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THE POTENTIAL USE OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AS AN ALTERNATIVE PREBIOTIC FOR NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

Nattanan Tiengtam

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Animal Production Technology

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THE POTENTIAL USE OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AS AN ALTERNATIVE PREBIOTIC FOR NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการใช้แก่นตะวันเป็นอาหารเสริมชีวนะทางเลือกสำหรับปลานิล โดยในการศึกษานี้ได้แบ่งการทดลองออกเป็น 3 การทดลอง

การทดลองที่ 1 เป็นการทดสอบเปรียบเทียบถึงผลการใช้อินูลิน และแก่นตะวัน เป็นสารเสริมพรีไบโอติกส์ต่อสมรรถนะการเจริญเติบโต และสุขภาพของปลานิลตั้งแต่ระยะลูกปลาเริ่มกินอาหาร จนถึงระยะปลานิ้ว การทดลองนี้มีกลุ่มทดลอง 5 กลุ่ม ประกอบด้วย กลุ่มทดลองที่ได้รับอาหารที่ไม่มีการเสริมพรีไบโอติกส์ (กลุ่มควบคุม) อาหารที่มีการเสริมอินูลินที่ระดับ 2.5 และ 5 กรัมต่อกิโลกรัมอาหาร และอาหารที่มีการเสริมแก่นตะวันที่ระดับ 5 และ 10 กรัมต่อกิโลกรัมอาหาร ผลการทดลองพบว่า ปลาที่เลี้ยงด้วยอาหารเสริมอินูลินที่ระดับ 5 กรัมต่อกิโลกรัมอาหาร และการเสริมแก่นตะวันที่ระดับ 5 และ 10 กรัมต่อกิโลกรัมอาหาร มีสมรรถนะการเจริญเติบโต และอัตรารอดสูงกว่าปลาที่ได้รับอาหารที่มีการเสริมอินูลินที่ระดับ 2.5 กรัมต่อกิโลกรัมอาหาร และปลากลุ่มควบคุม ($P<0.05$) นอกจากนี้ การเสริมอินูลิน และการเสริมแก่นตะวันในอาหารทำให้ปลามีค่าจำนวนเม็ดเลือดแดง ค่าโปรตีนในเลือด ค่าอิมมูโนโกลบูลินรวม ค่าการทำงานของไลโซไซม์ และค่า alternative complement haemolytic 50 activity (ACH50) เพิ่มสูงขึ้น การเสริมสารเสริมดังกล่าวช่วยปรับปรุงจุลสัณฐานของลำไส้ (เพิ่มความยาวของวิลไล และจำนวนเซลล์โกเบล็ท) และส่งผลต่อการเปลี่ยนแปลงประชากรจุลินทรีย์ในลำไส้ ($P<0.05$)

การทดลองที่ 2 เป็นการทดสอบถึงผลการใช้อินูลินและแก่นตะวันเป็นสารเสริมพรีไบโอติกส์ในอาหารปลานิลในระยะปลานิลวัยรุ่น การทดลองนี้มี 5 กลุ่มอาหารทดลองเช่นเดียวกับการทดลองที่ 1 ผลการทดลองพบว่า ปลาที่เลี้ยงด้วยอาหารที่เสริมอินูลินมีสมรรถนะการเจริญเติบโตดีกว่าปลาในกลุ่มควบคุม และปลาที่เลี้ยงด้วยอาหารที่เสริมแก่นตะวันมีสมรรถนะการเจริญเติบโตดีที่สุด การเสริมอินูลินและการเสริมแก่นตะวันในอาหารทำให้ปลามีค่าจำนวนเม็ดเลือดแดง ค่าชีวเคมีของโลหิต และค่าภูมิคุ้มกันเพิ่มสูงขึ้น (กลูโคส อัลบูมิน โปรตีน แมกนีเซียม แคลเซียม เหล็กในเลือด ค่าอิมมูโนโกลบูลินรวม ค่าการทำงานของไลโซไซม์ และค่า ACH50) จุลสัณฐานของลำไส้ดีขึ้น และส่งผลต่อการเปลี่ยนแปลงประชากรจุลินทรีย์ในลำไส้ ($P<0.05$)

การทดลองที่ 3 เป็นการทดสอบเปรียบเทียบถึงผลการใช้สารเสริมในอาหาร 3 ชนิด ได้แก่ แก่นตะวัน กากผลองุ่นแดง (red grape pomace) และกากไอโซไครซิส (defatted *Isochrysis*) ใน

ปลานิลระยะปลานิ้ว การทดลองนี้มีกลุ่มทดลอง 5 กลุ่ม ประกอบด้วย กลุ่มทดลองที่ได้รับอาหารที่ไม่มีการเสริมสาร (กลุ่มควบคุม) อาหารที่มีการเสริมแก่นตะวันที่ระดับ 20 และ 40 กรัมต่อกิโลกรัมอาหาร อาหารที่มีการเสริมกากผลอุ่นแดง 20 กรัมต่อกิโลกรัมอาหาร และอาหารที่มีการเสริมกากไอโซไครซิส 20 กรัมต่อกิโลกรัมอาหาร ผลการทดลองพบว่า ปลาที่เลี้ยงด้วยอาหารที่เสริมแก่นตะวัน กากผลอุ่นแดง และกากไอโซไครซิสที่ระดับ 20 กรัมต่อกิโลกรัมอาหาร มีสมรรถนะการเจริญเติบโต และค่าภูมิคุ้มกันเพิ่มสูงขึ้นเมื่อเปรียบเทียบกับกลุ่มควบคุม ($P<0.05$) จากนั้นนำปลาที่เลี้ยงด้วยอาหารแต่ละกลุ่มทดลองมาฉีดเชื้อ *Stretococcus iniae* เพื่อทดสอบความต้านทานโรคต่อเชื้อ *S. iniae* ที่ระยะเวลา 12 วัน ผลการศึกษาพบว่า ปลาที่เลี้ยงด้วยอาหารกลุ่มควบคุม และอาหารที่เสริมแก่นตะวันที่ระดับ 40 กรัมต่อกิโลกรัมอาหาร มีอัตราการตายสะสม 80% ปลาที่เลี้ยงด้วยอาหารที่เสริมกากผลอุ่นแดง และกากไอโซไครซิสที่ระดับ 20 กรัมต่อกิโลกรัมอาหาร มีอัตราการตายสะสม 30% อย่างไรก็ตามไม่พบการตายของปลาที่เลี้ยงด้วยอาหารที่เสริมแก่นตะวันที่ระดับ 20 กรัมต่อกิโลกรัมอาหาร แสดงให้เห็นว่าการเสริมแก่นตะวันที่ระดับ 20 กรัมต่อกิโลกรัมอาหารส่งผลให้ปลามีความต้านทานต่อเชื้อ *S. iniae* ได้

โดยสรุป ผลการศึกษานี้ได้แสดงให้เห็นว่าการเสริมอินูลินในอาหาร มีผลดีต่อสมรรถนะการเจริญเติบโต และสุขภาพของปลานิล นอกจากนี้แก่นตะวันสามารถนำมาใช้เป็นสารเสริมพรีไบโอติกส์ในอาหาร และมีผลดีกว่าการเสริมอินูลินและสารเสริมอาหารอื่น ๆ ที่ได้ทดสอบ

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2559 ลายมือชื่ออาจารย์ที่ปรึกษา _____

NATTANAN TIENGTAM : THE POTENTIAL USE OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AS AN ALTERNATIVE PREBIOTIC FOR NILE TILAPIA (*OREOCHROMIS NILOTICUS*). THESIS ADVISOR : ASSOC. PROF. SURINTORN BOONANUNTANASARN, Ph.D., 161 PP.

INULIN/JERUSALEM ARTICHOKE/NILE TILAPIA/PREBIOTIC/BLOOD PROFILES/IMMUNE/INTESTINAL MICROBIOTA

The present study aimed to investigate the use of Jerusarem artichoke (JA) as an alternative prebiotic for Nile tilapia. This study included three experiments.

Experiment I evaluated the comparable effects of a dietary supplementation of inulin and JA as prebiotics on growth performance and health status in Nile tilapia during first feeding to fingerlings. Five dietary treatments were designed to incorporate inulin at 0 (control), 2.5, and 5 g kg^{-1} and JA at 5 and 10 g kg^{-1}. The results showed that dietary inulin at 5 g kg^{-1} or JA at either level had better growth performance and survival rate than those fed on the 2.5 g kg^{-1} inulin or control diet ($P<0.05$). In addition, dietary inulin and JA increased the red blood cell number (RBC), total protein in blood (TP), total immunoglobulin (Ig), lysozyme activity (Lz) and alternative complement haemolytic 50 activity (ACH50), improved intestinal morphology (intestinal villi height and goblet cell number) and modulated intestinal microbiota ($P<0.05$).

Experiment II evaluated the prebiotic effects of dietary inulin and JA on Nile tilapia juveniles. Again, the same five dietary treatments as that of Experiment I were designed and fed to Nile tilapia juveniles. Fish fed the inulin diets exhibited better growth performance than fish fed the control diet, and fish fed the JA diets had the best

growth performances among all diets tested. Dietary inulin and JA increased RBC, blood chemistry and immune parameters (glucose, albumin, TP, magnesium, calcium, iron, Ig, Lz and ACH50), improved intestinal morphology and modulated intestinal microbiota ($P<0.05$).

Experiment III evaluated the comparable effects of three feed additives including JA, red grape pomace (RGP) and defatted *Isochrysis* (ISO) in Nile tilapia fingerlings. Five dietary treatments were designed to incorporate JA at 0 (control), 20 g kg^{-1} JA (20 JA), 40 g kg^{-1} JA (40 JA), RGP at 20 g kg^{-1} RGP (20 RGP) and ISO at 20 g kg^{-1} (20 ISO). Fish fed the 20 JA diet, 20 RGP diet and 20.0 ISO had better growth performance and immune parameters compared with fish fed on the control diet ($P<0.05$). The experimental fish were subjected to challenge with *Stretococcus iniae* for 12 days. The result showed that cumulative mortality of Nile tilapia fed on either the control diet or 40 JA was 80%. Fish fed on 20 RGP and 20 ISO had a 30% mortality rate. However, no fish fed on 20 JA died, suggesting that dietary JA at 20 g kg^{-1} could contribute to fish being resistant to pathogenic *S. iniae*.

In conclusion, the present study demonstrated that dietary inulin had benefit effects on growth performance and health status. In addition, Jerusalem artichoke could be used as a dietary prebiotic supplementation, and its beneficial effects were superior to inulin and other tested feed additives.

School of Animal Production Technology Student's Signature Nattanan

Academic Year 2016 Advisor's Signature

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LISTS OF ABBREVIATIONS

ACH50	=	Alternative complement haemolytic 50 activity
ADG	=	Average daily gain
ANOVA	=	Analysis of variance
BUN	=	Blood urea nitrogen
cm	=	Centimeter
°C	=	Degree celsius
CFU	=	Colony-forming units
CO_2	=	Carbon dioxide
D-bilirubin	=	Direct bilirubin
DO	=	Dissolved oxygen
DP	=	Degree of polymerisation
EU	=	European union
FCR	=	Feed conversion ratio
FE	=	Feed efficiency
FI	=	Feed intake
FOS	=	Fructooligosaccharides
× *g*	=	Gravity force
g	=	Gram
g kg^{-1}	=	Gram per kilogram
g L^{-1}	=	Gram per liter

LIST OF ABBREVIATIONS (Continued)

h	=	Hour
ha^{-1}	=	Hectare
Hb	=	Hemoglobin
HBSS	=	Hank's buffered salt solution
H_2O_2	=	Hydrogen peroxide
Ht	=	Hematocrit
IP	=	Intraperitoneal
ISO	=	Defatted *Isochrysis* sp.
IU	=	International unit
JA	=	Jerusalem artichoke
K_2EDTA	=	Dipotassium ethylenediaminetetraacetate
L^{-1}	=	Liter
$L\ L^{-1}$	=	Liter per liter
$L\ min^{-1}$	=	Liter per minute
m	=	Meter
mg	=	Miligram
$mg\ kg^{-1}$	=	Miligram per kilogram
$mg\ L^{-1}$	=	Miligram per liter
$mg\ mL^{-1}$	=	Miligram per milliliter
min	=	Minute
ml	=	Mililiter
mm	=	Millimeter

LIST OF ABBREVIATIONS (Continued)

mM	=	Millimolar
mmol L^{-1}	=	Millimole per liter
mol L^{-1}	=	Mole per liter
M	=	Molar
MCH	=	Mean corpuscular hemoglobin
MCHC	=	Mean corpuscular hemoglobin concentration
MCV	=	Mean corpuscular volume
MRS	=	de Man, Rogosa and Sharpe
µg mL^{-1}	=	Microgram per mililiter
µL	=	Microliter
µm	=	Micrometer
µmol L^{-1}	=	Micromole per liter
nm	=	Nanometer
NaCl	=	Sodium chloride
NBT	=	Nitroblue tetrazolium activity
OD	=	Optical density
ppm	=	Part per million
PBS	=	Phosphate buffer saline
PCA	=	Plate count agar
PER	=	Protein efficiency ratio
rpm	=	Revolutions per minute
RBC	=	Red blood cells

LIST OF ABBREVIATIONS (Continued)

RGP	=	Red grape pomace
SCFA	=	Short-chain fatty acids (acetate, propionate and butyrate)
SD	=	Standard deviation
SGOT	=	Serum glutamic oxaloacetic transaminase
SGPT	=	Serum glutamic pyruvic transaminase
SGR	=	Specific growth rate
T-bilirubin	=	Total bilirubin
TCBS	=	Thiosulfate citrate bile salts sucrose
TMB	=	Tetramethylbenzidine
Total Ig	=	Total immunoglobulin
TSB	=	Tryptic soy broth
U L^{-1}	=	Unit per liter
U mg^{-1}	=	Unit per miligram
WBC	=	White blood cell
WG	=	Weight gain

CHAPTER I

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is an important economic fish of Thailand. The Nile tilapia has been cultured for domestic consumption and for the export market. Among freshwater fish species, tilapia has been cultured with the highest production and value in Thailand and been exported among other freshwater fish species. Currently, most tilapia production has been obtained from an intensive culture system. In intensive fish farming, fish obtain significant nutrition from supplementary feed. Therefore, the commercial diet of tilapia is developed for complete nutrition which is required for fast growth and good health of fish. Tilapia can be grown and harvested in a short time, therefore, the industrial production of tilapia commercial diet is growing rapidly (Riche and Garling, 2003; Gupta and Acosta, 2004; Mensah and Attipoe, 2013). Various aspects of research and development of tilapia production in Thailand has been conducted continuously to allow tilapia to grow rapidly and healthy. The research and development also aims to find good quality alternative raw materials for lower costs of fish feeds. For example, the study on the use of various plants and grains as alternative ingredients in fish feed (El-Sayed, 1999; Mabahinzireki et al., 2001; El-Saidy and Gaber, 2003; Bhujel, 2013). There has been growing interest in the investigation of the optimum use of feed additives. In addition, the study on the optimum use of feed additives has been investigated to improve growth performance and immunocompetence of Nile tilapia.

Feed additives are important nutrients for fish feed production in an intensive culture system (Ajiboye et al., 2012). Because the fishes are raised intensively in the intensive culture system, the fishes may not get sufficient minerals and vitamins from feeds. Thus, the development of feed additives in fish feed is necessary for the tilapia culture, which mainly is a commercial aquaculture system. Prebiotic is another type of common feed additives used for fish feed. The use of prebiotic as fish feed addictives would enhance the growth of probiotics or microorganisms that are beneficial to fishes. These beneficial bacteria would improve growth performance, feed utilization, fish health and immunity (Yousefian and Amiri, 2009; Ringø et al., 2010; Ganguly et al., 2013).

Prebiotic is a compound of oligosaccharide, which is a source of carbohydrate that cannot be digested by the digestive enzymes of animals. However, it is beneficial to animals by stimulating the growth and performance of beneficial bacteria in the colon, which help promote the health of their hosts (Gibson and Roberfroid, 1995; Pool-Zobel et al., 2002; Roberfroid, 2002; Flickinger et al., 2003). Inulin is a type of carbohydrate called polysaccharide in the fructan group, which is one of the most common prebiotics used in feed for livestock and aquatic animals (Verdonk et al., 2005; Ringø et al., 2010). It improved growth performance of many aquatic species, and the immune system in aquatic animals (Mahious et al., 2006a; Ibrahem et al., 2010; Ortiz et al., 2013). However, the studies of several aquatic animals have demonstrated that it did not improve the performance of growth and immune response (Mahious et al., 2006b; Bakke-McKellep et al., 2007; Reza et al., 2009; Burr et al., 2010; Mourino et al., 2012; Eshaghzadeh et al., 2015). As a result, the study on the specific use of prebiotics such as inulin and fructooligosaccharide are needed in

aquatic animals in order to gain the precision use of prebiotic as a dietary feed additive.

For commercial use, inulin and fructooligosaccharide (FOS) have been produced from chicory roots, which mostly is imported from Europe. Thailand is predominantly an agricultural country and many plant species can grow throughout the year (Herath, 1999). Therefore, finding the source of inulin and fructooligosaccharide in Thailand for the use as feed additives to reduce the import of prebiotic will lead to lower cost feed production for a more self-reliant industry in the country. Jerusalem artichoke (*Helianthus tuberosus*; JA) is a root crop like the potato, native to North America (Kays and Nottingham, 2007), and can be grown year-round in tropical areas including Thailand. The JA tuber contains 160-200 g kg^{-1} inulin and 120-150 g kg^{-1} of FOS (Moshfegh et al., 1999); therefore, it would be a good source of oligofructose-enriched inulin. Although, raising Nile tilapia and growing JA can be done together in tropical areas, there has been no extensive study of the potential use of JA as a prebiotic directly for aquatic animal feeds.

In this study, the effect of dietary supplementation with inulin and JA in Nile tilapia were evaluated and compared. The effects of dietary inulin and JA on growth performance, body composition, intestinal microbiota, intestinal morphology and various parameters indicating fish health were examined in all phases of Nile tilapia production. In addition, JA was also compared to the use of other feed additives from abroad on Nile tilapia fingerlings, including red grape pomace (RGP) and defatted *Isochrysis* (ISO) to observe the growth performance, immune parameters and resistance to the *S. iniae* challenge. These studies would provide the information for further development of using JA as prebiotic feed additives in commercial fish feed.

1.1 Research objectives

1.1.1 To study the effects of dietary inulin and JA as prebiotic additives on the growth performance and health status of Nile tilapia during the feeding period from first feeding to fingerling size.

1.1.2 To study the effects of dietary inulin and JA as prebiotic additives on the growth performance and health status of Nile tilapia juveniles.

1.1.3 To investigate the effect of three feed additives including JA, RGP and ISO in the diets on growth performance, immune response and resistance of Nile tilapia fingerlings to the *Streptococcus iniae* challenge.

1.2 Research hypothesis

1.2.1 Inulin has beneficial prebiotic effects on Nile tilapia from first feeding to fingerling size, and JA has a comparable prebiotic effect when supplementation in the diet for Nile tilapia during the feeding period from first feeding to fingerling size.

1.2.2 Inulin has beneficial prebiotic effects on Nile tilapia juveniles, and JA has a comparable prebiotic effect when supplementation in the diet for Nile tilapia juveniles.

1.2.3 Three feed additives including JA, RGP and ISO could improve growth performance, immunological stimulation and resistance to the *S. iniae* challenge of Nile tilapia.

1.3 Scope of the study

This study will focus on the use of commercial inulin extract from chicory roots (PREBIOFEED 88) and direct supplementation with JA as prebiotic additives in

Nile tilapia. The level of inulin used in the experiment is to be set in the range that manufacturers have recommended for use in fish feed. In order to compare the level of supplementation of crude JA, JA powder contains an equal level of fructan as the commercial inulin used for dietary supplementation. The prebiotic effects of dietary inulin and JA on growth performance, survival rate, sex-reversal efficiency, body composition, intestinal morphology and intestinal microbiota were investigated. In addition, health status such as hematology, blood chemistry and immune parameters of Nile tilapia during the phases of first feeding to fingerling size and Nile tilapia juveniles were evaluated and compared. Moreover, JA was also compared to the use of other feed additives on Nile tilapia fingerlings including RGP and ISO to observe the growth performance, immune parameters and resistance to the *S. iniae* challenge.

1.4 Expected results

1.4.1 Information about dietary supplementation of inulin influences the growth performance and health status of fingerling Nile tilapia.

1.4.2 Information about dietary supplementation of inulin influences the growth performance and health status of Nile tilapia juveniles.

1.4.3 Information on the comparative effects between inulin and JA on the growth performance and health status in fingerling Nile tilapia.

1.4.4 Information on the comparative effects between inulin and JA on the growth performance and health status in Nile tilapia juveniles.

1.4.5 Information on the comparative effects of three feed additives including JA, RGP and ISO on growth performance, immune response and resistance to the *S. iniae* challenge of Nile tilapia.

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CHAPTER II

LITERATURE REVIEW

2.1 Culture of Tilapia

Tilapia is the generic name for three economically important genera and species of fish in the family Cichlidae, that is *Oreochromis*, *Sarotherodon* and *Tilapia* (Fitzsimmons, 2000). Tilapias are a freshwater group of fish species originating exclusively from Africa, the Mediterranean and the Middle East, but interest in their aquacultural potential led to worldwide distribution (Popma and Lovshin, 1996). The highest production occurs in tropical and subtropical areas in developing countries but they can also be cultured in temperate climates, where production must be carried out in indoor tanks. The species that are most important for aquaculture are in the genus *Oreochromis*, including the Nile tilapia (*O. niloticus*), Mozambique tilapia (*O. mossambicus*), Blue tilapia (*O. aureus*), Zanzibar tilapia (*O. urolepis hornorum*) and hybrids (Popma and Masser, 1999; Fitzsimmons, 2000; Lim and Webster, 2006). However, among these species, *O. niloticus* (common name, Nile tilapia) (Figure 2.1) is the most widely cultured tilapia in the world because its rapid growth, late age at sexual maturity and high tolerance to adverse environmental conditions (Twibell and Brown, 1998).

The culture of Nile tilapia can be traced back to ancient Egyptian times dating over 4,000 years ago. The first recorded scientifically oriented culture of tilapia was conducted in Kenya in 1924 and soon spread throughout Africa (Gupta and Acosta,

2004). While significant worldwide distribution of tilapias, generally Mozambique tilapia occurred during the 1940s and 1950s, distribution of the more desirable Nile tilapia occurred during the 1960s up to the 1980s. Nile tilapia from Japan were introduced to Thailand in 1965, and from Thailand they were sent to the Philippines. Nile tilapia from Ivory Coast were introduced to Brazil in 1971, and from Brazil they were sent to the United States in 1974. In 1978, Nile tilapia was introduced to China, which presently leads the world in tilapia production and consistently produced more than half of the global production since 1992 (Mjoun et al., 2010).

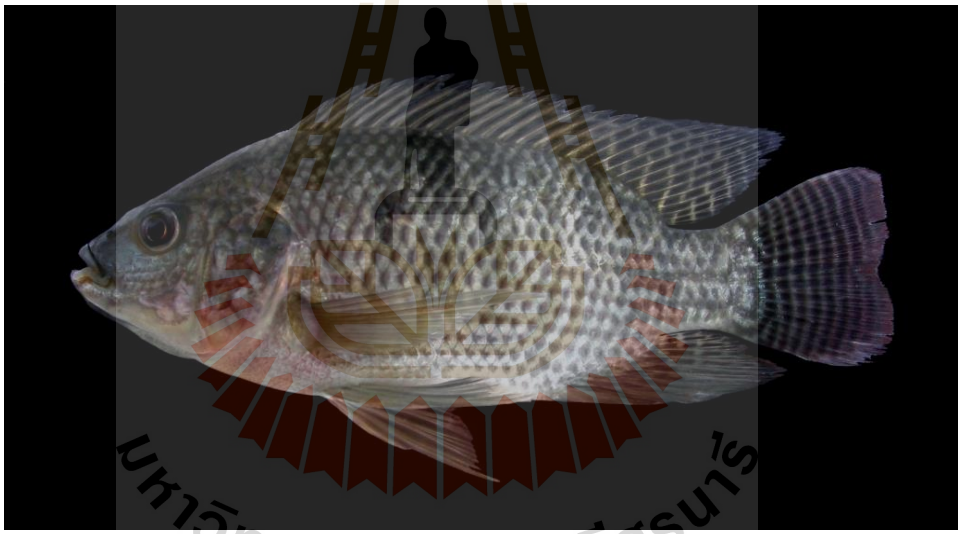

Figure 2.1 The morphological characteristics of Nile tilapia (*Oreochromis niloticus*).

