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INFLUENCE OF BITTER LEAF EXTRACT ON NILE TILAPIA GROWTH, HEMATOLOGY, AND HEAT STRESS RESPONSE

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INFLUENCE OF BITTER LEAF (*VERNONIA AMYGDALINA*) EXTRACT ON NILE TILAPIA, *OREOCHROMIS NILOTICUS* GROWTH, HEMATOLOGY, AND HEAT STRESS RESPONSE.

BY

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JUNE, 2021

DECLARATION

This thesis is the result of my own original work, and no part of it has ever been submitted for another degree at this University or elsewhere:

Candidate's Signature:…….. Date:……

Samuel Opoku Dandi

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Herbal supplements are suitable for improving growth, health of fish and increasing tolerance to environmental perturbations. Cost of formulated feed, disease outbreak and hypoxic related stress issues are major challenges confronting tilapia production. In this study, the effects of the bitter leaf (*Vernonia amygdalina)* extracts (BLE) on growth, hematology, liver health and resistance to heat stress were investigated in Nile tilapia (*Oreochromis niloticus*). Experimental fish of average weight 30 ± 1 g were puts into 4 groups each for a respective treatment and fed with diets to include a control (0 % BLE), and BLE supplemented diets at 1% BLE, 3% BLE and 5% BLE for 8 weeks in circular concreate tanks. Upon termination of the experiment, growth performance (i.e. mean weight gain (WG), viscerosomatic index (VSI), hepasomatic index (HSI) and feed conversion ratio (FCR) amongst others), hematology (i.e. red blood cell, white blood cells, leukocyte, haemoglobin, and hematocrit amongst others), liver enzyme activity (i.e. aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase), plasma chemistry to include total protein (TP) and albumin (ALB amongst others) and on hematological parameters also after heat stress treatments were sampled using appropriate tools and methods. One-way ANOVA and Duncan multiple range test were used to determine differences in growth, hematological, stress response, and toxic parameters using IBM SPSS at ($P<0.05$). The end results indicated that tilapia fed diets enriched with BLE in the range of 1% - 5% BLE were superior in the growth indices measured compared to the control group with 1% BLE exhibiting the best effects. Hematology and plasma chemistry showed similar results with 1% BLE presenting the best of results. However, it is worth nothing that increasing level of BLE showed a decreasing trend yet significantly high in comparism to the control group. Increased levels of AST, ALT and ALP were observed in the first 4 weeks but were significantly lower at 8 weeks in all BLE groups compared to the control suggesting initial tolerance and hepato-protectiveness of BLE in fish. The hematological indices investigated after exposure to heat stress test revealed that fish fed BLE supplemented diet could help increase tolerance to heat stress than those fed the control diet. In summation, the results suggest that BLE inclusion particularly at 1% BLE has the potential to improve growth and health of tilapia. Palatability and digestibility

test is recommended in future trials to help understand and improve feed acceptance of fish fed with BLE supplements.

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DEDICATION

This thesis is dedicated to my late father, Mr. Ernest Kwabena Sule Dandi, my mother, Comfort Abena Sule for their constant prayers.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

LIST OF ABBREVIATIONS

FAO ……Food and Agriculture Organisation

IPCC……International Panel on Climate Change

BLE…….. Bitter Leaf Extract

NEPAD……New Partnership for Africa Development

GDP…….. Gross Domestic Product

BOG…….. Bank of Ghana

MoFAD……Ministry of Fisheries and Aquaculture Development

FCA……. Financial Conduct Authority

SPADA……. Special Program for Aquaculture Development in Africa

WBC…….. White blood cell

RBC……Red blood cell

HGB……Hemaglobin

HCT……Hematocrite

LYM……Lymphocyte

NUE……. Nuetrophiles

MON……Monophiles

ESO…….. Esinophiles

BAS…….. Basophiles

TP……Total protein

ALB……. Albumin

AST……. Aspartate aminotransferase

ALT……Alanine transaminase

ALP……Alkaline phosphate

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Nile tilapia, *Oreochromis niloticus* (generally known as tilapia) is a major freshwater aquaculture species (Foyer & Noctor, 2005; Shitote et al., 2013; Emeka, 2014). Tilapia farming has always been successful and is mainly accredited to its ease of culture and desirable characteristics as a food fish (FAO, 2018; He et al., 2011). Tilapia is the next most significant cultured fish species following carps, and it is also the most widely grown (Mwanja & Nyandat, 2013; Ekelemu, 2017; FAO, 2018). Aquaculture, particularly tilapia culture, has the capacity to exhibit a key functional role in Ghana's and the world's fight against malnutrition, food insecurity, and poverty (Bene & Hecks, 2005;Subasinghe et al., 2009; FAO, 2018).

Tilapia can reproduce and acclimatize in a variety of environmental conditions and can withstand stress from handling (Siddik et al., 2014; Emeka, 2014). Today, tilapia has risen to prominence in aquaculture and is sometimes referred to as "aquatic chicken." Its consumption rate has risen all around the world (Fitzsimmons, 2005; Akpabio & Inyang, 2007; Okomoda, 2016). Global production in recent times of farmed tilapia has steadily expanded. (Ahsan et al., 2013). Tilapias, which originated from Africa and the Middle East, have been farmed in more than 90 nations for fisheries and aquaculture production (Subasinghe et al., 2009) in the twentieth century. Tilapia is now cultivated commercially in nearly ten nations, making it one of the world's most important food fishes (FAO, 2018). Among the resource poor, particularly in rural areas, due to its ease of breeding and

farming, low protein demand, and capacity to digest plant protein, tilapia has become the most interested culturing specie (He et al., 2011; Ekelemu, 2017).

1.2 Problem statement

Formulated feed is becoming expensive because majority of the ingredients are imported, and prices are constantly rising. Feed nutrient balance is critical for feed utilization and growth. To maximize its benefits, it is necessary to find a locally cost-effective way of supplementing these commercial feeds (Xie et al., 2008; Abdel-Tawwab et al., 2014; Miao, 2015). Intensification of various technological tilapia farming practices has resulted in farming misconduct such as high stocking density, excessive feeding from unapproved feed sources, and water quality issues (Ansah et al., 2014; Gupta & Acosta, 2016; Frimpong, 2018).

Disease outbreaks have been a major challenge from advanced technological practices to improve tilapia production, affecting the entire tilapia production before harvest (Okomoda, 2016; Udoh et al., 2017). Thus, disease is the most important factor among others that contribute significantly to economic losses in tilapia farming, either by affecting the quality of the flesh, morbidity and mortality, reducing growth rate, reducing trade, affecting general profit margin with no effect on the immunity of fishes (Ofori et al., 2009). Disease transmission is relatively easier in tilapia farming due to high density stocking and intensification of feeding in water-stressed areas, distribution of disease-causing organisms through common water sources in farms and ponds, and the supply of fish fry, fingerlings, and transporting of fish broodstock from other fish farms without adequate precaution (Ainoo-ansah, 2019). Agbede & Aletor, 2003 reported that under intensive culture, disease problems could result in financial losses.

Increasing temperature and dramatic changes in climatic conditions have resulted into increased concentrations of greenhouse gases and carbon dioxide in the atmosphere (Olusola et al., 2019). Moreover, accumulation of rising mean of annual temperatures, the frequency, duration, and severity of exceptionally hot periods are putting a strain on our aquatic ecosystems and their inhabitants (Van Rijn & Reina, 2010).

Nonetheless, hypoxic stress conditions associated with low levels of DO have been found to decrease feed intake, cause stress to fish physiology, inhibit fish immune response, and decrease their resistance to diseases in tilapia, and sometimes cause death because the acclimatory capacity of aquatic organisms is significantly exceeded under high temperatures (Van Rijn & Reina, 2010; Olusola et al., 2019).

1.3 Significance of study

Over the years, researchers have made significant efforts to reduce production costs resulting from cost of commercial feed, improving tilapia immunity, treating disease, and other climatic influences on tilapia culture around the world (Soediono, 1989; Kadiri & Olawoye, 2016).

Medicinal plants have become significant sources of therapeutic agents for use in the promotion of fish growth, health and resistance to stress and disease since they serve as un-conversional and less expensive sources for treatment and greater precision with no toxicity (Syahidah et al., 2015; Udoh et al., 2017; Abarike et al., 2018; Dorothy et al., 2018; Xu et al., 2020).

The presence of active compounds in herbal plants such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids, and essential oils has been linked to a number of its medicinal functions (Farombi & Owoeye, 2011; Dienye, 2017).

Vernonia amygdalina is a herbal plant (Achuba, 2018a) that holds a promise in supporting fish production by facilitating fish growth, resistance to heat and disease. Because of its bitter taste, *Vernonia amygdalina* is commonly known as bitter leaf (Egharevba, 2014; Sayed & Moneeb, 2015).

Bitter leaf has been reported to contain bioactive substances such as saponins and flavonoids which play important role in supporting the nutritional needs of man and animals, It has been purported to contribute in boosting the immune system of animals (Olusola et al., 2013; Awad & Awaad, 2017).

V. amygdalina phytochemical analysis revealed the presence of bioactive compounds such as sesquiterpene lactones, terpenoids, flavonoids such as luteolin, and luteolin 7-O-glucosides, which serve to improve the nutritional status and immunity of various farmed fishes (Dienye, 2017; Olowolafe & Mo, 2018). It has properties like hypoglycemic, anti-diabetic and anticholesterol which aids in the treatment of disease in Catfish (Udoh et al., 2017; Olowolafe & Mo, 2018), renders the fish more resistant to infectious diseases, stress and also help to kindle fish growth (Farombi & Owoeye, 2011; Kadiri et al., 2016).

Likewise, the leaf extract has been noted to improve the hematological parameters, and enhanced the fertility of giant African catfish, *Heterobranchus bidorsalis* (Udoh et al., 2017; Olowolafe & Mo, 2018).

Tilapia is the most culturable aquatic organism worldwide and it's been consumed almost by everyone, remedies sort for problems rising from tilapia culture maybe wide but the application of bitter leaf extract supplemented diet seems to be promising in Ghana. Bitter leaf is commonly found in the environs of a Ghanaian community and has a variety of properties that account for it numerous medicinal function, including antioxidant, antibacterial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, appetite enhancer, bile production stimulant, and digestive enzyme activity, to name a few among others (Frimpong & Fynn, 2014; Amenyogbe et al., 2018). A local treatment additive can be more acceptable and easier to be use when discovered than others (Fagbenro et al., 2013; Okomoda, 2016; Dienye, 2017; Anjusha et al., 2019).

This research aimed at evaluating the application of bitter leaf extract supplemented diet on growth, hematology and stress response of tilapia which could serve as reference to develop a promising alternative for improving growth and health of tilapia.

1.4 Research objectives

The objectives of the study were:

1. To assess the effect of bitter leaf extract (BLE) on growth of tilapia (weight gain (WG), specific growth rate (SGR), viscerosomatic index (VSI), hepasomatic index (HSI), and condition factor (CF)).
2. To evaluate the effect of bitter leaf extract (BLE) on hematological parameters namely; white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), and hematocrit (HCT) of tilapia.
3. To analyse the effect of bitter leaf extract (BLE) on Plasma chemistry (Total protein (TP), Albumin (ALB))

4. To determine the effect of bitter leaf extract (BLE) on Liver health (i.e Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphate (ALP)) of tilapia
5. To measure the effect of bitter leaf extract (BLE) on Heat stress response on white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), and hematocrit (HCT) of tilapia.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Global Overview of Tilapia Culture

Production from capture fisheries has plateaued, and its productivity is no longer thought to be capable of meeting the expanding worldwide demand for fisheries products (Subasinghe et al., 2001; Gupta & Acosta, 2016). Aquaculture, particularly tilapia aquaculture, has the potential to play a key role in Africa's fight against food insecurity, malnutrition, and poverty (Bene et al., 2005; Hartley-alcocer, 2007). Tilapia is the common name for several species of cichlid fish found in freshwater streams, ponds, rivers, and lakes, as well as brackish water (Boyd, 2004). Tilapias, once considered an invasive species, are increasingly becoming more important in aquaculture (Hartley-alcocer, 2007). Marketability is due to its aptitude for aquaculture, and consistent market pricing, The world's second most farmed fish is tilapia, with production quadrupling in the last decade (Wang et al., 2016). In tropical and sub-tropical aquaculture, *Oreochromis niloticus*, Nile tilapia, is one of the most significant fish species (FAO, 2018). It is a significant source of animal protein and generates income throughout the world (Gupta & Acosta, 2016). Tilapia can thrive and reproduce in a variety of environments and can withstand stress caused by handling (Siddik et al., 2014; Gupta & Acosta, 2016). Tilapia mono-sex males are well known for their high production potential and reduced management requirements. Today, tilapia is the shining star of aquaculture, widely known as "aquatic chicken," and its consumption is on the rise all over the world (Fitzsimmons, 2005).

In recent years, annual global production of farmed tilapia has steadily expanded (FAO, 2018). Tilapias, which are native to Africa and the Middle East, have been transplanted

into more than 90 nations for aquaculture and fisheries (Gupta & Acosta, 2016) during the 20th century. Currently, a large portion of worldwide tilapia production takes place outside of the fish's natural area (Ofori et al., 2009). Tilapias are currently cultivated commercially in nearly ten nations and have risen to prominence as one of the world's most significant food fish.

After carps, it is the second most important farmed fish species (Cai et al., 2017; FAO, 2018). Tilapias, unlike most other finfish species, are highly resilient fish that can adapt to a variety of culture methods and environmental conditions, including low-density ponds, cage culture systems, raceway systems, and super-intensive culture systems (FAO, 2018; Miao, 2015) . It became a species of interest among the resource poor, particularly in rural areas, due to its ease of breeding and farming, low protein demand, and capacity to digest plant protein (FAO, 2018).

In many Asian countries, this fish is also popular and highly prized (FAO, 2018), like in the case of Philippines and Indonesia, where it has become a national cuisine and a native species to this countries (Costa-Pierce and Rakocy, 1997). This fish caused International Development Agencies to label it "Aquatic Chicken" in the 1970s, and "fish of the 1990s" twenty years later. It is now known as the "Food Fish of the Twenty-First Century" (Costa-Pierce and Rakocy, 2000; Ramnarine, 2005). The largest producers and consumers of tilapia are Asian countries. China is the world's leading producer of Oreochromis niloticus aquaculture, accounting for almost 3.2 million tonnes of total global production (FAO, 2018; El-Sayed, 2006). Tilapias are also grown in Egypt, Indonesia, Thailand, the Philippines, Brazil, Bangladesh, Vietnam, Columbia, and Malaysia, among other places

(El-Sayed, 2006). The Bureau of Fisheries and Aquatic Resources/Freshwater Aquaculture Center (BFAR/FAC) was established in Muoz, Philippines, in 1988, initiated an international collaborative study to improve the genetic performance of farmed Nile tilapia, *Oreochromis niloticus* (Gjedrem et al., 2012).

2.2 Tilapia Culture in Africa

Tilapia, an African and Middle Eastern fish, has risen from obscurity to become one of the world's most productive and widely traded food fish. Tilapia farming is thought to have originated in Egypt over 4,000 years ago in its most primitive form (Kaunda, 2015; Rouhani & Britz, 2004). The first scientifically directed tilapia production was carried out in Kenya in 1924, and it quickly expanded throughout Africa (Shitote et al., 2013). By the late 1940s, a decade later, tilapia had been transplanted and had established itself as a promising farmed species in the far East, and had spread across most of African countries (Adeleke et al., 2020).

Significant advancements in tilapia farming have occurred in Africa during the previous three decades (El-Sayed, 2006). The commodity is the Africa's most important farmed fish, aquaculture species of the twenty-first century, due to rising commercialisation and continued growth of the tilapia business (Shelton, 2002). Around 85 African countries cultivate the fish, and approximately 98 percent of the tilapia produced in these countries is farmed outside of their natural habitats (Shelton, 2002). The primary culture businesses are in the Far East, but they are also farmed in the Caribbean, Latin America, and, more recently, temperate locations where warm water can be artificially obtained (thermal effluents or geothermal springs). The contribution of Africa to global aquaculture production is still negligible (2.7%) (Halwart, 2020).

Despite huge increases in fish production due to larger-scale investments in Egypt, Nigeria, Uganda, and Ghana, there is still a long way to go (Cai et al., 2017; FAO, 2018). From 1995 to 2018, the region's production increased twenty-fold, from 110,200 tonnes to 2,196,000 tonnes, with an annual compound growth rate of 15.55 percent (FAO, 2018; Hamzah et al., 2013). The increase of tilapia production was aided by the establishment and strengthening of the private sector, controlled by both small and medium sized businesses (Halwart, 2020). In addition, commercial growth of large tilapia enterprises has been aided by a blend of increasing public support, expertise, foreign direct investment, aquaculture interest, raising global awareness through the NEPAD Fish for All Summit in 2005, and implementing the FAO Special Program for Aquaculture Development in Africa (SPADA) (Halwart, 2020).

The vast majority of production (99 %) is derived from inland freshwater systems and is dominated by the cultivation of indigenous and prolific species of tilapia and African catfish, with mariculture accounting for only 1% of total production volume, despite being a growing and promising subsector (FAO, 2018). There were innovative systems tilapia production established, such as dugout and cages, as well as improvements to existing production systems (FAO, 2018). On Africa, the aquaculture and tilapia business employ over 6.2 million people, with a substantial number of women working in large-scale commercial farms (Satia, 2016a). In general, research at the region concentrated on species characterization, selective breeding, and the manufacture of low-cost diets in some centers. (Halwart, 2020). In the SPADA-administered target nations, an on-farm participatory research method involving farms modelling and private firms has resulted in

rapid aquaculture technology transfer via farmer-to-farmer routes (Cobbina & Eiriksdottir, 2010).

The top African tilapia producers are Egypt, Nigeria, Uganda, Ghana, Tunisia, Kenya, Zambia, Madagascar, Malawi, and South Africa (Halwart, 2020). Several factors, including capacity building in critical subject areas, embracing good governance, research and development, access to credit facilities, and, most importantly, the promotion of private sector-led aquaculture development, have contributed to this key aquaculture producer's remarkable growth over the last decade (FAO, 2018).

The FAO Global Aquaculture Production Statistics Dataset for 1950-2015, which was released in March 2017, identified a total of 201 nations and territories that have aquaculture-related statistical data. There are now 591 aquatic species represented in the new dataset and Inland freshwater, inland saline water, coastal brackish water, and marine water have all been farmed, with tilapia farming being the most common.

The fast increase rate in aquaculture production in African countries over the last quarter-century is being reflected in freshwater fish output in Sub-Saharan Africa. In 2014, just 550 000 tonnes of fish were grown, accounting for less than 1% of global production. Almost all of this is made up of freshwater fish, the majority of which being tilapia (Machena & Moehl, 2001). The leading producers in the region are Nigeria and Uganda. The development of marine tilapia in Africa is a gloomier story. In 2008, just 2000t of total production was reported, falling to 1000t in 2014. The White Spot Syndrome Virus in tilapia aquaculture was mostly to blame for the decline in output. Since 2011, the sector in Africa has been decimated as a result of this (Cai et al., 2017).

Tilapia has traditionally been grown on a modest scale to meet the subsistence and livelihood needs of families (Jamu & Ayinla, 2003). This is still very much the case in Africa, despite recent growing commercialization of the continent's produce. Improvements in husbandry skills, feed, and production methods have enabled higher levels of productivity in small-scale agriculture (Cai et al., 2017). Various challenges, such as a lack of inputs, limited government backing, and socioeconomic circumstances, have inhibited the commercialization of small-scale tilapia production in Africa. In general, African tilapia culture productivity has lagged behind that of Asia, and demand is outpacing supply as human populations rise (Anderson et al., 2010).

