VALUED OF MEAT QUALITIES AND MATURATION OF HYBRID CATFISH FOR FOOD INDUSTRY

SUPAPORN SATTANG

DOCTOR OF PHILOSOPHY IN FISHERIES TECHNOLOGY
AND AQUATIC RESOURCES
MAEJO UNIVERSITY
2022

VALUED OF MEAT QUALITIES AND MATURATION OF HYBRID CATFISH FOR FOOD INDUSTRY

SUPAPORN SATTANG

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN FISHERIES TECHNOLOGY AND AQUATIC RESOURCES
ACADEMIC ADMINISTRATION AND DEVELOPMENT MAEJO UNIVERSITY
2022

VALUED OF MEAT QUALITIES AND MATURATION
OF HYBRID CATFISH FOR FOOD INDUSTRY

SUPAPORN SATTANG

THIS DISSERTATION HAS BEEN APPROVED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN FISHERIES TECHNOLOGY AND AQUATIC RESOURCES

APPROVED BY **Advisory Committee**

Chair .....
(Professor Dr. Kriangsak Mengamphan)
...../...../.....

Committee .....
(Associate Professor Dr. Doungporn Amornlerdpison)
...../...../.....

Committee .....
(Assistant Professor Dr. Sudaporn Tongsiri)
...../...../.....

Committee .....
(Assistant Professor Dr. Theeraphol Senphan)
...../...../.....

Program Chair, Doctor of Philosophy .....
in Fisheries Technology and Aquatic Resources (Associate Professor Dr. Chanagun Chitmanat)
...../...../.....

CERTIFIED BY THE OFFICE OF .....
ACADEMIC ADMINISTRATION (Associate Professor Dr. Yanin Opatpatanakit)
AND DEVELOPMENT Vice President
...../...../.....

ชื่อเรื่อง การเพิ่มมูลค่าคุณภาพเนื้อและการเจริญพันธุ์ของปลาลูกผสมเพื่ออุตสาหกรรมอาหาร
ชื่อผู้เขียน นางสาวสุภาพร สัตตัง
ชื่อปริญญา ปรัชญาดุษฎีบัณฑิต สาขาวิชาเทคโนโลยีการประมงและทรัพยากรทางน้ำ
อาจารย์ที่ปรึกษาหลัก ศาสตราจารย์ ดร.เกรียงศักดิ์ เม่งอำพัน

บทคัดย่อ

เนื่องจากปัจจุบันมีปริมาณความต้องการปลาหนังน้ำจืดแล่เนื้อในตลาดโลกเพิ่มมากขึ้นทำให้เกิดความต้องการลูกปลาที่มีคุณภาพสำหรับการผลิตสัตว์น้ำสูงขึ้น แต่จำนวนพ่อแม่พันธุ์ปลาหนังน้ำจืดมีจำกัด ส่งผลต่อความต้องการในการปรับปรุงพันธุ์เพื่อการเจริญเติบโต เพิ่มจำนวนการเจริญพันธุ์ และคุณภาพเนื้อของปลาลูกผสม (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) โดยการเสริมน้ำมันปลาน้ำจืด 3 ระดับ คือ ที่ระดับร้อยละ 0 (ชุดควบคุม) 1 และ 2 (น้ำหนัก/น้ำหนัก) ในอาหารปลา เพื่อทดสอบประสิทธิภาพการเจริญเติบโต การเจริญพันธุ์ คุณภาพเนื้อ ได้แก่ คุณภาพสีของเนื้อ คุณค่าทางโภชนาการ องค์ประกอบของกรดไขมัน วิตามินอี คุณภาพซาก สารต้านอนุมูลอิสระ และภูมิคุ้มกันแบบไม่จำเพาะ ก่อนนำเนื้อปลาลูกผสมไปแปรรูปเป็นผลิตภัณฑ์ไส้อั่ว จากนั้นประเมินคุณลักษณะทางประสาทสัมผัสของเนื้อปลาและผลิตภัณฑ์ไส้อั่ว ตลอดจนต้นทุนการผลิตปลาต่อกิโลกรัม

