) and lowest FER (0.84). This indicated that fish in this treatment required more feed to achieve the same weight gain, leading to lower efficiency compared to T2.

Studies indicate that several factors influence FCR and FER, including dietary composition, feeding frequency, and environmental conditions. For instance, according to Nguyen & Tran (2025), an FCR between 0.8 and 1.5 is considered efficient in aquaculture; the values that are closer to 1.0 are ideal for sustainability and cost-effectiveness. Additionally, the study by Kagali *et al.* (2024) stated that a lower FCR leads to reduced feed waste and better economic returns in fish farming.

In this study, since T2 (0.98) had a lower FCR than T1(1.12) and T0 (1.18), the results suggested that PE-CMPs supplementation improved feed efficiency, making T2 the best-performing feed in terms of FCR and FCE.

D. Conditioning Factor

The condition factor (K) offers vital insights into an organism's body condition in its environment, benefiting researchers in evaluating growth rates, reproductive success, and susceptibility to stressors. For instance, a study by Abd. Hamid *et al.* (2015) suggest that the condition factors of fish can be used to demonstrate the relationship between fish species and their environmental variables.

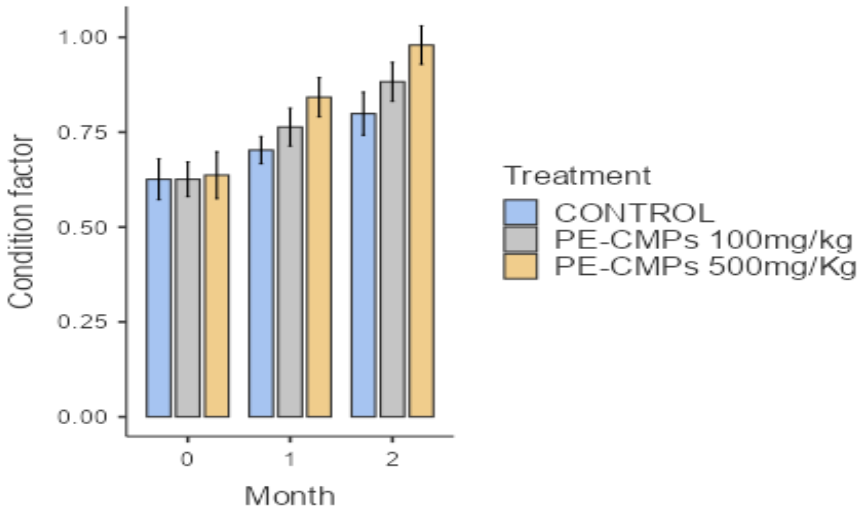

Figure 7. Condition Factor (K) of *Clarias gariepinus* ($p < 0.05$). Significant differences were observed among treatments ($p = 0.033$) and across months ($p < 0.001$), while the interaction between treatment and month was not significant ($p = 0.558$)

The data provided in Figure 7 presents the statistical analyses, including ANOVA and post hoc comparisons, examining the impact of "Month" and "Treatments" on the outcome variable. The data demonstrate a general increasing trend in the condition factor of African catfish over time across all treatments. Notably, the group supplemented with PE-CMPs at 500 mg/kg exhibited the highest condition factor across all months, followed by the 100 mg/kg group and the control. Error bars indicate some variability, but the differences appear consistent.

The ANOVA results revealed a statistically significant effect of treatment ($p = 0.033$) and month ($p < 0.001$) on the condition factor. This suggests that the condition factor of *C. gariepinus* was significantly influenced by the type of diet and duration of feeding. The interaction between treatment and month was not significant ($p = 0.558$), indicating

that the differences in condition factor over time were not significantly dependent on the specific treatment.

Further analysis through post hoc comparisons showed deeper observation into the significant differences among treatments and months. The PE-CMPs 500 mg/kg (T2) consistently showed significantly higher K values compared to the control ($p < 0.001$ in some comparisons). The PE-CMPs 100 mg/kg (T1) also exhibited improvement, but it was not as pronounced as the 500 mg/kg group. These results align with previous studies that emphasize the benefits of chitosan-based microparticles in improving fish growth and feed utilization efficiency (Asimi *et al*., 2020; Ali *et al*., 2018). It is shown in many studies that chitosan has been acknowledged in enhancing gut health and immune responses in fish, leading to better nutrient absorption and higher condition factors (Chen *et al*., 2019). The effect of the culture period on the condition factor is expected to be significant, as fish growth naturally progresses over time. However, the lack of significant interaction between treatment and month suggests that the effect of PE-CMPs is consistent over time rather than increasing or diminishing as fish age. The findings confirm that PE-CMPs supplementation, particularly at 500 mg/kg, enhances fish condition. This suggests a promising alternative for improving fish health and productivity in aquaculture.

Overall, the average condition factors for T0 is 0.89± 0.18, T1 is 0.87± 0.21, and T2 is 0.80± 0.22. However, Ragheb (2023) highlighted that Fulton's conditional factor greater than 1 does not necessarily indicate better health for the fish. The acceptable range can differ significantly depending on factors such as species' natural biology, habitat, and local environmental conditions (Bridle and Hoffman,2022). Therefore, Fulton's condition

factor of (T0) 0.89± 0.18, (T1) 0.87± 0.21 and (T2) 0.80± 0.22 not necessarily indicates a bad condition for the African catfish.

E. Survival rate of African catfish

Table 6 below presents the survival rate of African catfish (*Clarias gariepinus*) fingerlings reared under different dietary treatments. The survival rate was determined based on the total number of fish stocked and the recorded mortalities for each treatment group.

Table 6. Survival Rate of African Catfish (*Clarias gariepinus*) in 60-day

Treatment	Total Fish Stocked	Total Mortality	Survival Rate (%)
T0 (Control)	30	8	73.33%
T1 PE-CMPs (100mg/kg)	30	6	80.00%
T2 PE-CMPs (500mg/kg)	30	3	90.00%

The results in Table 6 show that the survival rate of African catfish increased with higher concentrations of PE-CMPs, with the control group having a 73.33% survival rate, T1 showing 80.00%, and T2 achieving the highest survival rate at 90.00%. This suggests that fish feeds infused with PE-CMPs enhance the survival of African catfish, potentially due to improved immune response or physiological benefits. Similar findings have been reported in studies on fish immune system boosters, where dietary supplementation with bioactive compounds improved the resilience of fish against stress and disease. For example, the study of Sribounoy (2020) evaluated the effect of chitosan-coated probiotic feed combined with plant extracts on the survival rate of Nile tilapia (*Oreochromis niloticus*), which showed there were higher survival rates in the fish fed with symbiotic

feed containing chitosan and natural extracts compared in the control group. The integration of chitosan and plant-derived compounds improved gut health, enhanced immune response, and reduced mortality, especially under stressful aquaculture conditions.

Innate immune system of African catfish

A. White Blood Cells

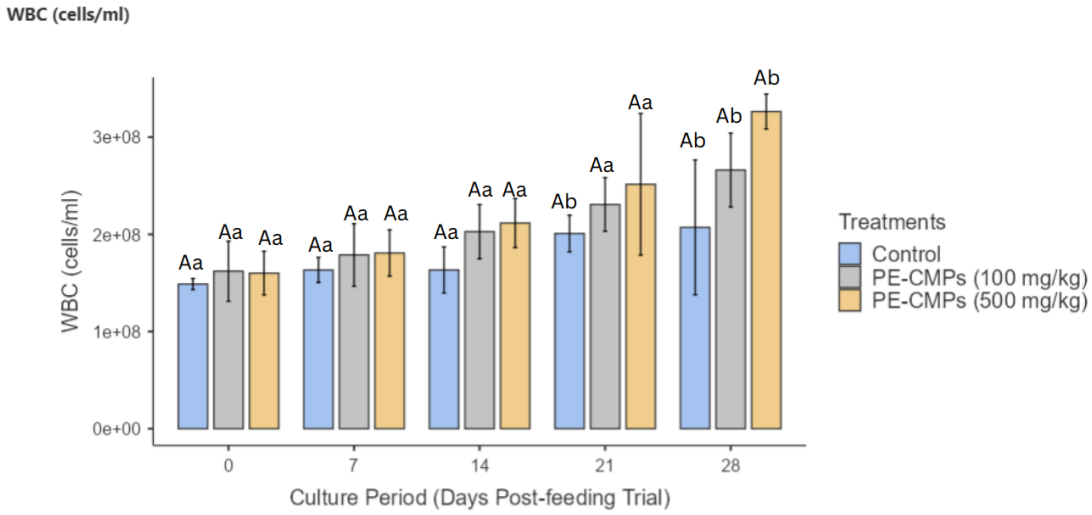

Figure 8. Effect of PE-CMPs on the White Blood Cells (WBC) of African catfish (*C. gariepinus*) for 28 days feeding trial (p-value<0.05). ***Note**: Treatment means with different capital letters denote significant difference among each treatment group in each sampling time. Means with different small letters denote significant difference of the treatments among the sampling/culture period.*

The result of the statistical analysis shown in Figure 8, showed that there was significant difference in the culture period (p value 0.004), while there is no significant difference on the treatment groups (p value 0.094) and in the combinations (Treatment groups and Culture period) (p value 0.919) on the white blood cells of African catfish for

28 days feeding trial with PE-CMPs. The initial WBC counts on all the treatments do not have a significant difference (p value >0.05), which suggests that the African catfish had similar white blood cell counts at the start of the feeding experiment. In 14 days, it showed that there is no significant difference among treatment groups, whereas in 21 days, there is an increase in PE-CMPs (500mg/kg) compared to the control group. Moreover, in 7 days to 28 days of feeding trial, a significant increase in WBC count in the PE-CMPs treatment 2 (500 mg/kg) was observed, in comparison to the control. The data for the white blood cells in African catfish fed for 28 days is normally distributed ($p > 0.05$); therefore, the null hypothesis is not rejected.

This finding shows that PE-CMPs are effective in regulating the WBC count of African catfish. The innate immune system is the first line of defense in fish, providing immediate protection against pathogens. WBCs play a critical role in recognizing and eliminating threats through phagocytosis, cytotoxicity, and the production of cytokines (Magnadóttir, 2005). Many studies have demonstrated the effect of immunostimulant plants on blood cells to improve immunity in aquatic animals. For instance, the study of Purbomartono *et al.* (2021) shows that the ginger and turmeric diets showed an increase in leukocytes. Similarly, the study of Onomu (2019) indicates that garlic-supplemented diets had a weekly progression on the WBC count. Likewise, the findings of Martins *et al*. (2002) stated that garlic inclusion in the diet can improve the WBC number of fish.