2.3 Tilapia Culture in Ghana

Rivers, lakes, dams, and dugouts abound in Ghana, making aquaculture a viable option across the country (FAO, 2018). With ideal geography and climate, sufficient human resources, an abundance of natural bodies of water, and significant fish demand, the environment and formal conditions are ideal for the production of fish to bridge the deficit from the marine stock (Amenyogbe et al., 2018; FAO, 2018). Ghana's engagement in aquaculture dates back to 1953, when the British colonial authority began building the first ponds as hatcheries to support culture-based fisheries development program (Frimpong & Fynn, 2014) and as a means of broadening the nation's appetite for fish and generating opportunities for employment (Chen et al., 2010; Amoah, 2019).

After-independence in 1957, according to Gropper (2012), the government started building irrigation dams under a program of converting 5% of the infrastructure to supplement fish farms. Nonetheless, recent times, the establishment of numerous cages in Volta Lake increased tilapia production (Amoah, 2019; Atindana et al., 2019).

Participation of Commercial investors in tilapia farming sector had automatically and permanently changed the face of aquaculture in Ghana (McCauley et al., 2003). Without a doubt, tilapia culture is a relatively young business in Ghana; however, its pattern is spreading throughout the country, particularly in the regions of Ashanti, Central, Eastern, Volta, and Western (Frimpong & Fynn, 2014). Many small-scale tilapia farmers employ the semi-intensive and intensive systems to raise tilapia in earthen ponds in the tilapia industry. The majority of farmers also used an extensive culture system for fish culture, which included dams, dugouts, ponds and reservoirs (FAO, 2018).

Commercial tilapia farmers make use of intensive culture systems and provide about 75% of Ghana's total production from aquaculture, despite being a minority (Hiheglo, 2008). Pond culture is the most common tilapia production system in the country's southern and central regions, summing for roughly 98 percent of tilapia farms. It is also predominantly small-scale and semi-intensive (Hiheglo, 2008; FAO, 2018). However, in recent years, the predominant culture strategy for tilapia production has altered, and the vast majority of tilapia cultured are intensely in cages, particularly on Lake Volta (FAO, 2018).

Concrete tanks, clay ponds, and floating cages some of the holding technologies used in tilapia production in Ghana. The majority of farmed tilapia in Ghana comes from cage culture systems, with the remainder coming from ponds (Hiheglo, 2008). Between 2010 and 2016, the cage farming system grew at a rate of roughly 73 percent each year on Lake Volta, making it the fastest-growing business activity. According to statistics, Ghana's first tilapia fish farm was created in 2001 (Appenroth et al., 2018). Most tilapia farms do not have their own hatcheries and instead purchase fingerlings from other hatcheries.

Fingerlings are typically purchased by medium-scale farmers from large-scale farmers and other sources such as Akosombo's Water Research Institute, Aquaculture Research, and Development Centre (WRIARDEC). Typically, they seek technical advice from WRI-ARDEC in Akosombo (Hiheglo, 2008; Cobbina & Eiriksdottir, 2010).

Although bycatch production is significantly higher, the cage system of tilapia farming currently accounts for roughly 2% of farms (FAO, 2018). The Eastern Region, namely the Asuogyaman District, is home to the majority of tilapia cage farms, between Akosombo Dam and Kpong Dam, the majority of small-scale cage farms are located. A number of small to medium-sized cage farms can also be found in these areas such as Kpeve in the Volta Region's South Dayi District, Akuse in the Lower Manya Krobo District and Akrusu in the Upper Manya Krobo District of the Eastern Region (Amenyogbe et al., 2018). The common tilapia culture practice in Northern, Upper East, and Upper West regions of Ghana, extensive or culture-based fisheries are done at irrigation sites, reservoirs, and dams. In Ghana's Lake Volta, the vast majority of commercial tilapia farmers on a large-scale use cage culture method, while a minority or few use earthen ponds and cage systems (Kaunda et al., 2015).

In Ghana, tilapia (*Oreochromis niloticus*) has long been the most popular and preferred fish species for both farmers and consumers. With a current annual production of slightly more than 52,000 tonnes, tilapia species account for more than 80% of the farmed fish catch (Alanis et al., 2005). Wheat bran, maize bran, rice bran, and cereal brans are among the most popular feeds used by tilapia fish farmers in Ghana, especially by many small-scale farmers (Amenyogbe et al., 2018). Commercial floating feed, which is relatively

expensive, is used by only a few farmers. Farmers continue to import commercial feeds into Ghana, despite the establishment of a feed mill in Ghana in 2011, because the mill was unable to meet farmer demand. High level of expenses in aquaculture (tilapia) production in Ghana are primarily as a result of the high cost of fish feed. Feed expenses account for 70% of overall production expenses, and imported feeds are typically 30% more expensive than locally produced feeds (Kaunda et al., 2015).

In Ghana, approximately 135,000 fish farmers operate approximately 699,000 fish ponds and cages (FAO, 2018). In 2013, tilapia production was slightly more than 30,000 metric tonnes of the total cultured fish, with roughly 88 percent of that coming from cages (FAO, 2018). To increase tilapia production, the Ministry of Fisheries and Aquaculture Development has prohibited the import of farmed fish, particularly flash-frozen tilapia (Amenyogbe et al., 2018).

The ministry also established the Ghana National Aquaculture Development Plan (GNADP), which set an ambitious production target of 100,000 metric tonnes of farmed tilapia by the end of 2016, which represents an estimated increase in production by 70,000 metric tonnes (McCauley, 2003). With an estimated cost of $85 million, the program's goal is to improve the practice, direction, and evolution of tilapia and aquaculture in general as a potential business activity. It was developed in partnership with the United Nations Food and Agriculture Organization (FAO), the Ghana National Aquaculture Development Plan (GNADP) (2013), and the National Aquaculture Strategic Framework (2006) (Amenyogbe et al., 2018).

2.4 Challenges facing tilapia culture

Fish infections by parasitic, bacterial, or fungi causing organisms, and viruses are to accountable for significant economic losses and tilapia mortalities in aquaculture farms (Dong et al., 2017; Amoah, 2019). Aside from mortalities, pathogenic circumstances have a significant impact on the food conversion ratio and the post-infection recovered fish's final body weight. Infectious disease-related mortality in aquaculture is common in tilapia throughout the year from June to October, the summer months, resulting in economic losses of around USD 100 million (Dong et al., 2017). The most common pathogens are bacteria in aquaculture farms, accounting for around 80% of fish disease cases (Akpabio & Inyang, 2007; Shaheen et al., 2013).

Infectious disorders triggered by several bacteria strains have also been recorded in Egyptian farms, with higher mortality rates than parasitic infections (Salem et al., 2010; Aly et al., 2013;). Because of a scarcity of a established surveillance program for the monitoring and control of viral infections in fish, there is a scarcity of information concerning viral infections and their transmission (Foyer & Noctor, 2005; He et al., 2011). Furthermore, the usage of antibiotics, chemicals, and other pharmacologically active agents can have negative consequences for consumers' health. Increased tilapia production in aquaculture is hampered by the spread of fish illnesses (FAO, 2018; Emeka, 2014). The collapse of shrimp aquaculture in the 1990s and carp aquaculture in the late 1980s are instances of what might happen if caution is not used (Mwanja & Nyandat, 2013).

Increased demand for tilapia will necessitate additional intensification and development of the culturing system, and special consideration must be given to the health management of cultured tilapia during the design stage (Mwanja & Nyandat, 2013).

Outbreaks of fish illness have a negative impact on total tilapia production (Subasinghe et al., 2001; Bondad Reantaso et al., 2005). In the tropics, where mitigation options are restricted, losses are extremely substantial. (Leung and Bates, 2013). Despite the fact that tilapia culture is booming throughout East Africa (Walakira et al., 2014), The possibility of monetary loss due to fish infections is present already (Akoll and Mwanja, 2012a). Regardless of the low profit margins enjoyed by Ugandan tilapia farmers (Hyuha et al., 2011).

Tilapia farming has the greatest potential to contribute to the nation's fish need (Bates et al., 2012; FAO, 2018). In Uganda and other African countries, outbreaks of aquatic illnesses have resulted in 60 percent mortality rates in hatcheries and grow-out systems (Akoll et al., 2012b). Infectious parasites and bacteria have been found in both private and public tilapia farms, causing negative consequences (Akoll et al., 2012b). As a result, worries about the danger of trans-boundary disease transmission in East Africa cannot be dismissed (Akoll and Mwanja, 2012a).

Under harsh environmental conditions, from farmed tilapia, bacterial pathogens (Flavibacterium sp., Pseudomonas sp., and Aeromonas sp.) were isolated (Ayieko et al., 2010). In wild fish taken from Lake Nyabihoko in the Ntungamo area, parasite transmission to tilapia and catfish was discovered (Nylund et al., 2015). Similarly, hatchery owners continue to struggle with fungal infections, particularly Saprolegnia and Branchiomyces, resulting in large financial losses.

Control tactics used by African tilapia farmers are ineffective and poorly understood, owing to a lack of data that might aid researchers, policymakers, and farmers in developing

control or prevention tactics against the possibility of aquatic diseases (Akoll and Mwanja 2012a). The development of fin fished commercialization in Africa in general is now looking at various options for dealing with infections as manufacturing risk factor that can significantly reduce product marketability.

2.5 Use of medicinal plants in aquaculture

The demand for plant-derived products for medicinal purposes has risen in recent years (Reverter et al., 2017). Aromatic herbs play an important role in primary health care in various nations around the world, particularly in rural areas, and a bigger population in emerging countries uses these traditional resources (Yilmaz et al., 2018). As a result, the utilization of essential oils produced from plants for medical purposes has become a hot topic in scientific research and industrial use. This is owing to oils' various biological actions, which include antibacterial, antioxidant, and anti-inflammatory properties (IARC, 2000; Olusola et al., 2013).

Aquaculture intensification has become a popular strategy in recent years as a way to maximize profits (Thirumal & Laavu, 2017). In both fish and shrimp production systems, high density stocking, water quality difficulties, stress leading in diseases, artificial feeds, and pond water fertilization have become widespread husbandry methods (Alam et al., 2014). Diseases caused by microorganisms (bacteria) have surfaced considerably in aquaculture culture systems as a result of the increase of culture procedures (Egharevba, 2014). To prevent and control the infections, several medications, synthetic compounds, antibiotics, and immunization programs have been used, but only partial success has been obtained (Achuba, 2018b). The use of various chemicals to strengthen or stimulate the immune systems of fish and other culturable organisms has been an alternative strategy

(Kadiri & Olawoye, 2016). Immunostimulants are a type of chemical that boosts the immune system. It has been demonstrated that these chemicals have a significant role in disease management in aquaculture systems (Dorothy et al., 2018).

For thousands of years, medicinal herbs have been used as immunostimulants. Plants used for medicinal purposes are natural and non-harmful substances as an alternative to antibiotics and immune-therapeutics has potential in aquaculture (Syahidah et al., 2015). These plants are gaining popularity around the world because they are easy to prepare, cheap, and have little negative impact on animals and the environment (Syahidah et al., 2015). Various aquatic animals have been examined with a variety of therapeutic plants, herbs, spices, seaweeds, herbal medications, herbal extracted chemicals, traditional Chinese medicines, and commercial plant-derived goods are all examples of plant-derived products. It is possible to use the entire plant or sections of it, such as extract, roots, leaves, seeds, flowers components (Mahadevi & Kavitha, 2020).

An immunostimulant is a drug that activates and amplifies by interacting directly with immune system cells, the innate or non-specific immune response. They're dietary supplements that boost the body's natural (non-specific) defense systems and boost resistance to certain diseases (Salou et al, 2020). There is no memory component developed, and the immunological reaction lasts only a few minutes. Immunostimulants are chemical compounds that cause leukocytes to become activated (Lunden & Bylund, 2000). Fatty acid adjuvant (FCA) was one of the first immunostimulants utilized in animals to boost a specific immune response, and it has also been used successfully in conjunction with fish bacterin injection (Anderson et al, 1992). Glucans, which are glucose polymers

found in the cell walls of plants, fungi, and bacteria, have so far been studied and seemed to be the most promising of all the immunostimulants examined in fish and shrimp production ponds by oral treatment, which was discovered to be the preferred method (Prusty et al., 2011).

Medical plants are as old as civilization, and their broad-spectrum medicinal effects have made them popular folk medicine throughout history. The endeavor to use them in aquaculture is a fresh idea that has gotten a lot of attention from all around the world, with Asia leading the way in terms of herb research (Citarasu, 2010). Moreover, the advantages of herbal extracts on aquaculture species have been extensively studied in tilapia species (Reverter et al., 2014).

These medicinal plants may be used whole or in part (for example, leaves, flowers, roots, seeds, or barks) in their natural state, or as extracts/compounds from whole plants or sections (Alam et al., 2014). In contrast, numerous medicinal herbs have been shown to have a broad spectrum of action, reducing the need for pharmaceutical medications. Several herbs in aquaculture, especially tilapia cultivation, have been shown in studies to promote growth, health, and disease resistance (Reverter et al., 2017). Herbs including It has been discovered that aloe vera, garlic, ginger lemon oils, and other ingredients can help tilapia grow and stay healthy (Abbasi Ghadikolaei et al., 2017). However, before herbs can be fully utilized in aquaculture, more research on their associated growth, health, and toxicity impacts, as well as preparation methods and optimum dosages, is required (Yilmaz et al., 2018).

The number of studies concluding that medicinal herbal extracts have the potential to eliminate the use of synthetic chemicals such as antibiotics and other chemotherapeutic medications in aquaculture has increased over the last two decades has increased dramatically (Schultz et al., 2014; Yilmaz et al., 2018). Medicinal herbal extracts stand out as viable alternatives to synthetic medications in aquaculture because they include physiologically active chemicals that have a variety of advantages, including immunological modulation (Shao et al., 2015; Leeuwis et al., 2019), antidepressant, growth-promoting, antioxidant-boosting, digestive-enhancement, and appetite-stimulating properties (Yu et al., 2010; Mahdavi et al., 2013) and hepatoprotective effects (Maji et al., 2015; Yilmaz & Ergun, 2018), if it's done correctly Other factors include the fact that medical plant extracts are readily available, affordable, and, in comparison to synthetic medications, tend to be more biodegradable in nature (Olusola et al., 2013; Reverter et al., 2014).

Pathogens that live in water come into touch with fish on a regular basis. Infection sensitivity is exacerbated as a result of high population density, poor hydrodynamic conditions, and poor nutrition. Various drugs are used to treat and prevent illnesses in order to avoid substantial economic losses caused by illness (Gabriel et al., 2015). Waterborne pathogens come into contact with fish on a regular basis. High population density, poor hydrodynamic conditions, and poor nutrition all promote infection sensitivity. To minimize significant economic losses caused by disease, several medications are utilized to cure and prevent illnesses.

2.6 Properties of *Vernonia amygdalina*

Vernonia amygdalina is an abundant tropical African shrub. They are widespread in Asia, where they can be found along drainage lines, in natural forests, and in sustainable plantations. It is known in Africa as 'African bitter leaf,' 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Ilo' in Igala, 'Oriwo' in Edo, 'Chusar-doki' in Hausa, 'Grawa' in Amharic, ‘Awonyono’ in Akan (Ghana). The leaves are usually green in color and have a distinct odor and bitterly flavor (Ajala et al., 2016). Leaves of *V. amygdalina* have been used as seasonings in soup after being washed and boiled to remove the bitter taste (Hamzah et al., 2013). In Nigeria, it can be used to make the bitter leaf soup "Onugbo" and as a spice in the Cameroonian dish "Ndole" (Egharevba, 2014). The herb is used as a tonic and drunk for therapeutic purposes in various parts of Africa, such as Nigeria (Udoh et al., 2017).

In Africa, *Vernonia amygdalina* is frequently used to treat diseases such as malaria, infertility, diabetes, gastrointestinal problems, and sexually transmitted infections (Farombi and Owoeye, 2011). *Vernonia amygdalina's* ethnomedical importance in the treatment of diseases such as veneral disorders, gastrointestinal problems, and malaria has also been described (Kokwaro, 2009; Afolabi et al., 2011; Igwe & harcourt, 2016). In Northern Nigeria, *V. amygdalina* has traditionally been used in horse feed to create a strengthening or fattening tonic known as 'Chusan Dokin' (Hamzah et al., 2013). *Vernonia amygdalina* had previously been used to treat parasite-related illness in Tanzanian wild chimps (Ikeda et al., 1999). *Vernonia amygdalina* extracts have been utilized for their anti-helminthic, antimalarial, antitumourigenic, bacteriostatic, and bactericidal effects on some microorganisms (Johnson et al., 2015; Igwe & Harcourt, 2016).

Nwajo (2005) and Kadiri et al. (2016) reported in an in vivo study, the *V. amygdalina* leaf extracts had a hypoglycemic and hypolipidaemic impact. Traditional health practitioners also praise the aqueous extract for treating a variety of illnesses, emesis, nausea, diabetes, loss of appetite, dysentery, gastrointestinal tract disorders, and sexually transmitted diseases, among others (Ajala et al., 2016; Okunlola et al., 2019). These findings necessitate further research into the efficacy of various plant parts in the treatment of a wide range of disease claims, as well as nutraceutical values (Madinah et al., 2015).

2.7 Phytochemical composition of *Vernonia amygdalina*

Phytochemicals are bioactive substances found in nature that provide a variety of health benefits (Dienye, 2017). They have an important role in the color, fruits and vegetables have a distinct flavor and aroma. Bioactive chemicals have been shown to be effective in the prevention of chronic diseases such as cancer, diabetes, heart disease, and Alzheimer's. Saponins, flavonoids, alkaloids, and hydrocyanic acids have been discovered in *Vernonia amygdalina* root and bark extracts. was revealed by (Dugenci et al., 2003). This study confirms Okunlola et al. (2019) report on the identification of phytochemicals in *Vernonia amygdalina* leaf extracts. *Vernonia amygdalina* phytochemicals include bioactive components that are antiviral in nature, as well as having anti-cancer and anti-inflammatory properties (Veber et al., 2002; Noumedem et al., 2013), while the report of Kadiri et al. (2016) found phytochemicals are present in According to some of the aforementioned phytochemicals, *Vernonia amygdalina* contained more bioactive components than Ocium gratissimum in terms of concentration (mg/100 g) for phytate and cyanogenic glycosides (Table 1).

Table 1: Phytochemical Components of Ethanoic Extracts of *Vernonia amygdalina* (mg/100g)*

Phytochemicals	*Vernonia amygdalina*	Ocimum gratissimum
Oxalate	3.84	0.75
Phylate	3.95	5.56
Tannins	9.62	2.84
Saponins	5.97	3.52
Flavonoid	4.89	1.74
Cynogenic glycoside	1.11	2.38
Alkaloids	2.16	1.07
Anthraquinone	0.14	0.31
Steriod	0.38	0.30
Phenol	3.24	0.73

Source: Okukpe et al. (2019)

2.8 Nutritional composition of *Vernonia amygdalina*

Vernonia amygdalina's nutritional value has been determined through a series of studies. *Vernonia amygdalina's* proximate composition as reported by Awah et al. (2012) that the presence of protein, carbohydrate, moisture, ash, fiber, and fat. Awah et al. (2012) reported a moisture content of 10.55%, which was higher than the moisture content (10.02%) reported by Okukpe et al. (2019). Differences have been attributed to soil nutrients and environmental conditions, both of which have an effect on plant nutrition availability (Olowolafe & Mo, 2018). *Vernonia amygdalina* has a crude fibre level of 8.78%, which is within the range of some Nigerian vegetables.