ผลการศึกษา พบว่าในอาหารปลาที่เสริมน้ำมันปลาน้ำจืดมีปริมาณกรดไขมันทั้งหมดสูงกว่าชุดควบคุม อย่างไรก็ตาม ปลาที่เลี้ยงด้วยอาหารปลาเสริมน้ำมันปลาน้ำจืดในช่วงฤดูวางไข่ (เมษายน-สิงหาคม) พบว่าปลาที่เลี้ยงด้วยอาหารปลาเสริมน้ำมันปลาน้ำจืดที่ระดับร้อยละ 1 มีน้ำหนักที่เพิ่มขึ้น และมีอัตราการเจริญเติบโตเฉลี่ยต่อวัน (ADG) ในช่วงฤดูวางไข่ดีที่สุด โดยมีค่าเท่ากับ 354.90 กรัม และ 2.96 กรัม/ตัว/วัน ตามลำดับ มากกว่าชุดควบคุมอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนี้ยังพบอีกว่าในปลาลูกผสมเพศเมียมีปริมาณฮอร์โมน 17 β-estradiol/E_2; 1,577 ± 5.26 pg/mL^{-1} และปลาลูกผสมเพศผู้มีปริมาณฮอร์โมน testosterone/T; 3.28 ± 0.41 ng/mL^{-1} ซึ่งมีความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$) สูงกว่าชุดควบคุมที่ 113.12 ± 43.33 pg/mL^{-1} และ 2.56 ± 0.95 ng/mL^{-1} ตามลำดับ อีกทั้งยังพบจำนวนเซลล์ไข่และน้ำเชื้อของปลาที่พัฒนาได้ดีในปลาที่เลี้ยงด้วยอาหารปลาที่เสริมน้ำมันปลาน้ำจืดที่ระดับร้อยละ 1 และ 2 สูงกว่าชุดควบคุม แต่ไม่แตกต่างทางสถิติ ($p>0.05$) ในช่วงหลังฤดูวางไข่ (กันยายน-ธันวาคม) ส่วนคุณภาพของเนื้อปลา ได้แก่ สีเนื้อ คุณค่าทางโภชนาการ องค์ประกอบของกรดไขมัน ปริมาณวิตามินอี และคุณภาพซากของปลา

ลูกผสมที่เลี้ยงด้วยอาหารปลาที่เสริมน้ำมันปลาน้ำจืดไม่มีความแตกต่างกันทางสถิติกับชุดควบคุม (p>0.05) ซึ่งผลของการเสริมน้ำมันปลาน้ำจืดในอาหารปลาต่อกิจกรรมต้านอนุมูลอิสระของระดับกลูตาไธโอน พบว่าไม่มีความแตกต่างกันทางสถิติ (p>0.05) แต่ระดับมาลอนไดแอลดีไฮด์ของปลาที่เลี้ยงด้วยอาหารปลาที่เสริมน้ำมันปลาน้ำจืดมีความแตกต่างกันทางสถิติ (p<0.05) กับชุดควบคุมในเดือนเมษายนและมิถุนายน และภูมิคุ้มกันแบบไม่จำเพาะ พบว่ามีการเพิ่มระดับของเอนไซม์ไลโซไซม์ของปลาที่เลี้ยงด้วยอาหารเสริมน้ำมันปลาน้ำจืดที่ระดับร้อยละ 1 สูงกว่าชุดควบคุม และมีความแตกต่างกันทางสถิติ (p<0.05) แต่ค่าไนโตรบลูเตตตราโซเลียมไม่มีความแตกต่างกัน (p>0.05) หลังจากการเลี้ยงนาน 8 เดือน ด้านการทดสอบความชอบทางประสาทสัมผัสของผลิตภัณฑ์เนื้อปลาและผลิตภัณฑ์ไส้อั่ว พบว่าผลิตภัณฑ์จากปลากลุ่มที่ได้รับอาหารปลาที่เสริมน้ำมันปลาน้ำจืดที่ระดับร้อยละ 1 มีคะแนนความชอบทางประสาทสัมผัสสูงสุด ตลอดจนอาหารปลาที่เสริมด้วยน้ำมันปลาน้ำจืดที่ระดับร้อยละ 1 และ 2 สามารถลดต้นทุนการผลิตได้ถึง 13.27 และ 8.47 บาท/กิโลกรัม ตามลำดับ เมื่อเปรียบเทียบกับอาหารชุดควบคุม

