UNIVERSITY FOR DEVELOPMENT STUDIES

PERFORMANCE OF *BACILLUS SUBTILIS* IN IMPROVING GROWTH, HAEMATOLOGY AND LIVER HEALTH OF THE AFRICAN CATFISH (*Clarias gariepinus*, BURCHELL 1822)

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2022

UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

FACULTY OF BIOSCIENCES

DEPARTMENT OF FISHERIES AND AQUATIC RESOURCES MANAGEMENT

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BY

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UDS/FIS/0002/19

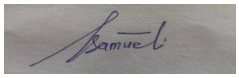
THESIS SUBMITTED TO THE DEPARTMENT OF FISHERIES AND AQUATIC RESOURCES MANAGEMENT, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN FISHERIES SCIENCE

SEPTEMBER 2022

DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere:

Candidate's Signature:  Date:……

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Dr. Akwasi Ampofo-Yeboah

ABSTRACT

The purpose of this research was to assess the effects of dietary probiotic (*Bacillus subtilis*) supplementation on haematology, growth and health of *Clarias gariepinus*. The feeding trial was conducted for 8 weeks. Average-weighted fish of 13.0 ± 0.22g were distributed into four groups, one of which was a control and the remaining three as treatment groups. The control diet (0 g/kg Bs) was made without the addition of probiotics, whereas the remaining three groups were prepared supplemented with the probiotic at different levels (10g/kg Bs, 20g/kg Bs and 30g/kg Bs). The length and weight of experimental fish were taken at intervals 0,15,30,45 and 60 days to evaluate growth parameters (initial weight, final weight, feed conversion ratio, weight gain) and body indices (condition factor, viscerosomatic index, and hepatosomatic index) of experimental fish. Blood samples were collected at the intervals 30 and 60 days for liver, plasma chemistry and haematological analysis. The haematological parameters such as red blood cells, haematocrit, haemoglobin concentration, and haematological indexes (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations) were examined. Total protein and albumin levels were measured in the plasma and also examined. Aspartate aminotransferase, Alanine transaminase and Alkaline phosphate were analysed to check the state of the liver. After eight weeks, the results showed that, fish groups fed with treated diets showed much significant improvement in growth and condition indices in comparison to the control group. The *Bacillus* fed group (10g/kg Bs) showed an increase significantly in haematological parameters than in other groups. An increase significantly in Aspartate aminotransferase, Alanine transaminase and Alkaline phosphate were observed at the end of four weeks in the Bs treated diets in comparison to the control group, decreasing significantly at the end of eight weeks to optimum ranges compared to the control. The result suggests that dietary administration of *Bacillus subtilis* at 10g/kg can be used effectively to improve growth and health status of fish.

ACKNOWLEDGEMENT

First and foremost, I would like to give thanks to God Almighty for His guidance and protection throughout the study in the University. I sincerely appreciate the effort, advice and supervision of Dr. Emmanuel Delwin Abarike and Dr. Akwasi Ampofo-Yeboah of the Department of Fisheries and Aquatic Resources Management. Had it not been for your guidance, motivation, encouragement and support, this work would not have been a success.

My sincere gratitude to the Dean of the Faculty of Bioscience, Prof. Elliot H. Alhassan for his consistent encouragement and advice. Likewise, the Head of the Department of Fisheries and Aquatic Resources Management, Dr. Daniel Nsoh Akongyuure. More to all the staff of the faculty of Bioscience for their encouragement, I am grateful.

Special thanks to my friends: Samuel Opoku Dandi and Emmanuel Owusu for assisting me in diverse ways towards the success of this work.

My heartfelt gratitude and appreciation go to my loving father and mother, as well as the rest of my family, for their love, patience, support, and understanding throughout my studies, and, most importantly, to the Almighty God who made it all possible.

DEDICATION

This thesis is dedicated to my parents, Mr. Wisdom Kwadwo Osei and my mother, Mrs Grace Osei for their constant prayers and efforts. More gratitude to my Senior Pastor Rev. Jonathan Amoto Sam (Oasis of life Chapel) and my beloved Sharon Esinam Dotse for the support throughout my educational journey, I am grateful for your support.

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LIST OF ABBREVIATIONS

ALP – Alkaline phosphatase

ALT – Alanine amino tranferase

AST – Aspartate amino transferase

BASO- Basophil

Bs – *Bacillus subtilis*

EOS- Eosinophils

FAO – Food and Agriculture Organisation

FCR – Feed conversion ratio

Hb- Haemoglobin

hct- Haematocrit

HSI – Hepasomatic index

LYM- Lymphocytes

MCV- Mean corpuscular volume

MCHC- Mean corpuscular Haemoglobin concentration

MCH- Mean corpuscular Haemoglobin

MON- Monocytes

NEU- Neutrophile

RBC- Red blood cell

SGR - Specific growth rate

VSI – Vicerosomatic index

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Nearly half of fish consumed worldwide is produced via aquaculture (FAO, 2020). According to food security forecasts, aquaculture has a lot of potential to produce more fish in the future and compensate for stagnant catch fisheries supply (Godoy et al., 2014).

The use of probiotics in aquaculture is seen to be a feasible option for improving animal health. and performance on growth (Tachibana et al., 2021). The concept “probiotic” originates in Greek pro and bios indicating “prolife” (Vrese, 2001), they are often referred to as "life promoters" since they aid the host organism's general health in a natural way. Due to their superior benefits in boosting fish development and wellbeing while maintaining a healthy ambience, probiotic bacteria has risen to the forefront of current research in fish culture (Su & Mao, 2014). The direct influence of probiotic bacteria on fish development performance, either by increasing nutrient intake or by delivering nutrients, is one of the most anticipated effects of utilizing probiotic bacteria (Nahid Akter et al., 2016). Numerous studies have reported growth enhancement (Avella et al., 2010; Azarin et al., 2014; Beck et al., 2015) following probiotic application in fish culture.

It is well known that haematological parameters have a strong influence on a fish's physiological status, and that these parameters can provide valuable information on the health of cultured fish (Harikrishnan et al., 2010). Several studies have found that probiotics have a positive effect on haematological indices, including white blood cell (WBC) count, red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (Hb) content in fish (Feliatra et al., 2018; Garcia-Marengoni et al., 2015; Ullah et al., 2020).

Activities of the enzymes AST and ALT can be utilized to detect harm to the tissues induced by toxicants (i.e. through the provided feed or in the surrounding environment) (Kunjiappan et al., 2015) therefore, they are essential enzymes for fish. *Bacillus* spp. has been discovered to play a crucial function in the modulation of AST and ALT in fish. Fish fed meals enriched with probiotic *Bacillus licheniformis* and *Bacillus subtilis*, for example, had decreased AST and ALT (Adorian et al., 2018).

Bacillus spp are one of the best prospects for use as feed additives among the various probiotic bacteria discovered, due to their good effects on growth, immunological response, and disease resistance (Salinas et al., 2008). *Bacillus subtilis*, *Bacillus amyloliquefaciens Saccharomyces cerevisiae*, and *Bacillus licheniformis* for example, have shown encouraging benefits on growth parameters, serum and mucosal immunology, and disease resilience in a variety of farmed fish (Kumar & Usha, 2012; Standen et al., 2016). *Bacillus* species are used successfully because they are microorganisms that produce spores that can endure heat during feed pelletization and continue to exist thereafter passing via the stomachs of fish in order to establish themselves in the intestines, where they can multiply and form a variety of digestive enzymes (amylase, protease and lipase) (Goda & Chowdhury, 2006).

1.2 Problem Statement and justification

Many countries throughout the world have been developing aquaculture technology in order to increase productivity, with a particular focus on feed and feed management procedures (Ali & Jauncey, 2004), because fish nutrition contributes for 70 percent to 80 percent of the total variable production costs on a fish farm, fish feed is a critical component (Munguti, 2021).

Clarias gariepinus, is one of the most important cultivated fish species and among the most widely employed produced fish to eat (Fasakin et al., 2003; Sutriana, 2007). Catfish farming is important

and is catching up with tilapia production, hence farmers are positioning themselves to its culture because it generates income, creates jobs, and alleviates food insecurity by providing low-cholesterol animal protein to the majority of Africans (Adebayo et al., 2013).

Despite the fact that *Clarias gariepinus* is a widely farmed fish, there have been few research examining its performance in response to probiotic addition in the diet. High stress levels, poor wellbeing and delayed performance of aquaculture species in terms of growth have resulted from fish farming intensified to supply the growing demand for fishes (Dalsgaard et al., 2012). As a result, a variety of chemicals and dietary additives have been utilized to help farmed fish grow and thrive (Reda & Selim, 2015). Probiotics are one of the most important of these supplements, which are utilized as a enhancer that is easy to use and safe to boost the host's growth by delivering nutrients and raising digestive enzyme activity, improving the utilization of feed and digestibility (Merrifield et al., 2010; Miranda et al., 2009; Reda & Selim, 2015).

1.3 Objectives of study

Therefore, the objectives of the study were to observe the effect of *Bacillus subtilis* (Bs) dietary supplementation on;

i. Growth of *Clarias gariepienus* (i.e initial weight, final weight, feed conversion ratio, weight gain and body indexes (condition factor, viscerosomatic index, and hepatosomatic index).
ii. Haematological parameters (i.e white blood cells, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations of *Clarias gariepienus.*
iii. Plasma chemistry (i.e Total protein, Albumin)
iv. Liver health (i.e Aspartate aminotransferase, Alanine transaminase and Alkaline phosphate) of *Clarias gariepinus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology and distribution of African catfish (*Clarias gariepinus*)

Clarias gariepinus, widely regarded as one of the most important tropical catfish species for aquaculture, has a nearly Pan-African distribution, from the Nile through West Africa and Algeria to South Africa. They can also be found in Asia Minor (Israel, Syria and South of Turkey) (FAO, 1996). The clariid genus is shaped like an eel, with an elongate cylindrical body and extraordinarily long dorsal and anal fins (almost reaching or reaching the caudal fin), and both fins comprising only soft fin rays. The pelvic fin generally has six soft rays, the head is flattened and strongly ossified, the skull bones (above and on the sides) form a casque, and the body is covered with a smooth, scaleless skin (FAO, 1996).

On the dorsal and lateral sections of the body, the skin is generally darkly coloured. The color is marbled and varies in tone from grayish olive to blackish depending on the substrate. When exposed to light, the skin's colour usually lightens (FAO, 1996).

They have four pairs of unbranched barbels, one on nasal, one maxillar (longest and most mobile) on the vomer and two mandibular (inner and outer) on the jaw. Tooth plates can be seen on both the jaws and the vomer. The barbels' primary purpose is to detect prey. A supra-brachial or auxiliary respiratory organ is present, consisting of a paired pear-shaped air-chamber with two arborescent structures (FAO, 1996b). Because the air chamber interfaces directly with the pharynx and gill chamber, this additional air breathing organ helps the fish to survive in muddy marshes for long periods of time. Males of the species are easily distinguishable because they have a conspicuous sexual papilla positioned immediately behind the anus. The sexual papilla is lacking in females (FAO, 1996).

Clarias species can be found in a variety of calm waters, including lakes, streams, rivers, marshes, and floodplains, with many of them being prone to seasonal drying. Floodplain, wetlands and pools are the most prevalent habitats, where the catfish may survive during the dry seasons thanks to the presence of their supplementary air breathing organs (Bruton, 1979).

Spawning occurs in rivers, lakes, and streams that are shallowly flooded. Males usually engage in highly aggressive encounters prior to courtship. In shallow waters, discrete pairs of males and females engage in courtship and mating. The mating posture, which is a type of amplexus in which the male rests in a U-shape curved around the female's head, is retained for a few seconds. After releasing a batch of milt and eggs, the female swishes her tail vigorously to disperse the eggs over a large region. After mating, the pair usually takes a short break (from a few seconds to several minutes) before returning to mating (FAO, 1996). Except for the careful selection of a good place, there is no parental care for guaranteeing the survival of the catfish offspring. At water temperatures of 23-28°C, eggs and larvae develop quickly, and larvae may normally swim 48 to 72 hours after fertilisation (Bruton, 1979).

The temperature has a significant impact on the development of oocytes in African catfish (as common with a large number of fish species) (Owiti & Dadzie, 1989).

2.2 Catfish culture in Ghana

Ghanaians' main source of animal protein is fish. Fish has accounted for over 70% of animal protein consumption over the years. Total marine fish production as at September 2019 stood at 235,276.06mt valued at US$3,607,576,286.44, whiles the inland sub-sector recorded 62,266.53mt of fish valued at GHS716,065,125.67 during the same period Currently, national fisheries production from combined capture and culture sectors is expected to be 391,694 tonnes, with aquaculture accounting for 0.3 percent of this total, valued at US$2.251 million (FAO, 2022). Over-exploitation, pollution, habitat damage, harmful fishing techniques, and the loss of foreign

fishing grounds have all contributed to a reduction in fish production in commercial and artisanal fisheries over the previous two decades (Ofori-danson et al., 2002), while per capita fish intake continues to increase from 22 kg/caput/ year in 1997 to 28 kg/caput/year in 2002 (FAO, 2006). Aquaculture's ability to bridge the gap between current demand and national supply has long been recognised around the world, including in Ghana (FAO, 2006).

Monoculture and polyculture are the two most common fish farming technologies in Ghana. The main species cultured in dugouts, pens, earthen ponds, and cages are tilapias (primarily *Oreochromis niloticus*) and catfish (*Clarias gariepinus*, *Heterobranchus longifilis*, and *Heterobranchus isopterus*) (Nguyen, 2008). In Africa, and for that matter Ghana, the potential of catfish in aquaculture to expand and sustain national fish production has long been acknowledged (Amisah et al., 2010). In Ghana, however, its culture is still on a small scale. Catfish, particularly the clariid catfish *Clarias* and Heterobranchus, have the ability to live in water with extremely low dissolved oxygen and high carbon dioxide levels, making them ideal culture fish. Catfish can be easily farmed at large densities and produce a lot of food per unit area. Catfish such as *Clarias* and *Heterobranchus* are prized delicacies. They are widely used as a "police fish" in tilapia ponds to keep undesired reproduction at bay (Owusu-Frimpong & Ampofo-Yeboah, 2008). The Directorate of Fisheries estimated aquaculture fish production at 950 tonnes in 2004. Although production has not been broken down by species, it is known that tilapia (mostly *Oreochromis niloticus*) is the most popular, accounting for about 88 percent of overall aquaculture output (FAO, 2006). The catfish is the second most widely cultivated species. Its cultivation accounts for around 10% of total national aquaculture production, with other species such as *Heterotis niloticus* accounting for the remaining 2%. Within the three decades, catfish production has generally ranged between 95 and 200 tonnes per year, with the biggest and lowest productions occurring in 1996 and 2003, respectively. (Nguyen, 2008)

2.3 Constraints to Catfish culture in Ghana

According to Craig et al. (2017), fish nutrition accounts for 40 percent to 50 percent of the total variable production costs on a fish farm, so fish feed is essential. The lack of efficient and affordable farm-made feeds for all stages of fish growth is one of Ghana's significant issues in aquaculture. Because of the increased demand for fish feed, the majority of the feed sold by vendors is substandard (Nguyen, 2008). Commercial tilapia feeds typically include 24 to 28 percent crude protein, however peasant farmers employ feeds with lower protein levels, such as rice bran, brewers waste, groundout brand, or maize bran, due to the high cost of fish meal. Among other things, anchovies are very popular (Kassam, 2014). The cost of components, rather than the nutritional requirements of the fish, drives the composition of farm-made meals (Cobbina, 2010).