Tilapias are of increasing importance in aquaculture globally and are only second to carps by volume of production. The total world tilapia production has increased annually from 2 million tons in 2004 to 4.5 million tons in 2012 and projected to reach 5.6 million tons in 2015, while global sales have also increased from US$1.6 billion to over US$10.0 billion in 2014 (Fitzsimmons, 2016). Of the

total world production of tilapias by weight and value, the Nile tilapia alone represents approximately 84%. Its production has accelerated from around 1,700,000 tons in 2005 to about 3.7 million tons in 2014 (Figure 2.2) (FAO, 2016). The production of tilapia has a wide distribution, and 72% are raised in Asia, 19% in Africa and 9% in America (FAO, 2012). Despite the fact that more than 140 countries have practised tilapia farming in 2015, only six countries (China, Indonesia, Egypt, the Philippines, Brasil and Thailand) have dominated world production. Those countries yielded 83% of global tilapia production in 2015. China alone produced 1,800,000 tons in 2015, representing 32% of total production, followed by Indonesia (20%), Egypt (14%), the Philippines (6%), Brasil (6%) and Thailand (5%) (Fitzsimmons, 2016). Nile tilapia were first introduced to Thailand in 1965 when 50 fry were imported (Pullin, 1988). These fish display the founding stock for Nile tilapia culture in Thailand, and are popularly known as the "Chitralada" strain. Currently, the aquaculture of Nile tilapia has expanded throughout the country, and become the first most important freshwater fish in Thailand with a volume of 204,787 tons in 2014 and exporting around 15,496 tons of total production (Figure 2.3) (Fisheries, 2015). The success of Nile tilapia farming is mainly attributed to it is ease of culture and desirable qualities as a food fish (Suresh, 2003). These include ease of breeding in captivity, tolerance to both crowding and relatively poor water quality and low susceptibility to diseases. According to El-Sayed (2006) Nile tilapia can survive well in water temperatures of 12-35°C, pH 6.5-8.5, dissolved oxygen 2.0-8.0 mg L^{-1} and salinity levels between 3 and 25 ppt.

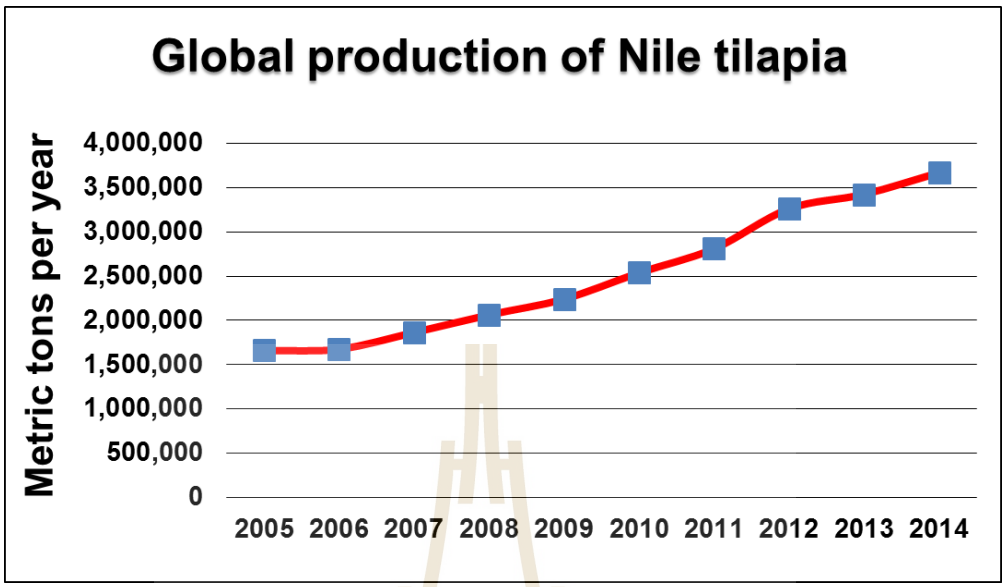

Figure 2.2 Global Nile tilapia aquaculture production.

Adapted from : FAO, 2016.

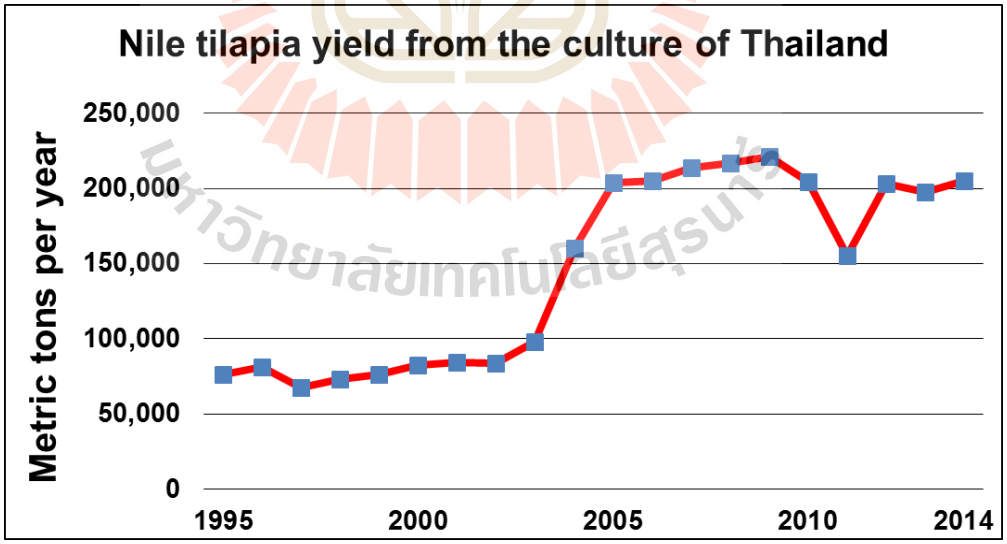

Figure 2.3 Nile tilapia yield from the culture of Thailand.

Adapted from : Fisheries, 2015.

2.2 Prebiotics

2.2.1 Definition of prebiotics

Gibson and Roberfroid (1995) first introduced the term prebiotics by exchanging the prefix “pro” from the term “probiotic,” meaning “for life” to “pre” which means “before” or “for”. They defined a prebiotic as “A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host health”. FAO (2007) proposed a recent definition for prebiotics as non-viable food components that confer health benefit on the host associated with modulation of the microbiota. Prebiotics are dietary carbohydrates that escape digestion in the upper gastrointestinal tract but change the bacterial composition of the gut by changing the type of substrate provided to the living gut microbiota (Gibson and Roberfroid, 1995; Mei et al., 2011). The effects of a prebiotic, according to Wood and Gorbach (2001), are characterized by an increase in beneficial bacteria and/or a decrease in harmful bacteria in the gut of the host, a decrease in intestinal pH through the production of short-chain fatty acids (SCFA) and changes in bacterial enzymes concentrations.

A number of health-related benefits have been attributed to prebiotic consumption, including relieving constipation (Roberfroid, 1993), reducing the risk of atherosclerosis by modulating lipid metabolism (Delzenne and Williams, 2002), decreasing the risk of osteoporosis by improving mineral absorption (Scholz-Ahrens et al., 2001), reducing the risk of colon cancer (Rumney and Rowland, 1995), preventing intestinal infections (Manning and Gibson, 2004) and stimulating the immune system of the body (Macfarlane and Cummings, 1999). The currently proposed mechanisms of health benefits by prebiotics are shown in Figure 2.4.

In particular, many food oligosaccharides and polysaccharides (including dietary fiber) have been claimed to have prebiotic activity, but not all fiber is prebiotic. According to Gibson et al. (2004), any food ingredient considered to be an effective prebiotic must demonstrate the following characteristics: 1) resists gastric acidity, hydrolysis by enzymes and gastrointestinal absorption, 2) is fermented by the intestinal microbiota, and 3) stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing.

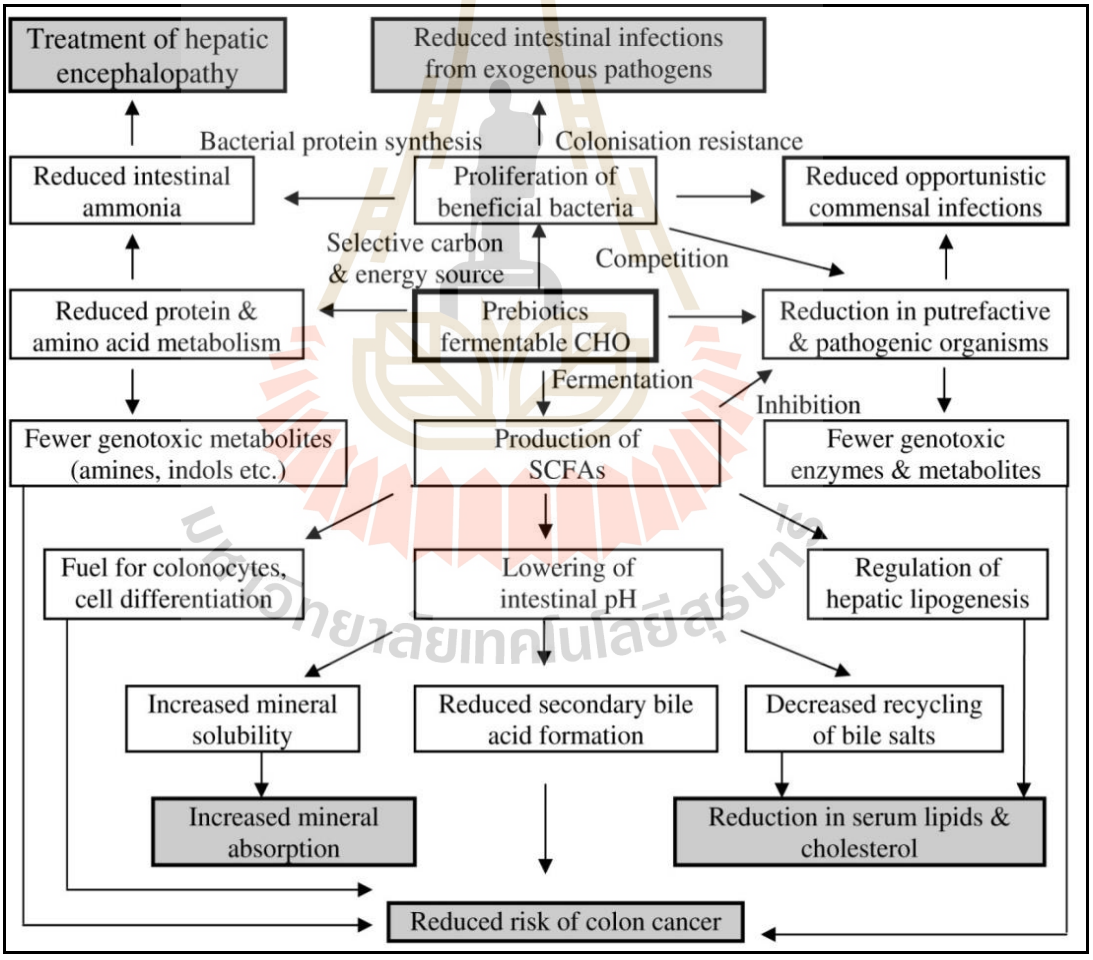

Figure 2.4 Proposed mechanisms of prebiotic effects on human health benefits.

Source : Crittenden, 1999.

2.2.2 The use of prebiotics in fish culture

New regulations, certification protocols and consumer liking are pushing the industry away from the use of antibiotics and other synthetic additives. The use of antibiotics in aquaculture has received considerable attention because their abuse has led to the development of drug-resistant bacteria, consequently, reducing drug capability. Moreover, the residues of antibiotics both in the environment and in aquaculture products can pose potential risk to consumers and the environment (Carrias et al., 2012). To meet the increasing consumer demands for fish and fish products that have not been treated with antibiotics while keeping good health and growth, fish farmers are turning to alternatives such as natural, cost-effective feed formulations that will decrease the effects of pathogenic bacteria on farm productivity. In recent years, prebiotics is under extensive investigation for their potential beneficial effects on fish health and growth. Prebiotics may have the role of increasing growth rate, improve immune system as well as stimulate selected beneficial indigenous microbiota.

2.2.3 The common prebiotics used in fish

Most prebiotics today are non-digestible oligosaccharides. They are obtained either by extraction from plants (e.g., chicory inulin), possibly followed by an enzymatic hydrolysis (e.g., oligofructose from inulin) or by synthesis (by trans-glycosylation reactions) from mono- or disaccharides such as sucrose (fructo-oligosaccharides) or lactose (trans-galactosylated oligosaccharides or galacto-oligosaccharides) (Crittenden and Playne, 1996). The common prebiotics used in fish to date include inulin, fructooligosaccharides (FOS), mannanoligosaccharides (MOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), arabinoxylooligo-

saccharides (AXOS) and GroBiotic®-A (Ringø et al., 2010b). Summaries of common prebiotics used in fish aquaculture are presented in Table 2.1. Results from several studies have indicated that prebiotics can improve growth performance and feed utilization of various fish species (Li and Gatlin, 2005; Mahious et al., 2006a; Hui-Yuan et al., 2007; Staykov et al., 2007; Torrecillas et al., 2007; Grisdale-Helland et al., 2008; Samrongpan et al., 2008; Ibrahem et al., 2010; Zhou et al., 2010; Soleimani et al., 2012; Akrami et al., 2013; Ortiz et al., 2013; Wu et al., 2013), enhance non-specific immune responses and resistance to bacterial infections (Li and Gatlin, 2005; Staykov et al., 2007; Buentello et al., 2010; Zhou et al., 2010; Soleimani et al., 2012), improve gut function and health by improving the ultrastructure of the intestine mucosa (Salze et al., 2008) and also activate health promoting bacteria in the intestine (Reza et al., 2009; Mourino et al., 2012; Akrami et al., 2013).

Table 2.1 The common prebiotics used in fish aquaculture.

Prebiotics	Fish species	References
Inulin	Grass carp (*Ctenopharyngodon idellus*)	Wang and Wang (1997)
	Tilapia (*Tilapia aureus*)	Wang and Wang (1997)
	Arctic charr (*Salvelinus alpinus*)	Olsen et al. (2001)
	Siberian sturgeon (*Acipenser baerii*)	Mahious et al. (2006a)
	Turbot (*Psetta maxima*)	Mahious et al. (2006b)
	Arctic charr	Ringø et al. (2006)
	Atlantic salmon (*Salmo salar*)	Refstie et al. (2006)
	Atlantic salmon	Bakke-McKellep et al. (2007)
	Gilthead sea bream (*Sparus aurata*)	Cerezuela et al. (2008)
	Beluga (*Huso huso*)	Reza et al. (2009)

Table 2.1 The common prebiotics used in fish aquaculture (Continued).

Prebiotics	Fish species	References
Inulin	Red drum (*Sciaenops ocellatus*)	Burr et al. (2009)
	Hybrid striped bass (*Morone chrysops* × *M. saxatilis*)	Burr et al. (2010)
	Nile tilapia (*Oreochromis niloticus*)	Ibrahem et al. (2010)
	Hybrid surubim (*Pseudoplatystoma* sp.)	Mourino et al. (2012)
	Rainbow trout (*Oncorhynchus mykiss*)	Ortiz et al. (2013)
	Gilthead sea bream	Cerezuela et al. (2013)
	Common carp (*Cyprinus carpio*)	Eshaghzadeh et al. (2015)
FOS	Hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂)	He et al. (2003)
	Turbot	Mahious et al. (2006b)
	Hybrid tilapia	Hui-Yuan et al. (2007)
	Atlantic salmon	Grisdale-Helland et al. (2008)
	Red drum	Buentello et al. (2010)
	Red drum	Zhou et al. (2010)
	Caspian roach (*Rutilus rutilus*)	Soleimani et al. (2012)
	Rainbow trout	Ortiz et al. (2013)
	Stellate sturgeon (*Acipenser stellatus*)	Akrami et al. (2013)
	Blunt snout bream (*Megalobrama amblycephala*)	Wu et al. (2013)
MOS	Hybrid tilapia	He et al. (2003)
	Gulf sturgeon (*Acipenser oxyrinchus desotoi*)	Pryor et al. (2003)
	Hybrid tilapia	Genc et al. (2007)
	Rainbow trout	Staykov et al. (2007)
	European sea bass	Torrecillas et al. (2007)

Table 2.1 The common prebiotics used in fish aquaculture (Continued).

Prebiotics	Fish species	References
MOS	Channel catfish (*Ictalurus punctatus*)	Welker et al. (2007)
	Rainbow trout	Yilmaz et al. (2007)
	Rainbow trout	Dimitroglou et al. (2008)
	Rainbow trout	Rodrigues-Estrada et al. (2008)
	Nile tilapia	Sado et al. (2008)
	Cobia (*Rachycentron canadum*)	Salze et al. (2008)
	Nile tilapia	Samrongpan et al. (2008)
GOS	Red drum	Burr et al. (2008)
	Hybrid striped bass	Burr et al. (2010)
	Atlantic salmon	Grisdale-Helland et al. (2008)
XOS	Crucian carp (*Carassius auratus gibelio*)	Xu et al. (2009)
AXOS	Siberian sturgeon	Rurangwa et al. (2008)
	African catfish (*Clarias gariepinus*)	Rurangwa et al. (2008)
GroBiotic®-A	Hybrid striped bass	Li and Gatlin (2005)
	Golden shiners (*Notemigonus crysoleucas*)	Sink and Lochmann (2008)
	Red drum	Burr et al. (2009)
	Hybrid striped bass	Burr et al. (2010)
	Kutum (*Rutilus frisii kutum*)	Yousefian et al. (2012)

Abbreviations : FOS = fructooligosaccharides, MOS = mannanoligosaccharides, GOS = galactooligosaccharides, XOS = xylooligosaccharides, AXOS = arabinoxylo-oligosaccharides.

2.3 Jerusalem artichoke (*Helianthus tuberosus*)

Jerusalem artichoke (JA; *Helianthus tuberosus*) or Kaen-ta-wan (Thai name) belongs to plants in the genus *Helianthus* L., in the family Asteraceae, and in the

order Asterales (USDA, 2006). It is a native plant of North America (Kays and Nottingham, 2007). It is presently cultivated in Europe, Australia and Asia (Baldini et al., 2004). In Thailand, JA can be harvested after 90-120 days, allowing a chance for farmers to produce 3 crops per year and crop yields of JA are typically 13-19 ton per hectare (Jogloy et al., 2006). The tubers of JA (Figure 2.5) generally contain about 80% water, 15% carbohydrate, and 1-2% protein (Kays and Nottingham, 2007). Its tuber contains high amount of dietary fiber that is inulin and FOS (15.28 and 5.96 g/100 g fresh weight, respectively) (Tanjor et al., 2012). On a dry weight basis, the tubers contain 68-83% fructans, 1.5-1.6% proteins, 13% insoluble fibre and 5% ash (Fleming and GrootWassink, 1979). Interestingly, the JA tubers do not contain starch. It has traditionally been used as food and animal feed and, more recently, as a raw material for the industrial production of fructose and fructans (Kosaric et al., 1984).

Figure 2.5 Jerusalem artichoke tubers (*Helianthus tuberosus*).

Generally, inulin and FOS are natural food components belonging to a class of carbohydrates knowns as fructans. The JA tubers accumulate high levels of fructans (68-83% on a dry weight) during their growth (Fleming and GrootWassink, 1979). The fructans are composed of linear β-D-(2→1)-linked fructose units and generally have a terminal unit (Waterhouse, 1993). They are characterised by the degree of polymerisation (DP_n) defined as the number of fructosyl units linked to the terminal glucose. By using this criterion, fructans are classified being either as inulin (DP_n>10) or FOS (DP_n<10) (Cabezas et al., 2002). Inulin and FOS are represented by the general formula GF_n and F_m, wherein G is glucosyl unit, F is fructosyl unit, n is an integer number of fructose units linked to the terminal glucose unit and m is an integer number of fructose units linked to each other in the carbohydrate chain. The molecular structures of inulin and FOS are shown in Figure 2.6.

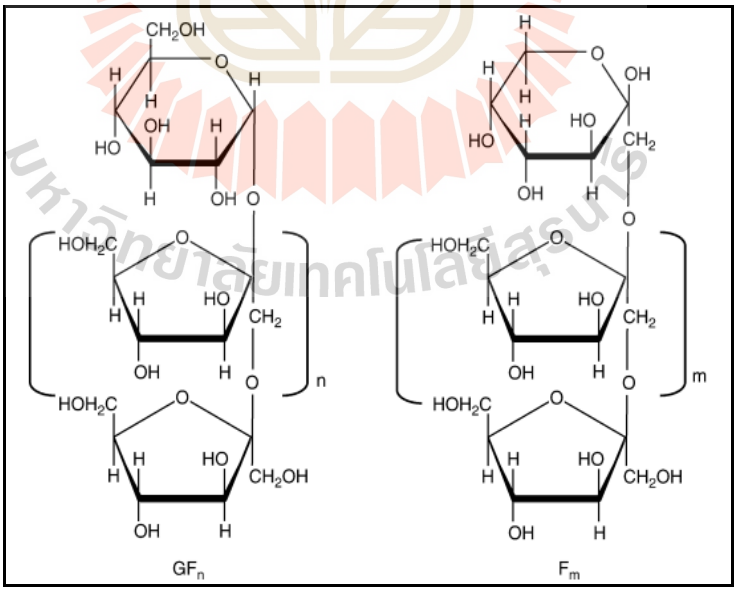

Figure 2.6 Chemical structures of GF_n-type inulin (left) and F_m-type FOS (right).

Source : Franck, 2000.

Inulin is a mixture of GF_n molecules with $2 < n < 60$ while FOS is a subgroup of inulin which is composed of GF_n and F_m with $2 \leq n$, and $m \leq 10$ (Franck, 2000). Actually, FOS and oligofructose are considered to be synonyms for the mixture of small inulin oligomers with $DP_{max} < 10$ (Roberfroid, 2004). The distribution of chain lengths in JA tubers is dependent on the cultivar and time of harvest (Baldini et al., 2004). Inulin and FOS are fructans that are not hydrolysed by pancreatic enzymes and escape digestion in the small intestine (Bach-Knudsen and Hessov, 1995). Beneficial bacteria especially *Bifidobacteria* have relatively high amounts of β-fructosidase that is selective for β 2-1 glycosidic bonds in fructans (De Vries and Stouthamer, 1967). After oligosaccharide hydrolysis, monomers then serve as a suitable growth substrate for the bifidus pathway of hexose fermentation (Scardovi, 1965). Fructan containing foods were reported to enhance mineral absorption, reduce cholesterol levels, stimulate the immune system of the body and decrease the levels of pathogenic bacteria in the intestine (Kaur and Gupta, 2002).

2.4 Use of inulin and FOS in fish aquaculture

Feed costs account for over 50% of the variable costs in most aquaculture activity, therefore applying the best feeding strategy can have a significant impact on optimizing profit, which is the primary goal of commercial aquaculture. Also, if fish are able to resist disease and survive until they are of marketable size, the subsequent cost of medication and overall production costs would be reduced drastically (Yousefian and Amiri, 2009). Inulin and FOS may have the role of increasing growth rate, improve immune system as well as change the community of bacterial in gastrointestinal track. Many scientists have worked to optimize the dosage of

supplementary inulin and FOS in feed to achieve better growth rate and survival. The studies of inulin and FOS in fish aquaculture have investigated the following parameters:

2.4.1 Effects of inulin and FOS on growth parameters

The addition of inulin and FOS to fish diets are metabolized by specific health-promoting bacteria such as *Lactobacillus* and *Bifidobacterium*. These bacteria are considered beneficial to the health and growth response of the host by decreasing the presence of intestinal pathogens and/or changing the production of health related bacterial metabolites (Roberfroid, 1993; Gibson and Roberfroid, 1995; Manning and Gibson, 2004; Ringø et al., 2010b). A review of the effect of dietary inulin and FOS supplementation on growth parameters in fish aquaculture is presented in Table 2.2 Mahious et al. (2006a) found that Siberian sturgeon fed a diet containing 20 g kg^{-1} inulin for 82 days had significantly improved growth performance, including final weight, SGR and FCR. Ibrahem et al. (2010) reported that dietary supplementation with 5 g kg^{-1} inulin for 8 weeks had significantly increased WG, SGR and survival rate in Nile tilapia. Ortiz et al. (2013) showed that rainbow trout fed inulin or FOS containing diets (5 and 10 g kg^{-1}) for 49 days exhibited significant WG improvements compared to the control group. In the study by Hui-Yuan et al. (2007), an 8 weeks feeding trial with hybrid tilapia showed that SGR, FI and FCR were significantly improved with increasing level of FOS (0.8 and 1.2 g kg^{-1}), while survival rate and condition factor were not affected compared to the control group. Zhou et al. (2010) reported that dietary supplementation with 10 g kg^{-1} FOS for 8 weeks had significantly increased WG. However, no significant effects on FE, PER and survival rate in red drum. Soleimani et al. (2012) also reported that Caspian roach fry fed 20 and 30 g kg^{-1} FOS containing

diets for 7 weeks had significantly improved final weight, WG, SGR and FCR. Wu et al. (2013) also reported that dietary supplementation of blunt snout bream fingerlings diets with 0.5, 1, 2, 4 and 8 g kg^{-1} FOS for 8 weeks showed that final weight, WG, SGR, FCR and survival rate were significantly improved with increasing level of FOS. Mahious et al. (2006b) also observed that dietary supplementation with 20 g kg^{-1} oligofructose, a FOS produced by partial enzymatic hydrolysis of inulin by hot water extraction of chicory roots, resulted in increased growth of turbot larvae, but 20 g kg^{-1} inulin itself had no effect on growth response.