According to Okorie-Kanu and Unakalamba (2014), the average WBC count of African catfish is 185,000-220,000 cells/$mm^{3,}$ which is $1.85x10^{8}$-$2.22x10^{8}$cells/ml. The leukocytes from the beginning of the study range from $1.07\ x10^{8}$ (control), $1.62\ x10^{8}$ (100

mg/kg PE-CMPs), and 1.60 x10^8 (500 mg/kg PE-CMPs) cells/ml, which is in the normal range.

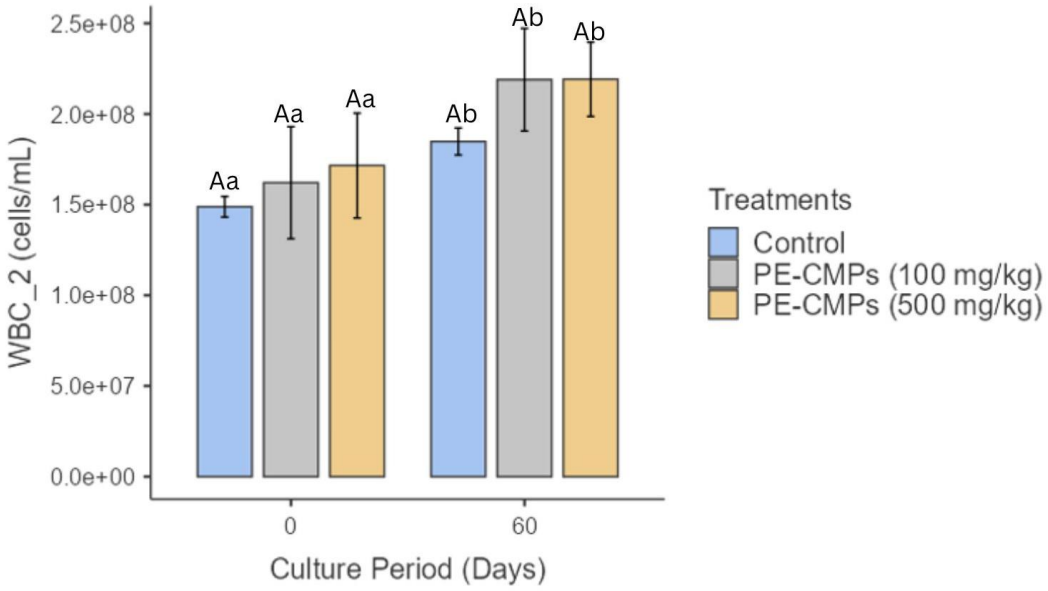

Figure 9. Effect of PE-CMPs on the White Blood Cells (WBC) of African catfish (*C. gariepinus*) for a 60-day feeding trial (p-value<0.05). ***Note**: Treatment means with different capital letters denote significant difference among each treatment group in each sampling time. Means with different small letters denote significant differences of the treatments among the sampling/culture period.*

At 7 days, the leukocytes range from 1.63 x10^8 (control), 1.79 x10^8 (100 mg/kg PE-CMPs), and 1.81 x10^8 (500 mg/kg PE-CMPs) was incorporated on the innate immune of African catfish, the same findings to 14 days of culture period where leukocytes range from 1.63 x10^8 (control) 2.03 x10^8 (100 mg/kg PE-CMPs), and 2.12 x10^8 (500 mg/kg PE-CMPs). During the 21 days, the leukocyte range was 2.01 x10^8 (control), 2.31 x10^8 (100 mg/kg PE-CMPs), and 2.51 x10^8 (500 mg/kg PE-CMPs), which was absorbed effectively. Furthermore, at the end of the culture period, the number of leukocytes increased into 2.07 x10^8 (control), 2.66 x10^8 (100 mg/kg of PE-CMPs) and 3.26 x10^8 (500 mg/kg of PE-CMPs)

which indicates that chitosan microparticles loaded with pili pulp extract is effective in increasing the white blood cells of African catfish.

Based on statistical analysis shown in Figure 9, there is a significant difference in culture period (p value 0.027), yet there is no significant difference in treatment (p value 0.432) and in culture period vs treatment (p value 0.901). The data is normally distributed ($p > 0.05$), meaning there is insufficient data to reject the null hypothesis and the data falls in the normality range. The WBC count during the 60-day feeding trial ranges from 1.85 $x10^8$ cells/ml (control), 2.19 $x10^8$ cells/ml (100 mg/kg PE-CMPs), and 2.19 $x10^8$ cells/ml (500 mg/kg PE-CMPs) compared to the control. This suggests that the white blood cell count of African catfish increases the longer it is exposed to the PE-CMPs feed additive.

B. Red Blood Cells

The result is based on statistical analysis shown on the Figure 10, which showed that there is a significant difference on the culture period (p value 0.012) and has no significant difference on treatments vs culture period (p value >0.05) to the red blood cells of African catfish for 4 weeks feeding trial with PE-CMPs. The initial RBC count in all treatments was found to have no significant value (p value >0.05), indicating that the African catfish that is used in this study had the same RBC count before the feeding trial started. The data of African catfish's RBC is normally distributed ($p > 0.05$). Investigated that there is no significant difference from 7 days up to 21 days of culture period. However, on 28 days, there is a significant difference (p value 0.043) in treatment 2 (PE-CMPs 500mg/kg) was observed relative to the control. This indicates that PE-CMPs are effective in improving the RBC of Nile Tilapia.

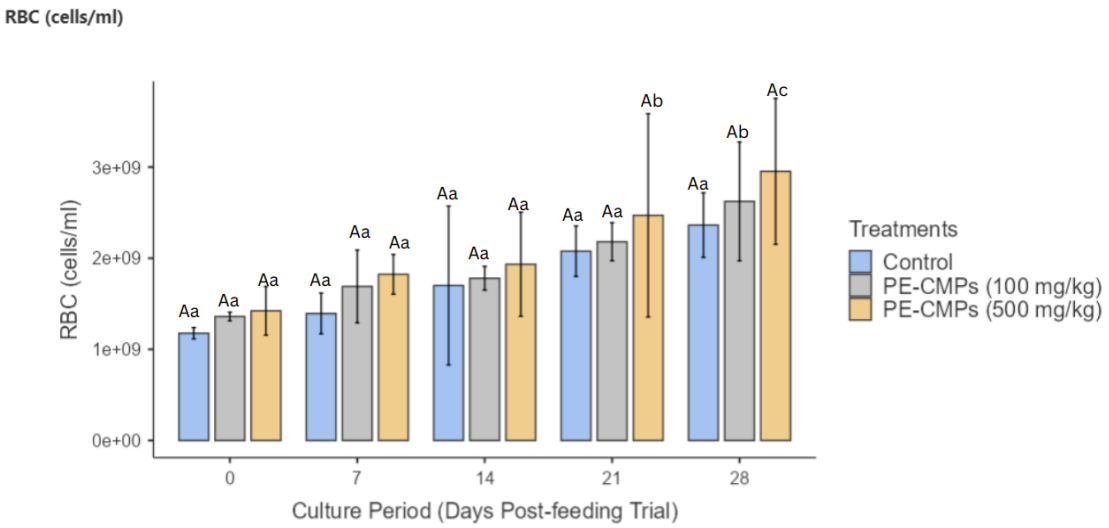

Figure 10. Effect of PE-CMPs on the Red Blood Cells (RBC) of African catfish (*C. gariepinus*) for 28 28-day feeding trial (p-value<0.05). ***Note**: Treatment means with different capital letters denote significant difference among each treatment group in each sampling time. Means with different small letters denote significant differences among the treatments during the sampling/culture period.*

In freshwater fish, normal RBC counts range from 0.4 to 5.2, with an average of 1.56 (×106/mm^3) (Esmaeili, 2021) or approximately 1.56 x10^9 cells/ml. In this study, the initial range of RBCs ranges from 1.12 x10^9 cells/ml (control), 1.34 x10^9 cells/ml (100 mg/kg PE-CMPs), 1.13 x10^9 cells/ml (500 mg/kg PE-CMPs), which still falls under the normal range. At week 1, the leukocytes range from 1.48 x10^9 cells/ml (control), 1.58 x10^9 cells/ml (100 mg/kg PE-CMPs), 1.95 x10^9 cells/ml (500 mg/kg PE-CMPs) which shows that there is no improvement on the feeding additive, likewise to week 2 culture period, RBC ranges from 1.42 x10^9 cells/ml (control), 1.66 x10^9 cells/ml (100 mg/kg PE-CMPs), 1.78 x10^9 cells/ml (500 mg/kg PE-CMPs). In addition, in week 3, the RBC counts range from 2.18 x10^9 cells/ml (control), 2.16 x10^9 cells/ml (100 mg/kg PE-CMPs), and 2.07 x10^9 cells/ml (500 mg/kg PE-CMPs). Moreover, in week 4, the RBC count from the control

ranged from 2.05 x10^9 cells/ml (control), 3.25 x10^9 cells/ml (100 mg/kg PE-CMPs), and 3.54 (500 mg/kg PE-CMPs).

Literature on the effect of pili-pulp in erythrocytes is scarce. However, a plant-based diet as a source of antioxidants of fish has been proven to increase the RBC count of fish. According to Mooraki *et al.* (2019), diets containing 0.3% turmeric proved to increase the RBC count of fish.