In addition, the ash content of 4.28 % is lower than the values reported by Okukpe et al. (2019) for bitter leaf (9.56 %) and smell leaf (4.28 %) (13.01 %). The presence of ash in bitter leaf indicates that mineral components are present. The crude protein level (18.75%) was higher than that of a variety of green vegetables, including *Momordica balsamina* (11.29 %) accordance to the findings of Emmanuel (2015), Kokwaro (2009), and Siwicki et al. (1994). The nutritional composition of dried *Vernonia amygdalina* is shown in Table 2.0. Singh et al. (2016) found the following mineral contents in *Vernonia amygdalina*: K > Na > Ca > Mg > Fe > Zn > Cu > Mn.

Table 2: Nutritional Analysis (mg/100g dry matter) of bitter leaf (*Vernonia amygdalina*)

Nutrient	Value (g/mg)
Crude protein	23.10g
Ash	17.13g
Cellullose	12.31g
Edible portion	100g
Fat	0.4g
Protein	5.2g
Water	82.0g
Energy	218g
Cabohydrate	10.0g
Dietary Fibre	1.5g
Calcium	145mg
Phosphorus	6.7mg
Iron	5.0mg
Zinc	85.0mg
Maganese	710.0mg
Ascorbic acid	5.1mg

*Source: Singh et al. (2016); Kokwaro et al. (2009)

The most common mineral element found was potassium, whereas manganese was the least common mineral element found (Kadiri et al., 2016). Potassium and calcium, for example, are known to play important roles in the maintenance of proper glucose tolerance and the release of insulin from beta cells in the islets of Langerhans, which aids in glucose control in the human body (Kadiri & Olawoye, 2016).

2.9 Pharmacological Properties of *Vernonia amygdalina*

Traditional medicinal practitioners have been using *Vernonia amygdalina* leaves to treat malaria for over a decade. In the absence of well-functioning public health care systems, Eknath et al. (1991), recognizes the significance of traditional medicine in rural communities in providing health care system. *Vernonia amygdalina* leaf extract was used to treat wister rats infected with rodent malaria, (Anoka and Njan, 2008). In mice, the leaf extract had analgesic efficacy as well as evident and strong anti-plasmodia properties.

Toxicity in rats was within control values calculated using historical reference ranges, as were incidental observations below or above normal reference levels (Adewole, 2015; Udoh et al., 2017). It was argued that the findings could explain the pharmacological basis for traditional healers' claims of efficacy of *Vernonia amygdalina* in treating pain and malaria (Onwuka et al., 1989; Okukpe et al., 2018).

2.10 Role of medicinal plants on fish growth

To investigate the effect of herbs on tilapia growth parameters, weight gain (WG), food conversion ratio (FCR), specific growth rate (SGR), condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) can all be used.

The Feed Conversion Ratio (FCR) is simply the amount of feed needed to grow one kilogram of fish. For example, if it takes two kilos of feed to grow one kilogram of fish, the FCR is two. This means that a low FCR feed requires less feed to produce one kilogram of fish than a higher FCR feed (Hassan et al., 2018). A low FCR indicates that the feed is of good quality. For the fish farmer, FCR is a valuable and powerful instrument. It provides for a rough estimate of how much feed will be needed during the growing cycle (Stratev et al., 2018). A farmer can calculate the profitability of an aquaculture business by knowing how much feed is required. This means that FCR allows farmers to make more informed decisions about feed selection and application in order to maximize profitability.

Similarly, in the work of Guroy et al. (2013b), the growth performance of 9.5 g of rainbow trout was unaffected by the average beginning weight of aloe vera extract. In a separate study, aloe vera given to feed in concentrations of 0.1 percent and 1% raised SGR and decreased rainbow trout with an average weight of 50.3 g of FCR (Hargreaves & Costa-Pierce, 2000). Another study found that adding aloe vera extract in ratios of 0.1 %, 0.5 %, and 2.5 % to the feed of carp (*Cyprinus carpio*) with an average weight of 29 g increased SGR and decreased FCR. (Mahdavi et al., 2013; Mohiseni et al., 2017). Uribe et al. (2011) reported that the addition of rosemary extract to carp (*Cyprinus carpio*) diet in concentrations of 0.25% and 0.5% as a result of which the weight increased and the FCR decreased This difference could be attributed to dietary differences between carnivorous and omnivorous fish.

Obesity is defined as an excessive increase in condition factor, whereas leanness is defined as a decrease in condition factor (Satheeshkumar et al., 2012).

In previous fish investigations, it was discovered that plants have little effect on the condition factor (Dügenci et al., 2003; Aly et al., 2008; Diab et al., 2008). When green tea byproduct and a high ratio of black cumin (2%) were used, the condition factor decreased (Chao & Krueger, 2007; Diab et al., 2008). A mixture of plants increased the condition factor in common sea bream (Jimoh et al., 2015). Gabriel et al. (2015) found that feeding aloe vera to tilapia had no influence on the condition factor.

It is well known that the livers of aquaculture fish are excessively fatty, and their color fades (Roberts et al., 2001). Excess fat from the feed is not used as energy by the fish; instead, it is stored in the organs and tissues, affecting the fish's health and reducing feed utilization. The HSI can detect an increase in the weight of the liver when the quantity of fat stored in the liver increases. According to Chao & Krueger (2007), fig extract, onion extract, and Indian fig were ineffective in reducing liver fat and HSI. However, oregano (*Origanum heracleoticum L.*) was found to lower HSI and VSI levels in channel catfish in a study (Zakes et al., 2008). The reduction in the rate of HSI in tandem with VSI in the same trial could be related to oregano's fat-burning impact on the liver. In numerous fish experiments, the use of *Quillaja saponin, Astragalus radix + Lonicera japonica*, and green tea plants resulted in a decrease in HSI ratings (Francis et al., 2002; Chao & Krueger, 2007; Zakes et al., 2008). Increased HSI was observed when genetically improved farmed tilapia juveniles fed feed containing 31.7 % protein and 7.3 % lipid with an average weight of 4 g, as well as feed containing aloe vera additives in ratios of 0.5 %, 1 %, 2 %, and 4 % per kilogram, while VSI remained unchanged (Gabriel et al., 2015).

The viscerosomatic index (VSI), which covers all internal organs, is used to determine whether a person has gained or lost weight. The HSI and VSI scores did not change when 0.5-4 g/100 g of Rheum officinale extract was added to the meal of *Cyprinus carpio* with an average weight of 5.39 g. In the control and experimental groups, there was no significant change in whole-body proximate composition (Xie et al., 2008).

Effects of different sweet potato (*Ipomea batatas*) peel quantities on cichlid growth, feed consumption, and certain metabolic reactions (*Oreochromis niloticus*). According to the findings, *Oreochromis niloticus* could withstand up to 15% sweet potato peel supplemented dietary. The importance of the study's findings is that to reduce toxicity, sweet potato peels can be added to fish meals. Adewolu, 2008 found the same thing in tilapia fingerlings, and Ferreira et al. (2009) found it in *Cyprinus carpio*. The effect of mushroom supplementation as a prebiotic ingredient in a super worm-based diet on red tilapia fingerling growth performance was investigated by (Mustafa & Sims, 2006; Falaye & Omoike, 2012). SGR and WG levels increased, and survival increased to 93.33 percent. MSM supplementation at a 10% level as a prebiotic for tilapia could be used in the insect-based diet of *Zophobas morio*. The use of mellon shell as a dietary energy source promotes nile tilapia (*Oreochromis niloticus*) weight gain (Orire & Ricketts, 2013).

The *Oreochromis niloticus* may successfully utilize up to 75% of the melon shell meal included in the diet of tilapia. The specific growth rate, protein intake, protein efficiency ratio, gross feed conversion efficiency, feed efficiency, mean feed intake, survival rate, and percentage weight gain all increase as the amount of dietary cowpea (*vigna unguiculata*) husk meal is increased. The replacement of maize meal in the diet with cowpea hull meal

improves the growth performance of *C. gariepinus* fry by 50% to 100% (Falaye et al., 2012). In an industrial recirculating aquaculture system, Dushay et al. (2010) investigated the effects of phytobiotics (thyme, seabuckthorn) on stellate sturgeon growth performance (*A. stellatus, pallas*, 1771). Thyme (*Thymus vulgaris*) and seabuckthorn (*Hippophae rhamnoides*) were used as phytobiotics and were imbedded in fodder at a concentration of 2% per kg fodder using gelatin. The feed used was *Alterna Storioni* with a crude protein content of 48%.

Finally, the two types of phytobiotics (*thyme and seabuckthorn*) provided at a concentration of 2%/kg fodder altered stellate growth performance. Guroy et al. (2012a) investigated the effect of partially substituting wet date for corn meal on growth performance in fingerling nile tilapia (*Oreochromis niloticus*) fed digestarom-supplemented diets. They discovered that a diet containing 30% WD and 0.3% Digestarom had the highest net profit and appears to be the most desirable level of WD and Digestarom (Ardo et al., 2008).

2.11 Effect of medicinal plants on hematology

Effect of medicinal plants on hematological parameters blood is the most commonly studied organ in vertebrates, including fish, to determine their health or physiological status (De Pedro et al., 2005; Bittencourt et al., 2003). As a result, hematological indices such as red blood cell (RBC), hemoglobin concentration (Hb), and the percentage of blood volume made up of red blood cells (RBC), hematocrit (Hct), have been used to directly measure health statuses such as oxygen carrying capacity (Hamzah et al., 1998; Harikrishnan et al., 2010; Jimoh et al., 2015). Secondary indices, also known as Wintrobe indices (Urrechaga

et al., 2015), such as mean corpuscular volume (MCV) = (Hct x 10/RBC), mean corpuscular hemoglobin (MCH) = (Hb x 10/RBC), and mean corpuscular hemoglobin concentration (MCHC) = (Hb x 100/Hct), can be derived from these indices for anemia classification (Gabriel et al., 2015).

Other hematological indices, such as white blood cell (WBC) counts and a number of their differential counts (i.e. leucocyte counts such as lymphocytes, neutrophils, eosinophils, monocytes, and basophils), have also been studied to account for an animal's innate immune status, particularly under stressful conditions (Harikrishnan et al., 2010; Reverter et al., 2014). Furthermore, the neutrophil-to-lymphocyte ratio has been shown to be a useful tool for estimating stress levels in vertebrates (Shane et al., 2017; Van Rijn & Reina, 2010).

Several aquaculture studies have demonstrated that a variety of medicinal plants can improve some of the aforementioned hematological parameters (Terry et al., 2000; Hassan et al., 2009; Anjusha et al., 2019). In tilapia culture, *aloe vera* supplemented food significantly increased RBC, Hct, Hb, WBC, and certain differential leukocyte counts of *O. niloticus* (GIFT-strain) before and after *Streptococcus iniae* challenge (Gabriel et al., 2015). Similarly, administration of *Rosmarinus officinalis*, *Trigonella foenum graecum, Thymus vulgaris*, and *Camellia sinensis* in *O. mossambicus* (Galeotti et al., 2014; Galluzzi et al., 2015; Abdel-Tawwab et al., 2014), *Citrus limon* in *O. mossambicus* (Baba et al., 2016), ginseng in *O. niloticus* (Adeyemo et al., 2003; Afolabi et al., 2011), *Ulva clathrata* in *O. niloticus* (Achuba, 2018b), (Adeparusi & Agbede, 2005), *Cynodon dactylon,* Aegle were reportedly improved some hematological parameters.

The ability of herbal extracts to stimulate erythropoiesis, thus improving oxygen transport capacity and defense mechanisms against physiological stress, is demonstrated by an increase in hematological indices (Rahman, 2008; Van Rijn & Reina, 2010; Prasad & Priyanka, 2011). This is thought to be due to their high nutritional value, which includes polysaccharides, important vitamins (such as riboflavin, thiamine, and folic acid), and non-essential amino acids, the majority of which are required for hemoglobin synthesis (Hamman, 2008; Abelli, 2013).

Furthermore, several herbal extracts have been shown to have no effect on the hematological characteristics of tilapia species. Crude extracts of *Cinnamomum camphora*, *Euphorbia hirta, Azadirachta indica*, and *Carica papaya*, for example, were found to have no effect on *O. niloticus* RBC, Hb, MCV, MCHC, Hct, and WBC levels (Kareem et al., 2016; Ajayi et al., 2018; Mahadevi & Kavitha, 2020). According to Gabriel et al. (2015), the ability or effects of herbs on hematological indices can only be well established if an animal is subjected to stressful conditions, which several studies have failed to do (Orun et al., 2003; Paez et al., 2004; Shalaby et al., 2006; Prasad & Priyanka, 2011; Qiu et al., 2018; Zaki et al., 2020). Furthermore, various studies in tilapia culture have been conducted (Gareau et al., 2011; Baba et al., 2016; Yimalz et al., 2018) and other fish species such as *Labeo rohita* (Sponchiado et al., 2016), *Cyprinus carpio* (Owen et al., 2011; Plengsuriyakarn et al., 2016), and *Oncorhynchus mykiss* (Harikrishnan et al., 2011) reported that herbal extract especially at higher dosage. The causes and mechanisms aren't well understood. They are thought to accomplish this via interfering with erythropoiesis, hemosynthesis, and osmoregulatory processes, as well as promoting erythrocyte death in hematopoietic organs (IARC, 2000; Jenkins et al., 2003; Rahman, 2008).

2.12 Toxicity of medicinal plants to fish

Numerous elements can be found in hematological parameters and blood serum that can be utilized to assess the health of fish. The amounts of serum total protein (WBC product) (Molofsky et al., 2013; Murugesu et al., 2019) and globulins (source of immunoglobulins or antibodies) (Naujokas et al., 2013) in the blood, for example, represent immune system activation (Siwicki et al., 1994). Whilst the presence of lysozyme, antimicrobial peptides, phagocytes, and complement factors in the blood indicates pathogen entry inhibition via pathogen cell wall lysis (lysozyme, antimicrobial peptides), phagocytosis (phagocytes), and neutralisation (complement factors), the presence of lysozyme, antimicrobial peptides, phagocytes, and complement factors in the blood indicates pathogen entry inhibition via pathogen (Udoh & Udoidiong, 2004; Verhaak et al., 2010).

Furthermore, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the key determinants of hepatoprotective action (Vedavathy, 2003; Yildiz et al., 2006; Cui et al., 2014), but glucose and cortisol blood level can represent stress (Chaklader et al., 2014; He et al., 2015; Xu et al., 2013; Xu et al., 2017). Several herbal extracts have been shown to significantly improve a variety of biochemical indices in fish, including tilapia species. Essential oils extracted from *C. sinensis* (Akoll et al., 2012b; Ajala & Owoyemi, 2016) and *C. limon* (Baba et al., 2016) were found to have improved innate immune biochemical parameters (total protein, and lysozyme), as well as resistance to the bacterial pathogens *Edwardsiella tarda* and *Streptococcus iniae* in *O. mossambicus*, respectively. In *O. niloticus*, improvements in innate immune biochemical markers were documented after dietary treatment of *A. vera* (Anthony et al., 2009; Gabriel et al., 2015), *A. sativum* (Shalaby et al., 2006), *Echinacea purpurea* (El-Sayed et al., 2014), *G. lucidum* (Yin et al., 2011),

ginseng (Goda, 2008), blackberry syrup respectively. In addition, dietary *E. purpurea* extracts (capsules) have been shown to activate immunoglobulin M in *O. niloticus* (El-Sayed et al., 2014), indicating that a specific immunological response has been triggered (Anjum et al., 2011; Anjusha et al., 2019).

Moreover, *A. vera* crude extracts were found to have increased antioxidant enzymes (CAT, SOD, GSH-Px), reduced stress (lower glucose and cortisol), and improved hepatoprotective activity (lower AST) in GIFT-tilapia, both pre- and post-*Streptococcus iniae* challenge (Gabriel et al., 2015). Qin et al. (2017) and Qui et al. (2018) obtained similar results in *O. niloticus* after feeding them a meal supplemented with *A. sativum* extracts, although these animals were not challenged (Citarasu, 2010; Rezania et al., 20016; Reynolds et al., 2008; Murugesu et al., 2019; Chahardehi et al., 2020). Similarly, unchallenged animals (*O. niloticus*) fed a meal containing acetone extracts from A. sativum showed increased hepatoprotective efficacy (Shalaby et al., 2006; Awah et al., 2012; Plengsuriyakarn et al., 2012; Sponchiado et al., 2016). Meanwhile, *O. mossambicus* was shown to be less stressed after being exposed to acetone extracts from *C. dactylon*, *A. marmelos*, *W. somnifera*, and *Z. officinale* (Manas & Takahashi, 1992; Immanuel et al., 2009; Asare et al., 2012; Brown, 2017).

2.13 Effect of Medicinal Plants against Temperature Stress in Cultured Fish

The environmental temperature range experienced by any animal can have a major impact on survival, performance and reproduction, and this is a particular problem for ectotherms that have limited capacity to regulate their own body temperature (Wootton-Beard, 2011). The rate of biochemical processes in cultured fishes roughly doubles for every 10^{o}C increase in temperature (Boyd and Tucker, 1998;). Temperature can also impact growth

rate and feed conversion ratio of freshwater fish (Sardella et al., 2004) and as a consequence, temperature can affect both specific and nonspecific processes including immune defence mechanism in fish (Le Morvan et al., 1998).

Temperature stressors affect many biomarkers in fish. For example, blood parameters such as hematocrit (HCT), red blood cells (RBCs), and haemoglobin (HGB) levels which are essential in oxygen uptake in organisms could be altered by stressful conditions (Bao et al., 2018).

In recent years, numerous studies demonstrated that medicinal plants exerted anti-stress effects in aquatic animals (Liu et al., 2016; Almadniare Motlagh et al., 2019). In freshwater shrimp *M. rosenbergii*, anthraquinone extract improved the capacity resist to high temperature (Liu et al., 2010) and *Moringa oleifera* leaf extract improved the anti-ammonia stress capacity (Kaleo et al., 2019). Meanwhile, anthraquinone extract from *R. officinale* Bail improved tolerance against hyperthermia in *Macrobrachium nipponense* (Song et al., 2020). Ulteriorly, as the active constituent of anthraquinone extract, emodin protected the herbivorous fish *M. amblycephala* from crowding stress (Liu et al., 2014) and dietary oxidised fish oil-induced oxidative stress (Song et al., 2019; Song et al., 2021). Analogously, *Radix Bupleuri* extracts protected against H_2O_2-induced oxidative stress in tilapia (Jia et al., 2019). Rosemary leaf mitigated the adverse effects of crowding stress in *C. carpio* (Yousefi et al., 2019). Moreover, dietary anthraquinone extract (1%–2%) mitigated the adverse effects of crowding stress in common carp (Xie et al., 2008) and turmeric improved the anti-stress ability of common carp during copper exposure (Rajabiesterabadi et al., 2020). In conclusion, herbal medicine positively affected the anti-stress capacity of aquatic animal.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Experimental plant material and preparation of leaf extract

Vernonia amygdalina (commonly called bitter leaf) was identified from the environs of the University for Development Studies, Nyankpala campus (Plate 1) with the aid of a herbal plant manual book by Odugbemi (2008). Fresh leaves of *V. amygdalina* of about 500 g were collected (Plate 2), washed and blended with 100 ml of distilled water at room temperature (i.e., 25 ± 2 °C) (Plate 3). After blending, the liquid extract was then sieved with a 100-micron sieve (Plate 4). After that, the aqueous solution was maintained in a plastic container at room temperature (i.e., 25 ± 2 °C) until it was used (Olusola et al., 2018).