Over-exploitation, pollution, habitat damage, harmful fishing techniques, and the loss of foreign fishing grounds have all contributed to a reduction in fish production in commercial and artisanal fisheries over the previous two decades (Nguyen, 2008; Ofori-danson et al., 2002), while per capita fish consumption has increased from 22 kg/caput/ year in 1997 to 28 kg/caput/year in 2002 (FAO, 2006; Owusu-Frimpong & Ampofo-Yeboah, 2008). Aquaculture has been identified as a viable means of bridging the gap between present demand and domestic supply (FAO, 2020). Probiotics have aided in the development of a variety of species. In improving growth performance, dietary administration of probiotics on different fish species has been recorded (Hai, 2015). Several reports have shown the effects of probiotics in enhancing growth performance increasing production.

Moreover, pollution from many other sources with creates hypoxic conditions in with fish will need alternatives so as to thrive in. In determining the quality of a fish diet, haematology is an important factor to be considered (Selim & Reda, 2015).

2.4 Usage of probiotics in aquaculture

Probiotics were first defined as organisms and chemicals that contribute to gut microbial balance by Lilly & Stillwell, (1965). As additional information became available, many definitions of probiotics were presented and amended. Probiotics are live microbial feed supplements or cultured products that have a positive effect on the host by enhancing the intestinal (microbial) balance (Fuller, 1989). A probiotic is a live microorganism culture, either mono or mixed, that improves the qualities of the native microflora (Havenaar et al., 1992).

Because the gut microbiota of aquatic animals is continually interacting with the environment and host processes, a probiotic is described as a live microbial supplement that has therapeutic effects, such as;

(i) altering the microbial community of the host or the environment

(ii) boosting the host's response to illnesses by improving the utilization of feed or increasing its nutritional value or

(iii) enhancing the quality of its surrounding environment (Verschuere et al., 2000).

A probiotic is a live, dead, or component of a microbial cell that is dietary fed to the animals or added to the rearing water, enhancing disease resistance, health status, growth performance, feed utilisation, stress response, or general vigour in the host, which is accomplished through improving the hosts microbial balance or the ambient environment's microbial balance (Merrifield et al., 2010). Probiotics are also commonly employed in both human and veterinary medicine. Lactic acid bacteria (*Lactobacillus* spp) make up the majority of them (Fuller., 1989). Probiotics are bacteria and non-bacteria that are used in aquaculture as a water routine and feed supplement. Probiotics aid the hosts by enhancing the growth of the hosts (Boonthai et al., 2011; Kumar et al., 2006) as well as lowering disease incidence (Irianto & Austin, 2002; Newaj-Fyzul et al., 2007), Furthermore, probiotics can thrive in a variety of aquatic habitats.: freshwater (Mujeeb Rahiman et al., 2010), brackish water and sea water (Vijayan et al., 2006). Probiotics are live and/or dead

microbial feed supplements or water additives that are administered to improve the rearing water quality, enhance the physiological and immune responses of aquatic animals, and reduce the use of chemicals and antibiotics in aquaculture. They come in the form of mono, multiple strains, or in combination with prebiotics or other immunostimulants.

2.5 Administration methods

Probiotics are administered in various ways: directly through the mouth/water or through feed additives, with the former being the most practical route for prawn probiotics (Hai, 2015). The latter, on the other hand, is the most widely utilized in aquaculture (Gildberg et al., 1995; Gildberg & Mikkelsen, 1998; Van Hai & Fotedar, 2009) as majority of probiotics are meant to be mixed with food (Gomes et al., 2009). Feed supplements such as probiotics (*Lactobacillus rhamnosus*) enhanced the fecundity of zebrafish (*Danio rerio*) (Gioacchini et al., 2010). Regardless of prawn size, oral administration offered benefits (Itami et al., 1998; Sakai, 1999), these prawns can be treated at any point during their culture.

2.5.1 Water and feed additives

Probiotics are administered in various ways: directly through the mouth/water or through feed additives, with the former being the most practical route for prawn probiotics (Hai, 2015). The latter, on the other hand, is the most widely utilized in aquaculture (Gildberg et al., 1995; Gildberg & Mikkelsen, 1998; Van Hai & Fotedar, 2009) as majority of probiotics are meant to be mixed with food (Gomes et al., 2009). Feed supplements such as probiotics (*Lactobacillus rhamnosus*) enhanced the fecundity of zebrafish (*Danio rerio*) (Gioacchini et al., 2010). Regardless of prawn size, oral administration offered benefits (Itami et al., 1998; Sakai, 1999), these prawns can be treated at any point during their culture. Probiotics are commonly introduced directly to culture water (Gibson et al., 1998; Gram et al., 1999; Queiroz & Boyd, 1998; Ringø & Vadstein, 1998;

Van Hai & Fotedar, 2009) as water additives (Cha et al., 2013; Zhou et al., 2009), dipped in bacterial suspension (Gram et al., 1999; Hansen & Olafsen, 1989).

2.6 Dosages

Varied receivers' responses to different dietary probiotic levels have been seen, and a probiotic dosage may produce good or negative outcomes (Bagheri et al., 2008; Panigrahi et al., 2004). The growth rate, lysozyme, antiprotease, serum peroxidase, and blood respiratory burst activities of Japanese flounder were all increased by a dietary supplement with *Lactococcus lactis* at 10^8 CFU g^{-1} (Heo et al., 2013). *Bacillus subtilis* and *Bacillus licheniformis* were added to rainbow trout fry diets at 10^9 CFU g^{-1}, which enhanced FCR, specific growth rate, weight gain, and protein efficiency ratio (Bagheri et al., 2008). *Aeromonas hydrophila* was resisted in hybrid tilapia (*Oreochromis niloticus* and *Oreochromis aureus*) fed a meal supplemented with *Lactobacillus brevis* at 10^9 cells g^{-1} (Liu et al., 2013). Although rainbow trout fed a probiotic meal at either 10^9 or 10^{11} CFU g^{-11} had increased head kidney leukocyte phagocytic activity, only the group given the probiotic at 10^{11} CFU g^{-11} had enhanced serum lysozyme and alternative complement activity when compared to the control group (Panigrahi et al., 2004).

Probiotics in a multi-strain blend at 10^7 CFU ml $^{-1}$ was the most effective probiotic concentration for Greenshell™ mussel (*Perna canaliculus*) larvae (Kesarcodi-Watson et al., 2012). Appropriate probiotic density is common at 10^5 CFU ml $^{-1}$g (Guo et al., 2009; Van Hai & Fotedar, 2010; Van Hai & Fotedar, 2009; Zhou et al., 2009). Probiotics at 10^7 CFU ml $^{-1}$ yielded stronger stimulatory effects due to an enhancement of cellular innate immune parameters (Salinas et al., 2006). A higher dose had no effect on the amount of protection (Pérez-Sánchez et al., 2013). Probiotic levels are determined by probiont species, fish species and their physiological health, rearing settings, and the application's specific objective (Merrifield et al., 2010).

2.7 Time duration

In the usage of probiotics, the duration of administration is also a crucial consideration. Studies have looked into potential probiotic applications for as little as six days (Jöborn et al., 1997), and more than 5 months (Aubin et al., 2005) or even 8 months (Aly et al., 2008). Probiotics can cause immune-suppression responses in non-specific immune systems if given for a long time (Sakai, 1999). After 3 days of feeding, probiotics had no effect on the microbial community composition associated with cultured rotifers (Qi et al., 2009). Probiotic supplementation has been shown to have short-term effects, however they were not identified in the GI tract for periods longer than 1–3 weeks (Balcázar et al., 2007; Kim & Austin, 2006; Robertson et al., 2000). While there is no research on long-term efficacy, (Merrifield et al., 2010), supplementation for a brief period of time has been shown to be effective (Brunt et al., 2007; Brunt & Austin, 2005; Newaj-Fyzul et al., 2007; Pieters et al., 2008). After 28 days of probiotic supplementation (*Shewanella xiamenensis* and *Aeromonas veronii*), the total number of grass carp that died (*Ctenopharyngodon idellus*) after being exposed to *Aeromonas hydrophila* for 14 days was reduced (Wu et al., 2015). Dietary supplementation with probiotics may be beneficial (Merrifield et al., 2010). In terms of long-term applications, Aubin et al. (2005) looked at how probiotic recovery levels changed over time, and also discovered that after 20 days, levels were higher than after 5 months.

The frequency with which probiotics are given has a big impact on how well they work. A daily probiotic addition is preferable to an application every other day during the culture period (Guo et al., 2009). As probiotic colonization was temporary in Atlantic cod larvae, probiotics must be administered to the fish larvae on a regular basis (Skjermo et al., 2015). Short-term-cyclic probiotic feeding techniques, like other immunostimulant agents, may be advantageous to the hosts (Bricknell & Dalmo, 2005), such treatments could include alternating probiotic-supplemented and unsupplemented diets for brief periods of time, cyclically. During the supplemental feeding phase,

this application may provide direct benefits of short-term use. During the stage with no supplementation, probiotics provided protection against transient infections and could continue to produce some degree of immunostimulation when stomach probiotic populations persisted for several weeks (Balcázar et al., 2007).

2.8 Improvement of growth and survival rate

It is now well acknowledged that feed prices account for a significant amount of a farmer's expenses, with estimates ranging from 50 to 60 percent (Sinha et al., 2011). As a result, multiple researchers are working on various techniques to reduce costs, including the use of various types of growth promoters (Hoseinifar et al., 2016; Ng & Koh, 2016). The use of probiotics in the diet has been shown to promote growth in a variety of fish species (Hai, 2015), probiotics produce exogenous enzymes, and enhancing gut physiology is one of the postulated mechanisms for growth improvement (Hoseinifar et al., 2016). There have been conflicting reports regarding various *Bacillus* species. Even though the probiotic and fish species were the same, different authors reported varying results. This is due to differences in target aquatic creatures' gut microbiome, life phases, and culture conditions (Moustafa & Mohamed, 2008)

Bacillus subtilis was originally used as a fish growth stimulator in a study by Kumar et al. (2006), by giving rohu different levels of feed (0.5, 1.0 and 1.5 $\times 10^7$ CFU g^{-1}) of *Bacillus subtilis* for 15 days and a considerable weight gain was noticed.. Later, Bagheri et al. (2008) added various doses of probiotic to the rainbow trout fry diet (4.8×10^8, 1.2×10^9, 2.01×10^9, 3.8×10^9, 6.1×10^9 CFU g^{-1}) of commercial *Bacillus subtilis*. After only 13 days of supplementation, probiotic-fed fish showed a significant increase in weight gain. When fish were fed 3.8×10^9 CFU g^{-1} probiotics, the highest growth promotion was seen. Liu et al. (2012) also evaluated the effects of oral delivery of *Bacillus subtilis* E20 in a 28-day feeding study (10^4, 10^6 and 10^8 CFU g^{-1}) on the orange-spotted grouper's growth performance factors, and discovered that supplementing feed with probiotics boosted

feeding efficiency and weight gain substantially. This improvement, according to the authors, can be ascribed to the supply of nutrients and exogenous enzymes. e.g. protease and lipase by *Bacillus subtilis*.

Bacillus sp. has been shown to provide vital nutrients to the host organism, such as amino acids and vitamins K and B12, which can improve growth performance. In the grass carp diet, Wu et al. (2012) employed a different strain (*Bacillus subtilis* Ch9). Different levels of feed were given to the fish. (1.0×10^9, 3.0×10^9 and 5.0×10^9 CFU kg^{-1}) of probiotic for 56 days, probiotic-fed fish gained considerably more weight at the end of the feeding period. Gobi et al. (2016) in a catfish experiment (*Pangasius hypophthalmus*) showed the usage of *Bacillus licheniformis* Dahb1 (10^5 CFU ml^{-1}) indicating host and probiotic particular species, lower dosage was more beneficial on growth than higher dosage. Furthermore, after 2 months of feeding young Asian sea bass (*Lates calcalifier*), fed diets supplemented with varied dosages of *Bacillus licheniformis* and *Bacillus subtilis* exhibited considerably greater growth at 1×10^6 CFU g^{-1} than higher or lower levels of the probiotics (Adorian et al., 2018). Similarly, when compared to fish fed a standard diet, dietary delivery of *Bacillus subtilis* (1×10^{10} CFU g^{-1}) significantly raised final weight, FCR, and protein efficiency ratio in olive flounder (Cha et al., 2013). Additionally, adding 5×10^6 CFU g^{-1} of *Bacillus subtilis* to the Nile tilapia diet increased growth performance indicators significantly (Telli et al., 2014). When Nile tilapia (65.5 g) were fed *Bacillus subtilis* at 1×10^7 CFU g^{-1} for two months, the survival rate and weight gain were considerably boosted (Aly et al., 2008).

In a recent study, Liu et al. (2017) found that feed treated with *Bacillus* subtilis HAINUP40 (10^8 CFU g^{-1}) improves growth performance, intestinal probiotic recovery, and digestive enzyme activity in Nile tilapia (95.8 g) over an 8-week period. In juvenile large yellow croaker (*Larimichthys crocea*) (7.82 g) in floating sea cages for 10 weeks, dietary administration of Bacillus subtilis (0.42-1.35 10^7 CFU g^{-1}) resulted in better growth performance at the higher

dosage of probiotic than at the lower dosage, indicating a significant effect by probiotic dosage optimization (Ai et al., 2011), similarly when Nile tilapia were fed a high dose of *Bacillus amyloliquefaciens* orally for two months, comparable outcomes were obtained (Reda & Selim, 2015).

Other Bacillus species have been studied as potential probiotics for fish growth in addition to *Bacillus subtilis*. Bandyopadhyay & Mohapatra. (2009) investigated the effects of *Bacillus circulans* PB7 isolated from *Catla catla's* gut as a feed supplement in the diet of *Catla catla* fingerlings. Fish were fed a variety of probiotic doses, including 2×10^4, 2×10^5, 2×10^6 CFU 100 g^{-1} for 2 months. The authors observed positive effects on growth performance indicators at the conclusion of the feeding study. When fish were fed 2×10^5 CFU g^{-1}, the best results were obtained. Sun et al. (2010) also used a single dose of two *Bacilli* species in the feed of orange spotted grouper during a 30-day research. Probiotic strain from the gut of orange spotted grouper was provided by the authors. These probionts, however, were unable to impact growth performance indicators, unlike prior investigations with *Bacilli* probiotics. In a 90-day feeding trial with Nile tilapia, the effects of *Bacillus amyloliquifaciens* were also investigated (Silva et al., 2014). Fish were given probiotics at concentrations of 1×10^6 CFU g^{-1}, 5×10^6 CFU g^{-1}, and 1×10^7 CFU g^{-1}. This probiotic had no effect on Nile tilapia, according to the findings. The effects of a six-week dietary administration of *Bacillus amyloliquefaciens* on fish growth were marginal, but digestive enzyme activities such as protease, amylase in the hepatopancreas, protease activity in the intestine, and lipase activity in the stomach of treated fish significantly increased when compared to control fish (Chen et al., 2010). When silver carp larvae were fed *Bacillus latrospores* and *Bacillus licheniformis* using rotifers (*Brachionus plicatilis*) as a live probiotic carrier, the treated fish outgrew the control fish (Sahandi et al., 2012). Probiotics have enhanced aquatic animal growth rates, feed utility, and survival rates by modulating digestive enzyme activities also increasing feed

digestibility (De Schrijver & Ollevier, 2000; Doeschate & Coyne, 2008) as a result of an increase in digestive enzymes (Zhou et al., 2009) such as alginate lyases, amylases and proteases (Zokaeifar et al., 2012). Probiotics help in digestion by producing extracellular enzymes like proteases, carbohydralases, and lipases, as well as providing growth factors (Arellano-Carbajal & Olmos-Soto, 2002; Leonel Ochoa-Solano & Olmos-Soto, 2006). The digestive protease activity, protein digestion and absorption levels, and growth rate of *Haliotis midae* were all improved by *Vibrio midae* SY9 (Huddy & Coyne, 2015). With an increase in lipase and cellulase activity, photosynthetic bacteria and Bacillus spp. boosted the growth of white leg prawns (Wang, 2007). The probiotic-fed *Fenneropenaeus indicus* had higher specific activities of amylase, total protease, and lipase (Ziaei-Nejad et al., 2006). Furthermore, the use of probiotics resulted in the production of vital nutrients such as fatty acids (Vine et al., 2006), biotin and vitamin B12 (Sugita et al., 1991). Probiotics can be used as a supplement to diet or to help with digestion (Verschuere et al., 2000), as deposit-feeding holothurians eat bacteria as one of its essential basic food items in natural settings (Moriarty, 1998). The survival of *Haliotis rufescens* was improved by *Vibrio* C21-UMA and *Vibrio midae*. (Silva-Aciares et al., 2011) and *Haliotis midae* (Macey & Coyne, 2006) respectively.

