SYNBIOTIC EFFECTS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AND *LACTOBACILLUS RHAMNOSUS*-SUPPLEMENTED DIET ON GROWTH PERFORMANCE, IMMUNOLOGICAL PARAMETERS AND PROTECTION AGAINST *AEROMONAS VERONII* CHALLENGE IN JUVENILE RED TILAPIAS (*OREOCHROMIS* SPP.)

Miss Mariya Sewaka

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Veterinary Pathobiology
Department of Veterinary Pathology
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2018

ผลของการเสริมซินไบโอติค เยรูซาเล็ม อาร์ติโชคและแลคโตบาซิลลัส แรมโนซัสในอาหารต่อการเจริญเติบโต การตอบสนองทางภูมิคุ้มกันและการป้องกันเชื้อแอโรโมแนส เวโรนิไอในปลาทับทิมวัยอ่อน

น.ส.มาริยา เสวกะ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตร์ดุษฎีบัณฑิต

สาขาวิชาพยาธิชีววิทยาทางสัตวแพทย์ ภาควิชาพยาธิวิทยา

คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2561

Thesis Title	SYNBIOTIC EFFECTS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AND *LACTOBACILLUS RHAMNOSUS*-SUPPLEMENTED DIET ON GROWTH PERFORMANCE, IMMUNOLOGICAL PARAMETERS AND PROTECTION AGAINST *AEROMONAS VERONII* CHALLENGE IN JUVENILE RED TILAPIAS (*OREOCHROMIS* SPP.)
By	Miss Mariya Sewaka
Field of Study	Veterinary Pathobiology
Thesis Advisor	Associate Professor Nopadon Pirarat, D.V.M., Ph.D., DTBVP
Thesis Co Advisor	Assistant Professor Channarong Rodkhum, D.V.M., Ph.D., DTBVP Associate Professor Nantarika Chansue, D.V.M., Ph.D., DTBVP

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial

Fulfillment of the Requirement for the Doctor of Philosophy

.....Dean of the Faculty of Veterinary Science

(Professor Roongroje Thanawongnuwech, D.V.M., M.Sc., Ph.D., DTBVP)

DISSERTATION COMMITTEE

.....Chairman

(Associate Professor Theerayuth Kaewamatawong, D.V.M., Ph.D., DTBVP)

.....Thesis Advisor

(Associate Professor Nopadon Pirarat, D.V.M., Ph.D., DTBVP)

.....Thesis Co-Advisor

(Assistant Professor Channarong Rodkhum, D.V.M., Ph.D., DTBVP)

.....Thesis Co-Advisor

(Associate Professor Nantarika Chansue, D.V.M., Ph.D., DTBVP)

.....Examiner

(Assistant Professor Sawang Kesdangsakonwut, D.V.M., M.Sc., Ph.D., DTBVP)

.....Examiner

(Associate Professor Chackrit Nuengjamnong, D.V.M., M.Sc., D.Tech.Sc.)

.....External Examiner

(Associate Professor Surintorn Boonanuntanasarn, Ph.D.)

มาริยา เสวกะ : ผลของการเสริมซินไบโอติค เยรูซาเล็ม อาร์ติโชคและแลคโตบาซิลลัส แรมโนซัสในอาหารต่อการเจริญเติบโต การตอบสนองทางภูมิคุ้มกันและการป้องกันเชื้อแอโรโมแนส เวโรนิไอ ในปลาทับทิมวัยอ่อน
SYNBIOTIC EFFECTS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AND *LACTOBACILLUS RHAMNOSUS*-SUPPLEMENTED DIET ON GROWTH PERFORMANCE, IMMUNOLOGICAL PARAMETERS AND PROTECTION AGAINST *AEROMONAS VERONII* CHALLENGE IN JUVENILE RED TILAPIAS (*OREOCHROMIS* SPP.)
อ.ที่ปรึกษาหลัก : รศ.น.สพ.ดร.นพดล พิฬารัตน์, อ.ที่ปรึกษาร่วม : ผศ.น.สพ.ดร.ชาญณรงค์ รอดคำ, รศ.สพ.ญ.ดร.นันทริกา ชันซื่อ

ซินไบโอติคคือการรวมของโพรไบโอติคและพรีไบโอติค ซึ่งมีการนำมาใช้อย่างแพร่หลายในการเสริมอาหารสำหรับปลาทับทิม วัตถุประสงค์ในการศึกษานี้คือ เพื่อศึกษาผลของการเสริมซินไบโอติค เยรูซาเล็ม อาร์ติโชคและแลคโตบาซิลลัส แรมโนซัสในอาหารต่อค่าเลือด การเจริญเติบโต รูปร่างของลำไส้ การตอบสนองทางภูมิคุ้มกัน แบคทีเรียในลำไส้และการป้องกันเชื้อแอโรโมแนส เวโรนิไอ ในปลาทับทิมวัยอ่อน โดยใช้ปลาทับทิมในการทดลอง 180 ตัว (น้ำหนักเฉลี่ยตัวละ 14.05±0.42 กรัม) แบ่งเลี้ยงในบ่อ 6 บ่อ ภายใต้สภาวะที่มีออกซิเจนและอุณหภูมิ 25–28 องศาเซลเซียส ให้อาหารปลาทับทิมแต่ละบ่อแตกต่างกันดังนี้ อาหารสูตรควบคุม อาหารเสริมแก่นตะวัน 5.0 กรัม/กิโลกรัม อาหารเสริมแก่นตะวัน 10.0 กรัม/กิโลกรัม อาหารเสริมแลคโตบาซิลลัส แรมโนซัส 10^8 CFU/กรัม อาหารเสริมแก่นตะวัน 5.0 กรัม/กิโลกรัมและแลคโตบาซิลลัส แรมโนซัส 10^8 CFU/กรัม อาหารเสริมแก่นตะวัน 10.0 กรัม/กิโลกรัมและแลคโตบาซิลลัส แรมโนซัส 10^8 CFU/กรัม หลังจากให้อาหารเป็นระยะเวลา 4 สัปดาห์เก็บตัวอย่างเพื่อศึกษาค่าเลือด การเจริญเติบโต รูปร่างของลำไส้ การตอบสนองทางภูมิคุ้มกัน แบคทีเรียในลำไส้ หลังจากนั้นแบ่งปลาแต่ละกลุ่ม มาฉีดด้วยเชื้อแอโรโมแนส เวโรนิไอ เข้มข้น 10^7 CFU/ตัว ด้วยวิธีการฉีดเข้าช่องท้อง ผลการทดลองพบว่าอาหารเสริมซินไบโอติคเพิ่มระดับ คอเลสเตอรอลและระดับโปรตีนในเลือด ค่าสมรรถนะการเจริญเติบโตของปลาที่กินอาหารเสริมซินไบโอติค โพรไบโอติคและพรีไบโอติคสูงกว่าปลาที่กินอาหารสูตรควบคุมอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ค่าพื้นที่การดูดซึมของลำไส้ส่วนต้นและลำไส้ส่วนปลายของปลาที่กินอาหารเสริมซินไบโอติคสูงกว่าปลาที่กินอาหารเสริม โพรไบโอติคและพรีไบโอติคอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) พบว่าเซลล์กอบเลทในลำไส้ส่วนต้นมีชนิดของเซลล์มิวคัสแบบกรดและเซลล์มิวคัสแบบกรด-กลาง ของปลาที่กินอาหารเสริมซินไบโอติคสูงกว่าปลาที่กินอาหารกลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) และพบว่าเซลล์กอบเลทในลำไส้ส่วนปลายมีชนิดของเซลล์มิวคัสแบบกรด เซลล์มิวคัสแบบกลางและเซลล์มิวคัสแบบกรด-กลาง ของปลาที่กินอาหารเสริมซินไบโอติคสูงกว่าปลาที่กินอาหารกลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ปลาที่กินอาหารเสริมซินไบโอติคมีไลโซไซม์และจำนวนโพรไบโอติคแบคทีเรียในลำไส้สูงกว่าปลาที่กินอาหารกลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) พบว่าอัตราการตายของปลาที่กินอาหารเสริมซินไบโอติคมีน้อยกว่าปลาที่กินอาหารกลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) จากผลการทดลองพบว่าการเสริมซินไบโอติค เยรูซาเล็ม อาร์ติโชคและแลคโตบาซิลลัส แรมโนซัสมีผลดีต่อค่าเลือด การเจริญเติบโต รูปร่างของลำไส้ การตอบสนองทางภูมิคุ้มกัน แบคทีเรียในลำไส้และการป้องกันเชื้อแอโรโมแนส เวโรนิไอ ในปลาทับทิมวัยอ่อน

สาขาวิชา พยาธิชีววิทยาทางสัตวแพทย์ ลายมือชื่อนิสิต.....
ปีการศึกษา2561 ลายมือชื่อ อ.ที่ปรึกษาหลัก.....
ลายมือชื่อ อ.ที่ปรึกษาร่วม.....
ลายมือชื่อ อ.ที่ปรึกษาร่วม.....

5775510131 : MAJOR VETERINARY PATHOBIOLOGY

KEYWORD : *Aeromonas veronii*, Jerusalem artichoke, Juvenile red tilapia, *Lactobacillus rhamnosus*, Synbiotic

Mariya Sewaka

SYNBIOTIC EFFECTS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AND *LACTOBACILLUS RHAMNOSUS*-SUPPLEMENTED DIET ON GROWTH PERFORMANCE, IMMUNOLOGICAL PARAMETERS AND PROTECTION AGAINST *AEROMONAS VERONII* CHALLENGE IN JUVENILE RED TILAPIAS (*OREOCHROMIS* SPP.)

Advisor: Assoc. Prof. Nopadon Pirarat, D.V.M., Ph.D., DTBVP Co-advisor: Asst. Prof. Channarong Rodkhum, D.V.M., Ph.D., DTBVP, Assoc. Prof. Nantarika Chansue, D.V.M., Ph.D., DTBVP

Synbiotics, a synergistic combination of probiotics and prebiotics, are now highlighted as one of the most practical nutritional supplements in red tilapia farms. The aim of the present study was to assess the effect of the dietary inclusion of Jerusalem artichoke, *Lactobacillus rhamnosus*, or their combination, on serum biochemical, growth performance, intestinal morphology, immune parameters, intestinal bacterial count and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.). One hundred and eighty male red tilapias (average body weight of 14.05±0.42 g) were distributed into six 1000-liter tanks containing water under continuous aeration and an average temperature of 25–28°C. Tilapias were fed a basal diet (control, C), a 5.0 g kg^{-1} Jerusalem artichoke-supplemented diet (5K), a 10.0 g kg^{-1} Jerusalem artichoke-supplemented diet (10K), a 10^8 CFU g^{-1} LGG-supplemented diet (LGG), a 5.0 g kg^{-1} Jerusalem artichoke +10^8 CFU g^{-1} LGG-supplemented diet (5K+LGG), and a 10.0 g kg^{-1} Jerusalem artichoke +10^8 CFU g^{-1} LGG-supplemented diet (10K+LGG) for 4 weeks. At the end of the feeding period, blood chemical, the growth performance, intestinal morphology, immune parameters, and intestinal bacterial count were analyzed. Then, fish from each diet were intraperitoneal-injected with 10^7 CFU/fish of *Aeromonas veronii*. The results showed that the synbiotic-supplemented diet increased the total cholesterol and total protein levels of red tilapia. The WG, SGR, and ADG of fish fed with the 5K + LGG, 10K + LGG, LGG, 5K, and 10K diets were significantly ($P < 0.05$) higher than that fish fed the control diet. The absorptive area of the proximal and distal intestine of fish fed 10K+LGG was significantly higher ($P < 0.05$) than those fed the probiotic-supplemented diets (LGG), the prebiotic-supplemented diets (5K and 10K) and the control diet. The goblet cell counts showed that the numbers of acid mucous cells and double-staining mucous cells of the fish fed the synbiotic-supplemented diet (5K+LGG) in the proximal intestine were significantly higher ($P < 0.05$) than those of fish fed other diets. Moreover the results found that the fish fed the synbiotic-supplemented diets (5K+LGG and 10K+LGG) showed the numbers of acid mucous cells, neutral mucous cells and double-staining mucous cells in the distal intestine were significantly higher ($P < 0.05$) than those of fish fed other diets. The fish fed with the synbiotic-supplemented diets (5K + LGG and 10K + LGG) experienced a significant increase ($P < 0.05$) in the lysozyme activity and the number of probiotic bacteria in comparison with other groups. The cumulative mortalities of fish fed the synbiotic-supplemented diets (5K+LGG, 10K+LGG) were significantly lower ($P < 0.05$) than those of fish fed C, 5K, 10K, and LGG. Results suggested that Jerusalem artichoke and LGG-supplemented diets had the beneficial effect on serum biochemical, growth performance, intestinal morphology, immune parameters, intestinal bacterial count and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.).

Field of Study: Veterinary Pathobiology

Academic Year: 2018

Student's Signature.....

Advisor's Signature.....

Co-advisor's Signature.....

Co-advisor's Signature.....

ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest gratitude to my advisor, Associate Professor Dr. Nopadon Pirarat for his excellent instruction and suggestion during the planning, development of this dissertation, and review the manuscripts. Particularly, his devotion which has enabled me to carry out my success.

I also would like to express my very great appreciation to my co-advisor, Assistant Professor Dr. Channarong Rodkhum for his consulting and encouraging me the knowledge, design of experiment, and problem-solving on the approach to this research work. His willingness to give his time for advising which made me to very appreciated.

I would like to offer my special thanks to my co-advisor, Associate Professor Dr. Nantarika Chansue for her kindness supporting and guiding me in the design of experiment and providing the material for the fish culture.

I also would like to thank Associate Professor Dr. Surintorn Boonanuntanasarn for her help in providing the Jerusalem artichokes and analyzing the immune parameters of the fish.

I am also very grateful to thank Dr. Clara Trullas, postdoctoral researcher for helping and consulting in experimental research, data analysis, and English academic writing. In addition, she is the person who encourage me in the difficult period of my study life. I really appreciate the time our spent together.

I also would like to thank the dissertation committee, Associate Professor Theerayuth Kaewamatawong, Assistant Professor Sawang Kesdangsakonwut, and Associate Professor Chackrit Nuengjamnong for their suggestion on the thesis writing.

I also would like to thank Miss Thanyathorn Hirankanokchot, Miss Thanyarat Ritrung, Miss Pannatika Tasuwan, Miss Suyanee Pradit, and Miss Kanorkorn Ananchotikul who helped me in culture the fish and counted the goblet cells.

I am especially appreciative for Aeromonas veronii was given by Ha Thanh Dong. I also would like to thank Mrs. Maligned Jongjaroanjai for helping me to providing the material for the fish culture.

I am also very appreciative to thank, Mrs. Waree Niyomtham for suggestion in microbiological techniques.

I also would like to thank Mr. Sitthichok Lacharoje, Miss Panitnan Puryathi, and Narisara Chimnakboon for helping me in histological techniques.

Furthermore, I would like to thank my friends, especially Mr. Cherdpong Phupolphan, Miss Piyanoot Fonghem, Miss Chanokchon Setthawongsin, Miss Poranee Banyatworakul, and Miss Chatvadee kornsuthisopon for supporting and cheering me. This work would not be success without their support.

I also would like to thank the Rajamangala University of Technology Srivijaya, the 90 th ANNIVERSARY OF CHULALONGKORN UNIVERSITY FUND (Ratchadaphiseksomphot Endowment Fund) and Overseas Academic Presentation Scholarship for Graduate Students, Graduate School, Chulalongkorn University for financial support.

Finally, I am grateful to my parents and friends for their encouragement and supporting in every aspect of my life.

Mariya Sewaka

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percent
°C	degree celcius
AB	alcian blue
AB-PAS	alcian blue - Periodic acid-Schiff
A. bestiarum	*Aeromonas bestiarum*
ACH50	alternative complement pathway hemolytic activity
A. baerii	*Acipenser baerii*
ADG	average daily gain
A. hydrophila	*Aeromonas hydrophila*
ALT	alanine transaminase
ANOVA	analysis of variance
AOAC	Association of Official Agricultural Chemists
A. sobria	*Aeromonas sobria*
AST	aspartate aminotransferase
A. veronii	*Aeromonas veronii*
AXOS	arabinoxylan oligosaccharide

B. circulans	*Bacillus circulans*
B. clausii	*Bacillus clausii*
B. coagulans	*Bacillus coagulans*
B. licheniformis	*Bacillus licheniformis*
B. subtilis	*Bacillus subtilis*
BUN	blood urea nitrogen
C3	complement component 3
C4	complement component 4
C5	complement component 5
C7	complement component 7
C. auratus	*Carassius auratus*
C. idella	*Ctenopharyngodon idella*
CFU g^{-1}	Colony forming units per gram
CUACUC	Chulalongkorn University Animal Care and Use Committee
cm.	Centimeter
CO_2	carbon dioxide
COS	Chitosan oligosaccharide

D-bilirubin	direct bilirubin
D. hansenii	*Debaryomyces hansenii*
D. rerio	*Danio rerio*
DO	dissolved oxygen
ECPs	extracellular products
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
F. columnare	*Flavobacterium columnare*
FCR	feed conversion ratio
FOS	fructo oligosaccharide
g kg^{-1}	gram per kilogram
GAP	Good Aquaculture Practice
GOS	galacto oligosaccharide
H	hours
H. cyanoguttatus	*Herichthys cyanoguttatus*
H. huso	*Huso huso*
ha	hectare
HPF	high-power field

HU	hemolytic unit
IL1β	Interleukin 1 beta
Ig	Immunoglobulins
JA	Jerusalem artichoke
L	liter
L. crocea	*Larimichthys crocea*
L. bulgaricus	*Lactobacillus bulgaricus*
L. crocea	*Lactobacillus crocea*
L. lactis	*Lactobacillus lactis*
L. sakei	*Lactobacillus sakei*
LGG	*Lactobacillus rhamnosus* G-G
L. plantarum	*Lactobacillus plantarum*
L. rohita	*Labeo rohita*
m^2	square meter
M. terminalis	*Megalobrama terminalis*
mg L^{-1}	milligram per liter
Min	minutes
mL	milliliter

MOS	mannan oligosaccharide
MRS	de Man, Rogosa and Sharpe Agar
M. rosacea	*Mycteroperca rosacea*
NaCl	sodium chloride
OD	optical density
O. mykiss	*Oncorhynchus mykiss*
O. niloticus	*Oreochromis niloticus*
PBS	Phosphate-buffer saline
PAS	Periodic Acid-Schiff
P. acidilactici	*Pediococcus acidilactici*
P. olivaceus	*Paralichthys olivaceus*
P. bocourti	*Pangasius bocourti*
P. corruscans	*Pseudoplatystoma corruscans*
P. olivaceus	*Panaeolus olivaceus*
P. pentosaceus	*Pediococcus pentosaceus*
P. scalare	*Pterophyllum scalare*
R. canadum	*Rachycentron canadum*
R. frisii	*Rutilus frisii*

S. aurata	*Sparus aurata*
S. salar	*Salmo salar*
SCFA	short chain fatty acids
scFOS	short chain fructo oligosaccharide
SGR	specific growth rate
TNFα	Tumor necrosis factor alpha
T. ovatus	*Trachinotus ovatus*
TSA	trypticase soy agar
TSB	trypticase soy broth
UV	ultraviolet
W. cibaria	*Weissella cibaria*
WG	weight gain

CHAPTER I
INTRODUCTION

Oreochromis sp. (red tilapia) is an important cultured freshwater fish in Thailand (Fisheries, 2005; Lin et al., 2008; Moffitt and Cajas-Cano, 2014). Red tilapia provides one of the sources of animal protein worldwide due to their fast growth, tolerance to harsh water quality and disease resistance (Yamprayoon and Sukhumparnich, 2010). However, intensive tilapia farming is influenced by infectious diseases, which are the major causes of economic losses in tilapia culture (Joseph and Carnahan, 1994; Kayansamruaj et al., 2014). Among bacterial diseases, the genus *Aeromonas* such as *A. veronii, A. hydrophila,* and *A. bestiarum* have been related to infections in various fish species including tilapia (Kozinska, 2007; Rey et al., 2009). Genus *Aeromonas* is gram-negative rods, facultatively anaerobic bacteria, and oxidase positive. *Aeromonas* spp. can be isolated from soil and water environments, a wide range of food products and from infected human and diseased fish (Janda et al., 1994; Janda and Abbott, 1998; Cui et al., 2007; Grobner et al., 2007; Sanchez-Cespedes et al., 2009). *Aeromonas* spp. have been associated with emerging food-borne disease in human (Martin-Carnahan and Joseph, 2005; Janda and Abbott, 2010). Several studies reported that wound infections, diarrhea, and septicemia in human illness were caused by the mesophilic aeromonads such as *A. veronii* and *A. hydrophila*. The symptoms were linked to the consumption of raw fish, seafood, and contaminated water or food (Pablos et al., 2009; Figueras and Beaz-Hidalgo, 2014). *A. veronii* septicemia has been reported in several fish species such as tilapia, carp, perch, catfish, and salmon (Joseph and Carnahan, 1994). Recently, the disease outbreak caused by concurrent infections with *A. veronii*

was reported with the high mortality losses in cultured tilapia farms in Thailand (Dong et al., 2015). Experimental infection of *A. veronii* isolated from the disease outbreak confirmed the high mortality in tilapia fingerling (Hoseinifar et al., 2015). The clinical signs included hemorrhagic septicemia, red sore disease, and ulcerative lesions in infected tilapias (Joseph and Carnahan, 1994). The various virulence factors associated with *A. veronii* have been described (Li et al., 2011; Tomas, 2012; Lowry et al., 2014; Liu, 2015). The diseased fish were contributed by the various virulence factors of *A. veronii* (Cai et al., 2012; Abolghait et al., 2013).

Disinfectants and antibiotics were used in the prevention or treatment of aquatic disease when disease outbreaks occur in aquaculture production. Using antibiotics for disease control may induce the emergence of bacterial resistance. Probiotics were used as feed additive and as biological control agents for a reduction in chemical agents and antibiotics used in aquaculture (Nikoskelainen et al., 2001).

Prebiotics are defined as non-digestible feed ingredients which beneficially affect to host health by selectively stimulating the growth and/or activity of microflora bacteria in the colon. Unique characteristics of feed ingredients as prebiotic properties are the limited hydrolysis and absorption in the upper gastrointestinal tract with selective stimulation of beneficial intestinal bacteria potentially related to health and well-being (Gibson and Roberfroid, 1995)**.** Prebiotics are found in several sources such as Jerusalem artichokes, leeks, asparagus, chicory, garlic, onions, wheat, oats, and soybeans (Vanloo et al., 1995). Jerusalem artichoke (*Helianthus tuberosus*), Kantawan, a member of family *Asteraceae* is a root vegetable and a native plant in central-eastern North America (Kleessen et al., 2007). Jerusalem artichoke has been introduced to Thailand and can be harvested after 100–140 days, and crop yields of Jerusalem

artichoke are approximately 13-19 ton per ha (Moshfegh et al., 1999). Jerusalem artichoke contains of the most common prebiotics such as inulin and fructooligosaccharide (FOS) (Moshfegh et al., 1999; Kleessen et al., 2007). Inulin is a low digestible carbohydrate and one of the most common prebiotics used in feed for livestock and aquatic animals (Grabitske and Slavin, 2009; Lied et al., 2011; Slavin, 2013). Previous studies have been reported that dietary supplement of inulin in fish feed enhanced growth performance, enhanced intestinal microbiota, modulate intestinal microbiota, and improved hematological and immunological parameters in fish (Mahious et al., 2006; Reza et al., 2009; Ibrahem et al., 2010; Ortiz et al., 2013). Fructooligosaccharide (FOS) are prebiotics utilized in animal feeds. FOS has been shown to significantly increase fecal bifidobacteria at fairly low levels of consumption (Costabile et al., 2010). However, using the inulin and FOS as a prebiotics feed additive in the animal feed industry is limited by the high cost of the inulin and FOS extraction process.

Probiotics are defined as a live microbial which have beneficial effects on the host by production of compounds for inhibition toward pathogens (Bruno and Montville, 1993; Perez et al., 2014), by enhancement of the host response to disease (Madsen, 2006; Preidis et al., 2011), by improving nutritional and microbial balance in the environment (Villamil et al., 2002). Lactobacilli are the most common use as probiotics in human beings and farm animals (Gatesoupe, 2007). Several studies found that Lactobacilli were isolated and selected from aquatic environments such as *L. rhamnosus, L. plantarum*, *L. bulgaricus* (Madsen, 2006). Among many *Lactobacillus* strains used as effective probiotics in human or animal. *L. rhamnosus* has been reported to control bacterial infection such as *A. salmonicida*, *Vibrio anguillarum* and

Flavobacterium psychrophilum in rainbow trouts and turbots (Nikoskelainen et al., 2001) and *Edwardsiella tarda* in tilapia (Pirarat et al., 2006). Therefore, supplementation of probiotics, of human origin in aquaculture feed may not only promote an aquatic animal's health but also benefit human health.

Synbiotics are nutritional supplements that combine probiotics and prebiotics in a form of synergistic effect (Gibson and Roberfroid, 1995). A synergistic effect occurs when the combined effects of the two ingredients are significantly greater than the amount of the effects of each ingredient alone. Previous studies have been reported the administration of synbiotics in fish aquaculture (Ai et al., 2011; Geng et al., 2011; Ye et al., 2011; Mehrabi et al., 2012). However, studies of the potential benefit of the synbiotic between *L. rhamnosus* GG and Jerusalem artichoke on its basal immune response and the increased immunity and protection against *A. veronii* infection on red tilapia are limited. Therefore, in this study, we investigate the synbiotic effects of dietary Jerusalem artichoke and *L. rhamnosus* and protection against *A. veronii* infection on red tilapia.

Research Hypothesis

1. The synbiotic has the beneficial effect on growth performance and the serum biochemical profiles in juvenile red tilapia.
2. The synbiotic has the beneficial effect on intestinal morphology, immunological parameters and intestinal bacterial count in juvenile red tilapia.
3. The synbiotic could protect juvenile red tilapia against *A. veronii* infection.