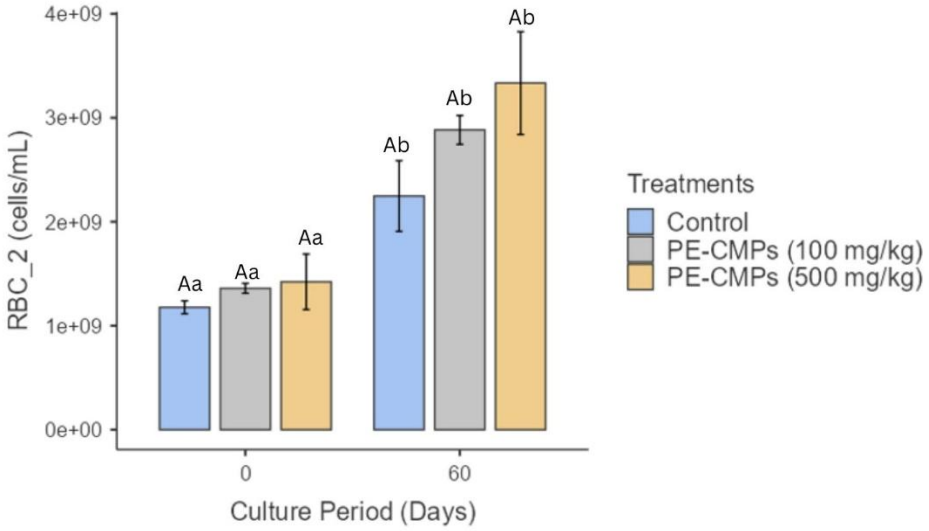

Figure 11. Effect of PE-CMPs on the Red Blood Cells (RBC) of African catfish (C. gariepinus) for 60 60-day feeding trial (p-value<0.05). ***Note**: Treatment means with different capital letters denote significant difference among each treatment group in each sampling time. Means with different small letters denote significant differences of the treatments among the sampling/culture period.*

In Figure 11, based on the statistical analysis shown, there is a significant difference in culture period (p value <.001), yet there is no significant difference in treatment (p value 0.090) and in culture period vs treatment (p value 0.345). The RBC of African catfish fed

for 60 60-day culture period is normally distributed ($p > 0.05$). The erythrocytes of the 60-day culture period range from 2.25 $x10^9$ cells/ml (control), 2.88 $x10^9$ cells/ml (PE-CMPs 100mg/kg), and 3.33 $x10^9$ cells/ml (PE-CMPs 500mg/kg), implicating that PE-CMPs are effective in increasing RBC in a longer period.

C. Differential Leukocytes

The result is based on the statistical analysis as shown in Table 7, innate immune response of African catfish *(C. gariepinus*) fed with PE-CMPs for 28 days feeding trials, showed that there is a significant difference in lymphocyte in culture period (p value <0.001) and treatment (p value 0.021) while in neutrophils, a significant difference (p value 0.034) was observed in treatment and monocytes, there is a significant difference observed in treatment (0.019) and in culture period (p value 0.001). However, basophils show no significant difference in any of the treatments or culture period. The different leukocytes, specifically the lymphocytes, neutrophils, monocytes, and basophils, during the 28-day culture period are normally distributed, having a p-value greater than 0.05, while eosinophils are not normally distributed ($p < 0.05$). A non-parametric test was conducted for eosinophils, and there is a significant difference observed in culture period ($p < 0.009$), while there is none in treatment as a grouping variable ($p > 0.05$).

Table 7. Effect of PE-CMPs on the Innate Immune Response of African catfish (*C. gariepinus*) for 28-day feeding trials.

Parameters	Control	PE-CMPs (100 mg/kg)	PE-CMPs (500 mg/kg)
Lymphocytes (%)	57.57±3.65	61.02±3.82	57.7±4.03
Neutrophils (%)	24.47±2.69	23.16±3.14	24.48±2.96
Monocytes (%)	15.67±1.99	14.02±3.47	13.34±4.00
Eosinophils (%)	1.65±2.14	1.32±0.46	1.84±1.09
Basophils (%)	1.55±2.26	1.05±0.36	1.47±1.06

Significantly, during the 60-day culture period, there is a significant difference observed in the lymphocytes during the culture period (p value 0.045) on treatment 2, PE-CMPs (500 mg/kg). Interestingly, data analyzed on neutrophil, monocyte, eosinophil, and basophil do not indicate any significant difference (p value>0.05) relative to the control. Data analyzed on lymphocytes, neutrophils, monocytes, basophils, and eosinophils during 60 60-day feeding trial are normally distributed (p value > 0.05).

White blood cells (WBC) and lymphocytes are reported to be the defense cells of the body. Porwal (2019) and Douglas and Jane (2010) demonstrated that the amount present in the body of the animal has implications in immune responses and the ability of the animal to fight infection. In this study, the PE-CMPs (100 mg/kg) 61.02±3.82% show the highest percentage of lymphocytes among other treatments. At 7 days to 21 days significant difference was found in PE-CMPs (100 mg/kg) and PE-CMPs (500mg/kg) compared to control, indicating improved adaptive immunity. Lymphocytes are indicators of the body's ability to mount specific immune responses and maintain immune memory, which are essential for fighting infections and diseases (Cano & Lopera, 2013). The average lymphocyte of African catfish is 49.33-70.33% (Porwal, 2019). According to

Owolabi (2010), it is numerous yet common in other fish. The observed lymphocytes in this study are within the normal range.

In this study, the neutrophil has a significant difference in treatments (p value 0.029), specifically between PE-CMPs (100 mg/kg) and PE-CMPs (500mg/kg). The percentage (%) of neutrophils in an African catfish range from 20.0-30.14%. In this study, the control 24.47±2.69% and the PE-CMPs (500 mg/kg) 24.48±2.96% have similar percentages, while the PE-CMPs (100 mg/kg) have slightly lower neutrophil percentage. A decrease in neutrophils may indicate a shift towards adaptive immunity as lymphocytes increase. Similarly, the findings of Adamu and Solomon (2015), the neutrophil keeps fluctuating as the lymphocyte increases, and the weight and length of the fish increase as well.

The results of monocytes in this study have a decreasing trend across treatments (refer to Table 7, Effect of PE-CMPs on the Innate Immune Response of African catfish (*C. gariepinus*) for 28 days feeding trial). However, there is no significant difference observed, making it relatively stable. In the same study by Adamu and Solomon (2015), the monocytes of African catfish range from 5-26 %. The lowest percentage of monocytes observed was at PE-CMPs (500 mg/kg), which is 13.34±4.00, while the highest percentage was observed in the initial data, 17.23±2.76. The percentage of monocytes analyzed is in the normal range. The reduction of monocytes indicates a lower inflammatory response due to the antioxidant property of the PE-CMPs feed additive.

The result, statistically analyzed in Table 7, the effect of PE-CMPs on the innate immune response of African catfish (*C. gariepinus*) for 28 28-day feeding trial, finds that eosinophils and basophils have no significant difference in culture period, treatment, and

treatment vs culture period. According to Okey *et al.* (2022), eosinophils range from 1.61% to 2.80%, and basophils from 1.07% to 2.27%. The PE-CMPs (100 mg/kg) 1.32±0.46 and PE-CMPs (500 mg/kg) 1.84±1.09 show a lower percentage of eosinophils compared to day 0. Similarly, the basophils do not have a major change across groups. The treatment PE-CMPs (100 mg/kg) 1.05±0.36 and PE-CMPs (500mg/kg) 1.47±1.06%is lower than the control 1.55±2.26%. Basophils play a minor role in fish immunity. Since these two parameters are stable, it indicates that the PE-CMPs do not induce an allergic or inflammatory response.

Table 8. Effect of PE-CMPs on the Innate Immune Response of African catfish (*C. gariepinus*) for a 60-day feeding trial.

60 days			
Parameters	**Control**	**PE-CMPs (100 mg/kg)**	**PE-CMPs (500 mg/kg)**
Lymphocytes (%)	60.99±7.02	57.12±3.85	66.89±4.04
Neutrophils (%)	22.62±7.45	24.99±4.04	18.32±2.04
Monocytes (%)	12.67±3.85	14.43±1.51	11.97±4.34
Eosinophils (%)	1.36±1.53	1.23±1.28	1.08±0.56
Basophils (%)	2.35±2.03	2.19±0.41	2.02±0.91

In Table 8, the effect of PE-CMPs on the innate immune response of African catfish (*C. gariepinus*) for the 60-day feeding trials, there is an increase in the percentage of lymphocytes in the treatment PE-CMPs (500 mg/g) as compared to the control, denoting a significant difference in the culture period. This indicates that the longer exposure to PE-CMPs led to a higher lymphocyte count, suggesting improved innate immune system over time. Similar to the result of the 28-day feeding trial, immune response parameters such as neutrophils, monocytes, eosinophils, and basophils decline over a long period of exposure

to the PE-CMPs feed additive, specifically on treatment 2 PE-CMPs (500 mg/kg). This shows the improvement of the innate immune response of African catfish, having a reduced allergic or inflammatory response, suggesting an adaptation in immune cell distribution.

Water Quality

Water quality plays a critical role in the growth and survival of African catfish (*C. gariepinus*) in aquaculture systems. The water parameters were checked every week with a multiparameter tester, and water change was done every day to maintain optimal conditions. In this study, the monitored water parameters in 60-day grow-out culture and 28-day culture for innate immune response remained within acceptable ranges for catfish culture, ensuring an optimal environment for fish growth and development.

Table 9. Monitored Water Quality Parameters for the grow-out culture of African Catfish.

Water parameters	Range
Dissolve Oxygen (DO)	0.5 – 5.9 mg/L
Oxidation Reduction Potential (ORP)	-2.9 – 108.3 mv
Temperature	24.9 – 28.6 C°
pH	5.71 - 6.91
Total Dissolved Solids	111 – 286 ppm

Table 10. Monitored Water Quality Parameters for the Innate Immune Response Culture of African Catfish

Water parameters	Range
Dissolved Oxygen (DO)	1.6 – 5.9 mg/L
Oxidation Reduction Potential (ORP)	-2.0 – 97.7 mv
Temperature	24.9 – 28.6 C°
pH	5.71 - 6.93
Total Dissolved Solids	114 - 235

CHAPTER 5

SUMMARY, CONCLUSION, AND RECOMMENDATION

This chapter presents the summary, conclusion, and recommendations for the study entitled "Effects of chitosan microparticles loaded with pili-pulp extract on the growth performance and innate immune response of African catfish (*Clarias gariepinus)*".

SUMMARY

The study evaluated the effects of Pili Extract-Loaded with Chitosan Microparticles (PE-CMPs) on the growth performance, feed utilization, survival, and condition factor of African catfish (*Clarias gariepinus*) over a 60-day feeding trial. Fish were subjected to three dietary treatments: Control (T0), PE-CMPs 100 mg/kg (T1), and PE-CMPs 500 mg/kg (T2).

The results on the growth performance showed a significant difference in weight gain and length gain for fish supplemented with PE-CMPs, particularly at the 500 mg/kg dose. The ANOVA test results confirmed that the treatment had a significant effect on both weight ($p < 0.001$) and length ($p < 0.001$), with a notable interaction between treatment and time ($p < 0.001$), suggesting that the impact of PE-CMPs increased progressively over the study period.