Although positive effects of dietary FOS/inulin were reported in a number of fish, dietary supplementation with inulin did not affect the growth response in several fish. In Atlantic salmon, Bakke-McKellep et al. (2007) reported that dietary supplementation with 75 g kg^{-1} inulin for 3 weeks did not significantly affect on body weight or body length compared with fish fed the control diet. Reza et al. (2009) also reported that supplementation of juvenile beluga diets with 10, 20 and 30 g kg^{-1} inulin for 8 weeks showed negative relationship between some performance indices including WG, SGR, PER, FE, which indicated that inulin is not appropriate for supplementation in the diets of juvenile beluga. Burr et al. (2010) also reported that hybrid striped bass fed 10 g kg^{-1} inulin containing diets for 8 weeks did not significantly affect on WG and FCR. Eshaghzadeh et al. (2015) also reported that common carp fed 5 and 10 g kg^{-1} inulin containing diets for 7 weeks did not significantly affect on growth performance and diet utilization. In hybrid tilapia, He et al. (2003) found that dietary supplementation with 2 and 6 g kg^{-1} FOS for 58 days did not affect on growth rate but increased survival rate compared with fish fed the control diet. In addition, Buentello et al. (2010) reported that supplementation with

10 g kg^{-1} FOS for 4 weeks did not significantly affect on WG, FCR and survival rate in red drum. The reasons for different results are not clear yet. It appears that the different basal diet, level of inulin or FOS supplementation, type of fructan, chemical structure (degree of polymerization), adaptation period, chemical structure, animal characteristics (species, age, stage of production) and period and hygienic conditions of the experiment can be causing these differences.

Table 2.2 Effects of inulin and FOS on growth parameters in fish aquaculture.

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
Inulin	20; 82 days	Siberian sturgeon (*Acipenser baerii*)	213.41	↑ Final weight, SGR and FCR	Mahious et al. (2006a)
	5; 60 days	Nile tilapia (*Oreochromis niloticus*)	11.00	↑ WG, SGR and survival rate	Ibrahem et al. (2010)
	5 and 10; 49 days	Rainbow trout (*Oncorhynchus mykiss*)	150.00	↑ WG	Ortiz et al. (2013)
	20; 55 days post hatching	Weaning turbot (*Psetta maxima*)	0.04	→ Final weight, SGR and survival rate	Mahious et al. (2006b)
	75; 3 weeks	Atlantic salmon (*Salmo salar*)	172.00	→ Final weight and body length	Bakke-McKellep et al. (2007)
	10, 20 and 30; 8 weeks	Beluga (*Huso huso*)	16.14	↓ Final weight, WG and SGR → FI, FCR and survival rate	Reza et al. (2009)
	10; 8 weeks	Hybrid striped bass (*Morone chrysops* × *M. saxatilis*)	344.40	→ WG and FCR	Burr et al. (2010)
	5 and 10; 7 weeks	Common carp (*Cyprinus carpio*)	0.55	→ Final weight, WG, SGR and FCR	Eshaghzadeh et al. (2015)

Table 2.2 Effects of inulin and FOS on growth parameters in fish aquaculture. (Continued).

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
FOS	20; 55 days post hatching	Weaning turbot (*Psetta maxima*)	0.04	↑ Final weight and SGR → Survival rate	Mahious et al. (2006b)
	0.8 and 1.2; 8 weeks	Hybrid tilapia (*Oreochromis aureus* ♂ × *O. niloticus* ♀)	5.55	↑ SGR, FI and FCR → Survival rate	Hui-Yuan et al. (2007)
	5 and 10; 49 days	Rainbow trout (*Oncorhynchus mykiss*)	150.00	↑ WG	Ortiz et al. (2013)
	10; 8 weeks	Red drum (*Sciaenops ocellatus*)	7.00	↑WG → FCR and survival rate	Zhou et al. (2010)
	10, 20 and 30; 7 weeks	Caspian roach (*Rutilus rutilus*)	0.67	↑ Final weight, WG, SGR and FCR → Survival rate	Soleimani et al. (2012)
	10 and 20; 75 days	Stellate sturgeon (*Acipenser stellatus*)	30.16	↑ Final weight, WG, SGR, PER and FCR → Survival rate	Akrami et al. (2013)
	0.5, 1, 2, 4 and 8; 8 weeks	Blunt snout bream (*Megalobrama amblycephala*)	1.42	↑ Final weight, WG, SGR, FCR and survival rate	Wu et al. (2013)
	2 and 6; 58 days	Hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂)	57.00	→ Growth rate ↑ survival rate	He et al. (2003)
	10; 4 weeks	Red drum	10.90	→ WG, FCR and survival rat	Buentello et al. (2010)

[1]Symbols represent an increase (↑), decrease (↓) or no effect (→) on the specified response.

Abbreviations : WG = weight gain, SGR = specific growth rate, FI = feed intake, FCR = feed conversion ratio, FE = feed efficiency, PER = protein efficiency ratio.

2.4.2 Effect of inulin and FOS on whole body composition

The approximate composition of fish body and fish fillet has generally been determined to assess the nutritional status of the fish. In beluga, no significant difference was reported with whole-body moisture, crude protein, total lipids and ash content among the control group and the dietary supplementation of 10, 20 and 30 kg^{-1} inulin (Reza et al., 2009). Similarly, Burr et al. (2010) reported that hybrid striped bass fed 10 kg^{-1} inulin did not affect the levels of moisture, crude protein, total lipids and ash in the whole body, and this is in accordance with previous reports on rainbow trout fed inulin or FOS (Ortiz et al., 2013), and Atlantic salmon (Grisdale-Helland et al., 2008), red drum (Buentello et al., 2010) and beluga (Hoseinifar et al., 2011a) fed FOS. In contrast, dietary supplementation with inulin at 10 g kg^{-1} significantly affected the carcass composition especially protein and lipid content of common carp fry (Eshaghzadeh et al., 2015). Changes in protein and lipid content in carcass of fish fed inulin and the control group may be due to changes in their synthesis, deposition rate in muscle (Abdel-Tawwab et al., 2008). Wu et al. (2013) also observed that increased with dietary FOS levels up to 4 g kg^{-1} resulted in both higher whole-body lipid and lower moisture contents, whereas ash and protein contents showed no significant differences among all the treatments of blunt snout bream fingerlings. Summaries of the effects of inulin and FOS on whole body composition in fish aquaculture are showed in Table 2.3.

Table 2.3 Effects of inulin and FOS on whole body composition in fish aquaculture.

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
Inulin	10, 20 and 30; 8 weeks	Beluga	16.14	→ Whole body crude protein, moisture, crude lipid and ash	Reza et al. (2009)
	10; 8 weeks	Hybrid striped bass	344.40	→ Whole body crude protein, moisture, crude lipid and ash	Burr et al. (2010)
	5 and 10; 49 days	Rainbow trout	150.00	→ Whole body crude protein, moisture, crude lipid and ash	Ortiz et al. (2013)
	5 and 10; 7 weeks	Common carp	0.55	→ Whole body moisture and ash ↑ Crude lipid ↓ Crude protein	Eshaghzadeh et al. (2015)
FOS	10; 16 weeks	Atlantic salmon	200.20	→ Whole body crude protein, moisture, crude lipid and ash	Grisdale-Helland et al. (2008)
	10; 4 weeks	Red drum	10.90	→ Whole body crude protein, moisture, crude lipid and ash	Buentello et al. (2010)
	10, 20 and 30; 7 weeks	Beluga	19.80	→ Whole body crude protein, moisture, crude lipid and ash	Hoseinifar et al. (2011a)
	5 and 10; 49 days	Rainbow trout	150.00	→ Whole body crude protein, moisture, crude lipid and ash	Ortiz et al. (2013)

Table 2.3 Effects of inulin and FOS on whole body composition in fish aquaculture. (Continued).

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
FOS	4; 8 weeks	Blunt snout bream	1.42	→ Whole body crude protein and ash ↑ Crude lipid ↓ Moisture	Wu et al. (2013)

[1]Symbols represent an increase (↑), decrease (↓) or no effect (→) on the specified response.

2.4.3 Effect of inulin and FOS on gastrointestinal microbial community

Dietary supplementation of inulin-type fructans, which are classified as non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of a limited number of health-promoting bacteria such as *Lactobacillus* and *Bifidobacteria* in the intestine, and thus improves host health (Gibson and Roberfroid, 1995; Van Loo, 2004). Inulin and FOS are selectively fermented by probiotic bacteria to produce SCFA (acetate, propionate and butyrate) (Rossi et al., 2005; Venter, 2007). Reducing the pH of the colon resulting from the production of SCFA is another prebiotic properties. Lower pH values inhibit the growth of certain pathogenic bacterial species while stimulating the growth of the bifidobacteria and other lactic acid species (Mussatto and Mancilha, 2007). In aquaculture, few reports are available on the influence of inulin and FOS on intestinal microbiota in fish (Table 2.4). Reza et al. (2009) reported that dietary supplementation with inulin (10.0 g kg^{-1}) for 8 weeks resulted in an increase in population of lactic acid bacteria in beluga compared with fish fed the control diet. Mourino et al. (2012) showed that

dietary supplementation with 5 g kg^{-1} inulin for 15 days had higher concentrations of lactic acid bacteria in hybrid surubim. Akrami et al. (2013) reported that stellate sturgeon fed 10 g kg^{-1} FOS containing diets for 75 days showed a significant increase in total heterotrophic autochthonous bacterial and lactic acid bacteria levels compared with fish fed the control diet. In addition, Ortiz et al. (2013) observed that supplementation with inulin (5.0-10.0 g kg^{-1}) for 49 days had reduced drastically the number of *Vibrio* spp. in the distal part of the intestine of rainbow trout. In fact, lactic acid bacteria and *Bifidobacteria* were well known that they could fermented inulin and FOS (Kaplan and Hutkins, 2000; Buddington et al., 2002; Roller et al., 2004). In addition, most of these bacteria would be categorized as beneficial bacteria for ecosystem of animal intestines by producing bacteriocins, lactic acid and anti-growth substance of other bacteria, which could inhibit the growth of pathogenic intestinal bacteria (Ringø and Gatesoupe, 1998; Ringø et al., 2010a)

Table 2.4 Effects of inulin and FOS on gastrointestinal microbial community in fish aquaculture.

Prebiotic	**Dose (g kg^{-1}); duration of trial**	**Fish species**	**Initial Wt. (g)**	**Response[1]**	**References**
Inulin	5; 15 days	Hybrid surubim (*Pseudoplatystoma* sp.)	73.60	↑ Lactic acid bacteria → Total bacteria, *Vibrio* spp. and *Pseudomonas* spp.	Mourino et al. (2012)
	10, 20 and 30; 8 weeks	Beluga	16.14	↑ Lactic acid bacteria → Total bacteria	Reza et al. (2009)
	10; 8 weeks	Red drum	111.70	→ Gastrointestinal tract microbial community	Burr et al. (2009)

Table 2.4 Effects of inulin and FOS on gastrointestinal microbial community in fish aquaculture (Continued).

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
Inulin	150; 4 weeks	Arctic charr (*Salvelinus alpinus*)	218.00	↓ Population level of adherent bacteria in the hindgut	Ringø et al. (2006)
	75; 3 weeks	Atlantic salmon	172.00	↓ Population composition of adherent bacteria in the distal intestine.	Bakke-McKellep et al. (2007)
	5 and 10; 49 days	Rainbow trout	150.00	→ *Aeromonas* spp., *Pseudomonas* spp. and Gram-positive bacteria ↓ Not detected *Vibrio* spp.	Ortiz et al. (2013)
FOS	20; 55 days post hatching	Weaning turbot	0.04	↑ *Bacillus* spp. ↓ *Vibrio* spp.	Mahious et al. (2006b)
	5 and 10; 49 days	Rainbow trout	150.00	→ *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp. and Gram-positive bacteria.	Ortiz et al. (2013)
	0.8 and 1.2; 8 weeks	Hybrid tilapia	5.55	→ *Aeromonas hydrophila* and *Lactobacillus* sp.	Hui-Yuan et al. (2007)
	10 and 20; 75 days	Stellate sturgeon	30.16	↑ Total bacterial and lactic acid bacteria	Akrami et al. (2013)

[1]Symbols represent an increase (↑), decrease (↓) or no effect (→) on the specified response.

2.4.4 Effect of inulin and FOS on hematological and blood chemical parameters

The analysis of hematological parameters is a valuable guide in assessing the health condition of aquatic organisms. Whereas blood chemical parameters can be used to interpret the blood metabolic response and nutritional status resulting from the use of dietary food additives. Reza et al. (2009) reported that supplementation of juvenile beluga diets with 10, 20 and 30 g kg^{-1} inulin for 8 weeks did not significantly affect on RBC, MCH, total cholesterol, glucose, triglycerides, albumin, SGOT and SGPT activities. Plasma total protein, WBC, Hb and Ht of fish fed with diets containing inulin were lower than that of the basal group. The highest value of WBC was observed in fish fed with the diet containing 10 g kg^{-1} inulin. Mourino et al. (2012) observed that dietary supplementation with 5 g kg^{-1} inulin for 15 days did not alter RBC, WBC and Ht in hybrid surubim. Řehulka et al. (2011) also found that rainbow trout fed 10 g kg^{-1} FOS containing diets for 108 days did not significantly affect on Ht, total protein, BUN, SGOT, SGPT and calcium contents. In addition, Hoseinifar et al. (2011b) reported that supplementation of juvenile beluga diets with 10, 20 and 30 g kg^{-1} FOS for 7 weeks did not significantly affect on RBC, MCV, MCH, MCHC, glucose, total protein, SGOT and SGPT activities. Although serum glucose and total protein levels remained unaffected compared to the control group, serum cholesterol was significantly lower in the 20 g kg^{-1} FOS group. It has been reported that FOS share many common physiological traits with soluble dietary fibre (Van Loo et al., 1999), and it is expected that it may also affect serum lipid profiles (Delzenne and Kok, 2001; Delzenne et al., 2002). Indeed, dietary oligofructose has been demonstrated to prevent the elevation of serum cholesterol in rats fed high fat diets

(Kok et al., 1998). Summaries of the effects of inulin and FOS on hematological and blood chemical parameters in fish aquaculture are showed in Table 2.5.

Table 2.5 Effects of inulin and FOS on hematological and blood chemical parameters in fish aquaculture.

Prebiotic	**Dose (g kg^{-1}); duration of trial**	**Fish species**	**Initial Wt. (g)**	**Response[1]**	**References**
Inulin	10, 20 and 30; 8 weeks	Beluga	16.14	↑ WBC in fish fed 10 g kg^{-1} inulin → RBC, MCH, Total cholesterol, glucose, albumin, triglycerides, SGOT and SGPT ↓ WBC, Hb, Ht and total protein in fish fed 30 g kg^{-1} inulin	Reza et al. (2009)
	5; 15 days	Hybrid surubim	73.60	→ RBC, WBC and Ht	Mourino et al. (2012)
FOS	10, 20 and 30; 7 weeks	Beluga	18.77	↑ Hb and Ht in fish fed 20 g kg^{-1} FOS → RBC, MCV, MCH, MCHC, glucose, total protein, SGOT and SGPT ↓ WBC in fish fed 30 g kg^{-1} FOS	Hoseinifar et al. (2011b)
	10; 108 days	Rainbow trout	240.00	→ Ht, total protein, BUN, SGOT, SGPT and calcium	Řehulka et al. (2011)

Table 2.5 Effects of inulin and FOS on hematological and blood chemical parameters in fish aquaculture (Continued).

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
FOS	10 and 20; 75 days	Stellate sturgeon	30.16	↑ WBC → RBC, Hb, Ht, MCV, MCH and MCHC	Akrami et al. (2013)

[1]Symbols represent an increase (↑), decrease (↓) or no effect (→) on the specified response.

Abbreviations : RBC = red blood cell, WBC = white blood cell, Hb = hemoglobin, Ht = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, BUN = blood urea nitrogen, SGOT = serum glutamic oxaloacetic transaminase, and SGPT = serum glutamic pyruvic transaminase.

2.4.5 Effect of inulin and FOS on mineral absorption

Prebiotics containing inulin and FOS enhance mineral bioavailability by improving the absorption of minerals in the colon, particularly calcium, magnesium and iron (Ohta et al., 1994; Delzenne et al., 1995; Caers, 2004; Roberfroid, 2004). The mechanism for this is possibly enhanced passive and active mineral transport across the intestinal epithelium, mediated by increased levels of butyrate and other SCFA and decreased pH (Scholz-Ahrens et al., 2001). Improvements in calcium and iron absorption may help prevent osteoporosis and anemia, respectively (Ohta et al., 1998; Weaver and Liebman, 2002). Prebiotic inulin and FOS added to the daily diet of animals significantly increase calcium absorption in animals (Ohta et al., 1994;

Coudray et al., 2003). This can increase mineralization and bone mineral density (Roberfroid et al., 2002). In rainbow trout, Ortiz et al. (2013) also observed that a diet supplemented with inulin or FOS (5 and 10 g kg^{-1}) for 49 days exhibited significant positive linear effect on the calcium content in the whole body, mean values increasing by 14.1% with the inclusion of inulin and by 3.4% with the inclusion of FOS compared with those recorded for the control group.

2.4.6 Effect of inulin and FOS on the immune system

Stimulation of the immune response of fish through dietary supplements is of high interest for commercial aquaculture (Staykov et al., 2007). The innate immune system is very important in this regard because aquatic animals are continually vulnerable to numerous opportunistic pathogens and this part of immune response provides the first line of defense for the host (Magnadóttir, 2006). A review of the effect of dietary inulin and FOS supplementation on immune system in fish aquaculture is presented in Table 2.6. The first preliminary study carried out using inulin in fish was conducted by Wang and Wang (1997). In this 14 day study, inulin was administered via intraperitoneal injection into grass carp (24.6±3.5 g) and tilapia (21.8±3.5 g). Although survival rates against *Aeromonas hydrophila* and *Edwardsiella tarda* were higher, the values were not significantly different from that of the control fish. Ibrahem et al. (2010) reported that dietary supplementation with 5 g kg^{-1} inulin for 8 weeks had significantly increased nitroblue tetrazolium (NBT) and lysozyme activity in Nile tilapia. He et al. (2003) reported that dietary supplementation with 2 and 6 g kg^{-1} FOS for 58 days had significantly enhanced activity of innate defence mechanisms lysozyme and ACH50 in hybrid tilapia. In a study with red drum, Buentello et al. (2010) observed that dietary FOS at 10 g kg^{-1} for 4 weeks had

significantly enhanced lysozyme activity and intracellular superoxide anion production. Zhou et al. (2010) found that red drum fed 10 g kg^{-1} FOS containing diets for 8 weeks had significantly enhanced lysozyme activity. Soleimani et al. (2012) reported that Caspian roach fed 20 and 30 g kg^{-1} FOS containing diets for 7 weeks had significantly enhanced Total Ig, lysozyme activity and ACH50. In stellate sturgeon, Akrami et al. (2013) also found that dietary supplementation with 10 g kg^{-1} FOS for 75 days had significantly enhanced serum lysozyme activity. The immunostimulatory nature of prebiotics may be attributed to stimulation of the growth of beneficial bacteria such as lactic acid bacteria and *Bacillus* spp. (Zhang et al., 2011; Sang et al., 2011), which possess cell wall components such as lipopolysaccharides which have immunostimulatory properties (Masahiro, 1999; Bricknell and Dalmo, 2005). Substantial experimental data suggest that prebiotics induce their immunological effects by several ways are presented in Table 2.7. However, Grisdale-Helland et al. (2008) reported that dietary supplementation with 10 g kg^{-1} FOS for 16 weeks did not significantly affect on NBT or serum lysozyme in Atlantic salmon. Mourino et al. (2012) also found that dietary supplementation with 5 g kg^{-1} inulin for 15 days had no effect on lysozyme activity and Total Ig in hybrid surubim. This contradictory result may be attributable to the low dosage, different duration of prebiotic administration, life stage and/or different fish species (Ibrahem et al., 2010).

Table 2.6 Effects of inulin and FOS on immune system in fish aquaculture.

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
Inulin	Intraperitoneal injection (10 mg kg^{-1} body weight; 2 weeks	Grass carp (*Ctenopharyngodon idellus*)	24.60	→ Susceptibility against *Aeromonas hydrophila* and *Edwardsiella tarda*	Wang and Wang (1997)
	Intraperitoneal injection (10 mg kg^{-1} body weight; 2 weeks	Tilapia (*Tilapia aureus*)	21.80	→ Susceptibility against *A. hydrophila* and *E. tarda*	Wang and Wang (1997)
	5; 60 days	Nile tilapia	11.00	↑ Nitroblue tetrazolium (NBT) and lysozyme activity	Ibrahem et al. (2010)
	5; 15 days	Hybrid surubim	73.60	→ Lysozyme activity and Total Ig	Mourino et al. (2012)
FOS	2 and 6; 58 days	Hybrid tilapia	57.00	→ Lysozyme and ACH50	He et al. (2003)
	10; 4 weeks	Red drum	10.90	↑ Lysozyme activity	Buentello et al. (2010)
	10; 8 weeks	Red drum	7.00	↑ Lysozyme activity	Zhou et al. (2010)
	20 and 30; 7 weeks	Caspian roach	0.67	↑ Lysozyme activity, Total Ig and ACH50	Soleimani et al. (2012)
	10; 16 weeks	Atlantic salmon	200.20	→ NBT and lysozyme activity	Grisdale-Helland et al. (2008)

[1]Symbols represent an increase (↑), decrease (↓) or no effect (→) on the specified response.

Abbreviations : Total Ig = total immunoglobulin, ACH50 = alternative complement haemolytic 50 activity, NBT = Nitroblue tetrazolium activity.

Table 2.7 Potential mechanisms of prebiotic-induced immunomodulation.

- Selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production.
- Increase in intestinal SCFAs production and enhanced binding of SCFA to G-coupled protein receptors on leukocytes.
- Interaction with carbohydrate receptors on intestinal epithelial cells and immune cells.
- Partial absorption of inulin/FOS resulting in local and systemic contact with the immune system.

Source : Seifert and Watzl, 2007.

2.4.7 Effects of inulin and FOS on intestinal histomorphology

It is well known that increased intestinal villi leads to increase surface area for nutrient absorption, thereby improving growth performance and feed utilization in animals (Caspary, 1992). Several researchers proposed that fermentation of inulin produced several substances that stimulate intestinal cell proliferation, which in turn resulted in increased villus height (Blottiere et al., 2003; Rehman et al., 2007). Inulin-type fructans were fermented by gut bacteria to yield SCFA, mainly acetate, propionate and butyrate, which had important physiological functions. In the digestive tract, butyrate could act directly (upper gastrointestinal tract or hindgut) or indirectly (small intestine) on tissue development and repair (Nabizadeh, 2012). The review of the effect of dietary inulin and FOS supplementation on intestinal histomorphology in fish aquaculture are summarized in Table 2.8. In blunt snout bream fingerlings, Wu et al. (2013) reported that dietary supplementation with 0.5, 1, 2, 4 and 8 g kg^{-1} FOS for 8 weeks had significantly increased microvilli heights in the mid-intestine. Similarly, Zhou et al. (2010) reported that red drum fed 10 g kg^{-1} FOS containing

diets for 8 weeks had significantly increased microvilli heights in pyloric caeca, proximal and mid-intestine. The increase in microvilli heights may convert into improved apical brush border integrity and absorptive surface area, which could result in better nutrient utilization. In addition, Refstie et al. (2006) found that Atlantic salmon fed the diet supplemented with 75 g kg^{-1} inulin did not damage the distal intestine, but stimulated intestinal growth resulting in increased relative mass of the gastrointestinal tract. However, Olsen et al. (2001) reported that high-level dietary inulin (150 g kg^{-1} dietary inclusion) had a negative effects on the intestinal morphology of Arctic charr, at the ultrastructural level, enterocytes were clearly damaged and the apical brush border showed signs of membrane loss and microvilli degradation. The negative effect may be attributed to the inability of intestinal microbiota to ferment excessive levels of inulin and subsequent accumulation in the intestine, which may be deleterious to the enterocytes. In addition, Cerezuela et al. (2013) found that gilthead sea bream fed diets supplemented with inulin at 10 g kg^{-1} for 4 weeks displayed shorter microvilli than those from the control group and the histological scores of intestine demonstrated significant damage in the intestines of fish fed the inulin supplemented diets. It is clear that optimizing prebiotics inulin or FOS dosage levels requires further attention as there is often a fine line between achieving benefits and achieving negative effects. Such negative effects may be attributed to the inability of intestinal microbiota to ferment excessive inulin levels and the subsequent accumulation of indigestible material in the intestine which may cause irritation to the gut (Olsen et al., 2001).

Table 2.8 Effects of inulin and FOS on intestinal histomorphology in fish aquaculture.

Prebiotic	Dose (g kg^{-1}); duration of trial	Fish species	Initial Wt. (g)	Response[1]	References
Inulin	75; 3 weeks	Atlantic salmon	172.00	→ Intestinal cell damage ↑ Intestinal growth and relative mass of the gastrointestinal tract	Refstie et al. (2006)
	150; 4 weeks	Arctic charr	218.00	↑ Intestinal cell damage	Olsen et al. (2001)
	10; 4 weeks	Gilthead sea bream (*Sparus aurata*)	50.00	↓ Microvillus height ↑ Microvilli disruption/damage	Cerezuela et al. (2013)
FOS	10; 8 weeks	Red drum	7.00	↑ Microvilli heights in pyloric caeca, proximal and mid-intestine	Zhou et al. (2010)
	0.5, 1, 2, 4 and 8; 8 weeks	Blunt snout bream	1.42	↑ Microvilli length in the mid-intestine	Wu et al. (2013)

[1]Symbols represent an increase (↑), decrease (↓) or no effect (→) on the specified response.

