



***EVALUATION OF GROWTH PERFORMANCE OF JUVENILE AFRICAN  
CATFISH [*Clarias gariepinus* (Burchell, 1822)] FED WITH STRIPED  
CATFISH [*Pangasius hypophthalmus* (Sauvage, 1878)] VISCERA AND  
PUTATIVE PROBIOTIC ISOLATE***

**SITI NADIA BINTI ABU BAKAR**

**FPV 2019 6**



**EVALUATION OF GROWTH PERFORMANCE OF JUVENILE AFRICAN  
CATFISH [*Clarias gariepinus* (Burchell, 1822)] FED WITH STRIPED CATFISH  
[*Pangasius hypophthalmus* (Sauvage, 1878)] VISCERA AND PUTATIVE  
PROBIOTIC ISOLATE**

By

**SITI NADIA BINTI ABU BAKAR**

**Thesis submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Master of Science**

**October 2018**

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**EVALUATION OF GROWTH PERFORMANCE OF JUVENILE AFRICAN CATFISH [*Clarias gariepinus* (Burchell, 1822)] FED WITH STRIPED CATFISH [*Pangasius hypophthalmus* (Sauvage, 1878)] VISCERA AND PUTATIVE PROBIOTIC ISOLATE**

By

**SITI NADIA BINTI ABU BAKAR**

**October 2018**

**Chairman : Assoc. Prof. Hassan Bin Hj Mohd Daud, PhD**  
**Faculty : Veterinary Medicine**

Post-harvest processing of fresh striped catfish (*Pangasius hypophthalmus*) has resulted in large volume of waste by-products such as viscera. Since it is not consumed by humans, utilization of discarded fish parts especially viscera may protect environment from the risk of disposal-related health problems and environmental pollution. Current research focus on the efficacy of utilizing discarded striped catfish (*Pangasius hypophthalmus*) viscera as feed ingredient to improve the growth and survival of juvenile African catfish (*Clarias gariepinus*). The discarded viscera were dried at 105°C prior for nutrient content identification. The proximate analysis of *P. hypophthalmus* dried viscera shows that it contains  $16.37 \pm 0.81\%$  of crude protein,  $48.1 \pm 10.77\%$  crude lipid,  $67.53 \pm 1.86\%$  of moisture and  $0.2 \pm 0.06\%$  of ash respectively. The indigenous putative probiont in the viscera were isolated and analysed based on its physiological and biochemical properties, adhesiveness, antibacterial activity and identified molecularly. The selected probiont were identified molecularly as *Weissella confusa* strain and possessed an outstanding performance from the identification of properties in the laboratory. It was found out to grow excellence in temperature ranged from 25 °C to 35 °C, salinity 0% until 3.5%, multiplied at pH 4, 6, 8 and 10, able tolerate to 0.15% and 0.30% presence of bile salts as well as possessed a good adhesive ability. Furthermore, current isolates shows a high inhibition zone towards some aquatic pathogenic bacteria tested; *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Vibrio harveyi* and *Vibrio parahaemolyticus*. The isolates also were found resistant to Vancomycin (30 µg) and Amoxilin (10 µg) while susceptible to Streptomycin (10 µg), Vancomycin (5 µg) and Ampicilin (10 µg) antibiotic disk. The suitability of dried fish viscera as feed ingredient in African catfish (initial size

2.71 ± 0.14 gm) were tested through feeding diet according to three different inclusion levels (10%, 20% and 30%) and it was found out that feed contained 20% of dried viscera yielded highest growth rate thus chosen for next feeding trial experiment. The efficacy of probionts isolate as well as dried viscera were tested to juveniles African catfish in 40 days of feeding trial according to four treatments; (T1) Commercial feed; (T2) Mix of commercial feed with dried viscera (T3) Mix of commercial feed with putative probiont isolated (T4) Mix of commercial feed with dried viscera and isolated probiont. Findings from feeding trial proven that T3 shows significantly high growth performance ( $P < 0.05$ ) in term of body weight, body weight gain, average daily gain and specific growth rate in compare to T1 but exhibited no significant difference ( $P < 0.05$ ) with T2 and T4. Feed intake and feed conversion ratio were not significantly difference ( $P > 0.05$ ) among all treatment but numerical value presented show high feed intake in group of fish fed probiotic T3. Survival rate of all experimental fishes were not significant difference ( $P > 0.05$ ) affected each other but relatively highest in T2 followed by T3, T4 and numerically lowest in T1. Further haematological assessment carried out proven that T3 shows better performance ( $P < 0.05$ ) in term of haemoglobin concentration, mean corpuscular volume and white blood cell count in compare with other treatment. Histological assessment made on intestinal epithelium height of villi between four treatments also proven that T3 shows significantly longer length ( $P < 0.05$ ) of villi in compare to T1, T2 and T4. Hence, African catfish diet composed of 20% *P. hypophthalmus* dried viscera or composed of putative probiont suspension from *P. hypophthalmus* mid-gut intestine at dosage of  $10^9$  cfu/ml diet was recommended to promote *Clarias gariepinus* growth performance.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENILAIAN KADAR PERTUMBUHAN KELI AFRIKA JUVANA [*Clarias gariepinus* (Burchell, 1822)] YANG DIBERI MAKAN VISERA IKAN PATIN [*Pangasius hypophthalmus* (Sauvage,1878)] DAN PUTATIF PROBIOTIK ASINGAN**

Oleh

**SITI NADIA BINTI ABU BAKAR**

**Oktober 2018**

**Pengerusi : Prof. Madya Dr Hassan Bin Hj Mohd Daud, PhD**  
**Fakulti : Perubatan Veterinar**

Ikan patin (*Pangasius hypophthalmus*) segar yang telah dipilih dan diproses telah menghasilkan jumlah sampingan ikan yang tidak digunakan di dalam skala besar seperti visera. Oleh kerana visera ikan ini tidak dimakan oleh manusia, penggunaan bahagian ikan yang dibuang terutamanya visera dapat melindungi alam sekitar daripada risiko masalah pencemaran. Penyelidikan ini memfokuskan tentang keberkesanan penggunaan visera ikan patin yang tidak digunakan sebagai salah satu bahan yang dapat membantu bagi meningkatkan pertumbuhan dan kelangsungan hidup ikan keli (*Clarias gariepinus*). Visera ikan yang dibuang akan dibersihkan dan dikering pada suhu 105°C sebelum dianalisis kandungan nutrien. Analisis proksimat visera ikan patin *P. hypophthalmus* yang kering menunjukkan bahawa ia mengandungi 16.37 ± 0.81% protein mentah, 48.1 ± 0.01% lemak mentah, 67.53 ± 1.86% kelembapan dan 0.2 ± 0.06% abu. Spesies probiotik yang dianggap sebagai asli dalam perut ikan telah diasingkan dan dianalisis berdasarkan sifat fisiologi dan biokimia, pelekatan, aktiviti antibakteria dan dikenalpasti secara molekular. Hasil daripada proses pengenalpastian secara molekular menunjukkan bakteria tersebut berasal daripada spesies *Weisella confusa* dan mempunyai ciri-ciri yang berkesan dan berguna. Bakteria tersebut dapat hidup pada kadar optima dalam suhu antara 25°C hingga 35°C, peratus keasidan air 0% hingga 3.5%, boleh bercambah pada pH 4, 6, 8 dan 10, mampu bertahan terhadap kewujudan 0.15% dan 0.30% kehadiran garam hempedu serta mempunyai keupayaan melekat yang baik. Tambahan pula, bakteria tersebut juga menunjukkan zon kesekatlaluan yang

tinggi terhadap bakteria akuatik berbahaya yang telah diuji seperti *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Vibrio harveyi* dan *Vibrio parahaemolyticus*. Bakteria tersebut juga didapati tahan terhadap cakera antibiotik Vancomycin (30 µg) dan Amoxilin (10 µg) namun terdedah terhadap Streptomycin (10 µg), Vancomycin (5 µg) dan Ampicilin (10 µg). Kesesuaian visera kering sebagai bahan makanan dalam ikan keli Afrika (saiz permulaan  $2.71 \pm 0.14$  gm) telah diuji melalui diet pemakanan mengikut tiga tahap kandungan sampingan yang berbeza (10%, 20% dan 30%) dan didapati makanan yang mengandungi kadar 20% visera kering telah menghasilkan kadar pertumbuhan tertinggi, lantas telah dipilih untuk percubaan makanan seterusnya. Keberkesanan bakteria yang telah diasingkan beserta visera kering tadi telah diuji kepada ikan keli juvena dalam tempoh 40 hari dengan memberi makan percubaan mengikut empat rawatan; (T1) Makanan komersil; (T2) Campuran makanan komersil dengan visera kering; (T3) Campuran makanan komersil dengan bakteria yang diasingkan; (T4) Campuran makanan komersil dengan visera kering dan bakteria asingan. Hasil daripada percubaan makanan membuktikan bahawa T3 menunjukkan prestasi pertumbuhan yang ketara ( $P < 0.05$ ) berat badan, purata pemakanan harian dan kadar pertumbuhan tertentu berbanding dengan T1 tetapi tidak menunjukkan perbezaan yang signifikan ( $P > 0.05$ ) dengan T2 dan T4. Nisbah pengambilan makanan tidak banyak perbezaan ( $P > 0.05$ ) di antara semua rawatan tetapi nilai berangka yang ditunjukkan menunjukkan pengambilan makanan yang tinggi dalam kumpulan ikan probiotik T3. Kadar kemandirian semua ikan eksperimen tidak mempunyai sebarang perbezaan ( $P > 0.05$ ) terjejas satu sama lain tetapi jumlah yang tinggi dalam T2 diikuti oleh T3, T4 dan T1. Penilaian haematologi yang lebih lanjut dijalankan membuktikan bahawa T3 menunjukkan prestasi yang lebih baik ( $P < 0.05$ ) dari segi kepekatan hemoglobin, jumlah kuantiti korpuskular dan kiraan sel darah putih berbanding dengan rawatan lain. Tinjauan histologi yang dibuat pada ketinggian dinding usus vili di antara empat rawatan juga membuktikan bahawa T3 menunjukkan ketinggian yang lebih panjang ( $P < 0.05$ ) daripada vili T1, T2 dan T4. Oleh itu, diet ikan keli Afrika yang terdiri daripada 20% visera *P. hypophthalmus* kering atau terdiri daripada bakteria daripada usus tengah *P. hypophthalmus* pada dos diet  $10^9$  cfu/ml disyorkan untuk mempertingkatkan kadar pertumbuhan *Clarias gariepinus*.

## ACKNOWLEDGEMENTS

### **With the name of Allah the Most Compassionate and Most Merciful**

All praise and thanks to Almighty Allah, with His blessing giving me the strength and passion, could manage to finish the research until this manuscript completed be compiled. I would like to express my most sincere and deepest gratitude to my supervisors, Associate Professor Dr Hassan Hj. Mohd Daud and Associate Professor Dr Orapint Jintataporn who is in Kasetsart University for his continuous supervision, constant support, useful advices and comments throughout my research. I also would like to extend my sincere appreciation to my supervisory committee members; Dr. Hasliza bt Abu Hassim, Dr Murni Marlina bt Abd Karim and Dr Srinoy Chunkam for their continuous support, guidances and advices.