Plate 1: Plant of a Bitter leaf

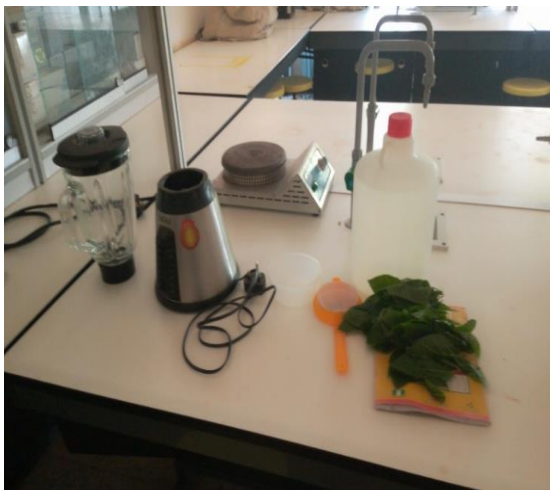

Plate 2: Fresh leaves *Vernonia amygdalina*

Plate 3: Akai blender

Plate 4: Sieving of BL extract

3.2 Preparation of experimental diets

A 3 mm commercial feed (Rannan company) of weight 25 kg was purchased from the open market in Tamale (Plate 5), Ghana. One Kilogram (kg) of the feed was measured (Plate 5) each into four different bowls. And to one-half of the 1 kg measured feed, 100 ml of distilled water was added and mixed thoroughly in a helical bowl and then dried under room temperature representing the control diet (CT). The remaining three halves of the feed, 1 % of the 1 Kg [(1% * 1000 = 10ml); (10ml BLE + 90ml of distilled water = 100ml in total volume)] was measured from the bitter leaf extract and was added to the 1 kg measured feed and mixed using my hands thoroughly in a helical bowl. 3 % of BLE and 5% BLE were measured and then mixed independently with a kilogram of the commercial feed until a uniform mixture was obtained (Plate 6) in a plastic container representing

control, (1 % BLE) as low dose, (3 % BLE) as medium dose and (5 % BLE) as high dose. The supplemented diets were then dried in a clean room under room temperature and later bagged and stored in an insect-proof bag for later use. The different codes used are found in table three.

Table 3: Dietary codes used in this study

Dietary Codes	Dietary combination
CT	A commercial pellet diet that is void of BLE extract
1% BLE	Supplementing a commercial diet with 1% BLE v/w
3% BLE	A commercial diet supplemented with 3% BLE v/w
5% BLE	Supplementing a commercial diet with 5% BLE v/w

Where: CT, control, BLE, bitter leaf extract, v/w, volume by weight

Plate 5: Mixing of the feed and extract

Plate 6: Ensuring homogeneity of feed

3.3 Experimental fish

A total of 200 healthy tilapia (Plate 7), of a mean weight of 30 ± 1.0 g (Plate 8) without any clinical signs (i.e., no abdominal distension, ragged fins, or hemorrhage) were obtained from Water Research Institute (Tamale, Ghana) for the experiment. The fish were split into eight groups (Plate 9) and acclimated for one week in 80L water capacity concrete circular tanks containing 50L of water (Plate 10). Throughout the acclimatisation period fishes were fed twice a day with the control diet at 2% their body weight in two equal rations at 8:30 am and 4:00 pm (Plate 12).

Plate 7: Healthy tilapia without symptoms

Plate 8: Initial weighing of fish

Fish were then assigned to the respective groups (i.e CT, 1% BLE, 3% BLE and 5% BLE) in duplicate with each tank containing 25 fishes. During the period of the experiment of 8 weeks of feeding, water quality was maintained by constantly measuring dissolved oxygen, temperature, total dissolve solids, pH, and conductivity by using hydrokits 2000 on weekly basis (Morning 8:00 am, afternoon 12:00 pm and evening 4:00 pm). By assessing the individual body weight of the fish in each group biweekly, the amount of food given out was modified to suit their body mass. About 30% of the water was exchanged on daily basis to maintain the quality, with an average temperature of 26 ± 2.0°C, pH of 6.5 ± 0.28, and dissolved oxygen concentration of 6.10 ± 0.35 mg/L (Plate 11)

Plate 9: initial stocking of fishes

Plate 10: Experimental tanks

Plate 11: Checking of water quality

Plate 12: Feeding trial of tilapia

3.4 Growth parameters

The initial weight of the experimental and control fishes was measured using a digital scale to the nearest 0.1 g (Plate 14). Body weight and total length of fish from each group were all measured and then six (6) fish from each treatment were dissected after 4 and 8 weeks of the feeding trial (Plate 13) to measure the weight of visceral mass and liver.

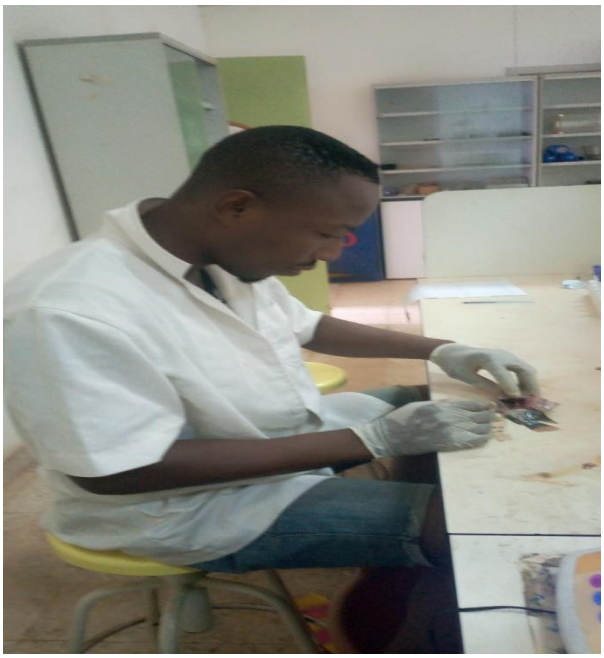

Plate 13: Viscera weighing

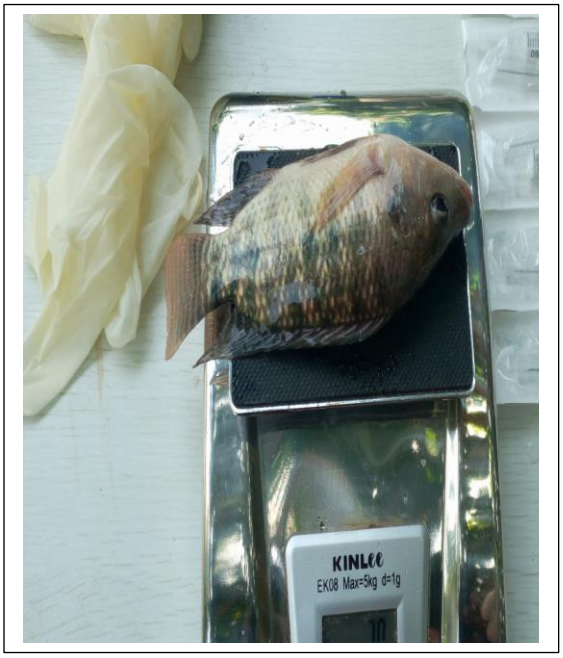

Plate 14: Final weighing of fish

Plate 15: Individual fish size

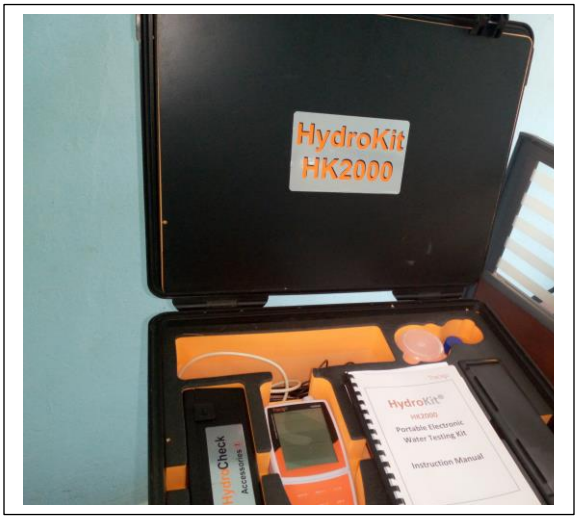

Plate 16: Water quality kits

3.5 Blood and tissue sample collection

Blood samples were taken from 6 fish (i.e., 3 gathered from each tank replicates, *n* =2) from each treatment group and the control group were taken at 4 and 8 weeks to assess the impact of feeding tilapia a bitter leaf supplemented meal on their health. A whole blood

(0.5–1 mL) was taken using 2mL disposable syringe from the caudal section (Plate 16) of the fish as described by (Strehl et al., 2014; Su et al., 2016). Blood samples were taken and decanted into a polypropylene specimen tube with dipotassium EDTA (Plate 15).

3.6 Analysis of data

3.6.1 Protocols for full blood count

A full blood count (FBC) measures the red blood cells (RBC), white blood cells (WBC), the platelets and the differentials of the RBCs and WBCs. The major principles involved in the automated unit 240 (Meditech, China) involves electrical impedance, optical flow cytometry and cytodemzal staining. The main reagents used in the analysis are the diluent, which creates an isotonic concentration to avoid lemolysis during the analysis. There is also a lysine solution that degrades the RBCs for the detection of the leme component. The blood sample is taken into an EDTA test tube and the probe of the analyser is immersed in it after vortexing to ensure homogeneity. After sucking by the probe, the agitator of the analyse agitates to evenly distribute the cells into channels depending on their size and their weight. The platelets and the differentials of the WBCs are then counted. For RBCs after agitation, the lysing reagent is added to the RBC chamber to be lysis. These chambers are fixed with flococytonetric sensors, which detect the cells as they flow in a fliud stream.

3.6.2 Protocol for toxicity test

The liver function test (LFT) determines the levels of aspartate aminotransferase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), alkaline phosphate (ALP), albumin globulin, total protein, Bilimbin, and other enzymes. Bilimbin, both direct and indirect. The major principle behind the measurement is spectrophotometry. The sample is taken into gel-activator test tube and allowed to stand for 20 minutes to clot.

After this, the sample is centrifuged at 1500rpm for 3minutes to separate the blood cells from the serum. The serum is then pipetted into a sample cup and inserted into the analyser for analysis.

3.6.3 Protocol for plasma chemistry

Bilimbin consist of direct bilinbin and indirect bilinbin. Direct bilinbin is conjugated to hens which makes it soluble whiles indirect bilinbin is unconjugated making it not soluble. For the test of bilinbin, Diazotised sulfanilic acid reacts differently with direct and indirect bilinbin, to form different quantities of chromophore azobilinbin. Azobilinbin red at 520nm to give an absorbance which is then converted to concentrations. The sum of the direct and the indirect bilinbin gives the total bilinbin.

3.6.4 Protocol for proteins analysis

The proteins consist of soluble osmotic albumin, Y-GT and Y-GT, structural insoluble globulin. The sum of the albumin and the globulin gives the total protein. Due to their differences in solubilities the biuret method is used to quantitatively determine them. During the analyses, the main reagent which is the bromocresol green reacts differently with the albumin and globulin to form a chromophore. The chromophore reacts at 450nm to give the absorbances which are then converted to concentration using the calibration curve.

3.7 Plasma chemistry

Blood samples were obtained at 4 and 8 weeks from 6 fish from the treatment group and the control group (i.e., 3 pooled from each tank replication, n =2) to examine the effect of the bitter leaf supplemented diet on the protein level of tilapia. As indicated by (Su et al., 2016) whole blood (0.5–1 mL) was taken from the caudal part of the fish using a 2mL

disposable syringe. Blood samples were collected and decanted into a plastic specimen tube containing dipotassium EDTA as an anticoagulant. The effect of the control and BLE supplemented diets on the protein content of tilapia was assessed using blood parameters such as TP, ALB, y-GT, and T-BIL.

3.7.1 Analysis of data for protein measurements

For data analysis for protein measurement, please see section 3.6

3.8 Toxic effect of dietary treatments in tilapia

and as a result of the toxic effect on the fish's liver, the liver functioning test (LFT) was developed, which uses enzymes found in the liver of tilapia as markers, such as AST, ALP, and ALT. These are enzymes that are found in the liver of the fish, when found streaming into the blood in appreciable levels denote the level of degradation to the liver. Their level talks about and it was done with Mindray Chemistry analyzer BS 240(Tamale teaching hospital, Ghana).

Plate 17: Dissecting equipment

Plate 18: Blood sampling

3.9 Heat stress

Eight (8) white rectangular plastic bowls of size of the 50cm by 10cm were filled with 5L of municipal water of average temperature 25 ± 2 ˚C (Plate 17). Mercury in glass thermometers were placed in each of the rectangular containers with initial temperature of 25 ± 2 ˚C (Plate 18) to monitor the increase in temperature using hot water boiled at 100 ˚C. As described by Fawzia et al. (2016), fish from each experimental group were transferred from the experimental tanks of 25 ± 2 ˚C, ambient temperature into containers with 5L of water. In the 50cm by 10cm rectangular plastic bowls, the water temperature was gradually raised from 25 ± 2 ˚C to 38 ± 0.5 ˚C at a rate of 2 ˚C per hour using 100 ˚C boiled water (Plate 19) and the stability was monitored using a mercury in glass thermometer. Fishes used for the heat stress in the plastic tanks were not fed throughout the heat stress test. Temperature-controlled fish (38 ± 0.5 ˚C) were maintained in that temperature for 2 h by constantly adding the boiled water before their blood was sampled from the caudal section using a 2mL disposable syringe and decanted thereafter into anticoagulant tubes to be analysed. Blood samples were collected before the commencement of the heat stress test and afterwards to look at the effect of heat stress on WBC, RBC, HGB and HCT (Plate 20).

Plate 19: Heat test bowls

Plate 20: initial temperature of water

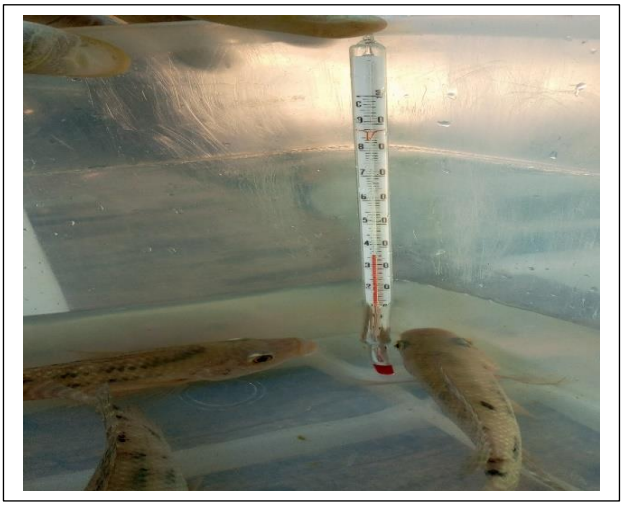

Plate 21: Monitoring of water temperature (38 ± 0.5 ◦C)

Plate 22: Blood sampling after hypoxic test

3.10 Data analysis

Growth parameters were analysed following the description in Xiao et al. (2018);

$$\text{Weight Gain(WG)} = 100 * \frac{[(\text{finalbody weight(g)} + \text{dead fish weight}) - \text{initial body}]}{\text{initial}} \text{body weight}]$$

$$\text{Feed conversion ratio(FCR)} = 100 * [\text{(Total feed intake(g)/ (final body weight(g) + dead body weight(g) – initial body weight(g))}]$$

Morphometric index as described by (Mustafa et al., 2006)

$$\text{Condition factor (CF)} = 100 * [\text{(total body weight(g)/ total body length(cm)}^3]$$

Viscerosomatic index (VSI) = 100 * [(viscera weight (g)/total body weight(g)]

Hepasomatic index (HIS) = 100 * [(liver weight (g)/total body weight(g)]

One-way ANOVA was used to determine differences ($P < 0.05$) in growth parameters, hematological, stress response, and toxic parameters by subjecting the measured indices in control and BLE groups using IBM SPSS statistical package for social sciences (SPSS version 16.0). In situations where there were differences in treatment means, Duncan multiple range test was further used to separate the difference in means ($P < 0.05$). The results were presented in the forms of means with standard error (± SE).

CHAPTER FOUR

4. RESULTS

4.1 Effect of diet on growth performance of tilapia

Test fish fed both control and BLE treated diets accepted the feed and responded well throughout the experimental period. The fish used at the beginning of the experiment were of an average weight of 30.10 g. At the end of the experiment, the average final weights of fish from the CT, 1%BLE, 3%BLE and 5%BLE were 49.42 g, 57.15 g, 62.33 g and 68.0 g, respectively. Fish feed BLE supplemented diets were significantly higher in comparison to the control ($P < 0.05$). Among BLE treated fish, those fed 1% BLE showed best weight gain and those fed 5%BLE showed the least weight gain. FCR of the control diet was significantly higher ($P < 0.05$) compared to the BLE diet at the end of the study period. Similarly, VSI and HSI showed similar trends, thus with BLE supplemented diets showing superiority in comparison to the control ($P < 0.05$). Control diet at the end of the study exhibited higher statistical value in the condition factor compared to the BLE treated diets (Table 4).

Figure 1 shows the growth pattern of tilapia fed with control and BLE inclusion diet. At the end of the experimental period, fish fed with BLE inclusion diet showed superiority in the pattern of growth compared to the control diet with 1% BLE presenting the best of result followed by 3% BLE and 5% BLE respectively.

Table 4: Effects of diets on growth of Nile tilapia fed control and BLE treated diet.

Growth Parameter	dietary Treatment			
	CT	1% BLE	3%BLE	5% BLE
Initial BW (g)	31.25±1.23^{a}	31.75±1.26^{a}	31.90±1.09^{a}	31.51±1.30^{a}
Final BW (g)	49.42±1.63^{d}	68.00±1.42^{a}	62.33±1.1.74^{b}	57.15±1.67^{c}
WG (g)	5.04±1.73^{c}	9.61±1.66^{a}	7.38±1.00^{b}	6.32±3.03^{c}
FCR(%)	3.52±0.80^{a}	1.57±0.04^{b}	1.62±0.18^{b}	1.51±0.22^{b}
VSI (%)	5.56 ±0.18^{b}	6.42±0.54^{a}	7.71±0.15^{a}	7.34±0.35^{a}
HSI (%)	0.08±0.01^{c}	0.74±0.01^{a}	0.67±0.06^{b}	0.64±0.06^{b}
CF	1.74±0.04^{a}	1.51±0.02^{b}	1.62±0.06^{b}	1.57±0.03^{b}

Note: Means in rows with the same superscript are not statistically different

Where: BW=body weight, WG=weight gain, FCR=food conversion ratio, VSI=viscerosomatic index, HSI=hepasomatic index and CF=condition factor.