Inulin and FOS are dietary fibers that cannot be digested in stomach and small intestine, but they are fermented in the large intestine or colon by beneficial intestinal bacteria, especially groups of bacteria with inulinase enzymes (Prosky, 1998; Roberfroid, 2002; Flickinger et al., 2003). As a result, these two compounds have been used as prebiotic additives in fish feed because they have a role in increasing feed efficiency, increasing growth performance and reducing enteric pathogens (Gibson et al., 2004), which gives health benefits since they can increase disease

immunity for the fish (Grisdale-Helland et al., 2008). However, most experiment is done with foreign fishes by testing various prebiotic substances, including inulin, FOS, MOS, GOS, XOS, AXOS and Brewers yeast (GroBiotic®-A) (Li and Gatlin, 2005; Refstie et al., 2006; Ringø et al., 2010b). These studies showed that prebiotic substances have potential to be used as additives in fish feed. In order to develop the use of prebiotic, to be additives in Nile tilapia that can be use practically, to be development of products produced within the country for actual practice, especially for agriculture as the main activity of Thailand. There should be a study on the use of healthy natural products from JA tuber, which consists of inulin and FOS produced within the country locally as a prebiotic addictive in Nile tilapia feed, which is an important economic fish of Thailand, to gain beneficial information for further development in the agricultural industry.

2.5 References

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CHAPTER III

EFFECTS OF DIETARY INULIN AND JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AS PREBIOTIC ADDITIVES ON NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

3.1 Abstract

This study evaluated the prebiotic effects of dietary inulin from chicory root and Jerusalem artichoke tuber (JA) on Nile tilapia (*Oreochromis niloticus*) during fingerling stage. Five dietary treatment (each diet in four replicates) were designed to incorporate inulin at 0 (control), 2.5, and 5.0 g kg^{-1} and JA at 5.0 and 10.0 g kg^{-1}. During the first feeding for 4 weeks, fish larvae were reared in cages which were located in earthen pond. Since during 4 weeks from first feeding, fry were fed diet incorporated with 17α-methyltestosterone for four weeks of sex-reversal phase. Dietary inulin had no effects on growth, FCR, survival rate and intestinal villi height and microbiota. Although dietary JA had no effect on growth, FCR, survival rate and intestinal villi height, it altered intestinal microbiota. Dietary JA increased lactic acid bacteria and *Bifidobacteria* whereas it decreased *Vibrio* and yeast and fungi. After 4 weeks of first feeding, fish larvae were continued to grow to reach fingerling stage for 12 weeks. Dietary inulin at 5.0 g kg^{-1} or JA at either level had better growth performance and survival rate than those fed on the 2.5 g kg^{-1} inulin or control diet

(P<0.05). The body chemical composition including moisture, protein, lipid and ash of fish in all groups appeared to be similar (P>0.05). Dietary inulin and JA increased the red blood cell number (P<0.05), but they had no effects on hemoglobin and hematocrit (P>0.05). Among the nine blood chemicals examined, dietary inulin or JA led to increase total protein in blood (P<0.05). However, dietary neither inulin nor JA affected glucose, cholesterol, triglyceride, albumin, blood urea nitrogen, total bilirubin, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (P<0.05). Dietary inulin or JA improved total immunoglobulin content, lysozyme activity and alternative complement haemolytic 50 (ACH50) activity (P<0.05). Dietary inulin or JA increased the height of intestinal villi and goblet cell number (P<0.05). Inulin or JA supplementation modulated the population of intestinal microbiota. Supplementation with inulin at 5.0 g kg^{-1} and JA at both levels increased intestinal lactic acid bacteria and *Bifidobacteria* and decrease *Vibrio* number. Taken together, dietary inulin (only at 5.0 g kg^{-1}) and JA at both levels had positive effects on growth, health status and intestinal bacteria in Nile tilapia fingerlings production.

Key words : prebiotic, inulin, Jerusalem artichoke, *Helianthus tuberosus*, Nile tilapia, *Oreochromis niloticus*, hematology, immune

3.2 Introduction

Inulin is a common prebiotic used in feed additives fed to livestock and aquatic animals. Inulin belongs to a class of carbohydrates known as fructans and comprises fructosyl residues linked by β-2, 1-linkages (Goodwin and Mercer, 1983;

Burr et al., 2005; Yousefian and Amiri, 2009; Ringø et al., 2010b). It is a dietary soluble fiber compound that cannot be digested by enzymes in the anterior gastrointestinal tract in monogastric animals. Thus, inulin is used as a substrate for fermentation in the posterior gastrointestinal tract. Inulin selectively stimulates the colonization and multiplication of beneficial microbiota, including bifidobacteria and other lactic acid-producing bacteria (Pool-Zobel et al., 2002; Roberfroid, 2002; Flickinger et al., 2003; Gibson and Roberfroid, 1995). Most commercially available inulin for use in animal feed has been obtained from chicory and has been demonstrated to improve growth performance, hematological and immune parameters in fish as well as to modulate intestinal microbiota (Mahious et al., 2006a; Ibrahem et al., 2010; Mourino et al., 2012; Nabizadeh, 2012; Ortiz et al., 2013).

Given the beneficial effects of dietary prebiotic inulin in the production of several species of fish, alternative low-cost sources of inulin-type fructans have been required to meet the demand from the fishery industry. Jerusalem artichoke (*Helianthus tuberosus*; JA) tuber was reported to contain 160-200 g kg^{-1} inulin and 120-150 g kg^{-1} fructooligosaccharide (FOS) (Moshfegh et al., 1999). JA, a root vegetable native to central-eastern North America (Rogers et al., 1982; Kays and Nottingham, 2007), is widely grown year-round in tropical areas. In Thailand, JA can be harvested after 90-120 days, and crop yields of JA are typically 13-19 ton ha^{-1} (Jogloy et al., 2006). Given its relatively high inulin and FOS content together with its high yielding year-round cultivation, JA powder is hypothesized to be an appropriate alternative source of fructans for the animal feed industry.

Tilapia is the second most important farmed fish after carp, and its production has increased intensely to meet the growing global demand from the whitefish market

(FAO, 2014). Among the species of tilapia, Nile tilapia (*Oreochromis niloticus*) commercially dominates the farm-cultured tilapia. Generally, Nile tilapia are easy to raise year round and grow fast in tropical areas. However, mass tilapia deaths in intensively farmed systems as a result of disease occasionally occur, particularly when fish are cultured at high density. Chemotherapeutic agents, such as antibiotics, have been used to control the risk of disease in tilapia farms, although without knowing the causative agents. The overuse of antibiotics in fish farms might pose a public health threat and also adversely impact the natural environment. An alternative application to the use of chemotherapeutic substances is the use of biotherapeutics, such as prebiotics, as a dietary supplements to help support more environmentally friendly tilapia farms. To investigate the effects of the use of prebiotics as a functional feed additive throughout the culture period of tilapia, the addition of prebiotics to feed during the culture of the first feeding larval stage is required.

3.3 Objective

This study, the effects of dietary supplementation with either inulin or JA on the growth performance during this sex-reversal phase were investigated. In addition, the effects of dietary inulin and JA on growth performance, body composition, intestinal morphology, intestinal microbiota, health status, such as hematological indices, blood chemical and immune parameters of the fingerling nursing stage were evaluated and compared.

3.4 Materials and methods

3.4.1 Jerusalem artichoke

JA samples were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. Proximate analyses of JA powder were performed according to the standard methods of AOAC (1990) for dry matter, protein, total lipid, fiber and ash (Table 3.1). In addition, the content of oligofructose in JA powder was measured according to Joye and Hoebregs (2000). Oxymation and silylation of extracted sugar was carried out and analyzed using high-temperature capillary gas chromatography method.

Table 3.1 Chemical composition and oligosaccharide contents of JA tuber.

Components	**g kg^{-1} (dry matter basis)**
Dry matter	934.4
Crude protein	57.8
Crude lipid	1.7
Crude fibre	126.0
Ash	80.8
Fructans	502.0

3.4.2 Experimental design, feed formulation and diets preparation

The experimental design was completely randomized with five treatment diets, each of which was replicated four times to test the validity of the conclusions. The five treatment diets were as follows: basal diet (control); 2.5 g kg^{-1} inulin-supplemented diet (2.5 inulin); 5.0 g kg^{-1} inulin-supplemented diet (5.0 inulin);

5.0 g kg^{-1} JA-supplemented diet (5.0 JA) and 10.0 g kg^{-1} JA-supplemented diet (10.0 JA). The 2.5 inulin and 5.0 inulin diets were prepared to incorporate inulin (PREBIOFEED 88; Warcoing, Belgium) to ensure supplementation levels of 2.5 g kg^{-1} and 5.0 g kg^{-1}, respectively. The 5.0 JA and 10.0 JA diets were prepared to incorporate JA at 5.0 g kg^{-1} and 10.0 g kg^{-1}, respectively, which were equal to inulin levels of 2.5 g kg^{-1} and 5.0 g kg^{-1}, respectively.

Two basal diets (sex-reversal and fingerling stages) were used in this study. During the sex-reversal period (first 4 weeks), the basal diet was fishmeal (crude protein 560 g kg^{-1}, crude lipid 100 g kg^{-1}) which was added 17α-methyltestosterone at 60 mg kg^{-1}. The basal dietary ingredients used for the fingerling stage are detailed in Table 3.2. The proximate composition (moisture, crude protein, crude fat and ash content) of the experimental diets was determined following standard AOAC methods (AOAC, 1990). All test ingredients were obtained from animal feedstuff companies. Before formulating the feed, all feed ingredients were analyzed to determine gross composition (moisture, crude protein, crude lipid, crude fiber and ash) according to AOAC methods (1990). All experimental diets were produced using a hammer grinder, mixer and extruder (Paktongchai Pasusat, Nakhon Ratchasima, Thailand). The dry ingredients were ground using a grinder and mixed using a ribbon screw mixer (22 rpm). The floating pellet was produced using a single screw extruder at an extruding temperature of 120-160°C. All experimental diets were stored at room temperature until use.

Table 3.2 Ingredients and chemical composition (g kg^{-1}) of the basal diet for the tilapia fingerlings.

Ingredients	**g kg^{-1}**
Fish meal	300
Soybean meal	270
Rice bran	150
Corn meal	145
Cassava chips	120
Premix[a]	10
Vitamin C	5
Proximate composition (g kg^{-1}dry weight)	
Dry matter	933.0
Crude protein	343.0
Crude lipid	79.3
Ash	104.0
Crude fiber	41.8
Nitrogen-free extract[b]	364.9

[a]Vitamin and trace mineral mix provided the following (IU kg^{-1} or g kg^{-1} diet): biotin, 0.25 g; folic acid, 0.003 g; inositol, 0.25 mg; niacin, 0.0215 g; pantothenic acid, 0.03 g; vitamin A, 5000 IU; vitamin B1, 0.0025 g; vitamin B2, 0.0012 g; vitamin B6, 0.0075 g; vitamin B12, 0.00005 mg; vitamin C, 1 g; vitamin D3, 1000 IU; vitamin E, 100 IU; vitamin K, 0.008 g; copper, 0.02 g; iron, 0.2 g; selenium, 0.3 mg; zinc, 0.32 g.

[b]Nitrogen-free extract = 1000-(moisture+crude protein+crude lipid+crude fiber+ash).

3.4.3 The experimental fish and fish culture

Several generations of Nile tilapia, *O. niloticus* strain Chitralada 3, were reared at the Suranaree University of Technology Farm (SUT Farm; Nakhon Ratchasima, Thailand). For larval production, five hundred males and females (sex ratio 1:2; two fish m^{-2}) were reared in an earthen pond (0.08 ha). The broodstock were fed a commercial feed (40% crude protein, 6% crude fat) daily at 2-3% body weight. The experimental Nile tilapia used in this study were fry (the swim-up stage) that were collected from the brooders.

Twenty hapas (cages; 2×2×0.9 m^3) were maintained in an earthen pond. Five hundred fry (0.03 g) were randomly distributed in the experimental hapas. During the sex-reversal period, experimental diets were hand-fed to the fry four times daily at a level 30% (week 1), 20% (week 2) or 15% (week 3-4) of their body weight. The water temperature, pH, and dissolved oxygen in the culture area ranged from 27.0 to 28.5°C, 7.50 to 7.78, and 5.05 to 5.27 mg L^{-1}, respectively. Any dead fish were recorded and removed daily. The growth performance and feed utilization were evaluated at the end of week 4. Fifty randomly sampled fish from each group were sacrificed to determine the sex ratio. The gonads were removed and prepared using the squash method (Guerrero and Shelton, 1974) with microscopic examination. The efficacy of sex reversal was acceptable at >98% (Phelps and Popma, 2000).

Then, 100 of the experimental fish with uniformed size were transferred into new hapas (2×4×0.9 m^3) for growth to the fingerling stage. For the culture of the fingerling phase, experimental diets were hand-fed twice daily until the end of week 12. Diets were fed ad libitum. The water temperature, pH, and dissolved oxygen in the culture pond ranged from 27.5 to 29.0°C, 7.48 to 7.73, and 5.09 to 5.41 mg L^{-1},

respectively. Any dead fish were recorded and removed daily. The growth performance and feed utilization were determined at the end of week 12.

3.4.4 Fish sampling, blood collection and body composition analyses

After 12 weeks of the experiment period, fish were not fed for 18 h before sampling. Four representative fish from each hapa (diet replication) were then removed and anesthetized with 2-phenoxyethanol (0.2%). A blood sample was collected from the caudal vein using a 21-gauge needle. The collected blood samples were divided into two sets. One set was mixed with K_2EDTA (at 1.5 mg mL^{-1} blood) as an anticoagulant for hematological examination and plasma collection. Another set was left to clot by being kept on ice for 3 h, followed by 1 h at room temperature. Serum was collected by centrifuging the clotted blood at 9000×*g* for 10 min at room temperature. In addition, plasma was collected by centrifuging K_2EDTA-blood at 9000×*g* for 10 min at 4°C and was stored at -80°C for further analysis. The specimens from each replication hapa were pooled for proximate composition analysis of the whole body according to the AOAC method (1990).

3.4.5 Hematological analysis

Analysis of the hematological parameters was conducted using K_2EDTA blood. The red blood cell (RBC) number was counted in duplicate using a Neubauer hemocytometer after dilution with Grower's solution (Voigt 2000). The hemoglobin content was determined using the photometrical cyanohemoglobin method. Hematocrit values was evaluated in duplicate by placing blood into glass capillary tubes and subjecting them to microhematocrit centrifugation 15,000×*g* for 5 min.

3.4.6 Blood chemistry analysis

To determine the effects of dietary supplementation with inulin and JA on fish health and nutritional status, content of the following compounds in the blood was measured : glucose, triglyceride, cholesterol, total protein, albumin, blood urea nitrogen (BUN), total bilirubin (T-bilirubin), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Immediately after blood sampling, the K_2EDTA-treated blood was used to measure the blood glucose levels using a hand-held glucometer (AccuTrend; Roche, Mannheim, Germany). Serum triglyceride content was measured using the glycerol-3-phosphate oxidase-sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (GPO-ESPAS) method described by Bucolo and David (1973). Serum cholesterol was quantitatively analyzed using the cholesterol oxidase-phenol+aminophenazone (CHOD-PAP) technique described by Flegg (1973). Plasma protein contents were determined using the Biuret method (Gornall et al., 1949). Serum albumin content was quantitatively estimated using the bromocresol green method (Doumas et al., 1971). BUN content was measured using a modified indophenol colorimetric method (Weatherburn, 1967). T-bilirubin contents were measured using the new diazo-DMSO method (Winsten and Cehelyk, 1969). SGOT and SGPT were analyzed using Reitman and Frankel's colorimetric method (Reitman and Frankel, 1957).

3.4.7 Immune assay

Immune parameters, including total immunoglobulin, lysozyme activity and alternative complement haemolytic 50 (ACH50) activity, were measured. Total immunoglobulin was measured according to Siwicki et al. (1994). Using fish serum,

lysozyme activity was estimated as described by Pitaksong et al. (2013), and ACH50 activity was measured according to Sunyer and Tort (1995).

3.4.8 Histological analysis

At the end of the experimental period (4 and 12 weeks), four fish from each replicate of each treatment were sampled and prepared for histological analysis as described previously (Phumyu et al., 2012) to investigate the effect of dietary supplementation with inulin or JA on intestinal morphology. Portions of the anterior, middle and posterior parts of the intestine were dissected and preserved in 10% phosphate-buffered formalin (pH 7.2). After dehydration, the tissue was embedded in paraffin wax, cut into slices 5 µm thick, and mounted on glass slides. After deparaffinization, the slides were dehydrated and stained with hematoxylin and eosin. Villus height was measured on stained sections under a microscope using an ocular micrometer at 100×magnification. The five longest intact villi in each intestinal position were measured in two cross-sections from each sample. In addition, the number of goblet cells along the selected intact villi were counted.

3.4.9 Intestinal microbiota analysis

At the end of the experimental period (4 and 12 weeks), four fish from each replication of each treatment were sampled for microbiological studies. The fish were then dissected under sterile condition and the gut remove, weighed, homogenized and suspended in sterile 0.9% NaCl. The suspension, serially diluted to 10^{-4} and 0.15 ml of the solution was spread onto plate count agar (PCA) culture medium (total bacteria), de Man, Rogosa and Sharpe (MRS) agar (lactic acid bacteria), bifidobacterium agar (*Bifidobacteria*), thiosulfate citrate bile salts sucrose (TCBS) agar (*Vibrio*) and Sabouraud agar (yeast and fungi). All of the plates were

incubated at 37°C for 2 days. After incubation, the total numbers of colony-forming units (CFU) g^{-1} were calculated from statistically viable plates (i.e., plates containing 30-300 colonies) (Rawling et al., 2009).

3.4.10 Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS for Windows (Release 10) (SPSS Inc., Chicago, IL, USA). When significant differences were found among the groups, Duncan's multiple range tests were used to rank the groups. The statistical model utilized was $y_{ij} = \mu+\tau_I+\varepsilon_{ij}$, where y_{ij} was the response; μ, the general means; τ_I, the dietary inulin or JA effect; and ε_{ij}, the random error. Throughout the experiment, effects and differences were declared to be significant at $P<0.05$.

3.4.11 Experimental location and period

The experiment was conducted at Suranaree University of Technology Farm, The Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology. The experiment was from January 2013 to July 2013.

3.5 Results

3.5.1 Effects of dietary inulin and JA as prebiotic additives on Nile tilapia during the first feeding for 4 weeks (sex-reversal period)

The growth performance and survival rates of Nile tilapia fry were determined (Table 3.3). During the first 4 weeks of the sex reversal period, dietary supplementation with either inulin or JA tended to improve the final weight, average daily gain (ADG), specific growth rate (SGR) and feed conversion ratio (FCR), although there were no significant differences in these parameters among

experimental groups. The survival rate of all experimental groups appeared to be similar. Furthermore, the efficiency check of sex reversal tilapia founded that there was no female tilapia in all groups of the experiment.

The effects of dietary supplementation with inulin and JA on intestinal morphology of Nile tilapia fry are shown in Table 3.4. There were no significant differences in the villus height in the anterior, the middle and the posterior parts of the intestine ($P>0.05$). However, the villus height in all parts of the intestine in fish fed the diet supplemented with either inulin or JA had tended to higher than those of fish in the control group. In addition, fish fed the JA diets had a higher villus height than the other groups, although there were no significant differences ($P>0.05$).

The effects of dietary inulin and JA on intestinal microbiota of Nile tilapia fry was analyzed in Table 3.5. There were differences in the population of intestinal microbiota among the experimental groups. Fish fed the diet supplemented with JA at 10.0 g kg^{-1} exhibited increased intestinal total bacteria, lactic acid bacteria and *Bifidobacteria* spp. compared with the fish fed the control diet ($P<0.05$). Meanwhile, fish fed the diet supplemented with JA (5.0 g kg^{-1}) and inulin at both levels (2.5 g kg^{-1} and 5.0 g kg^{-1}) did not show significant differences on these intestinal microbiota compare with the fish fed the control diet ($P>0.05$). Furthermore, the lowest number of *Vibrio* spp. and yeast and fungi were observed in fish fed the 10.0 JA diet, and significant differences from fish fed the control diet ($P<0.05$).

Table 3.3 Growth performance of Nile tilapia fry fed experimental diets for 4 weeks[1].

Diet	Final weight (g)	ADG[2] (mg d^{-1})	SGR[3] (%)	FCR[4]	Survival rate[5] (%)
Control	2.24	75.90	15.26	1.57	97.85
2.5 Inulin	2.26	79.60	15.45	1.48	97.85
5.0 Inulin	2.28	80.30	15.49	1.46	98.15
5.0 JA	2.63	93.00	15.99	1.27	98.10
10.0 JA	2.65	93.60	16.01	1.26	98.20
P-value	0.08	0.16	0.15	0.08	0.75
Pooled SEM	0.07	4.85	0.12	0.04	0.10

[1]Data represent means from four replicates per treatment.

[2]Average daily gain (ADG) = (final mean body weight - initial mean body weight)/ experimental days.

[3]Specific growth rate (SGR) = 100 × [(ln final body weight - ln initial body weight)/ experimental days].

[4]Feed conversion ratio (FCR) = dry feed fed/wet weight gain.

[5]Survival rate = 100 × (initial number of fish/final number of fish).

Table 3.4 Intestinal villus height in different parts of the intestine of Nile tilapia fry fed experimental diets for 4 weeks[1].

Diet	Anterior	Middle	Posterior
	Villus height (µm)	Villus height (µm)	Villus height (µm)
Control	257.49	181.41	153.26
2.5 Inulin	263.73	192.21	155.75
5.0 Inulin	289.43	199.87	164.07
5.0 JA	295.05	201.58	161.60
10.0 JA	311.85	205.72	169.66
P-value	0.19	0.43	0.24
Pooled SEM	8.20	4.26	2.50

[1]Data represent means from four replicates per treatment.

Table 3.5 Intestinal microbiota of Nile tilapia fry (log CFU g^{-1}) fed experimental diets for 4 weeks[1].

Diet	Total bacteria	Lactic acid bacteria	*Bifidobacteria* spp.	*Vibrio* spp.	Yeast and fungi
Control	5.65[a]	3.96[a]	5.62[a]	5.00[b]	3.82[b]
2.5 Inulin	5.70[a]	3.98[a]	5.66[a]	4.98[b]	3.81[b]
5.0 Inulin	5.72[a]	3.99[a]	5.66[a]	4.97[b]	3.79[b]
5.0 JA	5.75[ab]	4.04[ab]	5.72[ab]	4.93[b]	3.80[b]
10.0 JA	5.84[b]	4.11[b]	5.83[b]	4.85[a]	3.67[a]
P-value	0.03	0.04	0.02	0.00	0.04
Pooled SEM	0.02	0.02	0.02	0.01	0.02

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

3.5.2 Effects of dietary inulin and JA as prebiotic additives on Nile tilapia fingerlings

For fish examined after 5-12 weeks, fingerling Nile tilapia fed 5.0 Inulin, 5.0 JA or 10.0 JA showed significantly better growth responses, including final weight, ADG and SGR ($P<0.05$). In addition, compared with a control diet, the lower FCR was observed in Nile tilapia fed 5.0 Inulin, 5.0 JA or 10.0 JA ($P<0.05$). Nile tilapia fed 5.0 Inulin, 5.0 JA or 10.0 JA had significant higher survival rates compared with the fish fed the control diet ($P<0.05$) (Table 3.6).

The proximate chemical composition of the experimental Nile tilapia is shown in Table 3.7. There were no significant differences in moisture, crude protein, crude lipid or ash content among the experimental groups.

Table 3.8 details the hematological parameters of experimental Nile tilapia. Dietary supplementation with inulin (2.5 or 5.0) and JA (5.0 or 10.0) led to significantly increased RBC numbers compared with the fish fed the control diet ($P<0.05$). No significant differences in hemoglobin content or hematocrit were found.

The blood chemical parameters, including glucose, cholesterol, triglycerides, total protein, albumin, BUN, total bilirubin, SGOT and SGPT, of the experimental Nile tilapia were analyzed after 12 weeks of feeding (Table 3.9). The results showed that there were no significant differences in glucose, cholesterol, triglycerides, albumin, BUN, total bilirubin, SGOT and SGPT among the experimental groups ($P>0.05$). However, dietary supplementation with 5.0 kg^{-1} inulin or JA at either level significantly increased plasma protein levels ($P<0.05$).

The effects of dietary inulin and JA on humoral immune parameters are shown in Table 3.10. Compared with the control group, 5.0 kg^{-1} inulin resulted in a statistically significant increase in total Ig and lysozyme activity ($P<0.05$). In addition, JA inclusion diets (5.0 JA and 10.0 JA) significantly increased total Ig and lysozyme activity ($P<0.05$). Either inulin or JA inclusion diet resulted in a statistically significant increase in ACH50 activity, with dietary JA having the highest ACH50 among the experimental groups ($P<0.05$).

The effects of dietary supplementation with inulin and JA on intestinal morphology of Nile tilapia fingerlings are shown in Table 3.11. Dietary supplementation with JA at 10.0 g kg^{-1} had higher villus height in the anterior part of the intestine compared to that of fish fed the control diet ($P<0.05$). In the middle part of the intestine, fish fed the diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had higher villus height compared to that of fish fed the control diet ($P<0.05$).

However, there were no significant differences in the villus height in the posterior intestine of fish among treatment diets. In addition, in the anterior and the middle parts of the intestine, fish fed diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had higher goblet cell number compared with the fish fed the control diet ($P<0.05$). There were no significant differences in the number of goblet cells in the posterior intestine of fish among treatment diets ($P>0.05$).

