EFFECT OF *STREPTOCOCCUS THERMOPHILUS* ON PERFORMANCE, SURVIVAL AND GUT BACTERIAL FLORA OF *CLARIAS GALMAENSIS*AND*CLARIAS GARIEPINUS* HYBRID

BY

Martins Bohotolo IYAKO

DEPARTMENT OF BIOLOGY,

FACULTY OF LIFE SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

JULY, 2023

EFFECT OF *STREPTOCOCCUS THERMOPHILUS* ON PERFORMANCE, SURVIVAL AND GUT BACTERIAL FLORA OF *CLARIAS GALMAENSIS* AND *CLARIAS GARIEPINUS*HYBRID

BY

Martins Bohotolo IYAKO

[B.Sc. Biology (A.B.U) 2015]

P17LSBS8002

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOLOGY

DEPARTMENT OF BIOLOGY.

FACULTY OF LIFE SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

JULY, 2023

DECLARATION

I declare that this M.Sc. dissertation titled "**Effect of *Streptococcus thermophilus* onperformance, survival, and gut bacterial floralof *Clarias galmaensis* and *Clarias gariepinus*hybrid**" was undertaken by me in the Department of Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, under the supervision of Dr. M. Galadima, Dr. B. S. Bawa, and Dr. H. I. Atta. The information derived from literatures has been duly acknowledged in the text and list of references provided. No part of this project has previously been presented for another degree or diploma at this or any other institution.

Martins Bohotolo Iyako …….. ……..

Signature Date

CERTIFICATION

This is to certify that the research dissertation titled "EFFECT OF *STREPTOCOCCUS THERMOPHILUS* ON PERFORMANCE, SURVIVAL AND GUT BACTERIAL FLORA OF *CLARIAS GALMAENSIS* AND *CLARIAS GARIEPINUS*HYBRID" by Martins Bohotolo IYAKO, meets the requirements for the award of a Masterof Science in Biology (M.Sc. Biology), Ahmadu Bello University, Zaria, and is approved for its' contribution to knowledge and literature presentation.

…….. ……..……..

Dr. M. A. Galadima (Chairman, supervisory team) Date

(Department of Biology)

…….. ……..……..

Dr. B. S. Bawa (Member, supervisory team) Date

(Department of Fisheries and Aquaculture)

…….. ……..……..

Dr. H. I. Atta (Member, supervisory team) Date

(Department of Microbiology)

…….. ……..……..

Prof. I. M. K. Gadzama Date
(Head of Department, Biology)

…….. ……..……..

Prof. Sani A. Abdullahi Date
(Dean, School of Postgraduate Studies

DEDICATION

This dissertation is dedicated to my family for their supportand contribution to seeing the completion ofthis programme.

ACKNOWLEDGEMENT

I am grateful to Almighty God for the grace to complete this M.Sc. programme. The programme came with many difficulties and doubts, but in His mercy gave me the grit to finish. Our lives are a sum of God's mercy and love, and the contribution of people He brings into our lives.

Most importantly, I would like to express my appreciation to my team of supervisors for their dedication, patience and guidance during the course of this research. The chairman in person of Dr. M. Galadima,Dr. B. S. Bawa and Dr. H. I. Atta without whom this work will not have been possible. Their encouragement, unwavering support and adviceswere instrumental to the success of this dissertation.I could ask of no better supervisors and academic mentors.

I also want to appreciate the postgraduate seminar coordinator of the Department of Biology, Dr.A. Yusuf, for hisadvice, and for helping with my data analyses. Mr. Sunday Baba, the Technologist in charge of the fish laboratory for his help and assistance. Mallam Magaji of Hydrobiology lab for the analyses of my water samples.

My appreciation goes to the Director, National Agricultural and Extension Research Liaison and Services (NAERLS) for approving my application for a research space in their facility; Dr. Onimisi, the Head of Aquaculture and Fisheries unit, and Mr. Sanni Mohammed the technologist in charge ofFisheries and hatchery unit. I learnt a lot from your advice and experience working on fish. Thank you very much sir.

I also want to appreciate the staff of the Department of Microbiology, firstly the Head of Department, Prof. M. Aminu for approving my application for a bench space in theirlaboratory for my research. Mrs. Addai the chief Technologist of the Department, the following technologists; Mallam Shittu, Mallam Buhari, Abdullahi Tabari and Mustapha Yusuf. Their help was profound and I am very grateful.Also, the post graduate students of the Department of Microbiology who helped me with materials; Rose Madaki, Mr. Pemida Pascal Omeiza, Mr. Olamide Abdulkabiru Bidemi, Mr. Duke Effiom, Mrs. Linda Ameh andMr. Aaron Ogenyi.

To my amazing colleagues the M.Sc. programme brought my way;Godwin Ajah, Simon Ojodomo, Gonap Morgak, Williams Bitrus, Asiya Surajo, Salamatu Dalhatu, Ejiroghene Uyovbisere, Wase Gabriel, Maryam Bako,Mrs. Mariam Kabir who sadly passed away and couldn't finish the programme my gratitude to others who I cannot mention due to the limitation of space.

I would like to appreciate my parents (Bldr. and Mrs Musa A. Iyako) who supported me through this programme with their encouragement, prayers and financial support. I am very grateful. I also appreciate my amazing siblings (Maureen Tazamu, Millicent Tadushekwo, Mc'Donald Shekwonugemi and our cousin Elizabeth Tadushekwo.

Finally,this acknowledgementwill be incomplete without expressing my gratitude to the Federal Scholarship Boardof the Federal Ministry of Education,for the award given to me. The fundsindeed went a great length to cushioned my financialdifficulties during the course of the programme

ABSTRACT

The use of probiotics in aquaculture has shown great potentials in substituting for antibiotics in fish culture as growth promoters and in preventing fish mortality. However, the problem of antimicrobial resistance poses a great threat to human health and has therefore led to the ban of the use of medically important antibiotics in animals as growth promoters. This restriction in the use of antibiotics has led to the discovery and adoption of probiotics. This study was carried out to investigate the probiotic effect of *Streptococcus thermophilus* isolated from the rumen of cattle on the growth performance, nutrient utilization, survival rate and gut bacterial flora of23DPH hybrid using standard methods. Varying probiotic concentrations 1.5×10^{7}CFU/ml, 1.5×10^{8} CFU/mland 1.5×10^{9} CFU/ml of S. *thermophilus* was administered to three treatment groups ofthe hybrid for 56 days, while the control group was not administered with S. *thermophilus*. The results showed no significant effect ($p\geq0.05$) of the bacterium on the growth performance and nutrient utilization of the fry but showed significant association ($p\leq0.05$) with its survival rate. The fry survival rate was higher with*S.thermophilus* concentration of 1.5×10^{9} CFU/ml. The carcass proximate compositions of the fry revealed diminishing protein and lipid contents with increasing *S. thermophilus* concentrations, while carbohydrate content increased with increasing probiotic concentrations.*S. thermophilus* did not influence the gut viable bacterial count, however it influenced the gut bacterial isolates and composition. It was observed from this study that *S. thermophilus* does not have significant effect on the growth performance and nutrient utilization on *Clarias* hybrid fry but significantly increased its survival rate at 1.5×10^{9} CFU/ml. This study therefore recommend that *Streptococcus thermophilus* at 1.5×10^{9} CFU/ml can increase surival rate of fry.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS, SYMBOLS AND ACRONYMS

+	Positive
-	Negative
±	Plus or Minus
<	Less than
>	Greater than
%	Percentage
≥	Greater than or equal to
≤	Less than or equal to
°C	Degree Celsius
ADG	Average daily growth
AGP	Antibiotic Growth Promoters
AMPs	Antimicrobial Peptides
AOAC	Association of Analytical Chemist
B.C.	Before Christ
CFU/g	Colony forming unit/gram
CFU/ml	Colony forming unit/milliliter
Cm	Centimeters
DC	Dendritic Cells
DO	Dissolved Oxygen
DPH	Days Post Hatch
FCE	Feed conversion efficiency
FCR	Feed Conversion Ratio
FI	Feed intake

FW	Final weight
g/kg	Gram per kilogram
GIT	Gastrointestinal Tract
GRAS	Generally Recognized as Safe
GSI	Gonaldo somatic index
IECs	Intestinal Epithelial Cells
IW	Initial weight
K	Condition Factor
Kg	Kilogram
LAB	Latic acid Bacteria
mg/L	Milligram per litre
MIS	Mucosal Immune System
mL	Milliliter
mL/kg	Milliliter per kilogram
MRS	De-Man Rogosa and Sharpe
MRVP	Methyl Red
NA	Not applicable
NAERLS	National Agricultural Extension Research and Liaison Service
Nm	Nitrogen metabolism
PAMPs	Pathogen Associated Molecular Patterns
PER	Protein Efficiency Ratio
PI	Protein intake
PPM	Parts Per Million
PRPs	Pathogen Recognition Patterns

QPS	Quality Presumption of Safety
RGR	Relative Growth Rate
SCFA	Short chain fatty acids
SGR	Specific growth rate
SIM	Sulphate, Indole and Motility test
TDS	Total Dissolved Solids
TSI	Triple Sugar Iron
TSI	Triple Single Iron Test
VP	Voges Praukuer
WG	Weight Gain

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The growing demand for food has led to the intensification of aquaculture to meet global demand for aquatic food. This has led to the use of antibiotics as growth promoters and as measures to prevent or combat bacterial diseases. Fish diseases are one of the serious limiting factors of aquaculture and antibiotic therapy has been the most common strategy in combating this problem (Yazici *et al*., 2015). However, the sub-therapeutic use of antibiotics in disease prevention and as growth promoters in fish/animal feed has led to the development of antibiotic resistance resulting to their ban and restriction in many western European countries (Zilberg *et al.,* 2010; FAO, 2016). In recent years, the use of antibiotics in aquaculture has been reduced (Aydin and Cek-Yalniz, 2019) due to the diverse environmental problems generated in the ecosystem and probiotics have become suitable alternatives.For example the increased transfer of bacterial strains resistant to antibiotics, the incorporation of antibiotics to the culture species besides eliminating the pathogenic microbiota, and also the elimination of bacteria that are beneficial for the same organism (Sayes *et al.,* 2018).

The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Working Group defined probiotics as “live micro-organisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). This definition is widely accepted and adopted by the International Scientific Association for Probiotics and Prebiotics (Hill *et al.,* 2014). Fuller (1989) in his

definition attributed such beneficial effect in animals through improving the intestinal microbial balance.

The problem of antibiotics resistance has led the World Health Organisation (WHO) to recommend a complete restriction on the use of all classes of medically important antibiotics in food producing animals as growth promoters (WHO, 2017). To rectify this problem, emphasis has been placed on improved husbandry through better nutrition, lowering stocking density, improving water quality, the use of vaccines and biological control agents which work on the fact of microbial antagonism (Robertson *et al.*, 2000). This biological agent (probiotic) therefore, is suitable alternative for Antibiotic Growth Promoters (AGP) -based on the benefits they confer.

Probiotics (or direct fed microbials) are becoming increasingly popular as one of the alternatives to AGP and can be used as suitable alternatives to antibiotics (Molina *et al.*, 2009; Dawood *et al.*, 2019). The most important objectives for using probiotics in animal feed are to maintain and improve the performance (productivity and growth) of the animal, prevent and control the growth and multiplication of enteric pathogens, that is, to limit the growth of harmful microbes while encouraging the growth of beneficial ones. Due to this advantage to which they confer to the gut system, probiotics are been used as additive in animal feed and human food. The Nobel Laureate Ellie Metchnikoff as far back as 1907, published a seminar report linking longevity of Bulgarians with the consumption of fermented milk products containing viable *Lactobacilli.*This report by Metchnikoff therefore gave attention to the potential use of probiotics as inclusion in animal feed due to the advantage they proffer to the host.

In the context of the growing concern with the sub-therapeutic use of AGP in animal feed and greater appreciation of the role of the microbial ecology of the gastro-intestinal tract (GIT) in determining animal productivity, increasing numbers of probiotic products are being developed and used in animal nutrition (Ezema, 2013; FAO, 2016; AbdEl-Tawab *et al.,* 2016; Das *et al.*, 2017; Anee *et al.,* 2021

Different probiotics exert their effects through various mechanisms not yet fully understood and presumed to be due to their action either in the gastro-intestinal lumen or the wall of the GIT, however, the mechanism of action of these probiotics appears to be different (Fajardo *et al.,* 2012).

1.2 Statement of Research Problem

Bacterial diseases are the major cause of economic losses affecting fish farms, and this cause of mortality is especially high in the hatchery where catastrophic loss can be experienced as the fry are immunologically weak at this early stage of their development. The mortality is worsened by poor management practices such as poor water quality and pollution which encourage the growth of pathogenic bacteria and diminishes the oxygen content of the culture water. To combat bacterial diseases and their use as growth promoters, widespread use of broad-spectrum chemotherapeutics (antibiotics) has led to drug resistance problems through the development of antibiotics resistant strain of bacteria that are difficult to treat with established medication (Robertson *et al.*,2000, Marzouk*et al.*,2008) through the transfer of drug resistant zoonotic pathogens, and the presence of antibiotic residues in the environment and animal tissue (Nakano, 2007; Arsene *et al.,* 2021)leading to bioaccumulation of these chemicals in humans resulting to human health hazards.

The gut contents of slaughtered cattle usually constitute waste and litter the surroundings of abattoirs. This gut digesta when discarded decomposes and becomes a pollution source in the environment attracting vectors like flies, and rodents which constitute a nuisance and can transmit disease causing pathogens. The gut content of cattle and other ruminants have been studied to constitute a rich source of enteric bacteria, both pathogenic and non-pathogenic ones, albeit the potential ofdigesta as a rich and diverse microbial community has not been harnessed.

There is a paucity of information on the probiotic effect of *Streptococcus thermophilus* on the growth performance, nutrient utilizationand survivability offish fry.The bulk of researches on lactic acid bacteria (LAB) as probiotics on fish has focused on their effect on juveniles and not on the fry stage. There is a dearth of information on the influence of *Streptococcus thermophilus* on GIT bacterial flora of *Clarias*.

1.3 Justification

Clarias galmaensis is a hardy species of the *Clarias* genus(Aken'Ova,2007). This hardiness trait is a desired quality in fish breedingas it implies increased tolerance to stressful conditions. However, there is paucity of information about this species. This probably is due to their small sizes which makes them undesirable as aquaculture candidate for meat production, unlike the more preferred *Clarias gariepinus* and *Heterobranchus,* and the fact that it is a newly identified species. Hybridization of *Clarias galmaensis* and *Clarias gariepinus* was carried out to improve the offspring by combining the hardy trait of *Clarias galmaensis* and the large size and fast growth rate characteristic of *Clarias gariepinus*.

The use of probiotics in commercial aquaculture is relatively recent, however, interest in them has amplified due to their potential in disease control (Cruz *et al.,* 2012). Studies have stressed probiotics ability to stimulate appetite, improve absorption of nutrient and strengthen the host immune system (Wang *et al.,* 2008, Irianto and Austin, 2002). Probiotics provide a substitute that is more eco/consumer-friendly and more importantly sustainable (Standen*et al.*,2013; Lazado*et al.*,2015). They improve feed conversion rate and feed utilization of fish, they also improve water quality, serving as bioremediators (Marzouk*et al.,* 2008) hence reducing water wastage (bioremediation) and operational costs for fish farmers.In aquaculture, the dose of probiotics usually administered varies from10^6 to 10^{10} CFU/g feed (Nayak, 2010).

Beneficial bacteria occur naturally and hence are cheaper to use. They have been known to pose no harmful effect on the environment. In most recent studies, probiotic bacteria have been isolated from the gut of certain fish species and used on different species. The gastrointestinal tract of animals (especially ruminants) is a rich source of microbes and could be explored for use as probiotics. The intestinal microbiota is classified as the total collection of microbial organisms (bacteria and other microbes) within the gastrointestinal tract (GIT). It contains tens of trillions of microorganisms, including at least 1,000 different species of known bacteria, the vast majority of which belong to the phyla *Firmicutes*and *Bacteroidetes*(Ley *et al.,* 2008). Abattoirs generate large amounts of solid waste and effluents such as rumen contents, blood and wash water (Yitbarek*et al.*, 2016). These wastes can be utilized in value creation.

1.4 Aim

The aim of this study is to assess the effect of *Streptococcus thermophilus* from rumen of cattle on the performance, survival and gut bacteria flora of *Clarias gariepinus* and *Clarias galmaensis* hybrid.

1.5 Specific Objectives

The specific objectives of this research are to determine:

i. To isolate and identify *Streptococcus thermophilus* from rumen of cattle slaughtered at Zango abattoir.

ii. The effect of *Steptococcus thermophilus*on the growth performance and nutrient utilisation of *Clarias*fry (hybrid).

iii. The effect of *Steptococcus thermophilus*on the survival rate of *Clarias*fry (hybrid).

iv. The effect of *Steptococcus thermophilus*on the viable bacteria count and bacteria flora of the gut of *Clarias* fry (hybrid).

v. The effect of *Steptococcus thermophilus* on carcass proximate compositions of *Clarias* fry (hybrid)

1.6 Hypotheses

i. H*o*: *Steptococcus thermophilus* is not a microflora of the rumen of cattle.

ii. H*o*: *Steptococcus thermophilus*has no significant effect on the growth performanceand nutrient utilisation of *Clarias* fry (hybrid).

iii. H*o*: *Steptococcus thermophilus*has nosignificant effect on the survival rate of *Clarias* fry (hybrid).

iv. Ho: *Steptococcus thermophilus*no effect on the viable bacterial count and bacteria flora of the gut of *Clarias* fry (hybrid).

v. Ho: *Steptococcus thermophilus* has no effect on the carcass proximate composition of *Clarias* fry (hybrid).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrition in Animals

Feed is financially the single most important element of animal production, irrespective of species and production system (Makkar, 2016), and the optimization of feed use efficiency i.e. producing more with less feed decreases feeding costs and increases economic viability of the livestock operation (Makkar and Beever, 2013).Nutrition (also referred to as feeding) is a principal characteristic of living things, and is the process whereby organisms acquire energy and nutrients in order to continue with their normal metabolic processes. Nutrition is primary to the survival and growth of animals. The absorption and utilization of nutrients from feed ingested by an animal is crucial to the value to be derived from it. Food is broken down and absorbed by the digestive system which runs from the mouth down to the anus. Active absorption of nutrients however, occurs in the small and large intestines of the digestive track. Considerable research has been devoted during the last 30 years to characterisation of digestive ecosystems in terms of microbial composition and functional diversity, and has led to a better understanding of the influence of the gut microbiota to animal nutrition and health (Chaucheyras-Durand and Durand, 2010). Amongst the beneficial effects, GIT microbial communities are involved in digestion and fermentation of plant polymers which is of particular importance in herbivorous animals. The indigenous gut microbiota is also responsible for the synthesis of vitamins; bioconversion of toxic compounds to non-toxic residues; stimulation of the immune system; maintenance of gut peristalsis and intestinal mucosal integrity, and plays a barrier role against colonisation by pathogens (Chaucheyras-Durand and Durand, 2010).