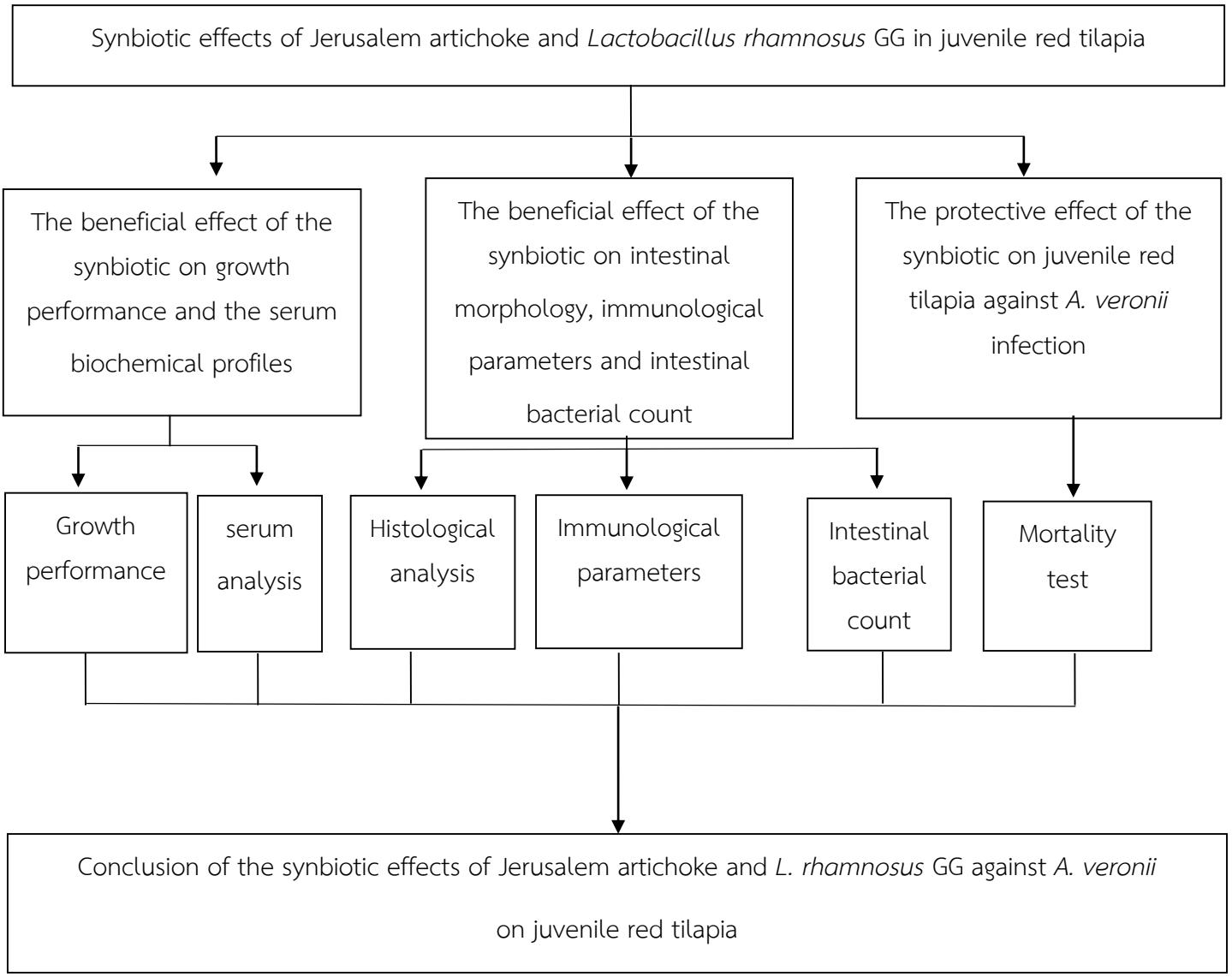
Research Objectives

1. To study the beneficial effects of the synbiotic on growth performance and the serum biochemical profiles in juvenile red tilapia.

2. To evaluate the beneficial effects of the synbiotic on intestinal morphology, immunological parameters and intestinal bacterial count in juvenile red tilapia.

3. To assess the protective effects of the synbiotic on juvenile red tilapia against *A. veronii* infection.

Conceptual framework



Advantages of Study

1. This study investigated the synergistic effect of symbiotic between Jerusalem artichoke or kantawan and probiotic *L. rhamnosus* GG on growth performance, gut mucosal immunity and immunomodulation of juvenile red tilapia.
2. This study leads to understanding about management to reduce mortality loss during the juvenile stage, increasing tilapia growth performance and eventual increasing profit of commercial tilapia farms.

Keywords

Aeromonas veronii, Jerusalem artichoke, juvenile red tilapia, *Lactobacillus rhamnosus*, synbiotic

CHAPTER II
LITERATURE REVIEW

2.1 The red tilapia (*Oreochromis* spp.)

Oreochromis spp. (red tilapia) is a member of phylum Chordata, class Actinopteri, order Cichliformes, family Cichlidae, genus *Oreochromis*. The red tilapia in Thailand was developed from hybridized with various pure and mixed strains of *Oreochromis niloticus* (Fisheries, 1983). Red tilapia provides one of the sources of animal protein worldwide due to their fast growth, tolerance to harsh water quality and disease resistance (Yamprayoon and Sukhumparnich, 2010). The areas for red tilapia production in Thailand include northeastern, central and northern regions. Red tilapia has been increased rapidly and economically important to Thailand in both domestic and export markets. In 2004, Production of red tilapia in Thailand had risen to nearly 20,711 tonnes with 13% of total tilapias and 4% of total freshwater aquaculture production (Fisheries, 2005). However, intensive red tilapia farming is influenced by infectious diseases, which are the major causes of economic losses in red tilapia culture. Among bacterial diseases, the *A. veronii* has been reported to be natural infection in Nile tilapia farms in Thailand and their pathogenicity in the red tilapia fingerling model. (Kozinska, 2007; Rey et al., 2009).

2.2 *Aeromonas veronii*

A. veronii is a member of phylum Proteobacteria, class Gammaproteobacteria, order Aeromonadales , family Aeromonadaceae, genus *Aeromonas*, species *A. veronii.* It is described as gram-negative rods, facultatively anaerobic, non-spore forming bacterium (Janda and Abbott, 2010). It can grow at the temperature ranging from 4 to 42 °C.

2.2.1 Virulence factors of pathogenic *Aeromonas*

Several virulence factors of pathogenic *Aeromonas* were reported such as extracellular products (ECPs), surface polysaccharides, biofilm formation, iron-binding systems, secretion systems, adhesins, motility, and flagella. Previous studies reported that the virulence factors are found in many *A. hydrophila* and *A. veronii* strains, but are absent in most *A. caviae* isolates. The virulence factors are the major components of bacterial septicemia associating with bacteria adhesion, colonization and invasion to the host cells after hematogenous dissemination before it causes diseases and organ damage.

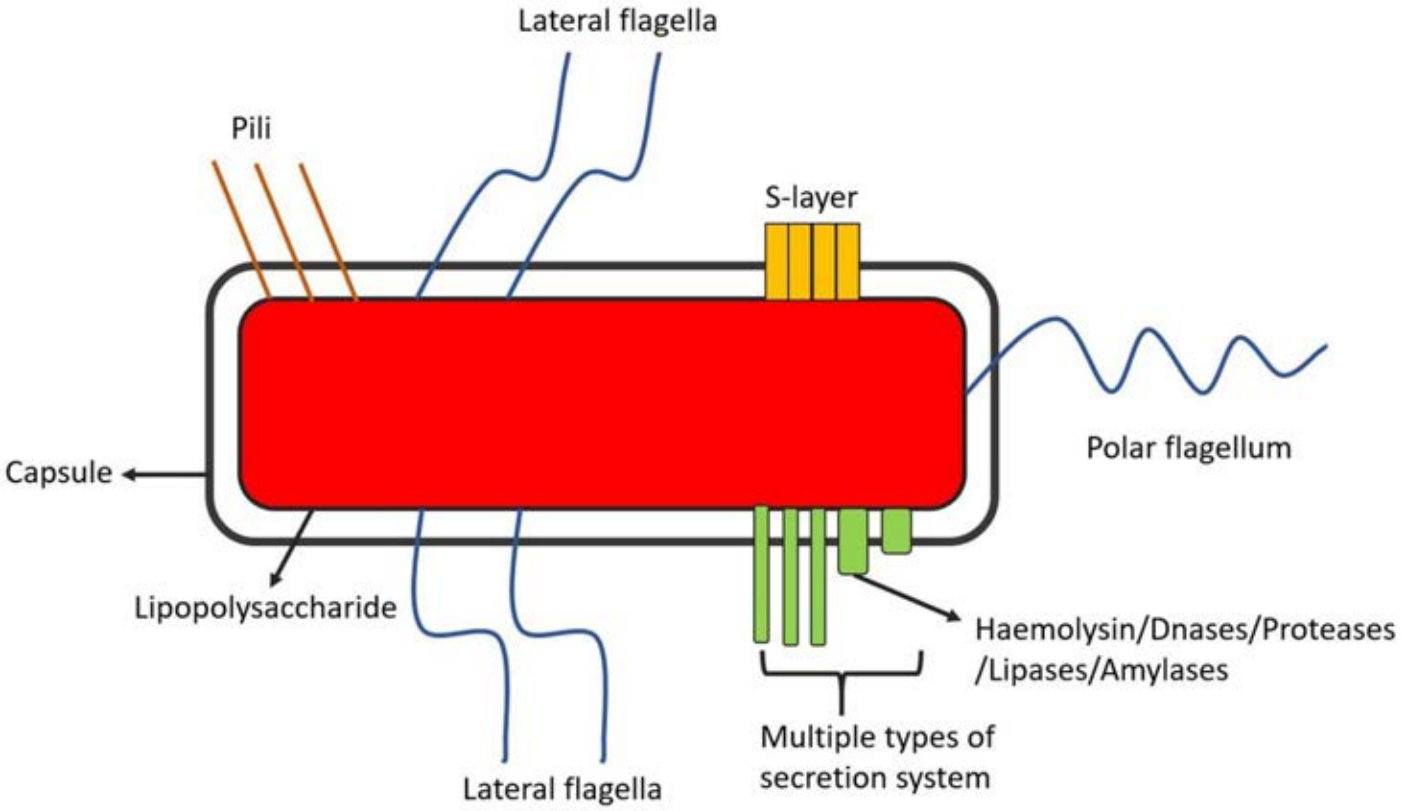


Figure 1 Virulence factors of pathogenic *Aeromonas* spp. include polar flagellum, lateral flagella, pili lipopolysaccharide, capsule, S-layer, multiple types of secretion system, and extracellular enzymes (Hemolysin/Dnases/Proteases/Lipases/Amylases) (Tomas, 2012; Lowry et al., 2014)

2.2.2 Pathogenic *Aeromonas* cause fish diesase

A. hydrophila and *A. veronii* cause hemorrhagic septicemia in tilapia, carp, perch, catfish, and salmon. Symptoms of septicemia including distended anus abdomen swelling, exophthalmia, detachment of the mucosa in the gastrointestinal tract, hemorrhages in internal organs, and jellylike discharge in the intestine were found (Rey et al., 2009; Kozinska and Pekala, 2012). *A. hydrophila* and *A. veronii* have been documented as a cause of red sore disease in bass and carp and ulcerative infections in catfish, cod, carp, and goby (Joseph and Carnahan, 1994). Mesophilic *Aeromonas* species infection in fish have been linked to a disease outbreak on fish farms and leading elevated mortality and economic losses in aquaculture (Monette et al., 2006). Previous studies have been shown that *A. veronii* bv. veronii caused the ulcerative syndrome in Chinese long snout catfish (*Leiocassis longirostris*) while *A. sobria* caused tail-rot disease in tilapia (Cai et al., 2012). The recent study reported that *A. veronii* and *F. columnare* are an emerging concurrent infection in cultured tilapia farms in Thailand (Dong et al., 2015). The data of experimental challenge showed that *A. veronii* isolates were highly pathogenic to tilapia fingerling and exhibited hemorrhages in the liver, kidney, and intestine and similar to the clinical signs of natural diseased fish (Hoseinifar et al., 2015).

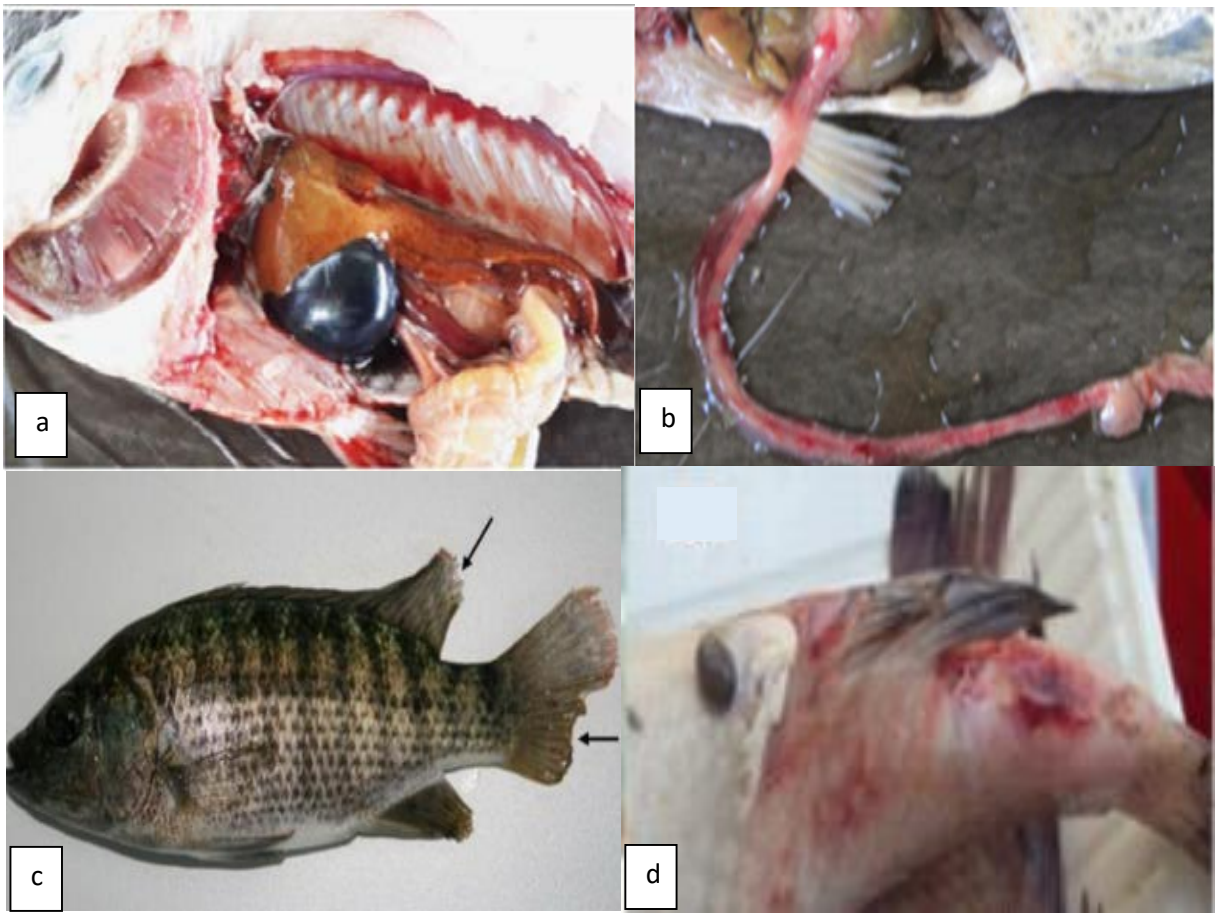


Figure 2 Gross finding of tilapia infected by *Aeromonas* spp. Diseased Nile tilapia exhibited necrotic gills, swollen gall bladder, hemorrhagic liver (a), enteritis (b), darkness in the skin with fin rot in caudal and dorsal fins (arrows) (c), and ulcerative lesions (d) (Yardimci and Aydin, 2011; Dong et al., 2015)

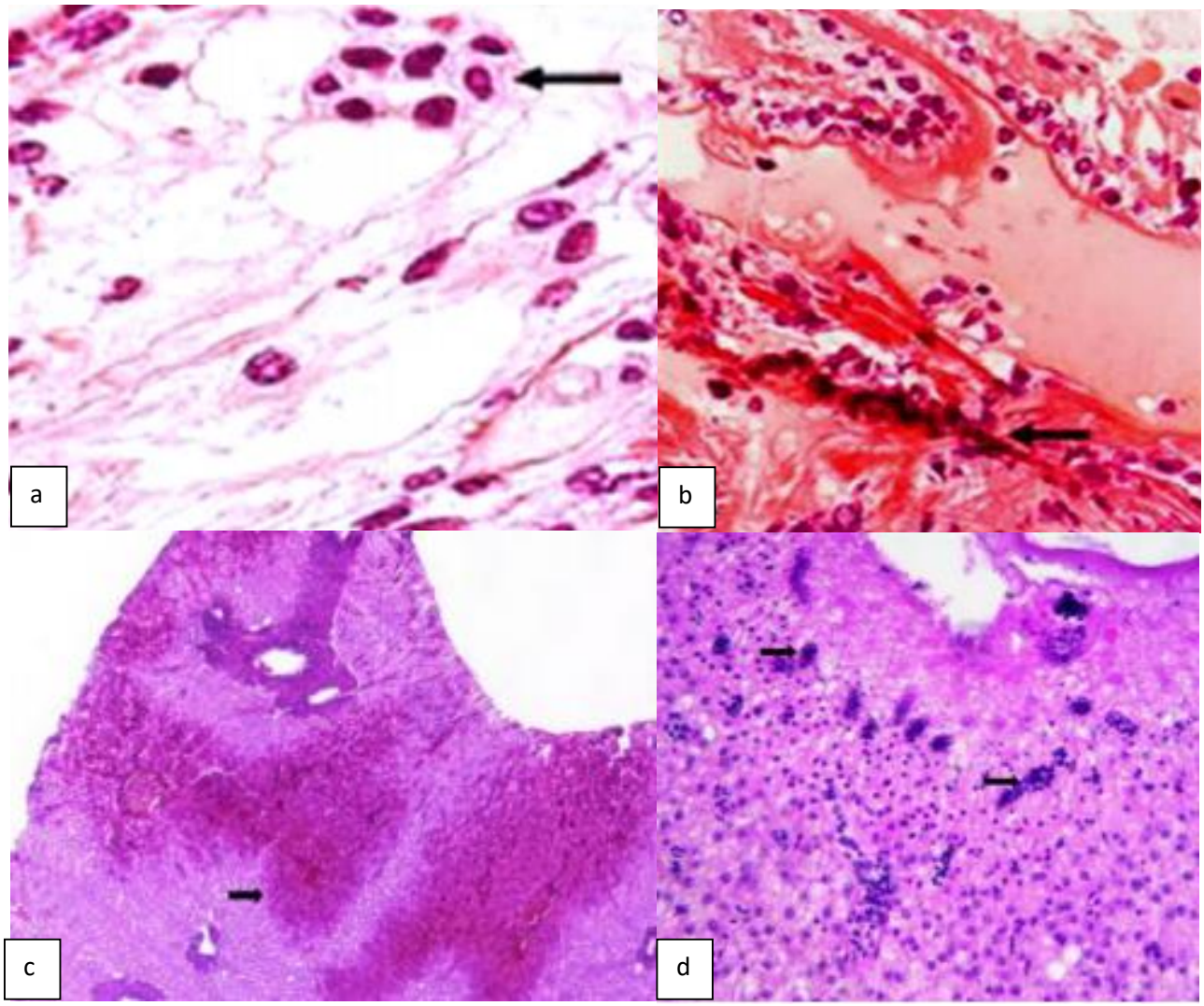


Figure 3 Histopathological finding of tilapia infected by *Aeromonas* spp. Infected gill lamella, hemocyte aggregation (arrow) (H&E, 100x) (a) Infected fish, Hemocyte infiltration in digestive system (arrow) (H&E, 100x) (b) Hemorrhage in liver (arrow) (H&E, 40x) (c) lymphocyte infiltration (arrow) (H&E, 200x) (Yardimci and Aydin, 2011; AlYahya et al., 2018)

2.3 Immune response in fish

The immune system of teleosts, or bony fish, is composed of innate and adaptive immune responses. Several studies have been described as innate and adaptive immune responses against pathogen *Aeromonas* infection in fish. Innate immune responses act as the first line of defense against all pathogens and also play an instructive role in the adaptive immune responses. Elements of innate immune components include antibacterial peptides, lysozyme, lectins, and the complement system. Many of the antibacterial peptides including defensins, cathelicidins, hepcidins, histone-derived peptides, and piscidins have been found in the skin secretion of fish species (Masso-Silva and Diamond, 2014). Previous studies showed the innate immune in fish mucus responses against pathogenic *Aeromonas* infection. The antimicrobial effect of histone-derived peptides in the skin mucus of Atlantic cod inhibit of *A. hydrophila* infection (Joseph and Carnahan, 1994). The lectins showed agglutination activity against the pathogenic bacterium *A. hydrophila* infection induces an inflammatory response such as TNFα, IL1β, and iNOS in infected zebrafish (Rodriguez et al., 2008). *A. salmonicida* induces mobilization of leucocytes in the gut of Atlantic salmon (Ringo et al., 2007).

2.3.1 Mucus cells

Mucus cells are a crucial first line of gut mucosal defense against pathogenic bacterial infection. Mucous goblet cells are composed of acid and neutral mucins, which aid in lubricating, trapping and removing pathogens (Ngamkala et al., 2010; Lazado and Caipang, 2014). Acid mucins are further differentiated based on their histochemical properties into sulfate-containing mucins (sulfomucins) and sialic acid-

containing mucins (sialomucins) (Filipe, 1979). Innate immune responses in a fish act as the first line of defense against all pathogens and also play an instructive role in the adaptive immune responses.

2.3.2 Lysozyme

Elements of innate immune components include lysozyme, the complement pathways and natural antibodies (Alexander and Ingram, 1992; Magnadóttir, 2006;; Gomez et al., 2013). Lysozyme is described as a mucolytic enzyme, which digests the β-(1,4) linkages between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layers of Gram-positive bacteria. In general, lysozyme are not directly damaged Gram-negative bacteria. However, lysozyme can against the Gram-negative bacteria after the outer membrane of Gram-negative bacteria was destroyed, then lysozyme can expose to the inner peptidoglycan layer of bacteria. Lysozyme is activated the polymorphonuclear leucocytes and macrophages and opsonin of the complement system for phagocytosis (Saurabh and Sahoo, 2008; Uribe et al., 2011).

2.3.3 Complement system

The complement system in fish have been documented to several isotypic such as C3, C4, C5, C7, factor B, and factor I (Holland and Lambris, 2002;). The expression of complement component 7 (C7) was up-regulated in the skin of grass carp (*Ctenopharyngodon idella*) during *A. hydrophila* infection (Shen et al., 2012). The expression of complement component 6 (C6) was also up-regulated in the gut of grass carp after challenge with *A. hydrophila* (Shen et al., 2011).

2.3.4 Immunoglobulin

The immunoglobulins (Ig) in fish are described as humoral immune response and importance of innate and adaptive immunity in fish. The Ig in teleost fish has been classified into three classes such as IgM, IgD and IgT (Salinas et al., 2011; Mashoof and Criscitiello, 2016). The presence of higher IgM titers in the intestine and serum at weeks 5^{th} and 6^{th} week of post-immunization has been described in *A. hydrophila* infection in carp (*C. auratus gibelio*) (Tu et al., 2010). However, studies of immune responses against *A. veronii* infection in red tilapia are still poorly understood.

2.4 Jerusalem artichoke (*Helianthus tuberosus*), Kantawan as prebiotic

2.4.1 Concept of prebiotics

Prebiotics are defined as non-digestible food ingredients which beneficially affect to host health by selectively stimulating the growth and/or activity of microflora bacteria in the gut. Unique characteristics of prebiotic properties are limited hydrolysis and absorption in the upper gastrointestinal tract, selective stimulation of beneficial intestinal bacteria potentially related to health and well-being (Gibson and Roberfroid, 1995). Prebiotics are found in several foods such as Jerusalem artichokes, leeks, asparagus, chicory, garlic, onions, wheat, oats, and soybeans (Vanloo et al., 1995).

2.4.2 Jerusalem artichoke (*Helianthus tuberosus*), Kantawan

Jerusalem artichoke (*Helianthus tuberosus*), Kantawan, a member of family *Asteraceae* is a root vegetable and a native plant in central-eastern North America (Kleessen et al., 2007). Jerusalem artichoke has been introduced to Thailand and can be harvested after 100–140 days, and crop yields of JA are approximately 13–19 ton per ha (Moshfegh et al., 1999). Jerusalem artichoke consists of the most common prebiotics, inulin, and fructooligosaccharide (FOS) (Moshfegh et al., 1999; Kleessen et al., 2007).

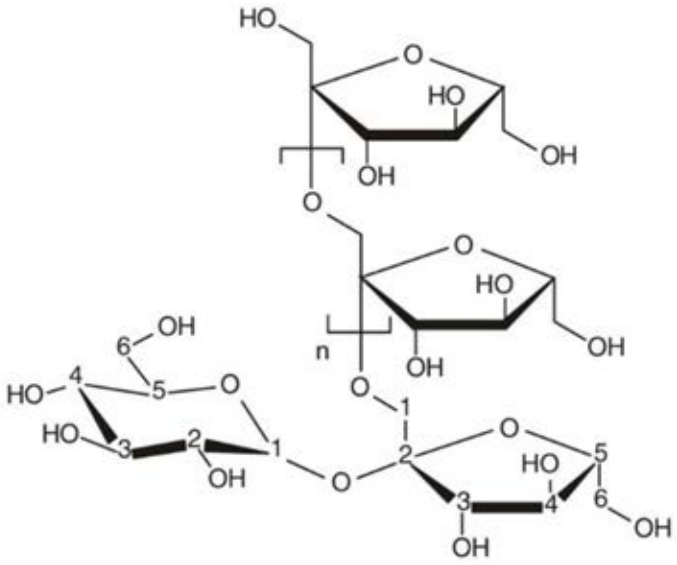
2.4.3 Inulin

Inulin is low digestible carbohydrates and one of the most common prebiotics used in feed for livestock and aquatic animals (Grabitske and Slavin, 2009; Lied et al., 2011; Slavin, 2013). Previous studies have been reported that dietary supplement of inulin in fish feed enhanced growth performance and intestinal microbiota, modulated intestinal microbiota, and improved hematological and immune parameters in fish (Mahious et al., 2006; Reza et al., 2009; Ibrahem et al., 2010; Ortiz et al., 2013). Inulin was found in many plants such as Jerusalem artichoke, Onion, Chicory, etc. Among the

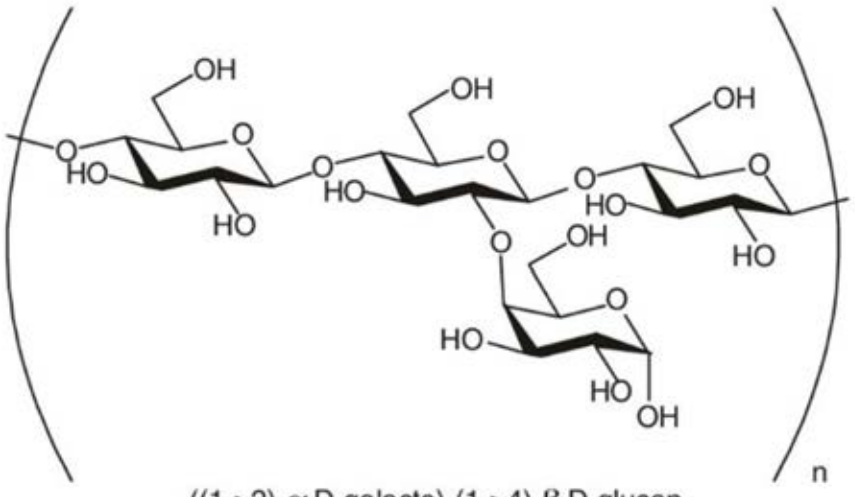
plant, Jerusalem artichoke contained high concentration of inulin compared with the other plants (Vanloo et al., 1995; Moshfegh et al., 1999).

2.4.4 Fructooligosaccharide (FOS)

Fructooligosaccharide (FOS) is prebiotic which has been utilized in animal feeds. FOS has been shown to significantly increase fecal bifidobacteria at fairly low levels of consumption (Costabile et al., 2010). However, the inulin and FOS were used as prebiotics feed additive in the animal feed industry has been limited by the cost of the inulin and FOS extraction process.



Inulin



Fructooligosaccharides

Figure 4 Chemical structure of inulin and fructooligosaccharides adapted from (Nilegaonkar and Agte, 2010).

2.5 *Lactobacillus rhamnosus GG* as probiotic

2.5.1 History and concept of probiotic

'Probiotic' is derived from the Greek word meaning 'for life'. The concept of probiotics was the first introduction in 1908 by Elie Metchnikoff, the Nobel prize winner in Medicine in 1908, at the Pasteur Institute laboratory in Paris. He observed that the bacteria present in yogurt was correlated to health and longevity in Bulgarian peasant (Anukam and Reid, 2007). The term probiotics was firstly described as one microorganism secreted the substances which stimulate the growth of another (Lilly and Stillwell, 1965).