The effects of dietary inulin and JA on intestinal microbiota of Nile tilapia fingerlings was analyzed in Table 3.12. Fish fed the diet supplemented with JA at both levels (5.0 JA and 10.0 JA) exhibited higher intestinal total bacteria compared with the fish fed the control diet ($P<0.05$). Dietary supplementation with inulin at 5.0 g kg^{-1} and JA at both levels resulted in a statistically significant increase in intestinal lactic acid bacteria compared with the fish fed the control diet ($P<0.05$). Fish fed the diet supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had significantly increased *Bifidobacteria* spp. compared with the fish fed the control diet ($P<0.05$), and the highest number of *Bifidobacteria* spp. was observed in fish fed the 10.0 JA diet. Furthermore, supplementation with JA at both levels resulted in a statistically significant decrease in *Vibrio* spp. ($P<0.05$), and the lowest number of *Vibrio* spp. was observed in fish fed the 10.0 JA diet.

Table 3.6 Growth performance of Nile tilapia fingerlings fed experimental diets for 12 weeks[1].

Diet	Final weight (g)	ADG[2] (mg d^{-1})	SGR[3] (%)	FCR[4]	Survival rate[5] (%)
Control	47.15[a]	575.00[a]	8.99[a]	1.02[b]	97.60[a]
2.5 Inulin	47.27[a]	575.00[a]	8.99[a]	1.01[b]	97.80[ab]
5.0 Inulin	50.89[b]	620.00[b]	9.08[b]	0.94[a]	98.10[b]
5.0 JA	51.29[b]	625.00[b]	9.09[b]	0.93[a]	98.10[b]
10.0 JA	53.60[b]	655.00[b]	9.14[b]	0.89[a]	98.20[b]
P-value	0.00	0.01	0.00	0.00	0.03
Pooled SEM	0.69	5.58	0.02	0.01	0.07

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]Average daily gain (ADG) = (final mean body weight - initial mean body weight)/ experimental days.

[3]Specific growth rate (SGR) = 100 × [(ln final body weight - ln initial body weight)/ experimental days].

[4]Feed conversion ratio (FCR) = dry feed fed/wet weight gain.

[5]Survival rate = 100 × (initial number of fish/final number of fish).

Table 3.7 Whole body composition of Nile tilapia fingerlings (g kg^{-1}) fed experimental diet for 12 weeks[1].

Diet	Moisture (g kg^{-1})	Crude Protein (g kg^{-1})	Crude lipid (g kg^{-1})	Ash (g kg^{-1})
Control	726.10	119.20	25.20	38.40
2.5 Inulin	727.40	121.00	25.30	39.00
5.0 Inulin	739.80	123.20	26.70	39.10
5.0 JA	732.20	121.40	26.10	39.10
10.0 JA	739.90	124.30	26.30	41.00
P-value	0.07	0.69	0.57	0.82
Pooled SEM	2.09	1.12	0.31	0.70

[1]Data represent means from four replicates per treatment.

Table 3.8 Hematological parameters of Nile tilapia fingerlings fed experimental diet for 12 weeks[1].

Diet	RBC[2] (cell x 10^{12} L^{-1})	Hemoglobin (g L^{-1})	Hematocrit (L L^{-1})
Control	2.13^{a}	71.40	0.25
2.5 Inulin	2.21^{b}	73.90	0.25
5.0 Inulin	2.23^{b}	74.70	0.25
5.0 JA	2.24^{b}	76.00	0.26
10.0 JA	2.26^{b}	77.40	0.26
P-value	0.01	0.12	0.09
Pooled SEM	0.02	0.76	0.27

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]RBC = red blood cell count.

Table 3.9 Blood chemical parameters of Nile tilapia fingerlings fed experimental diet for 12 weeks[1].

Blood chemical	**Diet**						
Parameter	**Control**	**2.5 Inulin**	**5.0 Inulin**	**5.0 JA**	**10.0 JA**	**P-value**	**Pooled SEM**
Glucose (mmol L^{-1})	2.97	2.69	3.45	3.10	3.32	0.11	0.10
Cholesterol (mmol L^{-1})	3.79	3.89	4.26	3.91	4.05	0.44	0.08
Triglycerides (mmol L^{-1})	1.62	1.61	1.79	1.75	1.80	0.48	0.04
Total protein (g L^{-1})	34.90^{a}	37.60^{ab}	40.00^{b}	39.80^{b}	40.70^{b}	0.02	0.69
Albumin (g L^{-1})	17.70	19.20	19.70	19.50	21.90	0.29	0.60
BUN[2] (mmol L^{-1})	0.84	0.81	0.79	0.73	0.75	0.08	0.04
Total bilirubin (µmol L^{-1})	2.91	2.57	2.22	2.39	1.88	0.77	0.17
SGOT[3] (U L^{-1})	35.29	35.12	30.49	27.70	29.68	0.08	1.09
SGPT[4] (U L^{-1})	21.37	21.03	19.26	17.59	18.71	0.06	0.49

[1]Means with different superscripts in each row differ significantly from each other ($P<0.05$).

[2]BUN = blood urea nitrogen; [3]SGOT = serum glutamic oxaloacetic transaminase; [4]SGPT = serum glutamic pyruvic transaminase.

Table 3.10 Immunological parameters of Nile tilapia fingerlings fed experimental diet for 12 weeks[1].

Immunological parameter	Diet						
	Control	2.5 Inulin	5.0 Inulin	5.0 JA	10.0 JA	P-value	Pooled SEM
Total Ig[2] (g L^{-1})	30.10^{a}	31.10^{ab}	34.70^{bc}	34.60^{bc}	36.10^{c}	0.02	0.74
Lysozyme activity (µg mL^{-1})	8.37^{a}	8.91^{ab}	9.79^{b}	9.98^{b}	10.12^{b}	0.04	0.23
ACH50[3] (units mL^{-1})	288.40^{a}	317.70^{b}	372.70^{c}	375.00^{c}	386.60^{c}	0.00	9.48

[1]Means with different superscripts in each row differ significantly from each other ($P<0.05$).

[2]Total Ig = total immunoglobulin.

[3]ACH50 = alternative complement haemolytic 50 activity.

Table 3.11 Intestinal villus height and number of goblet cells in different parts of the intestine of Nile tilapia fingerlings fed experimental diets for 12 weeks[1].

Diet	Anterior		Middle		Posterior	
	Villus height (µm)	No. of goblet cells	Villus height (µm)	No. of goblet cells	Villus height (µm)	No. of goblet cells
Control	355.36[a]	25.67[a]	282.33[a]	23.50[a]	180.01	18.50
2.5 Inulin	378.80[a]	26.75[a]	315.63[ab]	26.00[a]	185.69	18.67
5.0 Inulin	402.04[ab]	33.92[b]	345.63[b]	31.50[b]	210.37	20.75
5.0 JA	405.40[ab]	34.00[b]	349.19[b]	31.75[b]	208.43	21.17
10.0 JA	446.83[b]	35.50[b]	360.53[b]	31.83[b]	213.46	21.50
P-value	0.02	0.00	0.04	0.01	0.06	0.17
Pooled SEM	9.72	1.16	9.68	1.04	4.78	0.51

[1]Means with different superscripts in each column differ significantly from each other (P<0.05).

Table 3.12 Intestinal microbiota of Nile tilapia fingerlings (log CFU g^{-1}) fed experimental diets for 12 weeks[1].

Diet	Total bacteria	Lactic acid bacteria	*Bifidobacteria* spp.	*Vibrio* spp.	Yeast and fungi
Control	5.86^{a}	3.97^{a}	5.83^{a}	5.02^{c}	3.79^{b}
2.5 Inulin	5.87^{a}	4.00^{ab}	5.85^{a}	4.98^{bc}	3.76^{b}
5.0 Inulin	5.95^{ab}	4.07^{bc}	5.95^{b}	4.96^{bc}	3.74^{b}
5.0 JA	5.96^{b}	4.08^{bc}	5.97^{b}	4.92^{b}	3.75^{b}
10.0 JA	6.02^{b}	4.12^{c}	6.00^{b}	4.82^{a}	3.63^{a}
P-value	0.01	0.01	0.00	0.00	0.01
Pooled SEM	0.12	0.02	0.02	0.02	0.01

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

3.6 Discussion

The results from the current study showed that supplementation with inulin at 5.0 g kg^{-1} led to significantly improvement in growth performance in terms of body length, body weight, ADG, SGR and FCR in sex-reversed Nile tilapia during the fingerling stage. Similarly, supplementing the diet with inulin at 5.0 g kg^{-1} improved weight gain and SGR of both sexes of Nile tilapia (Ibrahem et al., 2010). In addition, supplementation with inulin (at 5.0 and 10.0 g kg^{-1}) was reported to improve weight gain and FCR in rainbow trout (*Oncorhynchus mykiss*) (Ortiz et al., 2013). Supplementation of dietary FOS was also demonstrated to improve growth performance in blunt snout bream (*Megalobrama amblycephala*) and Caspian roach (*Rutilus rutilus*) (Soleimani et al., 2012; Wu et al., 2013). However, neither dietary

inulin nor FOS had a significant effect on growth performance in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) or common carp (*Cyprinus carpio*) (Burr et al., 2010; Hoseinifar et al., 2014). Supplementation with FOS did not significantly increase the growth response in Atlantic salmon (*Salmo salar*), although the feed efficiency ratio was improved (Grisdale-Helland et al., 2008). Moreover, supplementation of inulin at 10.0-30.0 g kg^{-1} was observed to have negative effect on growth response in beluga (*Huso huso*), although it did not significantly affect FCR (Reza et al., 2009). Taken together, the effect of supplementation with functional prebiotics on the growth performance varies among fish species, suggesting that the appropriate utilization of functional prebiotics as the feed additives should be investigated for each fish species individually.

JA contains approximately 50% of the same amount of oligosaccharide as inulin; therefore, it was supplemented at twice the level of experimental inulin to examine the effects of a comparable functional amount of prebiotic. Although similar positive effects were expected between JA and inulin, supplementation with JA (at the same amount of functional prebiotic content) appeared to be superior to that of inulin. These findings were consistent with a previous study on the comparative effects of inulin and JA in Nile tilapia during the juvenile stage (Table 4.1-4.2). JA contains FOS (at a high proportion of 43-52%) and inulin (Moshfegh et al., 1999; for review, see Kays and Notthingham, 2007); therefore, the superior effect of JA might be partly the result of its FOS content. The better improvement in growth in response to FOS compared with dietary inulin was also observed in turbot (*Psetta maxima*) (Mahious et al., 2006b; Soleimani et al., 2012).

By the end of the experimental period, dietary supplementation with inulin at a high level or with either level of JA significantly increased the survival rate of fingerling Nile tilapia. Similar effects of supplementation with either inulin or FOS on the survival rate have previously been demonstrated in Nile tilapia (Ibrahem et al., 2010), blunt snot bream (Wu et al., 2013) and common carp (Hosenifar et al., 2014). Nevertheless, there were no significant increases in survival rate of turbot, beluga and rainbow trout following dietary supplementation with either inulin or FOS (Mahious et al., 2006b; Reza et al., 2009; Ortiz et al., 2013). Although supplementation with dietary FOS did not significantly increase the survival rate of Caspian roach, it did significantly enhance their survival rate when the fish were exposed to salinity stress (Soleimani et al., 2012). Therefore, dietary supplementation with prebiotic inulin or FOS tends to exert positive effects on the survival rate in fish. The commercial sex-reversed tilapia production, the efficacy of sex reversal is very important in tilapia farming. All male tilapia is more profitable for an intensive culture system, the acceptable efficacy of sex reversal should be higher than 98% (Phelps and Popma, 2000). The results showed that the sex reversal rate of all experimental groups were in the acceptable rate.

The present study demonstrated that dietary supplementation with either inulin and JA did not significantly affect on intestinal villus height of Nile tilapia during the first feeding period. However, fish fed the diets supplemented with inulin at 5.0 g kg^{-1} for growth to the Nile tilapia fingerling stage had higher villus height in the middle part of the intestine compare with the fish fed the control diet, and led to an increased the number of goblet cells in the anterior and the middle parts of the intestine. In addition, dietary supplementation with JA at both levels also resulted in an increased

the villus height and goblet cell numbers in the anterior and the middle parts of the intestine. The result of the study of intestinal morphology is accordance to the growth performance of fish that was fed the diets supplemented with inulin and JA, to increase in growth response, especially in the Nile tilapia fingerling stage. Caspary (1992) reported that increased intestinal villi leads to increased surface area for nutrient absorption, thereby improving growth performance and feed utilization in animals. Several researchers proposed that fermentation of inulin produces several substances that stimulate intestinal cell proliferation, which in turn results in increased villus height (Blottiere et al., 2003; Rehman et al., 2007; Nabizadeh, 2012). However, the effect of dietary inulin on carnivorous fish appears to be different. For example, Olsen et al. (2001) reported that a high level of dietary inulin (150 g kg^{-1} dietary inclusion) had negative effects on the ultrastructure of the gastrointestinal tract of Arctic char (*Salvelinus alpinus*). In addition, decreased microvillus height was observed in gilthead sea bream fed a diet that included 10.0 g kg^{-1} inulin (Cerezuela et al., 2013).

The intestinal microbiota was reported to colonize in the gastrointestinal tract of fish larvae immediately after hatching stage (Olafsen, 2001; Denev at al., 2009). The results demonstrated that the numbers of beneficial bacteria increased, and the numbers of potential pathogenic species decrease in the intestine of fry, which is beneficial to the increasing growth rate and immune system. The modulation of intestinal bacteria might be affected by dietary supplemented with inulin or JA. In fact, lactic acid bacteria and *Bifidobacteria* spp. were well known that they could fermented inulin and FOS (Kaplan and Hutkins, 2000; Buddington et al., 2002; Roller et al., 2004). In addition, most of these bacteria would be categorized as beneficial

bacteria for ecosystem of animal intestines by producing bacteriocins, lactic acid and anti-growth substance of other bacteria, which could inhibit the growth of pathogenic intestinal bacteria (Ringø and Gatesoupe, 1998; Ringø et al., 2010a). This study found that supplementation with JA at 5.0 g kg^{-1} significantly increased intestinal lactic acid bacteria and *Bifidobacteria* spp., and decreased the population of *Vibrio* spp. in Nile tilapia during the first feeding period. Dietary supplementation with JA at both levels had significantly increased intestinal lactic acid bacteria and *Bifidobacteria* spp., and decreased the population of *Vibrio* spp. in Nile tilapia fingerlings. Particularly dietary inulin (only at 5.0 g kg^{-1}) significantly increased intestinal lactic acid bacteria and *Bifidobacteria* spp., and decreased *Vibrio* spp. number in Nile tilapia fingerlings, which was consistent with the results of previous studies on various fishes. Reza et al. (2009) reported that dietary supplementation with inulin (10.0 g kg^{-1}) for 8 weeks resulted in an increase in population of lactic acid bacteria in beluga compared with fish fed the control diet. Mourino et al. (2012) showed that dietary supplementation with 5.0 g kg^{-1} inulin for 15 days had higher concentrations of lactic acid bacteria in hybrid surubim (*Pseudoplatystoma* sp.). In addition, dietary supplementation with inulin (5.0-10.0 g kg^{-1}) for 49 days had reduced drastically the number of *Vibrio* spp. in the distal part of the intestine of rainbow trout (Ortiz et al., 2013).

To determine whether improvement of in growth response by supplementation with either inulin or JA resulted from differences in body composition, including moisture, crude lipid, crude protein and ash, the proximate analysis of the whole body of the experimental fish was conducted. The results showed no significant differences in proximate whole-body composition, which were similar to the results of beluga, hybrid striped bass and rainbow trout (Reza et al., 2009; Burr et al., 2010; Ortiz et al.,

2013). However, supplementation with FOS led to an increase and decrease in crude lipid and moisture content, respectively, in the body of blunt snout bream (Wu et al., 2013).

The results of inulin on hematological indices may vary among fish species, might result from the level of inulin supplementation, and duration of feeding. For example, dietary inulin did not modulate RBC numbers in hybrid surubim that were fed an inulin-incorporated diet (5.0 g kg^{-1}) for 15 days or in beluga fed an inulin-supplemented diet (10.0-20.0 g kg^{-1}) for 8 weeks (Mourino et al., 2012; Reza et al., 2009). However, in this study demonstrated that dietary supplementation with either inulin or JA had significantly higher RBC numbers compared with the fish fed the control diet. While there were no significant differences in Hb and Ht among treatment diets. Similarly, Ibrahem et al. (2010) and Mourino et al. (2012) reported that the Ht of Nile tilapia and hybrid surubim were not affected by dietary inulin.

Blood chemical parameters can be used to interpret the blood metabolic response and nutritional status resulting from the use of dietary feed additives. The present results demonstrated that dietary inulin or JA did not effect any significant changes in several blood metabolites, including glucose, cholesterol, triglyceride, albumin, BUN, total bilirubin, SGOT or SGPT, in Nile tilapia fingerlings. A previous study in juvenile tilapia revealed that dietary inulin or JA had no effect on cholesterol, triglyceride, BUN, bilirubin, SGOT or SGPT; however, both increased glucose and albumin (Table 4.7-4.8). The differences between the results of these two studies terms of the effect of supplementation with inulin or JA on glucose and albumin could be because of different growth stages of Nile tilapia that of were used. Dietary inulin has been reported to have no effects on several blood chemicals in other fish. For

example, dietary inulin did not alter blood glucose, albumin, chloride, triglyceride, aspartate aminotransferase or alanine aminotransferase in juvenile beluga (Reza et al., 2009). In addition, dietary inulin provided for 15 days did not have any significant changes in blood glucose in hybrid surubim (*Pseudoplatystoma* sp.; Mourino et al., 2012). It was revealed that dietary inulin led to a decrease in cholesterol and triglyceride in mammals (Flickinger et al., 2003). The current results showed that both dietary inulin and JA led to an increase in blood protein, which is consistent with results from juvenile Nile tilapia (Table 4.7-4.8). Dietary inulin had no significant modulating effect on blood protein in hybrid surubim (Mourino et al., 2012). In the piscivorous beluga, dietary inulin had negative effects on growth response and resulted in a decrease in total protein in blood. Combined together, these results suggest that the variable effects of dietary functional prebiotics on the modulation of blood chemicals depend on the species, feeding habit and life stage. More studies are required to determine whether and how dietary functional prebiotics modulate blood metabolites relating to feeding habit and life stage and to provide relevant information for the use of prebiotics in aquaculture systems.

Dietary inulin and/or FOS was revealed to exert positive effects via direct and/or indirect mechanisms on the immune system of the host. Intake of inulin and/or FOS interacted with gut carbohydrate receptors, directly modulating the immune function of the host (reviewed in Bricknell and Dalmo, 2005; Seifert and Watzl, 2007). The current study demonstrated that supplementation of dietary inulin or JA for Nile tilapia from the first larval feeding to fingerling phases enhanced several immune parameters, including lysozyme, ACH50 and total Ig levels. Similarly, the positive effects of dietary prebiotics on these immune parameters were reported in

juvenile Nile tilapia fed the same prebiotics for 8 weeks (Table 4.9). While a short-term (1 month) intake of inulin did not significantly increase lysozyme activity in Nile tilapia, long-term (2 months) intake of diets supplemented with inulin led to the significant increase in lysozyme activity (Ibrahem et al., 2010). Supplementation with FOS for 8 weeks significantly increased lysozyme activity in red drum (*Sciaenops ocellatus*) (Zhou et al., 2010). Similarly, in Caspian roach (*Rutilus rutilus*) fry, supplementation with FOS for 7 weeks increased the total Ig level, lysozyme and ACH50 activities (Soleimani et al., 2012). However, dietary supplementation with inulin for 15 days had no significant effects on lysozyme activity and total Ig in hybrid surubim (*Pseudoplatystoma* sp.) (Mourino et al., 2012). Thus, dietary inulin, FOS, and/or JA could have positive effects on the immune system, and the resulting immunostimulatory effects could depend on the length of supplementation.

3.7 Conclusion

The current study demonstrated the beneficially prebiotic effects of inulin and JA on the growth performance and health status of Nile tilapia fingerlings. Although during the early first feeding stage, neither dietary inulin nor JA significantly improved growth performance and survival rate, these prebiotic additives did significantly improve the growth performance and survival rate of the fingerling stage. In addition, dietary supplementation with inulin at 5.0 g kg^{-1} or JA powder at 5.0 g kg^{-1} and 10.0 g kg^{-1} led to an increased blood protein, RBC number, the height of intestinal villi and goblet cell number, the immune parameters and modulated the population of the intestinal microbiota.

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CHAPTER IV

EFFECTS OF INULIN AND JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) AS PREBIOTIC INGREDIENTS IN THE DIET OF JUVENILE NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

4.1 Abstract

This study evaluated the prebiotic effects of dietary inulin and Jerusalem artichoke tuber (JA) on juvenile Nile tilapia (*Oreochromis niloticus*). Five dietary treatment (each diet in four replicates) were designed to incorporate inulin at 0 (control), 2.5, and 5.0 g kg^{-1} and JA at 5.0 and 10.0 g kg^{-1}. Fish were reared in concrete ponds for 16 weeks. Fish fed the inulin diets exhibited better growth performances than fish fed the control diet, and fish fed the JA diets had the best growth performances among all diets tested. There were not significant differences in survival rates among experimental diets (P>0.05). The body chemical composition including moisture, protein, lipid and ash of fish in all groups appeared to be similar (P>0.05). Dietary inulin and JA increased red blood cell number, and dietary JA at 10.0 g kg^{-1} for 16 weeks increased hemoglobin and hematocrit (P>0.05). Among the fourteen blood chemicals examined, dietary inulin or JA led to increase glucose, albumin, total protein, magnesium, calcium and iron content (p<0.05). However, dietary inulin or JA did not affect cholesterol, triglyceride, blood urea nitrogen, bilirubin, SGOT, SGPT

and chloride (P<0.05). Dietary inulin at 5.0 g kg^{-1} or JA at either level increased total immunoglobulin content, lysozyme activity and ACH50 activity (P<0.05). Dietary inulin or JA increased the height of intestinal villi and goblet cell number (P<0.05). Inulin or JA supplementation affected the population of intestinal microbiota. Supplementation of either inulin or JA led to increase intestinal lactic acid bacteria and *Bifidobacteria* and decrease *Vibrio* number. These findings indicate that inulin at 5.0 g kg^{-1} or direct supplementation with JA at 5.0-10.0 g kg^{-1} had positive effects on growth and health of Nile tilapia. Thus, both inulin and JA have great potential for use as prebiotics in fish feed.

Key words : prebiotic, inulin, Jerusalem artichoke, Nile tilapia, immune, intestinal morphology, intestinal microbiota

4.2 Introduction

Tilapia production has increased intensely to meet the growing global demand for fishery products (FAO, 2013). In particular, production of Nile tilapia (*Oreochromis niloticus*) has commercially dominated the farm-raised tilapia industry. Although Nile tilapia are easy to culture and fast growing in tropical areas, mass death in tilapia farms due to outbreaks of disease occasionally occurs, particularly when the water temperature is high during summer. Chemotherapeutic agents such as antibiotics have been used to control the risk of disease in tilapia farms. However, the overuse of antibiotics in fish farms may pose a threat to public health and also adversely impact the ecosystem. Application of biotherapeutics such as prebiotics as an alternative to chemotherapy may prove to be an environmentally friendly tool for use in fish farming.

Prebiotics are defined as non-digestible food ingredients that beneficially affect host health by selectively stimulating the growth and/or activity of healthful bacteria and by combating undesired bacteria in the intestinal tract (Gibson and Roberfroid, 1995). Inulin, which belongs to a class of carbohydrates known as fructans, is one of the most common prebiotics used in feed for livestock and aquatic animals. Inulin is composed of fructosyl residues, which are linked by β-2, 1-linkages (Goodwin and Mercer, 1983; Burr et al., 2005; Yousefian and Amiri, 2009; Ringø et al., 2010b). In humans and monogastric animals, fructans generally cannot be hydrolysed by the digestive enzymes in the proximal intestinal tract (Pool-Zobel et al., 2002). Instead, they are fermented in the large intestine or colon by beneficial bifidobacteria and other lactic acid producing bacteria, thereby enhancing their relative populations (Pool-Zobel et al., 2002; Roberfroid, 2002; Flickinger et al., 2003). Several dietary grades of inulin are available commercially, and their use as a dietary supplement in animal feed has been shown to enhance growth performance, modulate intestinal microbiota and improve hematological and immune parameters in fish, poultry and swine (He et al., 2002; Mahious et al., 2006a; Reza et al., 2009; Ibrahem et al., 2010; Mourino et al., 2012; Nabizadeh, 2012; Ortiz et al., 2013). Nevertheless, the use of inulin as a functional feed additive in the animal feed industry is limited by the cost of the inulin extraction process. Therefore, finding eco-friendly sources of fructan-type functional feed ingredients would contribute greatly to aquaculture productivity.

Jerusalem artichoke (*Helianthus tuberosus*; JA), which is a root vegetable native to central-eastern North America (Rogers et al., 1982; Kays and Nottingham, 2007), is widely grown year-round in tropical areas. In Thailand, JA can be harvested

after 90-120 days, and crop yields of JA are typically 13-19 ton ha^{-1}. The JA tuber contains 160-200 g kg^{-1} inulin and 120-150 g kg^{-1} fructooligosaccharide (FOS) (Moshfegh et al., 1999); therefore, it would be a good source of oligofructose-enriched inulin. Although Nile tilapia production and JA cultivation co-occur in the tropical zone, studies of the potential benefit of the direct use of JA as a prebiotic functional ingredient in aquafeed are limited.