One of the basic method of how to preserve animal health is to meet the requirements for all nutrients (Salobir *et al.,*2012). The restriction of the use of antibiotics as growth promoters in animal feed by the European Union in the year 2004, lead to the focus on the importance of the effect of nutrition on feed consumption, health status of the gastro-intestinal tract, function of the immune system, regulation of metabolism, prevention of negative effects of oxidative stress, removal and/or inhibition of antinutritive feed substances (Salobir *et al.,*2012). The knowledge of nutrients in feed has expanded the last decade for example, the knowledge of fiber in feed is very important because it serve as feed for the enteric microbes. Fiber is not digestible by the digestive enzymes of animals but can be broken down through the process of fermentation by the microflora in the GIT of the animal. The fermentation products of fiber provide a conducive environment and releases extra nutrients for absorption by the intestinal cells.

Some of the beneficial effects of probiotic consumption include improvement of intestinal health by the regulation of microbiota, and stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, and reducing the risk ofcertain diseases (Nagpal *et al.,* 2012). The action of probiotics in enhancing assimilation of nutrient in the intestine has been proven and explained by two methods, which are;

i. the digestive action of these probiotic bacteria in releasing certain nutrients from feed that are not normally releasable by the normal action of the digestive enzymes.
ii. by the enhancement and improvement of the assimilatory structure (villi) of the intestine. This is observed by histology of the intestinal architecture, such as the villi length.

2.2 History of Probiotic Use

Even though the use of the word probiotic in relation to feedsupplements only dates from 1974, the history of live microbial feed supplements goes back thousands of years. The first foods that contained living microorganisms were probably the fermented milks that are recorded in the Old Testament (Genesis 18:8) (Ezema, 2013). There is also evidence from wall paintings dating back to 2500 B.C. that Sumarians were in the habit of inoculating milk to induce fermentation (Kroger *et al.,* 1989). Metchnikoff is regarded as the godfather of probiotic. From his studies on probiotic and findings of his other coworkers, he wrote a book which in the original French edition published in 1907 was entitled "Essais optimistes". In the book, he discussed the philosophy, literature, religion, folklore and science of ageing. Only a small part of this discourse contained his views on the lower gut flora and the beneficial effects that fermented milk might have on it in humans. At the end of this section of the book, in the English edition, he concludes: "If it be true that our precocious and unhappy old age is due to poisoning of the tissues (the greater part of the poisoning coming from the large intestine inhabited by numberless microbes), it is clear that agents which arrest intestinal putrefaction must at the same time postpone and ameliorate old age. This theoretical view is confirmed by the collection of facts regarding races which live chiefly on soured milk and amongst which great ages are common. However, in a question so important, the theory must be tested by direct observations. For this purpose, the numerous infirmaries for old people should be taken advantage of and systematic investigation should bemade on the relation of intestinal microbes to precocious old age and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body. It can only be in the

future, near or remote, that we shall obtain exact information upon what is one of the chief problems of humanity".

In spite of these guarded statements, he is always quoted as having established a relationship between consumption of fermented milk and long life. This reputation was seemingly endorsed by the English translation of his book which was given the title "The Prolongation of Life". Theconsumption of fermented milk was given an added support by the publication in 1911 of a book by Londen Douglas called "The Bacillus of Long life". In the book, the author reiterated the connection between fermented milks and longevity. He also summarized what was known at that time of the Bacteriology of fermented milks (Douglas, 1911).

One of the most convincing demonstrations of the role of the gut microflora in resistance to diseases wasprovided by Collins and Carter (1978). They showed that the germ-free guinea-pig was killed by 10 cells of *Salmonella enteritidis* but it required 10^9 cells to kill a conventional grade animal with a complete gut microflora. There is thus, no doubt that animals have in their intestine a population of microorganisms that protects them against diseases.

2.3 *Streptococcus thermophilus* and Lactic Acid Production

S. thermophilus is a Gram positive cocci, lactic acid producing bacterium belonging to the Streptococci genus, currently known to have more than 40 species (Iyer *et al*., 2010) and to the Firmicutes (Bacillota) phylum consisting of the genera *Lactobacillus*, *Bacillus* and *Streptococcus*. The name "Streptococcus" is characteristic of the short chain arrangement of the bacterial cells, while "thermophilus" in Latin implies heat loving, meaning this

bacterium is adapted to high temperature. *S. thermophilus* is considered the second most important of industrial LAB after *Lactococcus lactis* and has the "Generally Recognized as Safe" (GRAS) status in USA and Quality Presumption of Safety (QPS) status in the European Union due to its long antiquity of safe use in food production, even though the genus has several pathogenic *Streptococcus* (Iyer *et al*., 2010). *S. thermophilus* is a normal micro flora of the GIT of animals and is also found in milk where it has been found to confer health benefit together with *Lactobacillus acidophilus*. It is well recognized as starter bacteria in the fermentation of dairy foods particularly dominant is in the production of yogurt, cheese and other fermented milk products. The main role of S. *thermophilus* in milk fermentation is in rapid acidification (Iyer *et al*., 2010).

The Firmicutes make up part of the complex diversity of the microbiome of the foregut of cattle (Godoy-Vitorino, *et al.,* 2012), where in ruminants, *Streptococcus* account for less than 1% of the bacteria abundance in the rumen microbiome (Jin *et al*. 2016). However their ability to metabolize starch and produce lactic acid has been reported to be associated with the onset of ruminal acidosis in cattle and sheep (Chen *et al*., 2016; Sousa *et al*., 2021). As a probiotic, S. *thermophilus* has been shown to survive gastro intestinal transit and to moderately adhere to intestinal epithelial cells (Elli *et al.,* 2006) which are conditions for important probiotic bacteria i.e. attach themselves to the mucosa lining, and to survive the high acidity of the stomach.

2.4 Probiotics and Gut Health

Maintaining gut health in animals, particularly in the context of AGP is being gradually phased out, through the manipulation of diet which is crucial to maintain or improve the performance of animals (Choct, 2009). One of the major determinants of a healthy GIT is

the composition of the microbial population. Probiotics can change the microbial population dynamics in the GIT eventually creating a more favourable microbial population due to a shift in the balance of beneficial over harmful microbes (Mountzouris *et al.,* 2007; An *et al.,*2008; Mountzouris *et al.,* 2009). Healthy microbial populations in the GIT are often associated with enhanced animal performance, reflecting more efficient digestion and improved immunity (Niba *et al.,* 2009; Hung *et al.,* 2012). The reduction in pathogenic micro-organisms in the GIT may be attributable to the production of antimicrobial substances such as bacteriocins (Shim *et al.,* 2012) and adhesion of the probiotic microbes to the intestinal epithelium, thereby excluding pathogens competitively or by inducing immune system response. Yeoman and White (2014) explained that the microbial density and diversity varies along the GIT with the greater number where fermentation is most active in the pregastric rumen in ruminants. Therefore, probiotic action can be categorized into four (4), viz; competitive exclusion, antimicrobial production (bacteriocins), aid nutrient digestion and absorption, and disease resistance (Hall and German, 2010)

For many years, studies focused on microorganism's characteristic from intestinal microbiota, and the term "probiotic" was mainly restricted to gram-positive lactic acid bacteria, particularly representative of the genera *Bifidobacterium, Lactobacillus, Lactococcus, Bacillus* and *Streptococcus* (Raja *et al.*, 2015; Anee *et al.*, 2021). Researches have been performed to isolate beneficial bacteria from various sources like soil, water and animal gut to control disease causing pathogens in aquaculture systems (Austin and Day 1990; Munro *et al.,* 1995; Gomez-Gil *et al.,* 2000; Ahmed *et al.,* 2005; Skrodenyte-Arbaciauskiene *et al.,* 2006; Kim *et al.,* 2007; Bestha Lakshmi *et al.,* 2013). Probiotics that currently used in aquaculture industry include a wide range of taxa, from

Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus and Carnobacterium spp. *Bacillus, Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Aeromonas, Enterococcus, Nitrosomonas, Nitrobacter, and Vibrio* spp., and *yeast Saccharomyces, Debaryomyc* (Irianto and Austin 2002; Sahu *et al.,* 2008; Hemaiswarya *et al.,* 2013). The best known probiotic strains such as *Bifidobacteria*, *Lactobacilli,* and *Streptococcus thermophilus* are employed as the dietary supplementation with probiotic bacteria in the aquaculture industry to increase the efficiency and sustainability of aquaculture production (Kim *et al.,* 2007). In aquaculture, probiotics are essential tools for fish health management (Akter *et al.,* 2016). Probiotics are also used as therapeutic, prophylactic and supplement growth in animal production and human health (Kesarcodi-Watson *et al.,* 2008). Generally, probiotics are included in the genera of *Lactobacillus*, *Bacillus, Bifidobacterium, Vibrio, Saccharomyces, Enterococcus* (Pannu *et al.,* 2014). According to El-Haroun *et al.,* (2006) *Bacillus* sp. and *Lactobacillus* spp. are "great candidates" as probiotics used in aquatic animals because they are able to survive in high temperature such as after the pelleting process in feed production. Research carried out has confirmed that application of probiotics in aquaculture has the potential to enhance health status of fish through modulating the immune system and thus increasing resistance to disease and improve the quality of the water through balancing microflora in the water (Fuller 1989; Verschuere *et al.,* 2000). Iribarren *et al.* (2012) stated that the use of probiotic bacteria is one of the internal solutions in reducing environmental damage due to the accumulation of wastes in aquaculture media, producing optimal growth and feed efficiency, and also reducing production costs.

The gastrointestinal microbiota of cattle is a complex population of microorganisms, which have significant roles in health and disease. These comprise archaea, bacteria,

fungi, protozoa and viruses (Suchodolski *et al.,* 2012; Guard *et al.,* 2015). According to Chloe *et al.* (2018), the rumen of bovine can be viewed as an anaerobic and methanogenic fermentation chamber that contains microorganisms that have the ability to utilise, and increase the productivity of, cellulolytic feeds (i.e. straw, hay, silage and grass) and the rumen dynamics is almost solely responsible for providing nutrient to the host animal (Krehbiel, 2014).

Ware *et al.,* (1998) reported *Lactobacillus platarium*, *L. casei*, *L. acidophilus*, *Bacillus stutilis*, *Enterococcus diacerylactis*, *Bifido bacterium* and *Saccharomyces cerevisiae* as microbes located at the lower part of cattle gut having probiotic activity. The normal bacterial flora of the intestine play significant roles in the overall health of the gastrointestinal tract. Commensal organisms for example, are known to promote gut health through the induction of mucus production and enterocyte turnover (Hall and German, 2010). Bacteria aid in food digestion, nutrient extraction and production of substances such as short chain fatty acids (SCFA) and vitamins K, B9, B12 (Hall and German, 2010). These organisms also play an important role in the immune status of an individual. Commensal organisms provide protection against invading organisms both within the GIT and also in the systemic circulation. These effects may be mediated through direct competition for nutrients, stimulation of antimicrobial peptide production by the enterocyte and host immune modulation (Sancak *et al.,* 2004).

2.5 Characteristics of a Good Probiotic

Fuller (1989) enumerated features that should characterize a good probiotic. They are;

1. It should be a strain, which is capable of exerting a beneficial effect on the host animal, for example increased growth or resistance to disease.

2. It should be non-pathogenic and non-toxic.
3. It should be present as viable cells, preferably in large numbers.
4. It should be capable of surviving and metabolizing in the gut environment for example, it should be resistant to low pH and organic acids.
5. It should be stable and capable of remaining viable for periods under storage and field conditions.

Probiotics can eliminate the pathogenic microorganisms from the GIT and change the host's microbial population density in the intestinal tract. Probiotics subsequently establish a better suited microbial population through a transformation in the equilibrium of beneficial and noxious microorganisms (Mountzouris *et al.*,2009). Probiotics prompts an immunologic response after they settle in the gut. The most common modulation of the GIT microflora by probiotics (for example in chickens) is an increase in the populations of *Lactobacillus* and *Bifidobacteria* while populations of coliforms particularly *Escherichia coli* decrease (FAO, 2016). This pattern of modification of the GIT microflora occurs with all the common types of bacteria used as probiotics, such as lactic acid bacteria (LAB) (Mountzouris *et al.,* 2010; Cao *et al.,* 2013; Mookiah *et al.,* 2014), spore forming bacteria (*Bacillus* spp.) (Shim *et al.,* 2012; Abdelqader *et al*., 2013; cited in FAO, 2016) and clostridial bacteria (*C. butyricum*) (Zhang *et al.,* 2011; Yang *et al.,* 2012b), and with both Gram positive and Gram negative strains (Hashemzadeh *et al.,* 2013). The intestinal cells can assemble a series of immuno regulatory molecules exhilarated by bacteria (Corcionivoschi *et al.,* 2010). Some probiotic metabolites can modulate various metabolic pathways in cells. Probiotic metabolic components such as bacteriocins, amines, and hydrogen peroxide interact with specific targets of multiple metabolic pathways to

regulate apoptosis, cell proliferation, inflammation, and differentiation (Plaza-Diaz *et al.*, 2019).

Probiotic microbes can withstand pathogenic bacteria by lowering luminal pH. Probiotic *Bifidobacterium breve* can lower the luminal pH, andcan trigger acetic acid production in higher concentrations in a deadly Shiga toxin-yielding *Escherichia coli* O157:H7 mouse experimental model (Asahara *et al.*,2004). Probiotics can shape the cell–cell communication of bacteria and host and maintain cellular consistency by strengthening the intestinal barrier function. Such consistency is achieved through the modulation of cytoskeletal and tight junctional protein phosphorylation. *Lactobacillus* can abide by the epithelial cells of the ileum of chickens (Jin *et al.*, 1996). Probiotics can competitively exclude pathogenic microorganisms from the host intestine by strengthening this intestinal communication system (Mookiah *et al.*, 2014). This anti-pathogenic mechanism (competitive exclusion) demonstrates that bacterial species rigidly battle for attaching to receptors at particular binding sites in the GIT and might integrate antimicrobial substances secretion and competition for accessible nutrients (van Zyl *et al.*, 2020). Probiotics can improve the immunity of the host by modulating the immune system. The consumed probiotics play a crucial role in stimulating the mucosal immune system (MIS) and induce a network of signals. The reaction of diverse probiotic microbes on dendritic cells (DC) has been investigated in different experimental approaches. Dendritic cells are antigen- displaying cells that have crucial roles in innate and adaptive immunity. Dendritic cells can identify and react to bacterial components besides launching primary immune responses, which lead to the straight development of T- and B-cell responses. Probiotics can directly govern intestinal dendritic cells with pathogen recognition patterns (PRPs) displayed on the surface, which can precisely recognize the pathogenassociated molecular

patterns (PAMPs) on the bacterial organism. This acknowledging method stimulates DC maturation regarding up-regulation of co-stimulatory molecular expression. As the immune system becomes active, cytokine secretion incites T-cell activation (Langenkamp *et al.,* 2000; Mellman and Steinman, 2001). DC-originated signals ascertain the type of T-cells responses such as T helper cells polarization or T regulatory response, which determines the B-cell responses against pathogenicity (Kapsenberg, 2003).

2.6 Modes of Probiotic Action

Several mechanisms have been proposed to explain the effects of probiotics and it is likely that the positive results reported in the different animal studies are due to a combination of some, if not all, of these mechanisms. The metabolic activities of the probiotic strains and survivability throughout the gut appear to be of great importance for an optimal efficacy (Chaucheyras-Durand *et al.,* 2008). Effects are also greatly dependent on the strain used (Newbold *et al.,* 1995). In monogastrics, the production of organic acids (lactic or acetic acid) by bacterial probiotics can help decrease the gut pH, create more favourable ecological conditions for the resident microbiota and decrease the risk of pathogen colonisation (Servin, 2004). The release of antimicrobial peptides, such as bacteriocins, which inhibit the growth of pathogenic bacteria, or production of enzymes able to hydrolyse bacterial toxins have been demonstrated (Buts, 2004). Some strains can competitively exclude pathogenic bacteria through their higher affinity for nutrients or adhesion sites (La Ragione *et al.,* 2003; 2004). Some probiotics produce nutrients and growth factors which are stimulatory to beneficial microorganisms of the gut microbiota. In addition to interacting and stimulating other microorganisms, probiotics also interact with the host, by influencing the immune response (Delcenserie *et al.,* 2008), or producing components able to positively affect mucosa development or the metabolism of the host's

intestinal cells (Johnson-Henry *et al.,* 2008). Some probiotics can also metabolize or aid in the detoxification of certain inhibitory compounds such as amines or nitrates or scavenge for oxygen (Chaucheyras-Durand *et al.,* 2008; Marden *et al.,* 2008), which is of great importance in gut anaerobic ecosystems. Most of these mechanisms have also been proposed to explain the effects of probiotics in the human gut, where benefits both in terms of nutrition and health have been demonstrated (Chaucheyras-Durand and Durand, 2010).