The amount of dry feed supplied per unit live weight growth is known as the feed conversion ratio (FCR). It is frequently used as a metric for determining how effective a diet is. The more growth-friendly the diet, the less food is required to achieve a unit weight gain, resulting in a lower FCR (Abarike, 2011).

According to Glencross, (2020), in order to analyze the nutrient utilization of a diet and by reference to a component, feed intake must be measured (typically stated as both an amount (g fish-1) and a rate (g fish-1) (g fish-1 day-1). The feed conversion ratio (FCR), which is defined as the dry weight of feed supplied divided by the weight gained by the animal, is used to determine

diet utilization. As feed usage efficiency improves, the feed conversion ratio (FCR) decreases. The FCR will rise at feeding rates that are above or below the optimal for the conditions present during the experiment, with the lowest FCR value happening at the feeding level where food conversion efficiency is best (Jha et al., 2015).

Bagheri et al. (2008) investigated the impact of adding different quantities of commercial *Bacillus subtilis* to the diet of rainbow trout fry. After only 13 days of dosing, probiotic-fed fish demonstrated a significant improvement in feed conversion ratio (FCR) for treatments compared to control. Wu et al. (2012) fed grass carp a different strain (*Bacillus subtilis* Ch9). Fish were fed different amounts of probiotic (1.0×10^9, 3.0×10^9, and 5.0×10^9 CFU kg^{-1}) for 56 days, and probiotic fed fish had considerably greater FCR at the end of the feeding phase. Similarly, nutritional delivery of *Bacillus Subtilis* (1×10^{10} CFU g^{-1}) increased FCR and protein efficiency ratio in olive flounder as compared to fish fed a control diet (Cha et al., 2013). Abdollahi-Arpanahi et al. (2018) showed in his study that all probiotics had a substantial impact on the feed conversion ratio (FCR) as compared to the control group.

The condition factor (K) is used to quantify the fatness or welfare (state of health) of the fishes, based on the idea that heavier fish of a given length are in better physiological condition. The condition factor is a valuable metric for assessing fish feeding intensity, age, and growth rates (Ndiaye et al., 2015). Roohi, (2015) in his study on the condition factor of *Rutilis rutilis* where four diets were prepared, fish fed supplemented diets experienced a considerable increase as compared to the control group. Abarike et al. (2018) in their investigation of commercial probiotics blend of *Bacillus subtilis* and *Bacillus licheniformis* reported that the condition factor was significantly higher in supplemented groups in comparison to the control within four weeks of his studies. Heo et al. (2013) in their study looked at the effects of a possible probiotic strain *Lactococcus lactis* on the immunological response and growth of olive flounder (*Paralichthys*

olivaceus) and a notable rise was seen at the end of the 5-week feeding study in the condition factor in comparison to the control. However, Jo et al. (2012) in his study evaluated the effects of probiotics on growth, stress tolerance, and non-specific immune response in Japanese flounder (*Paralichthys olivaceus*), finding no significant differences between the probiotic and control groups.

2.9 Improving haematological parameters

2.9.1 Probiotics' effect on haematocrit (hct)

Haematocrit (hct) is a measurement of blood capacity to carry oxygen, according to Gallaugher (1994) . The greater the hct level in an organism, the better the blood's ability to transport oxygen. The teleost hct value has been found to range from 0 percent to 70 percent (Fletcher & Haedrich, 1987; Graham & Fletcher, 1983, 1985; Wells & Baldwin, 1990; Wells & Weber, 1991). In rainbow trout, hct values have been found to range from 17 percent to 44 percent (Wells & Weber, 1991), 52.5 percent mackerel (*Auxis rochei*), 53 percent tuna (*Thunnus thynnus*), 43 percent blue marlin (*Makaira nigricans*) 8.5 percent hagfish (Eptatretus cirrhatus) (Fánge, 1992). Higher hct readings in the organism suggest better health (Gallaugher, 1994), It can also be an antistress reaction to compensate for the increased demand for oxygen for metabolic energy (Gallaugher, 1994). Nutrition in fish with primalac probiotics (Nikandishan Farjad Commerce Corporation, Tehran, Iran) containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium bifidium* has been shown to increase hct levels in Caspian roach fry exposed to salinity stress when compared to those fed a control diet (Roohi, 2015). In addition, Nile tilapia submerged in *Bacillus* sp. showed a rise in hct and survival after infection with the pathogenic bacteria *Streptococcus iniae*. The use of a mixed probiotic species has been reported in several studies of *Lactococcus rhamnosus* and *Lactococcus lactis* in red seabream (Dawood et al., 2017), *Bacillus* sp. in Nile tilapia (Feliatra et al., 2018), a combination of *Bacillus cereus* and *Bacillus*

subtilis in Nile tilapia (Garcia-Marengoni et al., 2015) and *Lactococcus rhamnosus* on rainbow trout (Panigrahi et al., 2010) have been shown to raise hct levels. Fish fed probiotic-supplemented diets had a better health status than fish on control diets, according to these studies. Taking all into account, fish on probiotic-treated diets may have greater hct levels and be better able to respond to stressors. This suggests that, as previously reported, feeding probiotic-supplemented meals to fish can boost their anti-stress response (Feliatra et al., 2018). Optimum ranges in African catfish was recorded from 32.64 - 45.74 % (Akinrotimi et al., 2011).

2.9.2 Probiotics' effects on haemoglobin (Hb)

Bolliger & Everds, (2012) defined haemoglobin (Hb) concentration as the quantity of total Hb per volume of whole blood, which is measured using a spectrophotometric technique after red blood cell lysis. Hb function seems to be to adapt to the different metabolic needs of animals living in constantly changing environments, as well as to play an important part in transporting oxygen from gas-exchange organs to peripheral tissues (de Souza & Bonilla-Rodriguez, 2007). When compared to terrestrial animals, Hb establishes a barrier between the organism and the environment in fish (Landini et al., 2013), but more particularly through environmental changes and modifications in temporal and geographical fluctuations, as well as oxygen availability (de Souza & Bonilla-Rodriguez, 2007). In African catfish haemoglobin has been reported to range from 10.02 - 18.64 g/dL (Akinrotimi et al., 2011). Accounts of the use of probiotic-supplemented diets in fish show promise in raise in Hb levels. In *Clarius batrachus* for example, the administration of a probiotic species of *Lactococcus sporogenes* outperformed the fish fed control diets (indian magur) (Dahiya et al., 2012), *Bacillus cereus* in juvenile Nile tilapia (Garcia-Marengoni et al., 2015), *Bacillus pumillus* in *Labeo rohta* (Rajikkannu et al., 2015), *Bacillus Licheniforms* and *Bacillus Subtilis* in *Rutilis frisii kutum* (Azarin et al., 2014) have shown to increase the haemoglobin values in fish.

According to these studies, fish fed probiotic-supplemented diets had a better health status than fish on control diets.

2.9.3 Probiotics' effects on red blood cells (RBCs)

An automated counter is used to determine the number of RBCs, also known as Erythrocytes (ERI), in a given volume of blood (Bolliger & Everds, 2012). RBCs in fish are elliptical or ovoid, although their diameters and length vary between species, ranging from 10–20 mm in length and 6–10 mm in breadth (Farrell, 2011). RBCs are primarily responsible for oxygen delivery via high quantities of the respiratory pigment haemoglobin, as well as pH regulation (Farrell, 2011). It was reported optimum values of red blood cells in African catfish ranges from 3.051 - 8.64 10^{12} /L (Akinrotimi et al., 2011). When compared to fish fed control or unsupplemented diets, fish fed probiotic-supplemented diets have higher RBC levels (Azarin et al., 2014). For instance, in *Clarias batrachus*, a mixed probiotic strain of *Lactobacillus sporogenes* was used (Dahiya et al., 2012), a combined dosage of *Lactobacillus sporogenes*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Saccharomyces cervirial* in *Clarias mrigal* in (Sharma & Sihag, 2013) and *Bacillus licheniformis* and *Bacillus subtilis* in *Rutilis frisii kutum* (Azarin et al., 2014), LAB in rainbow trout, *Lactobacillus plantarum* in Nile tilapia (Faramazi et al., 2011) were shown to have significantly higher RBCs compared to fish feed supplemented diets. Furthermore, different scientific studies show that supplementing with probiotics boosted the level of RBCs in fish species.

2.9.4 White blood cell responses to probiotics

WBCs count (leukocyte count) is defined by Medicine Net as the number of WBCs in the blood that can be determined as part of a complete blood count (CBC). WBCs in fish play a crucial role in cellular defense and immunity. WBCs present in tissues outside of the circulation (thymus,

spleen, and kidney) have functional effects that are also manufacturing locations (Farrell, 2011). Despite their minor presence in fish blood (Leucocrit (Lct) = 0.3–1.0 percent), fish regulate the number of circulating WBCs. Toxicants and stress, on the other hand, can lower leucocrit levels (Farrell, 2011). In African catfish WBC values is reported to range from 18.66 - 25.61 10^9/L (Akinrotimi et al., 2011). Probiotic-supplemented diets have been shown to increase the WBC levels of fish as compared to those fed control/unsupplemented diets. In contrast to the fish fed control diets, *Lactobacillus sporogenes* as probiotic in *Clarias batrachus* (Dahiya et al., 2012), the application of a mixed probiotic species *Lactobacillus sporogenes*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Saccharomyces cervirial* in *Cirrhinus mrigal* (Sharma & Sihag, 2013), *Bacillus subtilis* in rainbow trout (Kamgar & Ghane, 2014), mixed probiotic species of *Bacillus subtilis* and *Saccharomyces cerevisiae* in *Cirrhinus mrigala* (Ullah et al., 2018), *Lactobacillus acidophilus* and *Bacillus subtilis* in Nile tilapia (Aly et al., 2008) have been shown to increase WBC levels in fish.

When compared to fish fed control diets, fish fed probiotic supplemented diets had better immunological responses. Furthermore, fish fed probiotic-supplemented diets may have greater WBC levels and adapt to stressors more effectively. The immunological defense of fish fed probiotic-supplemented diets was improved (Munir et al., 2018).

2.9.5 Probiotics' effects on other blood parameters

The mean corpuscular haemoglobin concentration (MCHC), which represents the average haemoglobin concentration within erythrocytes and is calculated by dividing the whole blood haemoglobin value (in grams per decilitre) by the hct (as a percentage) and multiplying by 100 expressed as grams per decilitre of erythrocytes, it is the first blood derivative (Harvey, 2012). Akinrotimi et al. (2011) reported optimum range for mean cell haemogloin concentration (MCHC) from 38.21 - 46.74 g/dL in African catfish. The concentration of haemoglobin within an RBC is

usually represented as mean cell haemoglobin concentration (MCHC), and it varies greatly depending on the fish species and environmental factors (physiological and environmental) (Farrell, 2011). In an infected *Heteropnuestes fossilis* (Haniffa, 2015), the introduction of probiotics supplementing feed resulted in changes in MCHC, a mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae* in a *Cirrhinus mrigala* (Ullah et al., 2018), *Bacillus subtilis* in rainbow trout (Kamgar & Ghane, 2014) and *Bacillus pumilus* in *Labeo rohita* (Rajikkannu et al., 2015). The mean corpuscular haemoglobin is the second blood derivative (MCH).

The average quantity of haemoglobin detected in each RBC is calculated by dividing the haemoglobin by the number of RBCs (Bolliger & Everds, 2012). MCH is the least useful haematology parameter since it is insensitive to changes and provides less information than other RBC parameters (Bolliger & Everds, 2012). The use of probiotics as a feed-in for experimentally infected *Heteropnuestes fossilis* resulted in a change in MCH levels (Haniffa, 2015). Akinrotimi et al. (2011) reported optimum ranges (30.21 - 46.74 pg) for African catfish. A combination of *Bacillus subtilis* and *Saccharomyces cerevisiae* in *Cirrhinus mrigala* (Ullah et al., 2018), *Bacillus subtilis* in rainbow trout (Kamgar & Ghane, 2014) and *Bacillus pumilus* in *Labeo rohita* (Rajikkannu et al., 2015).

The Mean Corpuscular Volume is the ultimate blood derivative (MCV). MCV refers to the average size of red blood cells (Bolliger & Everds, 2012). MCV is calculated when a haemocytometer determines cell counts. Red blood cell volume is measured during the cell count mentioned above for instrument-generated cell counts, and the MCV is calculated using the histogram (Bolliger & Everds, 2012). When counting cells using a haemocytometer, the MCV is calculated by dividing the haematocrit by the RBC count (Bolliger & Everds, 2012). The administration of probiotics as feed-in infected *Heteropnuestes fossilis* (Haniffa, 2015), *Bacillus licheniformis* and *Bacillus subtilis* fed in Kutum (*Rutilus frisii kutum*) fry (Azarin et al., 2014), *Bacillus licheniformis* and *Bacillus subtilis* in matrinxa (*Brycon amazonicus*) breeders fed (Dias et al., 2012) and *Bacillus*

pumilus in *Labeo rohita* (Rajikkannu et al., 2015). Optimum range for Mean Corpuscular Haemoglobin in African catfish recorded 72.11 - 91.34 fl (Akinrotimi et al., 2011).

2.10 Probiotics' Effects on Liver Health

In recent years, aquatic systems have been severely impacted by the accumulation of pollutants/toxicants, prompting speculation that these chemicals may be the source of liver enlargement and gall syndrome disease in many cultured fish (Kunjiappan et al., 2015).

The enzymes aspartate transaminase (AST) and alanine transaminase (ALT) are responsible for various biochemical events in metabolism that interconvert amino acids with other metabolic intermediates, and a rise in their levels can indicate tissue damage, such as in chronic liver disease (Abdollahi-Arpanahi et al., 2018; Babazadeh et al., 2011) As stated by Chimela et al. (2014), because they are cytoplasmic in origin and are discharged into circulation (blood) following cellular injury, AST and ALT are sensitive biomarkers utilized in the detection of liver damage. AST is found in both mitochondrial and cytoplasmic forms in vertebrates, with the highest levels in the heart, liver, muscle, and kidney tissues, respectively (Abdollahi-Arpanahi et al., 2018). The activity of the enzymes AST and ALT can be utilized to detect tissue damage induced by toxicants (i.e. within feed administered or in the environment) (Kunjiappan et al., 2015) as a result, they are enzymes that are extremely important to fish. *Bacillus* spp. has been discovered to play a role in the modulation of AST and ALT in fish. Fish fed a diet enriched with probiotic *Bacillus licheniformis* and *Bacillus subtilis*, for example, had decreased AST and ALT (Adorian et al., 2018). In shrimps, AST and ALT activity were considerably lower in *Bacillus subtilis* and *Bacillus licheniformis*-treated groups than in control group (Abdollahi-Arpanahi et al., 2018). *Bacillus subtilis*, *Bacillus megaterium*, and *Bacillus licheniformis* fed to Nile tilapia produced similar results (Soltan & Abdo, 2017; Sutthi, 2018) same results was observed in *Labeo rohita* supplemented with *Bacillus amyloliquefaciens* CCF7 (Nandi & Banerjee, 2017). *Bacillus* was

found to be responsible for the removal of harmful substances and the improvement of liver functions, suggesting their hepatoprotective capability. However, there are few studies on their capacity to alleviate the developing liver-related issues in the fish farming industry.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Diet preparation

A commercial feed (Raanan) of 2 mm was purchased from the Tamale central market, with *Bacillus subtilis* probiotic obtained from China (Plate 1). A kilogram each of purchased feed was measured into four separate plastic bowls. 100 ml distilled water was added to one kg out the four measured feed, and were dried at ambient temperature, which served as a control diet (0g/kg), and to the remaining three portions, varied probiotic *Bacillus subtilis* dosages was added at 10g/kg, 20g/kg and 30g/kg. Normal starch was added as an adhesive to the diets and mixed thoroughly (Plate 2). The diets were then dried at room temperature and for the purpose of the experiment, were stored in an insect-proof bag. Table 1 shows the various codes that were used.