4.3 Objective

In this study, the prebiotic effects of dietary inulin and JA on juvenile Nile tilapia were evaluated and compared. Prebiotic effects on growth performance, body composition, intestinal morphology and intestinal microbiota were measured. In addition, hematological, blood chemistry and immune parameters were examined to better interpret the effects of prebiotic supplementation on the health status of the fish.

4.4 Materials and methods

4.4.1 Experimental design, feed formulation and diets preparation

The experimental design was completely randomized with five treatment diets, each of which was replicated four times. The five treatment diets were as follows: basal diet (control, C), 2.5 g kg^{-1} inulin-supplemented diet (2.5 inulin), 5.0 g kg^{-1} inulin-supplemented diet (5.0 inulin), 5.0 g kg^{-1} JA-supplemented diet (5.0 JA) and 10.0 g kg^{-1} JA-supplemented diet (10.0 JA). The 2.5 inulin and 5.0 inulin diets were prepared to incorporate inulin (PREBIOFEED 88; Warcoing, Belgium) to ensure supplementation levels of 2.5 g kg^{-1} and 5.0 g kg^{-1}, respectively. JA tubers were bought from the Phetchabun Research Station, Agro-Ecological System

Research and Development Institute, Kasetsart University, Phetchabun, Thailand. JA contained the proximate chemical and oligofructose compounds as same as that of chapter III (Table 3.1). The 5.0 JA and 10.0 JA diets were prepared to incorporate JA at 5.0 g kg^{-1} and 10.0 g kg^{-1}, respectively, which were equal to inulin levels of 2.5 g kg^{-1} and 5.0 g kg^{-1}, respectively. The basal dietary ingredients and the proximate composition (moisture, crude protein, crude fat and ash content) of the experimental diets were determined in the same way as described in chapter III (Table 3.2).

4.4.2 Experimental fish and fish culture

The Nile tilapia used in this study were reared at the Suranaree University of Technology Farm (SUT Farm; Nakhon Ratchasima, Thailand). The experimental Nile tilapia were all male fish that were produced by feeding the swim-up fry with a 50 mg kg^{-1} 17α-methyltestosterone-supplemented diet for 4 weeks and then with a diet consisting of 350 g kg^{-1} crude protein until the experiment started.

Twenty cement ponds (2×2×1 m^3) (i.e., four replicates of five treatments) were used for the experiment. They were randomly assigned to each treatment diet, and 30 fish (42-47 g) were randomly distributed into each cement pond containing water (depth, 0.7 m) under continuous aeration and with continuous water flow (5 L min^{-1}). In addition, a flow-through water change system was implemented by replacing one-third of the water in each pond with dechlorinated water every week. To acclimatize the Nile tilapia to the experimental conditions, the fish were fed the basal diet for 2 weeks. Throughout the experimental period, the fish were hand-fed ad libitum twice daily, and daily feed consumption by replicate was recorded to determine feed utilization. At the end of week 8 and 16, four fish from each pond

(replication) were sampled and weighed to assess growth performance. Air and water temperatures were measured daily and were 25-33°C and 24.5-28.4°C, respectively. Dissolved oxygen (DO) content and pH were measured weekly using a DO meter and pH meter, and values were within acceptable ranges of 5.24-5.99 mg L^{-1} and 7.45-8.16, respectively. Dead fish were removed daily and mortality was recorded.

4.4.3 Fish sampling and blood collection

At the end of the experimental period (8 and 16 weeks), fish were not fed for 18 h before being sampled. Four fish from each diet replicate were removed from the tank and anesthetized with 2-phenoxyethanol (0.2%). Blood samples were collected from the caudal vein using a hypodermic syringe. The collected blood samples were divided into two sets. One set was mixed with K_2EDTA (at 1.5 mg mL^{-1} blood) as an anticoagulant for hematological examination and plasma collection. The other set was left to clot at 4°C for at least 3 h. Plasma was collected by centrifugation of the K_2EDTA-treated blood at 9000×*g* for 10 min at 4°C and stored at -80°C for further analysis. The serum was collected by centrifuging the clotted blood at 9000×*g* for 10 min at room temperature.

4.4.4 Hematological analysis

Immediately after blood sampling, the K_2EDTA-treated blood was used to examine hematological parameters. The red blood cell count (RBC) was analyzed in duplicate for each sample using a Neubauer haemocytometer after dilution with Grower's solution (Voigt, 2000). Hematocrit values (Ht) were measured in duplicate by placing K_2EDTA-treated blood into glass capillary tubes and centrifuging them for 5 min by microhematocrit centrifugation. The hemoglobin (Hb) content was measured using the photometrical cyanohaemoglobin method.

4.4.5 Blood chemistry analysis

To determine the effects of dietary supplementation with inulin and JA on fish health and nutritional status, the amount of the following compounds in the blood was measured: triglyceride, cholesterol, total protein, albumin, blood urea nitrogen (BUN), total bilirubin (T-bilirubin), direct bilirubin (D-bilirubin), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) according to the same way as described in chapter III. In addition, immediately after blood sampling, the K_2EDTA-treated blood was used to measure the blood glucose levels using a hand-held glucometer (AccuTrend; Roche, Mannheim, Germany). The calcium content in the serum was estimated using the o-cresolphthalein direct method (Moorehead and Biggs, 1974). Blood chloride content was measured using the thyocyanate method (Hamilton, 1966). Serum magnesium was estimated by the colorimetric method (Smith, 1955) and iron ferene content was measured using iron quantitative determination in the serum (IDS, Liege, Belgium).

4.4.6 Immune assay

Immune parameters, including total immunoglobulin, lysozyme activity and alternative complement haemolytic 50 (ACH50) activity were determined in the same way as described in chapter III.

4.4.7 Histological analysis

At the end of the experimental period (8 and 16 weeks), four fish from each replicate of each treatment were sampled and prepared for histological analysis in the same way as described in chapter III.

4.4.8 Intestinal microbiota analysis

At the end of the experimental period (16 weeks), four fish from each replication of each treatment were sampled for microbiological studies in the same way as described in chapter III.

4.4.9 Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS for Windows (Release 10) (SPSS Inc., Chicago, IL, USA). When significant differences were found among the groups, Duncan's multiple range tests were used to rank the groups. The statistical model utilized was $y_{ij} = \mu+\tau_I+\varepsilon_{ij}$, where y_{ij} was the response; μ, the general means; τ_I, the dietary inulin or JA effect; and ε_{ij}, the random error. Throughout the experiment, effects and differences were declared to be significant at $P<0.05$.

4.4.10 Experimental location and period

The experiment was conducted at Suranaree University of Technology Farm, The Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology. The experiment was from June 2012 to December 2012.

4.5 Results

Table 4.1-4.2 shows the growth performances and survival rates of Nile tilapia fed the experimental diets. During 8 weeks of experiment (Table 4.1) showed that fish fed the diets supplemented with inulin had better growth responses and feed utilization efficiency, including final body weight, weight gain (WG), specific growth rate (SGR), feed intake (FI) and feed conversion ratio (FCR), compared with fish fed the control diet ($P<0.05$). The increase in growth responses was inulin supplementation

level-dependent. Furthermore, among the experimental diets, fish fed diets supplemented with JA exhibited the best growth performances ($P<0.05$). An increase in JA supplementation level led to improved growth performances, although there were no significant differences ($P>0.05$). In addition, the survival rate of fish in all groups did not differ significantly ($P>0.05$) (Table 4.1).

During 16 weeks of experiment (Table 4.2) found that the study is consistent with the result during 8 weeks that fish fed the diets supplemented with inulin had better growth responses and feed utilization efficiency, including final body weight, WG, SGR, FI and FCR, compared with fish fed the control diet ($P<0.05$). The increase in growth responses was inulin supplementation level-dependent. Furthermore, among the experimental diets, fish fed diets supplemented with JA exhibited the best growth performances compared with that of fish fed the control diet and 2.5 inulin diet ($P<0.05$). However, dietary supplementation with JA at both levels did not significant differences on growth performances compared with fish fed the 5.0 inulin diet ($P<0.05$). Throughout the experimental period, survival rate of fish in all groups did not differ significantly ($P>0.05$) (Table 4.2).

To study dietary inulin or JA effect on the nutrient deposition in experimental fish, the whole-body proximate composition of the fish was analyzed in Table 4.3-4.4. During 8 weeks of experiment (Table 4.3) showed that there were no significant differences in the moisture, crude protein, crude lipid and ash contents among experimental treatments ($P>0.05$). It was also found that during 16 weeks of experiment (Table 4.4), the experiment was accordance to the 8 weeks experiment. There were no significant differences in the moisture, crude protein, crude lipid and ash contents among treatment diets ($P>0.05$).

The hematological indices of Nile tilapia fed the experimental diets are shown in Table 4.5-4.6. During 8 weeks of experiment (Table 4.5) showed that fish fed the diet supplemented with either inulin or JA had significantly higher RBC compared with that of fish fed the control diet ($P<0.05$). While there were no significant differences in Hb and Ht among treatment diets ($P>0.05$). During 16 weeks of experiment (Table 4.6) showed that fish fed the diet supplemented with either inulin or JA had significantly higher RBC compared with that of fish fed the control diet ($P<0.05$). Fish fed the diet supplemented with either inulin or JA had higher Hb and Ht compared with that of fish fed the control diet, although a significant increase in Hb and Ht were observed only in fish fed the 10.0 JA diet ($P<0.05$).

Table 4.1 Growth performance of Nile tilapia juveniles fed experimental diets for 8 weeks[1].

Diet	Initial weight (g)	Final weight (g)	WG[2] (%)	SGR[3] (%)	FI (g day^{-1})	FCR[4]	Survival rate[5] (%)
Control	44.56	233.43^{a}	423.79^{a}	2.76^{a}	4.33^{c}	1.49^{d}	99.17
2.5 Inulin	44.44	257.91^{b}	480.53^{b}	2.93^{b}	4.35^{c}	1.33^{c}	100.00
5.0 Inulin	44.25	280.29^{c}	533.55^{c}	3.07^{c}	4.18^{b}	1.16^{b}	99.17
5.0 JA	44.33	308.03^{d}	594.99^{cd}	3.23^{d}	3.93^{a}	0.97^{a}	99.17
10.0 JA	44.33	325.50^{d}	634.32^{d}	3.32^{d}	4.00^{a}	0.92^{a}	99.17
P-value	0.58	0.00	0.00	0.00	0.00	0.00	0.91
Pooled SEM	0.06	8.14	18.67	0.05	0.04	0.05	0.31

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]Weight gain (WG) = 100 × (final mean body weight - initial mean body weight)/initial mean body weight.

[3]Specific growth rate (SGR) = 100 × [(ln final body weight - ln initial body weight)/experimental days].

[4]Feed conversion ratio (FCR) = dry feed fed/wet weight gain.

[5]Survival rate = 100 × (initial number of fish/final number of fish).

Table 4.2 Growth performance of Nile tilapia fed experimental diets for 16 weeks[1].

Diet	Initial weight (g)	Final weight (g)	WG[2] (%)	SGR[3] (%)	FI (g day^{-1})	FCR[4]	Survival rate[5] (%)
Control	44.56	394.52^{a}	785.33^{a}	1.82^{a}	6.05^{c}	1.64^{d}	96.41
2.5 Inulin	44.44	441.04^{b}	892.75^{b}	1.91^{b}	5.91^{b}	1.48^{c}	98.33
5.0 Inulin	44.25	481.03^{c}	987.04^{c}	1.99^{c}	5.90^{b}	1.31^{b}	98.33
5.0 JA	44.33	483.13^{c}	989.91^{c}	1.99^{c}	5.43^{a}	1.20^{a}	99.17
10.0 JA	44.33	504.69^{c}	1038.59^{c}	2.03^{c}	5.47^{a}	1.15^{a}	99.17
P-value	0.58	0.00	0.00	0.00	0.00	0.00	0.49
Pooled SEM	0.06	10.01	23.07	0.02	0.06	0.04	0.52

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]Weight gain (WG) = 100 × (final mean body weight - initial mean body weight)/initial mean body weight.

[3]Specific growth rate (SGR) = 100 × [(ln final body weight - ln initial body weight)/experimental days].

[4]Feed conversion ratio (FCR) = dry feed fed/wet weight gain.

[5]Survival rate = 100 × (initial number of fish/final number of fish).

Table 4.3 Whole body composition of Nile tilapia juveniles (g kg^{-1}) fed experimental diets for 8 weeks[1].

Diet	Moisture (g kg^{-1})	Crude Protein (g kg^{-1})	Crude lipid (g kg^{-1})	Ash (g kg^{-1})
Control	700.29	120.25	38.50	40.39
2.5 Inulin	700.49	122.10	38.64	40.90
5.0 Inulin	710.62	123.05	42.40	43.69
5.0 JA	700.62	124.52	39.81	42.57
10.0 JA	710.47	125.87	40.39	46.09
P-value	0.71	0.31	0.81	0.11
Pooled SEM	3.49	0.88	1.05	0.80

[1]Data represent means from four replicates per treatment.

Table 4.4 Whole body composition of Nile tilapia (g kg^{-1}) fed experimental diets for 16 weeks[1].

Diet	Moisture (g kg^{-1})	Crude Protein (g kg^{-1})	Crude lipid (g kg^{-1})	Ash (g kg^{-1})
Control	705.70	121.50	42.11	41.02
2.5 Inulin	706.54	123.22	43.91	44.78
5.0 Inulin	722.41	123.62	45.25	47.89
5.0 JA	707.36	124.67	44.07	49.03
10.0 JA	720.12	128.02	44.42	50.31
P-value	0.23	0.07	0.69	0.08
Pooled SEM	3.06	0.75	0.66	1.20

[1]Data represent means from four replicates per treatment.

Table 4.5 Hematological parameters of Nile tilapia juveniles fed experimental diets for 8 weeks[1].

Diet	RBC[2] (cell x 10^{12} L^{-1})	Hemoglobin (g L^{-1})	Hematocrit (L L^{-1})
Control	2.22[a]	84.80	0.34
2.5 Inulin	2.33[b]	86.70	0.35
5.0 Inulin	2.34[b]	88.30	0.35
5.0 JA	2.36[b]	88.60	0.36
10.0 JA	2.39[b]	88.80	0.36
P-value	0.00	0.24	0.07
Pooled SEM	0.01	0.63	0.01

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]RBC = red blood cell count.

Table 4.6 Hematological parameters of Nile tilapia fed experimental diets for 16 weeks[1].

Diet	RBC (cell x 10^{12} L^{-1})	Hemoglobin (g L^{-1})	Hematocrit (L L^{-1})
Control	2.41[a]	91.10[a]	0.35[a]
2.5 Inulin	2.52[b]	92.20[ab]	0.36[ab]
5.0 Inulin	2.55[b]	92.80[ab]	0.36[ab]
5.0 JA	2.56[b]	93.10[ab]	0.36[ab]
10.0 JA	2.58[b]	94.20[b]	0.37[b]
P-value	0.00	0.04	0.04
Pooled SEM	0.02	0.35	0.01

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]RBC = red blood cell count.

To study how dietary inulin or JA affects metabolic feed utilization, several blood chemical parameters in experimental fish were examined in Table 4.7-4.8. During 8 weeks of experiment (Table 4.7) showed that dietary supplementation with either inulin or JA did not affect blood triglyceride, cholesterol, BUN, T-bilirubin, D-bilirubin, SGOT, SGPT and chloride contents, they did modulate several other blood parameters. For instance, both inulin and JA supplementation led to significantly increased blood glucose and albumin levels ($P<0.05$) and increased total protein in blood, although significant enhancement in the latter was observed only in fish fed diets supplemented with JA ($P<0.05$). In addition, supplementation with the higher level of inulin and JA at both levels increased serum magnesium content ($P<0.05$). Moreover, blood calcium and iron levels were markedly increased in fish fed the 10.0 JA diet ($P<0.05$).

During 16 weeks of experiment (Table 4.8) showed that dietary supplementation with either inulin or JA did not affect blood triglyceride, cholesterol, BUN, T-bilirubin, D-bilirubin, SGOT, SGPT and chloride contents. However, dietary supplementation with inulin and JA had significantly increased blood glucose ($P<0.05$), and resulted in an increased total protein and albumin in blood, although significant enhancement in the latter was observed only in fish fed diets supplemented with inulin (5.0 g kg^{-1}) and JA (5.0 g kg^{-1} and 10.0 g kg^{-1}) ($P<0.05$). In addition, supplementation with JA at both levels increased serum iron content ($P<0.05$). Moreover, blood calcium and magnesium levels were markedly increased in fish fed the 10.0 JA diet ($P<0.05$).

The effects of dietary supplementation with inulin and JA on humoral immune parameters were assessed (Table 4.9-4.10). During 8 weeks of experiment (Table 4.9)

showed that dietary supplementation with inulin and JA led to increased total immunoglobulin content and lysozyme activity, although a significant increase in lysozyme activity was observed only in fish fed the 5.0 inulin diet, 5.0 JA diet and 10.0 JA diet ($P<0.05$). Compared with fish fed the control diet, higher ACH50 activity was also found in fish fed these three diets, and the highest ACH50 activity was observed in fish fed the 10.0 JA diet. During 16 weeks of experiment (Table 4.10) showed that dietary supplementation with inulin at 5.0 g kg^{-1} and JA at both levels had higher total immunoglobulin content, lysozyme activity and ACH50 activity compared to that of fish fed the control diet ($P<0.05$). Fish fed the 5.0 inulin diet and 5.0 JA diet had significantly higher ACH50 activity compare with that of fish fed the control diet ($P<0.05$). The highest ACH50 activity was observed in fish fed the 10.0 JA diet.

To evaluate whether prebiotic inulin and JA influence intestinal morphology, these villus height and goblet cell number in intestines of experimental fish in all groups were measured (Table 4.11-4.12). During 8 weeks of experiment (Table 4.11) showed that in the anterior and the middle parts of the intestine, fish fed diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had higher villus height compared to that of fish fed the control diet ($P<0.05$), and the highest villus height was observed in the anterior intestine of fish fed the 10.0 JA diet. There were no significant differences in the villus height in the posterior intestine of fish among treatment diets. In all parts of the intestine, the number of goblet cells in fish fed the diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels were higher than that of fish fed the control diet ($P<0.05$).

During 16 weeks of experiment (Table 4.12) showed that in the anterior, the middle and the posterior parts of the intestine of fish fed the diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had higher villus height compared to that of fish fed the control diet ($P<0.05$), and the number of goblet cells in all parts of the intestine of fish fed the diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels were higher than that of fish fed the control diet ($P<0.05$).

The effects of dietary inulin or JA on intestinal microbiota of Nile tilapia during 16 weeks of experiment are shown in Table 4.13. Fish fed the diet supplemented with inulin at 5.0 g kg^{-1} and JA at both levels exhibited increased intestinal total bacteria and lactic acid bacteria compared with that of fish fed the control diet ($P<0.05$). Furthermore, dietary supplementation with JA at both levels (5.0 JA and 10.0 JA) resulted in an increased the number of *Bifidobacteria* spp. compared with that of fish fed the control diet ($P<0.05$). Meanwhile, the population of *Vibrio* spp. and yeast and fungi would be decreased in fish fed the 5.0 inulin diet, 5.0 JA diet and 10.0 JA diet ($P<0.05$).

Table 4.7 Blood chemical parameters of Nile tilapia juveniles fed experimental diets for 8 weeks[1].

Blood chemical	Diet						
Parameter	**Control**	**2.5 Inulin**	**5.0 Inulin**	**5.0 JA**	**10.0 JA**	**P-value**	**Pooled SEM**
Glucose (mmol L^{-1})	2.71^{a}	4.09^{b}	4.81^{b}	4.10^{b}	4.19^{b}	0.01	0.24
Cholesterol (mmol L^{-1})	4.10	4.19	4.69	4.37	4.41	0.17	0.08
Triglycerides (mmol L^{-1})	1.70	1.66	1.71	1.75	1.89	0.82	0.04
Total protein (g L^{-1})	36.40^{a}	39.30^{ab}	40.40^{ab}	41.60^{b}	42.50^{b}	0.04	0.70
Albumin (g L^{-1})	16.90^{a}	20.40^{b}	20.90^{b}	21.20^{b}	23.10^{b}	0.00	0.60
BUN[2] (mmol L^{-1})	0.85	0.82	0.80	0.77	0.78	0.25	0.02
Total bilirubin (µmol L^{-1})	4.62	3.42	2.99	3.17	2.82	0.11	0.34
Direct bilirubin (µmol L^{-1})	2.39	1.71	1.50	1.64	1.46	0.12	0.17
SGOT[3] (U L^{-1})	34.52	33.18	32.04	29.49	30.29	0.53	0.99
SGPT[4] (U L^{-1})	21.00	20.86	19.90	19.58	19.79	0.51	0.31
Chloride (mmol L^{-1})	130.70	128.20	132.70	138.20	139.70	0.69	2.81
Calcium (mmol L^{-1})	3.48^{a}	3.46^{a}	3.59^{a}	3.71^{a}	4.05^{b}	0.01	0.07
Magnesium (mmol L^{-1})	1.00^{a}	0.96^{a}	1.14^{b}	1.15^{b}	1.17^{b}	0.00	0.03
Iron (µmol L^{-1})	12.00^{a}	13.73^{ab}	14.04^{ab}	14.52^{ab}	16.05^{b}	0.04	0.44

[1]Means with different superscripts in each row differ significantly from each other ($P<0.05$).

[2]BUN = blood urea nitrogen; [3]SGOT = serum glutamic oxaloacetic transaminase; [4]SGPT = serum glutamic pyruvic transaminase.

Table 4.8 Blood chemical parameters of Nile tilapia fed experimental diets for 16 weeks[1].

Blood chemical	Diet						
Parameter	**Control**	**2.5 Inulin**	**5.0 Inulin**	**5.0 JA**	**10.0 JA**	**P-value**	**Pooled SEM**
Glucose (mmol L^{-1})	2.81^{a}	4.47^{b}	4.35^{b}	4.22^{b}	4.27^{b}	0.03	0.20
Cholesterol (mmol L^{-1})	4.34	4.57	4.79	4.67	4.75	0.33	0.07
Triglycerides (mmol L^{-1})	1.76	1.71	1.81	1.91	1.94	0.85	0.05
Total protein (g L^{-1})	32.40^{a}	32.60^{a}	36.70^{b}	37.40^{b}	40.40^{b}	0.00	0.85
Albumin (g L^{-1})	20.70^{a}	21.20^{a}	23.10^{b}	23.10^{b}	24.00^{b}	0.00	0.30
BUN[2] (mmol L^{-1})	1.01	0.94	0.88	0.80	0.79	0.13	0.03
Total bilirubin (µmol L^{-1})	3.42	3.08	2.91	2.91	2.22	0.84	0.34
Direct bilirubin (µmol L^{-1})	1.88	1.88	1.71	1.71	1.54	0.94	0.17
SGOT[3] (U L^{-1})	39.96	37.43	34.71	34.54	33.29	0.35	1.12
SGPT[4] (U L^{-1})	26.99	23.83	23.67	23.50	22.52	0.45	0.76
Chloride (mmol L^{-1})	133.38	131.50	137.13	137.75	142.75	0.78	2.78
Calcium (mmol L^{-1})	3.66^{a}	3.56^{a}	3.67^{a}	3.70^{a}	4.19^{b}	0.02	0.07
Magnesium (mmol L^{-1})	1.04^{a}	1.04^{a}	1.17^{ab}	1.19^{ab}	1.32^{b}	0.00	0.03
Iron (µmol L^{-1})	12.84^{a}	13.87^{ab}	14.43^{ab}	16.05^{b}	16.73^{b}	0.04	0.48

[1]Means with different superscripts in each row differ significantly from each other ($P<0.05$).

[2]BUN = blood urea nitrogen; [3]SGOT = serum glutamic oxaloacetic transaminase; [4]SGPT = serum glutamic pyruvic transaminase.

Table 4.9 Immunological parameters of Nile tilapia juveniles fed experimental diets for 8 weeks[1].

Immunological parameter	Diet						
	Control	**2.5 Inulin**	**5.0 Inulin**	**5.0 JA**	**10.0 JA**	**P-value**	**Pooled SEM**
Total Ig[2] (g L^{-1})	32.00^{a}	34.10^{a}	35.60^{ab}	36.10^{ab}	38.80^{b}	0.03	0.74
Lysozyme activity (µg mL^{-1})	8.64^{a}	8.71^{a}	10.01^{b}	10.13^{b}	10.42^{b}	0.02	0.24
ACH50[3] (units mL^{-1})	311.97^{a}	327.50^{a}	354.87^{b}	363.55^{b}	387.68^{c}	0.00	5.49

[1]Means with different superscripts in each row differ significantly from each other (P<0.05).

[2]Total Ig = total immunoglobulin.

[3]ACH50 = alternative complement haemolytic 50 activity.

Table 4.10 Immunological parameters of Nile tilapia fed experimental diets for 16 weeks[1].

Immunological parameter	Diet						
	Control	**2.5 Inulin**	**5.0 Inulin**	**5.0 JA**	**10.0 JA**	**P-value**	**Pooled SEM**
Total Ig[2] (g L^{-1})	26.90[a]	27.20[a]	31.10[b]	31.90[b]	34.90[b]	0.00	0.85
Lysozyme activity (µg mL^{-1})	8.92[a]	9.00[a]	10.41[b]	10.62[b]	10.64[b]	0.02	0.26
ACH50[3] (units mL^{-1})	335.52[a]	338.05[a]	377.81[b]	382.94[b]	405.34[c]	0.00	6.69

[1]Means with different superscripts in each row differ significantly from each other (P<0.05).

[2]Total Ig = total immunoglobulin.

[3]ACH50 = alternative complement haemolytic 50 activity activity.