2.7 Functions of Probiotics

2.7.1 Probiotics increase of digestibility

Probiotics increase the rate of digestion in animals. Probiotics can ameliorate cecal microorganism constitution and nutrient digestion in broilers (Khalid *et al.,* 2021).The response in broiler chickens to dietary supplementation with probiotics (*L. bulgaricus*)varied with level of probiotic provided. At a rate of 2×10^6 cfu/g there was no significant effect on digestibility of crude protein or fat, but at 6×10^6 CFU/g and 8×10^6 CFU/g there was a significant increase, ranging from 7 to 11% for protein and 6.5 to 13.4% for fat, with 7.9 to 11.7% increase in weight gain (Apata, 2008). Probiotics boost the ileal digestibility of essential amino acids, with a 5 percent enhancement in chicken body weight, Zhang and Kim (2014) and can ameliorate the bioaccumulation of calcium in poultry (Chawla *et al.,* 2013). (Maas *et al.,* 2021) experimented with enzymes xylanase, phytase together with probiotics. They reported the impact of *Bacillus amyloliquefaciens* on digestion or metabolism and found better calcium absorption and improved microorganisms' interactions in the gut. Probiotics increase enzyme activity in the GIT and improve the digestibility of the food eaten by the host. For example, a study in buffalo

calves showed that probiotic feed containing *Lactobacillus acidophilus* could ensure more dry matter intake, daily feed conversion efficiency, and apparent digestibility of nutrients compared to the control group (Sharma *et al.*, 2018).

2.7.2 Probiotics improvement of the immune system

Probiotics can enhance immunity in the host in many ways. Multiple studies have proven the immunostimulatory properties of probiotics (Punetha *et al.*, 2018; Kong *et al.*, 2020; Terada *et al.*, 2020; Bilal *et al.*, 2021). Probiotics comprising *Lactobacillus fermentum* and *Saccharomyces cerevisiae* excited the gut T-cell immunity, highlighted by the enhanced yield of CD3+, CD4+, and CD8+ T-lymphocytes in the gastrointestinal tract of chickens (Bai *et al.*, 2013). In neonatal chicks of three-day- and sevenday- old expression of CD3+, IL-2, and IFN-γ-genes were higher in the small intestine when provided food with probiotics *Lactobacillus jensenii* TL2937 and *Lactobacillus gasseri* TL2919 than without probiotics (Sato *et al.*, 2009). Probiotics can also boost serum immunoglobulin levels in chickens. A probiotic feed supplement containing *Lactobacillus acidophilus, Bacillus subtilis,* and *Clostridium butyricum* enhanced IgA and IgM serum levels in chickens, Zhang and Kim (2014). The LAB (Lactic Acid Bacteria) can generate a broad range of antimicrobial compounds to reduce pathogenic attacks. These include antimicrobial peptides (AMPs), such as defensins, organic acids, bacteriocins, ethanol, carbon dioxide, and diacetyl, Liao and Nyachoti (2017). Organic acids like short-chain fatty acids, formic acids, and lactic acid have been proved to suppress potential harmful microbes of importance in farm animals. *Lactobacillus* bacteria can generate lactic acid as the main glucose metabolism product (Russo *et al.*, 2017). Organic acids work on the bacterial cell wall, cytoplasmic membrane, and precise metabolic functions such as replication and protein synthesis, which lead to the destruction and death of pathogenic microorganisms

(Surendran Nair *et al.,* 2017; Zhitnitsky *et al.,* 2017). *Salmonella Enteritidis, Lactobacillus monocytogenes,* and *Escherichia coli* could be effectively inactivated after exposure to 0.5% lactic acid for two hours (Wang *et al.,* 2015). Probiotics can influence both the innate and adaptive immunity of the host. Several immune cell types, comprising granulocytes, dendritic cells, macrophages, T lymphocytes, and B lymphocytes, are engaged with inflammatory responses, which are regulated by cytokines like TNFα, IL-8, IL-1β, IL-15, and IL-6 interleukins. The anti-inflammatory reactions are mediated by TGFβ, IL-10, IL-12 (Hardy *et al.,* 2013). Innate immunity provides physical and chemical barriers against pathogens for the host organism. For example, intestinal epithelial cells (IECs) prevent the spread of harmful microbes so that infections do not occur. It has been reported that *Lactobacillus* bacteria such as *Lactobacillus gasseri*, *Lactobacillus salivarius*, *Lactobacillus fermentum,* and *Lactobacillus crispatus* can regulate favorably the secretion level of the pro- and anti-inflammatory interleukins IL-6, IL-8, and IL-10 to manage the inflammation and rebuild the physiological balance in animals (Luongo *et al.,* 2013; Perez-Cano *et al.,* 2010; Rizzo *et al.,* 2015; Sun *et al.,* 2013). *Lactobacillus delbrueckii* strain can stimulate anti-inflammatory properties and capable of downregulating the pro-inflammatory cytokines IL-8 levels. These inflammatory responses were seen in *Aeromonas hydrophila* contaminated carp (*Cyprinus carpio* Huanghe var.) when supplemented with 1 × 107 CFU g/L *Lactobacillus delbrueckii* probiotics (Zhang *et al.,* 2017). The adaptive immune response is dependent on B-lymphocytes and T-lymphocytes, which stimulate an antigen-specific response. In poultry, feed supplementation with 1×10^9 CFU/kg of *Lactobacillus acidophilus* LA5 elevated the amount of CD8+, CD4+, TCR1+ T-cell in the gastrointestinal tract as well as in the peripheral blood system (Asgari *et al.,* 2016).

2.8 Probiotics Use in Aquaculture

Probiotics confer several beneficial effects to aquatic animals of which growth promoting effect through feed utilization and digestion, biological control of pathogen colonisation (Chaucheyras-Durand and Durand, 2010), strengthen immunity, improve water quality, and work as an alternative to antibiotics (Banerjee and Ray, 2017). Although several microorganisms, particularly bacteria and fungi, have demonstrated probiotic abilities, species belonging to the genera *Lactobacillus* [consisting of the following strains; L. *lactis* (Balcazar *et al.,* 2007), *and L. plantarum* VSG-3 (Giri *et al.,* 2013)], *Streptococcus*, *Lactococcus*, *Bifidobacterium* (Arsene *et al.,* 2021) and *Bacillus*(Hong *et al.,* 2005; Olmos *et al.,* 2020) are the most popularly used probiotics in aquaculture. *Bacillus* probiotics alone can mitigate various harmful microorganisms in fish such as *Vibrio*, *Pseudomonas*, *Aeromonas, Clostridium, Streptococcus*, *Flavobacterium*, *Acinetobacter*, and white spot syndrome virus (Kuebutornye *et al.,* 2020). Yeast (*Saccharomyces cerevisiae*) as a probiotic has also been proven useful for aquatic animals (Mo *et al.,* 2020; Wu *et al.,* 2020). Farmers supply the fishes with probiotic feed supplement either through water circulation or dietary supplements (Moriarty, 1998; Skjermo and Vadstein, 1999). A single bacterial strain or a combination of several bacterial strains can be used as probiotics along with other prebiotics or immunostimulants (Hai *et al.,* 2009a, 2009b). Achieving the expected outcomes in fish culture depends on the proper dosage and timing. Probiotics improve the health conditions of fish and other water-dwelling creatures *Bacillus pumilus* (Aly *et al.,* 2008) and *Lactobacillus plantarum* (Van Doan *et al.,* 2020) can ameliorate the health conditions of Nile tilapia. To mitigate the adverse impact of the Tilapia Lake Virus, dietary supplements of 1 percent *Bacillus* spp. were given orally for the red hybrid Tilapia fishes (Waiyamitra *et al.,* 2020). This feed supplement can reduce

the fatality rate by Lake Virus infection. Probiotic *Pseudomonas* I-2 can inhibit disease-causing *vibrio* microorganisms (Chythanya *et al.,* 2002). Probiotic *Pediococcus acidilactici* can resist vibriosis in white leg shrimp (Castex *et al.,* 2008). Feeding White leg shrimp larvae with a variety of probiotic feed supplements (*Lactobacillus*, *Saccharomyces*, *Bacillus*, effective microorganisms such as grampositive cocci and *Bifidobacterium*, and Photosynthetic Bacteria) can accelerate the growth speed and larval metamorphosis. It also encumbers incomplete molting during the development and minimizes the number of vibrio pathogens (Wang *et al.,* 2020). *Pediococcus pentosaceus* and *Staphylococcus hemolyticus* can decrease the frequency of white spot syndrome virus in white leg prawns (Leyva-Madrigal *et al.,* 2011). *Saccharomyces cerevisiae* can be used as a substitute for live food in the cultivation of clownfish, Catla,hybrid striped bass, Japanese flounder and Nile tilapia (Gunasundari *et al.,* 2013; Mohanty *et al.,* 1996;Li and Gatlin, 2004, 2005; Taoka *et al.,* 2006; Lara-Flores *et al.,* 2003 respectively). *Bifidobacterium animalis* and *Lactobacillus acidophilus*-rich feed additives can enable the growth and lifespan of *Hypophthalmichthys molitrix* fingerlings (Noor *et al.,* 2020). Probiotics can enhance immune responses in fish. *Bacillus* activates the humoral and cell-mediated immunologic response in fish (Kuebutornye *et al.,* 2020).

Bacillus pumilus and *Bacillus licheniformis* strengthen the immunity of Nile tilapia (Aly *et al.,* 2008) and *Bacillus pumilus* improves the immunity of *rohu* fish (Ramesh *et al.,* 2015). *B. licheniformis* assists in performing the immunomodulatory activities and raises *Oreochromis niloticus* production by up-regulating the Toll-like receptors (TLR-2) and anti-inflammatory cytokines (Midhun *et al.,* 2019). Probiotics can improve growth performance, feed intake, and digestive enzyme processes in aquatic animals. Probiotics generate extracellular enzymes such as protease, carbohydrase, and lipase and efficiently

engage in the nutrient digestion of aquatic animals (Arellano- Carbajal and Olmos-Soto, 2002; Leonel and Olmos-Soto, 2006). *Bacillus* generates different hydrolytic enzymes such as β-1,3-glucanases, proteases, and cellulases to improve the digestion in fish (Kuebutornye *et al.*, 2020). In a sea snail, *Haliotis midae*, the probiotic *Vibrio midae* SY9 can reinforce digestive protease activity, protein digestion, and growth rate activities (Huddy and Coyne, 2015). *Bacillus* spp. and photosynthetic bacteria can improve white leg prawns' growth through an increase in lipase and cellulase activity, (Wang, 2007). *Pseudomonas aeruginosa* and *Pseudomonas synxantha* can enhance the growth rate of western king prawns (van Hai *et al.*, 2010, van Hai *et al.*, 2009a, 2009b). Probiotics have shown their potency in nourishing water properties, controlling disease, and thus upgrading fish habitat (Chen *et al.*, 2020a, 2020b; Kewcharoen and Srisapoome, 2019; Soltani *et al.*, 2019). Probiotics enhance water quality by reducing the number of harmful microbes (Park *et al.*, 2000; Dalmin *et al.*, 2001), mitigate nitrogen (Wang *et al.*, 2005) and reduce phosphate contamination in the sediments, Wang and He (2009). Probiotics can alleviate metabolic wastes at the time of the fish school's transportation, cardinal tetra (*Paracheirodon axelrodi*) (Gomes *et al.*, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was conducted in Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

3.2 Collection of Bovine Rumen Digesta

Rumen content from ten (10) freshly slaughtered cows (female cattle) from Zango abattoir, in Sabon Gari Local Government Area of Kaduna State were collected in ten different sterile polythene bags and transported to the General Laboratory, Department of Microbiology, Ahmadu Bello University, Sabon Gari Local Government Area, Kaduna State.

3.3 Microbiological Analyses

3.3.1 Isolation and identification of Lactic Acid Bacteria

The rumen digesta samples were homogenized and a stock solution wasprepared by weighing 10g of rumen digesta into a 90ml volume of physiological saline. Tenfold dilution was performed on the stock solution by measuring 1ml from the stock solution into a test tube containing 9ml peptone water to make 10^{-1} dilution. One milliliterfrom the first dilution was inoculated into the second test tube containing 9ml peptone water to make 10^{-2}. The same process was done up to the tenth (10^{-10}) dilution. Using physical observation of the turbidity of the dilutions, three dilutions (10^{-3}, 10^{-4} and 10^{-5}) were selected for plating (Cheesbrough, 2006).

Aliquots of 0.1ml of dilutions from $10^{-3,}$ 10^{-4} and 10^{-5}diluentswere aseptically inoculated onto the surface of freshly prepared De-Man Rogosa and Sharpe (MRS) medium by

spread plate method using bent glass rod and incubated overnight at 37°C in candle jar to provide the anaerobic conditions needed by LAB to grow. After 24 hours,discrete colonies of bacteria were observed to have grown on the media. These colonies were selected based on similarities in their morphology andsubculturedindividually on fresh MRS medium on slants and incubated anaerobically at 37°C for 24 hours to achieve pure isolates. The colonial morphology of the isolates cultured were observed and recorded on the basis of colour, shape and size for their identification (Atta *et al*., 2020).

3.3.2 Gram staining

Gram staining was performed according to the procedure of laboratory manual by Cheesbrough (2006). Thin smears of isolates were made on glass slides by picking a colony with the wire loop and spreading it on a glass slide containing a drop of water.The slides are then allowed to air dry and then heat fixed by passing the slidesover the Bunsen flame three times. The reagents for the Gram's staining were then applied in this sequence; the slides were irrigated with crystal violet for one minute, the stain was then rinsed with a gentle running tap water. The slides was then flooded with Gram's iodine solution for one minute and rinsed off with running water. The smears were then decolorized with 95% alcohol until there is no more dripping of blue colour (for about 10 to 20 seconds). The smears was counter stained with safranin solution for 1 minute and then the slides washed in a gentle running tap water. They were then air dried and observed under oil immersion objectives using a compound microscope (Chessbrough, 2006).

3.3.3 Catalase test

Catalase test was performed on the isolates to identify their catalase reactions. 3% hydrogen peroxide solution was added to 1ml of overnight cultures. The isolates which did not give gas bubbles are catalase negative and selected presumptively as LAB (Cheesbrough, 2006).

3.3.4 Motility test

Motility test was performed using motility media by stabbing the inoculatedstraight wire into the motility medium in a test tube. The cultures were then incubated for 24 hoursat 37ºCand observed for motility. Motile bacteria showed growth and extended away from the stab line while non-motile bacteria growth were restricted along the stab line (Cheesbrough, 2006).

3.3.5 Endospore test

Endospore or spore test was carried out by making a thick smear of the bacterial isolate on a glass slides. The slides were allowed to air dry and then heat fixed. The slides were placed over boiling water in a beaker resting them on the rim with the bacterial film uppermost for 40 secand irrigated with methyl blue over steam for 10 minutes. The slides were then viewed under the microscope and checked for formation of spores in the cells (Cheesbrough, 2006).

3.3.6 Oxidase test

The oxidase reagent was placed on filter paper. 24 hours isolate of the bacteria culture was picked using a sterile wire loop and a smear was made on the filter paper. The appearance ofpurple colour of the smear indicates oxidase positive, otherwise, the reaction is negative(Cheesbrough, 2006).

3.3.7 Inclusion of *Streptococcus thermophilus* in *Clarias* fry feed and rearing water

The *Streptococcus thermophilus* isolated was subcultured on MRS medium for 24 hours and standardized in normal saline. MacFarland standard was used to achieve the required bacterial concentrations. Colonies of pure culture of *S. thermophilus* were aseptically emulsified in 5 ml of normal saline water and the turbidity of the suspension was compared to that of 0.5 and 5.0 MacFarland standard which is equivalent to 1.5×10^8 CFU/ml1and 1.5×10^9 CFU/ml respectively to achieve the *Streptococcus thermophilus* concentrationof 1.5×10^8 CFU/ml and 1.5×10^9 CFU/ml respectively. One serial dilution was performed on the 1.5×10^8 CFU/ml of *S. thermophilus*to achieve 1.5×10^7 CFU/ml. The *S. thermophilus*concentrationswere the different treatment groups,where applied both in the culture water and mixed with Coppens (0.02mm).Inclusion both in feed and water was done every three days to maintain bacterium viability and maintain bacterium population after water change of the plastic tanks. The negative control contained no *S. thermophilus* inclusion. 10mls of different culture concentrations were poured and thoroughly mixed with 100 grams of feed. The feeds were shade-dried at room temperature and fed to the fry *ad libitum* to satiety three time daily at 8:00AM, 12:00PM and 4:00PM for 56 days.

3.4 Experimental Fish

Broodstocks of *Clarias gariepinus* from a private fish farm (Aqua consult fishery hatchery, Sabo, Kaduna) and *Clarias galmaensis* from Galma River, Zaria, Kaduna State were purchased and taken to the aquaculture unit of the National Agricultural Extension Research and Liaison Service (NAERLS) for the breeding exercise. The broodstocks were acclimatized for three (3) days and starved for twenty four (24) hours prior to hormone (ovulin) injection following standard procedure for catfish.

3.4.1 Morphometrics parameters and breeding indicesof broodstock

Broodstock morphometrics which included; Total length, Standard length, total body weight and gonad weight were recorded using measuring board and a digital weighing balance. The gonad weight of the male broodstock was not detected by the digital scale.

Table 3.1: Morphometric parameters of broodstock of *Clarias gariepinus* and *Clarias galmaensis*

Species	Weight	Standard length (cm)	Total length (cm)	Gonad weight (g)	GSI	Relative fecundity
Clarias galmaenis♂	33.7g	16.2	18.1	ND	NA	NA
Clarias gariepinus♀	1.00kg	39.8	48.5	79.1	7.91%	36,979

ND= Not Detected, NA=Not Applicable, GSI= Gonadosomatic Index

3.4.2 Broodstock hormone induction and stripping

The *Clarias gariepinus* female was injected with ovulin intramuscularly at 0.5mL/kg according to the manufacturer instruction above the lateral line and kept separately in plastic ponds for a latency period of 11 hours at 29°C. Just before stripping of the female, the male *Clarias galmaensis*broodstockwas sacrificed and testes removed and kept. The femalebroodstock was wrapped in a moist towel for proper handling to minimize agitation and sudden movements. The female was then stripped of eggs into a clean dried plastic bowl through the gentle ante-posterior massage of the abdomen towards the anal opening according to standard procedure for breeding catfish.