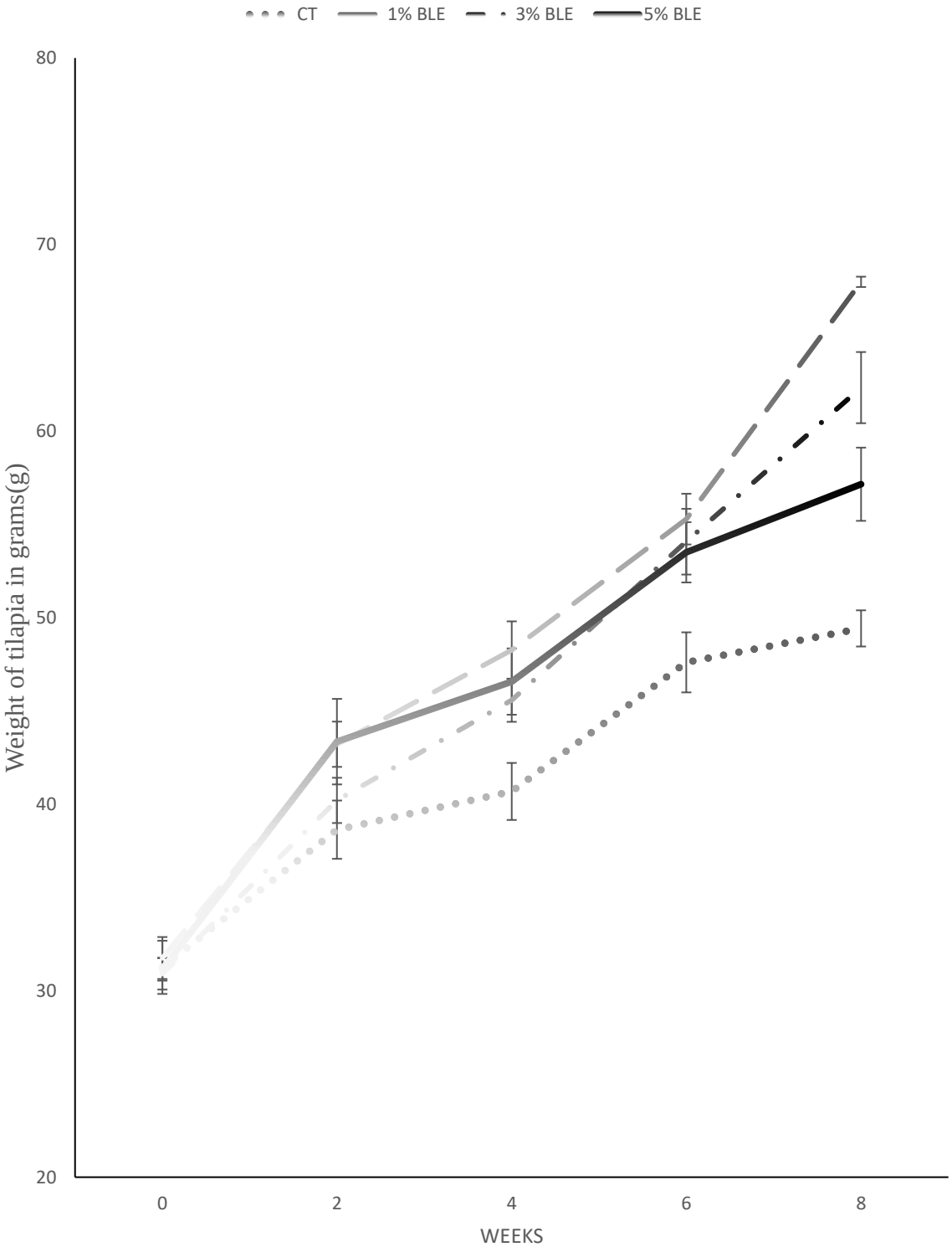

Figure 1: Growth pattern of Nile tilapia fed with control and BLE treated diet.

4.2 Effect of dietary treatment on hematological parameters

4.2.1 White blood cell (WBCs)

The effects of diets on the WBCs of tilapia after 4 and 8 weeks of feeding is shown in fig. 2. After 4 weeks of experimental feeding, fish fed BLE supplemented diets exhibited significantly higher levels of WBCs compared to the control group ($P < 0.05$). Among the BLE supplemented groups, fish fed 1% BLE supplementation showed significantly higher WBC compared to the others ($P < 0.05$).

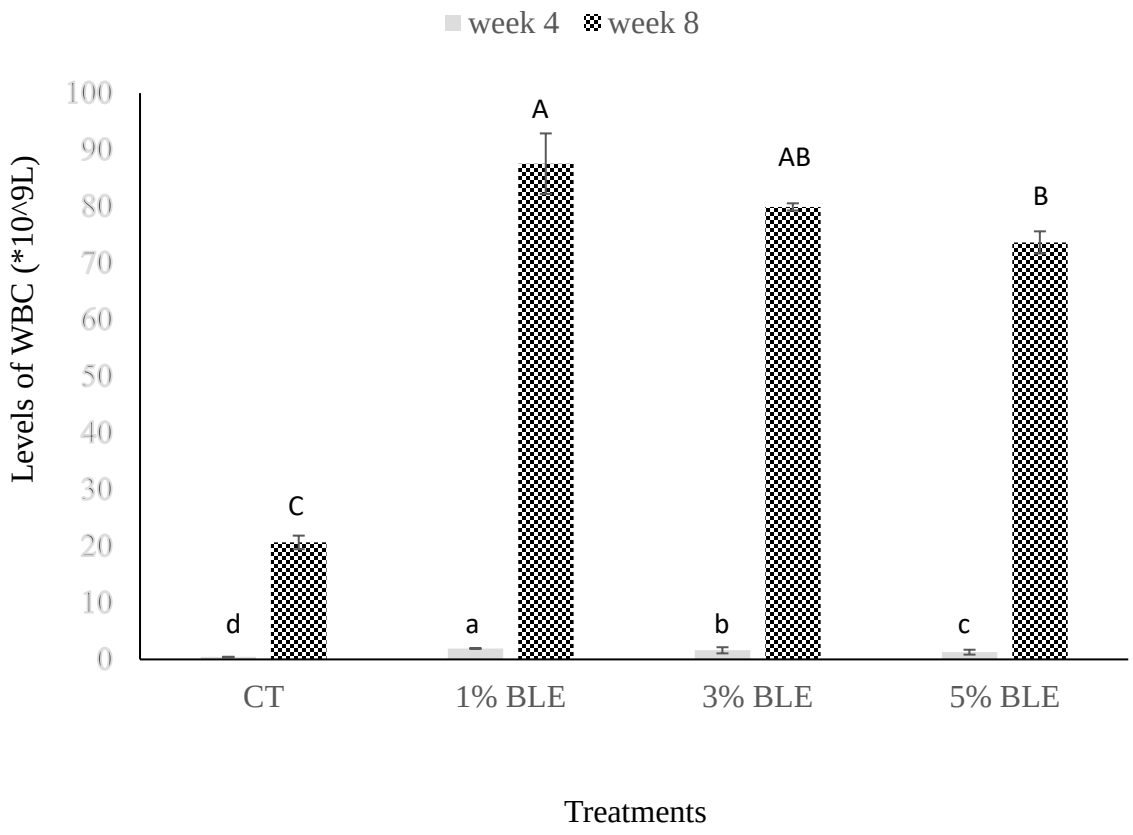

Figure 2: WBC levels of tilapia at 4 and 8 weeks fed with control and BLE diet

Note: Bars with the same alphabet per sampling time are not statistically different

At 8 weeks, a similar trend was observed thus the BLE supplemented groups still exhibiting significantly higher WBCs in comparison to the control ($P < 0.05$); however, it is worth noting that 1%BLE was similar to 3%BLE which was also similar to 5%BLE ($P > 0.05$). Generally, it was observed that the WBCs in the test fish increased as the experiment progressed from week four through to week eight. Although the results showed that BLE supplementation has the potential to increase significantly the WBCs in comparison to the control, there appears to be a decreasing trend with increasing BLE inclusion in the diets.

4.2.2 Differential WBC (LYM, NEU, MON, EOS and BASO) of Nile tilapia

In table 5 are presented the results of differential WBCs including LYM, MON, BASO, NEU and EOS in BLE treated diets were significantly increased compared to the control after 4 weeks of feeding. 1%BLE demonstrated significantly higher EOS followed by the control (0%BLE) which was also significantly higher in comparison to those fed 3%BLE and 5%BLE ($P < 0.05$). At 8 weeks, LYM was significantly higher in fish fed BLE diets compared to the control ($P < 0.05$). There were no observable effects of the dietary treatments on MON and NEU at 8 weeks. However, some variations were noticed on BAS thus with 1%BLE and 3%BLE showing some improvements and 0%BLE and 5%BLE showing no effects at all. It was observed that the differential WBCs with the exception of MON increased from 4 weeks to 8 weeks

Table 5: Differential WBCs of Nile tilapia fed with control and BLE treated diet.

Hematological Parameter	dietary Treatment							
	CT	1%BLE	3%BLE	5%BLE	CT	1%BLE	3%BLE	5%BLE
	WEEK 4				WEEK 8			
LYM (%)	14.80±1.46^{c}	48.92±3.15^{a}	38.22±5.31^{b}	44.79±4.93^{a}	33.13±19.40^{C}	51.39±26.30^{A}	51.44±15.02^{A}	49.96±8.96^{B}
MON (%)	14.93±7.47^{c}	38.08±1.28^{b}	50.99±3.74^{a}	37.13±2.84^{d}	12.77±4.62^{B}	38.08±1.28^{A}	14.26±1.86^{B}	10.88±1.42^{B}
BAS(%)	0.11±0.07^{d}	0.42±0.88^{a}	0.36±0.31^{b}	0.29±0.11^{c}	0.32±0.11^{C}	0.71±0.13^{A}	0.56±0.14^{B}	0.47±0.16^{C}
NUE (%)	24.93±3.68^{c}	41.29±4.54^{b}	47.46±5.99^{b}	61.45±5.78^{a}	50.47±18.96^{C}	54.38±24.67^{C}	72.34±16.52^{B}	91.91±1.82^{A}
EOS (%)	0.27±0.15^{b}	0.60±0.03^{a}	0.04±0.01^{c}	0.03±0.01^{c}	0.60±0.32^{C}	0.82±0.03^{A}	0.74±0.03^{A}	0.69±0.04^{B}

Note: Means in rows with the same superscript are not statistically different

Where; LYM=Lymphocyte, MON=Monocyte, BASO=Basophiles, NUE=Neutrophiles, ESO=Eosinophiles

4.2.3 Red blood cell (RBC) of tilapia

RBC ranged from 1.7 x 10^2 L to 3.6 x 10^2 L at 4 weeks with the least recorded in the control and the best recorded in 1%BLE fish group ($P < 0.05$) (Fig. 3). The RBC ranged from 2.07 x 10^2 L to 3.66 (x 102 L^{-1}) at 8 weeks with the 1 % BLE exhibiting the best effects compared to all other fish groups of study whilst 0 % BLE, 3 % BLE and 5 % BLE showed significantly lower RBCs ($P < 0.05$) (fig. 3). It was observed that RBC levels did not vary that much at 4 weeks and 8 weeks between the BLE supplemented diet. However, there was a slight increase of RBCs in the control group from 4 weeks to 8 weeks, a reverse trend was observed in the BLE treated groups as RBC was decreasing with increasing level of BLE. 1% BLE exhibited the best RBC levels within the BLE group with 5% BLE recording the least.

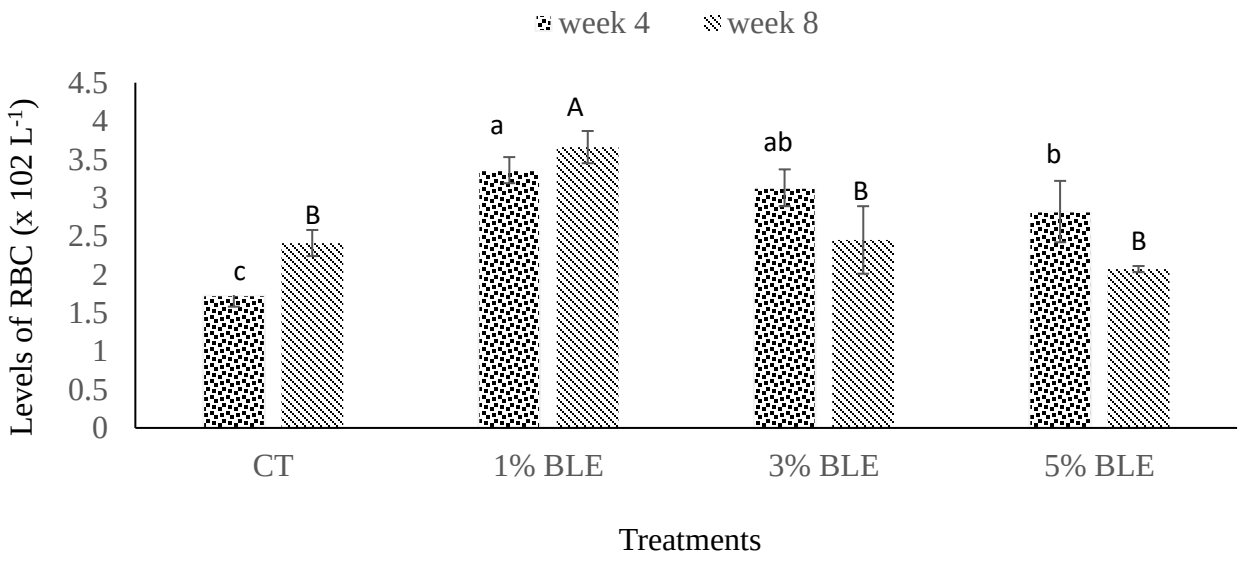

Figure 3: RBC levels of tilapia at four and eight weeks fed control and BLE diet

Note: Bars with the same alphabet per sampling time are not statistically different

4.2.4 Hemoglobin (HGB) level in Nile tilapia fed with control and BLE treated diet

Figure 4 presents average levels of HGB in tilapia fed control and BLE treated meal and it ranged from 10.70 g dL - 17.70 g dL and from 10.06 g dL - 14.34 g dL at 4 and 8 weeks respectively. At 4 weeks, all BLE supplemented dietary fish group recorded significantly higher HGB compared to the control ($P < 0.05$); likewise at week 8 with the exception of 5%BLE which showed statistical similarities with the control ($P > 0.05$). All in All, fish fed 1%BLE exhibited the best incremental effects ($P < 0.05$) on HGB compared to all other groups in the study both at 4 and 8 weeks of measure.

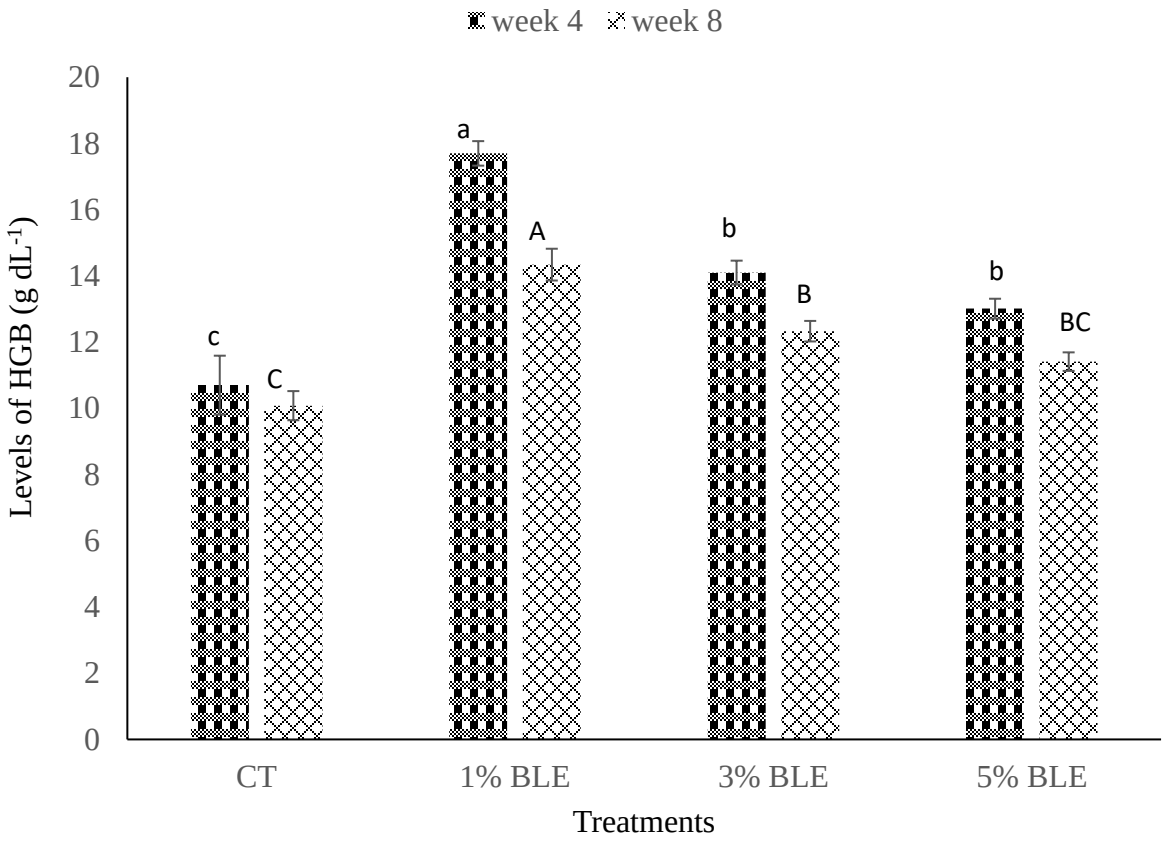

Figure 4: HGB levels of Nile tilapia at week 4 and 8 fed control and BLE diet

Note: Bars with the same alphabet per sampling time are not statistically different.

4.2.5 Levels of hematocrit (HCT) in Nile tilapia fed with control and BLE diet

Mean levels of HCT of the experimental fish ranged from 22.03 (L/L) - 42.36 (L/L) at week 4 of feeding trial and at week 8 ranged from 29 .42 (L/L) – 35.61 (L/L). It was clearly observed a declining trend in the BLE inclusion dietary at the 4th and 8th week. All BLE supplemented fish group at week 4 recorded significantly higher HCT levels in comparison to the control group ($P < 0.05$). At the 8th week, 1% BLE inclusion meal exhibited the best which was significantly higher ($P< 0.05$) within BLE treated groups and that of the control. 3% BLE, 5% BLE and the control meal elicited statistical similarities ($P > 0.05$) at week 8.