In terms of feed conversion efficiency, T2 exhibited the lowest Feed Conversion Ratio (FCR) of 0.98 and the highest Feed Efficiency Ratio (FER) of 1.02, indicating superior feed utilization compared to the control group, which had an FCR of 1.18 and FER of 0.84. The condition factor (K), a key indicator of fish health, also showed a significant increase over time ($p = 0.033$ for treatment effect), with T2 maintaining the highest K values, further confirming the positive effects of PE-CMPs supplementation. The

highest survival rate was observed in T2 (90%), followed by T1 (80%), and T0 (75%), indicating that dietary PE-CMPs may have enhanced fish immunity and resilience.

The effect of PE-CMPs on the innate immune response of African catfish was analyzed in microscopy, count in ImageJ software, and differentiated by using ANOVA. It was determined that the WBC and RBC have a significant value ($p<0.05$) in the culture period but none in the treatment and culture period treatment, during the 28-day and 60-day feeding trials. In addition, 500 mg/kg of PE-CMPs increases white blood cells and red blood cells, compared to the control, and has a significant difference in the culture period. During the 28-day feeding trials, there was a significant difference ($p<0.05$) observed in the lymphocytes and monocytes in terms of culture period and treatments, but none in culture period*treatments, while the neutrophil had a significant difference ($p<0.05$) in treatments. Basophil and eosinophils has no significant difference. In addition, all the different leukocytes is normally distributed except eosinophils. It was also determined that longer exposure to PE-CMPs feed additive increases the presence of lymphocyte, while lowering the neutrophils, monocytes, eosinophils, and basophils indicating the shift toward the adaptive immunity.

Additionally, the water quality parameters determined during the experimental period are maintained within acceptable ranges for catfish culture, ensuring an optimal environment for fish growth and development.

CONCLUSION

The study demonstrated that dietary supplementation with PE-CMPs at 500 mg/kg significantly enhanced growth performance, feed efficiency, survival rate, and condition

factor in *Clarias gariepinus*. The statistically significant differences between treatments confirm that higher doses of PE-CMPs led to better physiological responses and overall growth, suggesting that chitosan microparticles and bioactive compounds in Pili extract contributed to improved nutrient absorption and metabolic efficiency. The findings align with previous studies on chitosan-based feed additives in aquaculture, where similar improvements in growth rate, feed utilization, and immunity have been observed.

The effect of PE-CMPs on the innate immune response of African catfish was analyzed in microscopy, count in ImageJ software, and differentiated by using ANOVA. It was determined that the 500 mg/kg of PE-CMPs increases white blood cells and red blood cells, compared to the control, and has a significant difference in the culture period. It was also determined that longer exposure to PE-CMPs feed additive increases the presence of lymphocytes, while lowering the neutrophils, monocytes, eosinophils, and basophils, indicating the shift toward adaptive immunity.

This study shows that chitosan microparticles loaded with pili pulp extract or PE-CMPs on the innate immune response of African catfish (C. gariepinus). It demonstrated that the high concentration of PE-CMPs at 500 mg/kg is effective in the growth, white blood cells, red blood cells, and in improving the adaptive immune system.

Ultimately, the chitosan microparticles loaded with pili pulp extract have a favorable effect on the African catfish’s innate immunity, demonstrating its promise as a feed additive for aquaculture applications.

RECOMMENDATION

The researchers made the following recommendations for additional research based on the findings and conclusions of this study:

1. Analysis of the efficacy of PE-CMPs in exhibiting antimicrobial properties.
2. Effects of PE-CMPs on other aquatic organisms
3. Effects of PE-CMPs in terms of an increase and a decrease in dosage.

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APPENDICES

APPENDIX A. Analysis of Variance (ANOVA) of Growth Rate (length)

	Sum of Squares	df	Mean Square	F	p
MONTH	**2725.8**	**2**	**1362.89**	**657.03**	**<.001**
Treatment	**48.0**	**2**	**23.98**	**11.56**	**<.001**
MONTH ✻ Treatment	**18.9**	**4**	**4.73**	**2.28**	**0.064**
Residuals	**261.4**	**126**	**2.07**		

APPENDIX B. Post Hoc Comparisons on the Treatment ✻ MONTH for Length Gain of African Catfish in 60-day culture period

Comparison									
Treatment	MONTH		Treatment	MONTH	Mean Difference	SE	df	t	p_{tukey}
CONTROL	0	-	CONTROL	1	-30.000	2.57	126	-11.6774	<.001
		-	CONTROL	2	-65.527	2.57	126	-25.5061	<.001
		-	PE-CMPs 100mg/kg	0	0.467	2.57	126	0.1816	1.000
		-	PE-CMPs 100mg/kg	1	-34.393	2.57	126	-13.3875	<.001
		-	PE-CMPs 100mg/kg	2	-68.733	2.57	126	-26.7543	<.001
		-	PE-CMPs 500mg/Kg	0	-0.253	2.57	126	-0.0986	1.000
		-	PE-CMPs 500mg/Kg	1	-46.193	2.57	126	-17.9806	<.001
		-	PE-CMPs 500mg/Kg	2	-79.480	2.57	126	-30.9374	<.001
	1	-	CONTROL	2	-35.527	2.57	126	-13.8287	<.001
		-	PE-CMPs 100mg/kg	0	30.467	2.57	126	11.8591	<.001
		-	PE-CMPs 100mg/kg	1	-4.393	2.57	126	-1.7101	0.739
		-	PE-CMPs 100mg/kg	2	-38.733	2.57	126	-15.0769	<.001
		-	PE-CMPs 500mg/Kg	0	29.747	2.57	126	11.5788	<.001
		-	PE-CMPs 500mg/Kg	1	-16.193	2.57	126	-6.3032	<.001
		-	PE-CMPs 500mg/Kg	2	-49.480	2.57	126	-19.2600	<.001
	2	-	PE-CMPs 100mg/kg	0	65.993	2.57	126	25.6877	<.001

Comparison									
Treatment	MONTH		Treatment	MONTH	Mean Difference	SE	df	t	p_{tukey}
		-	PE-CMPs 100mg/kg	1	31.133	2.57	126	12.1186	<.001
		-	PE-CMPs 100mg/kg	2	-3.207	2.57	126	-1.2482	0.944
		-	PE-CMPs 500mg/Kg	0	65.273	2.57	126	25.4075	<.001
		-	PE-CMPs 500mg/Kg	1	19.333	2.57	126	7.5255	<.001
		-	PE-CMPs 500mg/Kg	2	-13.953	2.57	126	-5.4313	<.001
PE-CMPs 100mg/kg	0	-	PE-CMPs 100mg/kg	1	-34.860	2.57	126	-13.5692	<.001
		-	PE-CMPs 100mg/kg	2	-69.200	2.57	126	-26.9359	<.001
		-	PE-CMPs 500mg/Kg	0	-0.720	2.57	126	-0.2803	1.000
		-	PE-CMPs 500mg/Kg	1	-46.660	2.57	126	-18.1623	<.001
		-	PE-CMPs 500mg/Kg	2	-79.947	2.57	126	-31.1190	<.001
	1	-	PE-CMPs 100mg/kg	2	-34.340	2.57	126	-13.3668	<.001
		-	PE-CMPs 500mg/Kg	0	34.140	2.57	126	13.2889	<.001
		-	PE-CMPs 500mg/Kg	1	-11.800	2.57	126	-4.5931	<.001
		-	PE-CMPs 500mg/Kg	2	-45.087	2.57	126	-17.5499	<.001
	2	-	PE-CMPs 500mg/Kg	0	68.480	2.57	126	26.6557	<.001
		-	PE-CMPs 500mg/Kg	1	22.540	2.57	126	8.7736	<.001
		-	PE-CMPs 500mg/Kg	2	-10.747	2.57	126	-4.1831	0.002
PE-CMPs 500mg/Kg	0	-	PE-CMPs 500mg/Kg	1	-45.940	2.57	126	-17.8820	<.001
		-	PE-CMPs 500mg/Kg	2	-79.227	2.57	126	-30.8388	<.001
	1	-	PE-CMPs 500mg/Kg	2	-33.287	2.57	126	-12.9568	<.001

Note. Comparisons are based on estimated marginal means

APPENDIX C. Analysis of Variance (ANOVA) of Growth Rate (Weight)

Treatment	Sum of Squares	df	Mean Square	F	p
	2527	**2**	**1263.7**	**25.53**	**<.001**
MONTH	**114489**	**2**	**57244.4**	**1156.44**	**<.001**
Treatment ✻ MONTH	**1183**	**4**	**295.7**	**5.97**	**<.001**
Residuals	**6237**	**126**	**49.5**		

APPENDIX D. One-Way ANOVA (Non-parametric) on the Weight Gain of African Catfish 60-days Grow-out culture

Kruskal-Wallis

	χ^2	df	p
WEIGHT (Grams)	118	2	<.001

APPENDIX E. Post Hoc Comparison on the MONTH* Treatment for Weight Gain of African Catfish in 60-day culture period

Post Hoc Comparisons - MONTH ✻ Treatment

Comparison									
MONTH	Treatment		MONTH	Treatment	Mean Difference	SE	df	t	p_{tukey}
0	CONTROL	-	0	PE-CMPs 100mg/kg	0.467	2.57	126	0.1816	1.000
		-	0	PE-CMPs 500mg/Kg	-0.253	2.57	126	-0.0986	1.000
		-	1	CONTROL	-30.000	2.57	126	-11.6774	<.001
		-	1	PE-CMPs 100mg/kg	-34.393	2.57	126	-13.3875	<.001
		-	1	PE-CMPs 500mg/Kg	-46.193	2.57	126	-17.9806	<.001
		-	2	CONTROL	-65.527	2.57	126	-25.5061	<.001
		-	2	PE-CMPs 100mg/kg	-68.733	2.57	126	-26.7543	<.001
		-	2	PE-CMPs 500mg/Kg	-79.480	2.57	126	-30.9374	<.001
	PE-CMPs 100mg/kg	-	0	PE-CMPs 500mg/Kg	-0.720	2.57	126	-0.2803	1.000
		-	1	CONTROL	-30.467	2.57	126	-11.8591	<.001
		-	1	PE-CMPs 100mg/kg	-34.860	2.57	126	-13.5692	<.001