3.4.3 Reproductive parameters

Parameters including relative fecundity and the weight testes were taken using gravimetric calculationand digital weighing balance respectively.

3.4.4 Fertilization and breeding process

The testes were incisedlongitudinally and the milt squeezed out onto the eggs of the femaleand the gametes were stirred gently to mix. A little quantity of water was then added and mixed for 2 minutes to initiate activation (Nargesi *et al.*, 2019). Eggs were then kept for 10 minutes for water absorption and hardening (Contreras- Sanchez *et al*.,1998; cited in Nargesi *et al.*,2019). Fertilized eggs were then spread on breeding net in a flow through system and incubated (in darkness) till they hatched.

Percentage fertilization was evaluated by comparing the number of fertilized (eyed) eggs to unfertilized (uneyed) eggs, while percentage hatchability was evaluated by comparing and estimating the number of hatched eggs (hatchlings) to unhatched eggs on the breeding net.

Hatchlings were fedexogenously with coppens feed on the third day after hatching and had absorbed their yolk sac.

3.5 Experimental Design

Forty (40)fry of twenty three (23) dayspost hatch (dph) hybrid fry were allocated to each treatment group and control for 56 days (8 weeks). Fryof average weight 0.006g were sorted and allotted to the different treatment groups and the control. The treatments were set up in duplicates.

3.6 Monitoring Breeding and Culture Water physicochemical Parameters

The water parameters monitored include water pH and water temperature were both determined using a pocket sizedPhEP® pH meter (model HI 98107), total dissolved solids (TDS) was measured using TDS meter (TDS-3 HM Digital). The water parameters were collected every three days. The pH meter and TDS meter were standardized in the chemistry laboratory for accurate calibration and reading. Dissolved Oxygen (DO) was determined in Hydrobiology Laboratory, Department of Biology using the modified Winkler azide method (APHA, 1990) which employed the titration method.

3.7 Determination of Growth Parameters

At the end of the experiment, the lengthof the fish was measured by placing the fish lying straight on a measuring board with the tip of the head at the zero mark and the mark at which the caudal fin starts is recorded as the standard length, while the weight of the fry were measure as a pool (in group of more than three) and then the average weight was calculated by dividing the total weight by the number of fry. The weight of the fry were pooled because the digital scale could not detect the weight of the fry singly.

The growth parametersof the hybrid fry were evaluated and derived using the following formula;

- Length gain (cm)= $mean\ final\ length - mean\ initial\ length$
- Weight gain (g)= $mean\ final\ weight - mean\ initial\ weight$(Adikwu, 2003)
- % weight gain $= \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \times 100$ (Shalaby *et al*., 2006; Oso *et al*., 2011)
- $Specific\ growth\ rate\ (SGR) = 100(logW2 - logW1)/t$(Amisah *et al*., 2009)

Where: log = the natural log

W2 = final weight at a certain period (g)

W1 = initial weight at the same period (g)

t = the experimental period in days.

The body weights were measured using electronic weighing balance to the nearest 0.1g, total length of fry were measured from the maxilla to the end of the caudal tail.

- Average Daily Growth (ADG) $= \frac{mean\ weight\ gain}{Duration\ of\ feeding}$ (Fasakin *et al*., 2000)
- Relative Growth Rate (RGR) % $= \frac{Final\ weight - initial\ Weight}{Initial\ weight} \times 100$ (Fasakin *et al*., 2000)
- Condition Factor (K)$= \frac{100W}{L\text{^}3}$ (Le Cren, 1951; Wilson, 2002)

  W=weight, L= total length
- Production index (PI)$= \frac{Survival\ rate \times final\ weight - initial\ weight}{Duration\ of\ rearing\ (days)}$ (Mohanty, 2004)

3.8 Nutrient Utilization

Protein intake (PI) was determined using the formula of Mohanty, 2004;

PI=$Feed\ intake\ \times percentage\ protein\ in\ feed \div 100$

Feed conversion ratio (FCR) was determined using the formula of Jimoh *et al*., (2014)

FCR=$\frac{Feed\ \ intake}{Weig\,ht\ gain}$

Feed conversion efficiency (FCE) was determined using the formula of Olakunle, (2006)

(FCE) =$\frac{Weig\,ht\ gain}{Feed\ \ intake} \times 100$

Protein efficiency ratio (PER) was determined using the formula of Amoah, (2011)

PER= $\frac{Body\ \ weig\,ht\ gain}{Crude\ \ protein\ \ fed}$

The Nitrogen metabolism (Nm) index of feed utilization of the fry was determined using the following formula of Shalaby *et al*., 2006.

Nitrogen metabolism (Nm) = $\frac{0.549(b-a)h}{2}$

Where;

a = initial weight of fish (h)

b= final weight of fish (g)

h= experimental period in days

3.9 % Survival of Fish

The survival rate of the frywas calculated using the formula of Adesina *et al.*, (2013);

$$Survival\ Rate\ \% = \frac{Total\ number\ of\ fish\ harvested}{Total\ number\ of\ fish\ stocked} \times 100$$

3.10 Carcass Proximate Composition of Experimental Fish

Fish from all treatment were sacrificed and shade-dried for 48 hours, after which they were taken to the Food Science Laboratory in the Faculty of Agricultural Science for the proximate analysis where protein, lipid, ash, moisture content and Nitrogen free extract were determined by the method of Association of Analytical Chemist (AOAC) 2002.

3.10.1 Determination of moisture content (AOAC,2002)

Aluminum were washed and dried to a constant weight in an oven at 100°C. They were later removed and cooled in a desiccator and weighed (W1). 2grams of the grounded (powder) sample was placed in the weighed moisture dish (W2). The dish containing the sample was kept in an oven for about 3hours, the sample were removed and cooled in the desiccator and weighed W3.

The % of moisture was calculated as: $\frac{W2-W3}{W2-W1} \times 100$

3.10.2 Determination of ash content (AOAC, 2002)

Crucibles were cleansed and dried in the oven, after drying; they were cooled in the desiccator and weighed (W1). 2grams of the grounded (powder) sample was placed in the crucibles and weighed (W2). They were transferred into the Muffle Furnace for about 550^{o} C, then removed and cooled in the desiccator and weighed (W3).

The % of Ash was calculated as: $\frac{W3-W1}{W2-W1} \times 100$

3.10.3 Determination of fibre (AOAC, 2002)

2grams of the sample was placed in a beaker containing 1.2ml of H_2SO_4 per 100ml of solution and boiled for about 30minutes, the residue was filtered and wash with hot water, the residue was transferred to a beaker containing 1.2gram of NaOH per 100ml of solution and boiled for about 30minutes, the residue was washed with hot water and dried in an oven and weighed (C2), the weighed sample was incinerated in a Furnace for about 550°C, removed and allow to cool, and weigh (C3).

The % of fibre was calculated as: $\frac{C2-C3}{W} \times 100$

3.10.4 Determination of lipids (AOAC, 2002)

250ml clean boiling flask are dried in the oven and transfer into desiccator to cooling. Filter papers are weighed and labeled W1 to which 2 grams of samples are weighed into the labeled thimbles (filter paper) and the weight taken (W2). The boiling flasks of the soxhlet apparatus are filled with petroleum spirit or N-hexane. The soxhlet apparatus is assembled with the samples and allow refluxing for 8 hours after which the samples are removed and transferred into the oven to dry and then into the desiccator and allowed to cooled. The samples are then weighed (W3)

The % of Fat was calculated as: $\frac{W2-W3}{W2-W1} \times 100$

3.10.5 Determination of crude protein (AOAC, 2002)

3.10.5.1 *Digestion:*

0.5- 2gram of samples weigh into kjeldahl flasks and a catalyst (copper) and 15ml concentrated sulfuric acid (H_2SO_4) are added. The setup are heated in a fume cupboard till the solution assumes green colour. Allow to cool and rinse down any black particles at the

mouths and neck of the flask with distilled water. After cooling, the digested samples are transferred with several washings into a 100ml beaker and topped up with distilled water.

3.10.5.2 *Distillation:*

Before use, the Markham distillation apparatus is first steamed for about 15minutes. A 100ml conical flask containing 10ml of Boric indicator is placed under the condenser.10ml of the digest is pipetted into the body of the apparatus via the small funnel aperture. It is then rinsed down with distilled water followed by 10ml of 40% NaOH solution. It is steamed through for 5-7 minutes to collect ammonium sulphate (about 30-40ml). Thereceiving flask is removed and the tip of the condenser is rinsed into the flask.

3.10.5.3 *Titration:*

The solution was titrated in the receiving flask using N/100 (0.01N) hydrochloric acid and the Nitrogen content is calculated hence, the Protein content of the sample. During this process, a blank is always ran through along with the sample as a control.The % of protein was calculated as given by the formula below:

$$= \frac{Final\ reading - Initial\ reading - blank\ (0.2) \times standard\ number\ of\ Nitrogen\ (1.4)}{Initial\ weight\ (0.5) \times standard\ number\ of\ protein\ (6.25)}$$

3.10.6 Determination of carbohydrate (CHO) (Pearson 1976)

The carbohydrate content was determined by difference, in this method carbohydrate content was obtained by calculations having estimated all other fractions by proximate analysis and subtracting from 100 as given by the formula;

$$\%CHO = 100 - (\%\ Moisture + \%Ash + \%Protein + \%\ Fat)$$

3.11 Enumeration and Identification of Bacterial Isolates

3.11.1 Viable bacterial count of the gut of hybrid fry

At the end of the experiment, the experimental fish were sacrificed and dissected following aseptic method and their gut harvested. The guts of three fish per treatment were pooled and homogenized in sterile normal saline. This formed the stock solution from which two fold serial dilution was made. The second dilution was selected for spread plating on a freshly prepared nutrient agar using bent glass rod. The cultures were incubated at 37ºC for 24 hours and the number of colonies on each plate were counted using a colony counter. The viable count of bacterial (CFU/g) was calculated using the formula;

$$\frac{Total\ number\ \ of\ colonies}{Volume\ \ of\ innoculum} \times Dilution\ factor$$ (Chessbrough, 2006)

3.11.2 Biochemical tests and identification of bacterial isolates

The bacteria isolates were Gram stainedfollowing this procedure:For the Gram's positive cocci, catalase and coagulase tests were performed. The Gram positive rod was tested for catalase, spore and Methyl Red/Voges-Proskauer (MRVP), while the Gram negative rod were sub-cultured on freshly prepared Muller Hilton agar and incubated at 37°C for 24 hours. The lactose fermenters (pink colonies)were subjected to the following tests; Indole, methyl red, Voges Proskauer and citrate utilization. While the non-lactose fermenters(colourless colonies) were subjected to the following tests; Indole, Citrate, methyl red, Voges-Proskauer, motility, Triple Sugar Iron (TSI) and Urease following the procedure of (Cheesbrough, 2006).

3.11.2.1 *Catalase Test*

A drop of hydrogen peroxide was placed on a glass slide and using a sterile wooden stick, a little amount of theovernight cultured isolate were immersed into hydrogen peroxide solution. Isolate which gave out bubbles were positive, while if those which did not produce bubbles were negative (Atta *et al*., 2020).

3.11.2.2 *Coagulase Test*

A drop of water was placed on a glass slide and a smear of the isolate was made on a glass slide. Five drops of serum was added and the slide was rocked to mix the serum and the smear. It is observed and if it coagulates, appearing turbid, then it is coagulase positive. If no coagulation, then isolate was coagulase negative (Chessbrough, 2006).

3.11.2.3 *Methyl red and Voges Proskauer Test*

A loop-full of the isolate was inoculated into the Methyl red and Voges Proskauer medium and incubated at 37°C for 24 hours. The medium was then divided into two, for methyl red and Voges Proskauer tests. For Methyl red test, three drops of methyl red reagent was added into the tube and the setup was kept for a few minutes. If a red ring layer appears on the surface of the medium, then the isolate is methyl red positive. Where no ring layer is formed, the test is negative.

For VogesProskauertest, five drops of 40% KOH is added and 3-4 drops of Alpha naptol. A red layer appears if isolate is positive while the colour remains yellow if negative (Cheesbrough, 2006).

3.11.2.4 *Sulphate,Indole and Motility (SIM) Test*

A sterile test tube containing SIM medium was stabbed with a straight wire and incubated for 24 hours. The test tube is brought out and observed for motility of inoculum. Motility is observed if isolates form cloudy growths away from the stab line. If bacterial isolate

grow only alone the stab li e and do not dispersed into the motility medium, the isolate is not motile.The formation of black colouration in the test tube above reveals that the isolate is sulphate positive. If no formation of black colouration, then isolate is sulphate negative.

The motility medium is added with 3-4 drops of Kovac's reagent. It is then observed for the presence of rings. The appearance of red rings shows positive test result while the appearance of no red ring shows negative result (Cheesbrough, 2006).

3.11.3 Citrate Test

The bacterial isolates were streaked on Simmon- Citrate agar and incubated for 24 hours aerobically at 37°C after which the cultures were observed for colour change. Isolates that turned the Simmon- Citrate medium from green to blue are positive while those that did not change colour are negative (Atta *et al*., 2020).

3.11.4 Triple Sugar Iron (TSI) Test

The isolate is stabbed into the butt of the TSI medium using a straight wire aseptically and on removing the straight wire, a streak is made on the slant. It is then incubated for 24 hours and observed for colour change (Chessbrough, 2006).

3.11.5 Urease Test

Heavy inoculation was made into a sterile bijou containing 3ml of Christensen's modified urea broth and incubated aerobically at 37°C for 24 hours leaving the caps loosely covered to allow respiration by the bacteria. After 24 hours they were observed for colour change. Formation of pink colour implies a positive test for urease while if the broth remains yellow, it implies a negative test (Cheesbrough, 2006).

3.12 Data Analyses

Data collected were test for homogeneity and normality for parametric test. Data were summarized using descriptive statistics to calculate the means and were presented in tables. One Way Analysis of Variance (One way ANOVA) was used to compare between means of the different treatment groups of growth, nutrient utilization parameters and survival rate. Confidence interval was set at 95% and P-value was set at $P \leq 0.05$. Duncan's Multiple Range test (DMRT) was used to separate means where there were significant differences. The Statistical Package for Social Science (SPSS® version 26) was used for the analyses.

CHAPTER FOUR

4.0 RESULTS

4.1 Probiotic IsolatesfromRumen of Cattle

The bacteria isolated from the rumen of cattleare as presented in Table 4.1,five colonial morphologies were observed to have grownon the MRS medium (with codes; RS1, RT3, RT4, L2 and L4). The isolate RT3 was tentatively identified as *Streptococcus thermophiles*. RT4 and L2 showed similar result for all tests as RT3, however RT3 was selected over RT4 and L2on the basis of its better growth on MRS media.

4.2 Physico-chemical Parameters of Culture Water

Selected physico-chemical parameters (pH, TDS, water temperature and dissolved oxygen) of the experimental setup are shown in Table 4.2. There was no significant difference ($p>0.05$) for pH, TDS and water temperature among the treatments. However, significant difference exist among the treatment for dissolved oxygen ($p<0.05$).

Table 4.1:Gram Reaction and Biochemical tests of bacteria isolated fromcattle gut

Test	RS1	RT3	RT4	L2	L4
Morphology	Cocci	cocci	cocci	cocci	cocci
Gram's reaction	+	+	+	+	+
Catalase	+	-	-	-	+
Oxidase	+	-	-	-	+
Motility	-	-	-	-	-
Spore	+	-	-	-	+

Key
+ = Positive. - = Negative, RS, RT,L = isolates code

Table 4.2: Physico-chemical parameters of culture water administered *Streptococcus thermophilus* to fry *C. galmaensis* and *C. gariepinus*concentration of as probiotics

PARAMETER	CONTROL	A	B	C	P- Value
pH	7.23±0.82	7.21±0.82	7.12±0.82	7.16±0.82	0.788
TDS (ppm)	145.61±2.26	148.39±2.26	151.50±2.26	143.82±2.26	0.092
Temperature (°C)	25.54±0.622	25.54±0.622	25.96±0.622	25.71±0.622	0.956
Dissolved oxygen (mg/l)	1.61 ± 0.058^{d}	1.45 ± 0.058^{c}	1.26 ± 0.058^{b}	0.888 ± 0.058^{a}	0.00

TDS= total dissolved solid. Means with the same superscript along the rows have no significant difference ($p\geq0.05$), means with different superscript along rows are significantly different ($p\leq0.05$)

Key

A-1.5×10^{7}CFU/ml

B- 1.5×10^{8} CFU/ml

C- 1.5×10^{9}CFU/ml

4.3 Growth Parameters of Hybrid

The growth parameters of the fry as displayed on Table 4.3 shows no statistical difference($P \geq 0.05$) between the treatments for all parameters except for condition factor (K). The highest probiotic concentration inclusion (Treatment C) shows significant($p \leq 0.05$) differenceamong the groups for condition factor (K).

4.4 Nutrient Utilization Parameters of Hybrid

Streptococcus thermophilus administered as treatment didnot show significant difference ($P > 0.05$) for all nutrient utilization parameters displayed on Table 4.4 except for protein intake ($p < 0.05$). The protein intake differ significantly between the groups and the control. Protein intake increased steadily with probiotic inclusion until the highest concentration where it gave the lowest value.

4.5 Survival Rate of Hybrid

The percentage survival of hybrids for the control and treatment groups at the end of the experiment is represented on the Table 4.5. The result showssignificant effect of*Streptococcus thermophilus* on the survival rate of the hybrid. The highest survival rateof 28.75% was observed with Treatment C(*S. thermophilus*of 1.5×10^9 CFU/ml) was administered. The control group had the lowest survival rate andhighest mortality rate.