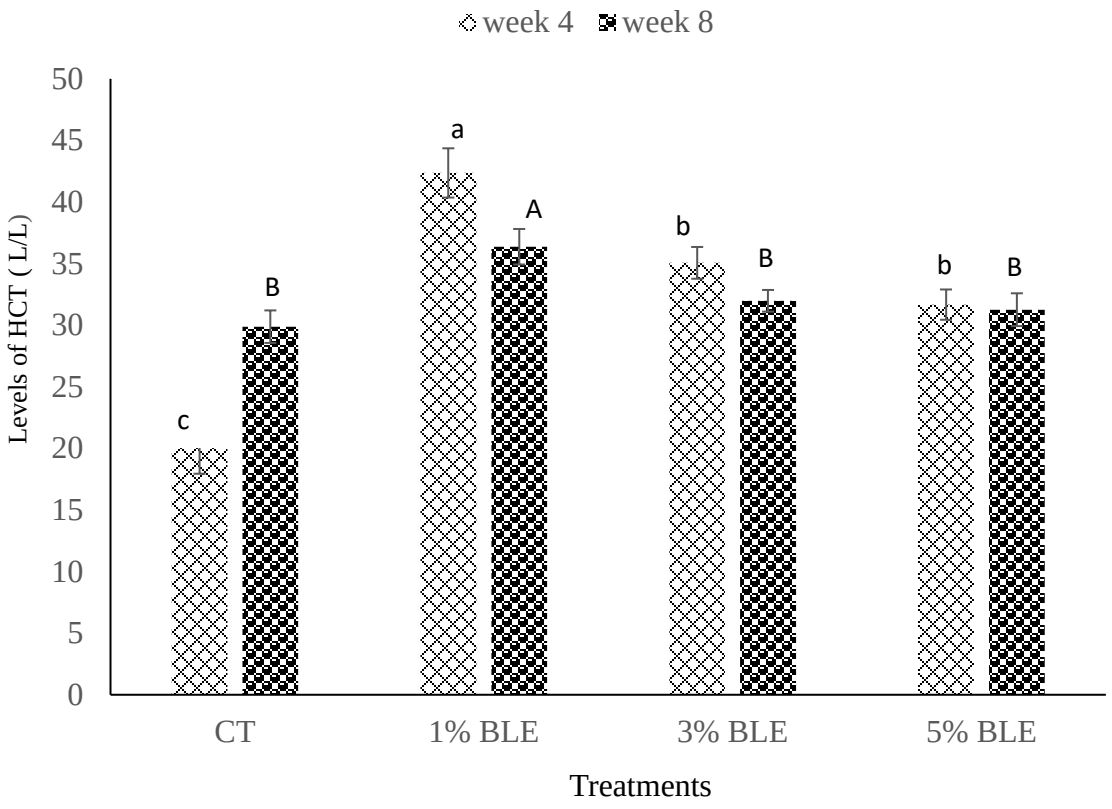

Figure 5: HCT levels of a Nile tilapia at 4 and 8 weeks fed control and BLE diet.

Note: Bars with the same alphabet per sampling time are not statistically different

4.2.6 MCV, MCH, MCHC of Nile tilapia fed with control and BLE diet

In table 6 is the results of MCV, MCH and MCHC. BLE treated diets increased significantly compared to the control after 4 weeks of feeding ($P < 0.05$). 1%BLE demonstrated significantly higher ($P < 0.05$) MCV followed by the control (0%BLE) which was also significantly higher in comparison to those fed 3%BLE and 5%BLE ($P < 0.05$) at the 8th week. MCH was significantly higher in fish fed BLE diets compared to the control ($P < 0.05$) at 4th and 8th week. MCHC at week 4 was improved statistically ($P < 0.05$) in fish fed 3%BLE and 5%BLE group compared to control and 1%BLE. MCHC at week 8 were statistically superior in BLE treated dietary group with 1%BLE exhibiting the best with control been the least ($P < 0.05$). At the end of the study, MCV, MCH, MCHC in fish fed with varying percentage of BLE were improved.

Table 6: MCV, MCH, MCHC of Nile tilapia fed with control and BLE diet

	dietary Treatment							
Parameter	**0% BLE**	**1% BLE**	**3% BLE**	**5% BLE**	**0%BLE**	**1%BLE**	**3%BLE**	**5% BLE**
	WEEK 4				WEEK 8			
MCV	96±13.29^{b}	125.36±4.54^{a}	123.53±2.34^{a}	127.26±6.04^{a}	131.23±0.89^{B}	142.93±6.22^{A}	104.13±27.5^{C}	10.76±0.52^{D}
MCH	40.76±0.77^{d}	52.83±30.77^{c}	146.36±20.90^{a}	122.93±9.28^{b}	42.0±2.74^{C}	57.90±3.72^{B}	106±4.75^{A}	50.30±1.12^{B}
MCHC	43.41±1.07^{c}	40.50±1.26^{c}	127.53±12.88^{a}	108.83±4.79^{b}	32.06±1.99^{D}	59.33±2.92^{B}	95.0±5.80^{A}	39.10±1.12^{C}

Note: Means in rows with the same superscript are not statistically different

Where MCV= mean corpuscular volume, MCH= mean corpuscular hemaglobin, MCHC= mean corpuscular hemaglobin concentration

4.3 Plasma chemistry

4.3.1 Total protein (TP) level of Nile tilapia fed control and BLE diet

Averages of TP level in fish fed both CT and BLE diets is shown in figure 6. At week 4, the TP level of tilapia fed BLE were enhanced and significantly higher compared to the control diet ($P < 0.05$). BLE treated diet exhibited statistical similarities at week 4 ($P > 0.05$). A similar trend was observed at week 8 where TP levels in the BLE treated groups were significantly superior compared to the control dietary ($P < 0.05$). Among the different BLE concentrations 1 % BLE, 3 % BLE and 5 % BLE were found to be highly significant within the BLE supplemented dietary group with a decreasing trend as the inclusion level of BLE increases ($P < 0.05$). BLE dietary elicited best TP levels at both 4 and 8 weeks of the period of study with the least been CT group.

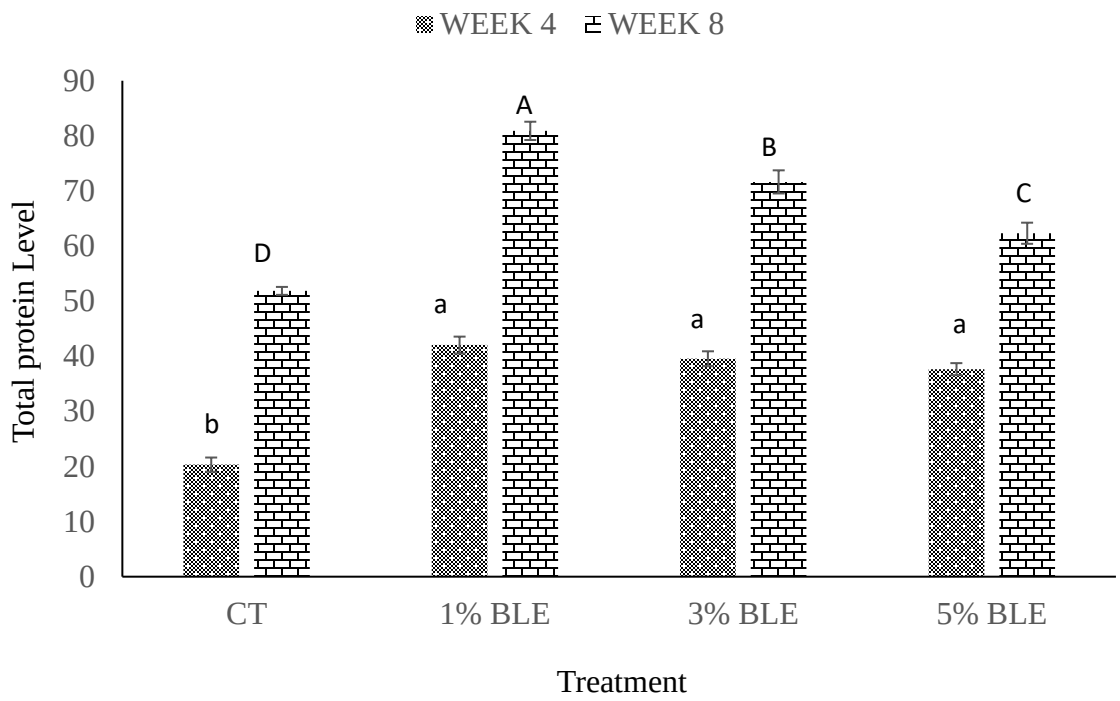

Figure 6: Total protein levels of Nile tilapia at 4 and 8 weeks of feeding trial

Note: Bars with the same alphabet per sampling time are not statistically different

4.3.2. Effects of treatments on Albumin levels.

Results from figure 7 shows the albumin level of fish fed both control and bitter leaf supplemented diets at 4 and 8 weeks. A similar trend was observed in the BLE supplemented diet at 4 and 8 weeks where all the BLE inclusion meals of fish had statistical similarities of albumin levels ($P < 0.05$) in comparison to the control. All in all, supplementing tilapia feed with 1% BLE – 5% BLE enhanced total protein levels.

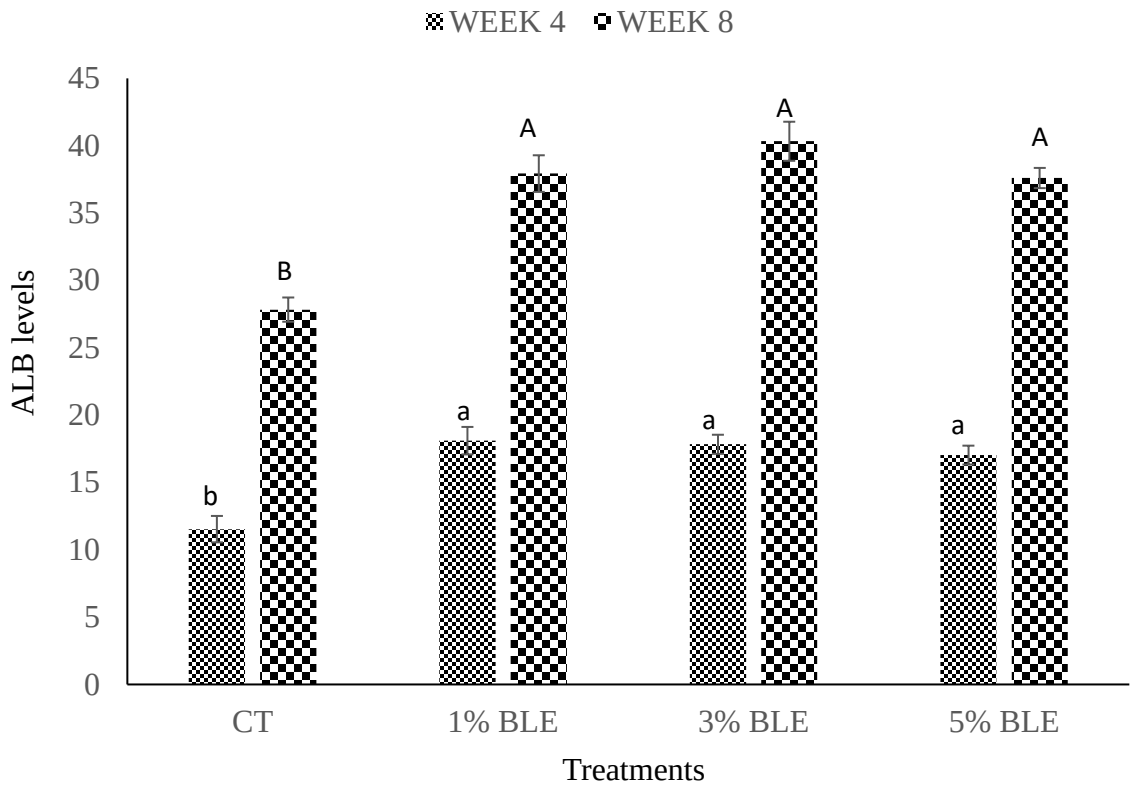

Figure 7: ALB levels of Nile tilapia at 4 and 8 weeks fed with control and BLE diets

Note: Bars with the same alphabet per sampling time are not statistically different

4.3.3 Y-GT levels of Nile tilapia fed with control and BLE diets

The sub protein (Y-GT) level of fish given control and BLE enriched diets at weeks 4 and 8 are shown in Table 7. Enriching tilapia diet with BLE helped improve significantly ($P < 0.05$) higher the levels of Y-GT of the experimental fish than the control group at week 4 and 8, with 3 % BLE and 1 % BLE supplemented diet recording the highest statistically ($P < 0.05$) within the BLE group at 4 and 8 weeks respectively with control diet being the lowest. Largely, the BLE diet boosted the level of Y-GT of tilapia.

Table 7**:** Y-GT of Nile Tilapia fed control and BLE supplemented diets

	dietary Treatment			
Week	**CT**	**1% BLE**	**3%BLE**	**5% BLE**
WEEK 4	0.33 ± 0.17^{c}	4.46 ± 0.63^{b}	7.23 ± 0.29^{a}	5.16 ± 0.31^{b}
WEEK 8	2.4 ± 1.39^{d}	48.06 ± 0.73^{a}	18.70 ± 6.7^{b}	5.16 ± 1.69^{c}

Note**:** Means in rows with the same superscript are not statistically different

4.3.4 T-BILL level of tilapia fed control and BLE diets

Effect of BLE dietary and control on T-BILL, sub-protein levels of Nile tilapia is indicated in table 8. Fish fed with a BLE enriched diet significantly improved ($P < 0.05$) higher level of T-BILL of fish compared to the control diet. 5 % BLE at the 4^{th} and 8^{th} week exhibited statistical superiority ($P < 0.05$) within the BLE dietary with the control group being the least at the termination of the experiment.

Table 8: T. BILL of Nile tilapia fed control and BLE diet after four and eight weeks

	dietary Treatment			
WEEK	**CT**	**1% BLE**	**3% BLE**	**5% BLE**
WEEK 4	1.39 ± 0.35^{c}	3.12 ± 0.25^{b}	$3.69\pm0.0.28^{b}$	5.48 ± 0.32^{a}
WEEK 8	1.77 ± 0.46^{c}	3.43 ± 0.17^{b}	3.67 ± 1.99^{b}	11.24 ± 3.33^{a}

Note: Means in rows with the same superscript are not statistically different

4.4 Toxic effect of dietary treatments in tilapia

4.4.1 ALT levels of a Nile tilapia fed control and BLE diets

In figure 9 is ALT levels of fish fed both control and bitter leaf inclusion meal. A clear observation was noticed with a rising trend of increasing percentage of BLE supplemented diet (i.e., 1 % BLE, 3 % BLE, and 5 % BLE) in each group. After week 4, the levels of ALT in the BLE supplemented diet group were highly different statistically ($P < 0.05$) than in the control group. Each BLE treated diet was statistically superior at week 4 ($P < 0.05$). At week 8, the control diet had a significantly higher ALT value than the BLE treatment groups ($P < 0.05$). There were no significant differences within the BLE treated diets ($P > 0.05$).

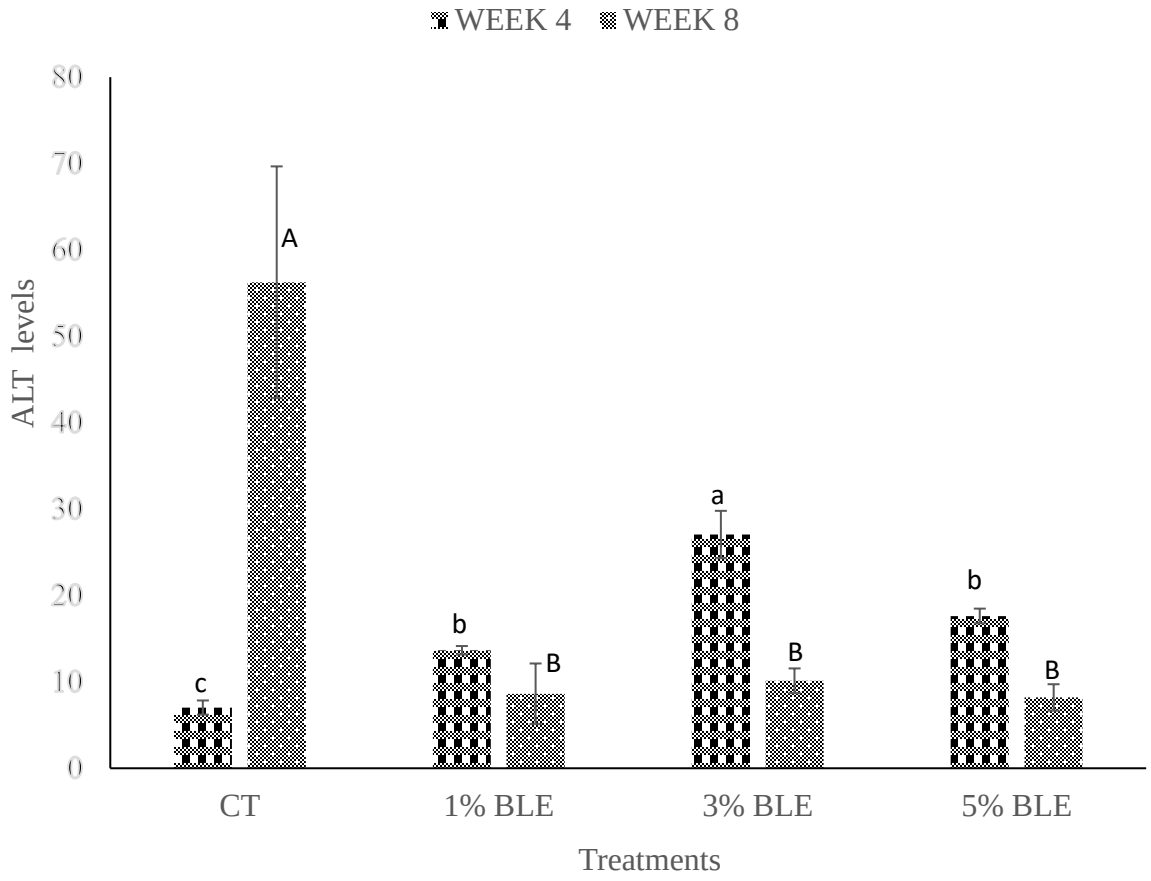

Figure 9: ALT levels of a Nile tilapia at 4 and 8 weeks fed control and BLE diets

Note: Bars with the same alphabet per sampling time are not statistically different

4.4.2 AST level of tilapia at 4 and 8 weeks fed with control and BLE diets

Figure 10 shows levels of AST in tilapia fed control and bitter leaf inclusion meal. The highest AST level was found in the BLE-treated diet at 5 % BLE, while the lowest was found in the control group at the 4th week. Week 8 exhibited a control diet and 5% BLE recorded the highest AST levels which were significant compared to 1%BLE and 3%BLE

treated diet ($P < 0.05$). All in all, AST levels in BLE dies were decreased at week 8 compared to the control group.