Throughout my master's research journey, many have contributed and helped. Thus, I would like to extend my deepest gratitude to my biggest advisors whom I considered as my sister, Nurhidayahanum binti Hamid, Supalug Katakdad and Siti Fairus binti Md Yusoff. Not forgotten to my other friends whose help me a lot throughout my research journey; Rachavut, Gun, Nora Faten Afifah, Sharifah Raina, Rashidah, Kumari Geetha, Zarith Sophea, Amer and many others whom I may forgot to list. Without the help of staffs from different faculties, I do not think I would be able to accomplish all objectives for my study, thus, I would like to acknowledge all staffs and students from Aquatic Animal Health Unit and Animal Nutrition of Faculty of Veterinary Medicine as well as Aquatic Animal Health of Faculty of Agriculture for helping me.

Never in my life imagined would be able to conduct my research at two different universities at the same time. Thus, I would like to send my deepest gratitude to all staff of School of Graduate Study especially to Mr Saiful Mohd Maskan whom helps me throughout my admission in Kasetsart University. I have gained so many new yet beneficial knowledge throughout my research in Kasetsart University, thus, I would like to thank to all staff and students of Department of Aquaculture, Faculty of Fisheries, Kasetsart University for their continuous support, help and understanding.

Last but not least, I would like to thank Ministry of Education of Malaysia as well as Southeast Asian Region Center for Graduate Study and Research in Agriculture (SEARCA) for providing me adequate funding and allowances for my research conducted at Universiti Putra Malaysia and Kasetsart University, Thailand.



This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Hassan Hj Mohd Daud, PhD**

Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Orapint Jintasataporn, PhD**

Senior Lecturer  
Faculty of Fisheries  
Kasetsart University  
(Member)

**Hasliza Abu Hassim, PhD**

Senior Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Murni Marlina Abd Karim, PhD**

Senior Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

**ROBIAH BINTI YUNUS, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name and Matric No.: Siti Nadia binti Abu Bakar (GS44060)

## Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to

Signature: \_\_\_\_\_

Name of Chairman of

Supervisory Committee: **Assoc. Prof. Dr Hassan Hj Mohd Daud**

Signature: \_\_\_\_\_

Name of Member of

Supervisory Committee: **Assoc Prof Dr Orapint Jintasataporn**

Signature: \_\_\_\_\_

Name of Member of

Supervisory Committee: **Dr Hasliza Abu Hassim**

Signature: \_\_\_\_\_

Name of Member of

Supervisory Committee: **Dr Murni Marlina Abd Karim**

## TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>APPROVAL</b>	vi
<b>DECLARATION</b>	vii
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF ABBREVIATIONS</b>	xvi
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
1.1 Objectives of the Study	2
<b>2 LITERATURE REVIEW</b>	<b>3</b>
2.1 World Food Consumption	3
2.2 Benefits of Fish Consumption	3
2.3 Aquaculture Status	4
2.4 Catfishes in General	5
2.5 Claridae Family	5
2.6 Biology of African catfish, <i>Clarias gariepinus</i>	6
2.7 Catfish Pathogens and Diseases	6
2.8 Probiotics	7
2.8.1 Selection of Probiotic	8
2.8.2 Mode of Action	8
2.8.3 Types of Probiotics	9
2.8.4 Lactic Acid Bacteria (LAB)	9
2.8.5 Probiotic Studies in Aquaculture	10
2.8.6 Probiotic Supplementation in Fish Diet	11
2.8.7 Growth Performance Evaluation of Fish	12
<b>3 EVALUATION OF NUTRIENT AND MICROBIAL CONTENT IN STRIPED CATFISH (<i>Pangasius hypophthalmus</i>) VISCERA AND ITS SUITAIBILITY AS INGREDIENTS IN FISH FEED FORMULATION</b>	<b>13</b>
3.1 Introduction	13
3.2 Materials and Methods	14
3.2.1 Sample Preparation	14
3.2.2 Nutrient Composition of Striped Catfish, <i>Pangasius hypophthalmus</i>	14
3.2.3 Microbial Count	16
3.2.4 Preliminary Feeding Trial on Efficiency of Dried Viscera Supplemented in juvenile African catfish diet	16
3.2.5 Statistical Analysis	19
3.3 Results	19
3.3.1 Proximate Composition of <i>Pangasius</i>	19

	<i>hypophthalmus</i> viscera	
3.3.2	Microbial Count	19
3.3.3	Preliminary Feeding Trial on Different Inclusion Level of Dried Viscera in African Catfish Diet	20
3.4	Discussions	21
3.5	Conclusion	24
<b>4</b>	<b>ISOLATION AND IDENTIFICATION OF THE PUTATIVE PROBIONT ISOLATED FROM INTESTINE OF THE STRIPED CATFISH (<i>Pangasius hypophthalmus</i>)</b>	<b>25</b>
4.1	Introduction	25
4.2	Materials and Methods	26
4.2.1	Collection of Samples	26
4.2.2	Serial Dilution and Bacterial Screening	26
4.2.3	Biochemical Test	26
4.2.4	Selection of Isolates by Antagonistic Test	27
4.2.5	Haemolysis Test	27
4.2.6	Putative Probiонт Properties	27
4.2.7	Identification of Putative Probiонт	29
4.3	Results	31
4.3.1	Serial Dilutions and Bacterial Screening	31
4.3.2	Biochemical Test	32
4.3.3	Selection of Isolated Probiонт by Antagonistic Effect Test	33
4.3.4	Putative Probiонт Properties	34
4.3.5	Identification of Probiонт	40
4.4	Discussions	41
4.5	Conclusions	45
<b>5</b>	<b>ASSESSMENT OF STRIPED CATFISH (<i>Pangasius hypophthalmus</i>) DRIED VISCERA AND ITS PUTATIVE PROBIONT (<i>Weisella sp.</i>) ON JUVENILE AFRICAN CATFISH (<i>Clarias gariepinus</i>) GROWTH PERFORMANCE</b>	<b>46</b>
5.1	Introduction	46
5.2	Materials and Methods	47
5.2.1	Fish Diet Preparation	47
5.2.2	Proximate Analysis of Experimental Feeds	48
5.2.3	Experimental Design	48
5.2.4	Feeding Trial	48
5.2.5	Growth Performances Analysis	48
5.2.6	Haematological Examination	48
5.2.7	Histological Examination	49
5.2.8	Statistical Analysis	49
5.3	Results	49
5.3.1	Proximate Analysis of Experimental Feeds	49

5.3.2	Efficacy of Striped Catfish ( <i>Pangasius hypophthalmus</i> ) Viscera and its Intestinal Putative Probiotic on Juvenile African Catfish ( <i>Clarias gariepinus</i> ) Growth Performance	50
5.3.3	Haematology	52
5.3.4	Histological Assessment	53
5.4	Discussions	55
5.5	Conclusion	57
<b>6</b>	<b>GENERAL DISCUSSIONS, RECOMMENDATIONS AND CONCLUSION</b>	<b>58</b>
	<b>REFERENCES</b>	<b>59</b>
	<b>APPENDICES</b>	<b>77</b>
	<b>BIODATA OF STUDENT</b>	<b>84</b>
	<b>LIST OF PUBLICATIONS</b>	<b>85</b>

## LIST OF TABLES

Table		Page
1.	Proximate composition of <i>Pangasius hypophthalmus</i> viscera	19
2.	Growth performance of juvenile African catfish ( <i>Clarias gariepinus</i> ) fed dried striped catfish ( <i>Pangasius hypophthalmus</i> ) viscera at 40 days	21
3.	Morphological, physiological and biochemical properties of screened putative probiont from mid-gut of <i>Pangasius hypophthalmus</i> .	33
4.	Growth of isolated putative probiont at different temperature 25°C, 45°C, 55°C, 15°C and 55°C after 24 hour.	35
5.	Growth of isolated putative probiont at different salinity (1.5%, 2.5%, 3.5%, 4.5%, 5.5%) after 24 hour of incubation	36
6.	Determination of optimum growth of isolated putative probiont from striped catfish intestines at pH 2, 4, 6, 8 and 10 at subsequent 3, 6, 12 and 24 hour of incubation	37
7.	Determination of optimum growth of putative probiont isolated from striped catfish intestines at different bile salt concentration at subsequent 0, 3, 6, 12 and 24 hour of incubation	38
8.	Determination of inhibition zone of putative probiont isolated from striped catfish intestines against pathogenous bacteria	39
9.	Antibiotic sensitivity of isolated putative probiont against several antibiotic disk treatment after 24 hour incubation on MH agar plate	40
10.	Proximate analysis content of the experimental feed used in the feeding trial of African catfish juvenile	50
11.	Growth performance of African catfish, <i>Clarias gariepinus</i> throughout the feeding trial at 45 days (n=10)	52
12.	Haematological parameters analysis of juvenile African catfish ( <i>Clarias gariepinus</i> ) after 45 days of feeding trial	53
13.	Measurement of intestinal epithelium height of African catfish after 45 days of feeding trial	55

## LIST OF FIGURES

Figure		Page
1.	Total Aquaculture Production of Malaysia in 2014 was lead by African catfish (38.9%) and Red Tilapia (25.76%)	4
2.	African catfish juvenile in the feeding trial which were stocked in 35L aquarium and optimum water quality were maintained throughout experiment time (n=12)	18
3.	Total plate count of Man de Rose agar isolated from fresh viscera of <i>Pangasius hypophthalmus</i> after 24 hour incubation	20
4.	Total plate count of Man de Rose agar isolated from dried viscera of <i>Pangasius hypophthalmus</i> after 48 hour of incubation	20
5.	MRS agar plate count of total colony forming units screen from mid intestine of <i>Pangasius hypophthalmus</i>	31
6.	The twelve isolated putative probiont from mid gut of <i>Pangasius hypophthalmus</i> on agar plates	32
7.	Selection of putative probiont based on the antagonistic test against pathogenic bacteria, <i>Aeromonas hydrophila</i> . Only one bacteria (circle) shows the antagonistic test among 12 bacteria.	34
8.	Haemolysis test of isolated putative probiont on blood agar which shows no clear halos ( $\gamma$ -hemolytic)	35
9.	Bile salt tolerance of the isolated putative probiont growth after 12 hour of incubation time	37
10.	Antagonism activity of isolated putative probiont against four pathogenic bacteria tested, <i>Vibrio parahaemolyticus</i> (A), <i>Streptococcus agalactiae</i> (B), <i>Vibrio harveyi</i> (C) and <i>Aeromonas hydrophila</i> (D) by spot assay method	39
11.	Antibacterial activity of the isolated putative probiont was found susceptible towards streptomycin, ampicillin and chloroamphenicol and shows resistancy towards amoxilin and vancomycin as circled.	40
12.	Agarose gel electrophoresis analysis of the PCR product showing that bacteria which isolated (arrow) were found to successfully amplified at 1500 bp	41