Plate 1: Probiotic (*Bacillus Subtilis* 200)

Plate 2: Mixing the feed and probiotics

Table 1: Dietary codes used in this study

Dietary Codes	Dietary combination
0g/kg	A commercial pellet diet that is void of Bs
10g/kg	Supplementing a commercial diet with 10g Bs w/w
20g/kg	A commercial diet supplemented with 20g Bs w/w
30g/kg	Supplementing a commercial diet with 30g Bs w/w

Where: Bs, *Bacillus subtilis*, w/w, weight by weight

3.2 Experimental set-up and fish management

Two hundred and twenty-eight (228) African catfish (*Clarias gariepinus*) fingerlings (Plate 3) without symptoms of disease (i.e., no abdominal distension, ragged fins, lethargic or hemorrhage) of average body weight 13.0 ± 0.22 g (mean ± SE), were obtained from Pilot Aquaculture centre, Kumasi, Ghana. Fish were distributed at random into (19 fish per tank) concrete tanks of capacity 80 L containing 50 L of water and a circumference of 3ft with height 2ft. Experimental fish were allowed to acclimatise for a week in which *Clarias gariepinus* were fed twice with the control diet on daily basis of their body mass at 2% in two equal ration at 8:30am and 4:00pm. The tanks were then randomly assigned groups thus (0g/kg Bs, 10g/kg Bs, 20g/kg Bs and 30g/kg Bs) in triplicates after acclimatization and sustained over the course of the eight-week trial. During the experiment, fish in the 0g/kg Bs group were fed diets without Bs supplementation while those in the 10g/kg Bs, 20g/kg Bs and 30g/kg Bs supplemented groups were fed control diet supplemented with Bs twice daily at 2 % of the body weight.

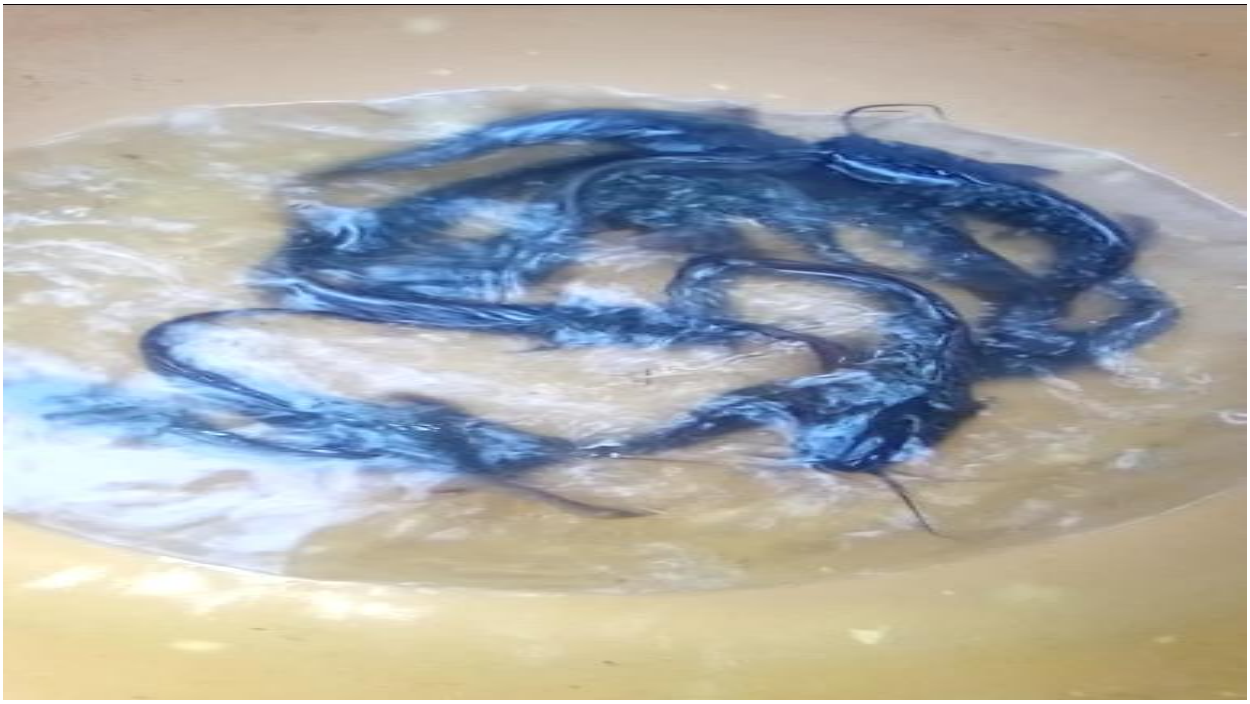

Plate 3: healthy catfish without symptoms of disease

3.3 Growth performance parameters and data analysis

Fish were weighed biweekly using an electronic scale till the final eight weeks before terminating the experiment. At the end of four and eight weeks six African catfishes were taken from each treatment and dissected to calculate body indices (condition factor (K), viscerosomatic index (VSI), and hepatosomatic index (HSI)). After eight weeks of feeding, the total amount of feed provided to the experimental and control groups was computed. Initial weight (Wi), Final weight (Wf), Feed conversion ratio (FCR) and Weight gain (WG), Abarike et al. (2013) revealed how the mentioned parameters were computed.

$$WG\ (\%) = 100 \times \frac{(Wf - Wi)}{Wi}$$

$$FCR = \frac{\text{total feed intake}}{\text{body weight gain}}$$

Body indices, condition factor (K), viscerosomatic index (VSI), and hepatosomatic index (HSI) were calculated as described by Adorian et al. (2018)

These parameters were calculated per tank as follows:

$$VSI = 100 \times \frac{\text{Viscera weight(g)}}{\text{Whole fish weight (g)}}$$

$$HSI\ (\%) = 100 \times \left(\frac{\text{liver weight}}{\text{final body weight}} \right)$$

$$K = \frac{Wf \times 100}{\text{final body length (cm)}3}$$ Where, Wi and Wf are initial and final weights (g), respectively

3.4 Blood sample collection

At four and eight weeks, blood samples were taken from fish fed diets treated with Bs and the control groups (i.e pooled into 9 EDTA tubes per treatment) to analyze the impact of giving *Clarias gariepinus* a supplemented diet of *Bacillus subtilis* at varied doses. Blood samples were taken from the caudal vein (Plate 4) of fish using a 2 ml disposable syringe and transferred to heparinized tubes as described by Adorian et al. (2018) which were taken to the Tamale Teaching Hospital lab for analysis. Hematological parameters, Plasma chemistry and liver functioning test were analysed at the Tamale teaching Hospital following the protocols below.

Plate 4: Blood sampling

Plate 5: Transfer of blood into EDTA tubes

3.5 Haematological analysis

3.5.1 Protocols for full blood count

The red blood cells, white blood cells, haemoglobin, haematocrit etc, were all measured in a full blood count (FBC). The blood sample was placed in an EDTA test tube and analysed using Urit – 5250 analyser at the Tamale teaching hospital lab.

3.5.2 Protocol for plasma chemistry

A working solution was prepared of three tubes. 1000 microlitre of reagent was added in the blank tube. 1000 microlitre of the reagent and 10 microlitre of standard was added to the standard tube, with 1000 microlitre of reagent and 10 microlitre of blood sample in third tube of test. The entire assay mixture was mixed and incubated for five minutes at 37°C. after the incubation was over, the whole mixture was aspirated into the flow cell and absorbance was measured. Blood indicators such as TP, ALB, y-GT, and T-BIL were analysed.

3.6 Protocol for toxicity of dietary treatments on *Clarias gariepinus*

Liver alanine aminotransferase and aspartate aminotransferase activities were assayed by the SGOT test based on the kinetic method. 800microlitre of the first reagent was added to 200 microlitre of the second reagent to get 1000 microlitre to acquire a working solution. 100 microlitre of blood was added to 1000 microlitre of the reagent. The reagent and the blood samples were mixed and aspirated into the flowcell. The absorbance was recorded at 60^{th} and 90^{th} seconds.

3.7 Statistical analyses

One way analysis of variance was carried out using IBM Statistical package for social sciences (SPSS version 21.0) to determine differences ($p < 0.05$) in growth, haematological, and toxic parameters. In cases where treatments showed a difference in means, Turkey HSD test, was further

used to determine which means were different ($p < 0.05$). Excel (2016) was used to present the data in the form of graphs and tables.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of Bs on Growth performance of *Clarias gariepinus*

Growth pattern of catfish (*Clarias gariepinus*) fed supplemented diets at different levels containing *Bacillus subtilis* is indicated in Figure 1. Initial mean weight recorded were 13.0 ± 0.22 g (mean ± SE). A significant increase was observed in all supplemented diets (10g/kg Bs, 20g/kg Bs and 30g/kg Bs) compared to the control (0g/kg Bs), with 10g/kg significantly higher amongst the supplemented diets at the end of week 8. Final weight at week 8 recorded were 44.65 ± 0.41g, 77.97 ± 0.89, 54.18 ± 0.95, 55.92 ± 0.30 for 0g/kg Bs, 10g/kg Bs, 20g/kg Bs and 30g/kg Bs respectively.

During the 8 weeks of study *Bacillus* fed diets and control were accepted by fishes. The development of *Clarias gariepinus* when given various doses of *Bacillus subtilis* is shown. in Table 2. A significant increase in final weight was observed in the fish fed supplemented diets (10g/kg Bs, 20g/kg Bs, 30g/kg Bs) in comparison to the control diet (0g/kg Bs), however the results showed 10g/kg Bs was significantly higher amongst the treated diet groups ($P < 0.05$). The *Bacillus* fed groups also showed a significant increase in weight gain in comparison to the control group, with 10g/kg Bs being significantly higher among the supplemented diets ($P < 0.05$). A significant decrease was observed in supplemented diet groups (10g/kg Bs, 20g/kg Bs, 30/kg Bs) compared to the control (0g/kg Bs) with FCR values. The FCR of fish fed the supplemented diets did not differ significantly ($P > 0.05$). Significant differences were observed in the HSI and VSI for *Bacillus* fed groups (10g/kg Bs, 20g/kg Bs, 30g/kg Bs) in comparison to 0g/kg Bs ($P < 0.05$). 10g/kg Bs showed a significant increase amongst the treated diet groups ($P < 0.05$). For K a significant increase was observed for 0g/kg in comparison to the *Bacillus* fed groups (10g/kg Bs,

20g/kg Bs, 30g/kg Bs). However, there was no significant difference in K among the treated diet groups (P > 0.05).

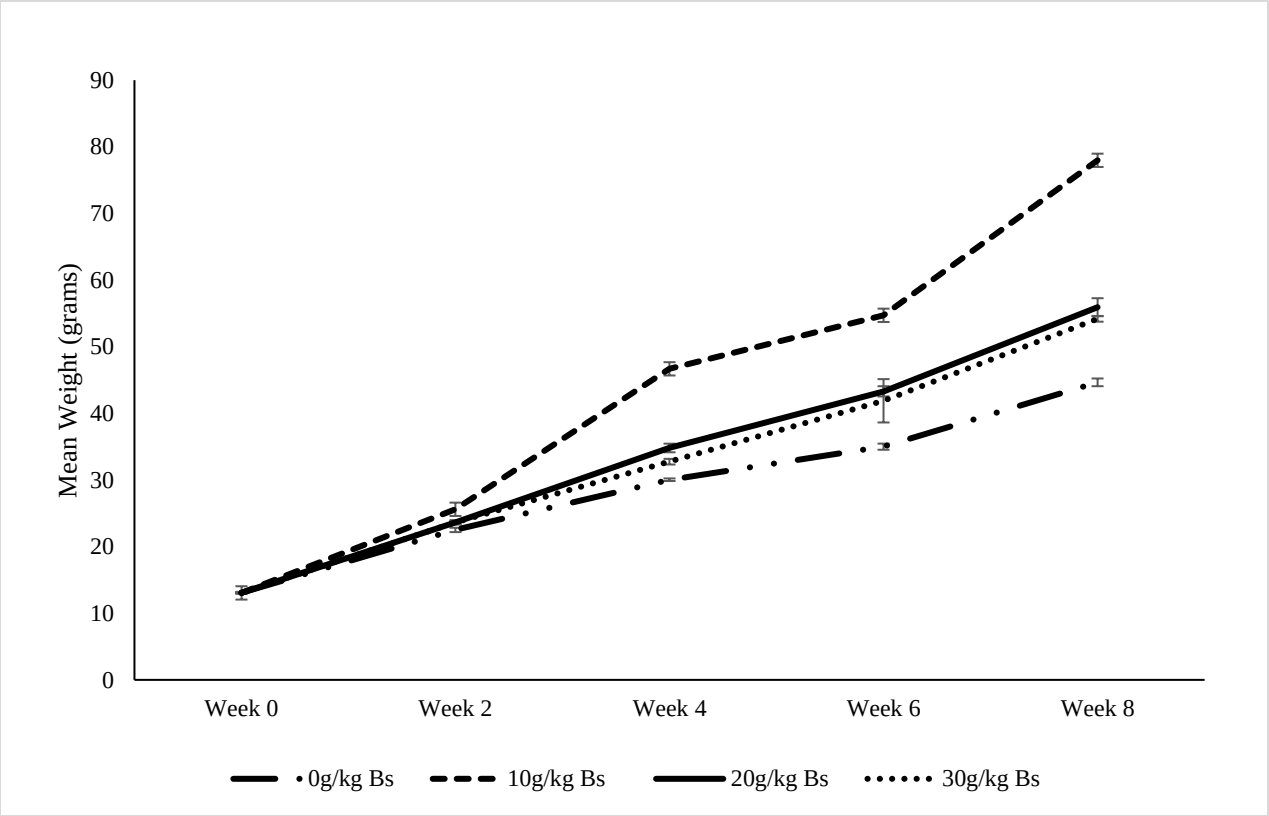

Fig 1: Growth pattern of Catfish for eight weeks

Table 2: Growth parameters of *Clarias gariepinus* fed test diets

Growth parameter	Dietary Treatments			
	0g/kg Bs	**10g/kg Bs**	**20g/kg Bs**	**30g/kg Bs**
Wi (g)	13.07 ^{a}	13.05 ^{a}	13.06 ^{a}	13.07 ^{a}
Wf (g)	44.65 ^{c}	77.97 ^{a}	55.92 ^{b}	54.18 ^{b}
WG (g)	31.07 ±1.71 ^{c}	64.92 ± 1.70 ^{a}	42.6 ± 0.95 ^{b}	41.11 ± 3.01 ^{b}
FCR (%)	3.72 ± 0.90 ^{a}	1.28 ± 0.05 ^{b}	1.25 ± 0.19 ^{b}	1.32 ± 0.24 ^{b}
VSI (%)	5.42 ± 0.62 ^{c}	8.24 ± 0.36 ^{a}	7.43 ± 0.15 ^{b}	7.21 ± 0.29 ^{b}

HS1 (%)	0.09 ± 0.01 [c]	0.87 ± 0.67 [a]	0.57 ± 0.54 [b]	0.54 ± 0.54 [b]
K	1.78 ± 0.04 [b]	2.52 ± 0.03 [a]	2.65 ± 0.03 [a]	2.58 ± 0.01 [a]

Note: The means in rows with the same superscript do not differ significantly

Where: Wi= initial weight, Wf= final weight, WG=weight gain, FCR=food conversion ratio, VSI=viscerosomatic index, HSI=hepasomatic index and K=condition factor.