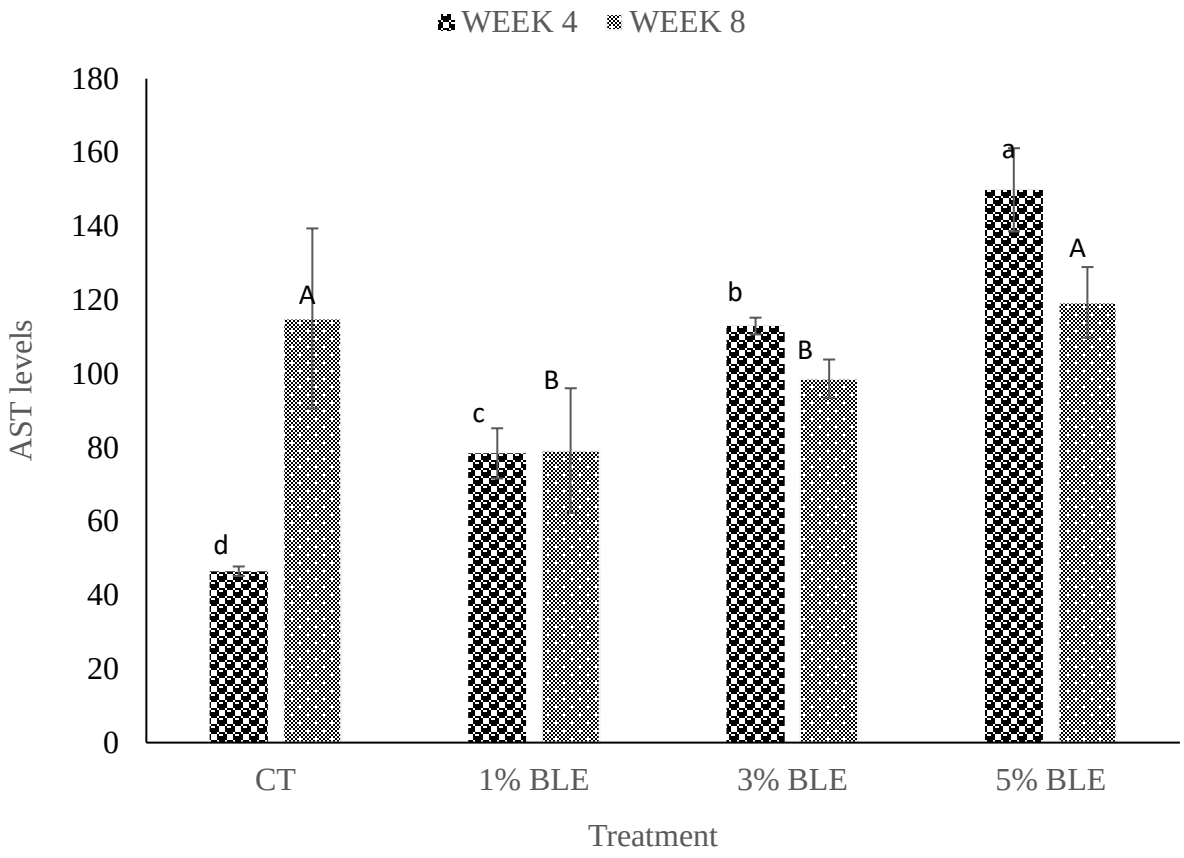

Figure 10: AST levels of Nile tilapia at week 4 and 8 fed control and BLE diets

Note: Bars with the same alphabet per sampling time are not statistically different

4.4.3 ALP level of tilapia fed control and BLE diets

Average levels of ALP in fish given control and bitter leaf supplemented diets is represented in figure 11. At week 4, ALP levels within the BLE supplemented diet was found to be significantly higher ($P < 0.05$) than the control. At week 8, the control group

had a superior significant difference ($P < 0.05$) than the BLE supplemented diet, while 1% BLE treated diet was different statistically ($P < 0.05$) among the BLE groups. It was observed at the end of week 8 that ALP levels were decreasing with increasing inclusion levels of BLE.

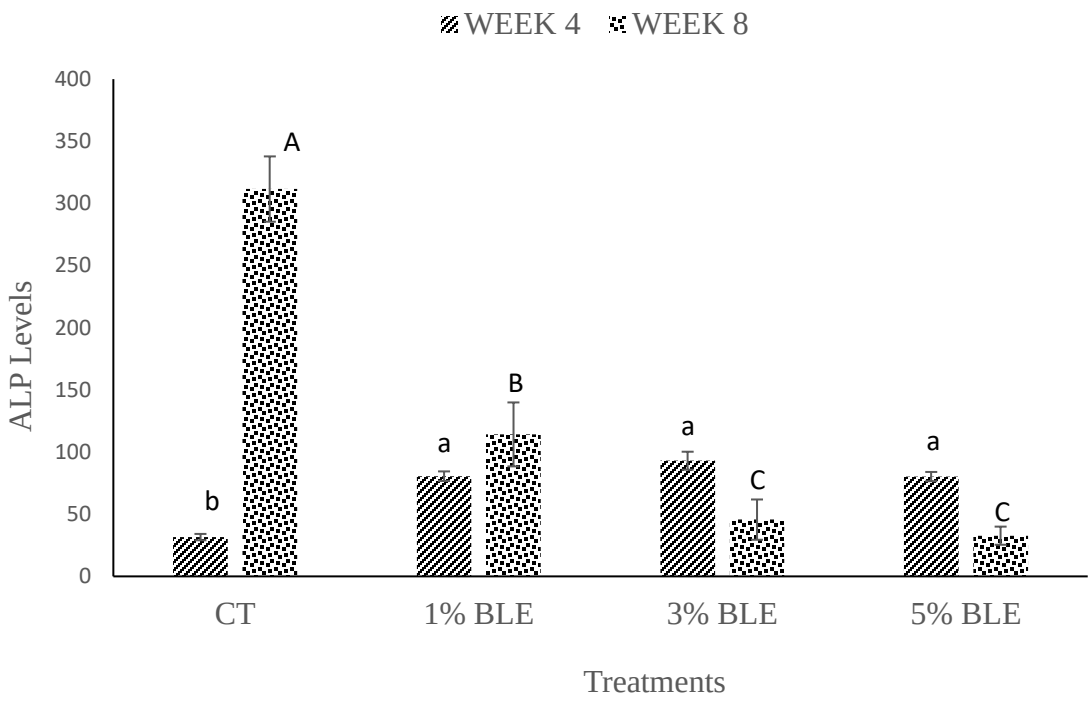

Figure 11: ALP levels of Nile tilapia after 4 and 8 weeks fed control and BLE diet

Note: Bars with the same alphabet per sampling time are not statistically different

4.5 Effect of Heat stress on tilapia after 8 weeks

4.5.1 Effect on WBC, HCT, HGB and RBC of tilapia fed control and BLE diet

Figure 12 is the influence of temperature (heat) stress on hematological parameters such as WBC, HCT, RBC, and HGB in fish fed with a diet enriched with BLE and a control. WBC in all the BLE groups was observed to have increased significant statistically compared to

the CT ($P < 0.05$). Observably, BLE treated meal highly elicited the levels of HCT compared to the control diet especially for 3 % BLE and 5 % BLE ($P < 0.05$) than the CT. BLE treated diet significantly enhanced HGB and RBC levels of tilapia in comparison to the control ($P < 0.05$). All in all, WBC, HCT, HGB and RBC were enhanced with increasing levels of BLE.

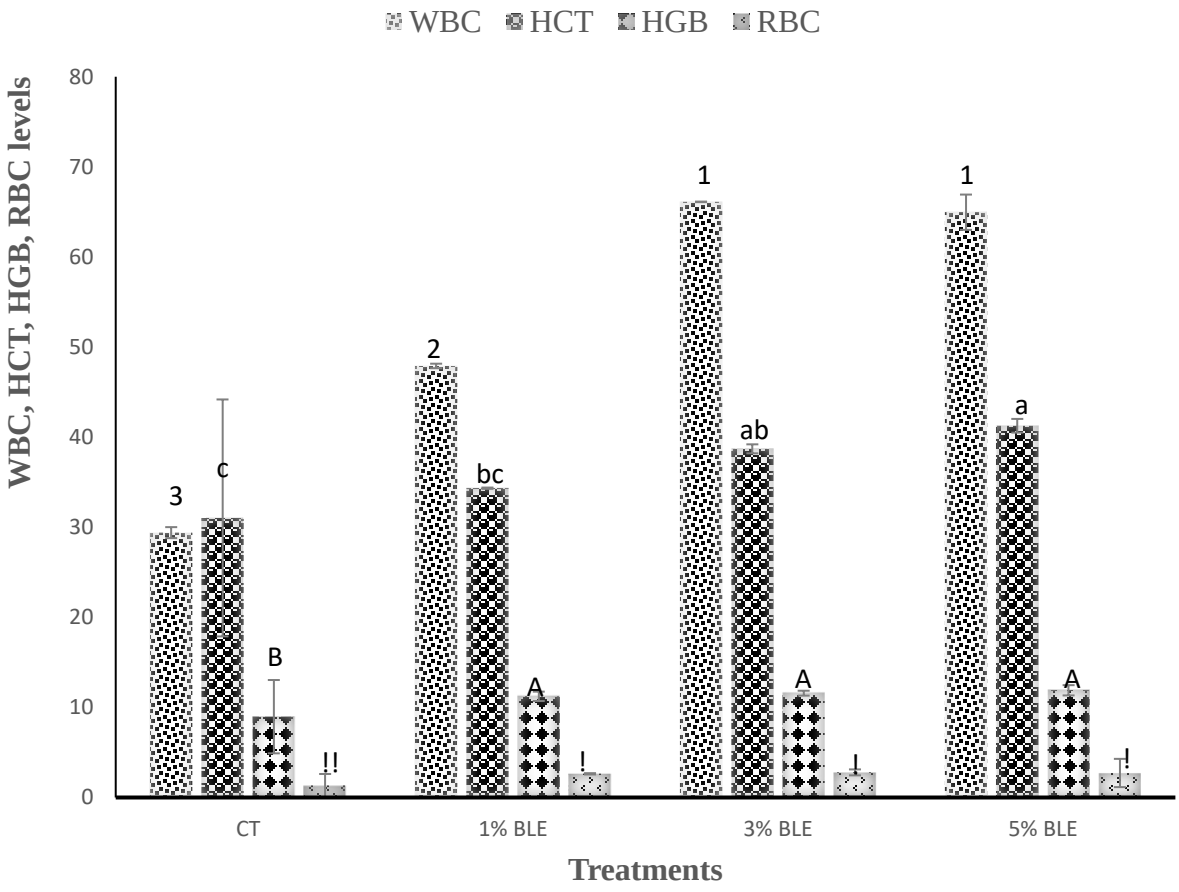

Figure 12: WBC, HCT, HGB and RBC levels of Nile tilapia after 8 weeks of heat stress

Note: Bars with the same alphabet numerals per sampling time are not statistically different

4.5.2 Differential WBCs of Nile tilapia following heat stress

Table 9 illustrates the effect of fish fed control and BLE-inclusion meals on differential WBCs (NEU, LYM, MON, EOS, BASO). LYM, MON and NUE at week 8 within the BLE experimental fish group were significantly higher than in the control ($P < 0.05$). Both BLE treated and a control diet did not effect fish in terms of BASO and EOS at the end of the study.

Table 9: Mean values of the differential WBCs hematological parameters of Nile tilapia after heat stress at week 8

	dietary Treatment			
Parameter	**CT**	**1% BLE**	**3%BLE**	**5%ml BLE**
LYM (%)	22.66±11.39^{c}	88.66±4.80^{a}	9.20±8.71^{b}	66.33±5.36^{ab}
MON (%)	1.40±0.70^{c}	3.03±0.06^{b}	10.45±1.60^{a}	9.00±0.73^{a}
BAS(%)	0.00±0.00^{c}	0.20±0.00^{b}	0.91±0.74^{a}	0.00±0.00^{c}
NUE (%)	36.90±31.51^{c}	98.47±1.53^{a}	86.33±5.78^{b}	100.00±0.00^{a}
EOS (%)	0.00±0.00^{c}	0.09±0.09^{b}	0.28±0.15^{a}	0.00±0.00^{c}

Note: Means in the same rows with the same superscript are not statistically different

Where LYM=lymphocyte, MON=monocyte, BAS=basophiles, NUE=nuetrophiles and EOS=eosinophiles

4.5.3 MCV, MCH, MCHC of Nile tilapia following the heat stress test.

Levels of MCV, MCH and MCHC in fish fed BLE and control diet are shown in table 10. Feeding tilapia with a BLE diet improved significantly the MCV, MCH and MCHC levels compared to the control group ($P < 0.05$). Within the BLE groups were also found to be highly significant ($P < 0.05$) with exception of the MCV which recorded statistical similarities ($P > 0.05$).

Table 10: MCV, MCH and MCHC of Nile tilapia following heat stress

	dietary Treatment			
Parameter	**CT**	**1% BLE**	**3% BLE**	**5% BLE**
MCV (x 109 L^{-1})	119.96 ± 11.63^{b}	140.76 ± 7.69^{a}	152.70 ± 4.89^{a}	126.43 ± 1.16^{a}
MCH (x 102 L^{-1})	43.26 ± 0.83^{d}	62.10 ± 7.35^{b}	95.96 ± 21.52^{a}	49.33 ± 0.27^{c}
MCHC (g dL^{-1})	43.41 ± 1.07^{d}	59.33 ± 2.92^{c}	127.53 ± 12.88^{c}	108.83 ± 4.79^{b}

Note: Means in rows with the same superscript are not statistically different

Where MCV= mean corpuscular volume, MCH= mean corpuscular hemaglobin, MCHC= mean corpuscular hemaglobin concentration.

CHAPTER FIVE

5. DISCUSSION

5.1 Growth parameters of Nile tilapia fed with BLE supplemented diets

In this study, the result suggests that including BLE in diets of tilapia from 1% - 5 % has the potential to improve weight gain and feed utilization better than control feed. However, lower supplementation of BLE thus at 1% is better than any dose above this. It therefore suggests that maintaining lower doses of BLE such as 1% inclusion is preferred to increase fish growth. The reasons for the decreasing performance with increasing BLE concentration could be ascribed to increasing bitterness of feed with a resultant low palatability, metabolic and digestive problems as having been explained earlier researchers (Lovell, 1989; Engin and Carter, 2001, 2005). However, the reason ascribed to the poorer performance with increasing BLE levels needs further investigation in future studies. In other studies, a similar improvement in growth and feed utilization have been reported using lower doses of medicinal plants (Guroy et al., 2012a; Güroy et al., 2013b).

5.2 Effect of BLE on hematology

The constituents of blood are affected by the quality, quantity, and toxicity of the food consumed by the animal and can be used to assess both the pathological and nutritional status of the animal. Generally, hematological parameters such WBCs, RBCs, HGB and HCT were significantly enhanced in fish fed the BLE diets compared to the control. This indicates that BLE included in the diets of tilapia has the potential to improve its' health status. This inference is drawn from Owen et al. (2011) who indicated that an increase in hematological parameters following herbal application is a good signal of improved health.

In this study, the data suggest that tilapia can fight diseases better due to the increase in WBCs following administration of BLE, particularly with 1 % supplementation. This is because Orun et al (2003) explained that organisms with an increase in WBCs could be a response to increasing resistance to infection on a pathological condition (Lunden & Bylund, 2000; Attia et al., 2017).

RBCs, in blood, aid in the transport of oxygen in the blood (Owen & Amakiri, 2011; Banuelos-vergas & Gamboa-mendoza, 2019; Satheeshkumar et al., 2012). HCT measures the mass percentage of RBCs in the blood, while HGB is the oxygen-carrying protein (Reverter et al., 2014) and it keeps the organism as stable as possible, including stress-related and hypoxic conditions caused by the presence of toxicants (Lunden & Bylund, 2000; Etim et al., 2013) or environmental stress, e.g., temperature(heat) (Udoh & Udoidiong, 2004; Kunjiappan et al., 2015).

In the present study, it was observed that BLE supplementation increased RBCs, HGB and HCT in tilapia suggesting tilapia can have a greater ability to take in oxygen in limited conditions due to the increase in the RBCs HGB and HCT levels. Therefore, fish fed BLE supplemented diets could survive better in low oxygen conditions than others (Anjusha et al., 2019; Leung & Bate, 2013; Liu, 2017).

5.3 Effect of BLE on plasma chemistry of tilapia

A total protein test is done on blood serum to aid diagnose medical conditions, such as kidney and liver diseases (Islam et al., 2017; Hang & Bertozzi, 2005). A decrease in total protein from normal levels may suggests poor feed intake and feed utilization while an increase in total protein in the blood of an organism may suggest better feed intake and

utilization (Nwajo, 2005). In the present study BLE supplementation significantly soared the plasma total protein. This suggests that BLE can improve the efficiency of protein use in feed. Gareau et al. (2011) reported improved protein utilization in catfish broodstock fed with various percentages of *V. amygdalina* and suggested that the active ingredients such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids, and essential oils in the bitter leaf, accounted for this.

5.4 Effect of BLE on liver health of tilapia

ALT, ALP and AST are essential enzymes in the liver of fish that aid in protein metabolism (Garcia et al., 2005; Hasan et al., 2006; Kunjiappan et al., 2015). When these enzymes are discovered in considerable amounts in the blood/serum, they may indicate tissue damage or organ dysfunction caused by toxicity (Feroz and Khan, 2011; Yin et al., 2011; Ming et al., 2012). Significant elevations in AST and ALT levels, for example, have been observed in pufferfish (*Takifugu obscurus*) (El-Dakar; Cheng et al., 2017), Yellow perch (*Perca flavescens*), (Elabd et al., 2017) and in tilapia (*Oreochromis niloticus*) (Bauer et al., 2000) after exposure to several herbal dietary inclusions. In contrast, feeding tilapia with probiotic-enriched diets results in a considerable reduction in AST and ALT (Aylward et al., 2014; Bajagai et al., 2016).

At week 4, tilapia exposed to various levels of BLE supplemented diet showed an increase in AST, ALT, and ALP with an increase in BLE concentrations compared to the control fish. It is believed this might be as a result of their initial tolerance to the BLE in the feed; however, at week 8, all of the BLE-treated diets had decreased levels of AST, ALT, and ALP compared to control, indicating that BLE-treated diets could aid in improving the health of the fish's liver, which can be linked to their potential to protect the liver of fish

instead of harming it. Support for this explanation is drawn from earlier reports (Ayoola et al., 2008).

5.5 Tilapia resistance to heat stress

Environmental stress (Xie et al., 2008; Larsson, 1989; Van Rijn & Reina, 2010) and temperature stress (Zhang et al., 2012) can cause stress in aquatic environments as well as significant losses in farmed fish (Zarychachanski & Houston, 2008; Yousefi et al., 2019). As a result, the primary goal of current research is to improve fish stress resistance so that they can endure the consequences of environmentally benign stress circumstances (Vedavathy, 2003; Liu et al., 2016).

During stressful conditions, levels of RBC, WBC, HGB and HCT have been reported to increase in fish (Harikrishnan et al., 2010b). Stressful conditions, such as HYS, can result in respiratory metabolic issues (Yun et al., 2014), CDS (Freeman et al., 2018; Freed et al., 2020) and HTS (Zhang et al., 2012) can alter the oxygen demand in organisms. A study in tilapia, *Oreochromis niloticus* by Qiang et al. (2013), tench, *Tinca tinca* (Bao et al., 2018; De Pedro et al., 2005) and in rainbow trout, *Oncorhynchus mykiss* depicted significant increase in the levels HCT, HGB, and RBSs, have been documented following exposure to water temperature and oxygen stressors (Garibaldi, 2012).

Furthermore, a significant increase in these parameters has been observed in Goldfish, Carassius auratus (Harikrishnan et al., 2010b) and *Paralichthys olivaceus* (Harikrishnan et al., 2011) due to the heightened response. In the present study, it appears that there was an increase in the above-mentioned hematological parameters in all fish groups in response to heat stress treatment. However, fish fed with BLE supplements appeared to respond better

than the control as the hematological parameters recorded were significantly higher. This means that, during heat stress, fishes when fed with BLE are in a better state to survive compared to those not fed with BLE.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, supplementing tilapia diet at 1% - 5% improves the growth of tilapia; however, 1% BLE appeared better compared to others.

Also, supplementing the tilapia diet at 1% - 5% significantly improve hematological parameters, plasma chemistry with 1 % BLE exhibiting the best influence on the above hematological indices in comparison to the other treatments.

Additionally, feeding tilapia with a 1% - 5% BLE inclusion levels seems not to have any negative effect (toxic) on the liver health of tilapia after 8 weeks of feeding.

More to it, tilapia fed with varying percentages of BLE ranging from 1% - 5% can improve resistance to heat stress in tilapia.

6.2 Recommendations

A palatability and digestibility test is recommended in future trials to help understand and improve feed acceptance of fish fed with BLE supplementation.

An artificial pathogenic infection test should be carried out on a fish fed BLE supplemented diet to ascertain its ability to increase resistance.

Outdoor culture trial may be necessary to understand the dynamics of BLE fed fish in real-time situations.

It is also needful to improve the processing methods to eliminate the anti-nutritional factors in *V. amygdalina* diets for fish.