Post Hoc Comparisons - MONTH ✻ Treatment

Comparison									
MONTH	Treatment		MONTH	Treatment	Mean Difference	SE	df	t	p_{tukey}
		-	1	PE-CMPs 500mg/Kg	-46.660	2.57	126	-18.1623	<.001
		-	2	CONTROL	-65.993	2.57	126	-25.6877	<.001
		-	2	PE-CMPs 100mg/kg	-69.200	2.57	126	-26.9359	<.001
		-	2	PE-CMPs 500mg/Kg	-79.947	2.57	126	-31.1190	<.001
	PE-CMPs 500mg/Kg	-	1	CONTROL	-29.747	2.57	126	-11.5788	<.001
		-	1	PE-CMPs 100mg/kg	-34.140	2.57	126	-13.2889	<.001
		-	1	PE-CMPs 500mg/Kg	-45.940	2.57	126	-17.8820	<.001
		-	2	CONTROL	-65.273	2.57	126	-25.4075	<.001
		-	2	PE-CMPs 100mg/kg	-68.480	2.57	126	-26.6557	<.001
		-	2	PE-CMPs 500mg/Kg	-79.227	2.57	126	-30.8388	<.001
1	CONTROL	-	1	PE-CMPs 100mg/kg	-4.393	2.57	126	-1.7101	0.739
		-	1	PE-CMPs 500mg/Kg	-16.193	2.57	126	-6.3032	<.001
		-	2	CONTROL	-35.527	2.57	126	-13.8287	<.001
		-	2	PE-CMPs 100mg/kg	-38.733	2.57	126	-15.0769	<.001
		-	2	PE-CMPs 500mg/Kg	-49.480	2.57	126	-19.2600	<.001
	PE-CMPs 100mg/kg	-	1	PE-CMPs 500mg/Kg	-11.800	2.57	126	-4.5931	<.001
		-	2	CONTROL	-31.133	2.57	126	-12.1186	<.001
		-	2	PE-CMPs 100mg/kg	-34.340	2.57	126	-13.3668	<.001
		-	2	PE-CMPs 500mg/Kg	-45.087	2.57	126	-17.5499	<.001
	PE-CMPs 500mg/Kg	-	2	CONTROL	-19.333	2.57	126	-7.5255	<.001
		-	2	PE-CMPs 100mg/kg	-22.540	2.57	126	-8.7736	<.001
		-	2	PE-CMPs 500mg/Kg	-33.287	2.57	126	-12.9568	<.001

Post Hoc Comparisons - MONTH ✻ Treatment

Comparison									
MONTH	Treatment		MONTH	Treatment	Mean Difference	SE	df	t	p_{tukey}
2	CONTROL	-	2	PE-CMPs 100mg/kg	-3.207	2.57	126	-1.2482	0.944
		-	2	PE-CMPs 500mg/Kg	-13.953	2.57	126	-5.4313	<.001
	PE-CMPs 100mg/kg	-	2	PE-CMPs 500mg/Kg	-10.747	2.57	126	-4.1831	0.002

Note. Comparisons are based on estimated marginal means

APPENDIX F. ANOVA for the Condition Factor of African Catfish in 60-day culture period

ANOVA - Condition factor

	Sum of Squares	df	Mean Square	F	p
Treatment	0.275	2	0.1374	3.509	0.033
Month	1.494	2	0.7468	19.072	<.001
Treatment ✻ Month	0.118	4	0.0295	0.753	0.558
Residuals	4.934	126	0.0392		

APPENDIX G. Post Hoc Analysis on the Comparisons of Treatments * Month of the Conditioning Factor of African Catfish in a 60-day culture period

Post Hoc Comparisons - Treatment ✻ Month

Comparison									
Treatment	**Month**		**Treatment**	**Month**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
CONTROL	**0**	-	**CONTROL**	**1**	-0.0767	0.0723	126	-1.061	0.979
		-	**CONTROL**	**2**	-0.1727	0.0723	126	-2.390	0.299
		-	**PE-CMPs 100mg/kg**	**0**	-1.89e−16	0.0723	126	-2.61e−15	1.000
		-	**PE-CMPs 100mg/kg**	**1**	-0.1373	0.0723	126	-1.901	0.615
		-	**PE-CMPs 100mg/kg**	**2**	-0.2567	0.0723	126	-3.552	0.015
		-	**PE-CMPs 500mg/Kg**	**0**	-0.0107	0.0723	126	-0.148	1.000
		-	**PE-CMPs 500mg/Kg**	**1**	-0.2160	0.0723	126	-2.989	0.078
		-	**PE-CMPs 500mg/Kg**	**2**	-0.3533	0.0723	126	-4.890	<.001
	1	-	**CONTROL**	**2**	-0.0960	0.0723	126	-1.329	0.921

Post Hoc Comparisons - Treatment ✻ Month

Comparison									
Treatment	**Month**		**Treatment**	**Month**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**PE-CMPs 100mg/kg**	**0**	0.0767	0.0723	126	1.061	0.979
		-	**PE-CMPs 100mg/kg**	**1**	-0.0607	0.0723	126	-0.840	0.995
		-	**PE-CMPs 100mg/kg**	**2**	-0.1800	0.0723	126	-2.491	0.246
		-	**PE-CMPs 500mg/Kg**	**0**	0.0660	0.0723	126	0.913	0.992
		-	**PE-CMPs 500mg/Kg**	**1**	-0.1393	0.0723	126	-1.928	0.596
		-	**PE-CMPs 500mg/Kg**	**2**	-0.2767	0.0723	126	-3.829	0.006
	2	-	**PE-CMPs 100mg/kg**	**0**	0.1727	0.0723	126	2.390	0.299
		-	**PE-CMPs 100mg/kg**	**1**	0.0353	0.0723	126	0.489	1.000
		-	**PE-CMPs 100mg/kg**	**2**	-0.0840	0.0723	126	-1.163	0.963
		-	**PE-CMPs 500mg/Kg**	**0**	0.1620	0.0723	126	2.242	0.386
		-	**PE-CMPs 500mg/Kg**	**1**	-0.0433	0.0723	126	-0.600	1.000
		-	**PE-CMPs 500mg/Kg**	**2**	-0.1807	0.0723	126	-2.500	0.242
PE-CMPs 100mg/kg	**0**	-	**PE-CMPs 100mg/kg**	**1**	-0.1373	0.0723	126	-1.901	0.615
		-	**PE-CMPs 100mg/kg**	**2**	-0.2567	0.0723	126	-3.552	0.015
		-	**PE-CMPs 500mg/Kg**	**0**	-0.0107	0.0723	126	-0.148	1.000
		-	**PE-CMPs 500mg/Kg**	**1**	-0.2160	0.0723	126	-2.989	0.078
		-	**PE-CMPs 500mg/Kg**	**2**	-0.3533	0.0723	126	-4.890	<.001
	1	-	**PE-CMPs 100mg/kg**	**2**	-0.1193	0.0723	126	-1.652	0.774
		-	**PE-CMPs 500mg/Kg**	**0**	0.1267	0.0723	126	1.753	0.712
		-	**PE-CMPs 500mg/Kg**	**1**	-0.0787	0.0723	126	-1.089	0.975
		-	**PE-CMPs 500mg/Kg**	**2**	-0.2160	0.0723	126	-2.989	0.078
	2	-	**PE-CMPs 500mg/Kg**	**0**	0.2460	0.0723	126	3.405	0.024

Post Hoc Comparisons - Treatment ✻ Month

Comparison									
Treatment	**Month**		**Treatment**	**Month**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**PE-CMPs 500mg/Kg**	**1**	0.0407	0.0723	126	0.563	1.000
		-	**PE-CMPs 500mg/Kg**	**2**	-0.0967	0.0723	126	-1.338	0.918
PE-CMPs 500mg/Kg	**0**	-	**PE-CMPs 500mg/Kg**	**1**	-0.2053	0.0723	126	-2.842	0.114
		-	**PE-CMPs 500mg/Kg**	**2**	-0.3427	0.0723	126	-4.742	<.001
	1	-	**PE-CMPs 500mg/Kg**	**2**	-0.1373	0.0723	126	-1.901	0.615

Note. Comparisons are based on estimated marginal means

APPENDIX I. ANOVA on the effects of PE-CMPs on the White Blood Cells (WBC) of African catfish (*C. gariepinus)* for 28 days of feeding trials

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
0	**Control**	-	**0**	**PE-CMPs (100 mg/kg)**	-1.32e+7	4.93e+7	30.0	-0.2687	1.000
		-	**0**	**PE-CMPs (500 mg/kg)**	-1.12e+7	4.93e+7	30.0	-0.2271	1.000
		-	**1**	**Control**	-1.45e+7	4.93e+7	30.0	-0.2940	1.000
		-	**1**	**PE-CMPs (100 mg/kg)**	-3.00e+7	4.93e+7	30.0	-0.6076	1.000
		-	**1**	**PE-CMPs (500 mg/kg)**	-3.19e+7	4.93e+7	30.0	-0.6475	1.000
		-	**2**	**Control**	-1.45e+7	4.93e+7	30.0	-0.2937	1.000
		-	**2**	**PE-CMPs (100 mg/kg)**	-5.39e+7	4.93e+7	30.0	-1.0929	0.998
		-	**2**	**PE-CMPs (500 mg/kg)**	-6.28e+7	4.93e+7	30.0	-1.2724	0.992
		-	**3**	**Control**	-5.20e+7	4.93e+7	30.0	-1.0541	0.999

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**3**	**PE-CMPs (100 mg/kg)**	-8.19e+7	4.93e+7	30.0	-1.6604	0.929
		-	**3**	**PE-CMPs (500 mg/kg)**	-1.03e+8	4.93e+7	30.0	-2.0808	0.737
		-	**4**	**Control**	-5.83e+7	4.93e+7	30.0	-1.1822	0.996
		-	**4**	**PE-CMPs (100 mg/kg)**	-1.17e+8	4.93e+7	30.0	-2.3792	0.546
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.78e+8	4.93e+7	30.0	-3.5996	0.061
	PE-CMPs (100 mg/kg)	-	**0**	**PE-CMPs (500 mg/kg)**	2.05e+6	4.93e+7	30.0	0.0416	1.000
		-	**1**	**Control**	-1.25e+6	4.93e+7	30.0	-0.0253	1.000
		-	**1**	**PE-CMPs (100 mg/kg)**	-1.67e+7	4.93e+7	30.0	-0.3390	1.000
		-	**1**	**PE-CMPs (500 mg/kg)**	-1.87e+7	4.93e+7	30.0	-0.3788	1.000
		-	**2**	**Control**	-1.23e+6	4.93e+7	30.0	-0.0250	1.000
		-	**2**	**PE-CMPs (100 mg/kg)**	-4.07e+7	4.93e+7	30.0	-0.8243	1.000
		-	**2**	**PE-CMPs (500 mg/kg)**	-4.95e+7	4.93e+7	30.0	-1.0037	0.999
		-	**3**	**Control**	-3.87e+7	4.93e+7	30.0	-0.7854	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-6.86e+7	4.93e+7	30.0	-1.3917	0.982
		-	**3**	**PE-CMPs (500 mg/kg)**	-8.94e+7	4.93e+7	30.0	-1.8121	0.875
		-	**4**	**Control**	-4.51e+7	4.93e+7	30.0	-0.9135	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-1.04e+8	4.93e+7	30.0	-2.1105	0.719