2.5.2 Mechanisms of actions of probiotics

Probiotics are defined as a live microorganism which have beneficial effects on the host by production of compounds for inhibition toward pathogens (Bruno and Montville, 1993; Perez et al., 2014), by enhancement of the host response to disease (Madsen, 2006; Preidis et al., 2011), by improving nutritional and microbial balance in the environment (Villamil et al., 2002). Probiotics bacterial bind with the binding sites in the intestinal mucosa and form a physical barrier for prevention from pathogenic bacteria binding. Probiotics produce antibacterial substances such as hydrogen peroxide and bacteriocins, which have antibacterial action mainly pathogenic bacteria (Mishra and Lambert, 1996; Ocana and Elena Nader-Macias, 2004; Ruiz et al., 2015). In addition, probiotic bacteria produce organic acids which lower the environment's pH of the gastrointestinal tract for prevention from the growth of various pathogens. Probiotics bacteria compete with the pathogenic bacteria for nutrients which are limited growth of pathogenic bacteria. Probiotics bacteria stimulate immune system

immune response, by increasing the production of antibodies, activation of macrophages, T-cell proliferation and production of interferon.

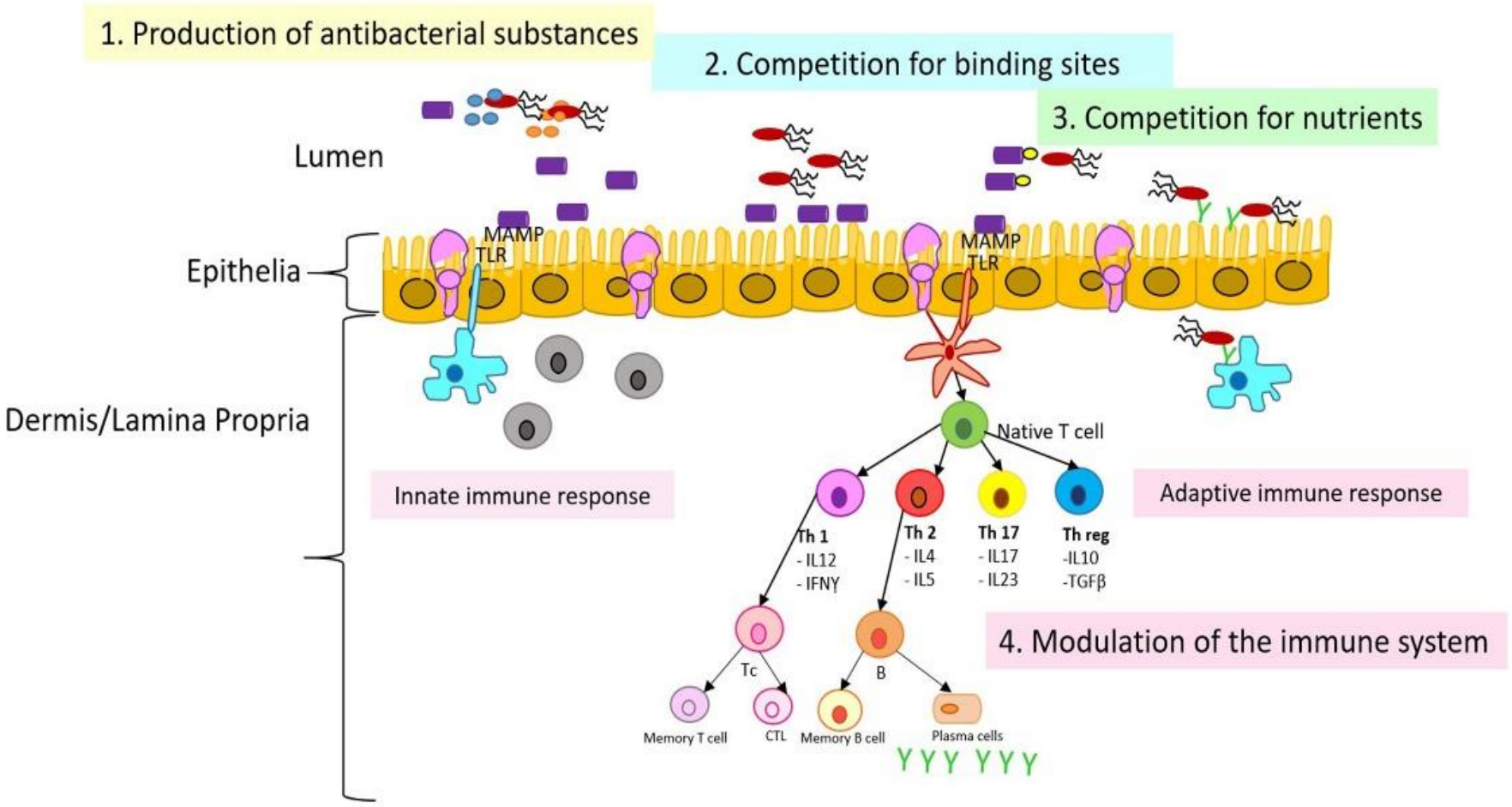


Figure 5 Mechanisms of action of Probiotics adapted from (Madsen, 2006; Bermudez-Brito et al., 2012; Newaj-Fyzul and Austin, 2014)

2.5.3 *Lactobacillus rhamnosus* GG

Lactobacilli are Gram-positive, non-spore-forming, non-flagellated rods, aerotolerant and strictly fermentative. Lactobacilli have been divided into the homofermentative and heterofermentative. In the homofermentative case, glucose was fermented predominantly to lactic acid. Whereas, the heterofermentative, glucose was fermented to lactic acid, CO_2 and ethanol (and/or acetic acid) (De Angelis and Gobbetti, 2016). Lactobacilli are important normal flora in the gastrointestinal and genital tracts of human and animals (Larsen and Monif, 2001; Tannock, 2004; Claesson et al., 2007). Research on probiotic Lactobacillus has increased exponentially during the last two decades from 180 research articles published in 1980 – 2000 to more than 5700 research articles published in 2000 – 2014 (Pandey et al., 2015). Lactobacilli are the most commonly used as probiotics, which have been reported in human such as the prevention of acute diarrhea in children, the prevention of antibiotic-associated diarrhea, the prevention and treatment of allergy, and as used in farm animals (Majamaa and Isolauri, 1997; Gatesoupe, 2007). Previous studies reported that Lactobacilli inhibited pathogens in aquatic environments such as *L. rhamnosus, L. plantarum*, *L. bulgaricus, L. acidophilus* (Madsen, 2006). *L. rhamnosus* has been reported to control bacterial infection. *Lactobacillus rhamnosus GG* (LGG) was isolated from fecal samples of a healthy human adult in 1983 by Sherwood Gorbach and Barry Goldin. The LGG is derived from their names. LGG is known to have a strong avidity for human intestinal cells and can survive in acid and bile environments. The important

factors of *L. rhamnosus* for adhesion to intestinal cells have been described including pili, lipoteichoic acid molecules, major secreted proteins, and galactose-rich exopolysaccharides (Segers and Lebeer, 2014). LGG has been used as probiotics in humans, animals, and aquaculture. Several studies reported that LGG has been used to control bacterial infections such as *A. salmonicida*, *Vibrio anguillarum* and *Flavobacterium psychrophilum* in rainbow trout and turbot (Nikoskelainen et al., 2001), as well as *Edwardsiella tarda* infections in tilapia (Pirarat et al., 2006). In addition, LGG was also found to promote the intestinal structure and mucosal immunity of Nile Tilapia (Pirarat et al., 2011).

2.6 Current data of synbiotics in aquaculture

2.6.1 Concept of synbiotic

Synbiotics are nutritional supplements that combine probiotics and prebiotics in a form of synergistic effect (Gibson and Roberfroid, 1995). In addition, the synergistic effect occurs when the combined effect of the two ingredients is greater significantly than the amount of the effects of each ingredient alone.

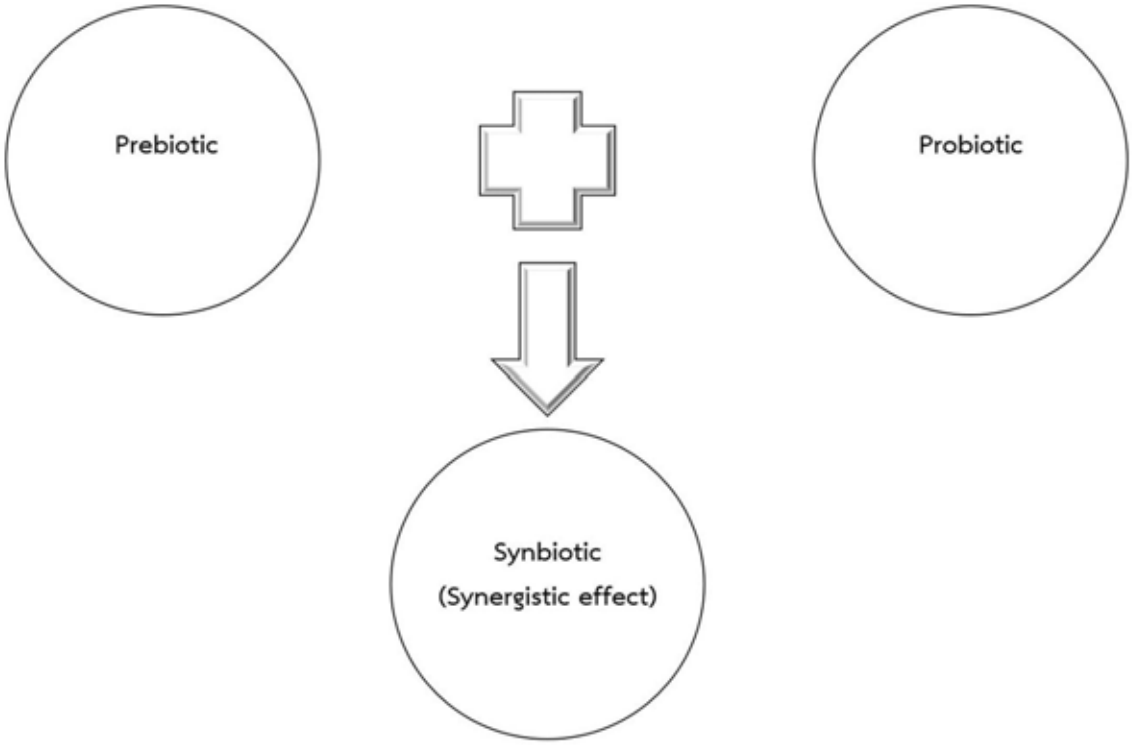


Figure 6 The concept of synbiotic adapted from (Huynh et al., 2017).

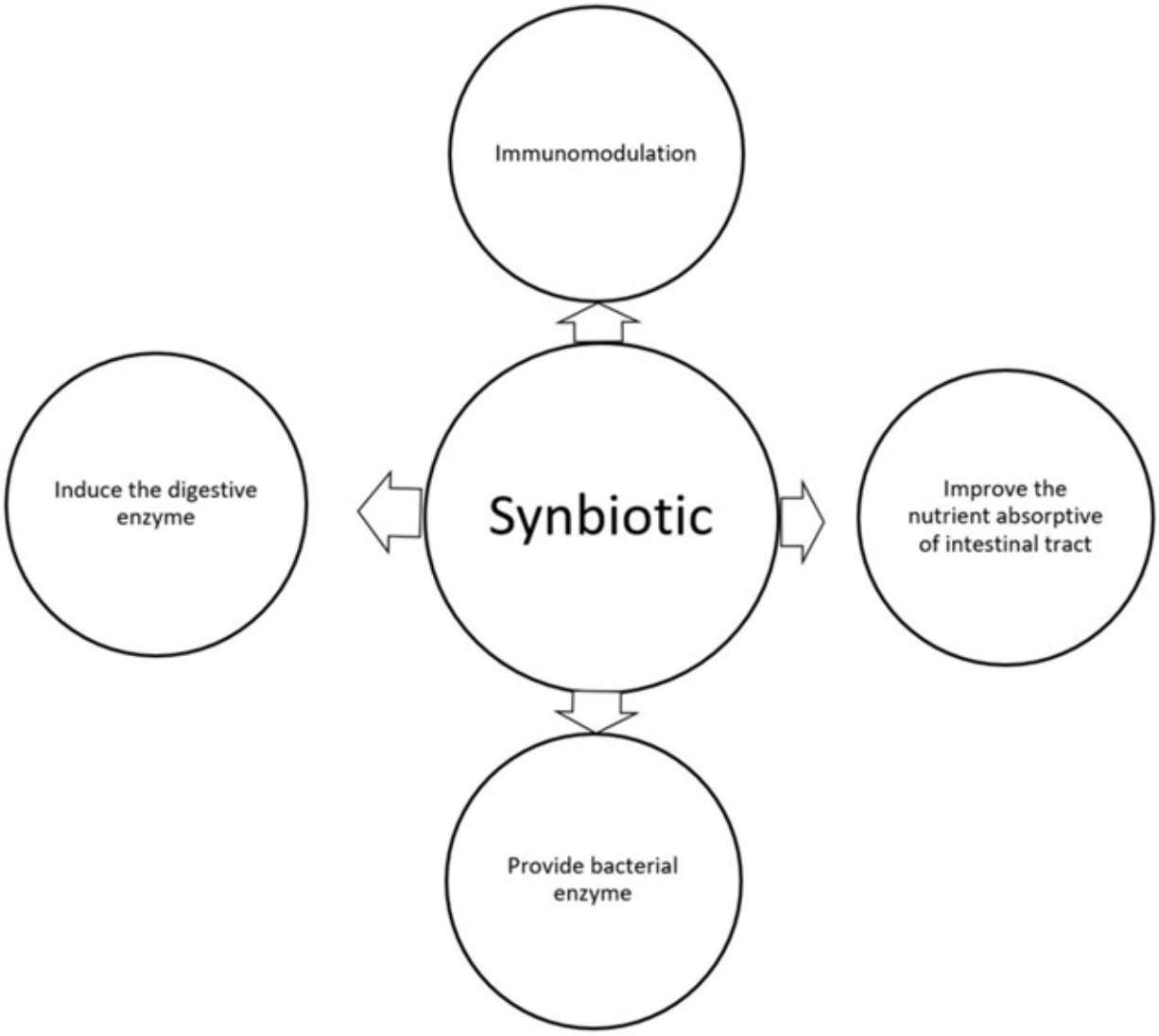


Figure 7 The function of synbiotics (Raman et al., 2015; Huynh et al., 2017)

2.6.2 Administration of synbiotics in fish farming

Previous studies have been reported the administration of synbiotics in fish farming (Ai et al., 2011; Geng et al., 2011; Ye et al., 2011; Mehrabi et al., 2012). Compounds with prebiotic (arabinoxylan oligosaccharide (AXOS), chitosan oligosaccharide (COS), fructo oligosaccharide (FOS), Jerusalem artichoke (JA), galacto oligosaccharide (GOS), mannan oligosaccharide (MOS), short chain fructo oligosaccharide (scFOS), chitosan, inulin, fulvic acid and biomin) have been combined with probiotics to be synbiotics for fish aquaculture (Table 1). In previous work, it has been reported that synergistic effect of Jerusalem artichoke and *L. plantarum* were shown significantly stimulated growth, immunity and disease resistance of *Pangasius bocourti* (Van Doan et al., 2016).

Table 1 The synbiotic were used in fish culture.

Probiotic	Prebiotic	Fish species	Reference
E. faecium	FOS	*O. mykiss*	(Mehrabi et al., 2012)
B. clausii	MOS, FOS	*P. olivaceus*	(Ye et al., 2011)
B. subtilis	FOS	*L. crocea*	(Ai et al., 2011)
B. subtilis	Chitosan	*R. canadum*	(Geng et al., 2011)
L. plantarum	JA	*P. bocourti*	(Van Doan et al., 2016)
E.faecalis	MOS	*O. mykiss*	(Rodriguez et al., 2009)
E. faecium	FOS	*O. mykiss*	(Firouzbakhsh et al., 2014)
LGG	MOS	*O. mykiss*	(Alak, 2012)
P. acidilactici	GOS	*O. mykiss*	(Hoseinifar et al., 2015)
P. acidilactici	scFOS	*S. salar*	(Abid et al., 2013)
B. lichenifomis	FOS	*M. terminalis*	(Zhang et al., 2013)
E. faecium	FOS	*Cyprinus carpio*	(Yarahmadi et al., 2014)
E. faecium	FOS	*C. auratus*	(Gharaei, 2014)
B.coagulans	COS	*Cyprinus carpio koi*	(Lin et al., 2012)
E. faecium	FOS	*P. scalare*	(Nekoubin et al., 2012b)
E. faecium	FOS	*D. rerio*	(Nekoubin et al., 2012a)
E. faecium	FOS	*H. cyanoguttatus*	(Montajami et al., 2012)
P. pentosaceus	Inulin and fulvic acid	*O. niloticus*	(Luna and Flores Miranda, 2013)
B. subtilis	MOS	*O. niloticus*	(Azevedo et al., 2015)
B. subtilis	FOS	*T. ovatus*	(Zhang et al., 2014)

Table 1 (Continued)

Probiotic	Prebiotic	Fish species	Reference
D. hansenii	Inulin	*S. aurata*	(Tapia Paniagua et al., 2011)
B. subtilis	Inulin	*S. aurata*	(Cerezuela et al., 2012)
B. clausii	MOS+FOS	*P. olivaceus*	(Ye et al., 2011)
L. sakei	Inulin	*M. rosacea*	(Reyes-Becerril et al., 2014)
B. subtilis	Chitosan	*R. canadum*	(Geng et al., 2011)
B. subtilis	FOS	*L. crocea*	(Ai et al., 2011)
W. cibaria	Inulin	*P. corruscans*	(MOURIÑO et al., 2012)
L. lactis	AXOS	*A. baerii*	(Geraylou et al., 2013)
E. faecium	Biomin	*H. huso*	(Akrami et al., 2015)

However, studies of the potential benefit of the synbiotic between *L. rhamnosus* and Jerusalem artichoke on growth performance, serum biochemical, intestinal morphology, immune parameters, intestinal bacterial count and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.) are limited. Therefore, in this study, we investigated the synbiotic effects of dietary Jerusalem artichoke and *L. rhamnosus* and their effect on protection against *A. veronii* infection in juvenile red tilapia.

CHAPTER III

MATERIALS AND METHODS

3.1 Disc diffusion assay between Jerusalem artichoke (Kantawan) and *Lactobacillus rhamnosus* GG (LGG)

Jerusalem artichoke (Kantawan) (5 g, 10 g, 15 g, 20 g) were extracted by distilled water (8/10 w/v) for 24 hours at 4°C. After that, the supernatant was collected and filtrated by a filter with a 0.22 µm pore size. The sterile blank paper discs were placed on the Muller Hilton agar which was inoculated with LGG. Then, 100 µl of the supernatant of Jerusalem artichoke (Kantawan) were applied on the paper discs. Plates were incubated at 37°C for 24 h and observed for their inhibition zone (Pirarat et al., 2009).

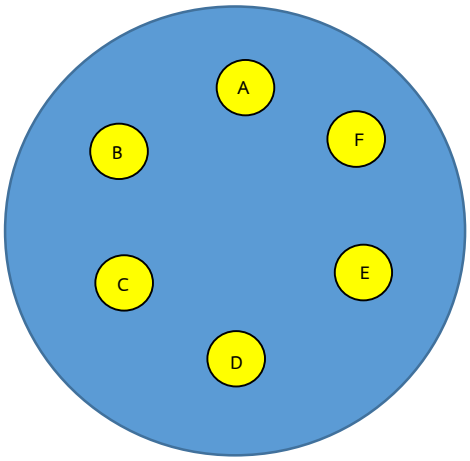


Figure 8 Disc diffusion assay between Jerusalem artichoke (Kantawan) and *Lactobacillus rhamonsus* GG on Muller Hilton agar : A : crude of Kantawan, B : the supernatant extracted from 5 g of Kantawan, C : the supernatant extracted from 10 g of Kantawan, D : the supernatant extracted from 15 g of Kantawan, E : the supernatant extracted from 20 g of Kantawan, F : antibiotic (Tetracycline)

3.2 Feed and Experimental design

The probiotic bacterium, *Lactobacillus rhamonsus* GG (ATCC 53103) was cultured in de Man, Rogosa and Sharpe Agar (MRS) broth at 37℃ for 48 h, centrifuged and washed with sterile phosphate-buffered saline (PBS) 3 times. Then, the density of the bacterial suspension in PBS was determined and bacteria were mixed into commercial dry pellets (10^8 CFU/g). Jerusalem artichoke (JA) samples were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. The JA tubers were cleaned and sliced into thin pieces from the middle of the tubers. After that, the samples were dried at 50 ℃ for 24 h into powder and kept at 4 ℃ until their used. The proximate composition of JA was analyzed by using the standard methods of AOAC (1990). The JA consists of oligofructose compounds, protein, lipid, fiber, dry matter and ash (Tiengtam et al., 2017).

Diets were divided into six groups as follows: basal (control, C), 5.0 g kg^{-1} Kantawan-supplemented (5K), 10.0 g kg^{-1} Kantawan-supplemented (10K), 10^8 CFU g^{-1} LGG-supplemented (LGG), 5.0 g kg^{-1} Kantawan+10^8 CFU g^{-1} LGG-supplemented (5K+LGG), and 10.0 g kg^{-1} Kantawan+10^8 CFU g^{-1}LGG-supplemented (10K+LGG). The proximate composition of the basal diet is composed of dry matter, crude protein, crude lipid, fiber, fructans and ash (Tiengtam et al., 2017).

The diets were prepared under sterile conditions. The 5K and 10K-supplemented diets were prepared to manually incorporated commercial dry pellets and Kantawan at rates of 5.0 g/kg and 10.0 g/kg, respectively. The LGG-supplemented diet was prepared to manually incorporate commercial dry pellets and LGG at rates of 10^8 CFU/g. The 5K+LGG-supplemented and 10K+LGG-supplemented diets were prepared to manually incorporated commercial dry pellets with Kantawan at rates of 5.0 g/kg and LGG at rates of 10^8 CFU/g and Kantawan at rates of 10.0 g/kg and LGG at rates of 10^8 CFU/g, respectively.

Table 2 Formula of the diets in this study.

Diets	Control	5K	10K	LGG	5K+LGG	10K+LGG
	Basal	Prebiotic		Probiotic	Synbiotic	
Basal diet	5% of body weight	5% of body weight	5% of body weight	5% of body weight	5% of body weight	5% of body weight
LGG	-	-	-	10^8 cfu/g of diet	10^8 cfu/g of diet	10^8 cfu/g of diet
Jerusalem artichoke or Kantawan	-	5g kantawan/ kg of diet	10g kantawan/ kg of diet	-	5g kantawan/ kg of diet	10g kantawan/ kg of diet

3.3 Enumeration of probiotic bacteria in the diet

One gram of all parts of the diets was transferred to tubes containing a 9.0 mL of 0.85% NaCl solution and homogenized on the stomacher. Ten-fold serial dilutions were performed in 0.85% NaCl solution from the 10^{-1} dilution until the 10^{-8} dilution. After that, the 1 mL of the suspension were pour plated with de Man, Rogosa and Sharpe Agar (MRS) for the enumeration of Lactobacilli bacteria. The plates were incubated at 28 °C for 24-48 h. The number of the colony on each plate was counted and calculated (Colony Forming Unit or CFU).

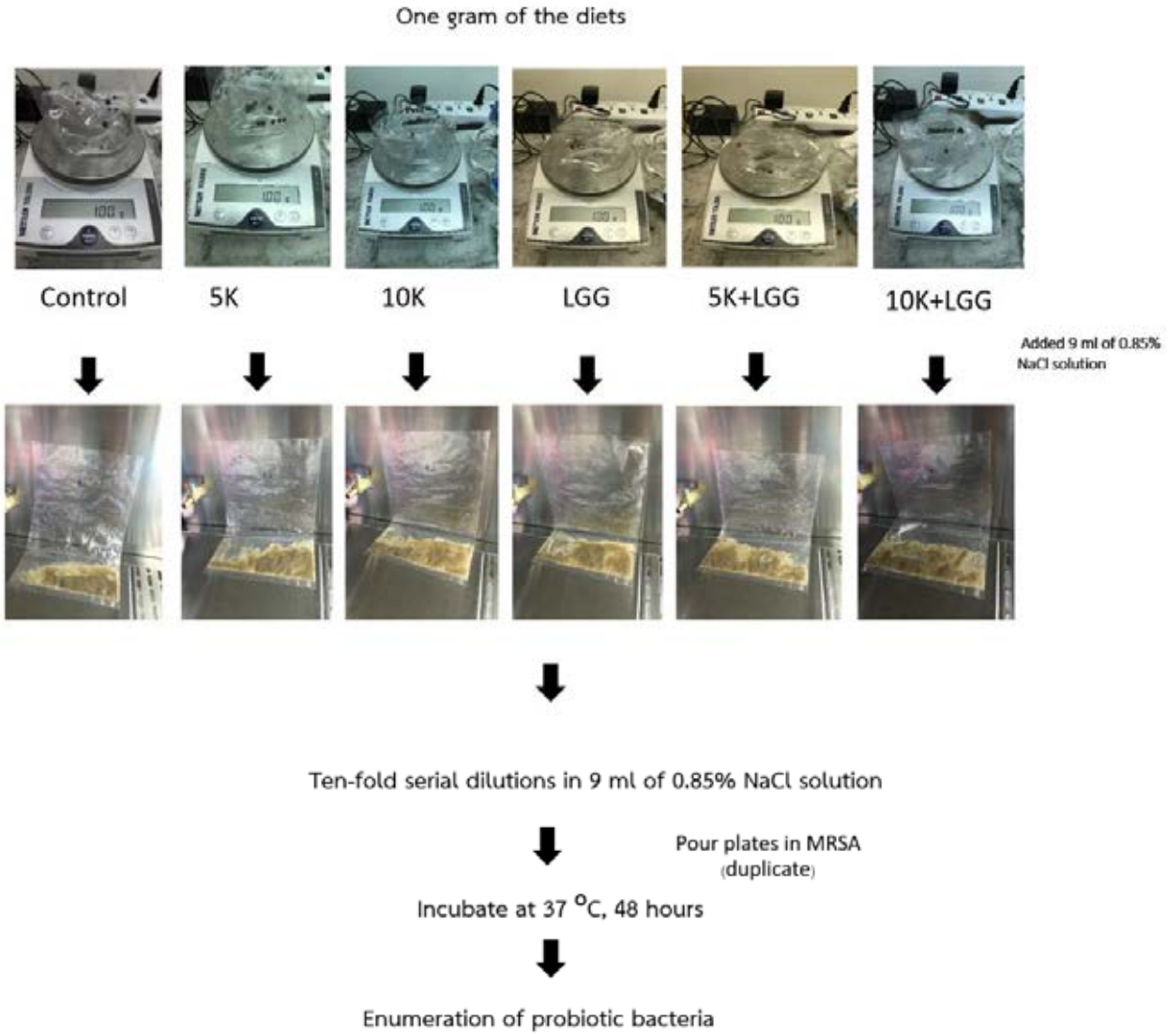


Figure 9 Enumeration of probiotic bacteria in the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control).

3.4 Fish culture

One hundred and eighty male fish (average body weight of 14.05±0.42 g) were obtained from Good Aquaculture Practice (GAP) certified farm, Thailand. The mono male of red tilapia in this experiment was produced by hormonal sex reversal. The fish were allowed to acclimatize for 2 weeks and divided into six groups containing water under continuous aeration with continuous water flow. Water temperature was measured (25–28°C), dissolved oxygen (DO), and pH were measured (5.24–5.98 mg $L^{-1}$and 7.48–8.16, respectively). Fish were hand-fed approximately 5% of body weight twice a day at 9 a.m. and 5 p.m. All protocols were approved by the ethics committee of Chulalongkorn University Animal Care and Use Committee (CUACUC; Approval No. 1731039).