Table 4.11 Intestinal villi height and number of goblet cells in different parts of the intestine of Nile tilapia juveniles fed experimental diets for 8 weeks[1].

Diet	Anterior		Middle		Posterior	
	Villus height (µm)	No. of goblet cells	Villus height (µm)	No. of goblet cells	Villus height (µm)	No. of goblet cells
Control	408.59^{a}	31.50^{a}	309.61^{a}	28.25^{a}	206.45	18.42^{a}
2.5 Inulin	421.37^{ab}	32.25^{a}	321.66^{ab}	29.00^{a}	213.76	18.67^{a}
5.0 Inulin	525.58^{bc}	38.42^{b}	392.37^{b}	35.67^{b}	225.19	23.59^{b}
5.0 JA	530.97^{bc}	39.00^{b}	394.59^{b}	36.83^{b}	229.60	24.00^{b}
10.0 JA	576.00^{c}	40.42^{b}	404.11^{b}	36.83^{b}	243.11	24.25^{b}
P-value	0.02	0.01	0.04	0.01	0.60	0.02
Pooled SEM	20.93	1.16	13.74	1.19	7.33	0.86

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

Table 4.12 Intestinal villi height and number of goblet cells in different parts of the in testine of Nile tilapia fed experimental diets for 16 weeks[1].

Diet	Anterior		Middle		Posterior	
	Villus height (µm)	No. of goblet cells	Villus height (µm)	No. of goblet cells	Villus height (µm)	No. of goblet cells
Control	480.13^{a}	35.25^{a}	343.86^{a}	33.00^{a}	231.53^{a}	19.75^{a}
2.5 Inulin	492.55^{a}	35.67^{a}	359.05^{a}	34.92^{ab}	242.89^{a}	21.83^{ab}
5.0 Inulin	619.82^{b}	42.75^{b}	447.436^{b}	40.67^{bc}	295.92^{b}	25.59^{b}
5.0 JA	621.14^{b}	43.08^{b}	449.20^{b}	41.17^{c}	302.42^{b}	25.75^{b}
10.0 JA	629.17^{b}	45.92^{b}	471.18^{b}	42.00^{c}	308.88^{b}	27.42^{b}
P-value	0.00	0.00	0.00	0.01	0.00	0.04
Pooled SEM	19.20	1.28	15.25	1.14	9.66	0.96

[1]Means with different superscripts in each column differ significantly from each other (P<0.05).

Table 4.13 Intestinal microbiota of Nile tilapia (log CFU g^{-1}) fed experimental diets for 16 weeks[1].

Diet	Total bacteria	Lactic acid bacteria	*Bifidobacteria* spp.	*Vibrio* spp.	Yeast and fungi
Control	5.92[a]	3.04[a]	5.93[a]	5.13[d]	3.36[d]
2.5 Inulin	5.96[a]	3.53[ab]	6.02[a]	5.01[cd]	2.93[cd]
5.0 Inulin	6.30[b]	3.80[bc]	6.12[ab]	4.76[bc]	2.60[bc]
5.0 JA	6.33[b]	4.08[bc]	6.30[b]	4.58[ab]	2.09[b]
10.0 JA	6.47[b]	4.34[c]	6.52[c]	4.33[a]	1.78[a]
P-value	0.00	0.00	0.00	0.00	0.00
Pooled SEM	0.06	0.13	0.06	0.08	0.18

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

4.6 Discussion

Several nutrition management studies have been conducted to quantitatively and qualitatively improve the productivity of commercial tilapia farming (Bhujel, 2001). Functional diet supplementation has recently become a topic of interest for improving not only growth rate and feed utilization but also health status of farmed fish. The recent development of industrial prebiotics requires evaluation of their use as a feed additive and their effects on animal production. Inulin has been shown to have beneficial effects on growth and health status in mammals (Coudray et al., 1997; Trautwein et al., 1998; He et al., 2002; Kaur and Gupta, 2002). However, little is known about its effects on Nile tilapia (Ibrahem et al., 2010). With this study I

provide valuable information about the incorporation of inulin in the diet of Nile tilapia at the juvenile stage until they reach market size adult tilapia. The use of commercial inulin as a feed additive inevitably leads to an increase in production cost. Therefore, development of alternative fructan-enriched sources of inulin would contribute to the eco-friendly use of this prebiotic as an animal dietary supplement. This study also demonstrated that JA directly incorporated into the diet of juvenile tilapia until they reach market size had effects comparable to those of inulin.

Dietary supplementation with inulin had a positive effect on growth responses, including final weight and SGR, in Nile tilapia. The improved growth response observed in the present study was similar to that reported previously in various fish species, including Nile tilapia, Siberian sturgeon (*Acipenser baerii*) and rainbow trout (*Oncorhynchus mykiss*) (Mahious et al., 2006a; Ibrahem et al., 2010; Ortiz et al., 2013). However, dietary supplementation with inulin did not affect the growth response in weaning turbot (*Psetta maxima*), Atlantic salmon (*Salmo salar*), hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) (Mahious et al., 2006b; Bakke-McKellep et al., 2007; Burr et al., 2010), and it had a negative effect on the growth response in beluga (*Huso huso*) (Reza et al., 2009). Thus, the effect of dietary inulin on growth responses in fish appears to vary among fish species, and more parameters need to be examined in order to better understand the metabolism of inulin.

The present results showed that dietary supplementation with inulin improved FCR in Nile tilapia, as was also true for Siberian sturgeon (Mahious et al., 2006a). However, several studies reported that dietary supplementation with inulin had no effect on FCR in rainbow trout, hybrid striped bass and beluga (Reza et al., 2009; Burr et al., 2010; Ortiz et al., 2013). Ibrahem et al. (2010) reported that dietary inulin

supplementation led to an increased survival rate in Nile tilapia. Dietary FOS supplementation led to increase survival rate of common carp fry although it did not significantly improve growth performance (Hoseinifar et al., 2014). However, in this study and in reports for other fish species, dietary inulin seemed to have no effects on survival rate (Mahious et al., 2006b; Reza et al., 2009). Thus, the effects of dietary inulin on growth performance and survival rate vary among fish species.

In this study, the growth performances (including final weight, SGR and FCR) of fish fed the 5.0 JA and 10.0 JA diets were superior to those of fish fed the 2.5 inulin and 5.0 inulin diets, respectively, even though latter two diets contained inulin and FOS at levels equivalent to the 2.5 inulin and 5.0 inulin diets, respectively. Therefore, the superior growth performances of fish fed the diets containing JA might be due to the differences in degree of polymerization of inulin sources and other substances in addition to inulin and FOS. JA contained high proportion of shorter fructan comparing to inulin from chicory. While high proportion (43-52%) of furctan in JA were short chain fructan (<9 degree of polymerization (dp)), 64-71% of fructan in inulin were medium chain fructan (10-40 dp) (Moshfegh et al., 1999; for review, see Kays and Nottingham, 2007). Although both inulin and FOS exert prebiotic effects, they showed several different prebiotic effects. For example, in fecal cultures, inulin and FOS affected the major fermentation products. Butyric acid was the major product of inulin fermentation whereas FOS fermentation mainly generated acetic acid and lactic acid (Rossi et al., 2005). Comparative study on prebiotic effects in vitro between inulin and FOS revealed that they influenced different microbial community and proteolytic activity (van de Wiele et al., 2006). Dietary supplementation with either inulin or FOS had similar effects on growth performance in rainbow trout (Ortiz et al.,

2013), whereas supplementation with FOS had a more positive effect than inulin on the growth rate of turbot larvae (Mahious et al., 2006b). Other substances such as micronutrients in JA also may have additional positive effects on growth response and feed utilization in Nile tilapia. In fact, JA contains various minerals and vitamins including iron, calcium, potassium, vitamin B complex, vitamin C and vitamin A (Van Loo et al., 1995; Kays and Nottingham, 2007). Thus, direct supplementation with JA had positive effects on growth performance in Nile tilapia that were comparable to those of inulin.

Generally, dietary effect on weight gain of fish results from body nutrient contents including water, protein, lipid and ash. The proximate composition of the fish body has been used as a parameter to determine and optimize the supplementation of functional feed ingredients in animal feed (Dumas et al., 2010). This study determined whether the differences in growth response among experimental fish would result from the variation in body chemical composition. The results showed that whole-body composition, including moisture, crude lipid, crude protein and ash, was not affected by either prebiotic inulin or JA supplementation. Dietary supplementation with inulin also did not significantly change the whole-body compositionin of hybrid striped bass and beluga (Reza et al., 2009; Burr et al., 2010).

Hematological parameters have been used to assess the health status of fish. In this study, dietary supplementation with either inulin or JA had significantly higher RBC compared with that of fish in the control group. Fish fed the diet supplemented with either inulin or JA for 16 weeks resulted in an increased Hb and Ht. However, RBC modulation was not observed in hybrid surubim (*Pseudoplatystoma* sp.) that were fed an inulin-incorporated diet (5.0 g kg^{-1}) for 15 days or in beluga fed an inulin-

supplemented diet (10.0-20.0 g kg^{-1}) for 8 weeks (Mourino et al., 2012; Reza et al., 2009). Ibrahem et al. (2010) and Mourino et al. (2012) reported that the Ht of Nile tilapia and hybrid surubim were not affected by dietary inulin. In addition, the Hb content and Ht were observed to decrease in juvenile beluga that were fed an inulin-incorporated diet at 20.0 g kg^{-1} and 30.0 g kg^{-1} (Reza et al., 2009). Taken together, these results suggest that the effect of inulin on hematological indices may vary among fish species, level of inulin supplementation and duration of feeding. Moreover, direct supplementation with JA in fish feed did exert additive effects on increases in hematological parameters.

Comparative information on blood metabolic responses would be necessary to investigate variably prebiotic effects in several fish. Blood chemical parameters were measured to help interpret the nutritional and health status of Nile tilapia that were fed dietary inulin and JA. Most of the blood chemical parameters showed the same trends for fish fed diets supplemented with inulin and JA, which suggests that JA could be used directly as a food ingredient, at least in Nile tilapia. However, limited information is available about the effect of dietary inulin on blood chemistry (Reza et al., 2009), and more studies are needed.

In general, prebiotics such as inulin have health benefits because they promote the proliferation of beneficial bacteria (usually bifidobacteria and lactobacilli) in the gut (Kolida et al., 2002; Manning and Gibson, 2004). Dietary FOS supplementation increased intestinal lactic acid bacteria number in common carp fry (Hoseinifar et al., 2014). Similarly, use of FOS as a prebiotic was found to enhance the intestinal digestive enzyme activities (amylase and protease) of blunt snout bream (*Megalobrama amblycephala*) (Wu et al., 2013) and Caspian roach (*Rutilus rutilus*)

(Soleimani et al., 2012). Others also have shown that probiotics led to increases in the activities of digestive enzymes such as amylase, protease and lipase (Ziaei-Nejad et al., 2006; Wang, 2007). Increased intestinal digestive enzyme activities would affect several blood chemical parameters. The results showed that total protein, glucose and albumin contents in fish fed diets supplemented with inulin and JA were significantly higher than those of fish in the control group. In contrast, Reza et al. (2009) reported that supplementation with inulin (10.0-30.0 g kg^{-1}) for 8 weeks had no effect on blood glucose and albumin levels in beluga. BUN seemed to be similar among experimental groups, whereas a dietary supplementation with inulin at the highest level and JA at the two levels tested led to increased total protein content. However, dietary inulin caused a decrease in total protein content in beluga (Reza et al., 2009). In some studies of mammals, inulin-incorporated feed was shown to decrease cholesterol and triglyceride levels (Trautwein et al., 1998; Flickinger et al., 2003), but dietary inulin and JA did not modulate triglyceride and cholesterol content in this study of Nile tilapia or in beluga (Reza et al., 2009). Bilirubin (both total and direct), SGOT and SGPT levels were similar among the experimental fish in this study. Similarly, inulin supplementation did not affect these blood parameters in beluga (Reza et al., 2009). The effects of inulin on several blood parameters were similar between Nile tilapia and beluga, whereas they differed for other blood parameters. The different effects might be due to differences in food habits between the two species, as Nile tilapia are omnivores and beluga are piscivores.

The results showed that dietary supplementation with inulin or JA significantly increased concentrations of several blood minerals in Nile tilapia, including magnesium, calcium and iron. Intestinal fermentation of inulin or JA might affect

intestinal acidification, and low pH would enhance mineral absorption. A number of mammal studies have shown that prebiotic oligosaccharides can modulate mineral metabolism, such as by stimulating mineral absorption, particularly of calcium, magnesium and iron (Chonan et al., 1995; Delzenne et al., 1995; Ohta et al., 1995; Coudray et al., 1997; Scholz-Ahrens et al., 2001). Gill absorption of monovalent salt regulates chloride homeostasis in fish, which may explain the lack of a significant effect of dietary inulin or JA on blood chloride levels in this study.

Prebiotics have potential for use as alterative biotherapeutics for fish production. Prebiotics are thought to enhance immunity in animals by selectively increasing the number of beneficial intestinal bacteria and/or interacting with carbohydrate receptors on intestinal epithelial cells and immune cells (reviewed in Seifert and Watzl, 2007). Consequently, the cell components (e.g., lipolysaccharides) of some beneficial microbiota can stimulate the immune system in host animals (Sakai, 1999; Bricknell and Dalmo, 2005). The results showed that fish fed inulin or JA for 8 and 16 weeks exhibited increased humoral innate immune responses, as indicated by increased levels of total immunoglobulin and increased lysozyme and ACH50 activities. Similarly, dietary inulin at 10.0 g kg^{-1} for 60 days was reported to increase serum lysozyme activity of Nile tilapia (Ibrahem et al., 2010). However, Cerezuela et al. (2008) reported that dietary inulin (5.0 g kg^{-1} or 10.0 g kg^{-1}) for 2 weeks had no effect on ACH50 activity of gilthead sea bream (*Sparus aurata*) compared to the control group (0 g kg^{-1}). Mourino et al. (2012) found that dietary supplementation with 5.0 inulin for 15 days had no effect on total immunoglobulin content and lysozyme activity of hybrid surubim. These contradictory effects of inulin on immune modulation might be explained by the differing periods of prebiotic

administration and fish species among different studies. Overall, the existing data indicate that dietary inulin can have beneficial effects on several immune parameters in several fish species. Thus, this finding that direct supplementation with JA (i.e., without extraction of inulin) had beneficial effects on several health parameters demonstrates that JA can be used directly as a functional feed ingredient.

Caspary (1992) reported that increased intestinal villi leads to increased surface area for nutrient absorption, thereby improving growth performance and feed utilization in animals. Several researchers proposed that fermentation of inulin produces several substances that stimulate intestinal cell proliferation, which in turn results in increased villus height (Blottiere et al., 2003; Rehman et al., 2007; Nabizadeh, 2012). The present study demonstrated that dietary supplementation with inulin (5.0 g kg^{-1}) and JA (5.0 g kg^{-1} and 10.0 g kg^{-1}) resulted in greater villus height in all parts of the intestine, although a significant increase in villus height was observed only in the anterior and middle parts. However, the effect of dietary inulin on carnivorous fish appears to be different. For example, Olsen et al. (2001) reported that a high level of dietary inulin (150 g kg^{-1} dietary inclusion) had negative effects on the ultrastructure of the gastrointestinal tract of Arctic char (*Salvelinus alpinus*). In addition, decreased microvillus height was observed in gilthead sea bream fed a diet that included 10.0 g kg^{-1} inulin (Cerezuela et al., 2013). Intestinal goblet cells synthesize mucin, which invades enteric pathogens. Thus, an increase in intestinal goblet cell numbers would help prevent colonization by pathogenic bacteria and promote beneficial bacteria. The present study demonstrated that the fish fed the 5.0 inulin diet and the 5.0 JA and 10.0 JA diets had a higher goblet cell number than the other groups. In another study, however, dietary supplementation with inulin at

10.0 g kg^{-1} had a negative effect on the number of goblet cells in gilthead sea bream (Cerezuela et al., 2013). These findings suggest that the beneficial effect of dietary inulin on intestinal morphology might differ among species, especially those with different feeding habits. The data also show that inulin and JA had comparable effects on villus height in Nile tilapia.

Because prebiotic would be used for promote the proliferation of beneficial bacteria in fish intestines, supplementation with prebiotic in fish feed could result in different kinds of intestinal microbial populations in comparison with fishes that did not receive prebiotic supplementation. This study found that fish fed the diet supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had significantly increased intestinal total bacteria, lactic acid bacteria and *Bifidobacteria* spp. On the other hand, the population of *Vibrio* spp. and yeast and fungi would be decreased in groups of fish that fed the three formula diets as well. This indicated that the use of inulin or JA as prebiotic feed additives might cause changes of intestinal microbiota of tilapia, which was consistent with the results of previous studies on various fishes. For example, Reza et al. (2009) reported that dietary supplementation with inulin (10.0 g kg^{-1}) for 8 weeks resulted in an increase in population of lactic acid bacteria in beluga compared with fish fed the control diet. Mourino et al. (2012) showed that dietary supplementation with 5.0 g kg^{-1} inulin for 15 days had higher concentrations of lactic acid bacteria in hybrid surubim. In addition, Ortiz et al. (2013) observed that supplementation with inulin (5.0-10.0 g kg^{-1}) for 49 days had reduced drastically the number of *Vibrio* spp. in the distal part of the intestine of rainbow trout. In fact, lactic acid bacteria and *Bifidobacteria* spp. were well known that they could fermented inulin and FOS (Kaplan and Hutkins, 2000; Buddington et al., 2002; Roller et al.,

2004). In addition, most of these bacteria would be categorized as beneficial bacteria for ecosystem of animal intestines by producing bacteriocins, lactic acid and anti-growth substance of other bacteria, which could inhibit the growth of pathogenic intestinal bacteria (Ringø and Gatesoupe, 1998; Ringø et al., 2010a)

4.7 Conclusion

This study demonstrated the beneficial effects of inulin on growth performance and health status in juvenile Nile tilapia. In addition, direct supplementation with JA had superior effects compared to those of inulin at the equivalent inulin levels. The recommended levels of dietary supplementation with inulin and JA are the maximal levels tested (i.e., 5.0 g kg^{-1} and 10.0 g kg^{-1}, respectively).

4.8 References

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CHAPTER V

THE USES OF JERUSALEM ARTICHOKE, RED GRAPE POMACE AND DEFATTED *ISOCHRYSIS* AS FEED ADDITIVES INGREDIENTS IN THE DIET OF NILE TILAPIA FINGERLINGS

5.1 Abstract

This study was conducted to evaluate and compare the effect of three additives including Jerusalem artichoke (JA), red grape pomace (RGP) and defatted *Isochrysis* (ISO) in the diets on growth performance, immune response and resistance of Nile tilapia fingerlings to *Streptococcus iniae* challenge. Five treatment diets included control, 20.0, and 40.0 g kg^{-1} JA, 20.0 g kg^{-1} RGP and 20.0 g kg^{-1} ISO. Each diet was fed to Nile tilapia fingerlings in aquaria for 7 weeks. Fish fed the 20.0 JA diet, 20.0 RGP diet and 20.0 ISO had better growth performance compared with fish fed the control diet. Comparing to the control diet, fish fed on 40.0 g kg^{-1} JA had similar growth performance. There were no significant differences in survival rate of fish in all groups. Dietary supplementation with 20.0 JA, 20.0 RGP and 20.0 ISO improved serum lysozyme activity, bactericidal activity and peroxidase activity. Daily cumulative mortality of Nile tilapia after 12 days of challenge with *S. iniae* showed that eighty percent of fish fed on control diet died at 4 days post challenge although similar mortality rate (80%) was observed in fish fed on 40 JA which was found at 6 days

post challenge. Fish fed on 20.0 RGP and 20.0 ISO had 30% mortality rate when fish was challenge with *S. iniae* for 7 days. However, no fish fed on 20.0 JA died during 12 days post challenge. These findings indicate that three feed additives including JA, RGP and ISO could exerted a positive effect on growth performance, innate immune responses and resistance of Nile tilapia fingerlings to the *S. iniae* infection. The recommended levels of dietary supplementation with JA, RGP and ISO are 20.0 g kg^{-1}.

Key words : Jerusalem artichoke, red grape pomace, defatted *Isochrysis*, Nile tilapia, growth performance, immune, *Streptococcus iniae*

5.2 Introduction

Tilapia is the second most important farmed fish after carp, and its production has increased intensely to meet the global demand for the whitefish market (FAO, 2014). Among tilapia species, Nile tilapia (*Oreochromis niloticus*) has commercially dominated the farm-cultured tilapia. Generally, Nile tilapia are easy to raise year round and grow fast in tropical areas. However, one major problem associated with an intensive tilapia culture is the increased susceptibility of fish to infectious diseases, including streptococcal disease in tilapia caused by *Streptococcus iniae* (Shoemaker and Klesius, 1997). The problem of streptococcal disease is worldwide in tilapias farming and contributing to an annual loss to the aquaculture industry (Amal and Zamri-Saad, 2011). Commercially, antibiotics have been used for treatment and prevention of bacterial disease of aquatic animals (Li and Gatlin, 2005). The overuse of antibiotics in fish farms might pose a public health threat and also adversely impact the natural environment. The use of antibiotics in animal production has been banned

in EU countries and is increasingly under public scrutiny and criticism in most other countries. Consequently, a wide variety of products ranging from polysaccharides, plant extracts and some nutrients, have been added in fish diets as immunostimulants to stimulate immune system function and/or their resistance to infectious diseases (Sakai, 1999; Gannam and Schrock, 2001).

Jerusalem artichoke (JA), which is a root vegetable native to central-eastern North America (Kays and Nottingham, 2007), is widely grown year-round in tropical areas and tend to increase planting both for human diet and also by-products or co-products for animals. The JA tuber was reported to contain total fructan at 502 g kg^{-1} dry matter as same as that of chapter III (Table 3.1). From the results of the chapter III-IV demonstrated the beneficially prebiotic effects of JA on growth performance and health status in Nile tilapia fingerlings and juvenile Nile tilapia.

Red grape pomace (RGP) is a wine making by-product of the red wine processing company containing various immunostimulating compounds such as polyphenols, mainly anthocyanins, hydroxycinnamic acids and flavanols (Kammerer et al., 2004). These compounds have also been reported to demonstrate antibacterial, antiviral and anticarcinogenic activities, protection against inflammation and protection against allergy (Fine, 2000; Li et al., 2001; Agarwal et al., 2002). In addition, dietary supplementation of grape seed proanthocyanidins in tilapia fingerlings was demonstrated to exert beneficial effects on growth performance, serum biochemical parameters and body composition (Zhai et al., 2014).

Defatted *Isochrysis* (ISO) is a by-product after fatty acid extraction process, *Isochrysis* is a marine microalgae belonging to family Prymnesiophyceae, *Isochrysis* contains high content of crude protein and essential fatty acids [Docosahexaenoic acid

(C22 : 6n-3)] (Brown, 1991; Otero et al., 1997; Becker, 2004; Patil et al., 2007), this characteristic together with the small cell size have made this species to produce enrich rotifer and artemia. In this study, the effect of dietary ISO by-product was investigated on growth and health status for Nile tilapia fingerlings.

Thus, the present study was conducted to evaluate and compare the effects of three feed additives including JA, RGP and ISO in the diets on growth performance, immune response and resistance of Nile tilapia fingerlings to the *S. iniae* challenge.

5.3 Objective

The objective of this experiment was to investigate the effect of three feed additives including JA, RGP and ISO in the diet on growth performance, non-specific immune parameters and diseases resistance of Nile tilapia fingerlings.

5.4 Materials and methods

5.4.1 Feed additives

JA samples were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. JA in this study contains fructans at 502 g kg^{-1} dry weight. RGP was obtained as powder from lab produced from (*Vitis vinifera*) agro-industrial residue from red wine processing companies, Gran Canaria, Spain. ISO was obtained from fatty acid extraction process by supercritical CO_2 extraction was obtained as powder from Biotechnology Department, Canarian Institute of Technology, Spain.

5.4.2 Experimental design, feed formulation and diets preparation

The experimental design was completely randomized with five treatment diets, each of which was replicated three times. The five treatment diets were as follows: basal diet (control, C), 20.0 g kg^{-1} RGP-supplemented diet (20.0 RGP), 20.0 g kg^{-1} ISO-supplemented diet (20.0 ISO), 20.0 g kg^{-1} JA-supplemented diet (20.0 JA) and 40.0 g kg^{-1} JA-supplemented diet (40.0 JA).

Table 5.1 shows the basal dietary ingredients and the proximate composition (moisture, crude protein, crude lipid and ash content) of the experimental diets as determined following standard AOAC methods (1990). All feedstuff were finely ground and sieved with a metallic mesh (500 µm/cm^2) to remove larger sized particles. The ingredients were weighed according to the proportion based on the corresponding diet formulation and mixed together using a mixer (DANAMIX BM 3 3 0 , Azpeitia, Gipuzcua, Spain). Mineral and vitamin premix was added and the mixture was mixed thoroughly. The feed prepared in powdered form was then passed through a pelletizer (CPM, California Pellet Mill, CA, USA) to obtain 1.5 mm size pellets. Pellets were then dried at 40ºC for 24 hours, after that, diets were stored at 4ºC and used throughout the experimental period.

Table 5.1 Ingredients and chemical composition (g kg^{-1}) of the basal diets.