4.2 Effects of supplemented diets on haematological parameters

4.2.1 White blood cells

The levels of WBC's of *Clarias gariepinus* fed supplemented diets for four and eight weeks is shown in figure 2. The results showed that the WBC's were significantly higher in fish fed supplemented diets (10g/kg Bs, 20g/kg Bs and 30g/kg Bs) diets than those fed on 0g/kg Bs diet at the end of four weeks ($P < 0.05$). However, among the supplemented diet groups, 10g/kg Bs showed significantly higher values ($P < 0.05$).

At eight weeks, Bs fed meals were significantly higher ($P < 0.05$) in comparison to the control, with 10g/kg Bs significantly higher among the supplemented diet groups. In general, as the experiment went from four to eight weeks the WBCs in the Bs supplemented diets dropped but were in optimal ranges. Also, although the results showed that *Bacillus subtilis* supplementation has the potential to increase significantly ($P < 0.05$) the WBCs in comparison to the control, there appears to be a decreasing trend with increasing doses of *Bacillus subtilis* in the diets.

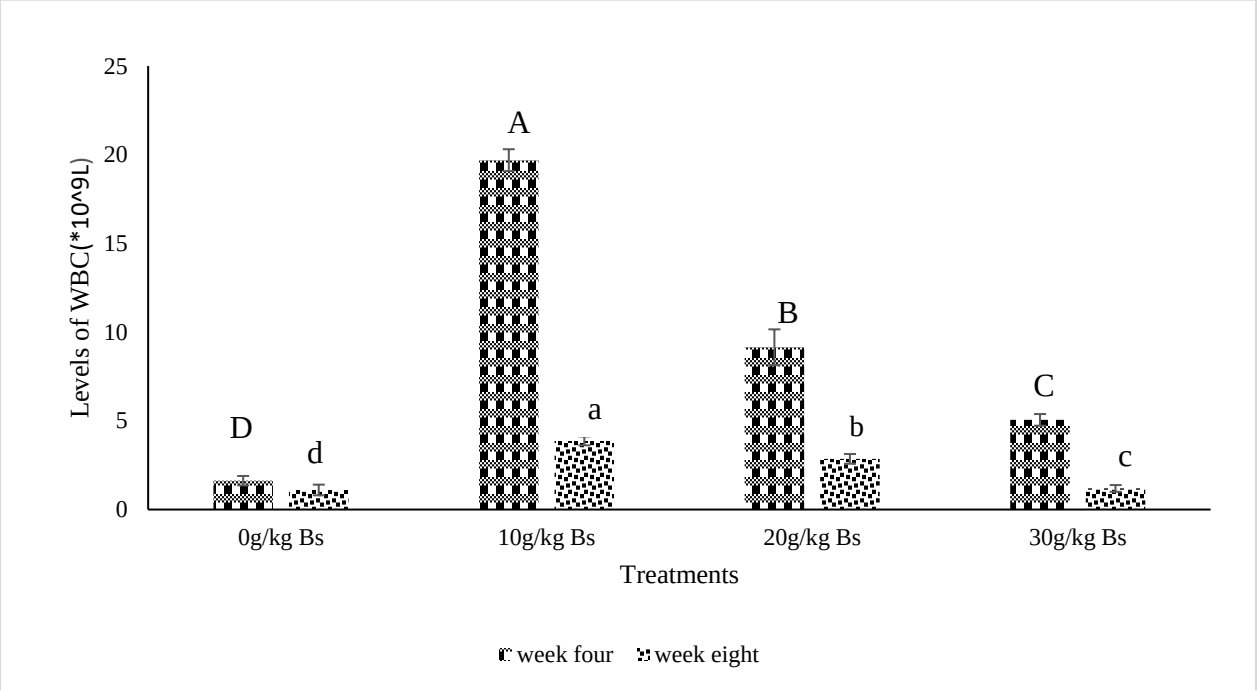

Fig 2: Bs supplemented diet on WBC after four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

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

The haematological parameters of individual White blood count (WBCs); Neutrophile (NEU), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS) and Basophil (BASO) of *Clarias gariepinus* fed *Bacillus subtilis* supplemented diets is shown in Table 3. Inclusion of *Bacillus subtilis* imparted significantly ($P < 0.05$) the blood parameters of the experimental fish. With a higher inclusion rate of supplemented diets, blood parameters showed a downward trend at both sampling times.

Table 3: Differential WBC's of *Clarias gariepinus* fed treated diets

Haematological Parameter	**Period**	**Dietary Treatments**			
		0g/kg Bs	**10g/kg Bs**	**20g/kg Bs**	**30g/kg Bs**
LYM	4 Wks	40.73 ± 0.40^{c}	73.63 ± 0.64^{a}	45.23 ± 0.30^{b}	41.68 ± 0.37^{c}
	8 Wks	34.46 ± 0.93^{c}	41.13 ± 0.68^{a}	37.59 ± 0.95^{b}	37.55 ± 0.72^{b}

MON	4 Wks	16.47 ± 2.50^{b}	21.07 ± 1.03^{a}	19.44 ± 2.41^{a}	20.19 ± 2.30^{a}
	8 Wks	25.27±1.03^{c}	36.76 ± 0.54^{a}	30.58±0.73^{b}	25.19± 0.92^{c}
NEU	4 Wks	39.15 ± 0.42^{b}	48.70 ± 0.16^{a}	46.66 ± 0.53^{a}	48.59 ± 0.62^{a}
	8 Wks	18.28±0.81^{d}	41.89 ± 0.26^{a}	30.63 ± 1.03^{b}	25.59 ± 0.74^{c}
EOSF	4 Wks	0.68 ± 0.60^{b}	2.77 ± 1.26^{a}	2.44 ± 1.25^{a}	2.01 ± 1.41^{a}
	8 Wks	1.46 ± 0.33^{c}	8.04 ± 0.20^{a}	4.13 ± 0.39^{b}	4.45 ± 0.28^{b}
BASO	4 Wks	0.73 ± 0.41^{b}	1.63 ± 1.24^{a}	0.83 ± 0.84^{a}	0.79 ± 0.66^{a}
	8 Wks	0.21 ± 0.11^{d}	1.84 ± 1.67^{a}	0.56 ± 0.05^{b}	0.32 ± 0.17^{c}

Note: The means in rows with the same superscript do not differ significantly

Where; LYM=Lymphocyte, MON=Monocyte, BASO=Basophiles, NEU=Neutrophiles, EOSF=Eosinophiles, weeks= Wks

4.2.3 RBC levels of *Clarias gariepinus* under different treatments

The effects of dietary supplemented diets on the level of RBC in *Clarias garepinus* for four and eight weeks is shown in Figure 3. The treated diet groups exhibited a considerable rise significantly in comparison to the control at the end of four weeks ($P < 0.05$). 10g/kg Bs was significantly higher among the supplemented diets with 20g/kg Bs and 30g/kg Bs showing similarities ($P > 0.05$). At the end of the eight-week period, the results showed a significant increase between *Bacillus* fed groups and the control, with a significant increase for 10g/kg Bs among the supplemented diet groups ($P < 0.05$).

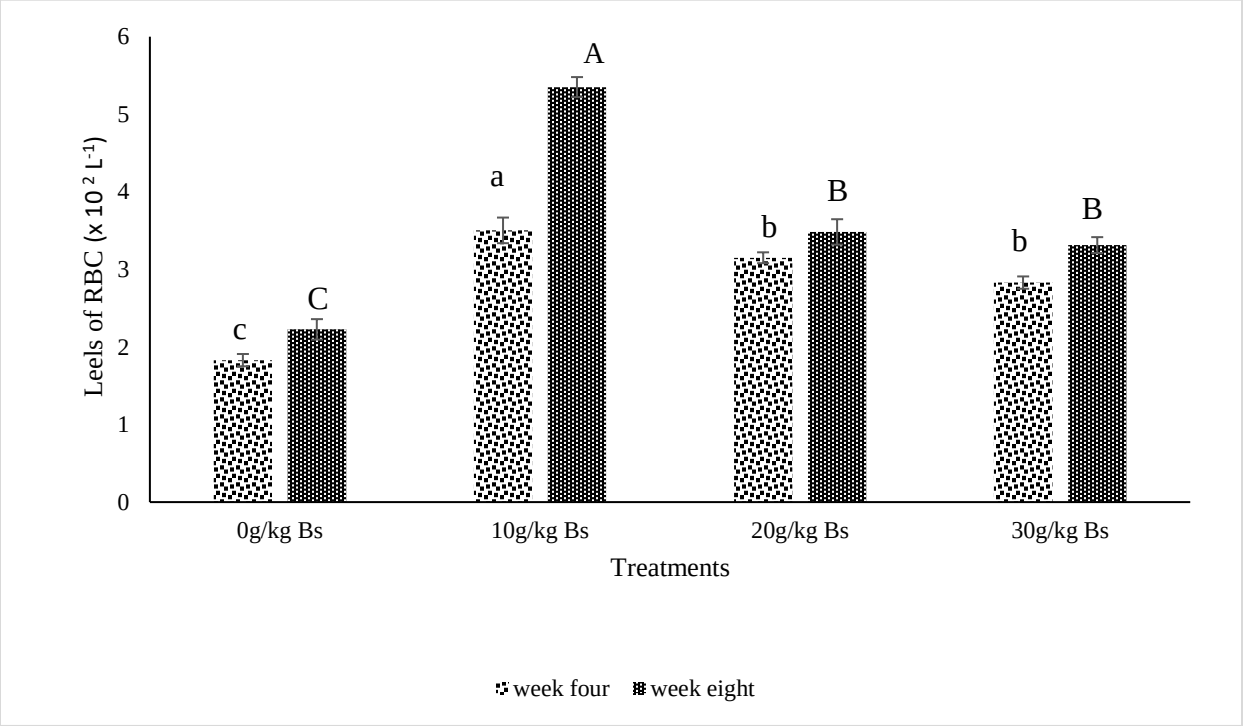

Fig 3: Bs supplemented diet on RBC after four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.2.4 Haemoglobin (Hb) levels of *Clarias gariepinus* under different treatments

As shown in Figure 4, there were significant differences in haemoglobin among the *Bacillus* fed diet group and control ($P < 0.05$). At four weeks, all *Bacillus subtilis* supplemented group had significantly higher results of Hb compared to the control ($P < 0.05$). Fish fed 10g/kg Bs showed the best increasing effects significantly on Hb compared to all other groups in the period of study ($P < 0.05$).

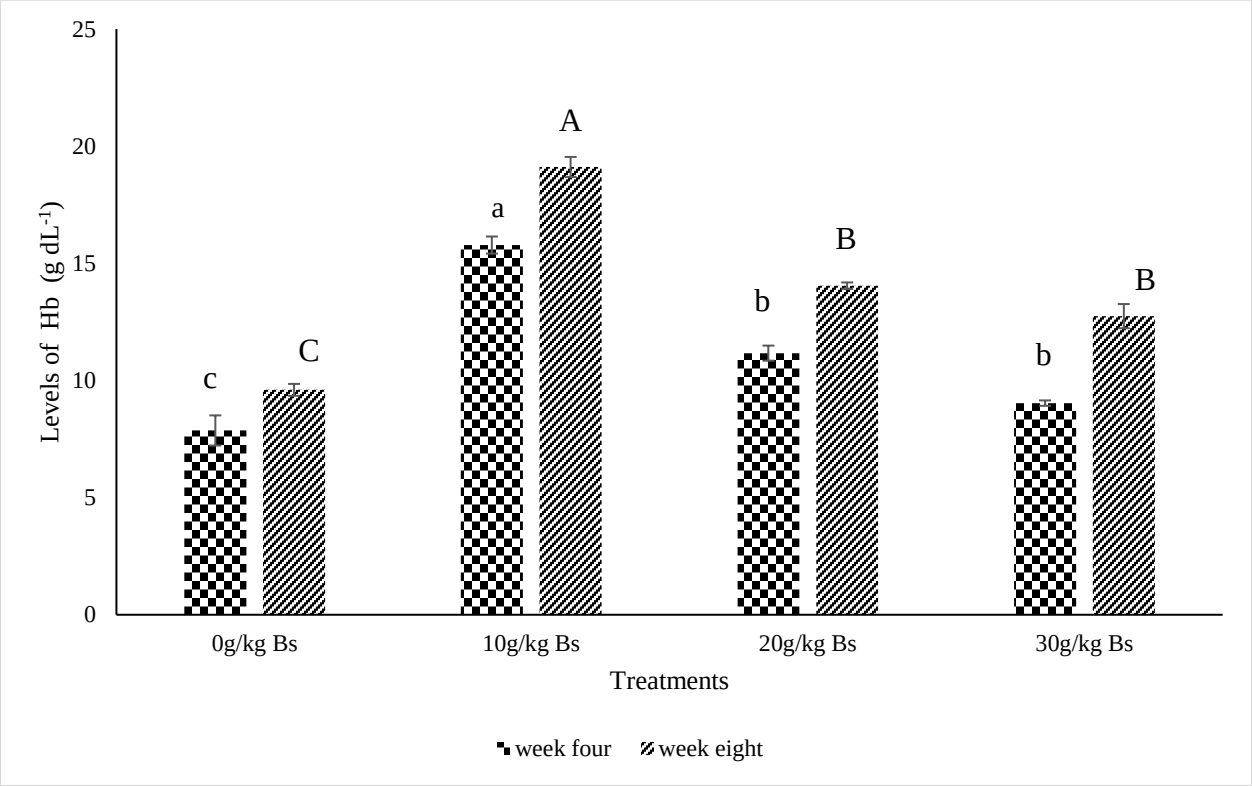

Fig 4: Bs supplemented diet on Hb after four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.2.5 Level of haematocrit (hct) in the blood of *Clarias gariepinus* under different treatments

The values of haematocrit under different doses for four and eight weeks is displayed in Figure 5. The haematocrit (hct) values were significantly lower in the fish in the control group compared to fish subjected to other treatments at the end of four weeks with 10g/kg Bs significantly higher among the supplemented diet group ($P < 0.05$). At the end of eight weeks a significant rise was observed with the supplemented diet group compared to the control where 10g/kg Bs showed a significant increase among the Bs supplemented diet group ($P < 0.05$).