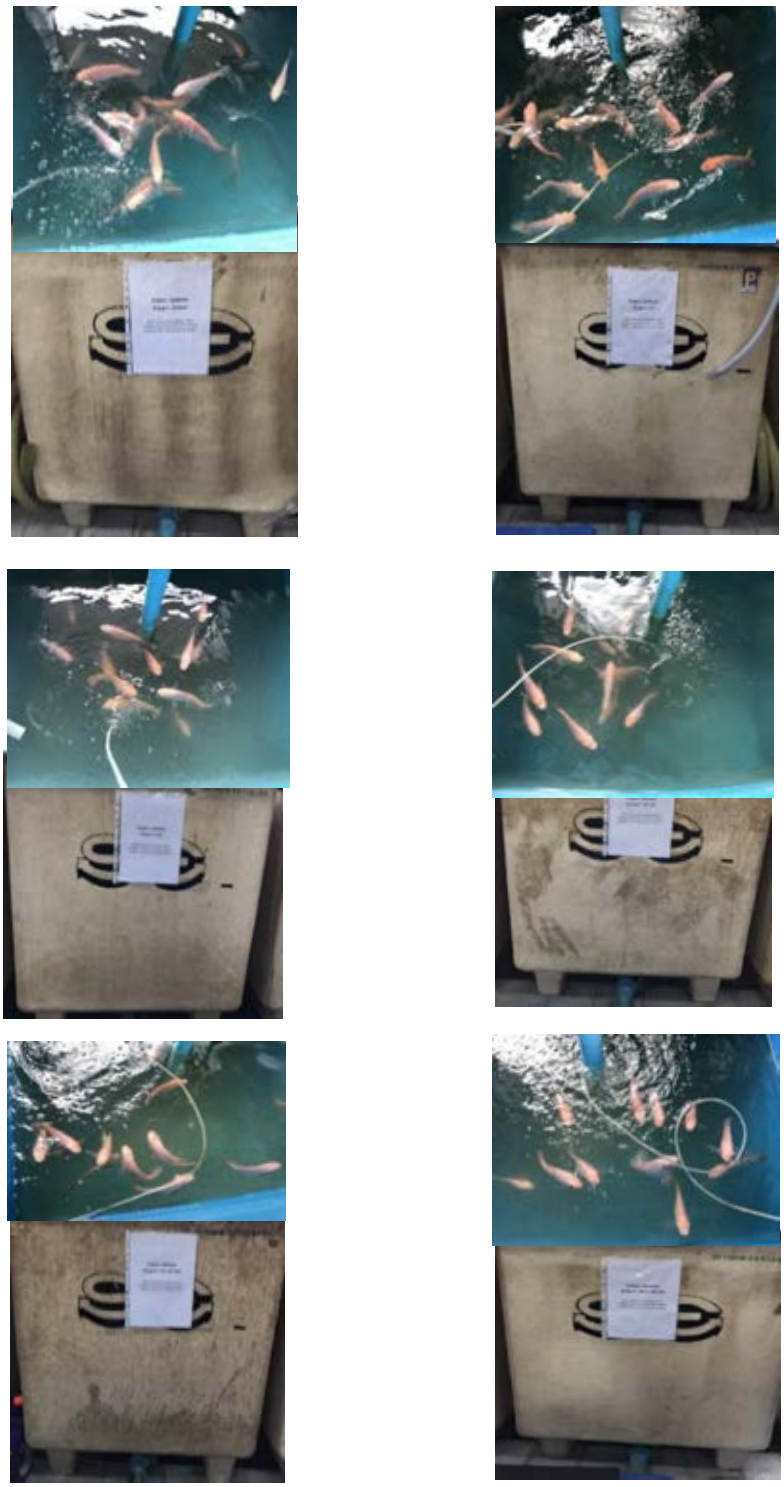


Figure 10 Fish cultured into six 1000-liter tanks containing water under continuous aeration with continuous water flow.

3.5 Blood collection and serum chemistry analysis

Blood samples were collected from six fish from each group from the caudal vein using a hypodermic syringe. Blood samples were allowed to clot at 4ºC for at least 3 h and were centrifuged at 2,600 × g for 10 min at room temperature to obtain serum samples. The samples were analyzed by using automated chemistry analyzer (AU400, Olympus, Tokyo, Japan). The following parameters were measured: glucose, triglyceride, cholesterol, total protein, albumin, blood urea nitrogen (BUN), total bilirubin (T-bilirubin), direct bilirubin (D-bilirubin), serum alanine transaminase (ALT), and serum aspartate aminotransferase (AST).

3.6 Growth performance

Final weight, weight gain (WG%), specific growth rate (SGR), and feed conversion ratio (FCR) were calculated according to standard formulae.

WG (%) = 100 x (final mean body weight-initial mean body weight) / initial mean body weight

SGR = [(ln (final body weight) – ln (initial body weight) / days] x 100

FCR= feed intake (g) / Weight gain

ADG = Weigh (g)/ Experimental period (days)

3.7 Measurement of villous height, villous width, absorptive area and goblet cells

Six fish from each group was sampled and anesthetized with clove oil. Three parts of the intestine, the foregut (after the pyloric part of the stomach to the spiral part of the intestines), the midgut (the spiral part of the intestines), and the hindgut (after the spiral part to 2 cm. before the anus) were collected and fixed in neutral buffered 10% formalin. Samples were processed routinely, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (H&E) and examined under light microscopy. Villous height and width were measured by the i-Solutions DT software (Image & Microscope Technology Inc., USA). For the villus height measurement, the 10 highest villi were selected per section and their height were measured from the tip to the bottom. The average was expressed as the mean villus height for each section (Pirarat et al., 2011). The absorptive area was calculated by means of the following calculation: absorptive area = villous height x villous width (Bello et al., 2012). In addition, the goblet cells in the intestinal were classify by special staining such as periodic acid-schiff (PAS) staining, alcian blue (AB) staining (pH 2.5), and AB-PAS double-staining. After that five fields were randomly selected from intestinal tissue section and counted at 400X magnification in a high-power field (HPF). The average of the goblet cells from each section (mucous cell numbers/HPF) was calculated.

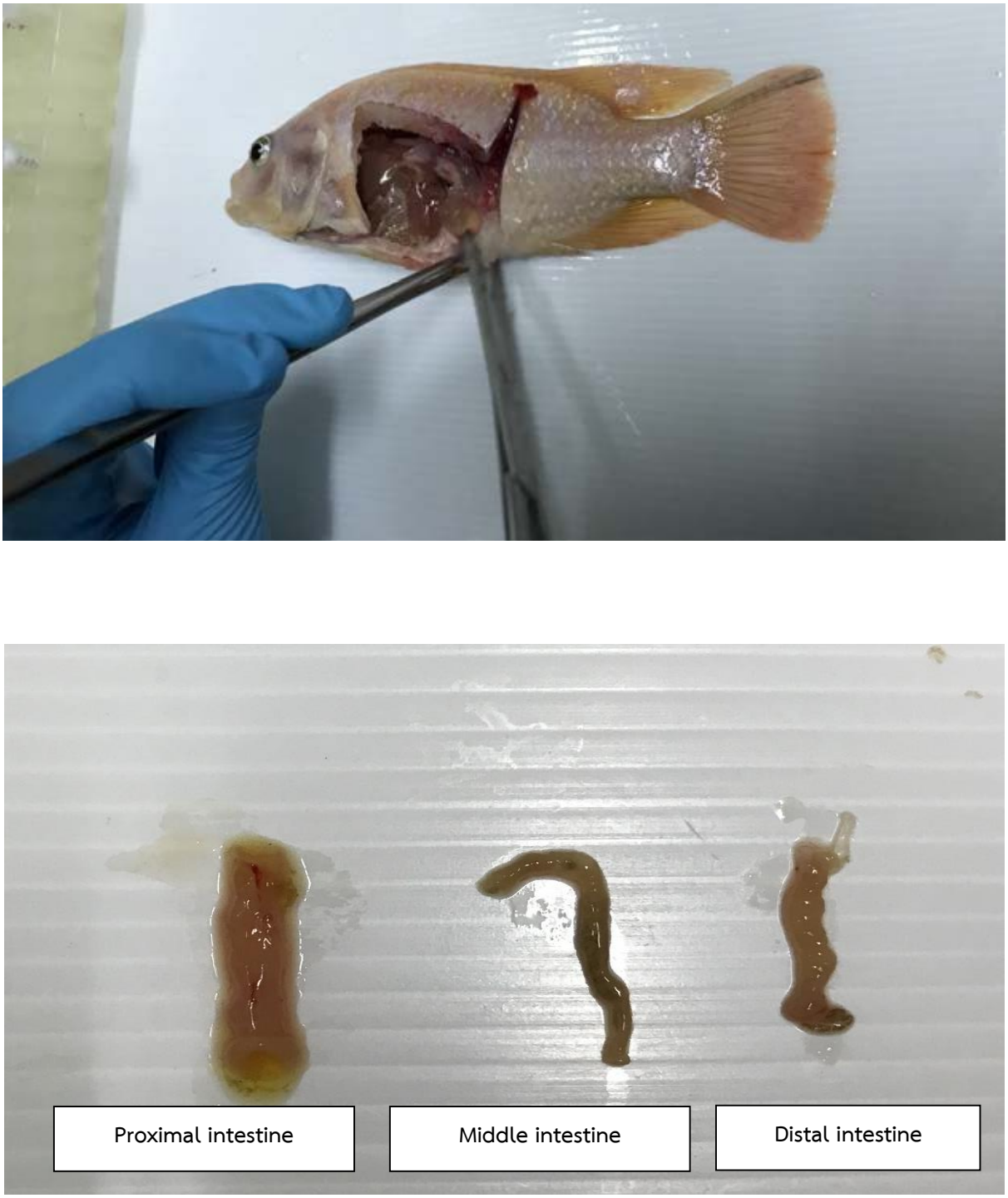


Figure 11 Three parts of the intestine such as proximal, middle, and distal intestine were collected from red tilapia.

3.8 Enumeration of intestinal bacteria

One gram of all parts of the intestines of each experimental unit was transferred to tubes containing a 9.0 mL of 0.85% NaCl solution and homogenized on the stomacher. Ten-fold serial dilutions were performed in 0.85% NaCl solution from the 10^{-1} dilution until the 10^{-8} dilution. After that, the 1 mL of the suspension were pour plated with trypticase soy agar (TSA), de Man, Rogosa, and Sharpe Agar (MRS) for the enumeration of total bacteria and Lactobacilli bacteria, respectively. The plates were incubated at 28 °C for 24-48 h. The number of the colony on each plate was counted and calculated (Colony Forming Units).

3.9 Hemolytic activity of *Aeromonas veronii* (In-vitro test)

A. veronii (NK01 and NK06 isolates) were isolated from two floating cage cultured farms in Nong Khai province, northeastern Thailand during disease outbreaks in August 2014 (Dong et al., 2015) and cultured in tryptic soy broth. The supernatant was collected. After that, extracellular products (ECPs) were precipitated from the supernatant with ammonium sulfate. Then, ECPs were collected by centrifugation. After that, the pellet was dissolved in phosphate buffered saline (PBS, pH 7.2) in the presence of 1mM proteinase inhibitors. The hemolytic activity of crude ECPs was performed using published procedures (Monfort and Baleux, 1991). Briefly, serial dilutions of ECPs mixed with an equal volume of a 1% suspension of sheep and tilapia erythrocytes. The plates were incubated at 37 °C for 2 h. After that, the samples were centrifuged at 800 g for 10 minutes to remove unlysed cells. The absorbance was measured on a microplate reader at 545 nm. One hemolytic unit (HU) was defined as the highest dilution of ECPs producing 50% hemolysis.

3.10 Mortality test

A single colony of *A. veronii* was grown in 5 ml TSB, incubated at 30 ℃ for 18 h. and the bacterial suspension was adjusted to OD600 equal 0.55 to 0.60. The colony forming units (CFU) of *A. veronii* were calculated by means of the plate count method. At the end of the experimental period (4 weeks), twenty-five fish from each diet received an intraperitoneal injection of 10^7 CFU/fish (Hoseinifar et al., 2015). Fish were maintained in tanks containing 20 L UV-treated water and fed twice per day with commercial feed (CP, Thailand). The temperature was maintained at 26±1℃. After the challenge, clinical signs, and mortalities were recorded for 15 days. The cumulative mortality was calculated by the following calculation:

(Total mortality in each treatment after challenge/Total number of fish challenged for same treatment) x 100 (Musthafa et al., 2016)

3.11 Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) using SPSS version 22 software for Windows (SPSS Inc., Chicago, USA). Statistical significant differences among the groups were determined by Duncan's multiple range test with significance at $P < 0.05$.

CHAPTER IV

RESULTS

4.1 Disc diffusion assay between Jerusalem artichoke (Kantawan) and *Lactobacillus rhamonsus* GG

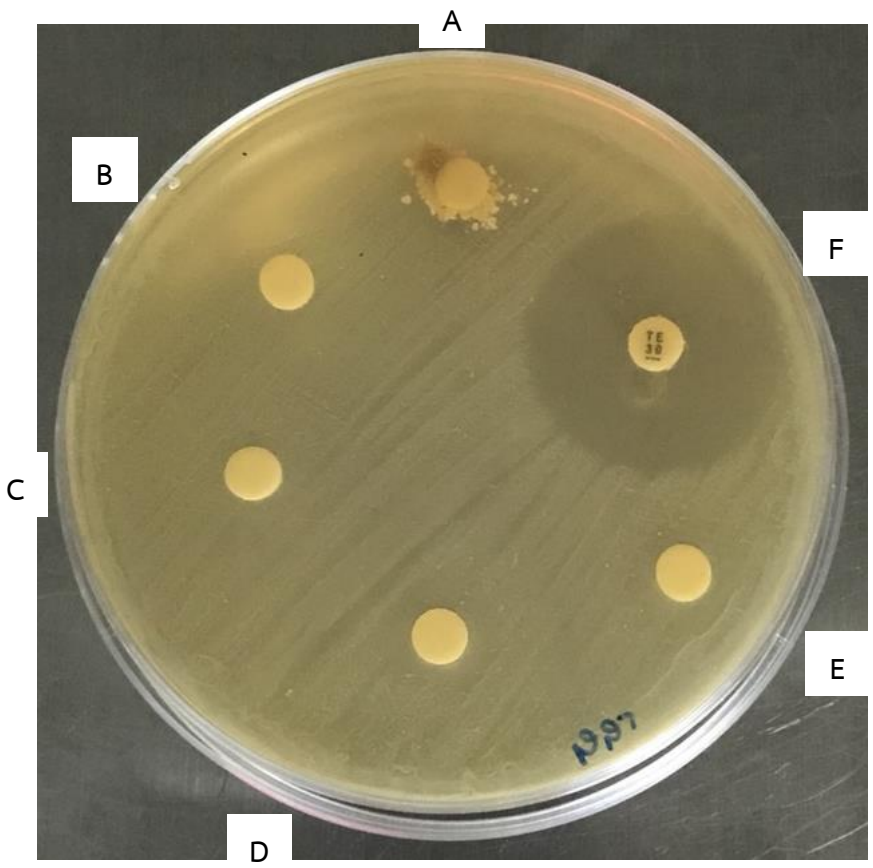


Figure 12 Disc diffusion assay between Jerusalem artichoke (Kantawan) and *Lactobacillus rhamonsus* GG on Muller Hilton agar : A : crude of Kantawan, B : the supernatant extracted from 5 g of Kantawan, C : the supernatant extracted from 10 g of Kantawan, D : the supernatant extracted from 15 g of Kantawan, E : the supernatant extracted from 20 g of Kantawan, F : antibiotic (Tetracycline)

Table 3 Inhibition zone of disc diffusion assay

	Disc diffusion assay					
	Crud Kantawan	**5g Kantawan**	**10g Kantawan**	**15 Kantawan**	**20 Kantawan**	**Antibiotic (Tetracycline)**
Inhibition zone	-	-	-	-	-	+
Diameter of disc (mm)	6.0	6.0	6.0	6.0	6.0	30.0

Discs are 6.0 mm in diameter and each disc

The results showed that Jerusalem artichoke (Kantawan) was not effect on the growth of LGG indicating that the mixture of JA and LGG could be done.

4.2 Enumeration of probiotic bacteria in the experimental diets

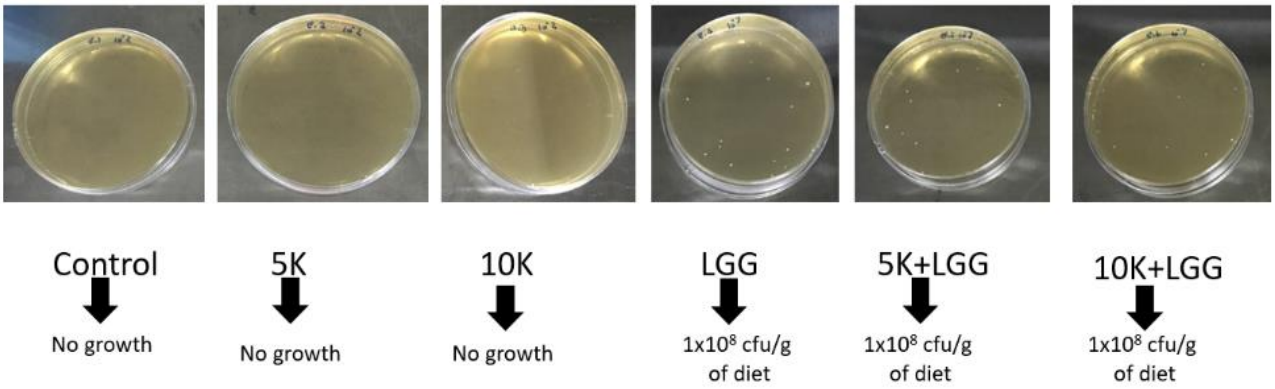


Figure 13 Enumeration of probiotic bacteria in the experimental diets

Table 4 Enumeration of LGG in the experimental diets

	Diets					
	C	5K	10K	10^8LGG	5K + 10^8LGG	10K + 10^8LGG
Probiotic bacteria (CFU/g)	0	0	0	1×10^8	1×10^8	1×10^8

The results showed that the probiotic and synbiotic diets have the number of LGG 1×10^8 CFU/g. There was no growth of LGG in the prebiotic and control diets.

4.3 Serum chemistry analysis

The results of serum biochemical parameters of juvenile red tilapias fed with the experimental diets are presented in Table 5

Table 5 Serum biochemical parameters of red tilapia fed with the experimental diets during 30 days

	Diets					
	C	5K	10K	10^8LGG	5K + 10^8LGG	10K + 10^8LGG
Parameters						
Glucose (mg/dl)	45.17±7.49[a]	75.67±36.43[b]	50.67±10.54[ab]	63.33±19.68[ab]	56.50±17.26[ab]	51.33±5.32[ab]
Triglycerides (mg/dl)	200±97.51	187.67±81.41	187±48.53	137.33±55.09	158.40±38.70	182.17±46.59
Total cholesterol (mg/dl)	161.33±10.54[a]	163.83±29.63[a]	227.83±24.60[c]	184.33±33.61[ab]	197.83±27.56[b]	196.00±9.47[b]
Total protein (g/dl)	2.60±0.22[a]	2.52±0.44[a]	2.83±0.38[ab]	2.80±0.09[ab]	2.68±0.42[ab]	3.12±0.56[b]
Albumin (g/dl)	0.97±0.27	0.77±0.12	0.97±0.21	0.87±0.08	0.90±0.10	1.00±0.40
BUN[a] (mg/dl)	1.80±0.40[b]	1.00±0.00[a]	1.50±0.55[ab]	1.50±0.55[ab]	1.67±0.94[ab]	1.17±0.41[ab]
Total bilirrubin (mg/dl)	0.02±0.01	0.01±0.00	0.01±0.00	0.01±0.00	0.05±0.07	0.01±0.00
Direct bilirrubin (mg/dl	0.02±0.01[b]	0.00±0.01[a]	0.00±0.00[a]	0.00±0.01[a]	0.01±0.01[a]	0.00±0.00[a]
ALT (IU/l)	9.50±6.53[b]	3.20±1.17[a]	3.33±0.82[a]	6.67±3.44[ab]	7.00±1.83[ab]	6.00±1.90[ab]
AST (IU/l)	13.00±2.76	14.80±5.38	17.33±7.69	19.83±10.53	27.00±23.94	11.60±3.88

Values are mean±SD of serum samples from six animals from each experimental group (n=6) fed the same diet. Values within the same row with different letters are significantly ($P<0.05$) different. TAG=triglycerides, BUN=blood urea nitrogen, ALT=alanine transferase, and AST=aspartate aminotransferase

The results showed that the total cholesterol and total protein levels of red tilapia fed with synbiotic-supplemented diet (10K + LGG) were significantly ($P < 0.05$) higher than prebiotic groups (5K) and control groups. The D-bilirubin values of fish from the control group was significantly ($P < 0.05$) higher than the probiotic group (LGG), prebiotic groups (5K and 10K), synbiotic groups (5K + LGG and 10K + LGG). Results showed no significant differences between the fish fed the control diet and those fed the diets with synbiotic in triglyceride, albumin, T-bilirubin and AST.

4.4 Growth performance

After feeding the fish for a month, the final weight of animals fed 10K+LGG was the highest, and was significantly higher ($p<0.05$) than those of fish fed C (Table 6).

Table 6 Growth performance and feed utilization of red tilapia fed with the different experimental diets during 30 days

	Diets					
	C	**5K**	**10K**	**10^8LGG**	**5K + 10^8LGG**	**10K + 10^8LGG**
Initial weight (g)	14.68±3.26	13.68±5.15	13.88±3.61	13.56±3.13	14.22±4.81	14.28±3.33
Final weight (g)	24.52±5.67^{a}	28.76±5.37^{b}	27.73±6.18^{b}	27.46±5.54^{ab}	26.88±4.81^{ab}	29.28±6.16^{b}
WG (%)	67.03±7.0^{a}	125.70±45.46^{d}	106.38±13.79^{c}	104.12±10.76^{bc}	91.61±6.41^{b}	106.05±7.39^{c}
FCR	2.32±0.55^{d}	1.36±0.11^{a}	1.61±0.35^{bc}	1.62±0.27^{bc}	1.76±0.35^{c}	1.52±0.32^{ab}
SGR (% day^{-1})	1.76±0.14^{a}	2.74±0.70^{d}	2.49±0.23^{c}	2.46±0.18^{c}	2.24±0.12^{b}	2.49±0.12^{c}
ADG (g day^{-1})	2.31±0.24^{a}	4.34±1.57^{d}	3.67±0.48^{c}	3.59±0.37^{bc}	3.16±0.22^{b}	3.66±0.25^{c}

Values represent mean± SD of values of 25 fish fed each experimental diet. Different letters indicate statistical significance obtained ($P<0.05$).

WG (%)=(final weight-initial weight)/(initial weight) × 100

Feed conversion ratio=(dry feed fed)/(wet weight gain)

Specific growth rate=[ln(final weight)-ln(initial weight)]/(number of days) × 100

ADG = Weigh (g)/ Experimental period (days)

The results showed that the WG, SGR, and ADG of fish fed with the 5K + LGG, 10K + LGG, LGG, 5K, and 10K diets were significantly ($P < 0.05$) higher than for fish fed the control diet. In addition, red tilapia fed with the JA supplemented diet had a significantly better growth performance than the control group.

4.5 Measurement of villous height, villous width, absorptive area, and goblet cells

Villous height in the proximal, middle, and distal parts of the intestine of fish fed LGG, 5K+LGG, and 10K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets (Fig.14), while the same was observed for the villous width (Fig.15) in the proximal part of the intestine. No significant differences in the villous width of the middle intestines of fish fed the different experimental diets were found ($P > 0.05$). The absorptive area of the proximal intestine of fish fed LGG, 5K+LGG, and 10K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets, and that of fish fed 10K+LGG was higher than in fish fed LGG (Fig.16). In the middle intestine, the absorptive area of fish fed LGG, 5K+LGG, and 10K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets. Finally, the distal intestine of fish fed LGG, 5K+LGG, and 10K+LGG presented a significantly higher ($P < 0.05$) absorptive area than those fed C, 5K, and 10K. Also, the absorptive area of the distal intestine of fish fed 10K+LGG was higher ($P < 0.05$) than in those fed LGG.

Table 7 Effect of experimental diets on intestinal morphology of red tilapias fed with the experimental diets during 30 days

Parameters	Diets					
	Control	5K	10K	LGG	5K+LGG	10K+LGG
1. Proximal part						
Villi height (μm)	288.22±6.96^{a}	293.82±6.34^{a}	295.23±5.98^{a}	510.14±8.33^{b}	515.03±11.46^{b}	518.21±4.88^{b}
Villi width (μm)	77.08±3.20^{a}	76.71±3.26^{a}	80.06±1.45^{a}	98.85±12.24^{b}	106.14±11.73^{bc}	111.02±9.95^{c}
Absorptive area (villi height x villi width (mm^2)	0.0222±0.00^{a}	0.0225±0.0005^{a}	0.0235±0.0002^{a}	0.0503±0.0055^{b}	0.0546±0.0050^{bc}	0.0575±0.0046^{c}
2. Middle part						
Villi height (μm)	262.21±2.72^{a}	263.92±9.47^{a}	265.61±8.39^{a}	320.13±5.92^{b}	320.19±5.96^{b}	332.48±7.01^{c}
Villi width (μm)	91.58±7.42	94.29±10.07	91.87±5.00	94.73±7.24	100.04±2.29	95.16±12.86
Absorptive area (villi height x villi width (mm^2)	0.0240±0.0017^{a}	0.0248±0.0017^{a}	0.0242±0.0006^{a}	0.0303±0.0018^{b}	0.0320±0.0003^{b}	0.0316±0.0035^{b}
3. Distal part						
Villi height (μm)	172.96±4.81^{a}	182.66±3.62^{a}	178.89±7.62^{a}	222.08±12.95^{b}	222.94±7.23^{b}	229.42±8.31^{b}
Villi width (μm)	89.20±4.73^{a}	101.46±4.66^{ab}	104.14±5.37^{b}	94.22±14.58^{ab}	101.90±10.13^{ab}	104.35±10.73^{b}
Absorptive area (villi height x villi width (mm^2)	0.0154±0.0006^{a}	0.0185±0.0005^{b}	0.0185±0.0009^{b}	0.0208±0.0024^{c}	0.0227±0.0016^{bc}	0.0239±0.0016^{c}

Values represent mean±SD of values of 6 fish fed each experimental diet. Different letters indicate statistical significance obtained ($P<0.05$), according to ANOVA. If significant differences were found among treatments, Duncan's multiple range tests were used to rank the means.