13. Growth performance based on body weight of African catfish, *Clarias gariepinus* throughout feeding trial (n=10) 51
14. Longitudinal sections based on villi length in posterior of intestinal epithelium of African catfish after 45 days of feeding trial (Villi height, VH; Muscularis mucosae, M). Mag. 100x; H&E. 54



## LIST OF ABBREVIATIONS

LAB	Lactic Acid Bacteria
GIT	Gastro Intestinal Tract
MRS	Man de Rose Agar
MH	Mueller Hinton
TSA	Tryptone Soy Agar
API	Analysing Profile Index
WG	Weight Gain
TL	Total Length
SGR	Specific Growth Rate
PER	Protein Efficiency Ratio
APNU	Apparent Net Protein Utilization
MIC	Minimum Inhibition Zone
GRAS	Generally Regarded As Safe
CFU	Colony-forming Unit
bp	Base pair
DoF	Department of Fisheries
µg	Microgram
µl	Microlitre
HCL	Hydrochloric Acid
rpm	Rotation per minutes
mg/ml	Milligram/mililitre
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
NCBI	The National Center for Biotechnology Information
MgCl <sub>2</sub>	Magnesium chloride
dNTP	Deoxynucleotide
TE	Tris-EDTA buffer
WBC	White Blood Cells
RBC	Red Blood Cells

## CHAPTER 1

### INTRODUCTION

Aquaculture is one of the most important sectors as it has major contribution in national economies. Aquaculture industry has been widely practiced in many parts of the world including in Asian regions. Aquaculture in the Asia-Pacific region has contributed at least 89% of the global production (FAO, 2012). The demand of aquaculture products are increasing several years as the community has realized the importance of good protein source in daily diet. The increasing demand of freshwater products also has improved the industrialization of aquaculture.

Freshwater aquaculture production in most Asian countries especially in Vietnam has been led by the production of *Pangasius catfish* in 2012 followed by Nile tilapia (Asia-Pacific Fishery Commission, 2014). Striped catfish or *Pangasius hypophthalmus* (Sauvage, 1878) is one of the most popular fish produced by most farmers in Asian countries. *P. hypophthalmus* is the second widely cultured fish species in Malaysia in 2010 according to Department of Fisheries Malaysia. Due to its desirable qualities such as low fat content as well as contain easily digestible protein content, it was commonly sold as frozen fillets for human consumption (Thammapat *et al.*, 2010). Thus, many processing companies were established in order to satisfy overwhelming demand from consumer for *P. hypophthalmus* fillet products.

Inevitably, post-harvest processing of fresh *P. hypophthalmus* has resulted large volume of waste by-products such as viscera, skin, head and bone which is not consumed by humans. The improper manage of these by products especially viscera could increase the risk of disposal-related health problems and environmental pollution including organic waste and microbial contamination. Approximately 20% of total fish weight were come from viscera that could produce more than 3, 000, 000 metric tons of waste yearly in Bangladesh (Hossain & Alam, 2015). Instead of being discarded thoroughly, these valuable viscera could be utilized in animal feed to reduce aquatic productions cost. Interestingly, the utilization of these waste by-product were found able to increase the economic value of fish (Thorpe, 2005).

The discarded *P. hypophthalmus* viscera could be utilized as one source of feeding for freshwater fish since it is very rich in microorganisms and enzymes (Feltes *et al.*, 2010), protein, amino acids and lipid (Villamil *et al.*, 2017). Moreover, due to its wide availability and cheap source, it could be use to be supplemented in the fish diet. The internal organs of fish such as heart, liver and gut contained nutritional value in terms of protein and essential fatty acids compound which might useful in production of fish meal and fish oil (Abdi *et al.*, 2011) that could be utilized as one of the aquaculture feed ingredients source.

Fish viscera could be utilized widely in feed processing industry as well as cosmetic industry as reported by some researcher. Bama *et al.* (2010) successfully extracted collagen from the carcass of catfish through its swim bladder and Shabanikakroodi (2014) has extracted oil from *P. hypophthalmus* for hand cream production. Besides, isolation of putative probiont from fish viscera particularly from gut of fish was also one of the most common and safe technique used for improving fish health. The presence of possible candidate of probiont in viscera may help to increase the digestibility of feed and nutrient bioavailability. Probiont was proven to be isolated from discarded part of fish viscera particularly fish gut in several fish species such as in *Catla catla*, *Labeo rohita*, *Cirrhinus mirigala* and *Cyprinus carpio* (Kandeepan & Muthukumar, 2015).

Probionts are one of the best and safe mitigation which could be used to treat any emerging disease occurred in fish farming which are very essential and beneficial for particular organism's health and nutrition. It commonly known as a live microbial adjunct which has a beneficial effect on the host by modulating the host associated or ambient microbial community, by enhancing use of the feed or improving its nutritional value, by elevating the host response towards disease, or by enhancing the quality of its ambient environment (Verschuere *et al.*, 2000).

Catfish has been widely distributed throughout the world and constituted a wide and unique group of marketably important freshwater fish especially in Southeast Asia. African catfish, *Clarias gariepinus* (Burchell, 1822) has been considered as one the most cultured freshwater fish and represented as widely produced food fish in the world particularly in Malaysian region (Fasakin *et al.*, 2003; Al-Dohail *et al.*, 2005). *C. gariepinus* was chosen as target species for the current study since it is widely available as well as high tolerance towards any condition. Diet which incorporated with dried fish viscera and putative probiont might be another factors which can enhance growth and survival of freshwater fish. Thus, current research focus on the efficacy of utilizing discarded *P. hypophthalmus* dried viscera as well as its intestinal putative probiont as feed ingredient to improve juvenile *C. gariepinus* growth and survival.

## 1.1 Objectives of the Study

In order to utilize the discarded viscera of *Pangasius hypophthalmus* efficiently in aquaculture industry, following objectives were outlined for current study:

1. To determine the proximate composition and putative probiont population of *P. hypophthalmus* viscera.
2. To identify and molecularly characterize the putative probiont present in intestines of *P. hypophthalmus*.
3. To determine the efficacy of *P. hypophthalmus* viscera and its intestinal probiont on juvenile *C. gariepinus* growth performance.

## REFERENCES

- Abasali, H., & Mohamad, S. (2010). Effect of dietary supplementation with probiotic on reproductive performance of female livebearing ornamental fish. *Research Journal of Animal Sciences*, 4(4), 103-107.
- Abdi, H., Christianus, A., Ramezani-Fard, E., Saad, C. R., & Hosseini, S. A. (2011). Proximate and Fatty Acid Composition of the Liver of Cultured Asian Redtail Catfish (*Hemibagrus nemurus*) and African Catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*, 6(7), 840-845.
- Abid, Z., Cross, A. J., & Sinha, R. (2014). Meat, dairy, and cancer-. *The American Journal of clinical nutrition*, 100(suppl\_1), 386S-393S.
- Adedeji F. A., Ibrahim, Z. B., Abdulsalami, S. A., Akinkunmi, A. A., (2014). Comparison of Microbiological and Proximate Analysis of *Syndontis nigrita*, *Chrysichthys nigrodigitatus* and *Mormyrus rume* in Olomore Market, Abeokuta, Ogun State, Nigeria. *Journal of Fisheries and Aquatic Science*, 1-5.
- Affandi, Farhana A. (2014). *Performance of Probiotic Bacillus subtilis G1 as a Dietary Supplement for Hemibagrus nemurus Valenciennes Fimgerlings*. Serdang: Thesis of Master Science, Universiti Putra Malaysia.
- Akhirebulu, C. & Okonji, V. (2013). Variation of Proximate Composition, Amino Acid and Fatty Acid profiles of parts of Cultured *Heterobranchus bidorsalis*. *Nigerian Journal of Agriculture, Food and Environment*, 7-12
- Akinjogunla, O. J., & Inyang, C. U. (2011). Bacterial species associated with anatomical parts of fresh and smoked Bonga Fish (*Ethmalosa fimbriata*): Prevalence and Susceptibility to Cephalosporins. *Research Journal of Microbiology*, 6(1), 87-97.
- Al- Dohail, M. A., Hashim, R., & Aliyu- Paiko, M. (2009). Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquaculture Research*, 40(14), 1642-1652.
- Allameh, S. K., Daud, H., Yusoff, F. M., Saad, C. R., & Ideris, A. (2012). Isolation, identification and characterization of *Leuconostoc mesenteroides* as new probiotic from intestine of snakehead fish. *African Journal of Biotechnology Vol 11(16)*, 3810-3816
- Allameh, S. K., Yusoff, F. M., Daud, H. M., Ringø, E., Ideris, A., & Saad, C. R. (2013). Characterization of a probiotic *Lactobacillus fermentum* isolated from snakehead, *Channa striatus*, stomach. *Journal of the World Aquaculture Society*, 44(6), 835-844.
- Aly SM, Ahmed YA, Ghareeb AA, Mohamed MF. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. *Fish Shellfish Immunol* 2008;25:128e36
- Andy Beumont, P. B. (2010). *Biotechnology and Genetics in Fisheries and Aquaculture*. United Kingdom: Blackwell Publishing.
- Angka S.L., Lam T. J. & Sin Y. M. (1995). Some virulence characteristics of *Aeromonas hydrophila* in walking catfish (*Clarias gariepinus*). *Aquaculture* 130, 103-112.
- Anonymous. (2010). *Commercial Catfish Production*. Retrieved 12 March, 2012, from Mississippi State University: <http://insucare.com/aquaculture/catfish/production.html>