ผลการวิจัยนี้สรุปได้ว่า การเสริมน้ำมันปลาน้ำจืดที่ระดับร้อยละ 1 ในอาหารปลาในช่วงฤดูกาลวางไข่ สามารถช่วยเพิ่มการเจริญเติบโต การเจริญพันธุ์ของพ่อแม่พันธุ์ปลา เพิ่มภูมิคุ้มกันแบบไม่จำเพาะ ตลอดจนการยอมรับผลิตภัณฑ์เนื้อปลาและไส้อั่ว ลดต้นทุนการผลิตปลาได้ และมีศักยภาพสู่การเพาะเลี้ยงสัตว์น้ำเชิงอุตสาหกรรมอาหารได้ นอกจากนี้ ได้จดอนุสิทธิบัตรสูตรอาหารปลาเสริมน้ำมันปลาและผลิตภัณฑ์ไส้อั่วจากปลาลูกผสม

คำสำคัญ : การเจริญเติบโต, การเจริญพันธุ์, การประเมินทางประสาทสัมผัส, คุณภาพเนื้อ, ต้นทุน, น้ำมันปลาน้ำจืด, ภูมิคุ้มกัน, อุตสาหกรรมอาหาร

Title	VALUED OF MEAT QUALITIES AND MATURATION OF HYBRID CATFISH FOR FOOD INDUSTRY
Author	Miss Supaporn Sattang
Degree	Doctor of Philosophy in Fisheries Technology and Aquatic Resources
Advisory Committee Chairperson	Professor Dr. Kriangsak Mengamphan

ABSTRACT

According to the increasing demand of freshwater catfish fillets in the world market, the demand of quality fry for fish production is expanding. A small amount of freshwater hybrid catfish broodstock effects on the breeding requirements to growth, increasing number, reproduction and meat quality of the hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*). This investigation was determined the effect of fish oil on various aspects of fish products. The brood fish was fed with fish feed pellet supplemented with 3 levels of freshwater fish oil including 0% (control), 1% and 2% (w/w) to improve growth efficiency, reproductive, meat qualities of fillet such as color quality, proximate composition, fatty acid composition, vitamin E content, carcass quality, antioxidants, nonspecific immunity and sensory evaluation of fillet and Sai Aua product (Northern Thai spicy sausage) as well as the cost of fish production.

The results showed that the fish diet supplemented with freshwater fish oil was higher fatty acid content than the control. The fish was fed with fish diet supplementd with freshwater fish oil during the spawning season (April-August). Fish diet supplemented with 1% freshwater fish oil had higher weight gain and average daily gain (ADG) in during the spawning season of 354.90 g and 2.96 g/fish/day, respectively, significantly more than the control (p<0.05). Female fish was 17 β-estradiol /E_2 of 1,577 ±. 5.26 pg/mL^{-1} and male fish was testosterone /T of 3.28 ± 0.41 ng/mL^{-1} which were higher values significant difference than the control (p<0.05) of 113.12 ± 43.33 pg/mL^{-1} and 2.56 ± 0.95 ng/mL^{-1}, respectively. In addition, the

number of well-developed oocytes and spermatocytes of fish diet supplemented with 1% and 2% freshwater fish oil were higher than the control. However, there were not significant differences during post-spawning season (September-December). The meat qualities such as color quality, proximate composition, vitamin E content in fish fillet and carcass quality in the fish sample treated with fish at diet supplemented with freshwater fish oil were not different with the control sample. The effects of freshwater fish oil supplementation in fish diet on antioxidants of glutathione was not different ($p>0.05$), but malondialdehyde levels of fish diet supplemented with freshwater fish oil were different with the control in April and June. Non-specific immunity was found that fish diet supplemented with freshwater fish oil at 1% was higher lysozyme value than the control sample and significantly different ($p<0.05$), but Nitroblue tetrazolium levels was not different after pisciculture 8 months. For the sensory evaluation of fillet and Sai Aua products, the product from fish feeding with fish diet supplemented with freshwater fish oil at 1% had the highest sensory score by panelists. In addition, fish diet supplemented with freshwater fish oil at 1% and 2% can reduce costs to produce fish product of 13.27 and 8.47 baht/kg, respectively, compared to control diet.

It can be concluded from results of this research that freshwater fish oil supplementation at 1% in fish diet during the spawning season can increase the growth, reproductive of the fish broodstock, increase non-specific immunity, meat qualities as well as acceptance of fish meat and Sai Aua products. The reducing cost of fish production and support for aquaculture food industrial were evaluate. The petty patents were accepted for fish diet supplemented with fish oil freshwater and Sai Aua.