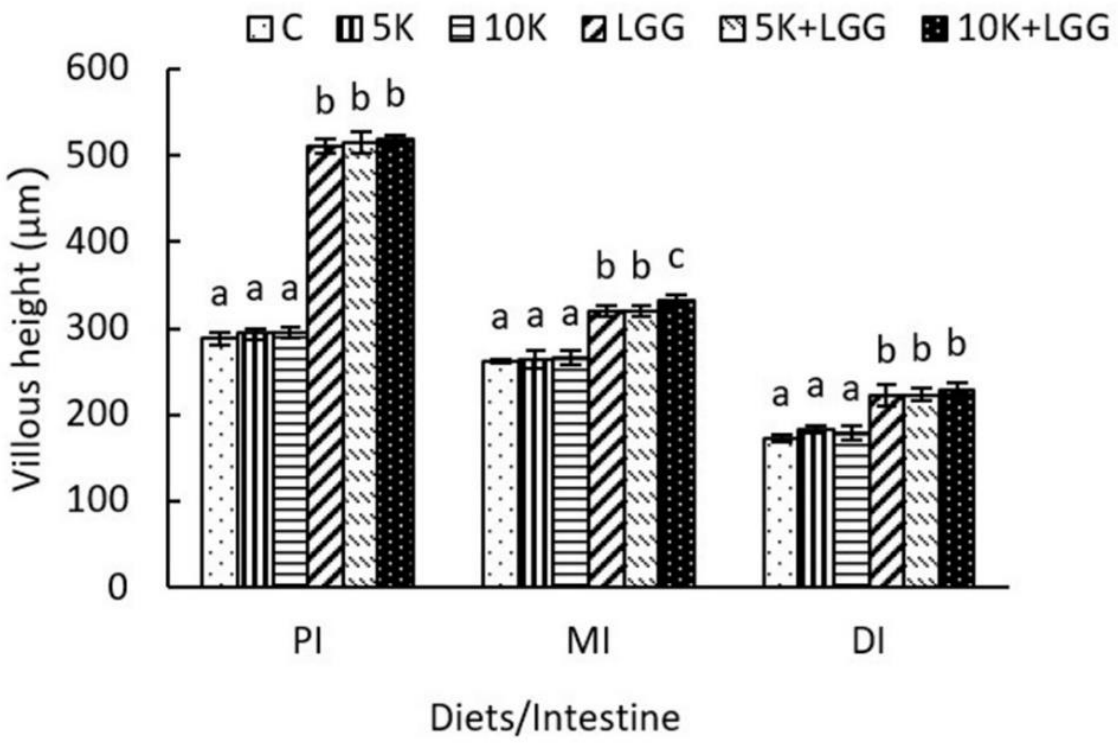


Figure 14 Villous height of the three parts of the intestine of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control).

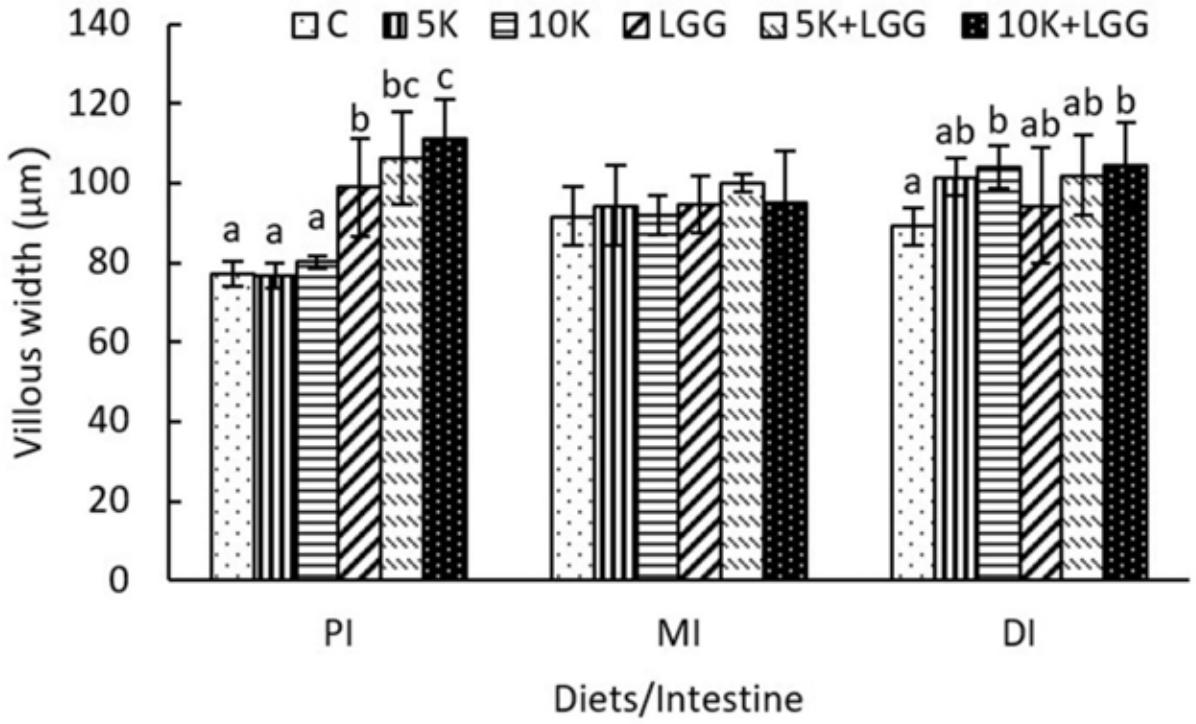


Figure 15 Villous width of the three parts of the intestine of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control).

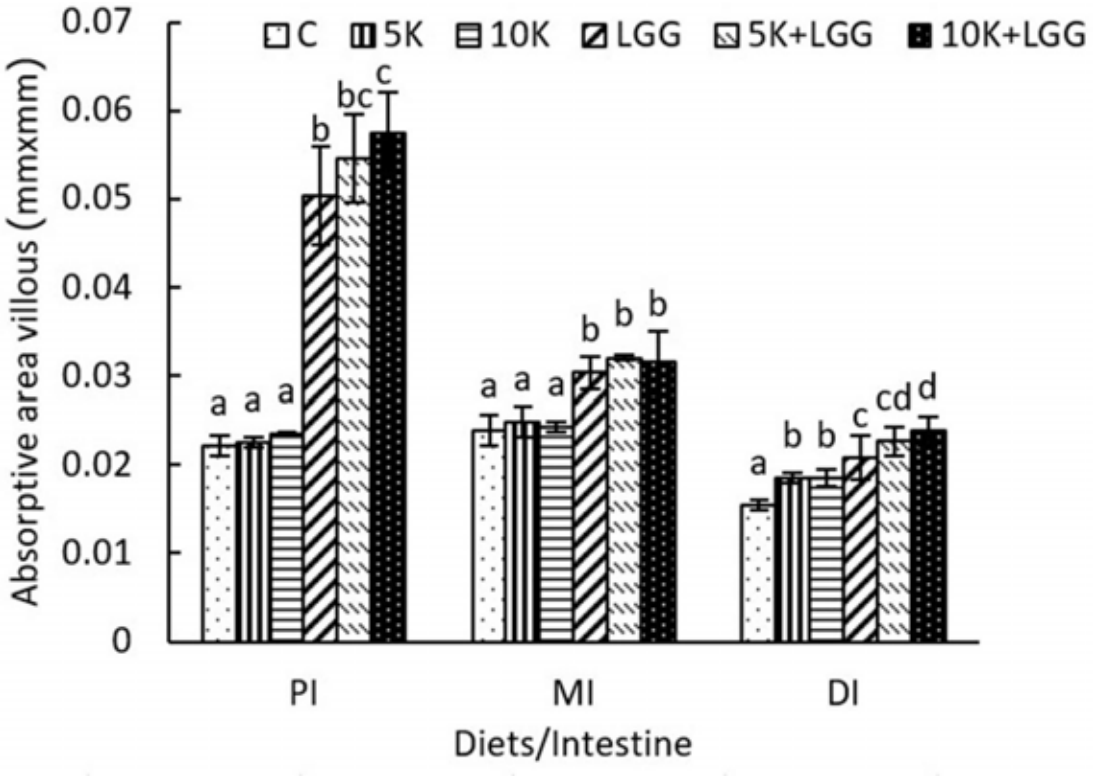


Figure 16 Absorptive area of the three parts of the intestine of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control).

The goblet cells counted in the proximal intestine of red tilapia showed that the acid mucous cells of the proximal intestine of fish fed 5K+LGG (Fig.17) was significantly higher ($P < 0.05$) than those of fish fed other diets, while the neutral mucous cells of the proximal intestine of fish fed LGG, 5K+LGG, and 10K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets. In addition, the double-staining mucous cells of the proximal intestine of fish fed 5K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets (Fig.17).

The goblet cells counted in the middle intestine of red tilapia presented that the acid staining mucous cells of the middle intestine of fish fed LGG was significantly higher ($P < 0.05$) than those of fish fed other diets, while the neutral mucous cells of the middle intestine of fish fed LGG, 5K+LGG, and 10K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets (Fig.18). In addition, the double-staining mucous cells of the middle intestine of fish fed 5K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets (Fig.18).

The goblet cells counted in the distal intestine of red tilapia showed that the acid, neutral, and double-staining mucous cells counted of the distal intestine of fish fed 5K+LGG and 10K+LGG was significantly higher ($P < 0.05$) than those of fish fed other diets (Fig.19)(Table 8).

Table 8 The average of goblet cells counted of red tilapia fed with experimental diets during 30 days

Parameters	Diets					
	Control	5K	10K	LGG	5K+LGG	10K+LGG
1. Proximal part						
Alcian blue staining	27.84±4.51[a]	46±2.68[b]	47.50±2.07[b]	61.00±2.45[c]	74.13±7.42[d]	60.23±5.69[c]
Periodic acid-schiff staining	30.75±3.88[a]	58.37±4.78[b]	61.83±1.72[b]	71.60±2.88[c]	75.90±3.63[cd]	76.83±3.97[d]
Alcian blue-Periodic acid-schiff staining	14.55±1.37[a]	30.97±2.23[b]	30.63±0.95[b]	42.40±2.63[c]	56.50±2.74[e]	46.25±2.56[d]
2. Middle part						
Alcian blue staining	57.00±2.74[a]	56.80±2.15[a]	56.80±1.79[a]	61.17±3.37[b]	58.80±1.72[ab]	58.00±1.87[ab]
Periodic acid-schiff staining	56.60±3.05[a]	57.40±2.15[ab]	60.00±1.58[bc]	61.50±1.87[c]	60.17±1.48[c]	61.80±2.17[c]
Alcian blue-Periodic acid-schiff staining	48.00±2.00[a]	51.00±2.19[bc]	49.50±1.29[ab]	51.83±2.32[bc]	52.17±0.75[b]	51.40±1.82[bc]
3. Distal part						
Alcian blue staining	49.00±3.54[a]	88.50±3.73[b]	89.23±7.03[b]	90.60±2.53[b]	111.96±4.83[c]	114.00±4.95[c]
Periodic acid-schiff staining	56.44±3.43[a]	100.17±3.92[b]	100.33±2.94[b]	119.54±2.36[c]	150.40±2.97[d]	154.44±3.36[e]
Alcian blue-Periodic acid-schiff staining	30.40±1.67[a]	53.04±1.93[b]	52.20±3.56[b]	52.17±3.19[b]	60.20±1.30[c]	57.60±4.88[c]

Values represent mean±SD of values of 6 fish fed each experimental diet. Different letters indicate statistical significance obtained ($P<0.05$), according to ANOVA. If significant differences were found among treatments, Duncan's multiple range tests were used to rank the means.

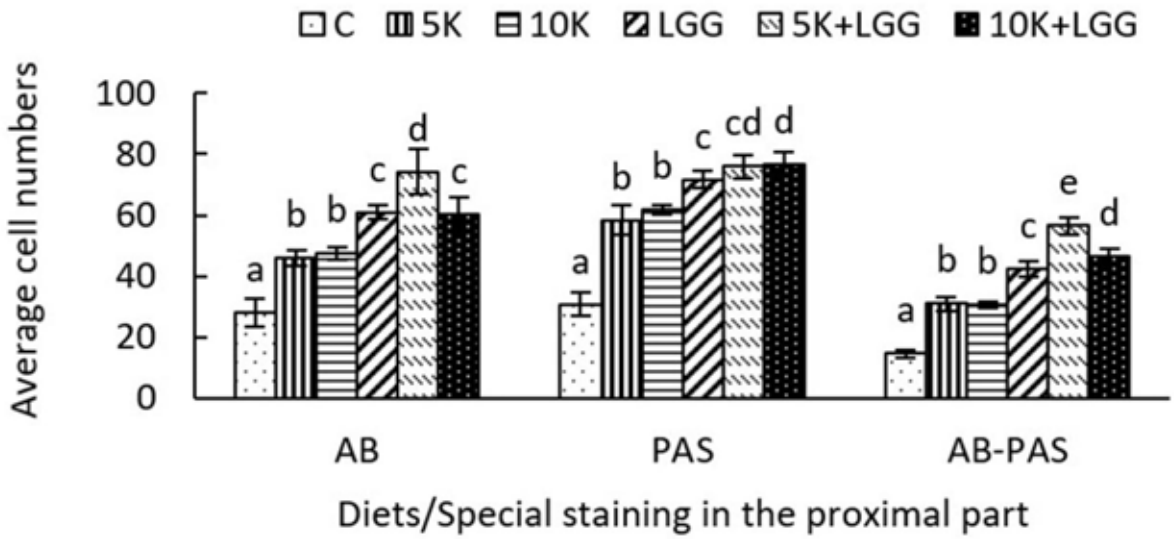


Figure 17 The average proximal intestinal goblet cells of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control) after special staining in to three types: AB staining (a), PAS staining (b), and AB-PAS double-staining (c). Statistical differences among groups were determined by Duncan's multiple range tests. Different letters indicate statistical significance ($P < 0.05$).

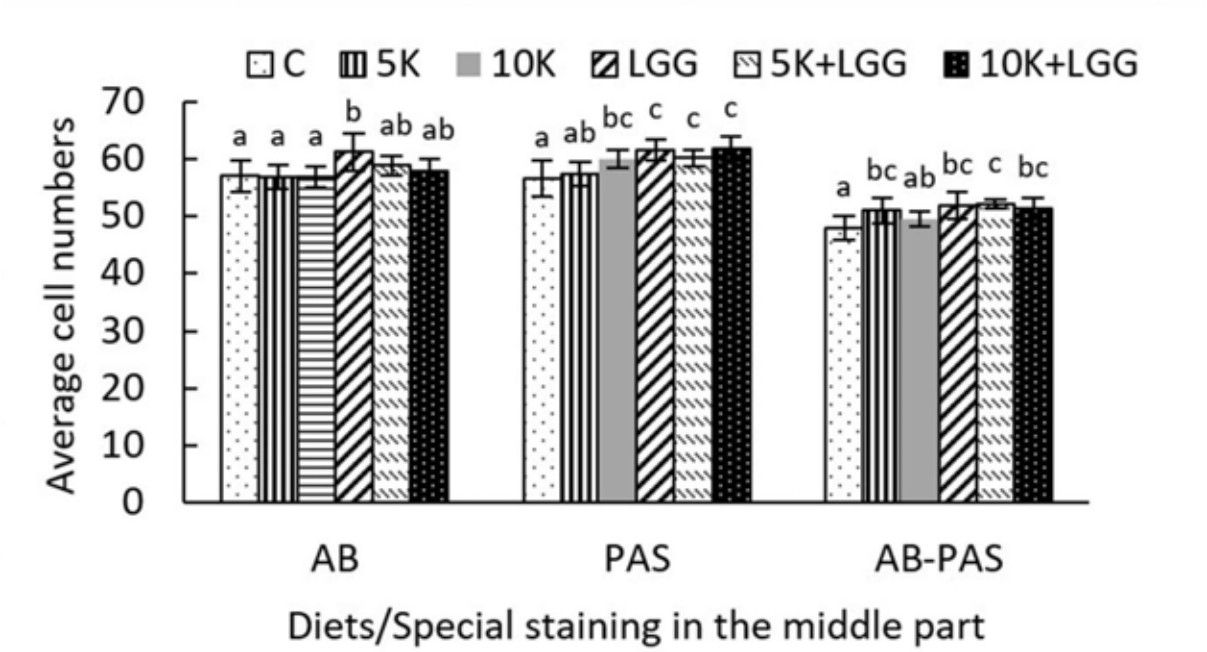


Figure 18 The average middle intestinal goblet cells of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control) after special staining in to three types: AB staining (a), PAS staining (b), and AB-PAS double-staining (c). Statistical differences among groups were determined by Duncan's multiple range tests. Different letters indicate statistical significance ($P < 0.05$).

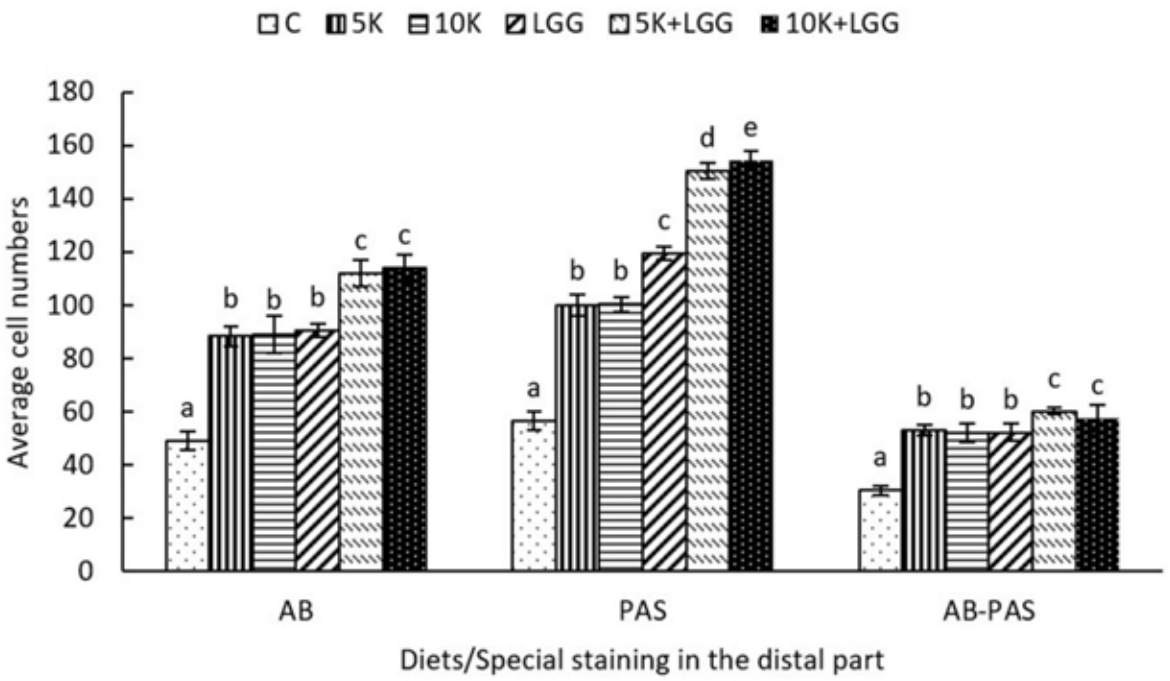


Figure 19 The average distal intestinal goblet cells of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control) after special staining in to three types: AB staining (a), PAS staining (b), and AB-PAS double-staining (c). Statistical differences among groups were determined by Duncan's multiple range tests. Different letters indicate statistical significance ($P < 0.05$).

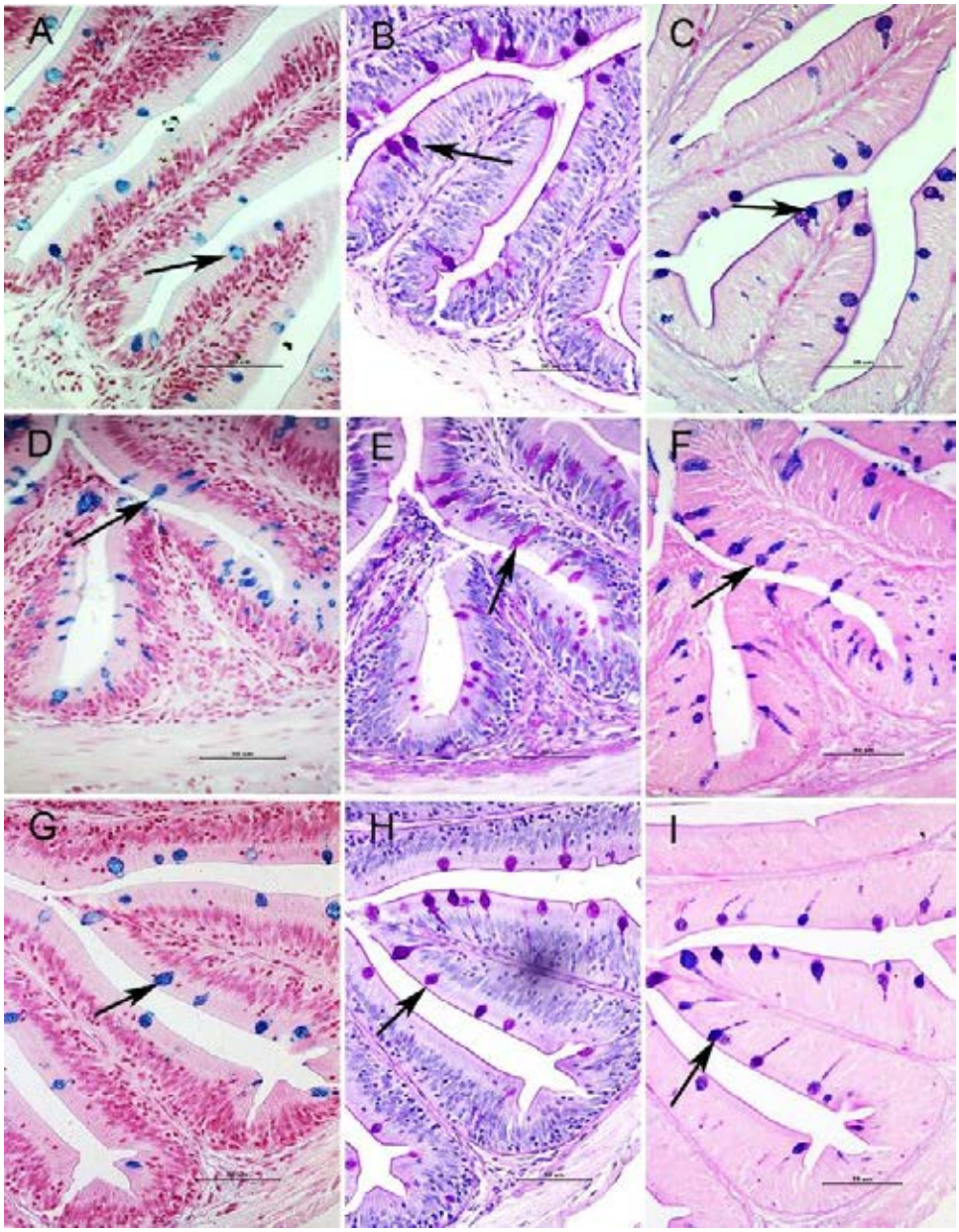


Figure 20 The proximal intestinal goblet cells (arrows) of red tilapia fed with the control, 5K, and 10K diets red tilapia fed with the control diet (A,B, and C) ; 5K diet (D, E, and F) ; 10K diet (G, H, and I) after special staining in to three types: AB staining (A, D, and G) PAS staining (B, E, and H) and AB-PAS double-staining (C, F, and I). *Bar* 50 µm

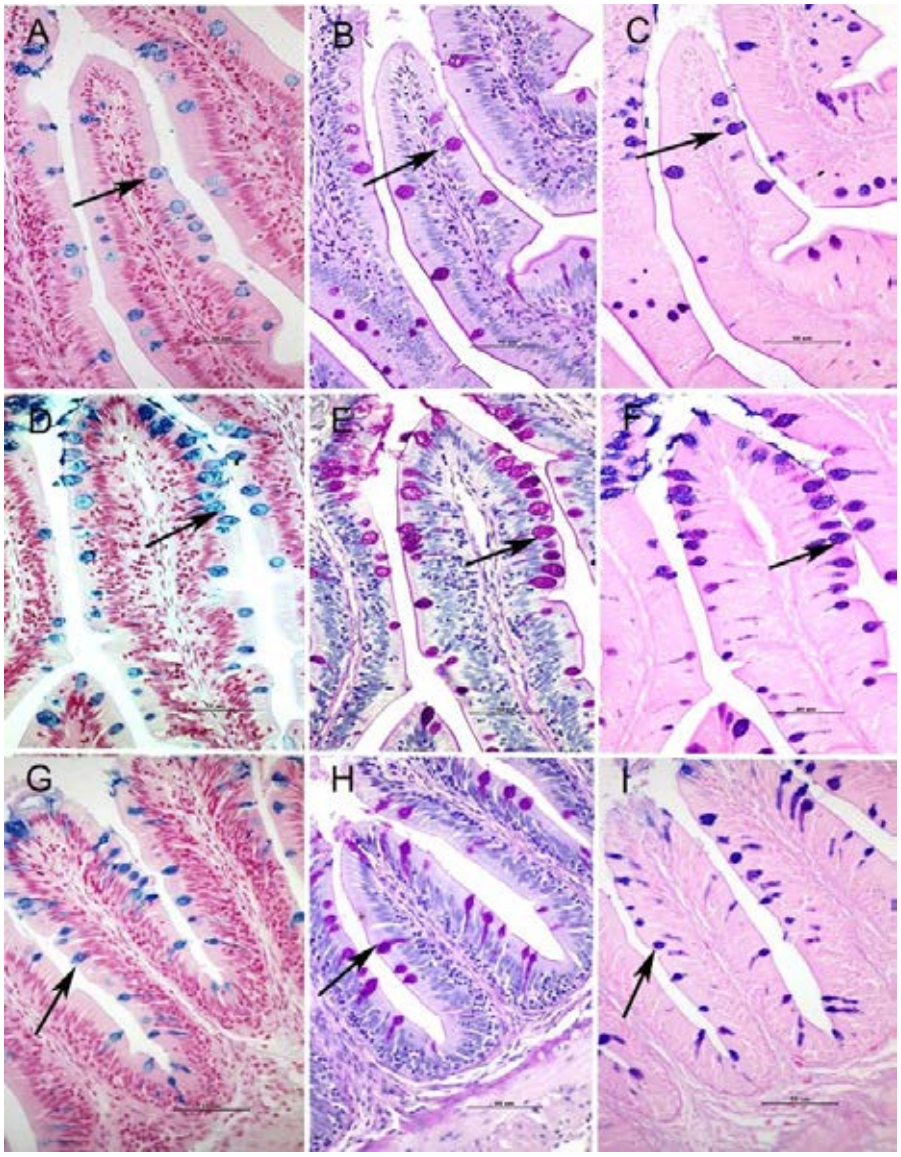


Figure 21 The proximal intestinal goblet cells (arrows) of red tilapia fed with the LGG, 5K+LGG, and 10K+LGG diets red tilapia fed with the LGG diet (A,B, and C) ; 5K+LGG diet (D, E, and F) ; 10K+LGG diet (G, H, and I) after special staining in to three types: AB staining (A, D, and G) PAS staining (B, E, and H) and AB-PAS double-staining (C, F, and I). *Bar* 50 μm