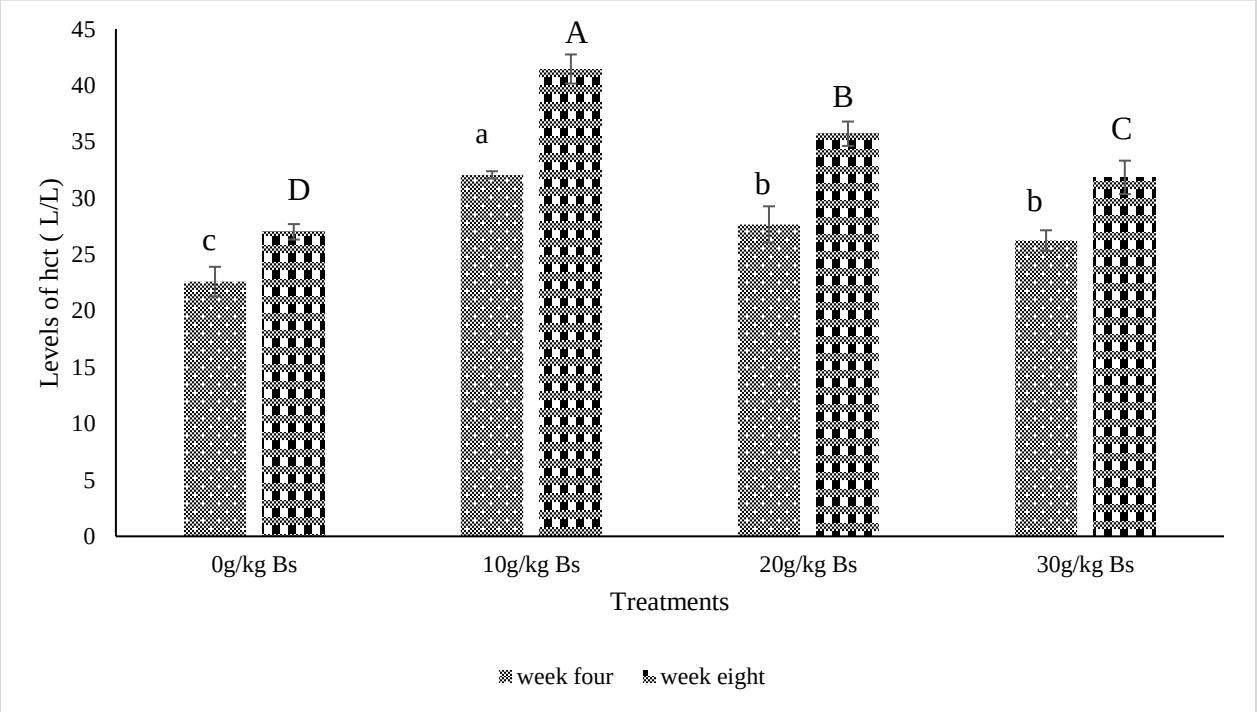

Fig 5: Bs supplemented diet on hct after four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.2.6 Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean Corpuscular haemoglobin concentrations (MCHC) of *Clarias gariepienus*

In Table 4, haematological parameters of *Clarias gariepinus* fed with *Bacillus subtilis* supplemented diets are shown, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentrations (MCHC). Supplementation of *Bacillus subtilis* imparted significantly ($P < 0.05$) on the blood of the *Clarias gariepinus*. The results for MCV revealed a significant difference in supplemented diet groups compared to the control with 10g/kg Bs showing a significant increase among the supplemented diet groups at the end of four and eight weeks ($P < 0.05$). At the end of four weeks, MCH significantly differed with the supplemented diet groups compared to the control ($P < 0.05$). However, among the Bs diet groups the was no difference ($P > 0.05$). Compared to the control group, the treated diet groups increased significantly with 10g/kg increasing significantly among

the supplemented diet group at the end of eight weeks ($P < 0.05$). MCHC values significantly showed no statistical difference between control and supplemented diet groups at the end of four weeks ($P > 0.05$). At the end of eight weeks, levels of MCHC in 10g/kg Bs increased significantly in comparison to other treatments including the control, with no difference significantly in other groups ($P > 0.05$).

Table 4: MCV, MCH, MCHC of *Clarias gariepinus*

Haematological Parameter	**Period**	**Dietary Treatments**			
		0g/kg Bs	**10g/kg Bs**	**20g/kg Bs**	**30g/kg Bs**
MCV	4 Wks	115.65 ± 2.75^{c}	132.60 ± 0.65^{a}	123.36 ± 1.60^{b}	122.16 ± 1.14^{b}
	8 Wks	130.01 ± 3.96^{c}	146.49 ± 0.63^{a}	137.65 ± 1.92^{b}	136.43 ± 1.94^{b}
MCH	4 Wks	40.80 ± 0.30^{b}	47.06 ± 0.41^{a}	46.96 ± 3.02^{a}	45.06 ± 1.81^{a}
	8 Wk	51.19 ± 1.11^{c}	63.03 ± 0.44^{a}	59.74 ± 1.14^{b}	57.35 ± 0.94^{b}
MCHC	4 Wks	33.43 ± 3.52^{a}	35.83 ± 1.02^{a}	34.86 ± 0.75^{a}	34.46 ± 0.26^{a}
	8 Wks	37.75 ± 0.45^{b}	46.24 ± 0.46^{a}	38.93 ± 0.91^{b}	39.73 ± 0.45^{b}

Note: The means in rows with the same superscript do not differ significantly

Where MCV= mean corpuscular volume, MCH= mean corpuscular haemaglobin, MCHC= mean corpuscular haemaglobin concentration, Wks= weeks

4.3 Plasma chemistry

4.3.1 Levels of total protein

Results of the total protein analysis are summarized in Figure 6. *Clarias gariepinus* fed on diets containing probiotics had higher total protein than the control group at four and eight weeks. Among the different *Bacillus* supplemented diets, 10g/kg Bs significantly was higher within the Bs treated group, with a declining tendency as the supplemented quantity of Bs increased ($P < 0.05$).

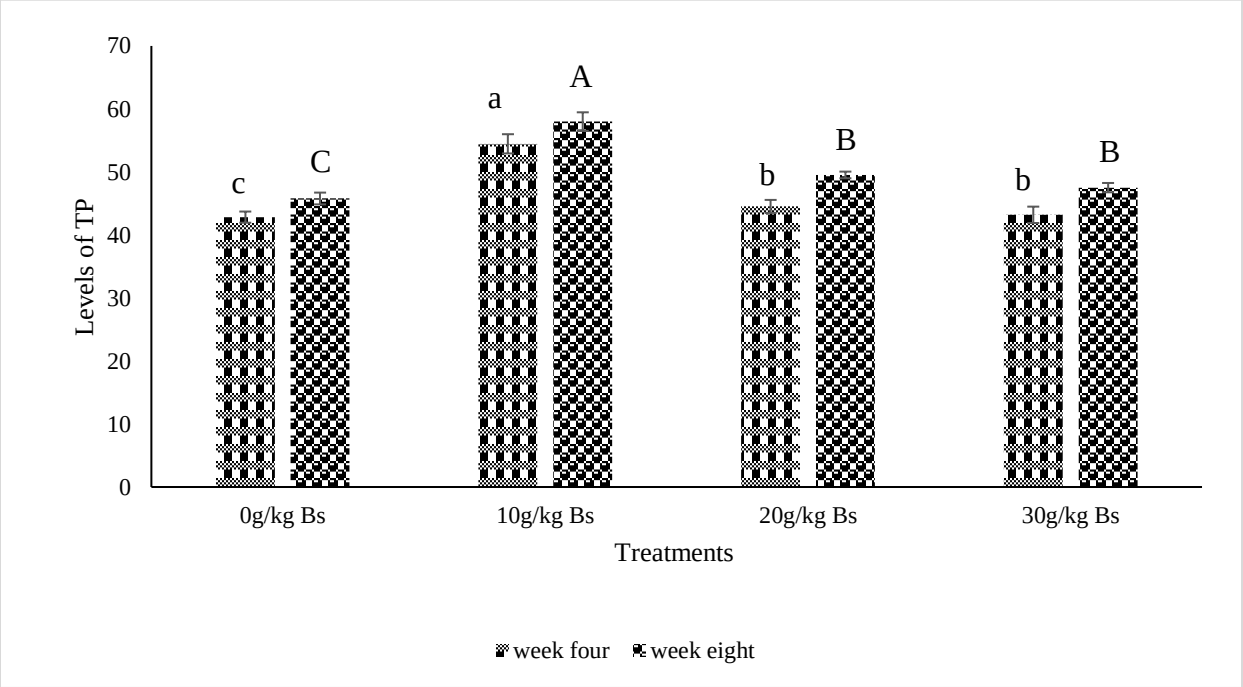

Fig 6: Bs supplemented diets on total protein (TP) for four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.3.2 Albumin levels

Figure 7 represents the effects of *Bacillus subtilis* supplementation on Albumin of *Clarias gariepinus*. At the end of four and eight weeks, the 10g/kg Bs supplemented diet group exhibited higher Albumin levels in comparison to groups receiving higher *Bacillus subtilis* addition (20g/kg Bs and 30g/kg Bs). The control group fed with 0g/kg Bs probiotic presented lower Albumin values than the supplemented diet groups ($P < 0.05$). There was statistical similarities between 20g/kg Bs and 30g/kg Bs. At the end of the experiment supplementing *Clarias gariepinus* with 10g/kg Bs – 30g/kg Bs enhanced the level of Albumin.

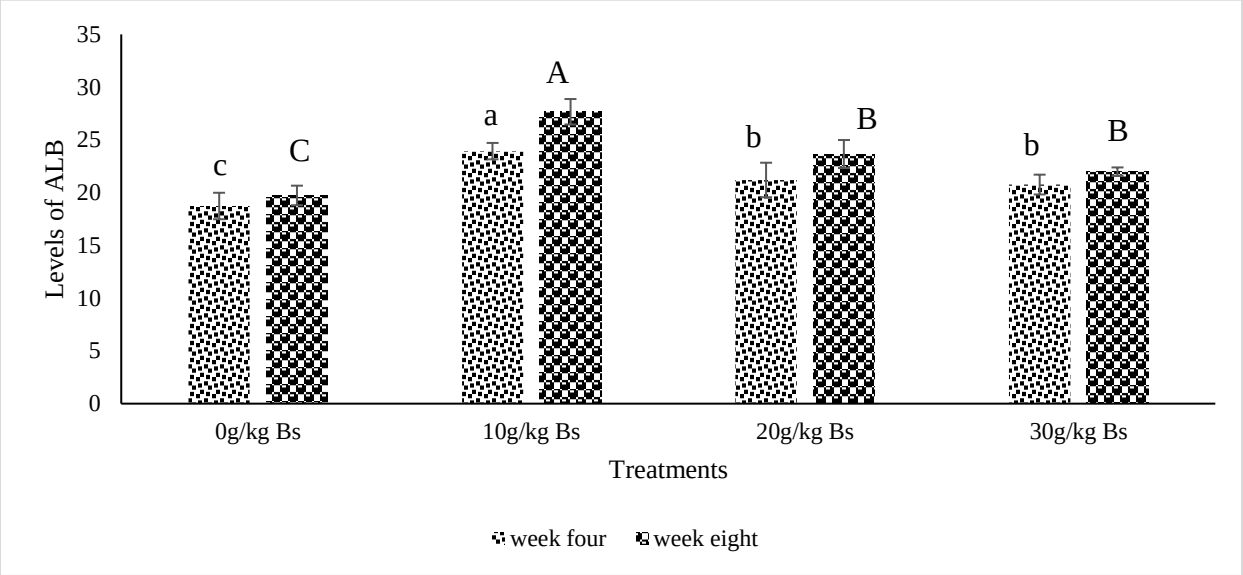

Fig 7: Bs supplemented diets on Albumin at four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.3.3 Y-GT and T-BIL levels

Table 5 shows the sub protein levels (Y-GT and T-BIL) of fish fed a control or Bs enhanced diet at four and eight weeks. At week four and eight, adding Bs to the diet of *Clarias gariepinus* enabled the experimental fish have considerably greater levels of Y-GT than the control group significantly ($P < 0.05$). At the end of four weeks, Y-GT levels was enhanced significantly in all Bs treated groups in comparison to the control group ($P < 0.05$). Statistically no significant difference was observed within the supplemented diet groups at the end of week eight ($P > 0.05$). When compared to the control group, Bs supplemented groups had significantly higher Y-GT levels, with 10g/kg Bs increasing significantly ($P < 0.05$).

Clarias gariepinus fed with Bs supplemented diet significantly improved the level of T-BIL of fish compared to control diet ($P < 0.05$). However, 20g/kg Bs and 30g/kg Bs showed no difference significantly at the end of four and eight weeks. With 10g/kg Bs increasing significantly among the treated groups ($P > 0.05$).

Table 5: Y-GT and T-BIL of *Clarias gariepinus*

Haematological Parameter	Period	Dietary Treatments			
		0g/kg Bs	10g/kg Bs	20g/kg Bs	30g/kg Bs
y-GT	4 Wks	0.76 ± 0.89^{b}	5.89 ± 0.41^{a}	6.83 ± 2.02^{a}	5.70 ± 1.81^{a}
	8 Wks	3.51 ± 1.67^{c}	13.61 ± 0.76^{a}	8.86 ± 1.82^{b}	7.02 ± 1.24^{b}
T-Bil	4 Wks	4.60 ± 0.34^{c}	13.53 ± 1.25^{a}	6.85 ± 1.25^{b}	6.16 ± 1.18^{b}
	8 Wks	5.19 ± 0.49^{c}	16.28 ± 0.38^{a}	9.92 ± 1.18^{b}	8.35 ± 0.70^{b}

Note: The means in rows with the same superscript do not differ significantly

4.4 Effects of Bs diets on *Clarias gariepinus* liver health

4.4.1 Alanine Amino transferase (ALT)

The ALT levels of fish given both control and *Bacillus subtilis* supplemented diets for four and eight weeks are shown in Figure 8. At four weeks, the results showed no significant difference between the control (0g/kg Bs) and 10g/kg Bs and also between (20g/kg Bs and 30g/kg Bs). However, a significant increase in ALT values in the control (0g/kg Bs) compared to the *Bacillus* fed diet group (10g/kg Bs, 20g/kg Bs, 30g/kg Bs) was observed at the end of eight weeks ($P < 0.05$). Among the treated diets groups, a significant decrease in ALT for 10g/kg Bs was observed ($P < 0.05$).

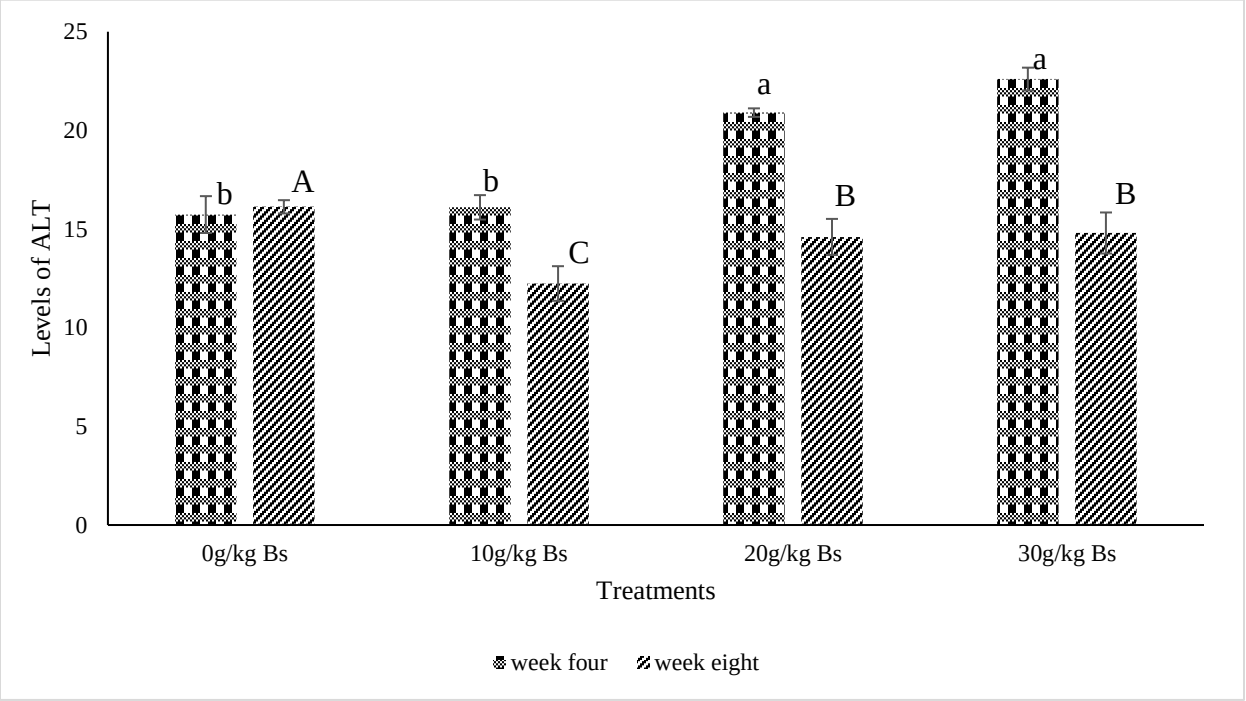

Fig 8: Bs supplemented diet on ALT at four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.4.2 Aspartate Aminotransferase (AST)

Among the different levels of *Bacillus* fed diets in each group. The greatest amount of AST was recorded in the Bs-supplemented diet at 30g/kg Bs with the control recording the lowest values at the end of four weeks. Eight weeks revealed the control diet, 20g/kg Bs and 30g/kg Bs, had the greatest AST levels, which was significantly higher ($P < 0.05$) when compared to the 10g/kg Bs supplemented diet ($P < 0.05$). Generally, in comparison to the control group, AST values in Bs supplemented meals were lower at eight weeks.