- AOAC. (1994). Official Methods of Analysis of the Association of Official Analytical Chemists. *Association of Analytical Chemist*, 1298.
- Areerat, S. O. P. A. (1987). Clarias culture in Thailand. *Aquaculture*, 63(1-4), 355-362.
- Asia-Pacific Fishery Commission. (2014). *Regional overview of aquaculture trends in the Asia-Pacific Region 2014*, RAP Publication 2014/26, 45 p
- Askarian, F., Zhou, Z., Olsen, R. E., Sperstad, S., & Ringø, E. (2012). Culturable autochthonous gut bacteria in Atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and in vitro growth inhibition of four fish pathogens. *Aquaculture*, 326, 1-8.
- Astawan, M. (2004). Ikan yang sedap dan bergizi. *Tiga Serangkai. Solo*, 1-7
- Ayoola, S. O. (2010). Haematological characteristics of *Clarias gariepinus* (Buchell, 1822) juveniles fed with poultry hatchery waste. *Iranian Journal of Energy & Environment 2 (1)*, 18-23.
- Badis, A., Guetarni, D., Moussa-Boudjemaa, B., Henni, D. E., Tornadijo, M. E., & Kihal, M. (2004). Identification of cultivable lactic acid bacteria isolated from Algerian raw goat's milk and evaluation of their technological properties. *Food Microbiology*, 21(3), 343-349.
- Bag, M. P., Mahapatra, S. C., Rao, P. S., & Debajyoti, C. (2011). Making aquatic weed as potential feed for Nile tilapia (*Oreochromis niloticus* L.) and its impact on fatty acid profile. *Int Res J Pharmac Pharmacology*, 1(8), 194-202. (Mukti PB)
- Bakke, A., M., Glover, C., Krogdahl, A. (2011) Feeding, digestion and absorption of nutrients. *The Multifunctional Gut of Fish: Volume 3, Fish Physiology*.
- Balcázar, J. L., De Blas, I., Ruiz-Zarzuola, I., Cunningham, D., Vendrell, D., & Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary microbiology*, 114(3-4), 173-186.
- Balcazar, J. L., Vendrell, D., Blas, I. D., Ruiz-Zarzuola, I., & Muzquiz, J. L. (2004). Probiotics: a tool for the future of fish and shellfish health management. *Journal of Aquaculture in the Tropics*.
- Balcázar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuola, I., Muzquiz, J. L., & Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture*, 278(1-4), 188-191.
- Bama, P., Vijayalakshimi, M., Jayasimman, R., Kalaichelvan, P. T., Deccaraman, M., & Sankaranarayanan, S. (2010). Extraction of collagen from cat fish (*tachysurus maculatus*) by pepsin digestion and preparation and characterization of collagen chitosan sheet. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(4), 133-137.
- Bandyopadhyay, P. & Mohapatra, P. K. D. (2009). Effect of a probiotic bacterium *Bacillus circulans* PB7 in the formulated diets: on growth, nutritional quality and immunity of *Catla catla*. *Fish physiology and biochemistry*, 35(3), 467-478.
- Bartley, D. M., Rana, K., & Immink, A. J. (2000). The use of inter-specific hybrids in aquaculture and fisheries. *Reviews in Fish Biology and Fisheries*, 10(3), 325-337.
- Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. (1966). Antibiotics susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology*. 45: 493-496.
- Beaumont, A., Boudry, P., & Hoare, K. (2010). *Biotechnology and genetics in fisheries and aquaculture*. John Wiley & Sons.
- Bednorz, C., Guenther, S., Oelgeschläger, K., Kinnemann, B., Pieper, R., Hartmann, S., ... & Bethe, A. (2013). Feeding the probiotic *Enterococcus faecium* strain

- NCIMB 10415 specifically reduces *Escherichia coli* pathotypes adherent to the gut mucosa of piglets. *Applied and environmental microbiology*, AEM-03138.
- Bhaskar, N., Benila, T., Radha, C., & Lalitha, R. G. (2008). Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease. *Bioresource technology*, 99(2), 335-343.
- Bhat, S., Amundsen, P. A., Knudsen, R., Gjelland, K. Ø., Fevolden, S. E., Bernatchez, L., & Præbel, K. (2014). Speciation reversal in European whitefish (*Coregonus lavaretus* (L.)) caused by competitor invasion. *PLoS One*, 9(3), e91208.
- Blaxhall, P. C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of fish biology*, 5(6), 771-781.
- Bomba, A., Nemcová, R., Mudroňová, D., & Guba, P. (2002). The possibilities of potentiating the efficacy of probiotics. *Trends in Food Science & Technology*, 13(4), 121-126.
- Boonyaratpalin S., & Kasornchan J. (1986). *Edwardsiella ictaluri*-like organism (EILO), a new fish pathogenic bacteria of walking catfish (*Clarias batrachus* Linn.) in Thailand. *Songklanakar Journal of Science and Technology* 8, 445-449.
- Boonyaratpalin, S. (1987). Bacterial pathogens involved in the epizootic ulcerative syndrome of fish in Asia. *Songklanakar Journal of Science and Technology* 9, 495-502.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., ... & Corner, R. (2010). Aquaculture: global status and trends. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365(1554), 2897-2912.
- Burgess, W. E. (1989). *An atlas of freshwater and marine catfishes: a preliminary survey of the Siluriformes*. Neptune City: TFH.
- Butprom, S., Phumkhachorn, P., & Rattanachaikunsopon, P. (2013). Effect of *Lactobacillus plantarum* C014 on Innate Immune Response and Disease Resistance against *Aeromonas hydrophila* in Hybrid Catfish. *The Scientific World Journal*, 1-6.
- Caballero, M. J., Izquierdo, M. S., Kjørsvik, E., Montero, D., Socorro, J., Fernández, A. J., & Rosenlund, G. (2003). Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture*, 225(1-4), 325-340.
- Cai, Y., Suyanandana, P., Saman, P., & Benno, Y. (1999). Classification and characterization of lactic acid bacteria isolated from the intestines of common carp and freshwater prawns. *The Journal of general and applied microbiology*, 45(4), 177-184.
- 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. *Poult. Sci.*, 92, 2949-2955.
- Cappucino J.G. & Sherman N. (2002). *Microbiology a laboratory manual (Vol 1)*. New York: Benjamin Cumming.
- Carnevali, O., Avella, M. A., & Gioacchini, G. (2013). Effects of probiotic administration on zebrafish development and reproduction. *General and comparative endocrinology*, 188, 297-302.

- Cebeci, A., & Gürakan, C. (2003). Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiology*, 20(5), 511-518.
- Cerezuela, R., Fumanal, M., Tapiá-Paniagua, S. T., Meseguer, J., Moríñigo, M. Á., & Esteban, M. Á. (2012). Histological alterations and microbial ecology of the intestine in gilthead seabream (*Sparus aurata* L.) fed dietary probiotics and microalgae. *Cell Tissue Res*, 350: 477-489.
- Chiu, C. H., Cheng, C. H., Gua, W. R., Guu, Y. K., & Cheng, W. (2010). Dietary administration of the probiotic, *Saccharomyces cerevisiae* P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. *Fish & shellfish immunology*, 29(6), 1053-1059.
- Collins, M. D., Ash, C., Farrow, J. A. E., Wallbanks, S., & Williams, A. M. (1989). 16S ribosomal ribonucleic acid sequence analyses of lactococci and related taxa. Description of *Vagococcus fluvialis* gen. nov., sp. nov. *Journal of Applied Bacteriology*, 67(4), 453-460.
- Collins, M.D., Samelis, J., Metaxopoulos, J. & Wallbanks, S. (1993) Taxonomic studies on some Leuconostoc-like organisms from fermented sausages - description of a new Genus *Weissella* for the Leuconostoc paramesenteroides group of species. *Journal of Applied Bacteriology*, 75(6), 595-603.
- Coward, K., & Bromage, N. R. (1998). Histological classification of oocyte growth and the dynamics of ovarian recrudescence in *Tilapia zilli*. *Journal of Fish Biology*, 53(2), 285-302
- Craig, S., Helfrich, L. A., Kuhn, D., & Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding.
- Dantas, D. V., Barletta, M., Costa, M. F., Barbosa- Cintra, S. C. T., Possatto, F. E., Ramos, J. A., ... & Saint- Paul, U. (2010). Movement patterns of catfishes (Ariidae) in a tropical semi- arid estuary. *Journal of Fish Biology*, 76(10), 2540-2557.
- Das, S., Ward, L. R. & Burke, C. (2010). Screening of marine *Streptomyces spp.* for potential use as probiotics in aquaculture. *Aquaculture*. 305(1-4): 32-41
- Dash, S. S., Das, B. K., Pattnaik, P., Samal, S. K., Sahu, S., & Ghosh, S. (2009). Biochemical and serological characterization of *Flavobacterium columnare* from freshwater fishes of Eastern India. *Journal of the World Aquaculture Society*, 40(2), 236-247
- De Pedro, N., Guijarro, A. I., López- Patiño, M. A., Martínez- Álvarez, R., & Delgado, M. J. (2005). Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. *Aquaculture research*, 36(12), 1185-1196.
- De, B. C., Meena, D. K., Behera, B. K., Das, P., Mohapatra, P. D., & Sharma, A. P. (2014). Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses. *Fish physiology and biochemistry*, 40(3), 921-971.
- Dacie, J. V. (2006). *Dacie and Lewis practical haematology*. Elsevier Health Sciences.
- Demarais, B. D., Dowling, T. E., Douglas, M. E., Minckley, W. L., & Marsh, P. C. (1992). Origin of *Gila seminuda* (Teleostei: Cyprinidae) through introgressive hybridization: implications for evolution and conservation. *Proceedings of the National Academy of Sciences*, 89(7), 2747-2751.
- Deplancke, B., & Gaskins, H. R. (2001). Microbial modulation of innate defense: goblet cells and the intestinal mucus layer-. *The American journal of clinical nutrition*, 73(6), 1131S-1141S.
- Desai, A. R., Links, M. G., Collins, S. A., Mansfield, G. S., Drew, M. D., Van Kessel, A. G., & Hill, J. E. (2012). Effects of plant-based diets on the distal gut



- microbiome of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 350, 134-142.
- Devaere, S., Jansen, G., Adriaens, D., & Weekers, P. (2007). Phylogeny of the African representatives of the catfish family Clariidae (Teleostei, Siluriformes) based on a combined analysis: independent evolution towards anguilliformity. *Journal of Zoological Systematics and Evolutionary Research*, 45(3), 214-229.
- Dewanti, R., & Wong, A.C.L. (1995). Influence of culture conditions on biofilm formation by *Escherichia coli* 0157:H7. *Food Microbiology* 67: 456-459
- Dhanasekaran, D., Saha, S., Thajuddin, N., Rajalakshmi, M. & Panneerselvam, A. (2011). Probiotic Effect of *Lactobacillus* isolates against bacterial pathogens in fresh water Fish. *Journal of Coastal Development*, 13(2), 103-112.
- Dimitrologue, A., Merrifield, D. L., Moate, R., Davies, S. J., Spring, P., Sweetman, J., & Bradley, G. (2009). Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of animal science*, 87(10), 3226-3234.
- Direkbusarakom, S., Yoshimizu, M., Ezura, Y., Ruangpan, L., & Danayadol, Y. (1998). *Vibrio* spp., the dominant flora in shrimp hatchery against some fish pathogenic viruses. *Journal of Marine Biotechnology*, 6, 266-267.
- Drury, R. A. B, and E. A. Wallington (1976). *Carleton's histological technique Forth edition*. London: Oxford University Press.
- Dunham, R. A. (2004). *Aquaculture and fisheries biotechnology: genetic approaches: CABI*. ISBN 0-85199-596-9.
- Duy, N., & Tram, Q. (2010). Evaluation of Local Feed Resources for Hybrid Catfish (*Clarias macrocephalus* x *C. gariepinus*) in Smallholder Fish Farming Systems in Central Vietnam. Agriculture. Nguyen Duy Quynh, T. (2010). *Evaluation of local feed resources for hybrid catfish (Clarias macrocephalus x C. gariepinus) in smallholder fish farming systems in central Vietnam* (Vol. 2010, No. 72).
- EFSA Scientific Committee. (2015). Statement on the benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood. *EFSA Journal*, 13(1), 3982.
- Eldar, A. A., & Ghittino, C. (1999). *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. *Diseases of Aquatic Organisms*, 36(3), 227-231.
- El-Rahman, A. M. A., Khattab, Y. A., & Shalaby, A. M. (2009). *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology*, 27(2), 175-180.
- El-Yazeed H.A. & Ibrahim M.D. (2009) Studies on *Edwardsiella tarda* infection in catfish and Tilapia nilotica. Beni-Suef. Veterinary Medical Journal 19,44–50.
- Enke, D. B. S., Lopes, P. S., Kich, H. A., Britto, A. P., Soquetta, M., & Pouey, J. L. O. F. (2009). Use of fish silage flour in diets for the jundiá in the juvenile phase. *Ciência Rural*, 39(3), 871-877.
- Fagbenro, O. A., & Sydenham, D. H. J. (1988). Evaluation of *Clarias isheriensis* (Sydenham) under semi-intensive management in ponds. *Aquaculture*, 74(3-4), 287-291.
- FAO. (2015). *The State of Food Insecurity in The World*, 348. Rome: FAO Fisheries and Aquaculture Department.