Keywords : Growth, Reproduction, Sensory evaluation, Meat quality, Feed cost, Freshwater fish oil, Immunity, Food industry

ACKNOWLEDGEMENTS

This research was supported by the Thailand Science Research and Innovation (TSRI) for awarding me a scholarship under the program of Research and Researcher for Industry (RRi) and S&P Syndicate Public Co., Ltd. as part of the Ph.D. Program (Project ID: PHD60I0002) and grateful for the financial support from Graduate School, Maejo University for a scholarship under products development grant for a Ph.D. Student (Project name: Buk Siam hybrid catfish (Pangasianodon gigas x Pangasianodon hypophthalmus, F2) ready to eat).

I would like to express my sincerest gratitude and appreciation, Professor Dr. Kriangsak Mengamphan, Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chairperson of my Advisory Committee Chairperson, for his continuous valuable guidance, constructive, criticism, excellent instruction, overwhelming support, kindness and encouragement throughout this research. This thesis would not have been accomplished without his help.

The author wishes to express his gratitude and deepest appreciation to My co-advisor committee Associate Professor Dr. Doungporn Amornlerpison, Assistant Professor Dr. Sudaporn Tongsiri from Faculty of Fisheries Technology and Aquatic Resources and Assistant Professor Dr. Theeraphol Senphan from Faculty of Engineering and Agro-Industry, Maejo University. I would like to thank them for all their suggestions, constructive, kindness, words of encouragement and all their help during my doctoral research.

I wish to express my gratitude to Professor Dr. Piamsak Menasveta, Chulalongkorn University, for serving as my chairperson of the examination committee and for his kindness, valuable guidance, and suggestions of research work that fulfilled my dissertation.

I would like to thank you Assistant Professor Dr. Nissara Kitcharoen, who served as a committee on my defenses examination and suggestions and constructive inputs in this study. I would like to thank you Associate Professor Dr. Chanagun Chitmanat, I am grateful for all his support in my research particularly for supplying all the needed

chemicals for my experiments immunity and review the English language accuracy of the dissertation.

To Professor Dr. Dusan Palic of the Chair for Fish Diseases and Fisheries Biology, the Faculty of Veterinary Medicine, Ludwig Maximilian University of Munich, my research supervisor during stay in Germany as a research exchange student, for his support and advice of this study and research visits on the immune system, distinguish fish blood leukocytes, striped cell culture and gene expression in fish, I am always grateful for all his support in my research particularly for supplying all the suggestion, check the correctness and edit the manuscript and published in a Aquaculture Reports Journal.

Special thanks Mr. Thiravat Rattanapot (Ph.D.) staff of Center of Excellence in Agricultural Innovation and Miss Arporn Panase, Ph.D. student of Faculty of Science, Maejo University, for all their intensive teaching in the part of antioxidant and non-specific immunity, for their support, expertise, suggestion, love and friendship.

I give thanks to Mr. Thanathat Suksri, Miss Kanidta Matan, Mr. Korn Mengumphan, Mr. Pongsakorn Noimoon, Miss Kanokwan Nakkham, Miss Rattanaporn Janthip, Mr. Uten Jamjai, Miss Thapanee Pholdee, Mr. Aphidet duangkhamming, Miss Jiraporn Sripum and Mr. Nattakul Wongka, Graduate students and staff of Faculty of Fisheries Technology and Aquatic Resources, Maejo University, for their helping in the part of fish culturing, their expertise, data storage of fish growth, fish diet production, support, suggestion, love and friendship.

And also thanks, Mrs. Wanvisa Hussein, Mr. Dominik Von La Roche, Miss Worrayanee Thammatorn and Mr. Moyan Hu, Graduate students at the laboratory, the Faculty of Veterinary Medicine, Ludwig Maximilian University of Munich, Germany, for their helping in the part of study and research on immune system, distinguish fish blood leukocytes, striped cell culture and gene expression in fish, their expertise, understanding and friendship.

Finally, I wish to express my deepest gratitude to my mother; Mrs. Buon Sattang and my father; Mr. Phasit Sattang and to all my family members for their love, encouragement, unfailing support and faith in me through to the end of my study.