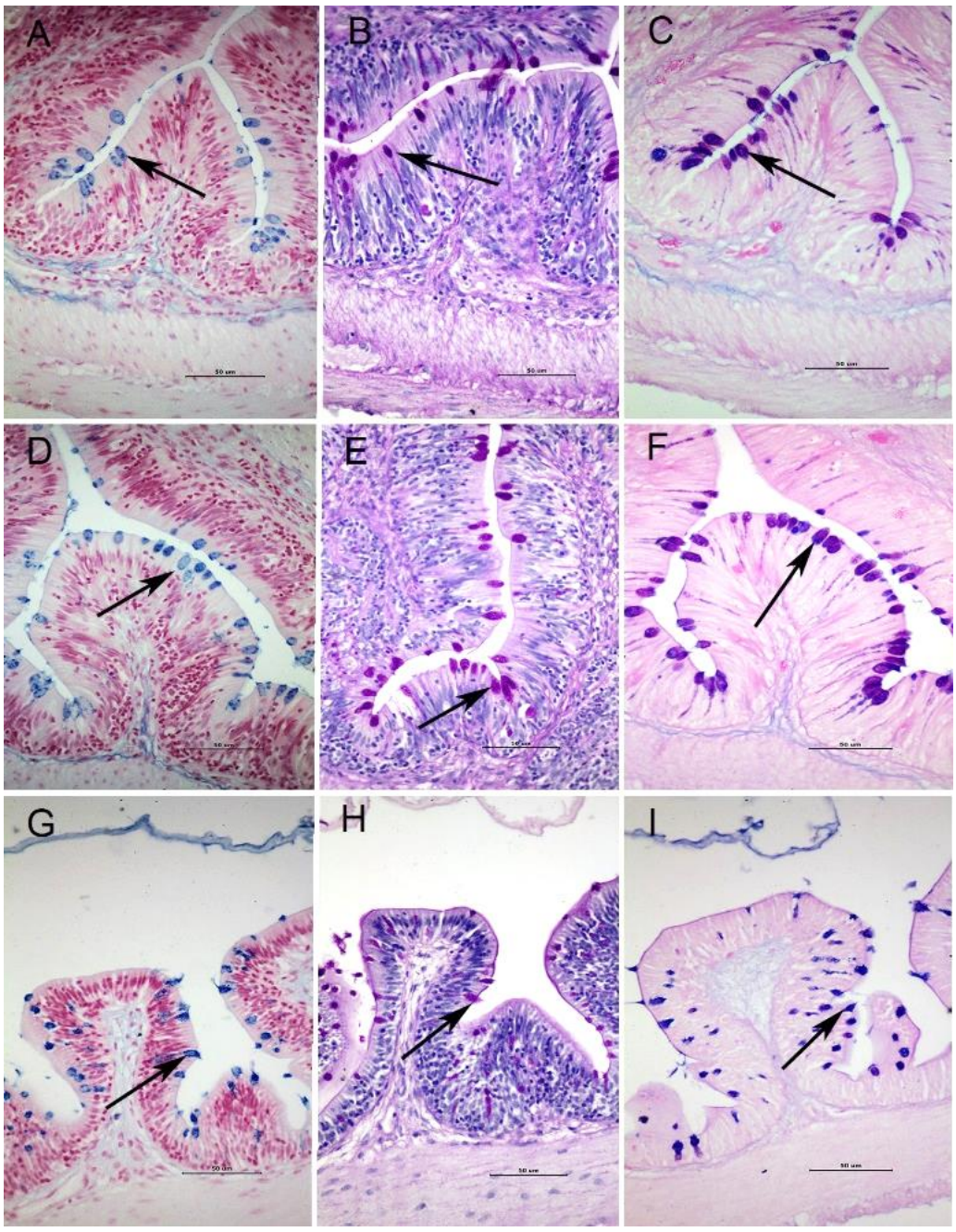


Figure 22 The distal intestinal goblet cells (arrows) of red tilapia fed with the control, 5K, and 10K diets red tilapia fed with the control diet (A,B, and C) ; 5K diet (D, E, and F) ; 10K diet (G, H, and I) after special staining in to three types: AB staining (A, D, and G) PAS staining (B, E, and H) and AB-PAS double-staining (C, F, and I). *Bar* 50 µm

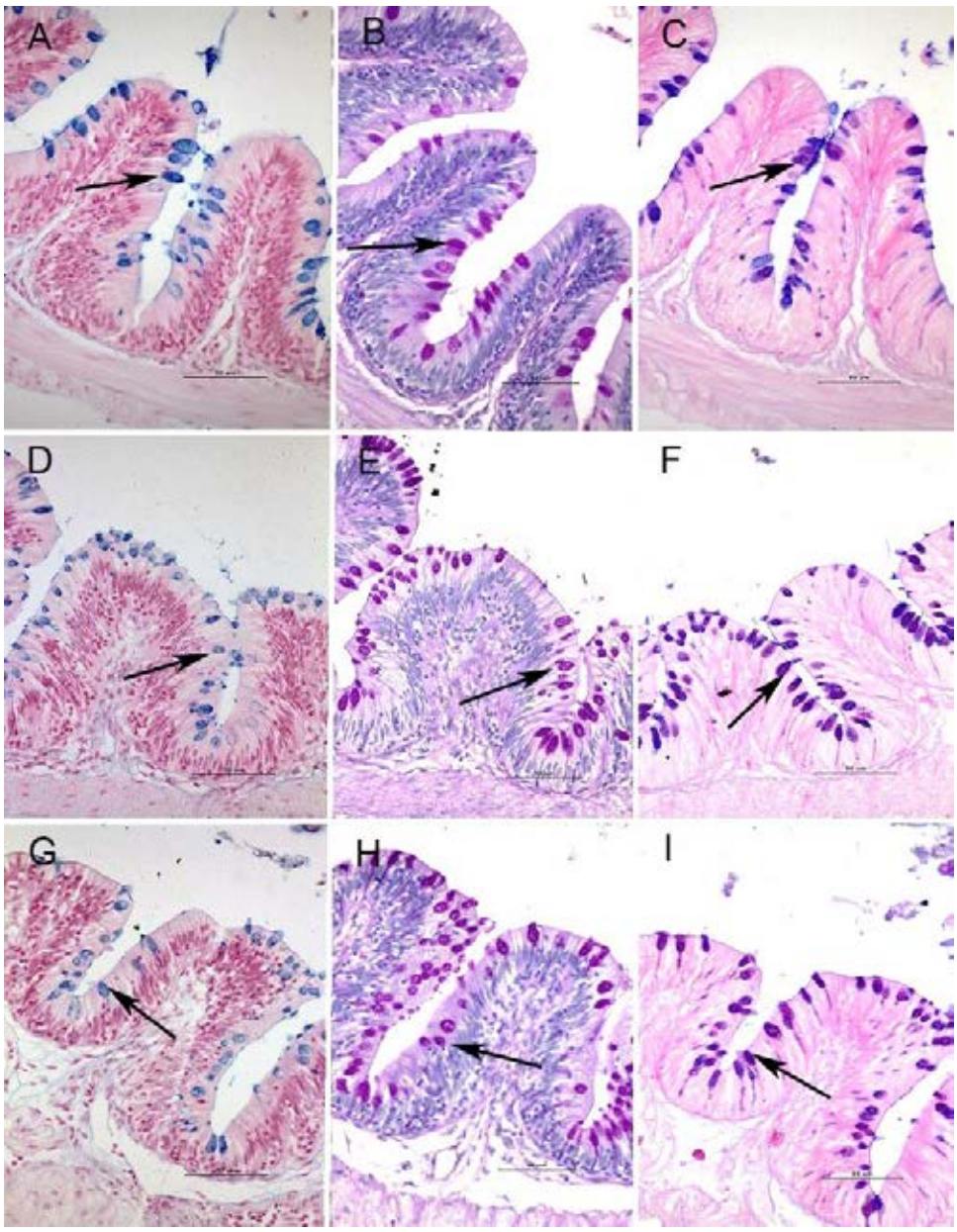


Figure 23 The distal intestinal goblet cells (arrows) of red tilapia fed with the LGG, 5K+LGG, and 10K+LGG diets red tilapia fed with the LGG diet (A,B, and C) ; 5K+LGG diet (D, E, and F) ; 10K+LGG diet (G, H, and I) after special staining in to three types: AB staining (A, D, and G) PAS staining (B, E, and H) and AB-PAS double-staining (C, F, and I). *Bar* 50 µm

4.6 Immune parameters

The results showed that fish fed with the synbiotic-supplemented diets (5K + LGG and 10K + LGG) experienced a significant increase ($P < 0.05$) in lysozyme activity in comparison with other groups (Fig. 25). There was no significant difference in ACH50 activity and Ig in fish fed with the different diets (Fig. 26, 27).

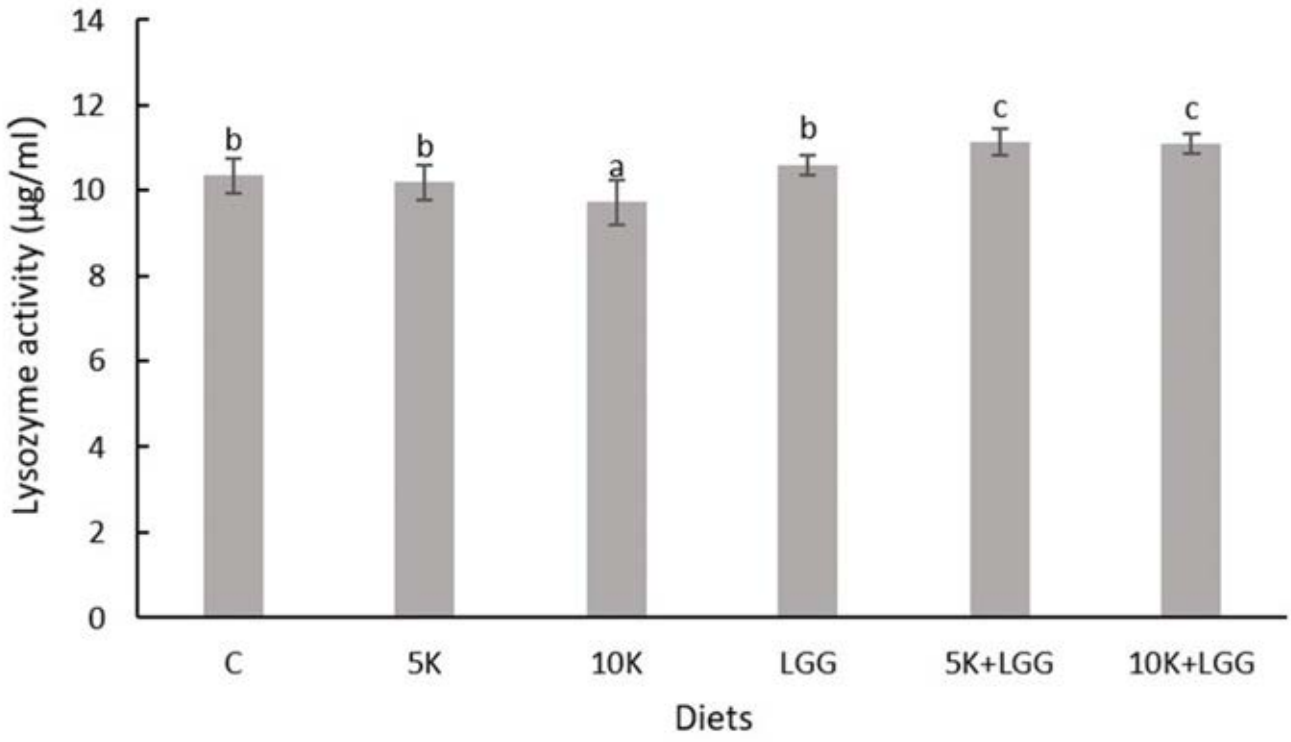


Figure 24 Lysozyme activity of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control). Statistical differences among groups were determined by Duncan's multiple range tests. Different letters indicate statistical significance ($P < 0.05$).

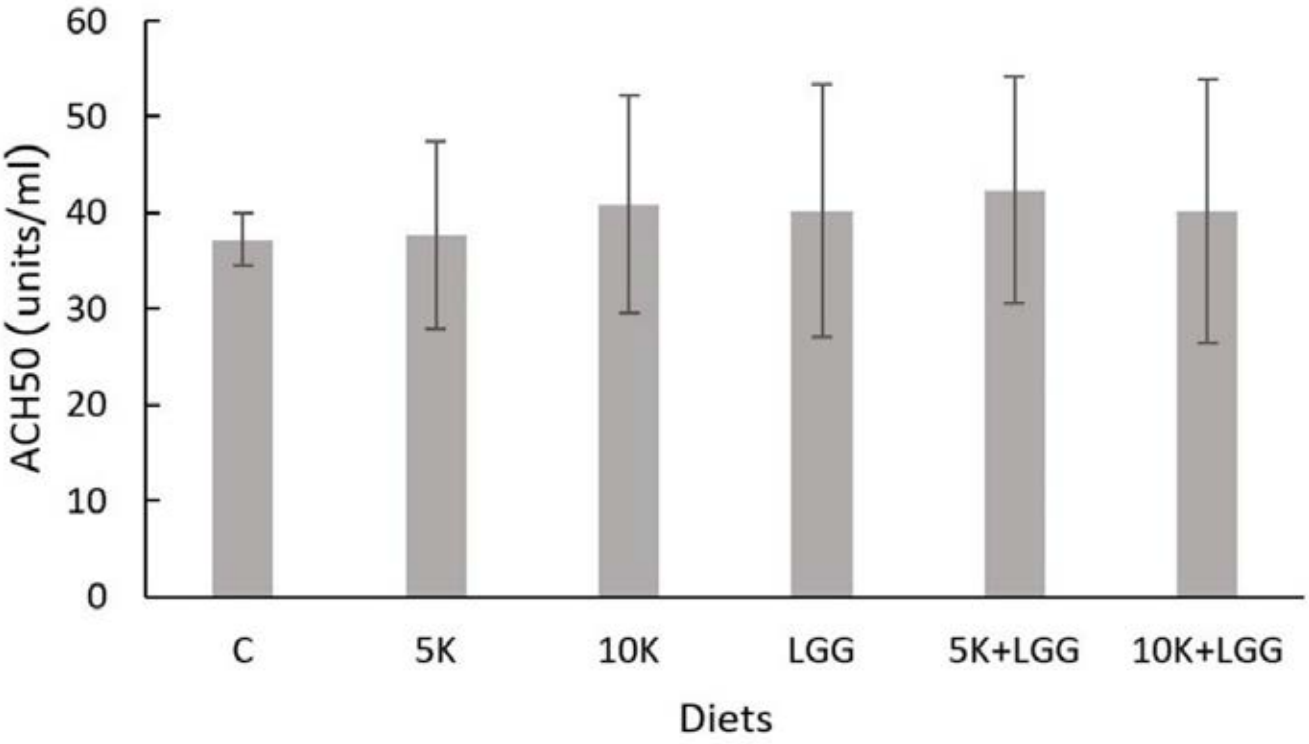


Figure 25 Alternative complement hemolytic 50 (ACH50) activity of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control). Statistical differences among groups were determined by Duncan's multiple range tests.

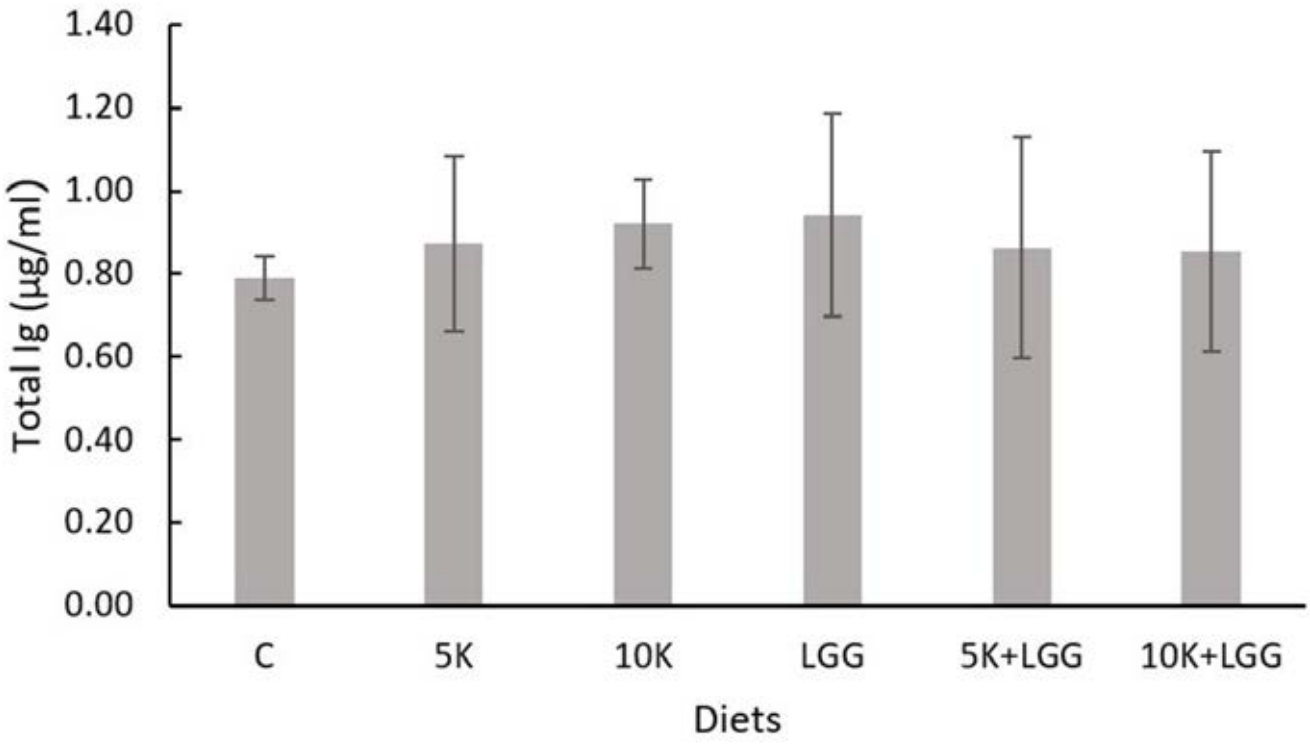


Figure 26 Total immunoglobulin of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control). Statistical differences among groups were determined by Duncan's multiple range tests.

4.7 Intestinal bacterial count

The results presented that fish fed with the LGG, 5K + LGG, and 10K + LGG a significant increase ($P < 0.05$) in the number of probiotic bacteria in comparison with other groups. There was no significant difference in the number of total bacteria in fish fed with the different diets (Table 9)

Table 9 Microbiological analysis of red tilapia fed with the different experimental diets during 30 days

	Diets					
	C	**5K**	**10K**	**10^8LGG**	**5K + 10^8LGG**	**10K + 10^8LGG**
Total bacteria (10^8 CFU/g)	2.40±0.14	2.52±0.12	2.53±0.15	2.35±0.07	2.33±0.04	2.30±0.14
Probiotic bacteria (10^4 CFU/g)	0.98±0.04[a]	1.06±0.21[a]	1.53±0.11[a]	4.05±0.28[b]	5.50±2.12[b]	5.9±1.27[b]

Values represent mean±std of values of 25 fish fed each experimental diet. Different letters indicate statistical significance obtained ($P<0.05$), according to ANOVA. If significant differences were found among treatments, Duncan's multiple range tests were used to rank the means.

4.8 Hemolytic activity of extracellular products of *Aeromonas veronii*

Hemolytic activity test of ECPs from *A. veronii* NK06 isolates revealed hemolytic activity both sheep and tilapia erythrocytes. HU of ECPs from *A. veronii* NK06 isolates against sheep and tilapia erythrocytes were 27.45, 57.57 µg, respectively. Similarly, *A. veronii* NK06 isolates were high mortality rate in tilapia (Dong et al., 2015). Therefore, hemolytic activity of ECPs involved virulence factors of *A. veronii* NK06 isolates. Although, we used the same condition for extraction the ECPs from *A. veronii* NK01 and NK06 isolates, the ECPs from *A. veronii* NK01 isolates not showed hemolytic activity both sheep and tilapia erythrocytes.

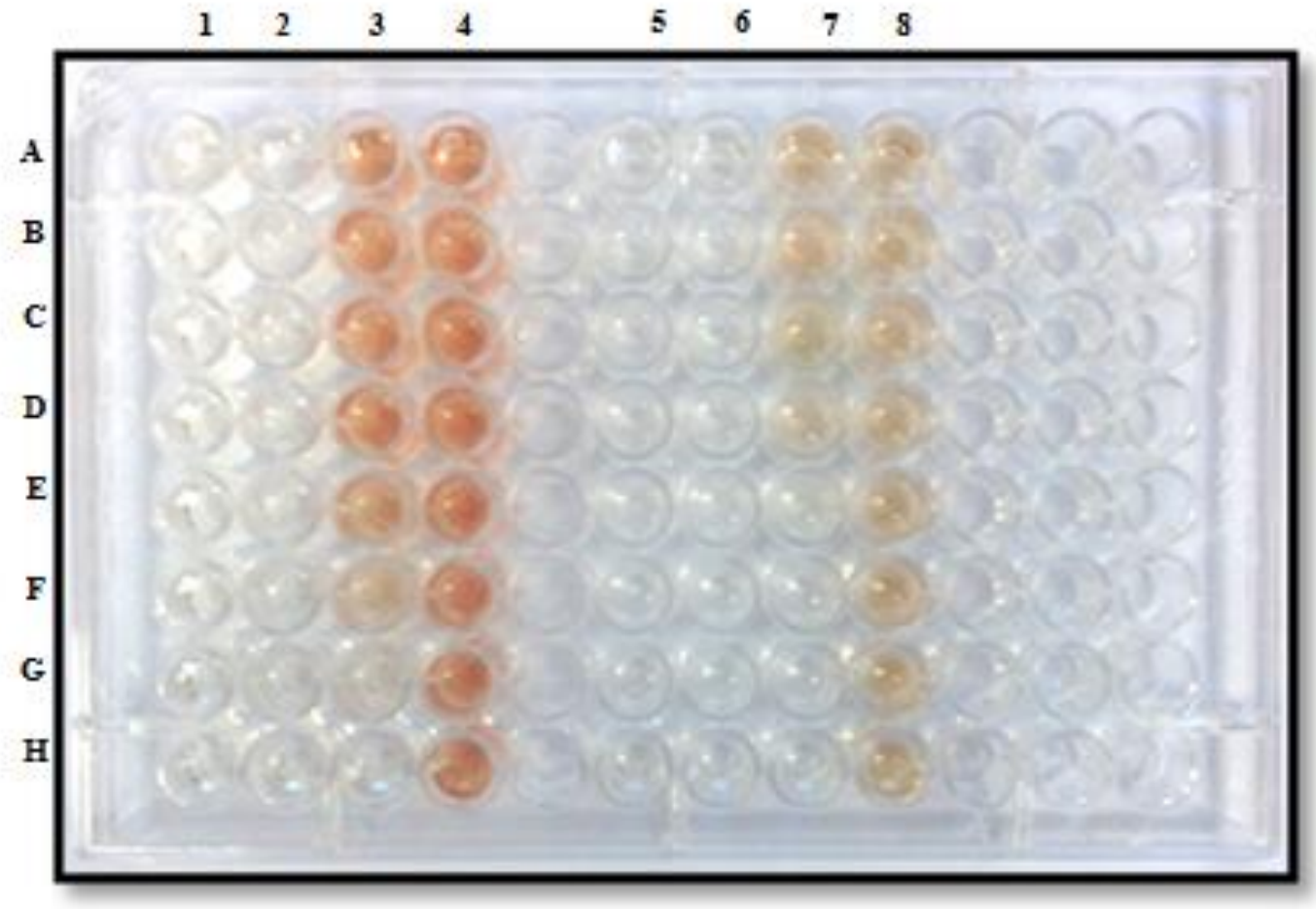


Figure 27 Hemolytic activity of the sample was incubated with sheep erythrocytes (lane 1-4) and tilapia erythrocytes (lane 5-8); lane 1, PBS (Negative control); lane 2, NK01; lane 3, NK06; lane 4, 0.1% TritonX-100 (Positive control); lane 5, PBS (Negative control); lane 6, NK01; lane 7, NK06; lane 8, 0.1% TritonX-100 (Positive control). The protein concentration of ECPs of the NK01 and NK06 in rows A-H were 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 µg, respectively

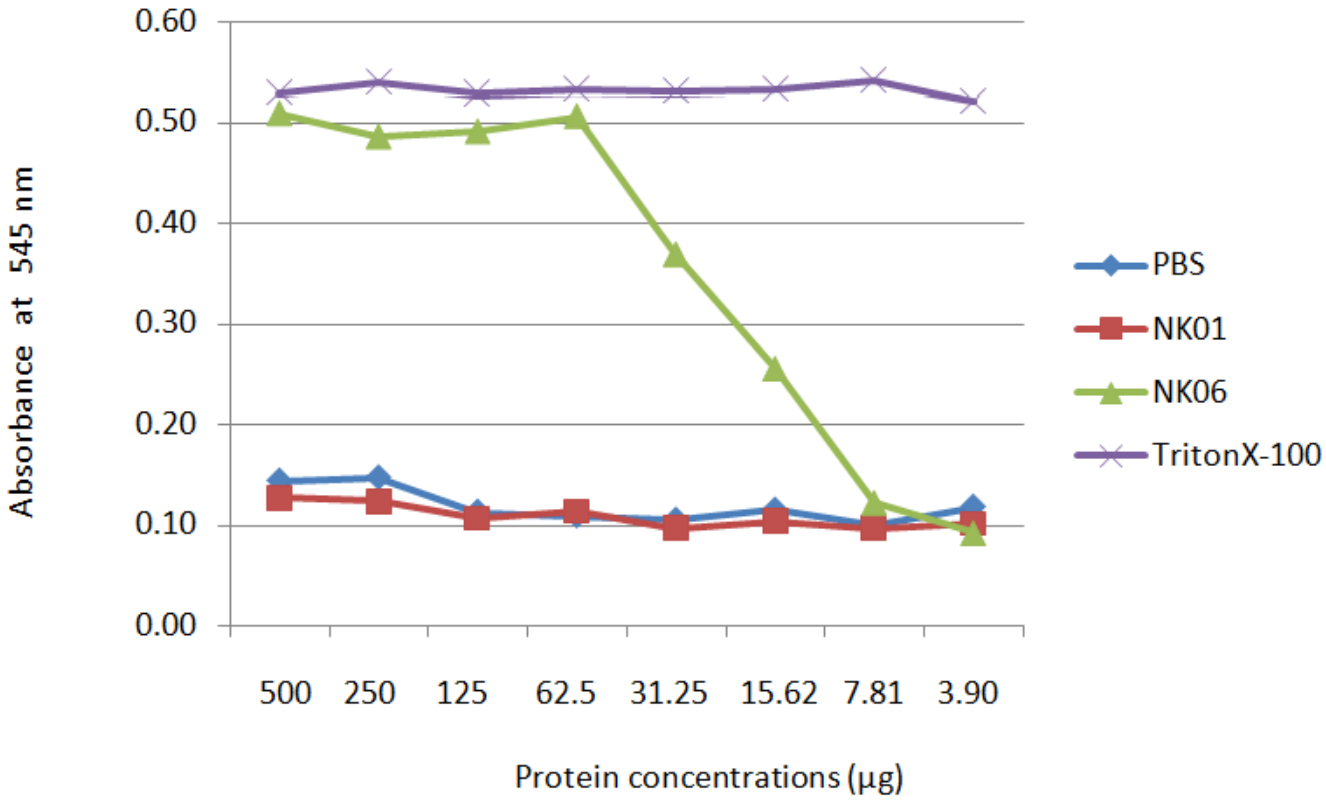


Figure 28 Hemolytic activity of the sample was incubated with sheep erythrocytes.

The activity was determined by measuring the microplate reader at 545 nm.