Table 4.3: Growth Parameters of hybrid between treatment groups fed varying levels of*Streptococcus thermophilus* as probiotics

PARAMETER	CONTROL	A	B	C	P-Value
Initial weight (g)	0.16±0.00	0.16±0.00	0.16±0.00	0.16±0.00	
Final weight (g)	0.678±0.203	0.822±0.203	0.856±0.203	0.766±0.203	0.928
Initial length (cm)	1.476±0.032	1.414±0.032	1.414±0.032	1.434±0.032	0.506
Final length (cm)	4.233±0.302	4.767±0.302	4.778±0.302	4.767±0.302	0.532
Length gain (cm)	2.757±0.305	3.353±0.305	3.364±0.305	3.333±0.305	0.464
Weight gain (g)	0.518±0.181	0.662±0.181	0.696±0.181	0.406±0.181	0.664
Condition factor (K)	2.301±0.234	1.671±0.234	1.638±0.234	1.239±0.234	0.068
Specific Growth Rate	0.923±0.362	1.182±0.362	1.244±0.362	1.081±0.362	0.927
RGR	323.543±113.151	413.963±113.151	434.793±113.151	253.963±113.151	0.664
ADG	0.0093±0.007	0.0118±0.007	0.0124±0.007	0.0073±0.007	0.956
Production index (PI)	0.185±0.087	0.310±0.087	0.357±0.087	0.200±0.087	0.466

RGR=Relative growth rate, ADG=Average daily growthFCR= Feed conversion ratio PER=Protein efficiency ratio.

Key
A- 1.5×10^{7} CFU/ml
B- 1.5×10^{8} CFU/ml
C- 1.5×10^{9} CFU/ml
D- 0

Table 4.4: Nutrient utilization of hybrid among treatment groups fed different concentrations of *Streptococcus thermophilus* as probiotics

PARAMETER	CONTROL	A	B	C	P-Value
FCR	2.87±0.587	2.15±0.587	2.54±0.587	3.16±0.587	0.939
FCE	1.581±0.477	1.604±0.477	1.697±0.477	0.894±0.477	0.648
PER	0.014+0.004	0.021±0.004	0.023±0.004	0.011±0.004	0.751
Protein intake	14.18 ± 0.306^{b}	17.033 ± 0.306^{c}	17.98 ± 0.306^{d}	16.10 ± 0.306^{a}	0.045
Nitrogen metabolism	7.960±2.783	10.183±2.783	10.693±2.783	6.243±2.783	0.663

FCR= Feed conversion ratio,FCE= Feed conversion efficiency, PER=Protein efficiency ratio; Means with the same superscript along columns have no significant difference, means with different superscript along columns are significantly different. Where A,B and C are treatment groups

Key

A- 1.5×10^{7} CFU/ml

B- 1.5×10^{8} CFU/ml

C- 1.5×10^{9} CFU/ml

D- 0

Table 4.5: Survival rate of hybrid among treatments administered with varying concentrations of *Streptococcus thermophilus* as probiotics

Treatment	% Survival	% Mortality
A	26.25	73.75
B	28.75	71.25
C	27.5	72.50
D	20	80

Key

A- 1.5×10^{7} CFU/ml

B- 1.5×10^{8} CFU/ml

C- 1.5×10^{9} CFU/ml

D- 0

4.6 Viable Gut Bacteria Count

The viable bacteria count from the gastrointestinal track of the hybridfor each treatment group is presented in Figure 4.1. The corresponding bacterial counts are 6.72×10^5CFU/g, 1.05×10^6 CFU/g, 2.08×10^5CFU/g and 4.40×10^5CFU/g for treatments A, B, C and D (control) respectively.

The Gram's reaction and morphology of the bacterial isolates from the gut of the hybrid are as presented on Table 4.6. on the basis of colony morphology of the isolates on the culture plates, three (3) colours of colonies was observed; yellow, gray, light gray (intermediate). The gray colonies appeared on all probiotic treatment groups. The yellow colonies appeared on treatment A, B and the control groups, but did not appear on treatment C. The light gray (intermediate) colony was observed only on the control group. The yellow and gray isolates of treatment A were rod shaped and gave a negative test to Gram's stain. The yellow isolates of treatment B gave a positive identification to Gram's test while the gray colony isolates gave negative test to Gram's stain. The bacteria isolate of treatment C had rod morphology and were negative to Gram's test. The yellow colonies of the Control group had cocci morphology and gave positive test to Gram's stain, the gray colonies gave negative test to Gram's and had rods morphology, while the intermediate gray colonies were positive to Gram's test and had cocci morphology.

The presumptive tests and identification of the bacterial isolates was presented on Table 4.7. The yellow and gray colonies of the gut of the hybrid of treatment A were identified to be *Enterobacter* sp. The yellow colonies of treatment B were identified to be *Bacillus* sp. (Dermikan *et al*., 2014). The gray colonies of treatment B and C were identified as E.

coli (Cheesbrough, 2006). The three bacterial isolates of the gastrointestinal tract of *Clarias*

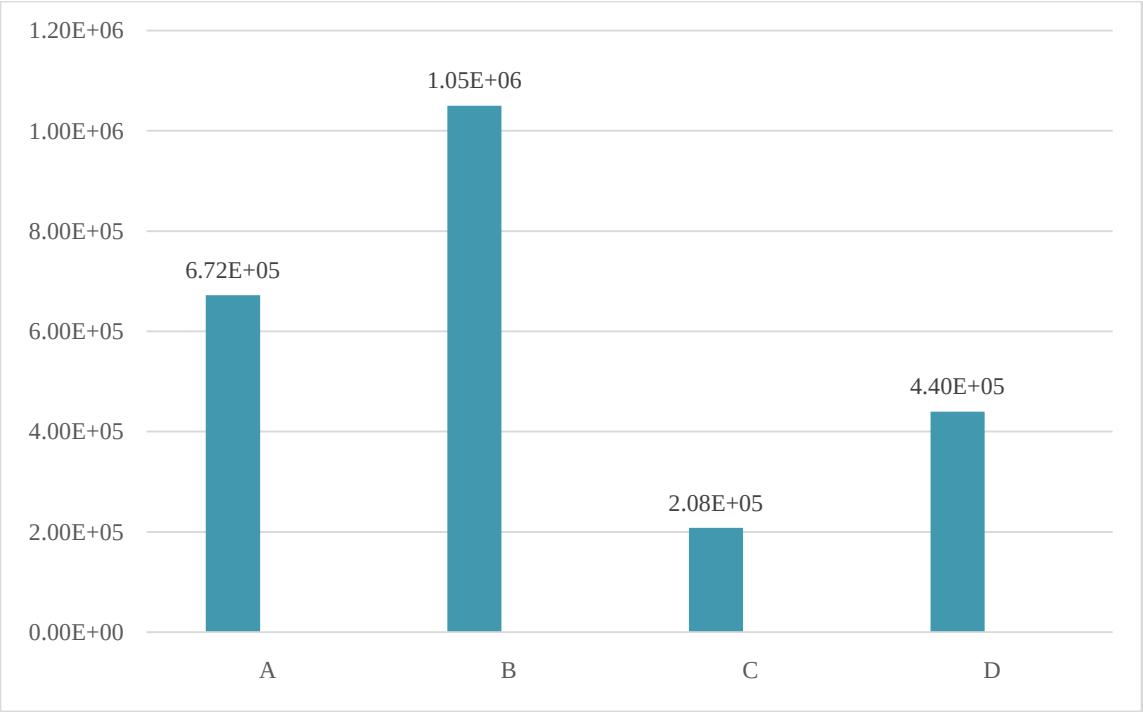


Figure 4.1:Viable bacterial count of the GIT of hybrid of the different treatment groups

Key

A- 1.5×10^{7} CFU/ml

B- 1.5×10^{8} CFU/ml

C- 1.5×10^{9} CFU/ml

D- 0

Table 4.6: Gram's reaction of bacterial isolates from the gut of hybrid

Identification	Control	A	B	C
Yellow Colonies	+ cocci	- rods	+ rods	Nil
Gray Colonies	- rods	- rods	- rods	- rods
Intermediate	+ cocci	Nil	Nil	Nil

Key

A- 1.5×10^{7} CFU/ml

B- 1.5×10^{8} CFU/ml

C- 1.5×10^{9} CFU/ml

D- 0

Table 4.7:Biochemical test, presumptive test and identification of bacterial isolates from the gut of hybridadministered concentrations of *S.thermophilus*

S/NO	Isolate	Morphology	Gram's reaction	Spore test	Catalase	MR	VP	Coagulase	Citrate	Motility	Indole	TSI	Urease	Sulphate	Suspected identification	Reference
1	By	Rod	+	+	+	-	-	NA	NA	NA	NA	NA	NA	NA	*Bacillus* sp.	Demirkan *et al.*, 2014
2	Dy	Cocci	+	NA	+	NA	NA	+	NA	NA	NA	NA	NA	NA	*Staphylococcus aureus*	Sizar and Unakal, 2021
3	Di	Cocci	+	NA	-	NA	NA	-	NA	NA	NA	NA	NA	NA	*Enterococcus faecalis*	Rahman *et al., 2009*
4	Ay	Bacilli	-	NA	NA	-	+	NA	+	+	-	NA	NA	-	*Enterobacter* sp.	Cheesbrough, 2006
5	Ag	Bacilli	-	NA	NA	-	+	NA	+	+	-	NA	NA	-	*Enterobacter* sp.	Cheesbrough, 2006
6	Bg	Bacilli	-	NA	NA	-	+	NA	-	+	+	K/A	-	-	E. *coli*	Cheesbrough, 2006
7	Cg	Bacilli	-	NA	NA	-	+	NA	-	+	+	K/A	-	-	E. *coli*	Cheesbrough, 2006
8	Dg	Bacilli	-	NA	NA	-	-	NA	-	-	-	K/A	-	-	*Shigella sp.*	Cheesbrough, 2006

Legend

A, B and C= Treatment (containing varying concentration of inoculum), D= control

TSI=Triple Sugar Iron, MR=Methyl Red, VP=Vogas-Proskauer, + = positive, -= negative, NA= Not applicabl

hybrid of the control group (D) were identified as the following; the yellow isolate as *Staphylococcus aureus* (Cheesbrough, 2006; Sizar and Unakal, 2021), the intermediate isolate as *Enterococcus faecalis* (Rahman *et al.*, 2009) and the gray isolate as *Shigella* sp. (Cheesbrough, 2006).

4.7 Carcass Proximate Compositions of Hybrid

The proximate compositions of the hybrid carcass for the different treatments is represented in Table 4.8. Treatment A had the highest protein content (36.45%) among all the Treatment groups, while Treatment C had the lowest protein content (24.79%). Treatment C on the other hand had the highest values for carbohydrate (18.93%), moisture (19.25%) and lipid content (23.66%) content, while the control group had the lowest value of lipid and carbohydrate content. Treatment B had the lowest moisture content (16.33%). There was increasing trend of carbohydrate composition with higher probiotic inclusion, while protein content increased on the first probiotic inclusion, but subsequently decreased with increasing probiotic inclusion.

Table 4.8: Proximate composition of carcass of hybrid of different treatment groups

Treatment	Moisture %	Ash %	Protein %	Lipid %	Carbohydrate %
A	17.46	14.97	36.45	21.60	9.52
B	16.33	13.95	30.62	23.55	15.55
C	19.25	13.37	24.79	23.66	18.93
D	18.77	22.55	32.04	21.28	5.36

Key

A- 1.5×10^{7} CFU/ml

B- 1.5×10^{8} CFU/ml

C- 1.5×10^{9} CFU/ml

D- 0

CHAPTER FIVE

5.0 DISCUSSION

The identification and isolation of *Streptococcusthermophilus* from the rumen of cattle in this studyprovide proof that it ispart of thenormal microflora of the rumen of cattle. This bacterium could have find their way into the cattle through feeding either on grasses and hay or from breast milk as calves from their mothers, since *S. thermophilus* together with *Lactobacillus acidophilus* are known to be present in milk.Xue *et al*., (2018) in their study of rumen bacteria reported that the predominant bacteria phyla was the Firmicutes, consisting of twenty three genera. As foregut fermenters(cattle),their rumen areexpected to have a rich population of fermenting bacteria to perform the fermentation activity. In addition, the anaerobic environment of the rumen provides a convenient environment for lactic acid bacteria to thrive, which *S. thermophilus*is one. Many studies have revealed that the genus *Streptococcus* is a normal bacterial flora of the gastrointestinal tract of cattle. Maki and Picard, (1964) found *Escherichiacoli* and *Streptococcusbovis* as the predominant bacteria inhabiting the duodenum of the intestinal. Wilssens and Buttiaux, (1958) also reported that the E. *coli*, *Streptococcus faecium, S. bovis, micrococci* and *Bacillus* spp. made up the most often present groups of bacteria inhabiting the gastrointestinal tract of cattle. Sypestyn (1949) isolated *Ruminococcus flavefaciens* from the rumen of cattle which is an anaerobic, Gram's positive *Streptococcus* bacterium that ferments cellulose. This bacterium characterization resembles *S. thermophilus*. *Streptococcus thermophilus* with probiotic properties as identified in this study is a normal microflora of the rumen of cattle.

The morphometric parameters of the broodstock is as expected the description for both species types. The morphometric parameters of *Clarias galmaensis*(male) agrees with the report of Aken'ova (2007), who reported in the identification and description of the species that the maximum weight and standard length of *C. galmaensis*♂ does not exceed 75g and 20.8cm respectively. The gonado-somatic index (GSI) of parent*C. gariepinus*female gave a relatively low value and consequently, a low relative fecundity for *C. gariepinus*. The gonad (testis) weight for the parent male*Clarias galmaensis* was undetectable by the weighing scale. The data in this study agrees to established knowledge where GSI and relative fecundityshows positive correlation to body size.

Hybrid cross between *Clarias gariepinus*♀and *Clarias galmaensis*♂has not been reported by any published literature. This could be because *Clarias galmaensis* is a newly identified species (Aken'Ova, 2007), and could be mistaken for stunted or slow growing *Clarias gariepinus*because it lack the large size trait desired in catfish. However, the cross breeds between these two species was successfully achieved. The successful breeding of the hybrid (*Clarias gariepinus*♀and *Clarias galmaensis*♂) shows compatibility between the two subspecies.Nevertheless, the hybridshows inferiorgrowth performance in comparison to the pure cross of *Clarias gariepinus*. Apparent observation of the hatchlings indicated larger fry size for the pure cross than the hybrid cross. This could be as a result of the inheritance of the smaller maternal morphometric data as oppose to the higher morphometric data for the pure crossbroodstock.The difference in the percentage fertilization and percentage hatchability of the pure cross and the hybrid could be as a result of phylogenetic divergence between the two subspecies.

Hybridization was carried out with the primary aim of improving the growth trait of *Clarias galmaensis* and to introduce the hardiness of *C. galmaensis* on the hybrid

offspring. This cross breed showed slow growth rate and small size that is characteristic of *Clarias galmaensis* reflecting paternal effect and by implication negative heterosis. The cross therefore showed dominance of the paternal trait over maternal effect, suggesting dominant expression of the male *C. galmaensis*♂ growth trait in the hybrid cross than the more desired large growth (Tave, 1986) of C. *gariepinus* or by increasing the number of paternal polymorphic loci in the offspring (Bartley *et al.*,2001). The results of inter-specific hybridization can be variable and depend on the genetic structure (including the sex) of the parent fish (Bartley *et al.*,2001). Dunham *et al*. (1982) reported the case of paternal dominance in Channel Blue hybrid fish.A study carried on channel catfish fry by Burgos *et al*. (2018) where fry were weighed from hatching to fingerling stage (150dph) revealed a faster growth rate. The channel catfish weighed significantly higher in 60dph (2.423±0.312) and were almost double that by 90dph (4.348±0.357) than the *C. galmaensis* and *C.gariepinus* hybrid in 80dph.

Streptococcus thermophilus had no significant effect on all observed water parameters except dissolved oxygen (DO). Cruz *et al*. (2012) reported that gram positive bacteria are able to improve water quality through minimizing organic matter accumulation in aquarium and transforming it to CO_2. Dissolved oxygen decreased with increasing *Streptococcus thermophilus* inclusion across the treatments. This decline could have been due to the increasing generation of the product of respiration incurred by the increasing *Streptococcus thermophilus* population across the treatments. This agreeswith the findings of Khalafalla *et al*. (2020) who reported no significant effect ($p>0.05$) on water quality parameters, but differed only on the data for dissolved oxygen. This difference on dissolved oxygen could be as a result of the use of a consortium of four different bacteria

as probiotics in their study, and each bacterial species possibly impacting the dissolved oxygen differently.

S. thermophilus isolated from the rumen of cattle showed no significant effect on the growth parameters and nutrient utilization on the hybrid fry. This result is not in agreement to the findings of Khalafallah *et al.* (2020) and Putra *et al*. (2020) who reported significant effect and improvement of growth and nutrient utilization parameters of Tilapia fry fed a mixture of probiotic bacteria, and probiotic *Bacillus* NP5 on the nutrient utilization (feed conversion ratio) of *Clarias gariepinus* respectively. Additionally, similar trend of increasing FCR with increasing probiotic inclusions in CFU/ml exist between this study and that of Putra *et al.* (2020). However, Khalafallah *et al*. (2020) indicated better feed conversion at less probiotic concentration. The differences in the findings of the studiescould be due to the low probiotic activity of the *Streptococcusthermophilus* strain used in this research. This could be the reason for their lesser use and status as probiotic in comparison to members of the*Lactobacillus*, *Bifidobacterium* and *Bacillus* genera. Barow and Feltham (1993) reported that lactic acid producing*Streptococcus saliverius* reported to be use as starter culture in the production of milk and Oropharyngeal *Streptococcussaliverius* should form a single species, however the oropharyngeal commensal *Streptococcus saliverius* is of no use as a starter culture. This may imply different activity of the same bacteria species or strain from different origin.This result does not agree with the study of Humphrey and McBain (2019) who reported that *Streptococcussalivarius* performed well (even better) than *Lactobacillus acidophilus* and *Lactobacillus plantarum* in its antimicrobial activity against *Streptococcus pyogenes* of human sample.However the findings of this study agreed with that of Perigon *et al*.,(1987)

who reported less effectiveness of *S. thermophilus* in the enhancement of the immune response of mice than *Lactobacillus acidophilus*.