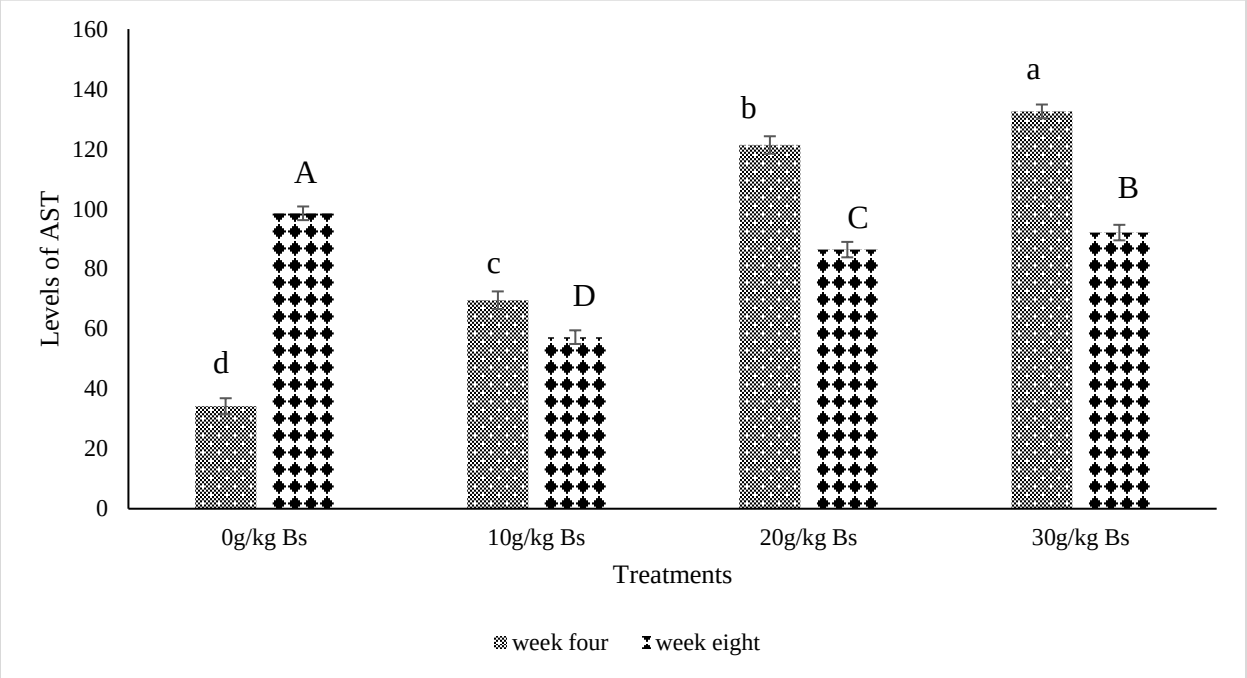

Fig 9: Bs supplemented diets on AST at four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

4.4.3 Alkaline Phosphatase (ALP)

Within the first four weeks, the ALP levels in *Clarias gariepinus* in the control group were substantially lower ($P < 0.05$) than those in the other treatments (Figure 10).

When compared to the treatment diets, the control group experienced a substantial increase ($P < 0.05$) in ALP levels after eight weeks. At the completion of this study, ALP levels were found to be substantially higher in the control group.

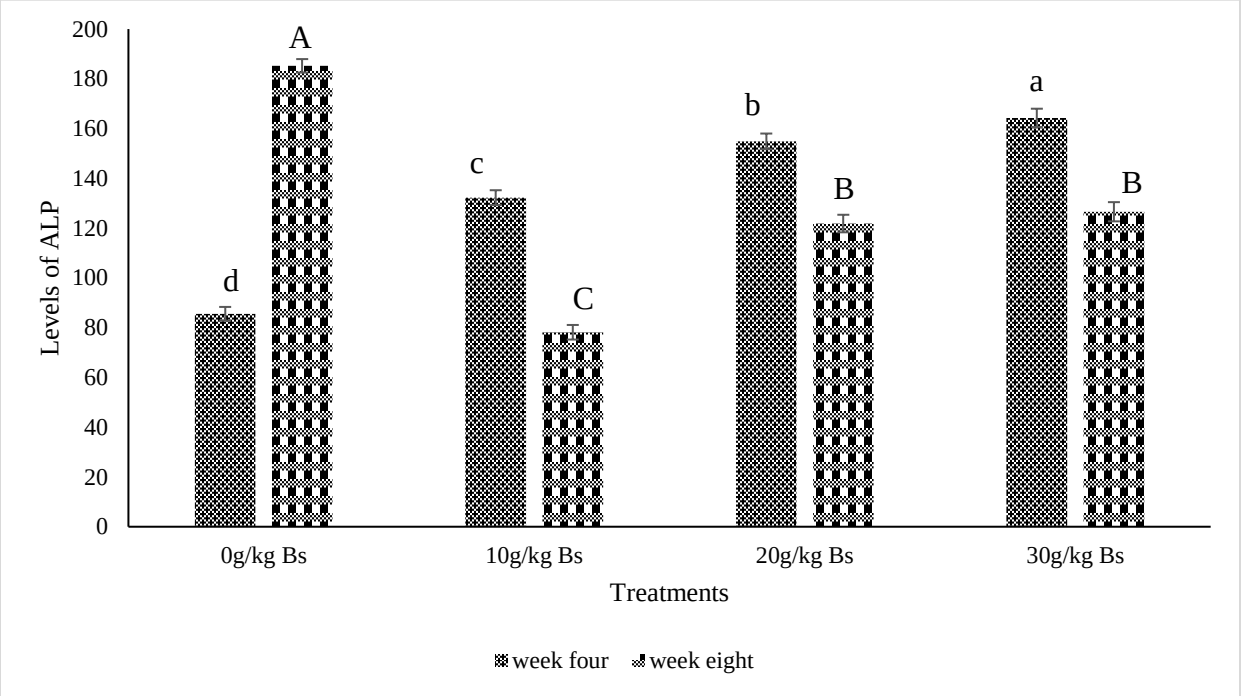

Fig 10: Bs supplemented diet on ALP at four and eight weeks

Note: Per sampling, bars with the same letter are not statistically different

CHAPTER FIVE

5.0 DISCUSSION

5.1 Growth of *Clarias gariepinus* fed Bs treated diet

Probiotics have been employed as a non-toxic and environmentally acceptable alternative for the enhancement of fish growth (Martínez Cruz et al., 2012). *Bacillus* probiotic looks to be widely utilized due to its several benefits, including its potential to promote fish health. and growth conditions (Soltani et al., 2019).

The recent study findings revealed that feeding *Clarias gariepinus* with *Bacillus* treated diets increased significantly, the final weight, weight gain, and feed conversion ratio of the fish.

Bacillus sp. has been shown to provide vital nutrients to the host organism, such as amino acids and vitamins K and B12, which can improve growth performance (Liu et al., 2012). Upon terminating the experiment at end of two months, the final weight of *Clarias garriepinus* increased significantly in probiotic groups particularly, with 10g/kg Bs group presenting the best of results. It therefore suggests that maintaining lower dose is preferred to give the best of result. This agrees with the findings in Cha et al. (2013) who supplemented dietary *Bacillus* spp at a lower dose on flounder. Fish fed enriched diets gained considerably more weight at various doses (10g/kg Bs – 30g/kg Bs) in comparison to the control, with 10g/kg Bs showing the best performance which also corroborated Aly et al. (2008) who fed tilapia a low dose of *Bacillus pumilus* when used for two months, it resulted in significant weight gain. This suggests that, feeding *Clarias gariepinus* with *Bacillus subtilis* within the ranges (10g/kg Bs – 30g/kg Bs) has the potential to improve weight gain within the stipulated eight weeks of study which will be desired for production and again 10g/kg presenting the best results.

Similarly, significantly lower FCR were observed in Bs treatment groups (10g/kg Bs, 20g/kg Bs and 30g/kg Bs) compared to the control is suggestive of supplemented diet groups' superior feed

and nutrient utilization at ranges (10g/kg Bs – 30g/kg Bs), converting to a higher final weights and weight gain observed in the Bs supplemented diets which agrees with previous studies (Van Doan et al., 2018; Liu et al., 2010). The quality of the feed is indicated by the body index, which includes VSI, HSI, and K (Munir et al., 2016).

The new study findings were consistent with those of previous studies (Abarike et al., 2018; Kuebutornye et al., 2019) as significantly HSI and VSI recorded were superior in the Bs diet groups than the control group. Similarly The current results also agreed with the work of Adorian et al., (2018) which showed the best performance in condition factor for *Lates calcarifer* fed *Bacillus* spp. The findings proved that catfish supplementation improves fish development and feed utilization with *Bacillus subtilis* at doses with 10g/kg Bs showing the best effect.

5.2 Effect of *Bacillus subtilis* treated diet on hematology

The quality, amount, and toxicity of the animal's ingested food alter the blood components, which can be used to determine the animal's pathological and nutritional status. At the end of 8 weeks blood parameters were boosted. This suggests that including *Bacillus subtilis* at 10g/kg Bs in *Clarias gariepinus* diet has the potential to boost its health. This conclusion is based on Dahiya et al. (2012) who indicated that an increase in hematological parameters following *Bacillus subtilis* supplementation is an indication of good health for fish. Farrell, (2011) stated that the primary role of the RBC's is for oxygen transport. The results of this study shows that RBC's were significantly higher in fishes fed with the supplemented diets at levels (10g/kg Bs – 30g/kg Bs) compared to the control in agreement to previous studies (Azarin et al., 2014; Faramazi et al., 2011; Sharma & Sihag, 2013). This indicates that, under stressful condition where oxygen is limited, fishes fed with *Bacillus* treated diets has the capacity to live well and survive.

According to Bolliger & Everds (2012), haemoglobin plays a big role in transport of oxygen to peripheral tissues from gas-exchange organs. The study findings revealed a rise in haemoglobin levels in fishes fed with Bs enhanced diets in comparison to the control group, indicating their high chance of survival in oxygen restricted conditions. This is in line with past research (Garcia-Marengoni et al., 2015; Rajikkannu et al., 2015) who supplemented Nile tilapia diets with lower doses of *Bacillus*. The haematocrit is a measurement of blood capacity to carry oxygen. The higher the oxygen-moving ability of the blood, the higher the haematocrit value (Gallaugher, 1994), and It tries to keep the organism stable as much as possible, even in stressful or hypoxic situations caused by toxicants. (Shah et al., 2006) or environmental stress, (Udoh & Udoidiong, 2004). The end results after eight weeks showed significantly increase in haematocrit levels for *Bacillus* treated diet groups compared to the control which agrees with previous reports (Feliatra et al., 2018; Garcia-Marengoni et al., 2015).

The findings of this investigation revealed that fishes fed *Bacillus subtilis* increased the WBC's levels significantly at the end of 8 weeks which fell in optimum ranges compared to the control agrees with previous work (Orun et al., 2003). This indicate that fishes fed *Bacillus* at ranges will have the tendency to increase their resistance to infection and disease conditions under pathological condition.

5.3 Effect of Bs on Plasma chemistry of *Clarias gariepinus*

A total protein test on blood serum is used to improve the detection of medical problems such as kidney and liver illness (Etim et al., 2013). A reduction in the values of total protein may indicate inadequate feed intake and utilization, whereas a rise in total protein in an organism's blood may indicate improved feed intake and utilization (Chowdhury & Roy, 2020). *Bacillus subtilis* supplementation significantly increased plasma total protein in the current investigation. This

suggests that Bs can increase the efficiency of protein utilization in diet. Chowdhury & Roy, (2020) reported an improvement in the protein utilization of *Clarias* broodstock fed with different doses of *Bacillus subtilis* which optimizes protein utilization for growth, lowering both feed and production costs.

5.4 Effect of *Bacillus subtilis* on liver health of *Clarias gariepinus*

AST, ALT, and ALP are enzymes involved in several biochemical metabolic activities that interconvert amino acids to other metabolic intermediates, and an increase in their levels can indicate tissue damage, such as in chronic liver disease (Abdollahi-Arpanahi et al., 2018; Babazadeh et al., 2011).

When compared to the control fish, *Clarias gariepinus* exposed to various dosages of Bs supplemented feed showed an increase in AST, ALT, and ALP. It is believed this might be as a result of their initial tolerance to the Bs in the feed. However, as compared to control meals, Bs-treated foods had lower levels of AST, ALT, and ALP after eight weeks, showing that Bs-supplemented diets could help improve the health of the fish's liver, which could be linked to their ability to protect rather than harm the fish's liver. One of the most well-known measures of liver function is the measurement of these enzymes (Abdollahi-Arpanahi et al., 2018).

Moreover, it was observed lower levels of ALT in groups fed the Bs treated diet in comparison to the control at the end of the 2 months experiment. This agrees with the findings of Adorian et al. (2018), who found that fish fed a diet supplemented with probiotic Bs had lower liver enzymes (AST, ALT, and ALP) than fish fed a control diet. (Abdollahi-Arpanahi et al., 2018). Similarly ALP was reduced significantly at the end of eight weeks in Bs groups compared to the control which agrees with the work of Abdollahi-Arpanahi et al. (2018) who administered *Bacillus subtilis* and *Bacillus licheniformis* in *Litopenaeus vannamei*.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Probiotics has emerged as an alternative for improving the growth and health of fish. From the current research work it can be concluded that *Bacillus subtilis* 200, a commercially available probiotic is a growth enhancer and can boost haematological parameters in fish. Supplementing *Clarias gariepinus* diet at 10g/kg – 30g/kg help improved the growth of *Clarias gariepinus*; however, 10g/kg in comparison to other groups proved to be superior. In addition, dietary supplements for *Clarias gariepinus* within 10g/kg – 30g/kg significantly improved hematological parameters, plasma chemistry with 10g/kg in comparison to the other treatments, having the greatest impact on hematological indicators.

Similarly, enhancing feed of *Clarias gariepinus* within levels 10g/kg – 30g/kg inclusion level appeared not to have unfavourable consequences on the state of liver health of *Clarias gariepinus* after two months of feeding.

6.2 Recommendation

It is recommended *Bacillus subtilis* is used for dietary supplementation at 10g/kg for the best of results. However, Histopathological study can be done to check the state of the liver after eight weeks.

It is also suggested that the study be extended beyond 8 weeks in order to guarantee that the liver is in good working conditions.

To determine its ability to improve resistance, pathogenic infection tests should be performed on fish fed a Bs supplemented diet.