- FAO. (1995). The Use of Ice on Small Fishing Vessels. *Food and Agriculture Organization*, 197.
- FAO. (2010). *Fisheries and Aquaculture*. Retrieved 19 June, 2011, from Food and Agriculture Organization (FAO) of United: [http://www.eoearth.org/article/Food\\_and\\_Agriculture\\_Organization\\_\(FAO\)](http://www.eoearth.org/article/Food_and_Agriculture_Organization_(FAO))
- FAO. (2012). *The State of World Fisheries and Aquaculture*. Rome: FAO Fisheries and Aquaculture Department.
- FAO. (2014). *The World Fisheries and Aquaculture*. Rome: FAO Fisheries and Aquaculture Department.
- Farhana, A. A. (2014). Performance of probiotic *Bacillus subtilis* G1 as a dietary supplement for *Hemibagrus nemurus* Valenciennes fingerlings. Universiti Putra Malaysia: Master Thesis
- Farzanfar, A. (2006). The use of probiotics in shrimp aquaculture. *FEMS Immunology and Medical Microbiology*, 43, 149-158.
- Fasakin, E. A., Balogun, A. M., & Ajayi, O. O. (2003). Evaluation of full- fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. *Aquaculture Research*, 34(9), 733-738.
- Felix, N. & Sudharsan, M. (2004). Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn *Macrobrachium rosenbergii*. *Journal of Aquaculture Nutrition* 10 (3), 193-197.
- Feltes, M., Correia, J. F., Beirão, L. H., Block, J. M., Ninow, J. L., & Spiller, V. R. (2010). Alternatives for adding value to fish processing residues. *Brazilian Journal of Agricultural and Environmental Engineering-Agriambi* , 14 (6).
- Fessard, A., & Remize, F. (2017). Why are *Weissella* spp. not used as commercial starter cultures for food fermentation?. *Fermentation*, 3(3), 38.
- Finegold, S. M., Sutter, V. L., & Mathisen, G. E. (1983). Normal indigenous intestinal flora. *Human intestinal microflora in health and disease*, 1, 3-31.
- Foran, J. A., Carpenter, D. O., Hamilton, M. C., Knuth, B. A., & Schwager, S. J. (2005). Risk-based consumption advice for farmed Atlantic and wild Pacific salmon contaminated with dioxins and dioxin-like compounds. *Environmental health perspectives*, 113(5), 552.
- Fukami, K., Nishijima, T., Ishida, Y. (1997). Stimulative and inhibitory effects of bacteria on the growth of microalgae. *Hydrobiologia* 358, 185-191.
- Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, 66: 365-378. AFRC, R. F. (1989). Probiotics in man and animals. *Journal of applied bacteriology*, 66(5), 365-378.
- Fuller, R. (1992). History and development of probiotics, . In F. R. (Ed.), *Probiotics: The Scientific Basis*. (pp. pp 1-8). London: Chapman and Hall.
- Fusco, V., Quero, G. M., Cho, G. S., Kabisch, J., Meske, D., Neve, H., Bockelmann, W., & Franz, C. M. (2015). The genus *Weissella*: Taxonomy, ecology and biotechnological potential. *Front. Microbiol*, 6, 1-22.
- Galindo-Villegas, J., Garcia-Moreno, D., De Oliveira, S., Meseguer, J. & Mulero, V (2012). Regulations of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development . *Proceedings of the National Academy of Sciences USA*, 109, E2605-E2614.
- Garriques, D., Arevalo, G., (1995). An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vannamei* post- larvae in Ecuador. In: In C. H. In: Browdy, S- *wimming through troubled water. Proceedings of the Special Session on Shrimp Farming, Aquaculture '95*. (pp. pp. 53-59). Baton Rouge: World Aquaculture Society.

- Gatesoupe, F. J. (1999). The use of probiotics in aquaculture. *Aquaculture*, 180 (1-2), 147-165.
- George-Gay, B., & Parker, K. (2003). Understanding the complete blood count with differential. *Journal of PeriAnesthesia Nursing*, 18(2), 96-117.
- Ghosh S., Sinha A. & Sahu C. (2007). Effect of probiotic on reproductive performance in female live bearing ornamental fish. *Aquaculture Research*, 38(5): 518-26.
- Giri, S.S., Sukumaran, V., & Oviya, M., (2013). Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity and disease resistance of tropical freshwater fish, *labeo rohita*. *Fish and Shellfish Immunology*, 34(1), 660-666.
- Gohila B, Damodaran, R., Bharathidasan, V. & Vinoth, M (2013). Comparative Studies on Growth Performance of Probiotic Supplemented Rohu Fingerlings . *International Journal of Pharmaceutical & Biological Archives* , 4910; 84-88
- Goldschmidt- Clermont, E., Wähli, T., Frey, J., & Burr, S. E. (2008). Identification of bacteria from the normal flora of perch, *Perca fluviatilis L.*, and evaluation of their inhibitory potential towards *Aeromonas* species. *Journal of fish diseases*, 31(5), 353-359.
- Gomez-Gil B, Herrera-Vega M. A., Abreu-Grobois F.A. & Roque A. (1998). Bioencapsulation of two different *Vibrio* species in nauplii of the brine shrimp (*Artemia franciscana*). *Applied and environmental microbiology*, 64(6), 2318-2322.
- Gomez-Gil, B., Roque, A. & Turnbull, J. F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture*, 191(1-3): 259-270.
- Gomez ,G.D, Balcazar, J.L. (2008) A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunol Med Microbiol* 52:145–54.
- Gonzalez, C. J., Encinas, J. P., Garcia-López, M. L., & Otero, A. (2000). Characterization and identification of lactic acid bacteria from freshwater fishes. *Food Microbiology*, 17(4), 383-391.
- Gram, L., and Ringo, E. (2005). Prospects of fish probiotics. In W. H. Naughton, *Microbial Ecology in Growing Animals* (pp. pp 379-417). Edinburgh: Elsevier.
- Greenwood, P. H., Rosen, D. E., Weitzman, S. H., & Myers, G. S. (1966). Phyletic studies of teleostean fishes, with a provisional classification of living forms. *Bulletin of the AMNH*; v. 131, article 4.
- Gupta, P., & Gupta, A. (2013). Effect of Fish and Chicken Viscera Incorporated Diets on Growth, Feed Utilization and Body Composition of Asian catfish, *Clarias batrachus* Fry. *Animal Nutrition and Feed Technology* 13, 196-203.
- Hammoumi, A., Faid, M., El yachoui, M., Amarouch, H. (1998). Characterization of fermented fish waste used in feeding trials with broilers. *Process Biochem.* 33, 423–427.
- Hansen, G.H., & Sørheim, R., (1991). Improved method for phenotypical characterization of marine bacteria. *J. Microbiol. Methods* 13(3), 231-241.
- Hardy, R.W. (2001). Nutritional deficiencies in commercial aquaculture: likelihood, onset, and identification. In C. L. Webster, *Nutrition and Fish Health* (pp. 131-147). London: Haworth Press Inc.
- Hassan, A. N., & Frank, J. F. (2001). Starter cultures and their use. In M. E. L., *Applied Dairy Microbiology, Second edition* (pp. 151-206). New York: Marcel Dekker, Inc.

- Hecht, T., Oellermann, L., Verheust, L., 1996. Perspectives on clariid catfish culture in Africa. *Aquat. Living Resour.* 9, 197–206 (Hors série).
- Hedrick, P. (2013) Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol Ecol* 22: 4606–4618. doi: 10.1111/mec.12415 PMID: 23906376
- Heikkinen, J., Vielma, J., Kemiläinen, O., Tirola, M., Eskilinen, P., Kiuru, T., Navia-Paldanius, D. & Von Wright, A. (2006). Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Onchorynchus mykiss*). *Aquaculture*, 261, 259-268.
- Hedrick, P. W. (2013). Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular ecology*, 22(18), 4606-4618.
- Hossain, U., & Alam, A. K. M. N. (2015). Production of powder fish silage from fish market wastes. *SAARC Journal of Agriculture*, 13(2), 13-25.
- Hossain, Z. (2014). Bacteria: *Streptococcus*. *Encyclop. Food Safety*, 1, 535-545.
- Hovda, M. B., Fontanillas, R., McGurk, C., Obach, A., & Rosnes, J. T. (2012). Seasonal variations in the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture Research*, 43(1), 154-159.
- Hovda, M. B., Lunestad, B. T., Fontanillas, R., & Rosnes, J. T. (2007). Molecular characterisation of the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 272(1-4), 581-588.
- Huss, H. H., Reilly, A. & Embarek, P. K. B., (2000). Prevention and Control of Hazards in Seafood. *Food Control*, 149-156.
- Ibrahim M.D., Atta A.H. & Shalaby M.A.. (2010). Bioavailability of orbifloxacin in African sharp-tooth catfish, *Clarias gariepinus*, and its efficacy in control of induced edwardsiellosis. *Journal of American Science* 6, 336-344.
- Ibrahim, M. D. (2015). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives. *Journal of Advanced Research*, 6, 765-791.
- IGFA, International Game Fish Association. (2001). Database of IGFA angling records until 2001. *IGFA, Fort Lauderdale, USA*.
- Irianto, A., B. Austin (2002). Probiotics in aquaculture. *Journal of Fish Dis*, 25: 633-642.
- Islami, S. N. E., Faisal, M., Akter, M., Reza, M. S., & Kamal, M. (2015). Comparative shelf life study of whole fish and fillets of cultured striped catfish (*Pangasianodon hypophthalmus*) during ice storage condition. *Research in Agriculture Livestock and Fisheries*, 2(1), 177-183.
- Islami, S. N. E., Reza, M. S., Mansur, M. A., Hossain, M. I., Shikha, F. H., & Kamal, M. (2014). Rigor index, fillet yield and proximate composition of, cultured striped catfish for its suitability in processing industries in Bangladesh. *Journal of Fisheries* 2(3), 157-162.
- Itoi, S., Yuasa, K., Washio, S., Abe, T., Ikuno, E & Sugita, H., (2009). Phenotypic variation in *Lactococcus lactis subsp. lactis* isolated derived from the intestinal tracts of marine and freshwater fish. *Journal of Applied Microbiology*, 107, 867-874.
- Jantrarotai, W., Sitasit, P., Jantrarotai, P., Viputhanumas, T. & Srabua, P. (1998). Protein and Energy Levels for Maximum Growth, Diet Utilization, Yield of Edible Flesh and Protein Sparing of Hybrid *Clarias* Catfish (*Clarias macrocephalus x Clarias gariepinus*). *Journal of the World Aquaculture Society* 29 (3), 281-289.