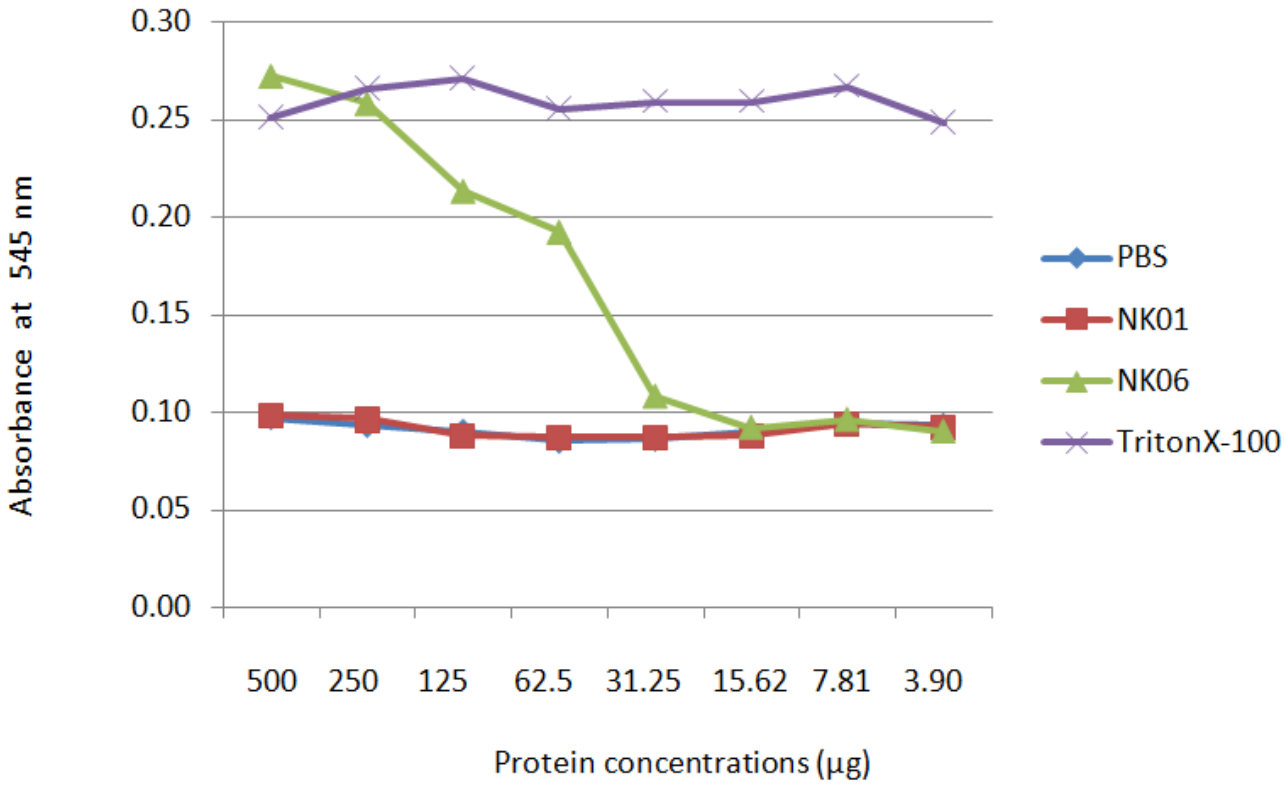


Figure 29 Hemolytic activity of the sample was incubated with tilapia erythrocytes.

The activity was determined by measuring the microplate reader at 545 nm.

4.9 Mortality test

The cumulative mortality of fish fed the synbiotic-supplemented diets (5K+LGG and 10K+LGG) was significantly lower ($P < 0.05$) than in fish fed 5K, 10K, and LGG. Fish fed 5K and 10K were significantly lower ($P < 0.05$) than those fed with control diet (Fig.31). All dead fish showed pale body surface, fin rot, cloudy eyes, hemorrhage in liver and spleen, and yellow liquid accumulation in the swollen intestine.

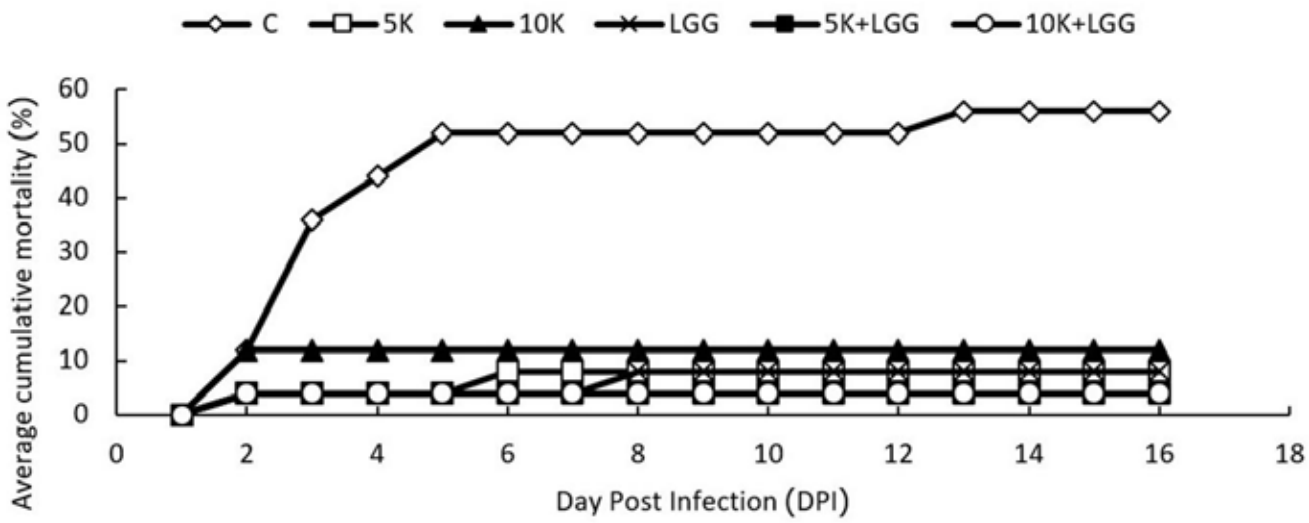


Figure 30 The average of cumulative mortality of red tilapia fed with the experimental diets (5K, 10K, LGG, 5K+LGG, 10K+LGG, and control) after a challenge with *Aeromonas veronii*. Statistical differences among groups were determined by Duncan's multiple range tests. Different letters indicate statistical significance ($P < 0.05$).

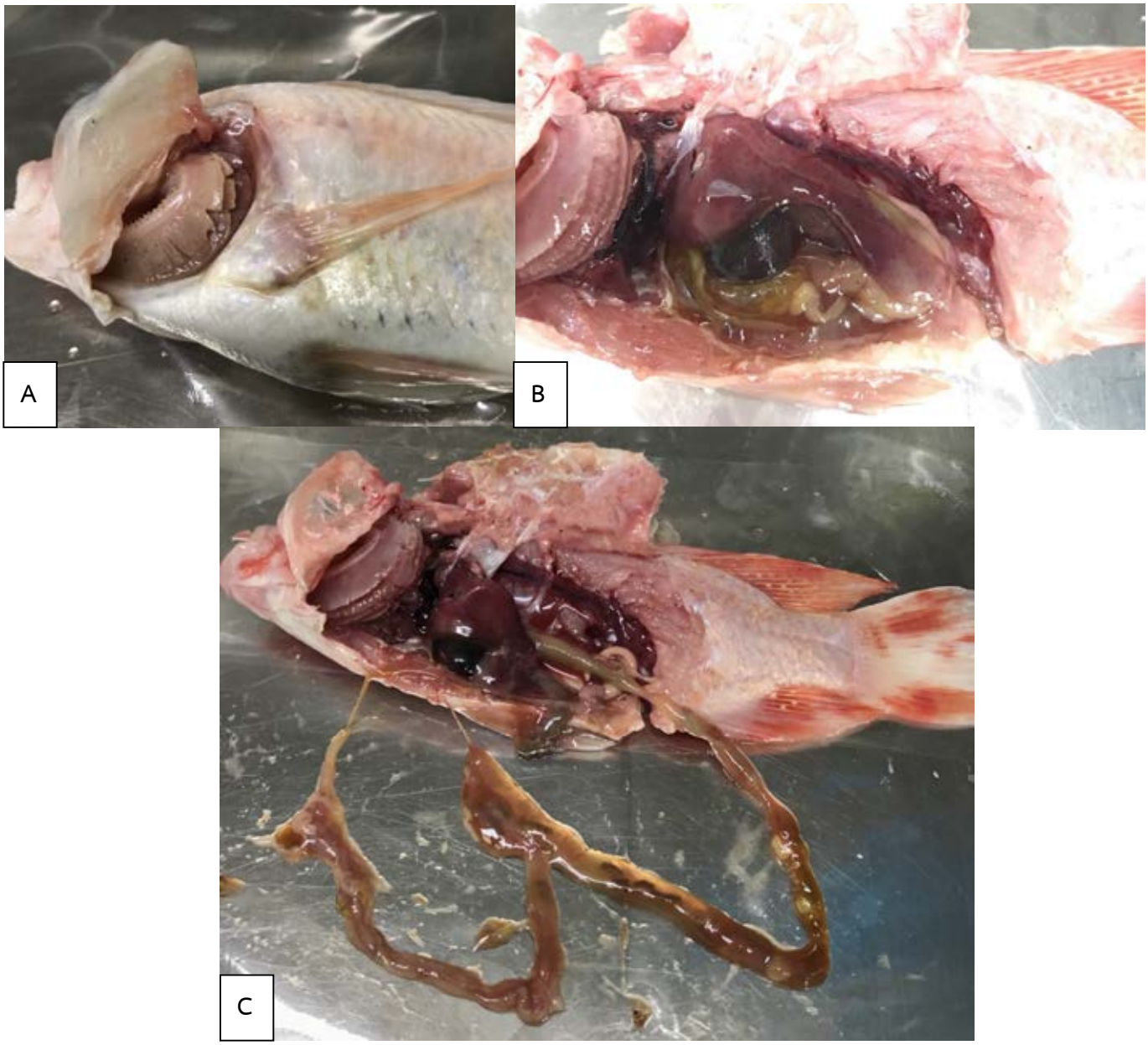


Figure 31 Red tilapia were challenged by *Aeromonas veronii*. Dead fish exhibited necrotic gills and pale body surface (a), hemorrhage in liver and yellow liquid accumulation in the swollen intestine (b) and enteritis (c).

CHAPTER V
GENERAL DISCUSSION AND CONCLUSION

5.1 Discussion

Different studies have reported that a dietary supplement of the prebiotic inulin in aqua feeds enhances the growth performance in fish (Mahious et al., 2006; Reza et al., 2009; Ibrahem et al., 2010; Ortiz et al., 2013), while others reported no effects (Grisdale-Helland et al., 2008; Burr et al., 2009). In the present study, the result showed that the final weight of fish fed the highest amount of JA together with LGG (10K+LGG) was the highest indicating a positive effect of the synbiotic on the growth performance in comparison with a previous study where Pangasius Catfish (Pangasius bocourti, Sauvage 1880) were fed a synbiotic (JA and *L. plantarum*) diet for 90 days (Doan et al., 2016), extending the beneficial improvement the growth performance and feed utilization by the JA and Lactobacili synbiotics in fish. The effect of supplementation with functional prebiotics on growth performance varies among fish species and prebiotic types (Ringø et al., 2010). The results of the current study revealed that red tilapia fed with the JA supplemented diet had a significantly better growth performance than the control group. Tiengtam et al., 2017 reported that supplementation with JA for 8 weeks increased the villus height in the proximal and middle intestine in juvenile Nile tilapia. In contrast, there was no statistical difference in villus height in the proximal and middle intestine of red tilapia supplemented with JA for 1 month in the current study, suggesting that the feeding duration of JA prebiotic and fish species influence

the feed efficiency and gut. The beneficial effect of JA on growth performance may be involved in the result of JA compounds such as inulin, FOS, carbohydrate, protein, Vitamin C, and minerals (Cardellina, 2015). Moreover, JA tubers contain natural antioxidants such as polyphenols involved in protecting against oxidative stress (Johansson et al., 2015; Dias et al., 2016), which could ultimately have a positive effect on the growth performance (Yuan et al., 2007).

The results showed that the numbers of probiotic bacteria in digestive tract of fish fed with the LGG, 5K + LGG, and 10K + LGG a significant higher ($P < 0.05$) than those of fish fed other diets. The amount of probiotic bacteria in digestive tract of fish fed with the probiotic and synbiotic indicated that LGG can colonized the digestive tract of the host (Balcazar et al., 2006). Probiotic bacteria are capable of producing digestive enzymes that help fish use feed nutrients and digest (Bairagi et al., 2002).

Prebiotics increase the amount of beneficial bacteria such as bifidobacteria and lactobacilli in the gut (Saad et al., 2013). Several studies reported that beneficial bacteria decomposed the nutrients in the gastrointestinal tract and provided physiologically active compounds such as enzymes, amino acids, and vitamins (Gatesoupe, 1999; Wang and Xu, 2006). Prebiotics such as inulin and fructooligosaccharide (FOS) from Jerusalem artichoke (Moshfegh et al., 1999; Kleessen et al., 2007) stimulate the growth of probiotics. For instance, Inulin stimulated the growth of beneficial bacteria in the gastrointestinal tract (Holscher, 2017).

Administration of synbiotic could have a double contribution in the pool of short chain fatty acids (SCFA) in the intestinal tract. Intestinal bacteria produce SCFA such as acetate, propionate and butyrate, the main energy source of intestinal epithelial cells, and so this may affect cell proliferation (Blottiere et al., 2003). They may increase the villi height resulting in an improved absorptive area, which relates to a higher absorption of available nutrients (Strocchi and Levitt, 1993; Cabello, 2006) and a better growth performance. In the present study, the fact that the absorptive area (Fig.16) in intestines of fish fed the synbiotic-supplemented diets (10K+LGG) was higher than those of fish fed other diets indicates that synbiotics could improve the absorptive area in the intestine of red tilapias. Also, the intestinal absorptive area in fish fed the probiotic-supplemented diets was higher than in those fed the prebiotic-supplemented and the control diets, which suggests the beneficial and widely reported positive effect of LGG in the intestine. Blood chemical parameter is one of the most common factors to assess the nutritional and health status of red tilapia. The synbiotic-supplemented diet increased the total cholesterol and total protein levels of red tilapia. These results suggest that synbiotic-supplemented diets are related with the energy and protein contribution in red tilapia (Kaushik and Seiliez, 2010; Panigrahi et al., 2010; Polakof et al., 2012). In addition, this study revealed that synbiotic-supplemented increased the intestinal absorptive area of red tilapia. Increased intestinal digestive enzyme activities and intestinal absorptive area would affect to the total cholesterol and total protein levels in serum. Fish from the control group had

the highest D-bilirubin values, which were significantly ($P < 0.05$) higher than the probiotic group (LGG), prebiotic groups (5K and 10K), synbiotic groups (5K + LGG and 10K + LGG). This may indicate that synbiotic-supplemented diets had effects in protecting the liver and kidney cells (Hassaan et al., 2014), as in the case of liver damage during which D-bilirubin is released into the blood, allowing for the early detection of liver problems (Chen, 2003; Kumar et al., 2011). The fish from the control group had the highest alanine transaminase (ALT) value, which was significantly ($P < 0.05$) higher than those of the fish fed with 5K and 10K diets. This could be indicating that the Jerusalem artichoke had positive effects in protecting the liver cells (Hassaan et al., 2014), because ALT is released into the blood in the case of liver damage being a suitable parameter for the early detection of liver problems (Kumar et al., 2011). In a similar way, fish from the control group showed the highest BUN value, being significantly ($P < 0.05$) higher than those of the fish fed with 5K. Results showed no significant differences between the fish fed the control diet and those fed the diets with synbiotic in triglyceride, albumin, T-bilirubin and AST, this indicating that the experimental diets had no effect on serum biochemical parameters in juvenile red tilapia (Ghobadi, 2015).

The immune system of teleosts, or bony fish, is composed of innate and adaptive immune responses. Mucus cell is a crucial first line of defense against pathogenic bacterial infection as an important defense mechanism and functions for

lubricating, trapping, and removing the pathogens (Ngamkala et al., 2010). The dominant mucous cell type in fish intestine epithelium is known to be goblet cell (Salinas and Parra, 2015). The goblet cells mucous secretion are composed of acid and neutral mucins (Sklan et al., 2004). In the present study, the fish fed the synbiotic-supplemented diet (5K+LGG) showed that the numbers of acid mucous cells and double-staining mucous cells in the proximal intestine had significantly higher ($P < 0.05$) than those of fish fed other diets. Moreover the results found that the fish fed the synbiotic-supplemented diets (5K+LGG and 10K+LGG) showed the numbers of acid mucous cells, neutral mucous cells and double-staining mucous cells in the distal intestine significantly higher ($P < 0.05$) than those of fish fed other diets. A similar results were reported from studies on *L. rhamnosus* supplemented-diet on Nile tilapia, *Oreochromis niloticus* (Ngamkala et al., 2010; Pirarat et al., 2011) and multi-species (*Bacillus* sp., *Pediococcus* sp., *Enterococcus* sp. and *Lactobacillus* sp.) supplemented-diet on Nile tilapia, *Oreochromis niloticus* (Ramos et al., 2017), which increased the number of goblet cells in the intestine. In addition, previous study suggested that *L. rhamnosus* (supplemented-diet) may be involved in the less severe damage of the tilapia intestine after the *Aeromonas* challenge (Ngamkala et al., 2010). This may indicate that the significantly higher numbers of goblet cells in the fish fed the synbiotic-supplemented diets (5K+LGG and 10K+LGG) in the present study may be related to the synergistic effect and the lower mortality of the fish fed the synbiotic-supplemented diets. In addition, current data suggests that acidic mucin are associated

with protect against bacterial translocation (Deplancke and Gaskins, 2001) while neutral mucin are related to digestion processes (Grau et al., 1992).

The current study revealed that synbiotic-supplemented diets enhance the innate immune response of red tilapia as indicated by increased levels of lysozyme activity. Similarly, a JA and *L. plantarum* synbiotic-supplemented diet was reported to increase levels of lysozyme activity on Pangasius Catfish after 90 days (Doan et al., 2016), confirming the enhanced activity of digestive enzymes against peptidoglycan layers of bacteria. However, there was no change in lysozyme activity for rainbow trout (*Oncorhynchus mykiss*) fed with FOS and *L. rhamnosus* supplemented diets for 30 days (Panigrahi et al., 2005). Lysozyme is capable of digestion the β-(1,4) linkages between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layers of Gram-positive bacteria. In general, lysozyme are not directly damaged Gram-negative bacteria. However, lysozyme can against the Gram-negative bacteria after the outer membrane of Gram-negative bacteria was destroyed, then lysozyme can expose to the inner peptidoglycan layer of bacteria. Lysozyme is activated the polymorphonuclear leucocytes and macrophages and opsonin of the complement system for phagocytosis (Saurabh and Sahoo, 2008; Uribe et al., 2011). The lack of differences in the total immunoglobulin and ACH50 activity obtained in the present study is in agreement with results obtained in the oral administration of *L. rhamnosus* supplemented diets (10^9CFU g^{-1}) in rainbow trout (*O. mykiss*) for 30 days (Panigrahi et

al., 2004). On the contrary, the difference in ACH50 activity was significant in rainbow trout fed the high dose of *L. rhamnosus* supplemented diet (10^{11}CFU g^{-1}), suggesting the beneficial effects of synbiotics on immune modulation might vary among fish species, level of supplementation, and duration of feeding. The cumulative mortalities of fish fed the synbiotic-supplemented diets (5K+LGG, 10K+LGG) showed significantly lower than the fish fed the supplemented diets (5K, 10K, LGG,), and those fed control diet. Similar results were observed in synbiotic supplemented-diet between Jerusalem artichoke and *L. plantarum* on Pangasius catfish (*Pangasius bocourti*) protected against *A. hydrophila* (Van Doan et al., 2016). In addition, prebiotic supplemented-diet of Jerusalem artichoke on Asian seabass (*Lates calcarifer*) showed protected against *A. hydrophila* (Bello et al., 2012). It has been reported that the lowest cumulative mortalities of fish fed synbiotic-supplemented diets may be caused by the synergistic effect between the prebiotic and the probiotic (Gibson and Roberfroid, 1995). Prebiotics stimulate the growth of probiotics, and these reduce the presence of pathogens in the host by means of the inhibition of their adherence and colonization (Bruno and Montville, 1993; Perez et al., 2014), enhancing the host response to diseases (Madsen, 2006; Preidis et al., 2011).

In-vitro test, *A. veronii* NK06 isolates showed hemolytic activity while *A. veronii* NK01 isolates not showed hemolytic activity. Previous study showed that *A. veronii* NK01 isolates (β-hemolysin) showed mortality rate in red tilapia challenging higher

than *A. veronii* NK06 isolates (α –hemolysin) (Dong et al., 2015) indicates that β-hemolysin and α –hemolysin of *A. veronii* are synthesized differential conditions (Thelestam and Ljungh, 1981). In addition, previous studies reported that *A. veronii* produced the extracellular products (ECPs) that contained various virulence factors such as hemolysin, enterotoxin, cytotoxin, gelatinase, collagenase, lecithinase, elastase, lipase, and lipopolysaccharide (Tomas, 2012; Lowry et al., 2014). Therefore, the several virulence factors such as protease, enterotoxin, and lipase activity requires further study.

5.2 Conclusion

The data confirmed the beneficial effect of synbiotic effects of Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical, intestinal morphology, immune parameters, intestinal bacterial count, and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.). Direct administration of JA and LGG in fish feed can be used as a practical nutritional supplement in red tilapia.

5.3 Suggestions for further investigation

In this study, the beneficial effect of synbiotic effect of Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets in juvenile red tilapia (*Oreochromis* spp.) had been investigated. Further studies on technological aspects should be investigated to determine antioxidant and immune gene expression for detection the antioxidative stress and immune response in juvenile red tilapia (*Oreochromis* spp.)

after feeding with synbiotic between Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets.

REFERENCES

Abid A, Davies SJ, Waines P, Emery M, Castex M, Gioacchini G, Carnevali O, Bickerdike R, Romero J and Merrifield DL. 2013. Dietary synbiotic application modulates Atlantic salmon (*Salmo salar*) intestinal microbial communities and intestinal immunity. Fish Shellfish Immunol. 35(6): 1948-1956.

Abolghait SK, Mohamed MF, Aly SM, Garbaj AM and Moawad AA. 2013. Attenuated virulence of pigment-producing mutant of Aeromonas veronii bv. sobria in HeLa cells and Nile tilapia (*Oreochromis niloticus*). IJVSM. 1(1): 43-47.

Ai Q, Xu H, Mai K, Xu W, Wang J and Zhang W. 2011. Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, Larimichthys crocea. Aquaculture. 317(1): 155-161.

Akrami R, Nasri-Tajan M, Jahedi A, Jahedi M, Razeghi Mansour M and Jafarpour SA. 2015. Effects of dietary synbiotic on growth, survival, lactobacillus bacterial count, blood indices and immunity of beluga (*Huso huso* Linnaeus, 1754) juvenile. Aquac Nutr. 21(6): 952-959.

Alak G. 2012. The Effects of Probiotics and Prebiotics on Rainbow Trout (*Oncorhynchus mykiss*) Intestinal Flora. Int J Aquac. 2(3).

Alexander JB and Ingram GA. 1992. Noncellular nonspecific defence mechanisms of fish. Annu Rev of Fish Dis. 2: 249-279.

AlYahya SA, Ameen F, Al-Niaeem KS, Al-Sa'adi BA, Hadi S and Mostafa AA. 2018. Histopathological studies of experimental *Aeromonas hydrophila* infection in blue tilapia, *Oreochromis aureus*. Saudi J Biol Sci. 25(1): 182-185.

Anukam K and Reid G. 2007. Probiotics: 100 years (1907-2007) after Elie Metchnikoff's Observation. In: Méndez-Vilas A (ed) Communicating Current Research and Educational Topics and Trends in Applied Microbiology Spain. 1: 466-474.

Azevedo RVd, Fosse Filho JC, Cardoso LD, Mattos DdC, Vidal Júnior MV and Andrade DRd. 2015. Economic evaluation of prebiotics, probiotics and symbiotics in juvenile Nile tilapia. Rev Ciênc Agron. 46(1): 72-79.

Bairagi A, Sarkar Ghosh K, Kumar Sen S and Ray A. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquacult Int. 10(2): 109-121.

Balcazar JL, de Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D and Muzquiz JL. 2006. The role of probiotics in aquaculture. Vet Microbiol. 114(3-4): 173-186.

Bello O, Emikpe B, Olaifa F, BELLO O, EMIKPE B and OLAIFA F. 2012. The body weight changes and gut morphometry of Clarias gariepinus juveniles on feeds supplemented with walnut (*Tetracarpidium conophorum*) leaf and onion (Allium cepa) Bulb residues. Int. J. Morphol. 30(1): 253-257.

Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, Gomez-Llorente C and Gil A. 2012. Probiotic mechanisms of action. Ann Nutr Metab. 61(2): 160-174.

Blottiere HM, Buecher B, Galmiche JP and Cherbut C. 2003. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. Proc Nutr Soc. 62(1): 101-106.

Bruno ME and Montville TJ. 1993. Common mechanistic action of bacteriocins from lactic Acid bacteria. Appl Environ Microbiol. 59(9): 3003-3010.

Burr G, Gatlin DM and Hume M. 2009. Effects of the Prebiotics GroBiotic((R))-A and Inulin on the Intestinal Microbiota of Red Drum, Sciaenops ocellatus. J World Aquacult Soc. 40(4): 440-449.

Cabello FC. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol. 8(7): 1137-1144.

Cai SH, Wu ZH, Jian JC, Lu YS and Tang JF. 2012. Characterization of pathogenic *Aeromonas veronii* bv. veronii associated with ulcerative syndrome from chinese longsnout catfish (*Leiocassis longirostris* Gunther). Braz J Microbiol. 43(1): 382-388.

Cardellina JH. 2015. Review of Biology and Chemistry of Jerusalem Artichoke, *Helianthus tuberosus* L. J Nat Prod. 78(12): 3083-3083.

Cerezuela R, Guardiola FA, Meseguer J and Esteban MA. 2012. Increases in immune parameters by inulin and *Bacillus subtilis* dietary administration to gilthead seabream (*Sparus aurata* L.) did not correlate with disease resistance to Photobacterium damselae. Fish Shellfish Immunol. 32(6): 1032-1040.

Chen CY. 2003. Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. Aquaculture. 218(1-4): 89-102.

Claesson MJ, van Sinderen D and O'Toole PW. 2007. The genus *Lactobacillus*-a genomic basis for understanding its diversity. FEMS Microbiol Lett. 269(1): 22-28.

Costabile A, Kolida S, Klinder A, Gietl E, Bauerlein M, Frohberg C, Landschutze V and Gibson GR. 2010. A double-blind, placebo-controlled, cross-over study to establish the bifidogenic effect of a very-long-chain inulin extracted from globe artichoke (*Cynara scolymus*) in healthy human subjects. Br J Nutr. 104(7): 1007-1017.

Cui H, Hao S and Arous E. 2007. A distinct cause of necrotizing fasciitis: *Aeromonas veronii* biovar sobria. Surg Infect (Larchmt). 8(5): 523-528.

De Angelis M and Gobbetti M. 2016. *Lactobacillus* SPP.: General Characteristics. In: Reference Module in Food Science (ed) Italy: Elsevier 1-12.

Deplancke B and Gaskins HR. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr. 73(6): 1131s-1141s.

Dias NS, Ferreira JFS, Liu X and Suarez DL. 2016. Jerusalem artichoke (*Helianthus tuberosus*, L.) maintains high inulin, tuber yield, and antioxidant capacity under moderately-saline irrigation waters. Ind Crops Prod. 94: 1009-1024.

Doan H, Doolgindachbaporn S and Suksri A. 2016. Effect of *Lactobacillus plantarum* and Jerusalem artichoke (*Helianthus tuberosus*) on growth performance, immunity and disease resistance of Pangasius catfish (*Pangasius bocourti*, Sauvage 1880). Aquacult Nutr. 22(2): 444-456.

Dong HT, Nguyen VV, Le HD, Sangsuriya P, Jitrakorn S, Saksmerprome V, Senapin S and Rodkhum C. 2015. Naturally concurrent infections of bacterial and viral

pathogens in disease outbreaks in cultured Nile tilapia (*Oreochromis niloticus*) farms. Aquaculture. 448: 427-435.