Supaporn Sattang

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1
INTRODUCTION

Recently, significant increasing in demand for freshwater catfish fillet was observed in global market, shifting from U.S. and EU to China, India and ASEAN markets (Food and Agriculture Organization of the United Nations (FAO), 2017). Thailand is one of most important ASEAN member countries importer of *Pangasius* spp. (most frequently *Pangasianodon hypopthalmus* traded as "Pangasius Dory fillet") from Vietnam, reaching over 10,600 tons of December 2019 (Department of Fisheries, 2019). Significant growth of the prices for freshwater catfish fillet market was recognized in period from 2017-2019, strongly driving the need for brood stock of high quality and quantity to produce enough fingerlings for catfish aquaculture. Even though COVID-19 pandemic caused an unexpected reduction in the 2020 demands, it is likely that freshwater catfish aquaculture industry will soon recover and continue to grow in longer term (FAO, 2020). Therefore, providing optimal growth and sexual maturation conditions with adequate diets and husbandry is crucial for achieving higher gamete production to meet both current and future demand of quality fingerlings catfish for aquaculture and food industry.

Industrial processing from whole catfish to frozen fish fillets produces 40-60% of by-products such as bone, fish fat/oil, viscera, and skin. The offal is not usable for human consumption and is frequently used as raw material to produce fish meal and oil, or in some cases fertilizer (Silva and Dean, 2001). Recently trending "added value" seafood products are emphasizing their content and benefits of omega 3, 6 and 9 fatty acids in human diet. It was shown recently that fish oil from the Maejo Buk Siam freshwater hybrid catfish (*Pangasianodon gigas* x *P. hypophthalmus*) contains favorable ratios of fatty acids (saturated: monounsaturated: polyunsaturated as 13.83: 76.64: 9.52 g/100 g, respectively). Furthermore, content of omega 3, 6 and 9 fatty acids was reported as 0.73, 8.42 and 42.28 g / 100 g, respectively (Sattang *et al*., 2018a). However, recent trends in "added value" seafood products are emphasizing their content and benefits of omega 3, 6 and 9 fatty acids

in human diet (Stoneham *et al*., 2018). Unsaturated fatty acids (HUFAs: docosahexaenoic acid, DHA, 22:6n-3; eicosapentaenoic acid, EPA, 20:5n-3; and arachidonic acid, ARA, 20:4n-6), are required for proper growth and reproduction of fish (Sargent *et al*., 1999; Luo *et al*., 2019). However, the downside of increased demand for fish meal/oil is reflected on the increased cost and decreasing sustainability of its use in commercial diet formulations (Alhazzaa *et al*., 2019). Using of fat from aquaculture to supplement fish diets may present as viable alternative ingredients (Rattanapot *et al*., 2018a).

Previously report, Tilapia fed with two different fish oil (Anchovy and cod liver oil) supplements showed differences in growth parameters (Hunt *et al*., 2018). Furthermore, male European sea bass (*Dicentrarchus labrax*) showed better survival rates and reproductive performance when fed two commercial pellet diet enriched with polyunsaturated fatty acid (PUFAs) during the reproductive season compared to wet diet (Asturiano *et al*., 2001). As arachidonic acid is a critical precursor for prostaglandin synthesis. It plays an important role in regulation of sex hormones involved in development and maturation of sperm and eggs in fish (Ann Sorbera *et al*., 2001; Wade and Van Der Kraak, 1993). Fish oil from the Maejo Buk Siam freshwater hybrid catfish (*P. gigas* x *P. hypophthalmus*) contains favorable ratios of fatty acids (saturated: monounsaturated: polyunsaturated as 13.83: 76.64: 9.52 g/100g, respectively) and content of omega 3, 6 and 9 fatty acids was reported as 0.73, 8.42 and 42.28 g/ 100 g, respectively (Sattang *et al*., 2018a). It was also reported that hybrid catfish responds to dietary supplementation of freshwater fish oil (1.5%, total fat 7.6%) with increased growth performance, but it had no effect on the prevention of oxidation. Because it had no effect on the oxidative defense and it could not reduce the level of lipid MDA in the fish plasma. (Rattanaphot *et al*., 2018a). However, growth performance of the Nile tilapia treated with feed supplemented with 1% and 1.5% freshwater fish oil dramatically increased and can be reduced oxidative stress, increased endogenous antioxidants and improved resistance to poor environmental conditions (Rattanaphot *et al*., 2018b; Amornlerdpison *et al*. 2019). In order to utilize fish oil efficiently, it is necessary to determine optimal, cost-effective and sustainable levels of supplementation that will enhance growth and reproductive performance in

the hybrid catfish broodstock. In addition, hybrid catfish crossbreeding between *Pangasius larnaudii* (male) and *P. hypophthalmus* (female) provided the highest fertilization rate (95.33%) and hatching rate (87.33%). After experimental raising in cage, it was found that good growth and high survival rate (93.78%) were observed, so it has the potential to produce hybrid catfish fry (Sattang, 2015).