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.64e+8	4.93e+7	30.0	-3.3309	0.109
	PE-CMPs (500 mg/kg)	-	**1**	**Control**	-3.30e+6	4.93e+7	30.0	-0.0669	1.000
		-	**1**	**PE-CMPs (100 mg/kg)**	-1.88e+7	4.93e+7	30.0	-0.3805	1.000
		-	**1**	**PE-CMPs (500 mg/kg)**	-2.07e+7	4.93e+7	30.0	-0.4203	1.000
		-	**2**	**Control**	-3.28e+6	4.93e+7	30.0	-0.0666	1.000
		-	**2**	**PE-CMPs (100 mg/kg)**	-4.27e+7	4.93e+7	30.0	-0.8658	1.000
		-	**2**	**PE-CMPs (500 mg/kg)**	-5.16e+7	4.93e+7	30.0	-1.0453	0.999
		-	**3**	**Control**	-4.08e+7	4.93e+7	30.0	-0.8270	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-7.07e+7	4.93e+7	30.0	-1.4333	0.977
		-	**3**	**PE-CMPs (500 mg/kg)**	-9.14e+7	4.93e+7	30.0	-1.8537	0.857
		-	**4**	**Control**	-4.71e+7	4.93e+7	30.0	-0.9551	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-1.06e+8	4.93e+7	30.0	-2.1521	0.693
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.66e+8	4.93e+7	30.0	-3.3724	0.100
1	**Control**	-	**1**	**PE-CMPs (100 mg/kg)**	-1.55e+7	4.93e+7	30.0	-0.3136	1.000
		-	**1**	**PE-CMPs (500 mg/kg)**	-1.74e+7	4.93e+7	30.0	-0.3534	1.000
		-	**2**	**Control**	16667	4.93e+7	30.0	3.38e-4	1.000
		-	**2**	**PE-CMPs (100 mg/kg)**	-3.94e+7	4.93e+7	30.0	-0.7989	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**2**	**PE-CMPs (500 mg/kg)**	-4.82e+7	4.93e+7	30.0	-0.9784	0.999
		-	**3**	**Control**	-3.75e+7	4.93e+7	30.0	-0.7601	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-6.74e+7	4.93e+7	30.0	-1.3664	0.985
		-	**3**	**PE-CMPs (500 mg/kg)**	-8.81e+7	4.93e+7	30.0	-1.7868	0.886
		-	**4**	**Control**	-4.38e+7	4.93e+7	30.0	-0.8881	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-1.03e+8	4.93e+7	30.0	-2.0852	0.734
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.63e+8	4.93e+7	30.0	-3.3055	0.115
	PE-CMPs (100 mg/kg)	-	**1**	**PE-CMPs (500 mg/kg)**	-1.96e+6	4.93e+7	30.0	-0.0398	1.000
		-	**2**	**Control**	1.55e+7	4.93e+7	30.0	0.3140	1.000
		-	**2**	**PE-CMPs (100 mg/kg)**	-2.39e+7	4.93e+7	30.0	-0.4853	1.000
		-	**2**	**PE-CMPs (500 mg/kg)**	-3.28e+7	4.93e+7	30.0	-0.6648	1.000
		-	**3**	**Control**	-2.20e+7	4.93e+7	30.0	-0.4464	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-5.19e+7	4.93e+7	30.0	-1.0527	0.999
		-	**3**	**PE-CMPs (500 mg/kg)**	-7.26e+7	4.93e+7	30.0	-1.4731	0.971
		-	**4**	**Control**	-2.83e+7	4.93e+7	30.0	-0.5745	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-8.74e+7	4.93e+7	30.0	-1.7716	0.892
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.48e+8	4.93e+7	30.0	-2.9919	0.214
		-	**2**	**Control**	1.74e+7	4.93e+7	30.0	0.3538	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**2**	**PE-CMPs (100 mg/kg)**	-2.20e+7	4.93e+7	30.0	-0.4455	1.000
		-	**2**	**PE-CMPs (500 mg/kg)**	-3.08e+7	4.93e+7	30.0	-0.6249	1.000
		-	**3**	**Control**	-2.01e+7	4.93e+7	30.0	-0.4066	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-5.00e+7	4.93e+7	30.0	-1.0129	0.999
	PE-CMPs (500 mg/kg)	-	**3**	**PE-CMPs (500 mg/kg)**	-7.07e+7	4.93e+7	30.0	-1.4333	0.977
		-	**4**	**Control**	-2.64e+7	4.93e+7	30.0	-0.5347	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-8.54e+7	4.93e+7	30.0	-1.7317	0.906
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.46e+8	4.93e+7	30.0	-2.9521	0.230
2	**Control**	-	**2**	**PE-CMPs (100 mg/kg)**	-3.94e+7	4.93e+7	30.0	-0.7993	1.000
		-	**2**	**PE-CMPs (500 mg/kg)**	-4.83e+7	4.93e+7	30.0	-0.9787	0.999
		-	**3**	**Control**	-3.75e+7	4.93e+7	30.0	-0.7604	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-6.74e+7	4.93e+7	30.0	-1.3667	0.984
		-	**3**	**PE-CMPs (500 mg/kg)**	-8.81e+7	4.93e+7	30.0	-1.7871	0.885
		-	**4**	**Control**	-4.38e+7	4.93e+7	30.0	-0.8885	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-1.03e+8	4.93e+7	30.0	-2.0855	0.734
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.63e+8	4.93e+7	30.0	-3.3059	0.115
	PE-CMPs (100 mg/kg)	-	**2**	**PE-CMPs (500 mg/kg)**	-8.85e+6	4.93e+7	30.0	-0.1795	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	p_{tukey}
		-	**3**	**Control**	1.92e+6	4.93e+7	30.0	0.0389	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-2.80e+7	4.93e+7	30.0	-0.5674	1.000
		-	**3**	**PE-CMPs (500 mg/kg)**	-4.87e+7	4.93e+7	30.0	-0.9878	0.999
		-	**4**	**Control**	-4.40e+6	4.93e+7	30.0	-0.0892	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-6.34e+7	4.93e+7	30.0	-1.2863	0.991
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.24e+8	4.93e+7	30.0	-2.5066	0.465
	PE-CMPs (500 mg/kg)	-	**3**	**Control**	1.08e+7	4.93e+7	30.0	0.2183	1.000
		-	**3**	**PE-CMPs (100 mg/kg)**	-1.91e+7	4.93e+7	30.0	-0.3880	1.000
		-	**3**	**PE-CMPs (500 mg/kg)**	-3.99e+7	4.93e+7	30.0	-0.8084	1.000
		-	**4**	**Control**	4.45e+6	4.93e+7	30.0	0.0902	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-5.46e+7	4.93e+7	30.0	-1.1068	0.998
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.15e+8	4.93e+7	30.0	-2.3272	0.580
3	**Control**	-	**3**	**PE-CMPs (100 mg/kg)**	-2.99e+7	4.93e+7	30.0	-0.6063	1.000
		-	**3**	**PE-CMPs (500 mg/kg)**	-5.06e+7	4.93e+7	30.0	-1.0267	0.999
		-	**4**	**Control**	-6.32e+6	4.93e+7	30.0	-0.1281	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-6.53e+7	4.93e+7	30.0	-1.3251	0.988
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.26e+8	4.93e+7	30.0	-2.5455	0.441
	PE-CMPs (100 mg/kg)	-	**3**	**PE-CMPs (500 mg/kg)**	-2.07e+7	4.93e+7	30.0	-0.4204	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	**4**	**Control**	2.36e+7	4.93e+7	30.0	0.4782	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-3.54e+7	4.93e+7	30.0	-0.7188	1.000
		-	**4**	**PE-CMPs (500 mg/kg)**	-9.56e+7	4.93e+7	30.0	-1.9392	0.816
	PE-CMPs (500 mg/kg)	-	**4**	**Control**	4.43e+7	4.93e+7	30.0	0.8986	1.000
		-	**4**	**PE-CMPs (100 mg/kg)**	-1.47e+7	4.93e+7	30.0	-0.2984	1.000
		-	**4**	**PE-CMPs (500 mg/kg)**	-7.49e+7	4.93e+7	30.0	-1.5188	0.963
4	**Control**	-	**4**	**PE-CMPs (100 mg/kg)**	-5.90e+7	4.93e+7	30.0	-1.1970	0.995
		-	**4**	**PE-CMPs (500 mg/kg)**	-1.19e+8	4.93e+7	30.0	-2.4174	0.522
	PE-CMPs (100 mg/kg)	-	**4**	**PE-CMPs (500 mg/kg)**	-6.02e+7	4.93e+7	30.0	-1.2204	0.994

Note. Comparisons are based on estimated marginal means

APPENDIX J. ANOVA on the effects of PE-CMPs on the Red Blood Cells (WBC) of African catfish (*C. gariepinus*) for 28 days of feeding trials.