S. thermophilus isolated from the rumen of cattle hadsignificant effect on the survival rate of hybrid fish. This could bebecause of the exclusion effect of the bacterium on pathogenic bacteria in the gut that could have led to suppression of the growth of pathogenic bacteria, while encouraging the growth of beneficial ones. The highest mortality observed in the control group could be as a result of lower population of beneficial bacteria inhabiting the GITof the fry compared to those in the*S. thermophilus*treated groups. The result agrees with the finding ofYakubu *et al*., (2016) who reported an increase in thesurvival rate,with increasing probiotics dose, where the highest dose (in grams per Kg of feed)of a commercial probiotic gave the highest survival rate in *Clarias gariepinus*.

Probiotic inclusion levels hadno effect on the viable bacterial count of the gut of the hybrid fish. The development of the gut microbiome in fish is considered a complex process and a reflection of the microbial composition of the aquatic environment and diet (Roeselers *et al.,* 2011).The viablebacteria count increased with the increasing probiotic concentration, until the highest inclusion level where it gave the lowest viable bacteria count. This indicated that increasing concentration of *S. thermophilus* increased the viable bacteria count until concentration of 10^9 CFU/mlwhich drastically show inhibitory effect on boththe population of gut bacteria, and the diversity (isolates) of bacteria.Findings of Panigrahi *et al*. (2005) and Dash *et al*. (2014) in rainbow trout (*Oncorhynchus mykiss*)and giant freshwater prawn (*Macrobranchium rosenbergii*), respectively show similar results of gut bacterial increase with probiotic inclusion. The decrease of viable bacteria count in the highest*S. thermophilus*inclusion level might have resulted due to the production of

very high levels of inhibitory substances in balance with the high *S. thermophilus* population fed to the fry. The control group which had no *S.thermophilus*inclusion had the highest diversity of bacteria, therefore showing anegative correlationbetween bacterial diversity and probiotic inclusion.

The bacterial isolates from the gut of the fry revealed possible activity of *S.thermophilus* in inhibiting *Stephylococcus aureus* and *Shigella sp.* which are pathogenic bacteria, andpresent in the control group but not in the treatment groups. The presence of these pathogenic bacteria is indicative of the presence of an enabling environment for their growth. The absence of these pathogenic bacteria in the*S. thermophilus*treatedgroups indicateactivity of *S.thermophilus* on these enteric pathogenic bacteria.*Enterococcus faecalis* which is a LAB and normal flora of the gut of fish was the only beneficial bacteria present in the control group. This reinstate the known fact ofthe cohabitation of pathogenic and non-pathogenic bacteria in the gastrointestinal tract of a normal fish. The occurrence of different bacterial isolates along the differenttreatment groups might be as a result of the differentinteractions of the bacteria to the varying acidifications of the gut of the fish created by the inclusion levels of*S.thermophilus*. The bacteria isolates of each treatment may correspond to their acid tolerance or pH requirement, implying *Bacillus* sp. possibly being the least tolerant tothe acidification of *S.thermophilus*at inclusion of 1.5×10^{7} CFU/ml, and *Escherichia coli*being the most tolerant bacteriafound both in treatments 1.5×10^{8} CFU/ml and the highest treatment with the *S.thermophilus* concentration of 1.5×10^{9} CFU/ml.

Carcass proximate compositions was influenced by probiotics. Crude protein content decreased with increasing probiotic inclusion while carbohydrate composition increased

with increasing probiotic concentration. There is an obvious inverse relationship between the protein and carbohydrate composition, and between lipid and water compositionamong the treatments groups. The presence of a complex and dynamic gut microbiome in teleosts has been recognized as an important factor in maintaining host health (Bacanu and Oprea 2013; Llewellyn *et al*., 2014). The spike of protein content in the first treatment above the control show possibly eitherbetter activity (utilization) of *Enterobacter* sp. ofthe gut bacteria on protein deposition for absorption by the intestine to the muscles of the fish(Aluwong *et al*., 2013), or the absence of *Staphylococcus aureus* and *E. coli* are responsible for the increase in feed absorption reflected in better protein content of the fish. Protein composition of the carcass declinedwithfurther increasing of probiotic concentrations. This could have resulted due to the low bacteria diversity as well as the isolates in the gut of the experimental fish. This might have reduced the contribution of the individual and/or synergistic interplay of a more diverse gut bacteria to release nutrients from the feed for assimilation by the digestive tract, resulting to decrease in protein deposition and availability in fish absorption with increasing probiotic inclusion.This finding does not agree with the work of Umaru and Agbugui (2021) who reported a steady increase in protein content in the carcass of *Clarias gariepinus* fed a commercial probiotics. The difference might be due to the different mechanism of action of yeast (*Sacharomyces cerevisiae*) that was used in that study as compared to *S. thermophilus* used in this study. The carbohydrate content as earlier stated was inversely related to protein content and correlates with the lipids content increased as protein content decreased but the least value was observed in the control group which means that *S. thermophilus*significantly increased the carbohydrate content with increase of *S. thermophilus* concentration along the treatments. This is seen as the gut isolate tended

towards a single bacteria (*E. coli*). This could have resulted as a result of E. *coli*getting involved to digest carbohydrate and therefore help release more of it for assimilation by the intestine.The increase in lipid content could be linked with the increase of carbohydrate contentof the fish carcass. The decline in ash content in the carcass of the fish with increasing probiotic levels could be as a result of less availability of minerals with increasing *S. thermophilus* concentration possibly resulting from the absorption of mineral by the gut bacteria.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

*Streptococcus thermophilus*is one of the constituent bacterium that makes up the microflora of the rumen of cattle. *S. thermophilus*was found to have no significant effect on the growth performance; final weight (p=0.928), final length (p=0.532), length gain (p=0.464), weight gain (p=0.664), SGR (p=0.927), Condition factor (p=0.068), RGR (p=0.664), ADG (p=0.956) and Production index (p=0.466),and nutrient utilization parameters; feed conversion ratio (p=0.939) feed efficiency ratio (p= 0.648), PER (p=0.751) and Nitrogen metabolism (p= 0.663) ofhybridfry. Only protein intake had significant effect (p=0.045), *S. thermophilus* had significant effect on the survival rate of the hybrid.Similarly, *S. thermophilus* had no effect on the viable bacterial count of the gut, but showed modulatory effect on the bacterial flora of the GIT of the hybrid.

6.2 Recommendations

I. *Streptococcus thermophilus* may be used as probiotic to arrest high mortality and increase survivability of fry in hatchery.

II. Similar studies of *Streptococcus thermophilus* on juvenile of pure breed of *Clarias* or other species of fish should be carried out to assess its effect on hematological parameters and intestinal structure.

III. The rumen of cattle should further be investigated for other possible probiotic bacterial candidates.

IV. Studies on the effect of probiotics on carcass proximate compositions should be carried out due to the importance of protein as a major nutrient of fish.

REFERENCES

Abasali, H., and Mohamad, S. (2010). Effect of dietary supplementation with probiotic on reproductive performance of female live bearing ornamental fish. *Research Journal of Animal Sciences*, 4(4):103-107.

Abd El-Tawab M. M., Youssef I. M. I., Bakr A. H., Fthenakis C. G. and Giadinis N. D. (2016). Role of probiotics in nutrition and health of small ruminants. A Review. *Polish Journal of Veterinary Sciences*, 19(4):893-906. DOI 10.1616/pjvs-2016-0114

Abdelqader, A., Irshaid, R. & Al-Fataftah, A.-R. (2013). Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production. *Tropical Animal Health & Production*, 45(4):1017–1024.

Ahmed H., Al-Harbi T. and Uddin M.N. (2005); Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture*, 250:566-572.

Aken'Ova T. O. (2007). A preliminary report on a new species of *Clarias* (Scopoli, 1777) in River Galma, Zaria, Nigeria. *Journal of Tropical Biosciences*, 7:45-48

Akter M. N., Parvez I. and Patwary Z. P. (2016). Beneficial effects of probiotics in aquaculture. *International Journal of Fisheries and Aquatic Studies*,4(5):494-499.

Aluwong, T., Kawu, M., Raji, M., Dzenda, T., Govwang, F., Sinkalu, V. and Ayo, J. (2013). Effect of Yeast Probiotic on Growth, Antioxidant Enzyme Activities and Malondialdehyde Concentration of Broiler Chickens. *Antioxidants*2**:**326-339.

Aly, S. M., Mohamed, M. F., and John, G. (2008). Effect of probiotics on the survival, growth and challenge infection in *Tilapia nilotica* (*Oreochromis niloticus*). *Aquaculture Research*. https://doi.org/10.1111/j.1365- 2109.2008.01932.x

An, B., Cho, B., You, S., Paik, H., Chang, H., Kim, S., Yun, C. and Kang, C. 2008. Growth performance and antibody response of broiler chicks fed yeast derived β-glucan and single-strain probiotics. *Asian-Australasian Journal of Animal Sciences*, 21(7): 1027–1032.

Anee I. J., Rowshan S. A., Begum A., Shahjahan R. M. and Khandaker A. M. (2021). The role of probiotics on animal health and nutrition. *Journal of Basic and Applied Zoology*, 82:52 https://doi.org/10.1186/s41936-021-00250-x

Apata, D**.** (2008).Growth performance, nutrient digestibility and immune response of broilerchicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*.*Journal of the Science of Food and Agriculture*, 88(7)*:*1253–1258.

Arellano-Carbajal, F., and Olmos-Soto, J. (2002). Thermostable α-1,4- and α-1,6-glucosidase enzymes from *Bacillus* sp. isolated from a marine environment. *World*

Journal of Microbiology and Biotechnology. https://doi.org/10.1023/A:1020433210432

Ariole, C.N., and Okpokwasili, G.C. (2012). The effect of indigenous probiotics on egg hatchability and larval viability of *Clarias gariepinus*. *Revista Ambiente and Água - An Interdisciplinary Journal of Applied Science*, **7**(1):81-88. https://doi.org/ 10.4136/ambi-agua.712.

Arsène M. M. J., Davares A. K. L., Andreevna S. L., Vladimirovich E. A., Carime B. Z., Marouf R. and Khelifi I. (2021) The use of probiotics in animal feeding for safe production and as potential alternatives to antibiotics, *Veterinary World*, 14(2): 319-328.

Asahara, T., Shimizu, K., Nomoto, K., Hamabata, T., Ozawa, A., and Takeda, Y. (2004). Probiotic *bifidobacteria* protect mice from lethal infection with Shiga toxin-producing *Escherichia coli* O157:H7. *Infection and Immunity*. https://doi.org/10.1128/IAI.72.4.2240-2247.2004

Asgari, F., Madjd, Z., Falak, R., Bahar, M. A., Heydari Nasrabadi, M., Raiani, M., and Shekarabi, M. (2016). Probiotic feeding affects T cell populations in blood and lymphoid organs in chickens. *Beneficial Microbes*. https://doi.org/10.3920/ BM2016. 0014

Atta, H. I., Gimba A. and Bamgbose T. (2020). Antimicrobial activity of lactic acid bacteria isolated from garri on *Escherichia coli* strains isolated from clinical and environmental samples. *Agricultural Science and Technology*, 12(4):357-363. DOI:10.15547/ast.2020.04.057

Austin B. and Day J. G. (1990). Inhibition of prawn pathogenic *Vibrio* ssp. By a commercial spray-dried preparation of *Tetraselmis suecica. Aquaculture*, 90:389-392

Aydin F., and Cek-Yalniz S. 2019. Effect of probiotics on reproductive performance of fish. A Review. *Natural and Engineering Science* 4(2):153-162.

Bai, S., Wu, A., Ding, X., Lei, Y., Bai, J., Zhang, K. and Chio, J. 2013. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Science*, 92(3): 663-670.

Balcazar, J. L., Rojas-Luna, T., and Cunningham, D. P. (2007). Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp (*Litopenaeus vannamei*) following immersion challenge with *Vibrio parahaemolyticus*. *Journal of Invertebrate Pathology*. https://doi.org/10.1016/j.jip.2007.04.008

Banerjee, G., and Ray, A. K. (2017). The advancement of probiotics research and its application in fish farming industries. *Research in Veterinary Science*, 115, 66–77. https://doi.org/10.1016/j.rvsc.2017.01.016

Bartley, D.M., Rana K. and Immink, A.J. 2001.The use of inter-specific hybrids in aquaculture and fisheries. *Reviews in Fish Biology and Fisheries* 10**:**325–337

Barow I. G. and Feltham K. A R. 1993. Cowan and Steel's manual for the identification of medical bacteria. 3rd edition. Edited and revised by G.I. Barrow and R.K.A. Feltham. *Cambridge University press*. BOOK

Batt A. C. (1999). *Lactobacillus* Introduction. *Academic Press.*

Bacanu, G., and Oprea, L. (2013) Differences in the gut microbiota between wild and domestic acipenser ruthenus evaluated by denaturing gradient gel electrophoresis. *Romania Biotechnology Letter* 18.

Bestha L., Buddolla V. and Sai-Gopal D.V.R. (2013). Probiotics as antiviral agents in shrimp aquaculture. *Journal of Pathogens*. Article ID 424123.

Bilal, M., Si, W., Barbe, F., Chevaux, E., Sienkiewicz, O., and Zhao, X. (2021). Effects of novel probiotic strains of *Bacillus pumilus* and *Bacillus subtilis* on production, gut health, and immunity of broiler chickens raised under suboptimal conditions. *Poultry Science*. https://doi.org/10.1016/j. psj.2020.11.048

Burgos, F. A., Ray, C. L. and Arias, C. R. (2018) Bacterial diversity and community structure of the intestinal microbiome of Channel Catfish (*Ictalurus punctatus*) during ontogenesis.*Systemic and Applied Microbiology*.

Buts, J.P. (2004). Exemple d'un médicament probiotique: *Saccharomyces boulardii* lyophilisé. In: Rambaud, J.C., Buts, J.P., Corthier, G. and Flourié, B. (eds.) Flore microbienne intestinale. John Libbey Eurotext, Montrouge, France, pp. 221-244.

Carnevali, O., Avella, M.A., and Gioacchini, G. (2013). Effect of probiotic administration on zebra fish development and reproduction. *General and Comparative Endocrinology*, 188:297–302.

Castex, M., Chim, L., Pham, D., Lemaire, P., Wabete, N., Nicolas, J. L., Schmidely, P., and Mariojouls, C. (2008). Probiotic *P. acidilactici* application in shrimp *Litopenaeus stylirostris* culture subject to vibriosis in New Caledonia. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2008.01.011

Cao, G. T., Zeng, X. F., Chen, A. G., Zhou, L., Zhang, L., Xiao, Y. P., & Yang, C. M. (2013). Effects of a probiotic, *Enterococcus faecium*, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with *Escherichia coli* K88. *Poultry Science*. https://doi.org/10.3382/ps.2013-03366

Chaucheyras-Durand F., Walker, N.D. and Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Animal Feed Science and Technology*, 145:5-26.

Chaucheyras Durand F. and Durand H. (2010). Probiotics in animal nutrition and health. *Beneficial Microbes,* 1(1):3-9. DOI 10.3920/BM2008.1002

Chawla, S., Katoch, S., Sharma, K. and Sharma, V. 2013. Biological response of broiler supplemented with varying dose of direct fed microbial. *Veterinary World,* 6(8): 521–524.

Cheesbrough, M. (2006). *District laboratory practice in tropical countries*. Part 2. Cambridge University Press. New York, pp;103

Chen, B., Peng, M., Tong, W., Zhang, Q., and Song, Z. (2020a). The Quorum Quenching Bacterium *Bacillus licheniformis* T-1 protects Zebrafish against *Aeromonas hydrophila* infection. *Probiotics Antimicrob Proteins*. https://doi.org/10.1007/s12602-018-9495-7

Chen, Y. Y., Wang, Y. L., Wang, W. K., Zhang, Z. W., Si, X. M., Cao, Z. J., Li, S. L., and Yang, H. J. (2020b). Beneficial effect of *Rhodopseudomonas palustris* on in vitro rumen digestion and fermentation. *Beneficial Microbes*. https:// doi.org/ 10. 3920/ BM2019. 0044

Chloe, M., Fiona, C., Eva, L., Michael, R., Paul, W. O. and Paul, D. C. (2018). The rumen microbiome: a crucial consideration when optimizing milk and meat production and nitrogen utilisation efficiency. *Gut Microbes*. DOI:10.1080/19490976.2018.1505176

Choct M. (2009). Managing gut health through nutrition. *British Poultry Science,* 50(1):9-15.

Chythanya, R., Karunasagar, I., and Karunasagar, I. (2002). Inhibition of shrimp pathogenic vibrios by a marine Pseudomonas I-2 strain. *Aquaculture*. doi:10.1016/S0044-8486(01)00714-1

Collins F. M, Carte P., B. (1978). Growth of salmonellae in orally infected germfree mice. *Infectious Immunology,* 21:41-7.

Corcionivoschi, N., Drinceanu, D., Pop, I. M., Stack, D., Ştef, L., Julean, C., and Bourke, B. (2010). The Effect of Probiotics on Animal Health REVIEW, Scientific Papers: *Animal Science and Biotechnologies.*

Cruz M. P., Ibanez L. A., Hermosillo M. A.O. and Saad R. C. H. (2012). Use of probiotics in aquaculture- *Review*. *International Scholarly Research network*. Doi:10.5402/2012/916845

Dalmin, G., Kathiresan, K., Purushothaman, A., 2001. Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem. *Indian Journal of Experimental Biology.*

Dawood O. A. M., Koshio S.and Esteban A. M. (2017). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture*.0:1-25. Doi: 10.1111/raq.12209

Das S., Lyla P. S. and Ajmal K. S. (2006). Application of Streptomyces as a probiotic in the laboratory culture of *Penaeus monodon (Faricius). Israeli Journal of Aquaculture*. 58(3):198-204.