Currently, there is no available information about effects of freshwater fish oil on hybrid catfish reproduction. Therefore, the aim of this study was to determine effects of low level (1 or 2%) freshwater fish oil dietary supplementation on growth, reproductive condition, antioxidant, non-specific immunity, fish flesh qualities and consumer acceptance of the hybrid catfish (*P. larnaudii* x *P. hypophthalmus*) in an effort to optimize brood stock husbandry in freshwater hybrid catfish aquaculture industry.

Objectives

1. To study the effect of freshwater fish oil dietary supplementation on growth and reproduction condition of the hybrid catfish (*P. larnaudii* x *P. hypophthalmus*) during and after spawning season.

2. To study the effect of freshwater fish oil dietary supplementation on antioxidant, non-specific immunity, fish flesh qualities and consumer acceptance of the hybrid catfish.

3. To develop valued fish products from hybrid catfish of 2 products, which are fish fillet and Sai Aua (Northern Thai Spicy Sausage).

4. To study the cost of fish diets.

5. To promote the aquaculture industry in the production of fish fry of freshwater hybrid catfish for sufficient quantities and sustainable.

Expected Benefits

1. Novel knowledge on reproductive and efficiency of brood stock and fry production of hybrid catfish.

2. The effect of freshwater fish oil dietary supplementation on growth, reproductive, fish flesh quality, antioxidant and non-specific immunity of hybrid catfish.

3. Acceptance fish fillet and Sai Aua products of fish healthy food, ready-to-eat menus for marketing.

4. Value added of fish diets using by product materials to develop health products from higher value from fish meat.

5. Industrial entrepreneurs apply the research results to increase the economy and stability for the business sector.

6. Farmers of hybrid catfish sustainably generate more income / year.

7. Submitted at least 1 petty patent.

8. Published 3 academic articles.

CHAPTER 2
REVIEW OF LITERATURE

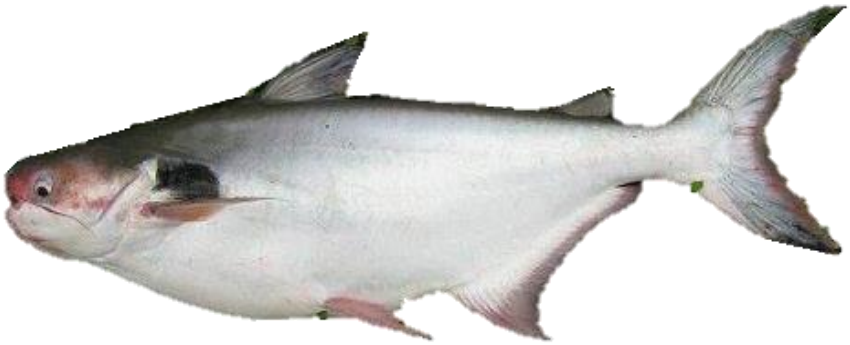
1. The biology of stripped catfish (*Pangasianodon hypophthalmus* Sauvage, 1878)