Ingredients	**g kg^{-1}**
Fishmeal	220
Corn flour	200
Corn gluten	53
Soybean meal	280
Wheat flour	70
Wheat gluten	50
Line oil	50
Vitamin mix[a]	10
Mineral mix[b]	20
Dicalcium phosphate	5
Carboxymethylcellulose	10
Bread	32
Proximate composition (g kg^{-1}dry weight)	
Crude protein	362
Crude lipid	94
Ash	70
Moisture	69

[a]Vitamin mix (mg kg^{-1}): thiamine, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; pantothenic acid, 58.33 mg; nicotinic acid, 100 mg; biotin, 0.67 mg; folic acid, 5 mg; vitamin B12, 0.33 mg; choline, 1350 mg; myoinositol, 1000 mg; vitamin C, 2500 mg; vitamin E, 125 mg; menedione, 10 mg; cholecalciferol, 2.67 mg; retinol acetate, 12.67 mg; ethoxyquin, 33.33 mg.

[b]Mineral mix (g kg^{-1}): $(H_2PO)_2Ca$, 1.6 g; $CaCO_3$, 4 g; $FeSO_4$, 1.5 g; $MgSO_4$, 1.6 g; KH_2PO_4, 2.8 g; Na_2PO_4, 1 g; $Al_2(SO_4)_3$, 0.02 g; $ZnSO_4$, 0.24 g; $CuSO_4$, 0.52 g; $MnSO_4$, 0.08 g; KI, 0.02 g; $CoSO_4$, 0.08 g.

5.4.3 Experimental fish and fish culture

The Nile tilapia (all male) used in this study were obtained from the Aquaculture research group broodstock (GIA broodstock; University of Las Palmas Gran Canaria, Spain). Nile tilapia fry (the swim-up stage) were held at 26°C and fed a commercial diet (42% crude protein, 8% crude fat) during 4 weeks, when fish reached the desired weight for the experiment (3.21±0.41 g). Nile tilapia were randomly distributed in glass aquariums at a density of 14 fish/aquarium.

The experimental system consisted of three replicates of five glass aquariums (60 L, flow rate 36 L/h) with top output connected to a glass overflow aquarium (50 L). Each triplicate of aquariums was connected to a filter (Eurojet; Mod. 3388, 40 L, 36 L/h flow rate) which recirculates the water from the overflow aquarium back to the fish rearing tanks. Filters were flushed once every week. 60% of the water was replaced once every week to maintain water quality within safety levels.

To acclimatize the Nile tilapia to the experimental conditions, the fish were fed the basal diet for 2 weeks. Throughout the experimental period, the fish were hand-fed to visual satiety three times every day, at 09:00, 12:00, and 15:00. Water temperature was maintained at 26.00-27.00°C using thermostatically controlled heaters. Dissolved oxygen (DO) and pH were measured weekly using a DO meter and pH meter, respectively. The values of DO and pH were within acceptable ranges of 6.30-6.50 mg L^{-1} and 7.50-8.00, respectively. Any dead fish were recorded and removed daily. The growth performance and feed utilization were determined at the end of week 7.

5.4.4 Fish sampling and blood collection

At the end of the experimental period (7 week), fish were not fed for 18 h before being sampled. Three fish from each diet replicate were removed from the tank. Blood samples were collected from the caudal vein with a 1 ml plastic syringe. The blood sample was transferred to 1.5 ml tubes and allowed to clot by being kept on ice for 2 h. Serum was separated by centrifuging the clotted blood at 9000×*g* for 10 min at room temperature and stored at -80°C for lysozyme activity, bactericidal activity and peroxidase activity determination.

5.4.5 Immunological assays

5.4.5.1 Lysozyme activity

Lysozyme level in blood serum was determined by turbidimetric assay according to the method described by Anderson and Siwicki (1994) using hens egg white lysozyme (Sigma) in PBS as a standard.

5.4.5.2 Bactericidal activity

Streptococcus iniae was used to determine the bactericidal activity present in serum samples. *Streptococcus iniae* was grown in agar plates at 25°C in the adequate media: tryptic soy (TSB, Sigma). Then, fresh single colonies were diluted in 5 ml of appropriate liquid culture medium and cultured at 25°C at 7×*g* for 16 h. The serum antimicrobial activity was determined by evaluating their effects on the bacterial growth curves using the method of Sunyer and Tort (1995) with some modifications. Aliquots of 100 µl of each one of the bacterial dilutions (1/10) were place in flat-bottomed 96-well plates and cultured with equal volumes of serum samples. The OD of the samples was measured at 600 nm at 30 min intervals during 180 min at 25°C. Samples without bacteria were used at blanks (negative control).

Samples without serum were used as positive controls (100% growth or 0% bactericidal activity).

5.4.5.3 Peroxidase activity

The peroxidase activity in serum was measured according to Quade and Roth (1997). Briefly, 30 µl of serum were diluted with 120 µl of Hank's buffered salt solution (HBSS) without Ca^{+2} or Mg^{+2} in flat-bottomed 96-well plates. As substrates, 50 µl of 20 mM TMB and 5 mM H_2O_2 were added. The colour-change reaction was stopped after 2 min by adding 50 µl of 2 M sulphuric acid and the OD was read at 450 nm in a plate reader (MultiskanFc, Thermo, Chicago). The tested sample without serum was used as blank. One unit was defined as the amount producing an absorbance change of 1 and the activity expressed as U mg^{-1} serum proteins.

5.4.6 Bacterial challenge experiment

Bacterial challenge was performed in another independent system. A frozen stock-culture of *Streptococcus iniae* (CIP 103769) isolated from brain tilapia lyophilized was grown in tryptic soy broth (TSB) at 26°C with shaking at 7×*g* for 24 h. The concentration of the culture was adjusted to an optical density of 1.0 measured on a Thermo Scientific Helios Epsilon spectrophotometer (Thermo Fisher Scientific Inc., USA) at 600 nm. After sampling for growth trial and immunological assays, Ten fish from each treatment diet were transferred into ten aquaria (40 L) (two replicates of five treatments) and challenged by intraperitoneal (IP) injection with 100 µL of *S. iniae* culture containing 6.0×10^7 cells/ml/fish. After injection, the fish were returned to their respective aquaria. Each group of fish continued to be fed twice daily with the same experimental diet that was assigned in the growth trial. Fish were

monitored for mortality, and dead fish were removed and recorded daily for 12 days following injection.

5.4.7 Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS for Windows (Release 10) (SPSS Inc., Chicago, IL, USA). When significant differences were found among the groups, Duncan's multiple range tests were used to rank the groups. The statistical model utilized was $y_{ij} = \mu+\tau_I+\varepsilon_{ij}$, where y_{ij} was the response; μ, the general means; τ_I, the dietary JA, RGP or ISO effect; and ε_{ij}, the random error. Throughout the experiment, effects and differences were declared to be significant at $P<0.05$.

5.4.8 Experimental location and period

The experiment was conducted at Aquaculture research group (GIA), University of Las Palmas de Gran Canaria, Spain. The experiment was from September 2015 to February 2016.

5.5 Results

Table 5.2 shows the growth performances and survival rates of Nile tilapia fingerlings fed the experimental diets. Fish fed the 20.0 JA diet, 20.0 RGP diet and 20.0 ISO had better final body weight, specific growth rate (SGR) and feed conversion ratio (FCR), compared with fish fed the control diet ($P<0.05$). Comparing to the control diet, fish fed on 40.0 g kg^{-1} JA had similar final body weight, SGR and FCR ($P>0.05$). Throughout the experimental period, survival rate of fish in all groups did not differ significantly ($P>0.05$).

The effects of dietary supplementation with JA, RGB and ISO on humoral immune parameters are shown in Table 5.3. Dietary supplementation with 20.0 JA, 20.0 RGP and 20.0 ISO led to increased serum lysozyme activity, bactericidal activity and peroxidase activity compared with fish fed the control diet ($P<0.05$). Fish fed on control diet and 40.0 JA appeared to have similar lysozyme activity, bactericidal activity and peroxidase activity ($P>0.05$).

Figure 5.1 demonstrated daily cumulative mortality of Nile tilapia after 12 days of challenge with *S. iniae*. Eighty percent of fish fed on control diet died at 4 days post challenge, although similar mortality rate (80%) was observed in fish fed on 40.0 JA, this mortality rate was found at 6 days post challenge. Fish fed on 20.0 RGP and 20.0 ISO had 30% mortality rate when fish was challenge with *S. iniae* for 7 days. No fish fed on 20.0 JA died during 12 days post challenge.

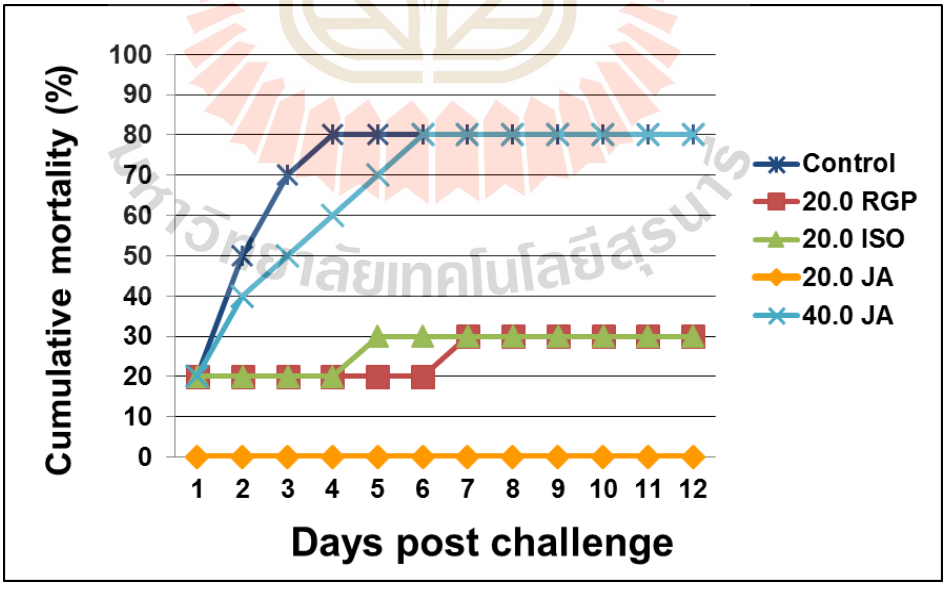

Figure 5.1 Daily cumulative mortality of Nile tilapia after 12 days of challenge with *Streptococcus iniae*.

Table 5.2 Growth performance of Nile tilapia fingerlings fed experimental diet for 7 weeks[1].

Diet	Initial weight (g)	Final weight (g)	SGR[3] (%)	FI (g day^{-1})	FCR[4]	Survival rate[5] (%)
Control	3.17	20.24^{a}	4.12^{a}	0.48^{b}	1.13^{b}	97.62
20.0 RGP	3.19	24.17^{b}	4.50^{b}	0.48^{b}	0.92^{a}	100.00
20.0 ISO	3.20	23.37^{b}	4.42^{b}	0.48^{b}	0.95^{a}	100.00
20.0 JA	3.32	25.33^{b}	4.51^{b}	0.48^{b}	0.87^{a}	100.00
40.0 JA	3.16	19.40^{a}	4.04^{a}	0.47^{a}	1.18^{b}	97.62
P-value	0.09	0.00	0.00	0.03	0.00	0.58
Pooled SEM	0.02	0.67	0.06	0.00	0.03	0.64

[1]Means with different superscripts in each column differ significantly from each other ($P<0.05$).

[2]Weight gain (WG) = 100 × (final mean body weight - initial mean body weight)/initial mean body weight.

[3]Specific growth rate (SGR) = 100 × [(ln final body weight - ln initial body weight)/experimental days].

[4]Feed conversion ratio (FCR) = dry feed fed/wet weight gain.

[5]Survival rate = 100 × (initial number of fish/final number of fish).

Table 5.3 Immunological parameters of Nile tilapia fingerlings fed experimental diet for 7 weeks[1].

Immunological parameter	Diet						
	Control	20.0 RGP	20.0 ISO	20.0 JA	40.0 JA	P-value	Pooled SEM
Lysozyme activity (μg mL^{-1})	8.28[a]	10.06[b]	9.89[b]	10.11[b]	8.06[a]	0.00	0.25
Bactericidal activity (%)	26.42[a]	33.20[b]	32.48[b]	33.51[b]	25.68[a]	0.00	0.96
Peroxidase activity (U mg^{-1} protein)	1.00[a]	1.80[b]	1.71[b]	1.81[b]	0.99[a]	0.00	0.10

[1]Means with different superscripts in each row differ significantly from each other ($P<0.05$).

5.6 Discussion

Oreochromis niloticus is one of the most important commercial species cultured all over the world. The use of natural immunostimulants is promising in aquaculture because they are safe for the environment and human health, and biodegradable (Ortun et al., 2002). JA, RGP and ISO have several biological activities including enhance growth performance, modulate intestinal microbiota, antioxidant, antiinflammatory, antibacterial and immunomodulatory effects (Kleessen et al., 2003; Rockenbach et al., 2011; Custódio et al., 2014). The results of the present study showed that dietary supplementation with 20.0 JA, 20.0 RGP and 20.0 ISO had better final body weight, SGR and FCR of Nile tilapia during the fingerlings stage. This is in agreement with the results from previous studies in some fish species. The positive effects of dietary JA on growth performance were consistent with those reported in Nile tilapia fingerlings and juvenile stages (chapter III-IV). Dietary supplementation with 0.2, 0.4, 0.6 and 0.8 g kg^{-1} grape seed proanthocyanidins was also demonstrated to improved growth performance in tilapia fingerlings (Zhai et al., 2014). Huang et al. (2012) showed that the fish fed the diet containing 10.0 g kg^{-1} grape seed extract had significantly improve the WG and FCR of hybrid Crucian carp. In addition, Palmegiano et al. (2009) found that gilthead sea bream (*Sparus aurata*) juveniles fed a diet including 700.0 g kg^{-1} dry weight of *Isochrysis* sp. had increased growth responses. However, the present study showed that growth responses were negatively affected when the level of JA increased to a level of 40.0 g kg^{-1}. This may be due to the significant reduction in FI (Table 5.2). FI reduction may have been due to the increased levels of soluble fiber in the 40.0 JA diet while the ability of tilapia to utilize them is limited. Additionally the diets with high soluble fiber intake had highly

viscous solution when dissolved, which difficult the enzyme substrate contact and interrupt nutrient absorption (Iji, 1999; Krogdahl et al., 2005).

The non-specific immune parameters are useful to determine the health status of fish and to evaluate the immunostimulants substances for fish farming as markers for diseases resistances (Sahoo et al., 2005). In the present study, the innate immunity which is important in affording protection against diseases, including serum lysozyme activity, bactericidal activity and peroxidase activity of tilapia fingerlings were significantly improved by dietary 20.0 JA, 20.0 RGB and 20.0 ISO supplementation. Similar results were found in the study of chapter III-IV demonstrated that the immune parameters could be significantly increased by dietary supplementation with JA in Nile tilapia fingerlings and juvenile Nile tilapia. Ibrahem et al. (2010) also reported that 10.0 g kg^{-1} inulin had significant increased serum lysozyme activity of Nile tilapia. In rainbow trout, supplementation with dietary inulin at 5.0 and 20.0 g kg^{-1} had significantly increased serum lysozyme activity and total IgM (Sheikholeslami et al., 2011). Dietary JA which mainly comprises inulin are thought to enhance immunity in animals by selectively increasing the number of beneficial intestinal bacteria and/or interacting with carbohydrate receptors on intestinal epithelial cells and immune cells (reviewed in Seifert and Watzl, 2007). Consequently, the cell components (e.g., lipolysaccharides) of some beneficial microbiota can stimulate the immune system in host animals (Sakai, 1999; Bricknell and Dalmo, 2005). Zhai et al. (2014) reported that dietary supplementation with 0.2, 0.4, 0.6 and 0.8 g kg^{-1} grape seed proanthocyanidins led to increased lysozyme activity of tilapia fingerlings. Schlicht (2003) also found that 1,000 ppm KPA® (grape seed extract) could increased lysozyme activity and the neutrophil respiratory burst activity of chinook salmon

(*Oncorhynchus tshawytscha*). The beneficial effects of grape seed proanthocyanidins on immunity were considered due to their have the capacity to act as powerful antioxidants by scavenging free-radicals and terminating oxidative reactions (Caillet et al., 2006; Brenes et al., 2008). Iqbal et al. (2015) suggested that immunity is influenced by oxidative stress and improving antioxidant status of the animal may enhance their immunity. In addition, Delaporte et al. (2003) reported that oyster *Crassostrea gigas* fed T-*Iso* (*Isochrysis* sp.) at 0.6×10^{9} cells animal^{-1} day^{-1} led to an increased phagocytic activity. They concluded that the increased phagocytic activity in oyster administered T-*Iso* which is rich in C22 : 6n-3 may be attributable to the potential positive effect of this fatty acid in stress responses.

An experimental infection provides an opportunity to determine the effect of the assayed treatments on the resistance of the fish species. *S. iniae* is an important pathogen tilapia and causes important losses in tilapias farming worldwide (Amal and Zamri-Saad, 2011). The results of the present study revealed that no fish fed on 20.0 JA died during 12 days post challenge with *S. iniae*. Cumulative mortality rates were also lower in the groups fed the 20.0 RGP and 20.0 ISO diet compared to the control group. The ability to increase resistance disease might be ascribed to the fact that the innate immune defenses of Nile tilapia were activated by 20.0 JA, 20.0 RGP and 20.0 ISO diets as consequently improve its resistance to the *S. iniae* infection. Similar to the present results, Sheikholeslami (2011) observed increase resistance of rainbow trout to *Streptococcus* sp. when fish were fed with 5.0 g kg^{-1} and 20.0 g kg^{-1} inulin for 15 days. Ibrahem et al. (2010) reported enhance the relative level of protection in Nile tilapia after challenged with *Aeromonas hydrophila* when fish were feed 10.0 g kg^{-1} inulin for 2 months. Wang et al. (2008) also obtained a significant

reduction of mortality after the infection with oocysts of *Eimeria tenella* in broiler chickens feed the 12.0 mg kg^{-1} grape seed proanthocyanidin extract for 21 days. They suggested that the decreased mortality of broiler chickens was due to the proanthocyanidins in grape seed extracts exerted an anti-inflammatory impact against the *E. tenella* infection. In addition, Molina-Cárdenas et al. (2014) found that *Vibrio alginolyticus*, *V. campbellii*, and *V. harveyi* which are common pathogens of mollusks, fishes and crustaceans were inhibited by *Isochrysis galbana* in batch cultures. They demonstrated that *I. galbana* synthesizes antibacterial fatty acids that inhibit the growth of these pathogenic *Vibrio* species.

5.7 Conclusion

The present study indicated that three feed additives including JA, RGP and ISO exerted a positive effect on growth performance, innate immune responses and resistance of Nile tilapia fingerlings to the *S. iniae* infection. The recommended levels of dietary supplementation with JA, RGP and ISO are 20.0 g kg^{-1}.

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CHAPTER VI

OVERALL CONCLUSION AND SUGGESTION

6.1 Conclusion

The purposes of the present study were to investigate the use of Jerusarem artichoke (JA) as an alternative prebiotic for Nile tilapia. The present studies were successfull.

The first experiment was conducted to evaluated and compared whether dietary inulin and JA can use as prebiotic additives on Nile tilapia during the feeding period from first feeding to fingerling size. The results showed that during the first feeding for 4 weeks, dietary inulin had no effects on growth, FCR, survival rate, intestinal villi height and microbiota. Although dietary JA had no effect on growth, FCR, survival rate and intestinal villi height, it altered intestinal microbiota. Dietary JA increased lactic acid bacteria and *Bifidobacteria* whereas it decreased *Vibrio* and yeast and fungi. In addition, dietary both prebiotic had no effects on sex reversal efficiency. After 4 weeks of first feeding, fingerlings fed on inulin at 5.0 g kg^{-1} or JA at either level had better growth performance and survival rate than those fed on the 2.5 g kg^{-1} inulin or control diet. Dietary supplementation with inulin or JA did not affect on whole body proximate composition of Nile tilapia fingerlings. Among nine blood chemistry parameters, inulin at 5.0 g kg^{-1} or JA (5.0 and 10.0 g kg^{-1}) led to increase total protein in blood. Dietary inulin at 5.0 g kg^{-1} and JA at either level increased total immunoglobulin and lysozyme activity. Inulin and JA inclusion diets

increased ACH50 activity and RBC number. Dietary inulin or JA increased the height of intestinal villi and goblet cell number. Supplementation of either inulin or JA increased intestinal lactic acid bacteria and *Bifidobacteria* and decrease *Vibrio* number. These findings demonstrated that dietary prebiotic inulin (only at 5.0 g kg^{-1}) and JA both positively influenced growth performance, health status and survival rate of Nile tilapia during nursing period, suggesting that dietary prebiotic inulin or JA could be used as feed additives since the first feeding of Nile tilapia larvae.

The second experiment was carried out to evaluated and compared the prebiotic effects of dietary inulin and JA on juvenile Nile tilapia. The results demonstrated the beneficial effects of inulin on growth performance and health status in juvenile Nile tilapia. Fish fed the inulin diets exhibited better growth performances than fish fed the control diet. Dietary supplementation with inulin did not affect on whole body proximate composition. Dietary inulin led to increased RBC number, blood glucose, albumin, total protein, magnesium, calcium and iron content. Inulin supplementation at 5.0 g kg^{-1} improved total immunoglobulin, lysozyme activity and ACH50 activity. Dietary inulin increased the height of intestinal villi and goblet cell number. Inulin supplementation affected the population of intestinal microbiota. Supplementation of inulin led to increase intestinal lactic acid bacteria and *Bifidobacteria* and decrease *Vibrio* number. In addition, direct supplementation with JA had superior effects compared to those of inulin at the equivalent inulin levels. These findings indicate that inulin at 5.0 g kg^{-1} or direct supplementation with JA at 5.0-10.0 g kg^{-1} had positive effects on growth and health of Nile tilapia. Thus, both inulin and JA have great potential for use as prebiotics in Nile tilapia feed.

The third experiment was conducted to evaluate and compare the effects of three feed additives including JA, RGP and ISO in the diet on growth performance, immune response and resistance of Nile tilapia fingerlings to *Streptococcus iniae* challenge. The results demonstrated that fish fed the 20.0 JA diet, 20.0 RGP diet and 20.0 ISO had better growth performance, serum lysozyme activity, bactericidal activity and peroxidase activity compared with fish fed the control diet. The experimental fish were subjected to the challenge with *S. iniae* for 12 days. The result showed that daily cumulative mortality of Nile tilapia which were fed on control diet was 80% at 4 days post challenge (dpc). The mortality rate (80%) was observed in fish fed on 40.0 JA at 6 dpc. Fish fed on 20.0 RGP and 20.0 ISO had 30% mortality rate at 7 dpc. However, through challenging period (12 dpc) no fish fed on 20.0 JA died, suggesting that dietary JA at 20.0 g kg^{-1} could contribute to fish being resistant to pathogenic *S. iniae*. Therefore, dietary supplementation with JA could be used comparable as feed additives from abroad, and can be supplementing the diet with JA levels up to 20 g kg^{-1}.

From the three present experiments, the results demonstrated the beneficial effects of inulin and JA on growth performance and health status in Nile tilapia, and could be used as feed additives in all phases of Nile tilapia production including the phases of first feeding to fingerling size and Nile tilapia juveniles. The results of this research will be beneficial to the quality improvement of practical diet for Nile tilapia production, to improve the growth performance and health status of Nile tilapia. Furthermore, direct supplementation with JA had superior effects compared to those of inulin at the equivalent inulin levels in the diet of Nile tilapia could lead to the development of the JA tuber usage, which is a plant that can be grown in the country,

as the prebiotic additive directly. This would reduce the imports of prebiotic from abroad, contributing to the self-reliant development of all stages of Nile tilapia culture industries for sustainable Nile tilapia culture of Thailand.

6.2 Suggestion

The present study suggests that the study of effects of dietary inulin and JA as prebiotic additives on Nile tilapia should be studied and practiced in sex reversal of tilapia farming for the commercial purpose, and continue to grow this fry to the marketable-size tilapia in the farming earthen ponds and/or cages. In addition, the study of effects of dietary inulin and JA as prebiotic additives on Nile tilapia should be studied and practiced in Nile tilapia farming that usually suffered from the outbreaks of disease.

BIOGRAPHY

Mr. Nattanan Tiengtam was born on March 16, 1982 in Trang, Thailand. In 2000, he finished high school from Wichienmatu School, Trang. In 2004, he graduated the Bachelor's degree of Science in Aquaculture from Faculty of Science and Fisheries Technology, Rajamangala Institute of Technology, Trang. In 2007, he graduated the Master's degree of Science in Fisheries Science from Faculty of Graduate School, Kasetsart University, Bangkok. He then worked as a lecturer in Department of Fisheries at Nakhon Phanom University, Nakhon Phanom. He began his Ph.D. studies in School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima and received a scholarship from the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0155/2553). During graduate study, he presented and published his research in several articles including:

Tiengtam, N., Khempaka, S., Paengkoum, P., and Boonanuntanasarn, S. (2016). The effects of dietary inulin on growth performance and health status of juvenile Nile tilapia. Oral presentation at EAAP 67th Annual Meeting of the European Federation of Animal Science, Belfast UK, 29 August - 2 September 2016.

Tiengtam, N., Khempaka, S., Paengkoum, P., and Boonanuntanasarn, S. (2015). Effects of inulin and Jerusalem artichoke as prebiotic ingredients in the diet of juvenile Nile tilapia (*Oreochromis niloticus*). Animal Feed Science and Technology. 207: 120-129.