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
0	**Control**	-	**0**	**PE-CMPs (100 mg/kg)**	**-1.83e+8**	**7.29e+8**	**30.0**	**-0.2513**	**1.000**
		-	**0**	**PE-CMPs (500 mg/kg)**	**-2.45e+8**	**7.29e+8**	**30.0**	**-0.3363**	**1.000**
		-	**1**	**Control**	**-2.17e+8**	**7.29e+8**	**30.0**	**-0.2970**	**1.000**

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	1	PE-CMPs (100 mg/kg)	-5.13e+8	7.29e+8	30.0	-0.7037	1.000
		-	1	PE-CMPs (500 mg/kg)	-6.47e+8	7.29e+8	30.0	-0.8865	1.000
		-	2	Control	-5.23e+8	7.29e+8	30.0	-0.7175	1.000
		-	2	PE-CMPs (100 mg/kg)	-6.03e+8	7.29e+8	30.0	-0.8271	1.000
		-	2	PE-CMPs (500 mg/kg)	-7.57e+8	7.29e+8	30.0	-1.0373	0.999
		-	3	Control	-9.00e+8	7.29e+8	30.0	-1.2338	0.994
		-	3	PE-CMPs (100 mg/kg)	-1.00e+9	7.29e+8	30.0	-1.3755	0.984
		-	3	PE-CMPs (500 mg/kg)	-1.29e+9	7.29e+8	30.0	-1.7731	0.891
		-	4	Control	-1.19e+9	7.29e+8	30.0	-1.6268	0.939
		-	4	PE-CMPs (100 mg/kg)	-1.45e+9	7.29e+8	30.0	-1.9833	0.792
		-	4	PE-CMPs (500 mg/kg)	-1.78e+9	7.29e+8	30.0	-2.4357	0.510
	PE-CMPs (100 mg/kg)	-	0	PE-CMPs (500 mg/kg)	-6.20e+7	7.29e+8	30.0	-0.0850	1.000
		-	1	Control	-3.33e+7	7.29e+8	30.0	-0.0457	1.000
		-	1	PE-CMPs (100 mg/kg)	-3.30e+8	7.29e+8	30.0	-0.4524	1.000
		-	1	PE-CMPs (500 mg/kg)	-4.63e+8	7.29e+8	30.0	-0.6352	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	2	Control	-3.40e+8	7.29e+8	30.0	-0.4661	1.000
		-	2	PE-CMPs (100 mg/kg)	-4.20e+8	7.29e+8	30.0	-0.5758	1.000
		-	2	PE-CMPs (500 mg/kg)	-5.73e+8	7.29e+8	30.0	-0.7860	1.000
		-	3	Control	-7.17e+8	7.29e+8	30.0	-0.9825	0.999
		-	3	PE-CMPs (100 mg/kg)	-8.20e+8	7.29e+8	30.0	-1.1242	0.997
		-	3	PE-CMPs (500 mg/kg)	-1.11e+9	7.29e+8	30.0	-1.5217	0.963
		-	4	Control	-1.00e+9	7.29e+8	30.0	-1.3755	0.984
		-	4	PE-CMPs (100 mg/kg)	-1.26e+9	7.29e+8	30.0	-1.7319	0.906
		-	4	PE-CMPs (500 mg/kg)	-1.59e+9	7.29e+8	30.0	-2.1844	0.673
	PE-CMPs (500 mg/kg)	-	1	Control	2.87e+7	7.29e+8	30.0	0.0393	1.000
		-	1	PE-CMPs (100 mg/kg)	-2.68e+8	7.29e+8	30.0	-0.3674	1.000
		-	1	PE-CMPs (500 mg/kg)	-4.01e+8	7.29e+8	30.0	-0.5502	1.000
		-	2	Control	-2.78e+8	7.29e+8	30.0	-0.3811	1.000
		-	2	PE-CMPs (100 mg/kg)	-3.58e+8	7.29e+8	30.0	-0.4908	1.000
		-	2	PE-CMPs (500 mg/kg)	-5.11e+8	7.29e+8	30.0	-0.7010	1.000
		-	3	Control	-6.55e+8	7.29e+8	30.0	-0.8975	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	3	PE-CMPs (100 mg/kg)	-7.58e+8	7.29e+8	30.0	-1.0392	0.999
		-	3	PE-CMPs (500 mg/kg)	-1.05e+9	7.29e+8	30.0	-1.4367	0.976
		-	4	Control	-9.41e+8	7.29e+8	30.0	-1.2905	0.991
		-	4	PE-CMPs (100 mg/kg)	-1.20e+9	7.29e+8	30.0	-1.6470	0.933
		-	4	PE-CMPs (500 mg/kg)	-1.53e+9	7.29e+8	30.0	-2.0994	0.725
1	Control	-	1	PE-CMPs (100 mg/kg)	-2.97e+8	7.29e+8	30.0	-0.4067	1.000
		-	1	PE-CMPs (500 mg/kg)	-4.30e+8	7.29e+8	30.0	-0.5895	1.000
		-	2	Control	-3.07e+8	7.29e+8	30.0	-0.4204	1.000
		-	2	PE-CMPs (100 mg/kg)	-3.87e+8	7.29e+8	30.0	-0.5301	1.000
		-	2	PE-CMPs (500 mg/kg)	-5.40e+8	7.29e+8	30.0	-0.7403	1.000
		-	3	Control	-6.83e+8	7.29e+8	30.0	-0.9368	1.000
		-	3	PE-CMPs (100 mg/kg)	-7.87e+8	7.29e+8	30.0	-1.0785	0.998
		-	3	PE-CMPs (500 mg/kg)	-1.08e+9	7.29e+8	30.0	-1.4760	0.971
		-	4	Control	-9.70e+8	7.29e+8	30.0	-1.3298	0.988
		-	4	PE-CMPs (100 mg/kg)	-1.23e+9	7.29e+8	30.0	-1.6863	0.921

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	4	PE-CMPs (500 mg/kg)	-1.56e+9	7.29e+8	30.0	-2.1387	0.701
	PE-CMPs (100 mg/kg)	-	1	PE-CMPs (500 mg/kg)	-1.33e+8	7.29e+8	30.0	-0.1828	1.000
		-	2	Control	-10.00e+6	7.29e+8	30.0	-0.0137	1.000
		-	2	PE-CMPs (100 mg/kg)	-9.00e+7	7.29e+8	30.0	-0.1234	1.000
		-	2	PE-CMPs (500 mg/kg)	-2.43e+8	7.29e+8	30.0	-0.3336	1.000
		-	3	Control	-3.87e+8	7.29e+8	30.0	-0.5301	1.000
		-	3	PE-CMPs (100 mg/kg)	-4.90e+8	7.29e+8	30.0	-0.6718	1.000
		-	3	PE-CMPs (500 mg/kg)	-7.80e+8	7.29e+8	30.0	-1.0693	0.998
		-	4	Control	-6.73e+8	7.29e+8	30.0	-0.9231	1.000
		-	4	PE-CMPs (100 mg/kg)	-9.33e+8	7.29e+8	30.0	-1.2795	0.991
		-	4	PE-CMPs (500 mg/kg)	-1.26e+9	7.29e+8	30.0	-1.7319	0.906
	PE-CMPs (500 mg/kg)	-	2	Control	1.23e+8	7.29e+8	30.0	0.1691	1.000
		-	2	PE-CMPs (100 mg/kg)	4.33e+7	7.29e+8	30.0	0.0594	1.000
		-	2	PE-CMPs (500 mg/kg)	-1.10e+8	7.29e+8	30.0	-0.1508	1.000
		-	3	Control	-2.53e+8	7.29e+8	30.0	-0.3473	1.000
		-	3	PE-CMPs (100 mg/kg)	-3.57e+8	7.29e+8	30.0	-0.4890	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	3	PE-CMPs (500 mg/kg)	-6.47e+8	7.29e+8	30.0	-0.8865	1.000
		-	4	Control	-5.40e+8	7.29e+8	30.0	-0.7403	1.000
		-	4	PE-CMPs (100 mg/kg)	-8.00e+8	7.29e+8	30.0	-1.0967	0.998
		-	4	PE-CMPs (500 mg/kg)	-1.13e+9	7.29e+8	30.0	-1.5492	0.957
2	Control	-	2	PE-CMPs (100 mg/kg)	-8.00e+7	7.29e+8	30.0	-0.1097	1.000
		-	2	PE-CMPs (500 mg/kg)	-2.33e+8	7.29e+8	30.0	-0.3199	1.000
		-	3	Control	-3.77e+8	7.29e+8	30.0	-0.5164	1.000
		-	3	PE-CMPs (100 mg/kg)	-4.80e+8	7.29e+8	30.0	-0.6580	1.000
		-	3	PE-CMPs (500 mg/kg)	-7.70e+8	7.29e+8	30.0	-1.0556	0.999
		-	4	Control	-6.63e+8	7.29e+8	30.0	-0.9094	1.000
		-	4	PE-CMPs (100 mg/kg)	-9.23e+8	7.29e+8	30.0	-1.2658	0.992
		-	4	PE-CMPs (500 mg/kg)	-1.25e+9	7.29e+8	30.0	-1.7182	0.911
	PE-CMPs (100 mg/kg)	-	2	PE-CMPs (500 mg/kg)	-1.53e+8	7.29e+8	30.0	-0.2102	1.000
		-	3	Control	-2.97e+8	7.29e+8	30.0	-0.4067	1.000
		-	3	PE-CMPs (100 mg/kg)	-4.00e+8	7.29e+8	30.0	-0.5484	1.000

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	3	PE-CMPs (500 mg/kg)	-6.90e+8	7.29e+8	30.0	-0.9459	1.000
		-	4	Control	-5.83e+8	7.29e+8	30.0	-0.7997	1.000
		-	4	PE-CMPs (100 mg/kg)	-8.43e+8	7.29e+8	30.0	-1.1562	0.997
		-	4	PE-CMPs (500 mg/kg)	-1.17e+9	7.29e+8	30.0	-1.6086	0.944
	PE-CMPs (500 mg/kg)	-	3	Control	-1.43e+8	7.29e+8	30.0	-0.1965	1.000
		-	3	PE-CMPs (100 mg/kg)	-2.47e+8	7.29e+8	30.0	-0.3382	1.000
		-	3	PE-CMPs (500 mg/kg)	-5.37e+8	7.29e+8	30.0	-0.7357	1.000
		-	4	Control	-4.30e+8	7.29e+8	30.0	-0.5895	1.000
		-	4	PE-CMPs (100 mg/kg)	-6.90e+8	7.29e+8	30.0	-0.9459	1.000
		-	4	PE-CMPs (500 mg/kg)	-1.02e+9	7.29e+8	30.0	-1.3984	0.981
3	Control	-	3	PE-CMPs (100 mg/kg)	-1.03e+8	7.29e+8	30.0	-0.1417	1.000
		-	3	PE-CMPs (500 mg/kg)	-3.93e+8	7.29e+8	30.0	-0.5392	1.000
		-	4	Control	-2.87e+8	7.29e+8	30.0	-0.3930	1.000
		-	4	PE-CMPs (100 mg/kg)	-5.47e+8	7.29e+8	30.0	-0.7494	1.000
		-	4	PE-CMPs (500 mg/kg)	-8.77e+8	7.29e+8	30.0	-1.2019	0.995

Post Hoc Comparisons - Culture Period (Weeks Post-Feeding) ✻ Treatments

Comparison									
Culture Period (Weeks Post-Feeding)	**Treatments**		**Culture Period (Weeks Post-Feeding)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
	PE-CMPs (100 mg/kg)	-	**3**	**PE-CMPs (500 mg/kg)**	**-2.90e+8**	**7.29e+8**	**30.0**	**-0.3976**	**1.000**
		-	**4**	**Control**	**-1.83e+8**	**7.29e+8**	**30.0**	**-0.2513**	**1.000**
		-	**4**	**PE-CMPs (100 mg/kg)**	**-4.43e+8**	**7.29e+8**	**30.0**	**-0.6078**	**1.000**
		-	**4**	**PE-CMPs (500 mg/kg)**	**-7.73e+8**	**7.29e+8**	**30.0**	**-1.0602**	**0.999**
	PE-CMPs (500 mg/kg)	-	**4**	**Control**	**1.07e+8**	**7.29e+8**	**30.0**	**0.1462**	**1.000**
		-	**4**	**PE-CMPs (100 mg/kg)**	**-1.53e+8**	**7.29e+8**	**30.0**	**-0.2102**	**1.000**
		-	**4**	**PE-CMPs (500 mg/kg)**	**-4.83e+8**	**7.29e+8**	**30.0**	**-0.6626**	**1.000**
4	**Control**	-	**4**	**PE-CMPs (100 mg/kg)**	**-2.60e+8**	**7.29e+8**	**30.0**	**-0.3564**	**1.000**
		-	**4**	**PE-CMPs (500 mg/kg)**	**-5.90e+8**	**7.29e+8**	**30.0**	**-0.8089**	**1.000**
	PE-CMPs (100 mg/kg)	-	**4**	**PE-CMPs (500 mg/kg)**	**-3.30e+8**	**7.29e+8**	**30.0**	**-0.4524**	**1.000**

Note. Comparisons are based on estimated marginal means

APPENDIX K. ANOVA on the Effects of PE-CMPs on the Red Blood Cells (RBC) of African Catfish (*C. gariepinus*) for 60 days feeding trials.