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APPENDICES

Appendix 1: Growth pattern of nile tilapia fed BLE and a control diet, week zero (0)

	dietary Treatment							
Week (0)	**0% BLE**		**1% BLE**		**3% BLE**		**5% BLE**	
	BW(g)	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**	**TL(cm**	**BW(g)**	**TL(cm)**
	29	13.6	31	12.9	29	13	30	12.9
	30	12.9	28	12.8	31	13.1	29	12.5
	31	12.8	29	13.2	30	12.9	30	11.9
	30	12.7	31	13	29	13.2	28	12.8
	29	12.9	29	12.9	32	12.9	31	12.9
	31	13.2	30	11.9	33	13.8	34	12.4
	29	12.9	28	13.1	27	12.9	32	13.1
	30	13.0	31	12.8	29	12.4	31	13.5
	29	12.6	30	12.9	28	13.1	29	13.4
	30	12.8	31	13.6	29	12.9	30	13.6
	31	12.9	29	13.8	31	13.6	29	13.5
	29	13.4	30	13.4	30	13.7	31	12.8
	30	13.2	28	13.8	31	13.4	29	13.2

	31	13.6	31	13.7	31	12.8	30	12.8
	30	13.7	30	12.8	32	11.6	30	12.5
	31	12.8	29	13.4	29	11.9	29	13.2
	29	12.6	30	13.3	30	12.6	31	12.8
	30	12.4	30	12.7	29	13.1	30	12.7
	31	11.9	29	12.5	31	12.8	29	12.9
	29	13.1	31	13.4	28	12.9	31	13.1
AVERAGE	**30.1**	**12.6**	**30.2**	**12.8**	**30.1**	**12.7**	**30.2**	**12.9**

Where BW is body weight, TL is total length

Appendix 2: Growth pattern of nile tilapia fed BLE and a control diet, week two

	dietary Treatment							
Week (2)	**0% BLE**		**1% BLE**		**3% BLE**		**5% BLE**	
	BW(g)	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**	**TL(cm)**
	53	13.5	50	14.2	39	13	52	13.6
	43	14	38	14.9	45	13.5	48	12.4
	48	13.8	47	13.9	47	12.8	53	13.4
	45	13.4	53	13.4	46	12.5	74	12.4
	42	13.9	43	12.7	50	13.5	38	13.4

	27	14.2	50	12.4	39	12.8	56	13.4
	41	15.3	43	13.4	48	13.4	48	13.4
	37	13.7	50	12.9	40	12.4	47	13.8
	47	12.9	42	11.9	44	13.4	45	13.4
	36	14.1	46	13.3	37	12.4	38	13.9
	42	13.6	45	14.2	37	13.7	45	14.1
	34	12.9	36	13.6	36	12.6	36	13.7
	40	12.4	50	14.2	39	13.2	41	14.3
	32	13.2	35	13.4	45	12.9	35	14.6
	33	12.6	44	14.1	36	13.6	42	13.7
	37	13.4	40	13.7	42	14.1	38	13.4
	35	12.4	41	13.8	33	14.2	32	12.8
	33	12.9	36	13.4	31	14.3	33	13.4
	29	13.4	44	13.7			34	12.9
AVERAGE	**38.63**	**13.2**	**43.21**	**13.8**	**40.2**	**13.6**	**43.35**	**13.7**

Where BW is body weight, TL is total length

Appendix 3: Growth pattern of nile tilapia fed BLE and a control diet, week 4

	dietary Treatment						
Week 4	**0% BLE**		**1% BLE**		**3% BLE**		**5% BLE**
	BW(g)	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**
TL(cm)							
	42	14.2	56	14.4	50	14	58
15.5							
	41	14	52	15	42	14	48
14.7							
	30	13	51	15	44	14.5	58
15.6							
	42	13.6	49	14.5	51	14.5	59
15.5							
	30	13	43	14.5	42	14.5	56
15.5							
	50	15	52	15	44	14.5	53
15							
	45	15.1	60	16	33	13.5	38
13.5							
	41	13.2	47	14.5	46	14.7	48
14.5							
	51	14.5	53	14.6	45	14.5	47
14.9							
	43	15.2	48	320	42	15	49
15							
	36	12.6	57	15	53	15.5	36
13.4							
	52	14	56	15	51	15	45
14.5							
	42	14.5	45	15	45	14.5	40
13.5							
	41	13.5	49	14.5	50	15	40
13.5							

	45	14.8	43	14.5	50	14.5	36
13.5							
	41	14.5	40	13.6	46	14.5	36
13.5							
	36	13.5	39	13.6	40	13	49
14.5							
	36	13	40	13.5	41	13.5	42
13.4							
	29	13	37	13.2			47
14.3							
AVERAGE	**40.68**	**13.9**	**48.26**	**14.54**	**45.57**	**14.35**	**46.57** **1442**

Where BW is body weight, TL is total length

Appendix 4: Growth pattern of nile tilapia fed BLE and a control diet, week six

	dietary Treatment						
Week (6)	**0% BLE**		**1% BLE**		**3% BLE**		**5% BLE**
	BW(g)	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**	**TL(cm)**	**BW(g)**
TL(cm)							
	53	15	58	16	57	15	64
16							
	51	15	63	16	64	15.5	66
16							
	41	13.5	62	15.7	61	15	53
15							
	52	15	53	14	59	14.6	44
13.7							
	49	14	55	15	60	14.8	58
15.5							
	58	15	61	15	49	14	60
15.5							
	51	14.7	52	14.5	57	14.7	56
15							

	45	14	56	14.5	55	14.7	55	14.5
	45	14.5	58	15	55	15.3	47	14.5
	54	15.3	59	15	46	13.4	54	14.5
	37	12.8	50	14.8	45	14	53	15
	48	14.2	47	14.5	47	13.2	50	14
	50	14.4	49	14	48	13.6	44	13.5
	44	14	51	13.5				
	36	13						
AVERAGE	**47.61**	**14.29**	**55.28**	**14.82**	**54.07**	**14.44**	**53.52**	**14.63**

Where BW is body weight, TL is total length

Appendix 5: Growth pattern of nile tilapia fed BLE and a control diet, week 8

	dietary Treatment							
Week (8)	**0% BLE**		**1% BLE**		**3% BLE**		**5% BLE**	
	BW(g)	**TL(cm)**	**BW(g)**	**TL(g)**	**BW(g)**	**TL(cm)**	**BW(g)**	**TL(cm)**
	52	15	67	16	68	16	66	16.3
	51	15	68	16	69	16	54	14
	53	16	67	15	69	15.8	68	15
	52	15.5	66	15.5	55	14	62	16
	51	15.1	67	15.5	69	16	60	15

	42	14.5	68	15.2	66	16	65
15							
	49	15	68	15	64	15.4	47
13.9							
	53	14	69	14	66	15.1	46
14.2							
	51	15	70	13.7	59	14.4	50
14.5							
	45	13.5	69	13.9	56	14	59
15							
	52	15	68	15.5	57	13.5	54
14							
	44	14	68	13.6	50	14.7	58
13.6							
	46	13.7	68	15.6			54
14.2							
	51	14.6	69	14.8			
AVERAGE	**49.4**	**14.72**	**68**	**14.95**	**62.3**	**15.07**	**57.15**
14.66							

Where BW is body weight, TL is total length

Appendix 6: Blood profile of tilapia at week 4

Hematological Parameter	**dietary Treatment**											
	0% BLE			**1% BLE**			**3%BLE**			**5%ml BLE**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
WBC	0.37	0.42	0.51	1.92	1.86	1.96	1.52	1.71	1.62	1.2	1.35	1.29
RBC	1.46	1.98	1.76	3.3	3.9	3.7	3.58	2.72	3.11	2.89	2.75	2.84

HGB	9.2	10.6	12.3	17	18.3	17.8	13.9	14.8	13.6	12.99	13.56	12.5
HCT	18.2	22.5	25.4	38.5	43.4	25.4	35.07	37.3	32.81	29.65	33.9	31.46

Appendix 7: Differential WBC of nile tilapia at week 4

Hematological	**dietary Treatment**										
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**	
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**
C											
LYM(%)	17.4	12.33	14.67	46.25	52.41	48.11	41.61	52.88	59.85	74.21	62.66	57.52
MON(%)	0	21.95	22.85	40.21	38.26	35.78	43.56	53.97	55.46	42.72	35.25	33.42
NUE(%)	27.62	17.64	29.54	52.14	57.24	41.8	84.37	75.61	63.69	53.63	57.98	72.76
EOS(%)	0	0.29	0.52	3.1	2.85	2.01	3.08	2.33	3.66	15.23	13.54	17.48
BAS(%)	0	0.09	0.24	0.4	0.78	0.64	0	0.99	0.86	1.56	1.86	1.48

Appendix 8: MCV, MCH and MCHC of nile tilapia at week 4

Hematological Parameter	dietary Treatment										
	0% BLE			1% BLE			3%BLE			5%ml BLE	
	A	B	C	A	B	C	A	B	C	A	B
C											
MCV	90.85	102.2	97.45	116.7	127.3	132.1	127.7	119.6	123.3	123.5	119.2
139.1											
MCH	39.8	42.3	40.2	51.5	54.2	52.8	178.6	153.3	107.2	108.2	140.1
120.5											
MCHC	41.5	45.23	43.5	54.1	59.7	64.2	152.8	119.3	110.5	100.4	117
109.1											

Appendix 9: TP, ALB, y-GT and T-BIL of nile tilapia at week 4

Hematological Parameter	dietary Treatment										
	0% BLE			1% BLE			3%BLE			5%ml BLE	
	A	B	C	A	B	C	A	B	C	A	B
C											
TP	18.4	22.8	19.8	41.8	44.8	39.5	37.06	41.7	39.9	37.8	39.5
35.7											
ALB	13.2	9.8	11.6	78.4	16.2	19.7	19.2	17.5	16.8	17.3	15.7
18.1											
y-GT	0.4	0	0.6	3.3	4.6	5.5	7.1	6.8	7.8	5.2	4.6
5.7											
T-BIL	1.18	0.91	2.09	3.41	2.61	3.35	4.19	3.68	3.22	6.09	5.38
4.97											

Appendix 10: Toxic effect of BLE supplemented and a control diet of a nile tilapia at week 4

Hematological Parameter	dietary Treatment										
	0% BLE			1% BLE			3%BLE			5%ml BLE	
	A	B	C	A	B	C	A	B	C	A	B
C											
ALT	8.6	5.8	6.7	12.9	14.6	13.5	22.2	27.3	31.7	17.1	19.3
16.5											
AST	46.5	44.2	48.7	91.8	69.5	73.9	113.6	108.7	116.5	167.9	152.6
129.1											
ALP	27.9	36.5	30.4	73.6	86.9	81.3	102.6	78.8	97.7	86.6	82.2
73.1											

Appendix 11: Blood profile of tilapia fed both control and a BLE diet at week 8

Hematological Parameter	dietary Treatment										
	0% BLE			1% BLE			3%BLE			5%ml BLE	
	A	B	C	A	B	C	A	B	C	A	B
C											
WBC	20	23	19	97.29	78.85	86.49	80.63	78.18	80.45	73.41	
70.38 77.15											
RBC	1.46	1.98	1.76	3.3	3.9	3.7	3.58	2.72	3.11	2.89	2.75
2.84											
HGB	9.2	10.6	12.3	17	18.3	17.8	13.9	14.8	13.6	12.99	
13.56 12.5											

HCT	18.2	22.5	25.4	38.5	43.4	45.2	35.07	37.3	32.81	29.65	33.9	31.46

Appendix 12: Differential WBC of nile tilapia fed BLE and a control diet at week 8

Hematological	**dietary Treatment**											
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
LYM(%)	67.21	32.19	0	0	67.33	86.84	53.69	8.18	52.81	0.05	0.04	0.06
MON(%)	6.64	21.83	9.84	9.53	12.1	0.13	17.74	11.35	13.71	8.33	11.06	13.25
NUE(%)	25.95	45.96	90.14	90.44	20.55	12.9	28.55	80.39	33.45	91.61	88.92	95.21
EOS(%)	0.2	0.02	0.02	0.03	0.02	0.13	0.02	0.08	0.03	0.05	0.02	0.04
BAS(%)	0	0	0	0	0	0	0	0.09	0.86	0	0	0

Appendix 13: MCV, MCH and MCHC of nile tilapia at week 8

Hematological	**dietary Treatment**											
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
MCV	129.5	132.5	131.7	150.6	147.6	130.6	49.1	129.7	133.6	11.6	9.8	10.9

MCH	40.2	47.4	38.4	59.5	63.4	50.8	37.2	51.1	51.8	48.4	50.2	52.31
MCHC	31.1	35.9	29.2	39.6	43	38.9	56.6	39.5	38.9	37.1	39.2	41.01

Appendix 14: TP, ALB, y-GT and T-BIL of nile tilapia at week 8

Hematological	**dietary Treatment**											
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
TP	51.7	53.2	50.7	78.5	84.1	80.1	75.8	70.1	69.02	58.7	65	60
ALB	26.4	27.6	29.5	37.1	40.6	36.1	43	38	40	36.4	37.4	39
y-GT	55.1	58.4	30.7	20.7	69	25.3	21	29.1	6	6.3	7.9	2.3
T-BIL	2.7	1.21	1.4	0.09	0.7	0.5	1.78	7.67	1.58	19.6	9.8	6.33

Appendix 15: Toxic level of nile tilapia fed BLE and a control diet at week 8

Hematological	**dietary Treatment**											
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
ALT	10.9	21.7	46.2	1.8	10.7	13.4	13	8.9	8.5	11.2	7.3	6.1

AST	72.4	72.8	99.8	57.7	47.6	50.8	107.1	89.1	99.7	37.8	10.6
14.4											
ALP	148.1	441.1	345.5	20.7	243.2	79.3	19.6	42	75.4	45.9	31.6
20.7											

Appendix 16: Blood profile of tilapia after heat stress at week 8

Hematological	**dietary Treatment**										
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**	
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**
C											
WBC	30.18	28.18	29.85	47.42	45.92	50.37	65.09	59.66	73.66	80.18	
38.77	75.95										
RBC	1.04	1.07	1.08	2.78	2.61	2.52	1.98	3.76	2.63	2.84	2.58
2.76											
HGB	9	8.9	9	10.8	11.9	11	11.62	12.04	11.09	11.3	11.5
12.9											
HCT	33	27.1	33	31.4	34.8	36.8	38.4	37.9	39.8	39.8	
41.54	42.4										

Appendix 17: Differential WBC of nile tilapia after heat stress fed BLE and a control diet at week 8

Hematological	**dietary Treatment**										
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**	
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**
C											

LYM(%)	32	0	36	98	86	82	50.71	76.64	50.26	56	69	74
MON(%)	2.1	0	2.1	3.1	2.9	3.1	13.31	7.75	10.31	8.7	7.9	10.4
NUE(%)	5.47	100	5.47	100	100	95.41	87	96	76	100	100	100
EOS(%)	0	0	0	0	0	0.28	0	0.32	0.52	0	0	0
BAS(%)	0	0	0	0	0	0	0	0.35	0.38	0	0	0

Appendix 18: MCV, MCH and MCHC of nile tilapia at week 8 after heat stress

Hematological	**dietary Treatment**											
Parameter	**0% BLE**			**1% BLE**			**3%BLE**			**5%ml BLE**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
MCV	161.6	126.7	161.6	154.6	139.7	128	143.2	155.4	159.5	124.3	126.2	128.3
MCH	44.1	41.6	44.1	76.5	57.5	52.3	53.6	110.5	123.8	49.5	49.7	48.8
MCHC	27.3	32.8	27.3	49.7	41.2	41	68.8	71.1	78	39.9	39.3	38.1

Appendix 19: VSI, HSI and CF of nile tilapia at week 8

Dietary Treatment							
0% BLE							
BW	**TL**	**TL^3**	**VW**	**LW**	**VSI**	**HS1**	**CF**
49	14.2	2863.288	2.8	0.04	5.714286	0.081633	1.711319
45	13.5	2460.375	2.6	0.05	5.777778	0.111111	1.828989
52	14.5	3048.625	2.7	0.03	5.192308	0.057692	1.705687
AVRG							
48.66667	14.06667	2790.763	2.7	0.04	5.561457	0.083479	1.748665
3.511885	0.51316	300.7565	0.1	0.01	0.321265	0.026757	0.06962
2.027588	0.296273	173.6418	0.057735	0.005774	0.185482	0.015448	0.040195

Where BW is body weight, TL is total length, VW is visceral weight, LW is liver weight, VSI is viscerosomatic index, HIS is hepasomatic index, CF is condition factor.

Appendix 20: VSI, HIS, and CF of nile tilapia at week 8

Dietary Treatment							
1% BLE							
BW	**TL**	**TL^3**	**VW**	**LW**	**VSI**	**HS1**	**CF**
62	16	4096	3.6	0.47	5.806452	0.758065	1.513672
53	15	3375	3.5	0.41	6.603774	0.773585	1.57037
55	15.5	3723.875	4	0.39	7.272727	0.709091	1.476956
AVRGE							
56.66667	15.5	3731.625	3.7	0.423333	6.560984	0.746913	1.520333
4.725816	0.5	360.5625	0.264575	0.041633	0.734074	0.033662	0.047062
2.728451	0.288675	208.1708	0.152753	0.024037	0.423818	0.019435	0.027171

Where BW is body weight, TL is total length, VW is visceral weight, LW is liver weight, VSI is viscerosomatic index, HSI is hepasomatic index, CF is condition factor.

Appendix 21: VSI, HIS and CF of nile tilapia at week 8

Dietary Treatment							
3%BLE							
BW	**TL**	**TL^3**	**VW**	**LW**	**VSI**	**HS1**	**CF**
52	14.5	3048.625	4	0.3	7.692308	0.576923	1.705687
50	14	2744	4	0.4	8	0.8	1.822157
52	15	3375	3.88	0.33	7.461538	0.634615	1.540741
AVRG							
51.33333	14.5	3055.875	3.96	0.343333	7.717949	0.670513	1.689528
1.154701	0.5	315.5625	0.069282	0.051316	0.270145	0.11579	0.141402
0.666667	0.288675	182.1901	0.04	0.029627	0.155968	0.066851	0.081639

Where BW is body weight, TL is total length, VW is visceral weight, LW is liver weight, VSI is viscerosomatic index, HIS is hepasomatic index, CF is condition factor.

Appendix 22: VSI, HSI and CF of nile tilapia at week 8

Dietary Treatment							
5% BLE							
BW	**TL**	**TL^3**	**VW**	**LW**	**VSI**	**HS1**	**CF**
51	15	3375	4.1	0.36	8.039216	0.705882	1.511111
55	15	3863	3.8	0.28	6.909091	0.509091	1.62963
48	14.5	3048.625	3.4	0.34	7.083333	0.708333	1.57448
AVRG							
51.33333	14.83333	3266.208	3.766667	0.326667	7.34388	0.641102	1.57174
3.511885	0.288675	188.4327	0.351188	0.041633	0.608448	0.114332	0.059307
2.027588	0.166667	108.7917	0.202759	0.024037	0.351288	0.066009	0.034241

Where BW is body weight, TL is total length, VW is visceral weight, LW is liver weight, VSI is viscerosomatic index, HIS is hepasomatic index, CF is condition factor.

INFLUENCE OF BITTER LEAF (VERNONIA AMYGDALINA) EXTRACT ON NILE TILAPIA, OREOCHROMIS NILOTICUS GROWTH, HEMATOLOGY, AND HEAT STRESS RESPONSE

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