- Johnson C.W., Timmons D.L. & Hall P.E. (2002). *Essential Laboratory Mathematics: Concepts and Applications for the Chemical and Clinical Laboratory Technician*. Florence, KY, USA: Cengage Learning.
- José Luiz Pedreira Mourão, Gabriella V. P., Felipe N. V., Adolfo B. J., Thiago T. U., Bruno C. d. S., Walter Q. S., Gabriel F. A. J. & Maurício L. M. (2016). Isolation of probiotic bacteria from the hybrid South American catfish (*Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae): A haematological approach. *Aquaculture Reports* 3, 166 – 171. Mourão, J. L. P., do Vale Pereira, G., do Nascimento Vieira, F., Jatobá, A. B., Ushizima, T. T., da Silva, B. C., ... & Martins, M. L. (2016). Isolation of probiotic bacteria from the hybrid South American catfish *Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae): A haematological approach. *Aquaculture Reports*, 3, 166-171.
- Kandeepan C. & P. Muthukumar (2015). Isolation, Identification and Characterization of Probiotic Organisms From Intestines of Fresh Water Fishes. *International Journal of Current Microbiology and Applied Sciences*, Volume 4 Number 3 pp 607-616.
- Kasornchandra J., Rogers W.A. & Plumb J.A. (1987). *Edwardsiella ictaluri* from walking catfish, *Clarias batrachus* L., in Thailand. *Journal of Fish Disease* 10, 137-138.
- Keddie, R. M. (1959). The properties and classification of *lactobacilli* isolated from grass and silage. *Journal of Applied Bacteriology*, 22, 403-416.
- Kesarcodi-Watson, A., Kaspar, H., Lategan, M. J., & Gibson, L. (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, 274(1), 1–14. <http://doi.org/10.1016/j.aquaculture.2007.11.019>
- Kim, D. H. & Austin B. (2008). Characterization of probiotic carnobacteria isolated from rainbow trout intestine. *Lett. Appl. Microbiology*, 47 (3) : 141-147.
- Kopermsub, P., & Yunchalard, S. (2010). Identification of lactic acid bacteria associated with the production of plaasom, a traditional fermented fish product of Thailand. *International journal of food microbiology*, 138(3), 200-204.
- Kraus, H. (1961). Kurze mitteilung uber das vorkommen von *Lactobazillien* auf fischen heringen. *Archiv fur Lebensmittelhygiene*, 12, 101-102.
- Kvasnikov, E. I., Kovalenko, N. K. & Materinskaya, L.G (1977). Lactic acid Bacteria of freshwater fish. *Microbiology*, 46, 619-624.
- Lall, S., P. & Tibbetts, S., M., (2009) Nutrition, Feeding and Behavior of Fish. *Veterinary Clinics of North America Exotic Animal Practice* 12, 361-372
- Lara-Flores M, Olvera-Novoa MA, Guzman-Mendez BE, 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* ;216:193–201.
- Lara-Flores, M., & Aguirre-Guzman, G. (2009). The use of probiotic in fish and shrimp aquaculture. A review. *Probiotics: production, evaluation and uses in animal feed*. *Research Signpost, Kerala*, 75-89.
- Lauzon, H. L., Pérez- Sánchez, T., Merrifield, D. L., Ringø, E., & Balcázar, J. L. (2014). Probiotic applications in cold water fish species. *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*, 223-252.
- Legendre M., Teugels G. G., Cauty C., & Jalabert B. (1998). The Biological Diversity and Aquaculture of Clariid and Pangasiid Catfishes in South-East Asia.

- Proceedings of the Mid-term Workshop of the "Catfish Asia Project" Jakarta (IDN), Can Tho: IRD; Can Tho.*
- Leonard, J. B. K., & McCormick, S. D. (1999). Changes in haematology during upstream migration to American shad. *Journal of fish biology*, 54(6), 1218-1230.
- Li Z., Zhang Q., & Yang H. (1997). The affect of the probiotics to the shrimp pond. *Aquaculture China [in chinese]*, 5: 30-1.
- Ling S., Hashim R., Kolkovski S., & Chong Shu-Chien A. (2006). Effect of Varying Dietary Lipid and Protein Levels on Growth and Reproductive Performance of female swordtails (*Poeciliidae*). *Aquaculture research*, 37(13), 1267-1275.
- Ljungh, Å., & Wadström, T. (2006). Lactic Acid Bacteria as Probiotics. *Current Issues in Intestinal Microbiology Volume 7*, 73-90.
- Llobrera A.T. & Gacutan, R. Q. (1987). *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. *Aquaculture* 67 (3-4), 273-278.
- Magnadottir, B. (2006). Innate Immunity of Fish (overview). *Fish and Shellfish Immunology Vol 20*, no 2, pp 137-151.
- Mahboob S., Haider S., Sultana S., Al-Ghanim K A, Al-Misned F, Al-Kahem Al-Balawi H. F., & Ahmad Z. (2014). Isolation and Characterisation of Collagen from the Waste Material of Two Important Freshwater Fish Species. *The Journal of Animal & Plant Sciences*, 1802-1810.
- Marlida, R., Suprayudi, M., A., Widanarni & Harris, E. (2014). Isolation, Selection and Application of Probiotic Bacteria for Improvement the Growth Performance of Humpback Groupers (*Cromileptes altivelis*). *International Journal of Sciences: Basic and Applied Research*, Volume 16, No 1. Pp 364-379
- Marzouk, M. S., Moustafa M. M. & Mohamed, N. M (2008). Evaluation of Immunomodulatory Effects of Some Probiotics on Cultured *Oreochromis niloticus*. *International Symposium on Tilapia in Aquaculture 2008*, 1043-1057.
- Maslow J. N., Dawson D., Carlin E. A., & Holland S. M.. (1999). Haemolysin as a virulence factor for systemic infection with isolates of *Mycobacterium avium* complex. *Journal Clin. Microbiol.*, 37 (2): 445-446.
- Mathieu Castex, Carly D. & Liet C. (2014). Probiotic Applications in Crustaceans. In D. M. Ringo, *Aquaculture Nutrition : Gut Health, Probiotics and Prebiotics* (pp. 290-327). United States: John Wiley & Sons Ltd.
- Mauguin, S., & Novel, G. (1994). Characterization of lactic acid bacteria isolated from seafood. *J. Appl. Bacteriol*, 76(6), 616-625.
- Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T., Bøgwald, J., ... & Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, 302(1-2), 1-18.
- Meyer, F. P. (1991). Aquaculture disease and health management. *Journal of Animal Science* 69, 4201-4208.
- Microbe, M. (28 February, 2013). *MetaMicrobe.com/Lactic Acid Bacteria*. Retrieved from Metamicrobe Website: <http://www.metamicrobe.com/lactobacteria/>
- Mizushima, S., Moriguchi, E. H. Ishikawa, P., Hekman, P., Nara, Y., Mimura, G., ... & Yamori, Y. (1997). Fish intake and cardiovascular risk among middle-aged Japanese in Japan and Brazil. *J. Cardiovasc. Risk*, 4(3), 191-199.
- Mondal, K., Kaviraj, A., & Mukhopadhyay, P., K. (2008). Evaluation of fermented fish-offal in the formulated diet of the freshwater catfish, *Heteropneustes fossilis*. *Aquaculture Research* 39, 1443-1449

- Moriarty, D. J. W. (1998). Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture* 164 (1-4), 351-358.
- Mouriño, J. L. P., Pereira, G. D. V., Vieira, F. D. N., Jatobá, A. B., Ushizima, T. T., Silva, B. C. Da, Martins, M. L. (2016). Isolation of probiotic bacteria from the hybrid South American catfish *Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae): A haematological approach. *Aquaculture Reports*, 3, 166–171. <http://doi.org/10.1016/j.aqrep.2016.03.001>
- Mulder, R.W., Havenaar, R., Huis in & Veld, J.H.. (1997). Intervention strategies: the use of probiotics and competitive exclusion microfloras against contamination with pathogen in pigs and poultry. In R. Fuller, *Probiotics 2: Applications and practical aspects* (pp. pp 187-207). London: Chapman and Hall.
- Mumford S. L. (2004). Histology of finfish. USFWS, Olympia Fish Health Center. Olympia. Washington.
- Munir M.B., Roshada H., Yam H.C., Terence L.M.,and Azizah S. (2016). Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striatus*) fingerlings. *Aquaculture* 460, 59-68.
- Na-Nakorn, U. (1999). Genetic factors in fish production: a case study of the catfish *Clarias*. In S. (In: Mustafa, *Genetics in Sustainable Fisheries Management* (pp. 175-187). Malden: Fishing New Books.
- Na-Nakorn, U. (2013). Interspecific hybrid catfish in Thailand. In J. D. Ruane, *Biotechnologies at Work for Smallholders: Case Studies From Developing Countries in Crops, Livestock and Fish* (pp. pp 149-155). Rome: FAO. Ruane, J., Dargie, J. D., Mba, C., Boettcher, P., Makkar, H. P. S., Bartley, D. M., & Sonnino, A. (2013). Biotechnologies at work for smallholders: case studies from developing countries in crops, livestock and fish. Food and Agriculture Organization of the United Nations (FAO).
- Na- Mondal, K., Kaviraj, A., & Mukhopadhyay, P. K. (2008). Evaluation of fermented fish- offal in the formulated diet of the freshwater catfish *Heteropneustes fossilis*. *Aquaculture Research*, 39(13), 1443-1449.
- Nakorn, U., Kamornrat, W., & Ngamsiri, T. (2004). Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed *C. gariepinus*. *Aquaculture* 240145-163.
- Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., ... & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceeding of the National Academy of Sciences* (pp. 15103–15110). United States of America: National Academy of Sciences.
- Nelson, D. L., Lehninger, A. L., & Cox, M. M. (2008). *Lehninger principles of biochemistry*. Macmillan.
- Ng, W. K. & Chen, M. L. (2002). Replacement of Soybean Meal with palm Kernel Meal in Practical Ciets for Hybrid Asian-African Catfish, *Clarias macrocephalus* x *C. gariepinus*. *Journal of Applied Aquaculture*, 12 (4), 67-76.
- Nguyen T. D. T., Kang J.H.,& Lee M.S. (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. *Int. J. Food Microbiol.* 113(113): 358- 361.
- Nikoskelainen, S., Ouwehand, A., Salminen, S., & Bylund, G. (2001). Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*? *Aquaculture* 198, 229-236.