Figueras MJ and Beaz-Hidalgo R. 2014. AEROMONAS | Introduction. In: Encyclopedia of Food Microbiology 2nd ed Tortorello Carl A. Batt Mary Lou (ed) Oxford: Academic Press. 24-30.

Filipe MI. 1979. Mucins in the human gastrointestinal epithelium: a review. Invest Cell Pathol. 2(3): 195-216.

Firouzbakhsh F, Mehrabi Z, Heydari M, Khalesi MK and Tajick MA. 2014. Protective effects of a synbiotic against experimental Saprolegnia parasitica infection in rainbow trout (*Oncorhynchus mykiss*). Aquac Res. 45(4): 609-618.

Fisheries Do. 1983. Thai Red Tilapia. National Inland Fisheries Institute, Department of Fisheries, Bangkok. 12.

Fisheries Do. 2005. Fisheries Statistics of Thailand. Bangkok: Fishery Statistics Analysis and Research Group. Fishery Information Technology Center, Department of Fisheries, Ministry of Agriculture and Cooperatives.

Gatesoupe FJ. 1999. The use of probiotics in aquaculture. Aquaculture. 180(1-2): 147-165.

Gatesoupe FJ. 2007. Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture. 267(1-4): 20-30.

Geng X, Dong XH, Tan BP, Yang QH, Chi SY, Liu HY and Liu XQ. 2011. Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, Rachycentron canadum. Fish Shellfish Immunol. 31(3): 400-406.

Geraylou Z, Souffreau C, Rurangwa E, De Meester L, Courtin CM, Delcour JA, Buyse J and Ollevier F. 2013. Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (*Acipenser baerii*). Fish Shellfish Immunol. 35(3): 766-775.

Gharaei A. 2014. Dietary synbiotic improves the growth performance, survival and innate immune response of Gibel carp (*Carassius auratus gibelio*) juveniles. IJAB. 2(2): 99-104.

Ghobadi S. 2015. Effect of dietary prebiotic mannan oligosaccharide (MOS) on growth performance, intestinal microflora, body composition, haematological and blood serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) Juveniles. J Fish Aquat Sci. 10(4): 255-265.

Gibson GR and Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 125(6): 1401-1412.

Gomez D, Sunyer JO and Salinas I. 2013. The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 35(6): 1729-1739.

Grabitske HA and Slavin JL. 2009. Gastrointestinal effects of low-digestible carbohydrates. Crit Rev Food Sci Nutr. 49(4): 327-360.

Grau A, Crespo S, C. Sarasquete M and Canales M. 1992. The digestive tract of the amberjack *Seriola dumerili*, Risso: A light and scanning electron microscope study. J Fish Biol. 41(2): 287-303.

Grisdale-Helland B, Helland SJ and Gatlin DM. 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture. 283(1-4): 163-167.

Grobner S, Bissinger AL, Raible A, Heeg P, Autenrieth IB and Schmidt SM. 2007. Severe diarrhoea caused by *Aeromonas veronii* biovar sobria in a patient with metastasised GIST. Pol J Microbiol. 56(4): 277-279.

Hassaan MS, Soltan MA and Ghonemy MMR. 2014. Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). Egypt J Aquat Res. 40(2): 199-208.

Holland MC and Lambris JD. 2002. The complement system in teleosts. Fish Shellfish Immunol. 12(5): 399-420.

Holscher HD. 2017. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut microbes. 8(2): 172-184.

Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Sharifian M and Esteban MA. 2015. Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. Fish Shellfish Immunol. 45(1): 27-32.

Huynh TG, Shiu YL, Nguyen TP, Truong QP, Chen JC and Liu CH. 2017. Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. Fish Shellfish Immunol. 64: 367-382.

Ibrahem MD, Fathi M, Mesalhy S and Abd El-Aty AM. 2010. Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 29(2): 241-246.

Janda JM and Abbott SL. 1998. Evolving concepts regarding the genus Aeromonas: An expanding panorama of species, disease presentations, and unanswered questions. Clin Infect Dis. 27(2): 332-344.

Janda JM and Abbott SL. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin Microbiol Rev. 23(1): 35-73.

Janda JM, Guthertz LS, Kokka RP and Shimada T. 1994. *Aeromonas* species in septicemia: laboratory characteristics and clinical observations. Clin Infect Dis. 19(1): 77-83.

Johansson E, Prade T, Angelidaki I, Svensson SE, Newson WR, Gunnarsson IB and Hovmalm HP. 2015. Economically viable components from Jerusalem artichoke (*Helianthus tuberosus* L.) in a biorefinery concept. Int J Mol Sci. 16(4): 8997-9016.

Joseph S and Carnahan A. 1994. The isolation, identification, and systematics of the motile *Aeromonas* species. Annu Rev Fish Dis. 4: 315-343.

Kaushik SJ and Seiliez I. 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. Aquac Res. 41(3): 322-332.

Kayansamruaj P, Pirarat N, Hirono I and Rodkhum C. 2014. Increasing of temperature induces pathogenicity of Streptococcus agalactiae and the up-regulation of inflammatory related genes in infected Nile tilapia (*Oreochromis niloticus*). Vet Microbiol. 172(1-2): 265-271.

Kleessen B, Schwarz S, Boehm A, Fuhrmann H, Richter A, Henle T and Krueger M. 2007. Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy volunteers. Br J Nutr. 98(3): 540-549.

Kozinska A. 2007. Dominant pathogenic species of mesophilic aeromonads isolated from diseased and healthy fish cultured in Poland. J Fish Dis. 30(5): 293-301.

Kozinska A and Pekala A. 2012. Characteristics of disease spectrum in relation to species, serogroups, and adhesion ability of motile aeromonads in fish. Sci World J. 2012: 1-9.

Kumar V, Makkar HPS and Becker K. 2011. Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus mykiss*) juveniles fed detoxified Jatropha curcas kernel meal. Aquacult Nutr. 17(4): 451-467.

Larsen B and Monif GRG. 2001. Understanding the bacterial flora of the female genital tract. Clin Infect Dis. 32(4): e69-e77.

Lazado CC and Caipang CMA. 2014. Mucosal immunity and probiotics in fish. Fish Shellfish Immunol. 39(1): 78-89.

Li Y, Liu Y, Zhou Z, Huang H, Ren Y, Zhang Y, Li G, Zhou Z and Wang L. 2011. Complete genome sequence of *Aeromonas veronii* strain B565. J Bacteriol. 193(13): 3389-3390.

Lied GA, Lillestol K, Lind R, Valeur J, Morken MH, Vaali K, Gregersen K, Florvaag E, Tangen T and Berstad A. 2011. Perceived food hypersensitivity: A review of 10 years of interdisciplinary research at a reference center. Scand J Gastroenterol. 46(10): 1169-1178.

Lilly DM and Stillwell RH. 1965. Probiotics: Growth-promoting factors produced by microganisms. Science. 147(3659): 747-748.

Lin S, Mao S, Guan Y, Luo L, Luo L and Pan Y. 2012. Effects of dietary chitosan oligosaccharides and Bacillus coagulans on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi). Aquaculture. 342-343: 36-41.

Lin Y-H, Lin S-M and Shiau S-Y. 2008. Dietary manganese requirements of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture. 284(1–4): 207-210.

Liu D. 2015. Chapter 61 - Aeromonas. In: Molecular Medical Microbiology (Second Edition). ed. Schwartzman Yi-Wei TangMax SussmanDongyou LiuIan PoxtonJoseph (ed.). Boston: Academic Press. 1099-1110.

Lowry R, Balboa S, Parker JL and Shaw JG. 2014. Aeromonas flagella and colonisation mechanisms. Adv Microb Physiol. 65: 203-256.

Luna A and Flores Miranda M. 2013. Effect of *Pediococcus pentosaceus*, inulin, and fulvic acid, added to the feed, on growth and survival of *Oreochromis niloticus*. Afr J Microbiol Res. 7(48): 5489-5495.

Madsen K. 2006. Probiotics and the immune response. J Clin Gastroenterol. 40(3): 232-234.

Magnadóttir B. 2006. Innate immunity of fish (overview). Fish Shellfish Immunol. 20(2): 137-151.

Mahious AS, Gatesoupe FJ, Hervi M, Metailler R and Ollevier F. 2006. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquac Int. 14(3): 219-229.

Majamaa H and Isolauri E. 1997. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol. 99(2): 179-185.

Martin-Carnahan A and Joseph S. 2005. Aeromonadales ord. nov. In: Bergey's Manual® of Systematic Bacteriology. ed. Brenner DonJ, Krieg NoelR, Staley JamesT, Garrity GeorgeM, Boone DavidR, De Vos Paul, Goodfellow Michael, Rainey FredA, and Schleifer Karl-Heinz (ed.). Springer US. 556-587.

Mashoof S and Criscitiello MF. 2016. Fish Immunoglobulins. Biology. 5(4): 45.

Masso-Silva JA and Diamond G. 2014. Antimicrobial peptides from fish. Pharmaceuticals (Basel). 7(3): 265-310.

Mehrabi Z, Firouzbakhsh F and Jafarpour A. 2012. Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. J Anim Physiol Anim Nutr (Berl). 96(3): 474-481.

Mishra C and Lambert J. 1996. Production of anti-microbial substances by probiotics. Asia Pac J Clin Nutr. 5(1): 20-24.

Moffitt CM and Cajas-Cano L. 2014. Blue Growth: The 2014 FAO State of World Fisheries and Aquaculture. Fisheries. 39(11): 552-553.

Monette S, Dallaire AD, Mingelbier M, Groman D, Uhland C, Richard JP, Paillard G, Johannson LM, Chivers DP, Ferguson HW, Leighton FA and Simko E. 2006. Massive mortality of common carp (*Cyprinus carpio carpio*) in the St. Lawrence River in 2001: diagnostic investigation and experimental induction of lymphocytic encephalitis. Vet Pathol. 43(3): 302-310.

Monfort P and Baleux B. 1991. Haemolysin occurrence among *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* strains isolated from different aquatic ecosystems. Res Microbiol. 142(1): 95-102.

Montajami S, Hajiahmadyan M, Forouhar Vajargah M, Sadat A, Zarandeh H, Shirood Mirzaie F and Abbas Hosseini S. 2012. Effect of synbiotic (*Biomin imbo*) on growth performance and survival rate of Texas Cichlid (*Herichthys cyanoguttatus*) Larvae. Glob Vet. 9(3): 358-361.

Moshfegh AJ, Friday JE, Goldman JP and Ahuja JK. 1999. Presence of inulin and oligofructose in the diets of Americans. J Nutr. 129(7 Suppl): 1407S-1411S.

Mourino JLP, Do Nascimento Viera F, Jatoba AB, Da Silva BC, Jesus GFA, Seiffert WQ and Martins ML. 2012. Effect of dietary supplementation of inulin and W. cibaria on haemato-immunological parameters of hybrid surubim (*Pseudoplatystoma* sp). Aquac Nutr. 18(1): 73-80.

Musthafa MS, Ali ARJ, Mohamed MJ, Jaleel MMA, Kumar MSA, Rani KU, Vasanth K, Arockiaraj J, Preetham E, Balasundaram C and Harikrishnan R. 2016. Protective efficacy of Azomite enriched diet in *Oreochromis mossambicus* against *Aeromonas hydrophila*. Aquaculture. 451: 310-315.

Nekoubin H, Gharedaashi E, Reza Imanpour M, Nowferesti H and Asgharimoghadam A. 2012a. The Influence of Synbiotic (*Biomin imbo*) on Growth Factors and Survival Rate of Zebrafish (*Danio rerio*) Larvae via Supplementation with Biomar. Glob Vet. 9(3): 358-361.

Nekoubin H, Hatefi S, Javaheri S and Sudagar M. 2012b. Effects of synbiotic (*Biomin Imbo*) on growth performance, survival rate, reproductive parameters of Angelfish (*Pterophyllum scalare*). WJST. 9(4): 327-332.

Newaj-Fyzul A and Austin B. 2014. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. J Fish Dis. 38(11): 937-955

Ngamkala S, Futami K, Endo M, Maita M and Katagiri T. 2010. Immunological effects of glucan and *Lactobacillus rhamnosus* GG, a probiotic bacterium, on Nile tilapia *Oreochromis niloticus* intestine with oral *Aeromonas* challenges. Fisheries Sci. 76(5): 833-840.

Nikoskelainen S, Salminen S, Bylund G and Ouwehand AC. 2001. Characterization of the properties of human- and dairy-derived probiotics for prevention of infectious diseases in fish. Appl Environ Microbiol. 67(6): 2430-2435.

Nilegaonkar S and Agte V. 2010. Prebiotics. In: comprehensive food fermentation biotechnology ed. Asiatech publishers, inc: 466-487.

Ocana VS and Elena Nader-Macias M. 2004. Production of antimicrobial substances by lactic acid bacteria II: screening bacteriocin-producing strains with probiotic purposes and characterization of a *Lactobacillus* bacteriocin. Methods Mol Biol. 268: 347-353.

Ortiz LT, Rebole A, Velasco S, Rodriguez ML, Trevino J, Tejedor JL and Alzueta C. 2013. Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (*Oncorhynchus mykiss*). Aquac Nutr. 19(4): 475-482.

Pablos M, Rodriguez-Calleja JM, Santos JA, Otero A and Garcia-Lopez ML. 2009. Occurrence of motile *Aeromonas* in municipal drinking water and distribution of genes encoding virulence factors. Int J Food Microbiol. 135(2): 158-164.

Pandey KR, Naik SR and Vakil BV. 2015. Probiotics, prebiotics and synbiotics- a review. J food Sci Technol. 52(12): 7577-7587.

Panigrahi A, Kiron V, Kobayashi T, Puangkaew J, Satoh S and Sugita H. 2004. Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential

probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Veterinary Immunol Immunopathol. 102(4): 379-388.

Panigrahi A, Kiron V, Puangkaew J, Kobayashi T, Satoh S and Sugita H. 2005. The viability of probiotic bacteria in rainbow trout *Oncorhynchus mykiss*. Aquaculture. 243(1-4): 241-254.

Panigrahi A, Kiron V, Satoh S and Watanabe T. 2010. Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Physiol Biochem. 36(4): 969-977.

Perez RH, Zendo T and Sonomoto K. 2014. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Fact. 13 Suppl 1: S3.

Pirarat N, Kobayashi T, Katagiri T, Maita M and Endo M. 2006. Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental *Edwardsiella tarda* infection in tilapia (*Oreochromis niloticus*). Vet Immunol Immunopathol. 113(3-4): 339-347.

Pirarat N, Pinpimai K, Chankow K, Malila K, Chansue N, Niyomtham W and Rodkhum C. 2009. In vitro efficacy of human-derived probiotic, *Lactobacillus rhamnosus* against pathogenic bacteria in fish and frogs. TJVM. 39(4): 305-310.

Pirarat N, Pinpimai K, Endo M, Katagiri T, Ponpornpisit A, Chansue N and Maita M. 2011. Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Res Vet Sci. 91(3): e92-97.

Polakof S, Panserat S, Soengas J and Moon T. 2012. Glucose metabolism in fish: A review. J Comp Psysiol B. 182(8): 1015-1045

Preidis GA, Hill C, Guerrant RL, Ramakrishna BS, Tannock GW and Versalovic J. 2011. Probiotics, enteric and diarrheal diseases, and global health. Gastroenterology. 140(1): 8-14.

Raman M, Ambalam P and Doble M. 2015. Short-chain fatty acids In: Probiotics and bioactive carbohydrates in colon cancer management India : Spirnger 1-120.

Ramos MA, Batista S, Pires MA, Silva AP, Pereira LF, Saavedra MJ, Ozorio ROA and Rema P. 2017. Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. Animal. 11(8): 1259-1269.

Rey A, Verjan N, Ferguson HW and Iregui C. 2009. Pathogenesis of *Aeromonas hydrophila* strain KJ99 infection and its extracellular products in two species of fish. Vet Rec. 164(16): 493-499.

Reyes-Becerril M, Ascencio F, Gracia-Lopez V, Macias ME, Roa MC and Esteban MA. 2014. Single or combined effects of Lactobacillus sakei and inulin on growth, non-specific immunity and IgM expression in leopard grouper (*Mycteroperca rosacea*). Fish Physiol Biochem. 40(4): 1169-1180.

Reza A, Abdolmajid H, Abbas M and Abdolmohammad AK. 2009. Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile Beluga, *Huso huso* (Linnaeus, 1758). J World Aquacult Soc. 40(6): 771-779.

Ringø E, Olsen R, Gifstad T, Dalmo R, Amlund H, HEMRE GI and Bakke A. 2010. Prebiotics in aquaculture: a review. Aquac Nutr. 16(2): 117-136.

Ringo E, Salinas I, Olsen RE, Nyhaug A, Myklebust R and Mayhew TM. 2007. Histological changes in intestine of Atlantic salmon (*Salmo salar* L.) following in vitro exposure to pathogenic and probiotic bacterial strains. Cell Tissue Res. 328(1): 109-116.

Rodriguez I, Novoa B and Figueras A. 2008. Immune response of zebrafish (*Danio rerio*) against a newly isolated bacterial pathogen *Aeromonas hydrophila*. Fish Shellfish Immunol. 25(3): 239-249.

Rodriguez U, Satoh S, Haga Y, Fushimi H and Sweetman J. 2009. Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. AGRIS 57: 609-614.

Ruiz FO, Pascual L, Giordano W and Barberis L. 2015. Bacteriocins and other bioactive substances of probiotic lactobacilli as biological weapons against *Neisseria gonorrhoeae*. Pathog Dis. 73(3).

Saad N, Delattre C, Urdaci M, Schmitter JM and Bressollier P. 2013. An overview of the last advances in probiotic and prebiotic field. Food Sci Technol. 50(1): 1-16.

Salinas I and Parra D. 2015. Fish mucosal immunity: intestine. In: Mucosal Health in Aquaculture. Peatman Eric (ed) San Diego: Academic Press. 135-170.

Salinas I, Zhang YA and Sunyer JO. 2011. Mucosal immunoglobulins and B cells of teleost fish. Dev Comp Immunol. 35(12): 1346-1365.

Sanchez-Cespedes J, Figueras MJ, Aspiroz C, Aldea MJ, Toledo M, Alperi A, Marco F and Vila J. 2009. Development of imipenem resistance in an *Aeromonas veronii* biovar sobria clinical isolate recovered from a patient with cholangitis. J Med Microbiol. 58(4): 451-455.

Saurabh S and Sahoo PK. 2008. Lysozyme: An important defence molecule of fish innate immune system. Aquac Res. 39(3): 223-239.

Segers ME and Lebeer S. 2014. Towards a better understanding of *Lactobacillus rhamnosus* GG-host interactions. Microb Cell Fact. 13 Suppl 1: S7.

Shen Y, Zhang J, Xu X, Fu J and Li J. 2012. Expression of complement component C7 and involvement in innate immune responses to bacteria in grass carp. Fish Shellfish Immunol. 33(2): 448-454.

Shen YB, Zhang JB, Xu XY and Li JL. 2011. Molecular cloning, characterization and expression analysis of the complement component C6 gene in grass carp. Vet Immunol Immunopathol. 141(1-2): 139-143.

Sklan D, Prag T and Lupatsch I. 2004. Structure and function of the small intestine of the tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Teleostei, Cichlidae). Aquac Res. 35(4): 350-357.

Slavin J. 2013. Fiber and prebiotics: mechanisms and health benefits. Nutrients. 5(4): 1417-1435.

Strocchi A and Levitt MD. 1993. Role of villous surface area in absorption. Science versus religion. Dig Dis Sci. 38(3): 385-387.

Tannock GW. 2004. A special fondness for lactobacilli. Appl Environ Microbiol. 70(6): 3189-3194.

Tapia Paniagua ST, Reyes-Becerril M, Ascencio F, Esteban M, Clavijo E and Balebona M. 2011. Modulation of the intestinal microbiota and Immune system of farmed Sparus aurata by the administration of the Yeast Debaryomyces hansenii L2 in conjunction with inulin. J Aquac Res Dev. S1.

Thelestam M and Ljungh A. 1981. Membrane-damaging and cytotoxic effects on human fibroblasts of alpha- and beta-hemolysins from *Aeromonas hydrophila*. Infect Immu. 34(3): 949-956.

Tiengtam N, Paengkoum P, Sirivoharn S, Phonsiri K and Boonanuntanasarn S. 2017. The effects of dietary inulin and Jerusalem artichoke (*Helianthus tuberosus*) tuber on the growth performance, haematological, blood chemical and immune parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquac Res. 48(10): 5280-5288

Tomas JM. 2012. The main Aeromonas pathogenic factors. ISRN Microbiol. 2012: 1-22.

Tu FP, Chu WH, Zhuang XY and Lu CP. 2010. Effect of oral immunization with *Aeromonas hydrophila* ghosts on protection against experimental fish infection. Lett Appl Microbiol. 50(1): 13-17.

Uribe C, Folch H, Enríquez R and Moran G. 2011. Innate and adaptive immunity in teleost fish: A review. Vet Med. 56(10): 486-503.

Van Doan H, Doolgindachbaporn S and Suksri A. 2016. Effect of *Lactobacillus plantarum* and Jerusalem artichoke (*Helianthus tuberosus*) on growth performance, immunity and disease resistance of Pangasius catfish (*Pangasius bocourti*, Sauvage 1880). Aquac Nutr. 22(2): 444-456.

Vanloo J, Coussement P, Deleenheer L, Hoebregs H and Smits G. 1995. On the presence of inulin and oligofructose as natural ingredients in the Western diet. Crit Rev Food Sci Nutr. 35(6): 525-552.

Villamil L, Tafalla C, Figueras A and Novoa B. 2002. Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin Diagn Lab Immunol. 9(6): 1318-1323.

Wang YB and Xu ZR. 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim Feed Sci Tech. 127(3-4): 283-292.

Yamprayoon J and Sukhumparnich K. 2010. Thai aquaculture: achieving quality and safety through management and sustainability. J World Aquacult Soc. 41(2): 274-280.

Yarahmadi P, Moradi N and Ghysvandi N. 2014. The effect of dietary supplemented with Synbiotic (Biomin IMBO®) on growth performance, carcass composition, hematological and serum biochemical parameters of common carp (*Cyprinus carpio* Linnaeus, 1758, Cyprinidae). JCBPSC. 4(3): 2129-2139.

Yardimci B and Aydin Y. 2011. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). Ankara Üniv Vet Fak Derg. 58: 47-54.

Ye JD, Wang K, Li FD and Sun YZ. 2011. Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder Paralichthys olivaceus. Aquac Nutr. 17(4): e902-e911.

Yuan S-b, Chen D-w, Zhang K-y and Yu B. 2007. Effects of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidative enzymes of weanling pigs. Asian-Australas J Anim Sci. 20(10): 1600-1605.

Zhang CN, Li XF, Xu WN, Jiang GZ, Lu KL, Wang LN and Liu WB. 2013. Combined effects of dietary fructooligosaccharide and Bacillus licheniformis on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*). Fish Shellfish Immunol. 35(5): 1380-1386.

Zhang Q, Yu H, Tong T, Tong W, Dong L, Xu M and Wang Z. 2014. Dietary supplementation of *Bacillus subtilis* and fructooligosaccharide enhance the growth, non-specific immunity of juvenile ovate pompano, *Trachinotus ovatus* and its disease resistance against *Vibrio vulnificus*. Fish Shellfish Immunol. 38(1): 7-14.

APPENDIX

Media, buffer and solution preparation

1. 10X PBS (Phosphate-buffer saline)

Sodium chloride 40 g

Potassium chloride 1 g

Sodium hydrogen phosphate 4 5.75 g

Potassium dihydrogen phosphate 1 g

Deionized distilled water 1000 ml

Sterilized the solution by autoclaving 121 °C 15 minutes

2. 1X PBS

10X PBS 100 ml

Sterilized deionized distilled water 900 ml

3. MRS broth

Proteose peptone 10 g

Beef extract 10 g

Yeast extract 5 g

Dextrose 20 g

Polysorbate 80 1 g

Ammonium citrate 2 g

Sodium acetate 5 g

Magnesium sulfate 0.1 g

Manganese sulfate 0.05 g

Dipotassium phosphate 2 g

Adjust to pH 6.5 and sterilized the solution by autoclaving 121 °C 15 minutes

4. MRS agar

Proteose peptone 10 g

Beef extract 10 g

Yeast extract 5 g

Dextrose 20 g

Polysorbate 80 1 g

Ammonium citrate 2 g

Sodium acetate 5 g

Magnesium sulfate 0.1 g

Manganese sulfate 0.05 g

Dipotassium phosphate 2 g

Agar 15 g

Adjust to pH 6.5 and sterilized the solution by autoclaving 121 °C 15 minutes

5. Tryptic Soy Broth (TSB)

Pancreatic digest of casein 17 g

Papaic digest of soybean 3 g

Dextrose 2.5 g

Sodium chloride 5 g

Dipotassium phosphate 2.5 g

Distilled water 1000 ml

Adjust to pH 7.3 and sterilized the solution by autoclaving 121 °C 15 minutes

6. TSA

Pancreatic digest of casein 15 g

Papaic digest of soybean 5 g

Sodium chloride 5 g

Agar 15 g

Adjust to pH 7.3 and sterilized the solution by autoclaving 121 °C 15 minutes

7. Normal saline

Sodium chloride 8.5 g

Distilled water 1000 ml

VITA

NAME	Mariya Sewaka
DATE OF BIRTH	14 December 1985
PLACE OF BIRTH	Nakhon Si Thammarat
INSTITUTIONS ATTENDED	Veterinary Microbiology and Fish Nutrition
HOME ADDRESS	46/3 Village No.3, Naphu, Praprom, Nakhon Si Thammarat, Thailand. 80000
PUBLICATION	**-** Mariya Sewaka, Clara Trullas, Arranee Chotiko, Channarong Rodkhum, Nantarika Chansue, Surintorn Boonanuntanasarn, Nopadon Pirarat. 2019 Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.). Fish and Shellfish Immunology. 86 : 260-268 (Impact Factor 3.185, Tier1-Q1)

List of publications

- Mariya Sewaka, Clara Trullas, Arranee Chotiko, Channarong Rodkhum, Nantarika Chansue, Surintorn Boonanuntanasarn, Nopadon Pirarat. 2019 Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.). Fish and Shellfish Immunology. 86 : 260-268 (Impact Factor 3.185, Tier1-Q1)

List of conference proceedings

- M. Sewaka, C. Rodkhum, H.T. Dong, S. Senapin, T. Sornwattana, N. Pirarat, 2016. Hemolytic activity of extracellular products of *Aeromonas veronii* recovered from disease outbreaks in farmed Nile tilapia. Proceeding of the 15th Chulalongkorn University Veterinary Congress, April 20-22, 2016 Bangkok, Thailand.

- M. Sewaka, C. Rodkhum, N. Chansue, C. Trullàs, N. Pirarat, 2017. Synbiotic effects of Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diet on blood biochemical parameters in juvenile red tilapias. Proceeding of the 16th Chulalongkorn University Veterinary Congress, March 22-24, 2017 Bangkok, Thailand.

- Mariya, S., Channarong, R., Nantarika, C., Thanyathorn, H., Thanyarat, R., Pannatika, T. Suyanee, P., Kanorkorn, A., Clara, T., Nopadon, P., 2017. Synbiotic effect of Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on intestinal morphology in red tilapias. Asian Veterinary Pathologists Congress, November 9-11, 2017 Karnataka, India.

- M. Sewaka, C. Trullàs, C. Rodkhum, N. Chansue, S. Boonanuntanasarn, N. Pirarat, 2018. Prebiotic effects of Jerusalem artichoke supplemented diet on growth performance and intestinal morphology in juvenile red tilapias (*Oreochromis* sp.). Proceeding of the 17th Chulalongkorn University Veterinary Congress, April 25-27,2018 Nonthaburi, Thailand.