Post Hoc Comparisons - Culture Period (Days) ✻ Treatments

Comparison									
Culture Period (Days)	**Treatments**		**Culture Period (Days)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
0	**Control**	-	**0**	**PE-CMPs (100 mg/kg)**	**-1.83e+8**	**3.90e+8**	**12.0**	**-0.470**	**0.996**
		-	**0**	**PE-CMPs (500 mg/kg)**	**-2.45e+8**	**3.90e+8**	**12.0**	**-0.629**	**0.986**

Post Hoc Comparisons - Culture Period (Days) ✻ Treatments

Comparison									
Culture Period (Days)	**Treatments**		**Culture Period (Days)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	60	Control	-1.07e+9	3.90e+8	12.0	-2.743	0.137
		-	60	PE-CMPs (100 mg/kg)	-1.71e+9	3.90e+8	12.0	-4.375	0.009
		-	60	PE-CMPs (500 mg/kg)	-2.16e+9	3.90e+8	12.0	-5.528	0.001
	PE-CMPs (100 mg/kg)	-	0	PE-CMPs (500 mg/kg)	-6.20e+7	3.90e+8	12.0	-0.159	1.000
		-	60	Control	-8.87e+8	3.90e+8	12.0	-2.273	0.276
		-	60	PE-CMPs (100 mg/kg)	-1.52e+9	3.90e+8	12.0	-3.905	0.020
		-	60	PE-CMPs (500 mg/kg)	-1.97e+9	3.90e+8	12.0	-5.058	0.003
	PE-CMPs (500 mg/kg)	-	60	Control	-8.25e+8	3.90e+8	12.0	-2.114	0.342
		-	60	PE-CMPs (100 mg/kg)	-1.46e+9	3.90e+8	12.0	-3.746	0.026
		-	60	PE-CMPs (500 mg/kg)	-1.91e+9	3.90e+8	12.0	-4.900	0.004
60	Control	-	60	PE-CMPs (100 mg/kg)	-6.37e+8	3.90e+8	12.0	-1.632	0.595
		-	60	PE-CMPs (500 mg/kg)	-1.09e+9	3.90e+8	12.0	-2.786	0.128
	PE-CMPs (100 mg/kg)	-	60	PE-CMPs (500 mg/kg)	-4.50e+8	3.90e+8	12.0	-1.154	0.850

APPENDIX M. ANOVA on the Effects of PE-CMPs on the White Blood Cells (WBC) of African Catfish (*C. gariepinus*) for 60 days feeding trials.

Post Hoc Comparisons - Culture Period (Days) ✻ Treatments

Comparison									
Culture Period (Days)	**Treatments**		**Culture Period (Days)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
0	Control	-	0	PE-CMPs (100 mg/kg)	-1.32e+7	3.21e+7	12.0	-0.41222	0.998
		-	0	PE-CMPs (500 mg/kg)	-2.27e+7	3.21e+7	12.0	-0.70674	0.977
		-	60	Control	-3.60e+7	3.21e+7	12.0	-1.11897	0.865
		-	60	PE-CMPs (100 mg/kg)	-7.01e+7	3.21e+7	12.0	-2.17986	0.313

Post Hoc Comparisons - Culture Period (Days) ✻ Treatments

Comparison									
Culture Period (Days)	**Treatments**		**Culture Period (Days)**	**Treatments**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**
		-	60	PE-CMPs (500 mg/kg)	-7.03e+7	3.21e+7	12.0	-2.18712	0.310
	PE-CMPs (100 mg/kg)	-	0	PE-CMPs (500 mg/kg)	-9.47e+6	3.21e+7	12.0	-0.29452	1.000
		-	60	Control	-2.27e+7	3.21e+7	12.0	-0.70674	0.977
		-	60	PE-CMPs (100 mg/kg)	-5.68e+7	3.21e+7	12.0	-1.76764	0.518
		-	60	PE-CMPs (500 mg/kg)	-5.70e+7	3.21e+7	12.0	-1.77490	0.514
	PE-CMPs (500 mg/kg)	-	60	Control	-1.32e+7	3.21e+7	12.0	-0.41222	0.998
		-	60	PE-CMPs (100 mg/kg)	-4.73e+7	3.21e+7	12.0	-1.47312	0.686
		-	60	PE-CMPs (500 mg/kg)	-4.76e+7	3.21e+7	12.0	-1.48038	0.682
60	Control	-	60	PE-CMPs (100 mg/kg)	-3.41e+7	3.21e+7	12.0	-1.06089	0.888
		-	60	PE-CMPs (500 mg/kg)	-3.43e+7	3.21e+7	12.0	-1.06815	0.885
	PE-CMPs (100 mg/kg)	-	60	PE-CMPs (500 mg/kg)	-233333	3.21e+7	12.0	-0.00726	1.000

Note. Comparisons are based on estimated marginal means

PLATES

Plate 1: Start of Feeding Trial

Plate 2: Data collection on the weight and length of African catfish

Plate 3: Data collection on Water Quality Parameters

Plate 4: Blood collection of African catfish

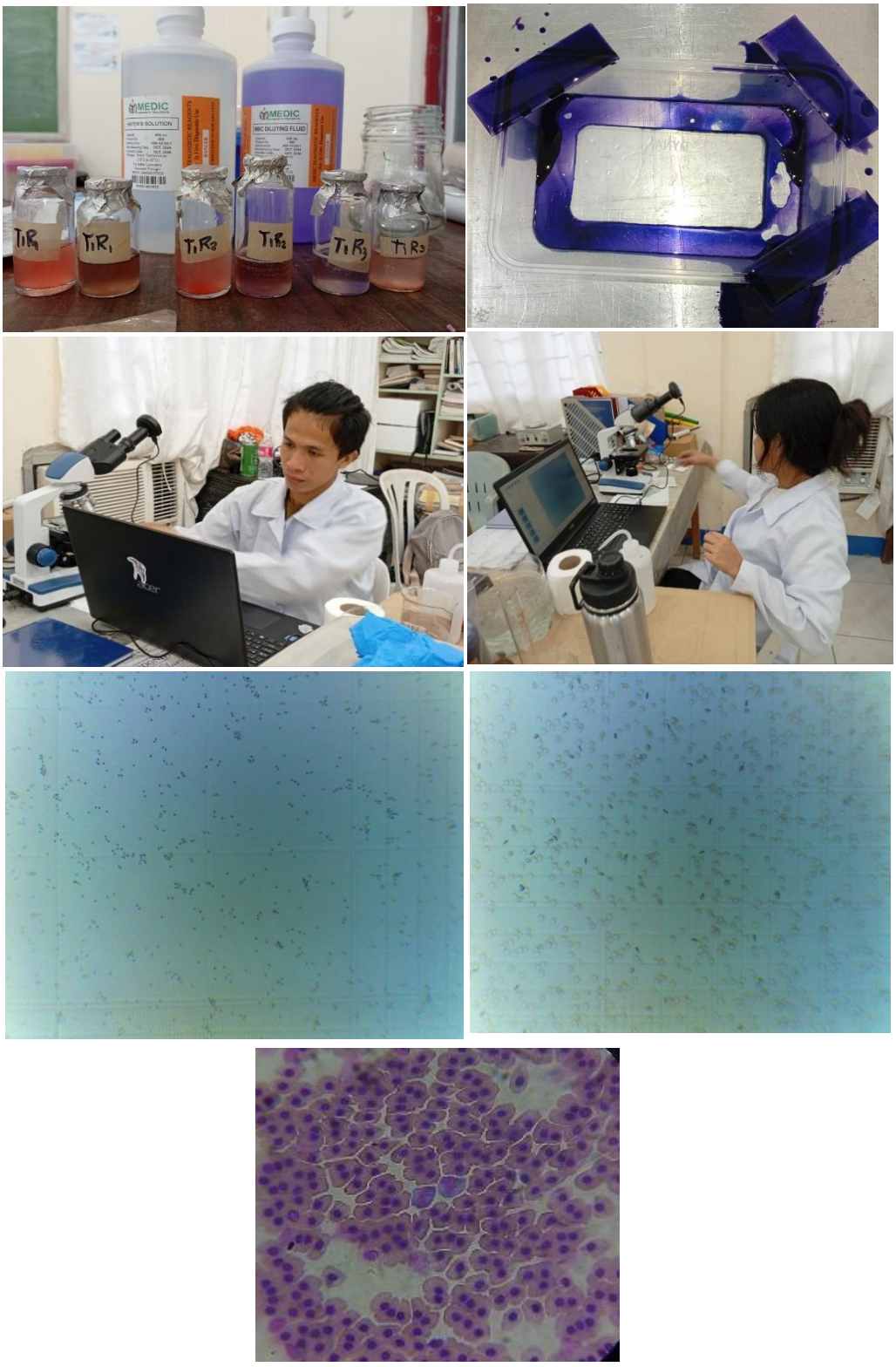

Plate 5: Preparation and analysis of blood samples for the red blood cells and white blood cells under microscopy.

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CURRICULUM VITAE

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