- NRC (National Research Council). (1993). *Nutrient Requirement of Fish*. Washington D. C. USA: National Academy Press.
- Nurhidayu, A., Ina-Salwany, M. Y., Daud, H. M., & Harmin, S. A. (2012). Isolation, screening and characterization of potential probiotics from farmed tiger grouper (*Epinephelus fuscoguttatus*). *African Journal of Microbiology Research*, 6(9), 1924-193.
- Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases*, 35(2), 83–108. <http://doi.org/10.1111/j.1365-2761.2011.01333.x>
- Onarheim, A.M., Wiik, R., Burghardt, J. & Stackebrandt, E., (1994). Characterization and identification of two *Vibrio* species indigenous to the intestine of fish in cold sea water; description of *Vibrio iliopiscarius* sp. *Syst. Appl. Microbiol.*, 17, 370-379.
- Otémé, Z.L., Hem, S., Legendre, M., 1996. Nouvelles espèces de poissons-chats pour le développement de la pisciculture africaine. *Aquat. Living Resour.* 9, 207–217 (hors série)
- Ouwehand, A. C., Salminen, S. & Isolauri, E (2002). Probiotics: an overview of beneficial effects. *Antonie van Leeuwenhoek*, 82, 279-289.
- Park, J-H., & Lee, Y-D. (2017) Genomic analysis of WCP30 phage of *Weissella cibaria* for Dairy Fermented Foods. *Korean Journal for Food Science at Animal Resources*. 37(6): 884-888
- Perez-Sanchez, T., Ruiz-Zarzuela, I., de Blas, I. & Balcazar, J.L. (2014). Probiotics in aquaculture: a current assessment. *Reviews in Aquaculture*, 6(3), 133-146 DOI 10.1111/raq.12033.
- Planas M., Vazquez J., Marques J., Perez R., Gonzalez M. & Murado M. (2004). Enhancement of rotifer (*Brachionus plicatilis*) growth by using terrestrial lactic acid bacteria. *Aquaculture*, 240(1-4): 313-29.
- Pond, M.J, Stone, D.,M., Alderman, D.,J. (2006). Comparison of conventional and molecular techniques to investigate the intestinal microflora of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 261(1):194-203.
- Popovic, N.T., Coz-Rakovac, R., Strunjak-Perovic, I. (2007). Commercial phenotypic tests (API 20E) in diagnosis of fish bacteria, a review. *Veterinary Medicine*. 52, 49–53
- Purushothaman, K., Lau, D., Saju, M. J., Syed Nusthaq, SK., Lunny, P., D., Vij, S. Orban, L. (2016), Morpho-histological characterisation of the alimentary canal of the alimentary canal of an important food fish, Asian seabass (*Lateolabrax niloticus*), *Peer Journal*, DOI 10.7717/peerj.2377
- Rai, A. K., Swapna, H. C., Bhaskar, N., Halami, P. M., & Sachindra, N. M. (2010). Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. *Enzyme and Microbial Technology*, 46 (1), 9-13.
- Ranzani-Paiva, M. J. T., Pádua, S. D., Tavares-Dias, M., & Egami, M. I. (2013). Métodos para análise hematológica em peixes. *Maringá: Eduem*, 140p.
- Rawls, J.F., Samuel, B. S. & Gordon, J.I. (2004). Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proceedings of the National Academy of Sciences USA*, 101 (13), 4596-4601.
- Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S., & Menasaveta, P. (2000). Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiotic bacterium (*Bacillus S11*). *Aquaculture*, 191(4), 271-288.
- Rengpipat, S., Rueangruklikhit, T., & Piyatiratitivorakul, S. (2008). Evaluations of lactic acid bacteria as probiotics for juvenile seabass *Lates calcarifer*. *Aquaculture Research*, 39(2), 134–143. <http://doi.org/10.1111/j.1365-2109.2007.01864.x>



- Ringø, E., & Gatesoupe, F. J. (1998). Lactic acid bacteria in fish: a review. *Aquaculture*, 160 (3-4), 177-203.
- Ringo, E., Wesmajervi, M.S., Bendiksen, H. R., Olsen R.E., Jansen, P.A. & Mikkelsen, H. (2000). Lactic Acid Bacteria associated with the digestive tract of Atlantic salmon (*Salmo salar*, L.). *Journal of Applied Microbiology*, 89, 317-322.
- Ringø, E., Lødemel, J. B., Myklebust, R., Kaino, T., Mayhew, T. M., & Olsen, R. E. (2001). Epithelium- associated bacteria in the gastrointestinal tract of Arctic charr (*Salvelinus alpinus* L.). An electron microscopical study. *Journal of Applied Microbiology*, 90(2), 294-300.
- Ringo, E., Zhou, Z., He, S. & Olsen, R.E. (2014). Effect of stress on intestinal microbiota of Arctic charr, Atlantic salmon, rainbow trout and Atlantic cod: a review. *African Journal of Microbiology Research* , 8, 609-618.
- Ringo, E., Olsen, R.E., Gifstad, T.O., Dalmo, R.A., Amlund, H., Hemre, G.I. & Bakke, A.M. (2010) Prebiotics in aquaculture: a review. *Aquaculture Nutrition*, 16, 117–136.
- Rinkinen, M., Jalava, K., Westermarck, E., Salminen, S., & Ouwehand, A. C. (2003). Interaction between probiotic lactic acid bacteria and canine enteric pathogens: a risk factor for intestinal *Enterococcus faecium* colonization. *Vet. Microbiol*, 92, 111-119.
- Riquelme, C., Araya, R., Vergara, N., Rojas, A., Guaita, M., & Candia, M. (1997). Potential probiotic strains in the culture of the *Chilean scallop Argopecten purpuratus* (Lamarck, 1819) . *Aquaculture*, 154 (1): 17-26.
- Robertson, P. A. W., O'Dowd, C., Burrells, C., Williams, P., & Austin, B. (2000). Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Aquaculture*, 185(3-4): 235-243.
- Robins, C. R., Bailey, R. M., Bond, C. E., Brooker, J. R., Lachner, E. A., Lea, R. N. and Scott, W. B. (1991). World fishes important to North Americans. Exclusive of species from the continental waters of the United States and Canada. *Am. Fish. Soc. Spec. Publ.* 21, 243.
- Rowley A. F. (1990). Collection, separation and identification of fish leukocytes. In: van Muiswinkel WB (ed) *Techniques in Fish Immunology*-. *SOS Publications*, 1-6.
- Ruiz-Moyano S., Martín A., Benito M.J., Nevado F.P. & Córdoba M.D.G. (2008). Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in Iberian dry fermented sausages. *Meat Sci.* 80:715-721
- Sabaj M. H., Page L. M., Lundberg J. G., Ferraris C. J., Armbruster J. W. Jr, Friel J. P. and Morris P. J. (2004). *All Castfish Species Inventory Website*. Retrieved from Available at: <http://clade.acnatsci.org/allcatfish>
- Sahoo, P. K. & Saurabh, S. (2008). Lysozyme: an important defence molecule of fish innate immune system. *Aquaculture Research*, 39, 223-239.
- Sakai, M. (1999). Current reseach status of fish immunostimulants. *Aquaculture* 172: 63-92
- Sakata, T. (1990). Microflora in the digestive tract of fish and shellfish. In R. Lesel, *Microbiology in Poecilotherms* (pp. 171-176). Elsevier: Amsterdam.
- Samelis, J., Maurogenakis, F. & Metaxopolous, J. (1994). Characterization of lactic acid bacteria isolated from naturally fermented Greej dry salami. *International Journal of Food Microbiology*, 23 (2): 179-196.
- Schleifer, K. H., Kraus, J., Dvorak, C., Kilpper-Balz, R., Collins, M.D. & Fischer, W. (1985). Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen. nov. . *Systematic and Applied Microbiology*, 6, 183-195.

- Sealey, W. M., Barrows, F.T., Casten, M. & Hardy, R.W. (2009). Dietary Protein Source and Level affects Growth in Neon Tetras. *N Am J Aquac* , 71 (4) : 320-324.
- Seehausen O. (2006). Conservation: losing biodiversity by reverse speciation. *Curr Biol* 16, 334-337.
- Seiverd C. E., (1964). *Haematology for medical technologists*'s. Philadelphia: Lea and febiger.
- Senanan, W., Kapuscinski, A. R., Na-Nakorn, U., & Miller, L. M. (2004). Genetic impacts of hybrid catfish farming (*Clarias macrocephalus* x *C. gariepinus*) on native catfish populations in central Thailand. *Aquaculture*, 235(1-4), 167-184.
- Shabanikakroodi, S. (2014). Characterization of Oil from liver and visceral fats of Patin (*Pangasianodon hypophthalmus* Sauvage) and its use in hand cream preparation. Universiti Putra Malaysia: Master Thesis
- Shakibazadeh, S., Saad, C. R., Christianus, A., Kamarudin, M. S., Sijam, K., & Sinaian, P. (2011). Assessment of possible human risk of probiotic application in shrimp farming. *International Food Research Journal*, 18(1).
- Sharp, M. (1981). The genus *Lactobacillus*. In M. S. Starr, *The Prokaryotes. A Handbook on Habitat, Isolation and Identification of Bacteria, Vol. I.* (pp. pp 1653-1679). Berlin: Springer.
- Shirahigue, L., Silva, M. O., Camargo, A. C., Sucasas, L. F. D. A., Borghesi, R., Cabral, I. S. R. & Oetterer, M. (2016). The feasibility of increasing lipid extraction in Tilapia (*Oreochromis niloticus*) waste by proteolysis. *Journal of Aquatic Food Product Technology* 25, 265-271.
- Shukla, S., & Goyal. A. (2011). 16S rRNA-based identification of a glucan glucan hyper producing *Weisella confusa*. *Enzyme Res* 2011, 250842 doi:10.4061/2011/250842.
- Sica, M. G., Brugnoli, L.I., Marucci, P.L. & Cubitto, M. A. (2012). Characterization of probiotic properties of lactic acid bacteria isolated from an estuarine environment for application in rainbow trout (*Onchorynchus mykiss*, Walbaum) farming. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology*, 101, 869-879.
- Siwicki, A. K., Anderson, D. P., & Rumsey, G. L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veteri. Immunolo. & Immunopatholo.*, 41: 125-139.
- Skelton, P. H. (2001). A complete guide to the freshwater fishes of Southern Africa. *Struik Publishers*.
- Skrodenyte-Arbaciauskiene, V., Sruoga, A., Butkauskas, D. & Skrupskelis, K (2008). Phylogenetic analysis of intestinal bacteria of freshwater salmon *Salmo salar* and sea trout *Salmo trutta trutta* and diet. *Fisheries Science*, 74, 1307-1314
- Slang, V. C. (1973). Comparison on the economic potentials of agricultural land, animal husbandry and oceans fisheries, the case of Aiwa Agriculture. *FAO Technical Conference on Aquaculture Kyoto, Japan*.
- Šližytė, R., Daukšas, E., Falch, E., Storror, I., & Rustad, T. (2005). Characteristics of protein fractions generated from hydrolysed cod (*Gadus morhua*) by-products. *Process Biochemistry* 40(6), 2021-2033.
- Snieszko, S. F., Camper, J., Howard, F., & Pettijohn, L. L. (1960). *Microhaematocrit as a tool in fisheries management. Special Scientific Report-Fisheries*. Washington DC: Fish and Fisheries Wildlife