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APPENDICES

Appendix 1: Growth pattern of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet, week zero (0)

Dietary treatments								
Week(0)	**0% Bs**		**1% Bs**		**2% Bs**		**3% Bs**	
	BW(g)	**TL (cm)**	**BW(g)**	**TL (cm)**	**BW(g)**	**TL (cm)**	**BW(g)**	**TL (cm)**
	13	10.4	18	14.2	16	13.6	12	11.6
	14	12.5	9	9.3	23	14.2	23	14.2
	15	14.5	10	9.7	20	14.1	29	14.8
	15	11.8	7	8.2	8	9.2	6	7.3
	12	10.2	6	7.3	15	13.6	7	7.9
	12	10.1	6	7.3	15	13.7	8	8.9
	9	9.3	21	14.2	18	14.8	19	15
	13	10.1	19	14.9	9	9.8	8	8.9
	17	13.7	17	13.5	11	12.2	14	11.8
	16	13.5	7	7.9	8	5.8	16	13.4
	12	11	16	13.8	7	7.4	8	8.8
	15	13.9	20	14.2	8	9.1	22	14.2
	16	13.2	7	8.2	12	11.8	9	10.2
	12	11	19	14.7	12	7.2	9	10.2
	6	7.2	21	14.3	8	9.1	21	14.2
	14	12.8	10	9.9	8	9.2	6	7.4
	16	13.2	8	9.1	8	8.9	12	11.4
	15	12.4	6	7.3	12	7.4	15	13.2
	13	11.6	5	6.8	9	5.2	18	14.5
Average	**13.07**	**11.7**	**13.05**	**11.25**	**13.06**	**10.34**	**13.07**	**11.4684**

Where BW is body weight, TL is total length

Appendix 2: Growth pattern of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet, week two

Dietary treatments

Week(2)	0% Bs		1% Bs		2% Bs		3% Bs	
	BW(g)	TL (cm)	BW(g)	TL (cm)	BW(g)	TL (cm)	BW(g)	TL (cm)
	52	19	29	16.1	53	19	29	15.1
	38	18	15	13.2	33	16.2	36	15.7
	14	13	27	16.1	10	11	12	12.8
	17	13	20	14	23	15.5	18	13.5
	44	17.5	39	17.4	11	12.4	22	14.8
	12	11.7	22	15	23	15	27	15.6
	12	11.8	41	17.4	13	12	13	12.6
	28	16	18	13.2	18	13.5	33	16.7
	29	15	15	13.2	26	15.4	27	15.7
	11	11	29	16.3	46	19	28	16.5
	15	12.5	19	14	21	14.5	27	14.5
	33	15.9	26	15.9	17	13.7	23	14.2
	12	13	20	14.2	24	15	16	12.5
	27	16	23	14.3	18	13	27	16.5
	15	12	31	15.4	17	13	35	17
	24	14.5	21	14.4	29	15	22	15
	14	12	31	16.4			16	13
	11	12.4					12	12.3
	20	14					26	15.7
Average	**22.33**	**14.12**	**25.6**	**15.08**	**23.87**	**14.57**	**23.63**	**14.72**

Where BW is body weight, TL is total length

Appendix 3: Growth pattern of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet, week four

Dietary treatments

Week(4)	0% Bs		1% Bs		2% Bs		3% Bs	
	BW(g)	TL (cm)	BW(g)	TL (cm)	BW(g)	TL (cm)	BW(g)	TL (cm)
	41	18	40	17.7	85	22	36	15
	45	18	45	17.7	60	21	50	19.4
	49	19	74	21.5	50	18	90	23
	45	18	66	20	50	18	43	18
	50	20	60	20	40	16	48	19
	29	14.5	60	20	30	17	50	19
	16	11	55	19	50	18	42	18
	22	14	45	17.5	30	16	30	16
	56	21	54	19	30	15	25	15.5
	24	14.7	54	18.5	33	15	41	15.5
	27	17	40	17.7	35	16	21	14
	28	17	45	18.2	29	15	17	13
	24	17	35	16.5	22	13	18	13
	14	12	35	17	29	14.7	18	14
	16	13	25	14	29	14.5	33	15
	18	11.7	25	14	18	14.5	24	14
	9	10	25	14	13	12	16	13
					18	15		
Average	**30.03**	**15.64**	**46.67**	**17.78**	**36.16**	**16.15**	**35.41**	**16.14**

Where BW is body weight, TL is total length

Appendix 4: Growth pattern of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet, week six

	Dietary treatments							
Week(6)	**0% Bs**		**1% Bs**		**2% Bs**		**3% Bs**	
	BW(g)	**TL (cm)**	**BW(g)**	**TL (cm)**	**BW(g)**	**TL (cm)**	**BW(g)**	**TL (cm)**
	71	21	67	21.1	80	21.5	52	20
	52	19	57	20	61	20	79	22
	47	18.4	29	15.5	58	20	60	19.8
	26	15.2	95	23.2	54	18.4	54	19.5
	40.5	18.5	63	20.3	50	18.9	51	19
	19	13.6	88	23.5	35	18.4	30	15.8
	41	17.3	64	21	52	18.5	44	19.1
	44	18.2	50	19.9	41	17.5	39	17
	31	14.5	52	20	23	15.9	15	13
	30	16.3	42	18.1	25	15.5	24	16
	37	17.9	54	19.9	23	15.9	32	15.9
	25	16.2	39	18	30	17	36	17.1
	21	15	25	15.4	32	16	31	17
	10	11.1	26	15.3	30	15.5	27	15
Average	**34.99**	**16.58**	**54.71**	**19.37**	**42.44**	**17.78**	**41**	**17.58**

Where BW is body weight, TL is total length

Appendix 5: Growth pattern of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet, week eight

	Dietary treatments							
Week(8)	**0% Bs**		**1% Bs**		**2% Bs**		**3% Bs**	
	BW(g)	**TL (cm)**	**BW(g)**	**TL (cm)**	**BW(g)**	**TL (cm)**	**BW(g)**	**TL (cm)**
	66	21.5	61	20.5	58	19.5	44	18.2
	55	19.5	123	25	42	17.5	85	23
	32	16.9	76	21.5	69	21.2	83	22.5
	34	17.9	129	25	43	18	77	21
	59	21.5	84	22	80	21.5	67	21
	65	21.9	87	23	43	17	39	18.4
	32	16.2	34	16.5	60	20.2	38	19
	89	22.6	78	21.5	33	16.2	63	20
	47	19.2	90	23.2	46	17.6	41	17
	30	16.1	60	19.8	56	20.1	63	19.9
	53	16.8	50	18.5	50	18.4	49	19.2
	41	16.9	40	17.5	46.2	17.7	38	17
	25	15.5	90	23			29	15.5
	11	11.4						
Average	**44.65**	**18.13**	**77.97**	**21.3**	**55.92**	**18.36**	**54.18**	**19.36**

Where BW is body weight, TL is total length

Appendix 6: Blood profile of *Clarias gariepinus* at week four

	Dietary treatment

Hematological	0% Bs			1% Bs			2% Bs			3% Bs		
parameter	A	B	C	A	B	C	A	B	C	A	B	C
WBC	1.43	1.82	1.61	13.36	14.82	18.89	9.43	8.34	9.67	4.67	5.65	4.83
RBC	1.26	1.96	2.27	2.89	3.57	4.05	2.89	3.18	3.38	2.55	2.98	2.96
HGB	6.59	7.47	9.55	13.78	15.67	17.89	11.27	11.23	10.98	7.65	9.45	9.99
HCT	18.47	21.82	27.52	29.44	32.12	34.62	22.45	26.43	34.11	21.34	25.67	31.68

Appendix 7: Differential WBC of *Clarias gariepinus* at week 4

Hematological	Dietary treatment											
parameter	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
LYM%	39.87	41.23	41.09	69.87	72.72	78.31	42.88	46.75	46.06	39.82	40.76	44.46
MON%	2.54	1.94	3.89	9.45	12.45	11.31	7.92	8.31	9.09	4.98	5.89	7.71
NEU%	38.78	36.87	41.8	46.3	46.93	52.87	41.78	45.21	46.99	49.57	49.76	45.67
EOS%	0.72	0.54	0.78	2.56	3.14	2.61	2.33	3.12	1.87	1.87	2.32	1.84
BASO%	0.65	0.75	0.79	1.77	1.32	1.81	0.78	0.92	0.79	0.64	0.82	0.91

Appendix 8: MCV, MCH and MCHC of *Clarias gariepinus* at week 4

Hematological	Dietary treatment											
parameter	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
MCV	110.66	117.76	118.58	129.88	131.45	136.47	121.76	125.77	123.56	119.32	125.54	121.2
MCH	39.65	41.32	41.43	42.33	45.69	44.16	40.17	43.77	44.94	44.76	39.87	41.45
MCHC	31.22	29.78	39.29	33.55	36.78	37.16	31.43	35.66	37.49	32.45	36.68	34.55

Appendix 9: TP, ALB, y-GT and T-BIL of *Clarias gariepinus* at week 4

Hematological parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
TP	41.65	39.76	47.08	51.34	49.54	62.51	40.64	50.12	44.03	45.62	47.44	36.57
ALB	17.15	16.63	22.41	22.78	24.01	25.01	20.84	19.56	23.23	19.85	18.98	23.47
y-GT	0.67	0.76	0.85	4.79	6.12	6.76	5.22	6.23	9.04	5.56	5.92	5.62
T-BIL	4.87	3.77	5.16	15.21	12.89	12.49	4.97	7.33	8.25	5.87	7.65	4.96

Appendix 10: Toxic effect of *Bacillus subtilis* supplemented and a control diet of a *Clarias gariepinus* at week 4

parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
ALT	14.64	15.56	16.99	15.97	16.34	15.99	21.24	19.88	21.58	21.9	23.76	22.14
AST	33.67	36.87	32.24	68.54	70.67	69.47	149.67	125.65	88.64	128.78	135.75	132.97
ALP	78.98	86.77	90.25	127.45	135.87	133.88	134.78	167.34	162.49	173.45	148.77	170.74

Appendix 11: Blood profile of *Clarias gariepinus* fed both control and a *Bacillus subtilis* diet at week 8

Hematological parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
WBC	1.29	1.02	0.99	3.45	4.12	4.04	2.34	2.59	3.64	0.78	0.97	1.82
RBC	1.99	2.31	2.38	4.92	5.32	5.81	3.21	3.12	4.11	3.21	3.41	3.32
HGB	8.76	8.79	11.28	16.78	19.17	21.38	13.25	14.21	14.66	11.45	13.43	13.37
HCT	23.45	25.56	32.02	34.56	39.67	50.15	28.45	34.45	44.23	29.45	30.15	35.92

Appendix 12: Differential WBC of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet at week 8

Hematological parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
LYM%	33.98	32.64	36.76	38.65	42.13	42.61	35.21	38.93	38.63	35.42	36.83	40.4
MON%	26.32	23.21	26.28	32.78	37.41	40.09	27.68	31.42	32.64	28.52	24.89	29.96
NEU%	16.25	19.77	18.82	42.78	39.33	43.56	28.62	31.53	31.74	26.76	23.56	26.45
EOS%	1.34	1.56	1.44	5.11	4.95	5.06	3.08	3.33	2.98	2.46	2.22	2.67
BASO%	0.22	0.21	0.21	2.24	1.42	1.86	0.57	0.61	0.51	0.29	0.33	0.34

Appendix 13: MCV, MCH and MCHC of *Clarias gariepinus* at week 8

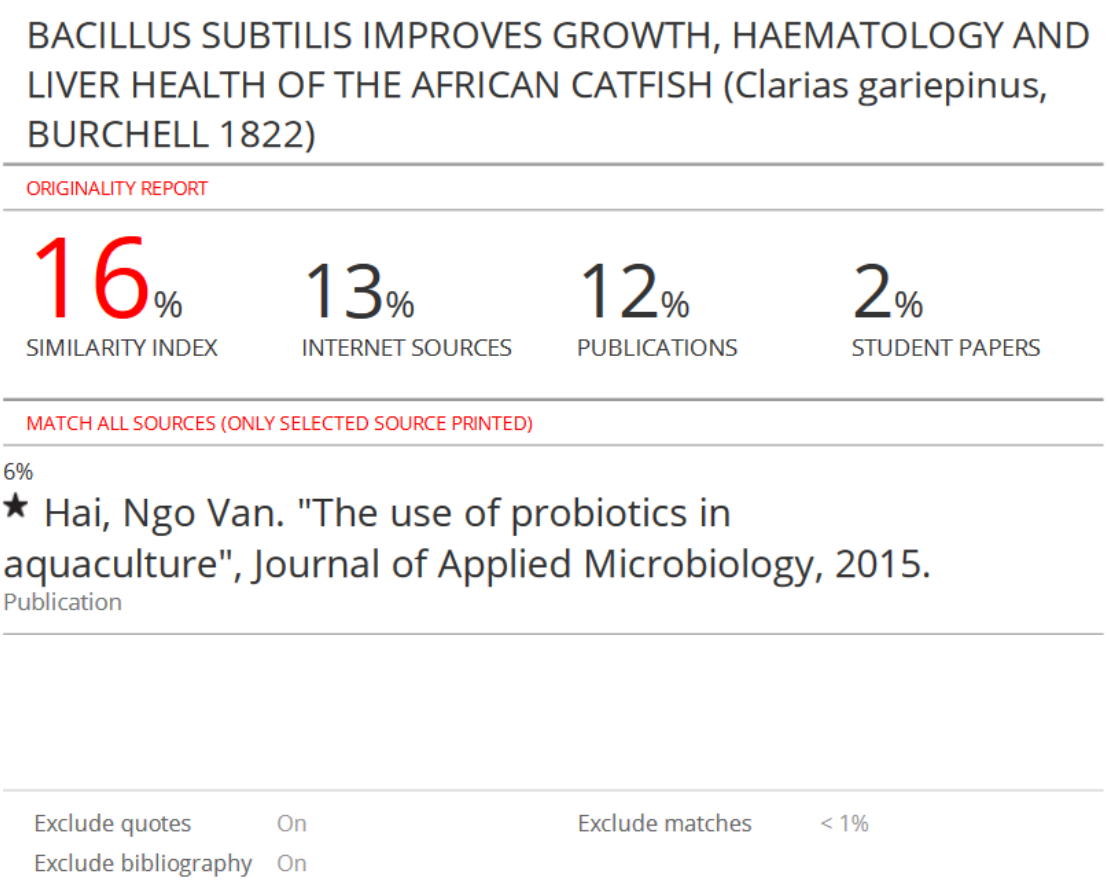
Hematological parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
MCV	128.44	136.55	140.09	135.67	140.21	141.81	128.45	139.41	145.09	132.33	134.98	141.98
MCH	49.76	54.19	52.62	60.13	65.76	63.2	55.32	61.34	62.56	55.66	55.78	60.61
MCHC	38.89	35.67	47.72	38.98	42.87	53.87	36.87	43.78	45.14	35.78	47.74	41.67

Appendix 14: TP, ALB, y-GT and T-BIL of *Clarias gariepinus* at week 8

Haematological parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
TP	44.25	41.23	51.85	52.89	51.26	69.82	44.27	53.51	50.81	47.53	50.89	44.11
ALB	18.62	17.01	23.56	24.82	26.46	31.72	23.22	21.43	26.31	20.87	20.11	24.98
y-GT	2.75	3.77	4.21	14.78	15.22	10.83	8.56	9.32	8.7	8.55	6.97	5.54
T-BIL	5.34	4.18	6.05	16.65	15.07	11.12	6.44	9.92	10.41	6.87	9.31	5.87

Appendix 15: Toxic level of *Clarias gariepinus* fed *Bacillus subtilis* and a control diet at week 8

parameter	Dietary treatment											
	0% Bs			1% Bs			2% Bs			3% Bs		
	A	B	C	A	B	C	A	B	C	A	B	C
ALT	15.01	16.21	17.16	11.67	12.14	12.91	15.67	16.65	11.48	14.23	15.66	14.51
AST	96.67	98.83	100.12	55.67	48.92	67.71	78.56	87.78	92.95	89.34	95.67	91.32
ALP	167.98	192.56	195.15	67.86	79.56	87.03	118.78	120.66	126.08	132.65	125.78	121.31



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