Das S., Mondal K. and Haque S. A. (2017). Review on application of probiotic, prebiotic and symbiotic for sustainable development of aquaculture. *Journal of Entomology and Zoology Studies,* 5(2):422-429.

Das S., Ward L. R. and Burke C. (2010). Screening for marine *Streptomyces* spp. For potential use as probiotics in aquaculture. *Aquaculture,* 305:32-41.

Dash, G., Raman, R. P., Prasad, K. P., Makesh, M., Pradeep, M.A. and Sen S. (2014). Evaluation of*Lactobacillus plantarum* as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture* 432(2014); 225-236

Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y. and Roy, D. (2008). Immunomodulatory effects of probiotics in the intestinal tract. *Current Issues in Molecular Biology*, 10:37-54.

Dermikan, E. S., Baygin, E. and Usta A. 2014. Screening of phytate hydrolysis *Bacillus* sp. isolated from soil and optimization of the certain nutritional and physical parameters on the production of phytase.*Turkish Journal of Biochemistry* 39(2):206-214

Dunham, R.A. Smitherman, R.O., Brooks, M.J., Benchakan M. and Chappell, J.A. (1982). Paternal predominance in reciprocal Channel-Blue catfish. *Aquaculture* 29:389-306.

El-Haroun E. R., Goda A.M.A.S. and Chowdhury M.A.K. (2006). Effect of dietary probiotic biogens supplementation as growth promoter on growth performance and feed utilization on Nile tilapia *Oreochromis niloticus* (L.). *Aquaculture Research,* 37:1473-1480.

Ezema C. 2013. Probiotics in animal production- A review. *Journal of Veterinary Medicine and Animal Health*. 5(11); 308-326. Doi:10.5897/jvmah2013.0201

Fajardo, P., Pastrana, L., Mendez, J., Rodriguez, I., Fucinos, C. and Guerra, N. P. 2012. Effects of feeding of two potentially probiotic preparations from lactic acid bacteria on the performance and faecal microflora of broiler chickens. *Scientific World Journal, Art. No. 562635*.

Food and Agriculture Organization/World Health Organization. 2001. Health and nutritional properties of probiotics in food includingpowder milk with live lactic acid bacteria. Cordoba, Argentina:American Cordoba Park Hotel. Rome, Italy.

FAO. *2016.* Probiotics in animal nutrition – Production, impact and regulation.*FAO Animal Production and HealthPaper*. No. *179.*

Fuller R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, 66(5):365-367.

Gatesoupe F. J. (1999). The use of probiotics in aquaculture. *Aquaculture,* 180(2):147-165.

Ghosh S., Sinha A., and Sahu C. (2007). Effect of probiotics on reproductive performance in female live bearing ornamental fish. *Aquaculture Research*, 38(5):518–526.

Giri, S. S., Sukumaran, V., and Oviya, M. (2013). Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish Shellfish Immunology*. doi:10.1016/j.fsi.2012.12.008

Gomes, L. C., Brinn, R. P., Marcon, J. L., Dantas, L. A., Brandao, F. R., De Abreu, J. S., Lemos, P. E. M., McComb, D. M., and Baldisserotto, B. (2009). Benefits of using the probiotic Efinol®L during transportation of cardinal tetra, *Paracheirodon axelrodi* (Schultz), in the Amazon. *Aquaculture Research*. doi:10.1111/j.1365-2109.2008.02077.x

Gomez-Gil B., Roque A. and Turnbull J. F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms, *Aquaculture,* 191:259–270.

Gunasundari, V., Ajith Kumar, T.T., Ghosh, S., Kumaresan, S., 2013. An ex vivo loom to evaluate the brewer's yeast Saccharomyces cerevisiae in clownfish aquaculture with special reference to Amphiprion percula (Lacepede, 1802). *Turkish Journal of Fish and Aquatic Science*.doi: 10.4194/1303-2712-v13_3_01

Hagi T. and Hoshino T. (2009). Screening and characterization of potential potential probiotic lactic acid bacteria from culture common carp intestine. *Bioscience, Biotechnology and Biochemistry,* 73(7):1479-1483.

Hai, N. V., Buller, N., and Fotedar, R. (2009a). Effects of probiotics (*pseudomonas synxantha* and *pseudomonas aeruginosa*) on the growth, survival and immune parameters of juvenile western king prawns (*penaeus latisulcatus kishinouye*, 1896). *Aquaculture Research*. doi:10.1111/j.1365-2109.2008.02135.x

Hanning I. and Daiz-Sanchez S. (2015). The functionality of the gastrointestinal microbiome in non-human animals. A review. *Microbiome.* 3(2015):51-62. DOI 10.1186/s40168-015-0113-6

Hardy, H., Harris, J., Lyon, E., Beal, J., and Foey, A. D. (2013). Probiotics, prebiotics and immunomodulation of gut mucosal defences: Homeostasis and immunopathology. *Nutrients*. https://doi.org/10.3390/ nu506 1869

Hashemzadeh, F., Rahimi, S., Amir, M., Torshizi, K., Akbar, A., Torshizi, M.A.K. and Akbar, A., (2013). Effects of Probiotics and Antibiotic Supplementation on Serum Biochemistry and Intestinal Microflora in Broiler Chicks. *International Journal of Agriculture and Crop Science.*

Hemaiswarya S, Raja R, Ravikumar R, Carvalho I. S. (2013). Mechanism of Action of Probiotics. *Brazilian Archives of Biology and Technology*, 56:113-119.

Hong, H. A., Le, H. D., and Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews*. doi:10.1016/j.femsre.2004.12.001

https://www.fisheriessciences.com/fisheries-aqua/assessment-of-gametes-in-tilapia-oreochromis-niloticus-effects-of-body-weight-in-a-new-lineage.php?aid=9103

Huddy, R. J., and Coyne, V. E. (2015). Characterization of the role of an alkaline protease from *Vibrio midae* SY9 in enhancing the growth rate of cultured abalone fed a probiotic-supplemented feed. *Aquaculture*. doi:10.1016/j.aquaculture.2015.05.048

Humphreys G.J. and McBain A.J. (2019). Antagonistic effects of Streptococcus and Lactobacillus probiotics in pharyngeal biofilms. *Letters in Applied Microbiology* 68, 303-312 doi:10.1111/lam.13133

Hung, A.T., Lin, S.-Y., Yang, T.-Y., Chou, C.-K., Liu, H.-C., Lu, J.-J., Wang, B., Chen, S.-Y. and Lien, T.-F. 2012. Effects of *Bacillus coagulans* ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens. *Animal Production Science,* 52(9): 874–879.

Irfan S. Y., Olcay H., Sevdan Y., and Murat Y. (2015). Effects of different probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss* under sublethal water temperature. *Marine science and technology bulletin*, 4(2):21-28.

Irianto A. and Austin B. (2002). Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 25:333-342.

Iriaton A. and Austin B. (2002). Probiotics in aquaculture. Journal *of Fish Diseases,* 25(11):633-642.

Iribarren D., Daga P., Moreira M. T. and Feijoo G. (2012). Potential environmental effects of probiotics used in aquaculture. *Aquaculture Internaltional*, 20(4):779-789.

Jin, L. Z., Ho, Y. W., Abdullah, N., Ali, M. A., and Jalaludin, S. (1996). Antagonistic effects of intestinal Lactobacillus isolates on pathogens of chicken. *Letters in Applied Microbiology*. doi:10.1111/j.1472-765X.1996.tb00032.x

Johnson-Henry, K.C., Donato, K.A., Dhen-Tu, G., Gordanpour, M. and Sherman, P. M. (2008). *Lactobacillus rhamnosus* strain GG prevents enterohemorragic *Escherichia coli* O157:H7-induced changes in epithelial barrier function. *Infection and Immunity,* 76:1340-1348.

Kapsenberg, M. L. (2003). Dendritic-cell control of pathogen-driven T-cell polarization. *Nature Reviews Immunology*. doi:10.1038/nri1246

Kesarcodi-Watson A., Kaspar H., Lategan M. J. and Gibson L. (2008). Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture*, 274(1):1-14.

Kewcharoen, W., Srisapoome, P., 2019. Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei)* on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). *Fish Shellfish Immunology*. doi:10.1016/j.fsi.2019.09.013

Khalafalla, M. M., Ibrahim, A. S., Zayed M. M., Awad N. M. and Mohamed A. R. (2020). Effect of dietary mixture of beneficial bacteria on growth performance, health condition, chemical composition and water quality of Nile Tilapia, *Oreochromis niloticus* fingerlings. *Journal of Aquatic Food Product Technology*. DOI:10.1080/10498850.2020.1764685

Khalid, A. H., Ullah, K. S., Naveed, S., Latif, F., Pasha, T. N., Hussain, I., and Qaisrani, S. N. (2021). Effects of spray dried yeast (Saccharomyces cerevisiae) on growth performance and carcass characteristics, gut health, cecal microbiota profile and apparent ileal digestibility of protein, amino acids and energy in broilers. *Tropical Animal Health and Production*. https:// doi. org/ 10. 1007/ s11250- 021- 02684-5

Kim D. H., Brunt J, and Austin B. (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology*, 102:1654-1664.

Kong, Y., Gao, C., Du, X., Zhao, J., Li, M., Shan, X., and Wang, G. (2020). Effects of single or conjoint administration of lactic acid bacteria as potential probiotics on growth, immune response and disease resistance of snakehead fish (Channa argus). *Fish and Shellfish Immunology*. https://doi.org/10.1016/j.fsi.2020.05.003

Krehbiel C. R. Invited Review: applied nutrition of ruminants: fermentation and digestive physiology. (2014). *The Professional Animal Scientist*, 30(2):129–139.

Kroger M., Kurmann J. A., Raic J.L. (1989). Fermented milks – past and present. *Food Technology*, 43:92-99.

Kuebutornye, F. K. A., Abarike, E. D., Lu, Y., Hlordzi, V., Sakyi, M. E., Afriyie, G., Wang, Z., Li, Y., and Xie, C. X. (2020). Mechanisms and the role of probiotic *Bacillus* in mitigating fish pathogens in aquaculture. *Fish Physiology and Biochemistry*. doi:10.1007/s10695-019-00754-y

La Ragione, R.M. and Woodward, M.J., (2003). Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype enteritidis and *Clostridium perfringens* in young chickens. *Veterinary Microbiology*, 94:245-256.

La Ragione, R.M., Narbad, A., Gasson, M. J. and Woodward, M. J., (2004). *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. *Letters in Applied Microbiology*, 38:197-205.

Langenkamp, A., Messi, M., Lanzavecchia, A., and Sallusto, F. (2000). Kinetics of dendritic cell activation: Impact on priming of TH1, TH2 and nonpolarized T cells. *Nature Immunology*. doi:10.1038/79758

Lara-Flores, M., Olvera-Novoa, M. A., Guzman-Mendez, B. E., and Lopez-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*. doi:10.1016/ S0044-8486(02)00277-6

Leonel Ochoa-Solano, J., and Olmos-Soto, J. (2006). The functional property of *Bacillus* for shrimp feeds. *Food Microbiology*. doi:10.1016/j.fm.2005.10.004

Leyva-Madrigal, K. Y., Luna-Gonzalez, A., Escobedo-Bonilla, C. M., Fierro-Coronado, J. A., and Maldonado-Mendoza, I. E. (2011). Screening for potential probiotic bacteria to reduce prevalence of WSSV and IHHNV in whiteleg shrimp (*Litopenaeus vannamei*) under experimental conditions. *Aquaculture*. doi:10.1016/j.aquaculture.2011.09.033

Li, P., Gatlin, D.M., (2004). Dietary brewer's yeast and the prebiotic Grobiotic^{TM} AE influence growth performance, immune responses and resistance of hybrid striped bass (Morone chrysops x M. saxatilis) to Streptococcus iniae infection. *Aquaculture*. doi:10.1016/j.aquaculture.2003.08.021

Li, P., Gatlin, D.M., (2005). Evaluation of the prebiotic GroBiotic®-A and brewer's yeast as dietary supplements for sub-adult hybrid striped bass (Morone chrysops x M. saxatilis) challenged in situ with Mycobacterium marinum. *Aquaculture*. doi:10.1016/j.aquaculture.2005.03.005

Liao, S. F., and Nyachoti, M. (2017). *Using probiotics to improve swine gut health and nutrient utilization*. Anim: Nutr. https://doi.org/10.1016/j.aninu.2017.06.007

Llewellyn, M.S., Boutin, S., Hoseinifar, S.H., Derome, N. (2014) Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology*, 5, 207.

Luongo, D., Miyamoto, J., Bergamo, P., Nazzaro, F., Baruzzi, F., Sashihara, T., Tanabe, S., and Rossi, M. (2013). Differential modulation of innate immunity in vitro by probiotic strains of *Lactobacillus gasseri*. *BMC Microbiology*. https://doi.org/10.1186/1471-2180-13-298

Maas, R. M., Verdegem, M. C. J., Debnath, S., Marchal, L., and Schrama, J. W. (2021). Effect of enzymes (phytase and xylanase), probiotics (*B. amyloliquefaciens*) and their combination on growth performance and nutrient utilisation in *Nile tilapia*. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.736226

Maki, L. R. and Picard K. (1965) Normal intestinal flora of cattle fed high roughage rations. *Journal of Bacteriology*,89(5)

Makkar P.S. H. (2016). Animal nutrition; beyond the boundaries of feed and feeding. *Broadening Horizons.* www.feedipedia.org

Makkar, H.P.S. and Beever, D. (2013). Optimization of feed use efficiency in ruminant production systems. Proceedings of the FAO Symposium, 27 November 2012, Bangkok, Thailand. *FAO Animal Production and Health Proceedings*, No. 16. Rome, FAO and Asian-Australasian Association of Animal Production Societies. http://www.fao.org/docrep/018/i3331e/i3331e.pdf).

Marden, J. P., Julien, C, Monteils, V., Auclair, E., Moncoulon, R. and Bayourthe, C. (2008). How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? *Journal of Dairy Science,* 91:3528-3535.

Mellman, I., and Steinman, R. M. (2001). Dendritic cells: Specialized and regulated antigen processing machines. *Cell*. doi:10.1016/S0092-8674(01)00449-4

Midhun, S. J., Neethu, S., Arun, D., Vysakh, A., Divya, L., Radhakrishnan, E. K., and Jyothis, M. (2019). Dietary supplementation of *Bacillus licheniformis* HGA8B improves growth parameters, enzymatic profile and gene expression of *Oreochromis niloticus*. *Aquaculture*. doi:10.1016/j.aquaculture.2019.02.064

Mo, W. Y., Choi, W. M., Man, K. Y., and Wong, M. H. (2020). Food waste-based pellets for feeding grass carp (*Ctenopharyngodon idellus*): Adding baker's yeast and enzymes to enhance growth and immunity. *Science of the Total Environment*. doi:10.1016/j.scitotenv.2019.134954

Mohanty S. K.,Tripathi, S. D., and Swai, S. N. (1996). Rearing of catla (*Catla catla* Ham.) spawn on formulated diets. *Journal of Aquaculture in the Tropics.*

Molina A. P. J, Miranda S. A., Gonzalez L. A., Diaz M. F. S., and Contreras R. M. (2009) *Aquaculture Research*. 40:887-894. Doi:10.1111/j.1365-2109.2009.02172.x

Mookiah, S., Sieo, C. C., Ramasamy, K., Abdullah, N., and Ho, Y. W. (2014). Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *Journal of the Science of Food and Agriculture*. doi:10.1002/jsfa.6365

Moriarty, D. J. W. (1998). Control of luminous Vibrio species in penaeid aquaculture ponds. *Aquaculture*. doi:10.1016/S0044-8486(98)00199-9

Mountzouris, K. C., Balaskas, C., Xanthakos, I., Tzivinikou, A., and Fegeros, K. (2009). Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with Salmonella enteritidis. *British Poultry Science*. https:// doi. org/ 10.1080/ 000716609031109 35

Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., and Fegeros, K. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*. doi:10.3382/ps.2009-00308

Mountzouris, K. C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G. and Fegeros, K. 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science,* 86(2): 309–317.

Munro P. D., Barbour A. and Birkbeck T. H. (1995). Comparison of the growth and survival of larval turbot in the absence of culturable bacteria with those in the presence of *Vibrio anguillarum, Vibrio alginolyticus*, or a marine *Aeromonas* sp. *Applied and Environmental Microbiology*, 61:4425–4428

Nagpal R., Kumar A., Kumar M., Behare P. V., Jain S., and Yadav H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. *Federation of European Microbilogical Society,* 334:1-15

Nakano T. (2007). Microorganism. En dietary supplements for the health and quality of culture fish. *Centre for Agriculture and Bioscience international.*

Nargesi E. A., Falahatkar B. and Sajjadi1 M. M. (2019). Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. *Aquaculture Nutrition,* 2019;00:1–11.DOI: 10.1111/anu.12970

Nayak S. K. (2010). Probiotics and immunity: A fish perspective: A Review. *Fish and Shellfish Immunology,* 29:2-14. Doi:10.1016/j.fsi.2010.02.017

Newbold, C. J., Wallace, R. J. and McIntosh, F. M. (1996). Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *British Journal of Nutrition,* 76:249-261.

Niba, A., Beal, J., Kudi, A. and Brooks, P. 2009. Bacterial fermentation in the gastro-intestinal tract of non-ruminants: influence of fermented feeds and fermentable carbohydrates. *Tropical Animal Health and Production,* 41(7): 1393–1407.

Noor, Z., Noor, M., Khan, I., and Khan, S. A. (2020). Evaluating the lucrative role of probiotics in the aquaculture using microscopic and biochemical techniques. *Microscopy Research and Technique,* 83:310–317.
doi: 10.1002/jemt.23416

Olmos, J., Acosta, M., Mendoza, G., and Pitones, V. (2020). *Bacillus subtilis*, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution. *Archives of Microbiology,* 202:427–435. doi:10.1007/s00203-019-01757-2

Panigrahi, A. Kiron, V., Puangkaew, J., Kobayashi, T., Satoh, S. and Sugita H. (2005). The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 243(2005) 241-254

Pannu R., Dahiya S., Sabholk V. P., Kumar D., Sarsar V. and Gahlawat S. K. (2014). Effect of probiotic, antibiotic and herbal extracts against fish bacterial pathogens. *Ecotoxicology, Environment and Contamination*, 16(1):13-20.