- Solomon, S. G., Ataguba, G. A., & Imbur, I. (2012). ISSN 2277-7729 Original Article Growth Performance Of Juvenile Clarias Gariepinus Fed Different Dietary Lipid Sources. *International Journal of Research in Fisheries and Aquaculture*, 2(4), 52–55.
- Son, V. M., Chang, C.-C., Wu, M.-C., Guu, Y.-K., Chiu, C.-H., & Cheng, W. (2009). Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. *Fish & Shellfish Immunology*. <http://doi.org/10.1016/j.fsi.2009.02.018>
- Spanggaard, B., Huber, I., Nielsen, J., Sick, E.B., Pipper, C.B., Martinussen, T., Slierendrecht, W. J. & Gram, L (2001). The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. *Environmental Microbiology*, 3, 755-765.
- Stanier, R. Y., Doudoroff, M. & Adelberg, E. A (1975). *General Microbiology Third Edition*. Basingstoke: Macmillan.
- Stone, N. J. (1996). Fish consumption, fish oil, lipids and coronary heart diseases. *Nutrition Committee of American Heart Association Circulation*, 94: 2337-2340.
- Stoskopf, M. K. 1993. Fish medicine Ed., W.B. Sainders Company, London. 27.
- Strom, E. (1988). *Melkesyrebakterier i fisketarm: isolasjon, karakterising og egenskaper [in Norwegian]*. MSci thesis. University of Tromso, Norway: The Norwegian College of Fishery Science.
- Suanyuk, N., Rogge, M., Thune, R., Watthanaphromssuanyuakul, M., Champhat, N., & Wiangkum, W. (2014). Mortality and pathology of hybrid catfish, *Clarias macrocephalus* (Günther) × *Clarias gariepinus* (Burchell), associated with *Edwardsiella ictaluri* infection in southern Thailand. *Journal of Fish Diseases*, 37(4), 385–395. <http://doi.org/10.1111/jfd.12127>
- Subasinghe, R. P. (2005). *Fish Health Management in Aquaculture*. Rome: FAO Fisheries and Aquaculture Department.
- Sugita, H., Okano, R., Suzuki, Y., Iwai, D., Mizukami, M., Akiyama, N., and Matsuura, S. (2002). Antibacterial abilities of intestinal bacteria from larval and juvenile Japanese flounder against fish pathogens. *Fish Sci.*, 68(5): 1004-1011.
- Sugiura, S. H., Dong, F. M., Rathbone, C. K., & Hardy, R. W. (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture*, 159(3-4), 177-202
- Sun, Y. Z.; Yang, H. L.; Ma, R. L.; Lin, W. Y., 2010: Probiotic applications of two dominant gut Bacillus strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*. *Fish Shellfish Immunol*, 29, 803–809
- Suzer, C., Çoban, D., Kamaci, H. O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., & Küçüksari, H. (2008). *Lactobacillus spp.* bacteria as probiotics in gilthead sea bream (*Sparus aurata* L.) larvae: Effects on growth performance and digestive enzyme activities. *Aquaculture*, 280 (1-4): 140-145.
- Tacon, A. G., Metian, M., & Hasan, M. R. (2009). *Feed ingredients and fertilizers for farmed aquatic animals: sources and composition* (No. 540). Food and Agriculture Organization of the United Nations (FAO).
- Talpur, A. D. & Ikhwanuddin, M. (2013). *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian Seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. *Fish Shellfish Immunol*, 34, 254-264.

- Talpur, A. D., Munir, M. B., Mary, A., & Hashim, R. (2014). Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. *Aquaculture*, 426–427, 14–20. <http://doi.org/10.1016/j.aquaculture.2014.01.013>
- Tannock, G. W. (1988). The normal microflora: new concepts in health promotion. *Microbiol. Sci.*, 5(1), 4-8.
- Tannock, G.W., Szylit, O., Duval, Y., & Raibaud, P., (1982). Colonization of tissue surfaces in the gastrointestinal tract of gnotobiotic animals by *Lactobacillus* strains. *Can. J. Microbiol.*, 28, 1196-1198.
- Teugels, G. G. (1986). A systematic revision of the African species of the genus *Clarias*. *Annales Musee Royale de l'Afrique Central Sciences Zoologiques*, 247.
- Teugels, G. G. (2003). State of the art of recent *Siluriform* systematics. In Arratia, G., Kapoor, B. G., Chardon, M., Diogo, R. (Eds). *Catfishes*, 317-352.
- Teugels, G. G., & Adriaens, D. (2003). Taxonomy and phylogeny of Clariidae: an overview. *Catfishes*, 1, 465-487.
- Thammapat, P., Raviyan, P., & Siriamornpun, S. (2010). Proximate and fatty acids composition of the muscles and viscera of Asian catfish (*Pangasius bocourti*). *Food Chemistry*, 122(1), 223–227. <http://doi.org/10.1016/j.foodchem.2010.02.065>
- Thanikachalam, K., Kasi, M., & Rathinam, X. (2010). Effect of garlic peel on growth, hematological parameters and disease resistance against *Aeromonas hydrophila* in African catfish *Clarias gariepinus* ( Bloch ) fingerlings, 3(8), 614–618. [http://doi.org/10.1016/S1995-7645\(10\)60149-](http://doi.org/10.1016/S1995-7645(10)60149-)
- Thapa, N., Pal, J., & Tamang, J. P. (2006). Phenotypic identification and technological properties of lactic acid bacteria isolated from traditionally processed fish products of the Eastern Himalayas. *International Journal of Food Microbiology*, 107: 33-38.
- Thayumanavan T., Vivekanandhan G, Savithamani K., Subashkumar R., & Lakshmanaperumalsamy P. (2003). Incidence of haemolysin-positive and drug-resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. *FEMS Immunol. Med. Microbiol.*, 36: 41-45.
- Thomson, I. S. I. (2014). FA Adedeji, ZB Ibrahim, SA Abdulsalami and AA Akinkunmi. *Journal of Fisheries and Aquatic Science*, 9(6), 496-500.
- Thorpe, A., Reid, C., Van Anrooy, & Brugere, C. (2005). When fisheries influence national policy-making: an analysis of the national development strategies of major fish-producing nations in the developing world. *Marine Policy* 29, 211-222.
- Thy, H. T. T., Thinh, N. H., Tri, N. N., Quy, O. M., Kannika, K., Unajak, S., & Areechon, N. (2017). Identification and Characterization of Potential Probiotic *Bacillus* spp. for Application in Striped Catfish (*Pangasianodon hypophthalmus* [Sauvage, 1878]). *Journal of fisheries and environment*, 41(2), 20-36.
- Thymer J, M. & Simberloff. D. (1996). Extinction by hybridization and introgression. *Annu Rev Ecol Syst* 27:, 83-109.
- Trust, T. J. & Sparrow, R. A. H.. (1974). The bacterial flora in the alimentary tract of freshwater salmonid fish. *Canadian Journal of Microbiology*, 20(9), 1219-1228

- Tucker, C. S., & D'Abramo, L. R. (2008). *Managing high pH in freshwater ponds*. Southern Regional Aquaculture Center.
- Uma, A., Abraham, T. J., Jeyaseelan, M. J. P., & Sundararaj, V. (1999). Effect of probiotic feed supplement on performance and disease resistance of Indian white shrimp *Penaeus indicus*. *H. Milne Edwards. J. Aqua. Trop.*, 14: 159-164
- UNICEF. (2006). United Nation Children Development Fund. Vitamin and mineral deficiency micronutrient initiated Canada. *KIR*, 1-5.
- Vázquez G.R. & Guerrero G.A. (2007). Characterization of blood cells and haematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). *Tissue Cell*, 39(3), 151-160.
- Velasco-Santamaría, Y., & Corredor-Santamaría, W. (2011). Nutritional Requirements of Freshwater Ornamental Fish : A review. *Rev MVZ Cordoba*, 16(2), 2458-2469.
- Vendrell, D., Balcazar, J.L, Ruiz-Zarzuela, I., de Blas, I., Girones, O. & Muzquiz, J.L (2006). *Lactococcus garvieae* in fish: a review. *Comparative Immunology, Microbiology and Infectious Diseases*, 29(4), 177-198.
- Venkat, H. K., Sahu, N. P., & Jain, K. K. (2004). Effect of the feeding *Lactobacillus* based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research* 35(5), 501-507.
- Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews* : *MMBR*, 64(4), 655–71. <http://doi.org/10.1128/MMBR.64.4.655-671.2000>
- Villamil, O., Váquiro, H., & Solanilla, J. F. (2017). Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties. *Food Chemistry*, 224, 160–171 <http://doi.org/10.1016/j.foodchem.2016.12.057>
- Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., & Sanchez-Vazquez, F. J. (2011). Effects of light during early larval development of some aquacultured teleosts. *Aquaculture*, 86-94.
- Vine, N. G., Leukes, W. D., & Kaiser, H. (2004) In vitro growth characteristics of five candidate aquaculture probiotics and two fish pathogens grown in fish intestinal mucus. *FEMS Microbiol. Lett*, 145-152.
- Vine N.G., Leukes W.D., & Kaiser H.. (2006). Probiotics in marine larviculture. *FEMS Microbiol. Rev*, 30(3): 404-427.
- Vlková, E., Kalous, L., Bunešová, V., Rylková, K., Světlíková, R., & Rada, V. (2012). Occurrence of bifidobacteria and *lactobacilli* in digestive tract of some freshwater fishes. *Biologia*, 67, 411-416.
- Wedemeyer, G. A. (1976). Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. *Journal of the Fisheries Board of Canada*, 33(12), 2699-2702.
- Welker, T.L. & Lim, C., (2011). Use of probiotics in diets of tilapia. *Journal of Aquaculture Research and Development.*, S1:014. doi:10.4172/2155.
- Yi, Y., Lin, C. K., & Diana, J. S. (2003). Hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) and Nile tilapia (*Oreochromis niloticus*) culture in an integrated pen-cum-pond system: growth performance and nutrient budgets. *Aquaculture* 217(1-4), 395-408.
- Yogendra, P. (2009). Prevalence, surveillance and virulence characterization of *Flavobacterium columnare* and *F. psychrophilum* in Indian catfishes of sub Himalayan region. *Biochemical and Cellular Archives* 9, 71-82

Yue, G. H., Kovacs, B., & Orban, L. (2003). Microsatellites from *Clarias batrachus* and their polymorphism in seven additional catfish species. *Molecular Ecology Notes*. <http://doi.org/10.1046/j.1471-8286.2003.00486>.