Park, S. C., Shimamura, I., Fukunaga, M., Mori, K. I., and Nakai, T. (2000). Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. *Applied and Environment Microbiology*. https://doi.org/10. 1128/AEM. 66.4.1416-1422.2000

Paulino S. M., Veras C. G., Felizardo O. V., Solis-Murgas D. L. and Freitas R.T.F. (2016). Assessment of gametes in tilapia *Oreochromis niloticus*: effects of body weight in a new lineage, *Journal of Fisheries Sciences.*

Perez-Cano, F. J., Dong, H., and Yaqoob, P. (2010). In vitro immunomodulatory activity of *Lactobacillus fermentum* CECT5716 and *Lactobacillus salivarius* CECT5713: Two probiotic strains isolated from human breast milk. *Immunobiology*, https://doi.org/10.1016/j.imbio.2010.01.004

Piamsomboon P., Mehl S. N., Sirivaidyapong S. and Wongtavatchai J. (2019). Assisted reproduction in Nile tilapia Oreochromis niloticus: Milt preservation, spawning induction and artificial fertilization. *Aquaculture*, (507):*139–143.* doi:10.1016/j.aquaculture.2019.04.019

Perdigon, G., Nader de Maclas, M. E., Alvarez, S., Oliver, G., and Pesce de Rulz Holgado A. A. (1987). Enhancement of Immune Response in Mice Fed with *Streptococcus thermophilus* and *Lactobacillus acidophilus*. *Journal of Dairy Science70*:919-926.

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., and Gil, A. (2019). Mechanisms of action of probiotics. *Advances in Nutrition,* 10**,** 549–566. doi:10.1093/advances/nmy063

Putra, N.A., Syamsunarno, B. M., Ningrum, W. and Mustahal J. (2020). Effect of the administration of probiotic *Bacillus* NP5 in the rearing media on water quality, growth, and disease resistance of African catfish (*Clarias gariepinus*). *Biodiversitas*, 21(6):2566-2575. DOI:10.13057/biodiv/d210629

Punetha, M., Roy, A. K., Ajithakumar, H. M., Para, I. A., Gupta, D., Singh, M., and Bharati, J. (2018). Immunomodulatory effects of probiotics and prilled fat supplementation on immune genes expression and lymphocyte proliferation of transition stage Karan Fries cows. *Vet World*. https://doi.org/10.14202/vetworld.2018.209-214

Raja S., Nandhin, E., Sahana K. and Dhanakkodi B. (2015). Benefitial and destructive effects of probiotics in aquaculture systems- A review. *International Journal of Fisheries and Aquatic Studies*, 2(3):153-159.

Rahman *et al.,* (2009). Enterococcus [Characterization of clinical bacteria and their control by a medical plant (Cassia sophera) L extract]

Ramesh, D., Vinothkanna, A., Rai, A. K., and Vignesh, V. S. (2015). Isolation of potential probiotic spp and assessment of their subcellular components to induce immune responses in *Labeo rohita* against *Aeromonas hydrophila*. *Fish and Shellfish Immunology*. doi:10.1016/j.fsi.2015.04.018

Rizzo, A., Fiorentino, M., Buommino, E., Donnarumma, G., Losacco, A., and Bevilacqua,N. (2015). *Lactobacillus crispatus* mediates anti-inflammatory cytokine interleukin-10 induction in response to *Chlamydia trachomatis* infection in vitro. *International Journal of Medical Microbiology*. https://doi.org/10.1016/j.ijmm.2015.07.005

Robertson, P. A. W., Odowd, C., Williams P., and Austin, B. (2000). Use of *Carnobacterium* sp. as a probiotic for Atlantic salmo (*Salmo salar*) and rainbow trout *Oncorhyncus mykiss* (Walbaum). *Aquaculture*, 185:235-243.

Roeselers, G., Mittge, E.K., Stephens, W.Z., Parichy, D.M., Cavanaugh, C.M.,Guillemin, K., Rawls, J.F. (2011) Evidence for a core gut microbiota in the zebrafish. ISME J. 5, 1595–1608.

Russo, P., Arena, M. P., Fiocco, D., Capozzi, V., Drider, D., and Spano, G. (2017). *Lactobacillus plantarum* with broad antifungal activity: A promising approach to increase safety and shelf-life of cereal-based products. *International Journal of Food Microbiology*. https://doi.org/10.1016/j.ijfoodmicro.2016.04.027

Sahu M. K., Swarnakumar N. S., Sivakumar K., Thangaradjou T. and Kannan L. (2008). Probiotics in aquaculture: importance and future perspectives. *Indian Journal of Microbiology*, 48:299-308.

Salobir J., Frankic T., and Rezar V. (2012). Animal nutrition for the health of animals, human and environment. *20th International Symposium*. "Animal Science Days", Kranjska gora, Slovenia.

Sato, K., Takahashi, K., Tohno, M., Miura, Y., Kamada, T., Ikegami, S. and Kitazawa, H. 2009. Immunomodulation in gut-associated lymphoid tissue of neonatal chicks by immunobiotic diets. *Poultry Science*, 88(12): 2532–2538.

Sayes, C., Leyton, Y. and Riquelme, C., 2018. Probiotic bacteria as a healthy alternative for fish aquaculture. *Antibiotic Use in Animals*. http://dx.doi.org/10.5772/intechopen.71206

Servin, A. L., (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiology Reviews*, 28: 405-440.

Sharma, A. N., Kumar, S., and Tyagi, A. K. (2018). Effects of mannan-oligosaccharides and Lactobacillus acidophilus supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *Journal of Animal Physiology and Animal Nutrition (berlin), 102*, 679–689. https://doi.org/10.1111/jpn.12878

Shim, Y. H., Ingale, S. L., Kim, J. S., Kim, K. H., Seo, D. K., Lee, S. C., Chae, B. J., and Kwon, I. K. (2012). A multi-microbe probiotic formulation processed at low and high drying temperatures: Effects on growth performance, nutrient retention and caecal microbiology of broilers. *British Poultry Science*. doi:10.1080/00071668.2012.690508

Sitorus H., EL Ramija K. and Akhir D. I. S. (2018). Effect of probiotic em-4 in feeds on the growth and survival rate of goldfish (*Carrasius auratus*). *International Journal of Fisheries and Aquatic Studies*, 6(3):108-111.

Sizar O. and Unakal C. G. (2021). Gram positive bacteria. *Statpearl* (internet)

Skjermo, J., and Vadstein, O. (1999). Techniques for microbial control in the intensive rearing of marine larvae. *Aquaculture*. doi:10.1016/S0044-8486(99)00096-4

Skrodenyte-Arbaciauskiene V., Sruoga A. and Butkauskas D. (2006). Assessment of microbial diversity in the river trout *Salmo trutta fario* L. intestinal tract identified by partial 16S rRNA gene sequence analysis. *Fisheries Science*, 72:597-602.

Soltani, M., Ghosh, K., Hoseinifar, S. H., Kumar, V., Lymbery, A. J., Roy, S., and Ringo, E. (2019). Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Review of Fish Science and Aquaculture*. doi:10.1080/23308 249.2019.15970 10

Suchodolski S. J., Markel E. M., Garcia-Mazcorro F. J., Unterer S., Heilmann M. R., Dowd E. S., Kachroo P., Ivanov I., Minamoto Y., Dillman M. E., Steiner M. J., Cook K. A., Toresson L. (2012). The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease, *Public Library of Science*, 7(2012). doi:10.1371/journal.pone.0051907

Sun, K., Xie, C., Xu, D., Yang, X., Tang, J., and Ji, X. (2013). Lactobacillus isolates from healthy volunteers exert immunomodulatory effects on activated peripheral blood mononuclear cells. *Journal of Biomedical Research*. https://doi.org/10.7555/JBR.27.20120074

Surendran Nair, M., Amalaradjou, M. A., and Venkitanarayanan, K. (2017). Antivirulence properties of probiotics in combating microbial pathogenesis. *Advances in Applied Microbiology*. https:// doi.org/10.1016/bs.aambs.2016.12.001

Sypestyn, A. K. Cellulose decomposing bacteria from the rumen of cattle. *Antonie van leeuwenhoek*, 15:49-52.

Taoka, Y., Maeda, H., Jo, J. Y., Jeon, M. J., Bai, S. C., Lee, W. J., Yuge, K., and Koshio, S. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. *Fisheries Science*. doi:10.1111/j.1444-2906.2006.01152.x

Tave D. (1986) Genetics for Fish Hatchery Managers. AVI Publishing Co. Inc. Westport Connecticut, 299 pp

Terada, T., Nii, T., Isobe, N., and Yoshimura, Y. (2020). Effects of probiotics *Lactobacillus reuteri* and *Clostridium butyricum* on the expression of toll-like receptors, pro- and anti-inflammatory cytokines, and antimicrobial peptides in broiler chick intestine. *The Journal of Poultry Science*. https://doi.org/10.2141/jpsa.0190098

Umaru, J. and Agbugui, M. O. (2013). Influence of Antox probiotic as water additive on growth performance, nutrient utilisation and body composition of the African catfish, *Clarias garienpinus* (Burchel, 1822) fingerlings. *Bayero Journal of Pure and Applied Sciences*. 14(2):178-183.

Van Doan, H., Hoseinifar, S. H., Tapingkae, W., Seel-audom, M., Jaturasitha, S., Dawood, M. A. O., Wongmaneeprateep, S., Thu, T. T. N., and Esteban, M. A. (2020). Boosted growth performance, mucosal and serum immunity, and disease resistance Nile Tilapia (*Oreochromis niloticus*) Fingerlings Using Corncob-Derived Xylooligosaccharide and *Lactobacillus plantarum* CR1T5. *Probiotics Antimicrobial Proteins*. doi:10.1007/s12602-019-09554-5

Van Hai, N., Buller, N., and Fotedar, R. (2009b). The use of customised probiotics in the cultivation of western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). *Fish and Shellfish Immunology*. doi:10.1016/j.fsi.2009.05.004

Van Hai, N., Buller, N., and Fotedar, R. (2010). Effect of customized probiotics on the physiological and immunological responses of juvenile western king prawns (*Penaeus latisulcatus* Kishinouye, 1896) challenged with *Vibrio harveyi*. *Journal of Applied Aquaculture*. doi:10.1080/10454 438.2010.527580

Van Zyl, W. F., Deane, S. M., and Dicks, L. M. T. (2020). Molecular insights into probiotic mechanisms of action employed against intestinal pathogenic bacteria. *Gut Microbes*. doi:10.1080/19490976.2020.1831339

Verschuere L., Rombaut G., Sorgeloos P. and Verstraete W. (2000). Probiotic bacteria as biological control agents in aquaculture: A Review. *Microbiology and Molecular Biology*, 64(4):655-671.

Waiyamitra, P., Zoral, M. A., Saengtienchai, A., Luengnaruemitchai, A., Decamp, O., Gorgoglione, B., and Surachetpong, W. (2020). Probiotics modulate tilapia resistance and immune response against tilapia lake virus infection. *Pathogens*, 9: 1–15. doi:10.3390/pathogens9110919

Wang B. Y., Li R. J. and Lin J. (2008). Probiotics in aquaculture: challenges and outlook. *Aquaculture*, 281:1-4.

Wang, C., Chang, T., Yang, H., and Cui, M. (2015). Antibacterial mechanism of lactic acid on physiological and morphological properties of *Salmonella enteritidis*, *Escherichia coli* and *Listeria monocytogenes*. *Food Control*. https://doi.org/10.1016/j.foodcont.2014.06.034

Wang, R., Guo, Z., Tang, Y., Kuang, J., Duan, Y., Lin, H., Jiang, S., Shu, H., and Huang, J. (2020). Effects on development and microbial community of shrimp *Litopenaeus vannamei* larvae with probiotics treatment. *AMB Express*. doi:10.1186/s13568-020-01041-3

Wang, Y. B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*. doi:10.1016/j.aquaculture.2007.05.035

Wang, Y. B., Xu, Z. R., and Xia, M. S. (2005). The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. *Fisheries Science*. doi:10.1111/j.1444-2906.2005.01061.x

Wang B. Y., Li R. J. and Lin J. 2008. Probiotics in aquaculture: challenges and outlook. *Aquaculture*. 281(1-4):1-4

Wang, Y., and He, Z. (2009). Effect of probiotics on alkaline phosphatase activity and nutrient level in sediment of shrimp, *Penaeus vannamei*, ponds. *Aquaculture*. doi:10.1016/j.aquaculture.2008.10.022

Wilssens, A., and Buttiaux R. (1958). Les bacteries dela flore fecale de la vache saine. *Ann Inst. Pasteur,* 94:332-340

World Health Organization (WHO), (2017). Who guidelines on use of medically important antimicrobials in food-producing animals- Policy brief.

Wu, R., Shen, J., Tian, D., Yu, J., He, T., Yi, J., and Li, Y. (2020). A potential alternative to traditional antibiotics in aquaculture: Yeast glycoprotein exhibits antimicrobial effect in vivo and in vitro on Aeromonas caviae isolated from *Carassius auratusgibelio*. *Veterinary Medicine and Science*. doi:10.1002/vms3.253

Yazici S. I., Hisar, O., Yilmaz, S. and Yigit M. (2015). Effects of differenct probiotic bacteria on growth, body composition, immune response and hematological parameters of rainbow trout (*Oncorhynchus mykiss*) under sublethal water temperature. *Marine Science and Technology Bulletin*,4(2):21-28

Yang, C., Cao, G., Ferket, P., Liu, T., Zhou, L., Zhang, L., Xiao, Y. & Chen, A. 2012a. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and caecal microflora in broiler chickens. *Poultry Science*, 91(9): 2121-2129.

Yang, H., Liu, Y., Xu, S., Li, Y. & Xu, Y. 2012b. Influence of symbiotics on the bacterial community in the caecal contents of broilers analysed by PCR-DGGE. *Acta Agri. Zhejiang*, 24(1): 26–31.

Yeoman J. C. and White A. B. (2014). Gastrointestinal tract microbiota and probiotics in production animals. *Annual Review of Animal Biosciences*, 2:469–486.

Yitbarek B. M., Mersso T. B., Wosen M. A. (2016). Effect of Dried Blood-Rumen Content Mixture (DBRCM) on feed intake, body weight gain, feed conversion ratio and mortality rate of SASSO C44 broiler chicks. *Journal of Livestock Science*, 7:139-149.

Zhang, B., Yang, X., Guo, Y. and Long, F. 2011. Effects of dietary lipids and *Clostridium butyricum* on the performance and the digestive tract of broiler chickens. *Archives of Animal Nutrition*, 65(4): 329–339.

Zhang, C. N., Zhang, J. L., Guan, W. C., Zhang, X. F., Guan, S. H., Zeng, Q. H., Cheng, G. F., and Cui, W. (2017). Effects of *Lactobacillus delbrueckii* on immune response, disease resistance against *Aeromonas hydrophila*, antioxidant capability and growth performance of *Cyprinus carpio* Huanghe var. *Fish and Shellfish Immunology*. https://doi.org/10.1016/j.fsi.2017.07.012

Zhang, Z. and Kim, I. 2014. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry Science*, 93(2): 364–370.

Zilberg, D., Tal, A., Froyman, N., Abutbul, S., Dudai, N. and Golan- Goldhirsh A. (2010). Dried leaves of *Rosmarinus officinalis* as a treatment for streotococcosis in tilapia. *Journal of Fish Diseases*, 33:361-369.

Zhitnitsky, D., Rose, J., and Lewinson, O. (2017). The highly synergistic, broad spectrum, antibacterial activity of organic acids and transition metals. *Science Reports*. https://doi.org/10.1038/srep44554

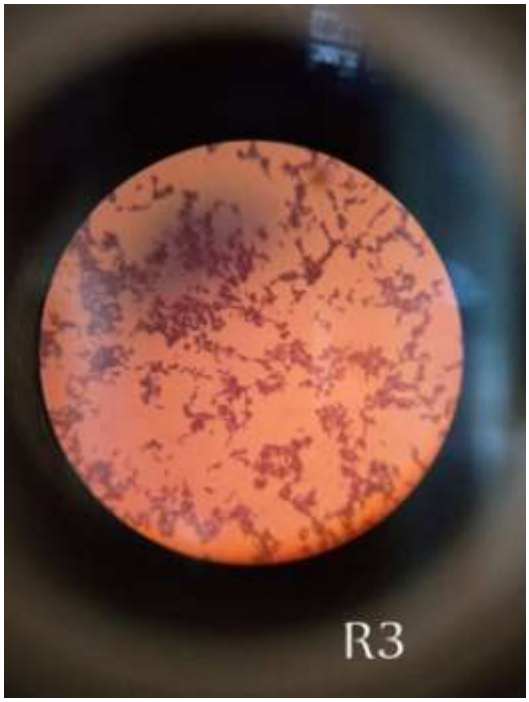
APPENDICIES

Appendix I: Breeding indices of broodstock

	Fertilization time	Latency period	Hatching time	Incubation period	% fertilization	% hatchability
C. *gariepinus*♀ × C. *galmaensis*♂ (cross)	8:55AM	11 hrs	2:00AM	17hrs	85%	90%
C. gariepinus (Pure)	8:40 AM	11 hrs	1:47AM	17hrs	95%	97%

Appendix II: C. *galmaensis* broodstock

Appendix III: Microscopic view of probiotic isolate RT3



Appendix IV: Methyl red test showing negative reactionof bacterial isolates of hybrid gut

Appendix V: Voges Prokauer test showing positive reaction of bacterial isolates of hybrid gut

Appendix VI: Indole, motility and sulphate Test of gut bacterial isolates

Appendix VII: Triple Suger Iron and Urease tests of gut bacterial isolates

Appendix VIII: Bacterial Isolate from gut of fry showing positive reaction

Appendix IX: Hybrid fry