Pangasianodon hypophthalmus is a member of the Pangasiidae family in the order Siluriformoes which includes all catfish (Roberts and Vidthayanon 1991). Originally, known as a riverine freshwater fish, this species is limited to the Mekong River, the Chaopraya River and possibly the Mekong Basins in Cambodia, as well as the Lao People's Democratic Republic, Thailand and Viet Nam, together with the Ayeyarwady Basin of Myanmar. The species has a variety of common English names including Sutchi catfish, iridescent shark-catfish, and striped catfish. It is called 'Pa sooai' and 'Pa sooai khaeo' in Laotian, 'Pla Sawai' in Thai language, 'Pra' and 'Trey pra' in Khmer and 'Cá Tra' in Vietnamese (Ish and Doctor, 2005). The traditional development of capture-based aquaculture for this species, particularly in Viet Nam and to a lesser extent in Thailand and Cambodia, probably began because it is a prolific spawner, producing a relatively large numbers of larvae that are easily harvested from flowing rivers. Mature fish can reach a maximum standard total length of 130 cm and up to 44 kg in weight. This species is benthopelagic, typically living within the range of pH values of 6.5-7.5 and 22-26 °C. Females take at least two or three years to reach sexual maturity in captivity (being over 1.5-3 kg in weight), while males often mature in their second year, probably taking about the same amount of time as in the wild (Ish and Doctor, 2005). The seasonal spawning takes place at the beginning in of the season May-June (Poulsen *et al*., 2008). The *P. hypophthalmus* in Puerto Rico may be spawned from June through September when water temperatures are above 27 °C. Individuals may be induced to spawn more than once during this time. Females can produce as many as 60,000 eggs/kg at this stage. At the time of ovulation eggs are around 1.5 mm in diameter (McGee, 2014).

Figures 1 Characteristics of *Pangasianodon hypophthalmus* (Sauvage, 1878).
Source: Sattang (2015)

2. The biology of black ear catfish (*Pangasius larnaudii* Bocourt, 1866)

Catfishes of the genus Pangasius are the members of the family Pangasiidae (Nelson, 1984). *P. larnaudii* is a species of catfish native to the Mekong and Chao Phraya river basins in Myanmar, Cambodia, Laos, Thailand, and Vietnam. This species migrates up and down the Mekong with the wet and dry seasons. Characteristics of *P. larnaudii* have anal soft rays 28 - 32. A large black spot above the base of the pectoral fin and a black longitudinal stripe along each caudal lobe. Dorsal and pectoral fins with a strong spine and a long, filamentous rays with 13-17 gill rakers in first arch. This *P. larnaudii* feeds on shrimps, small fishes, gastropods and plants (Roberts and Vidthayanon, 1991). *P. larnaudii* is used, along with hybrids, in aquaculture in the native range, but no records of aquaculture use outside of Southeast Asia were found (Froese and Pauly, 2018). Mature fish can reach a maximum standard total length of 130 cm SL (Baird *et al*. 1999). Furthermore, Bardach (1958) suggests that their spawning habitats are in the Mekong River near Stung Treng whereby larvae reach the Bassac River (southern Cambodia) within 6 – 8 days. *P. larnaudii* was observed to migrate over the Khone Falls in spawning conditions leading to speculation of spawning grounds above the Khone Falls. Young fish feed in floodplain habitats during the rainy season. During the dry season, they inhabit deep pools in the Mekong mainstream (Kratie-Stung Treng reaches).



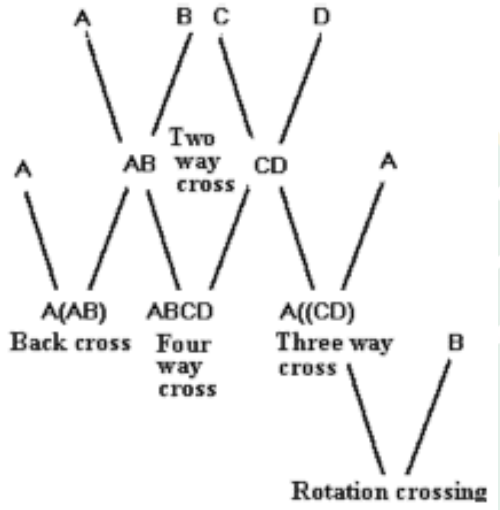
Figures 2 Characteristics of *Pangasius larnaudii* (Bocourt, 1866).
Source: Sattang (2015)

3. The crossbreeding and hybridization in aquaculture

3.1 Hybridization and crossbreeding

Hybridization is the mating of genetically differentiated individuals or groups and may involve crossbreeding within a species (also known as line crossing or strain crossing) or crosses between separate species (Rahman *et al*., 2013). This breeding technique is used by aqua culturists to produce higher aquatic animal quality, increase disease resistance, improve environmental tolerance, meat qualities, and manipulate sex ratios, as well as various other traits (overall improvement, hybrid polyploidization, experimental hybridization, and unplanned or accidental hybridization) to make fish more profitable to raise (Rahman *et al*., 2013; Bartley *et al*., 2000). At times, the goal of crossing species or lines is not to capitalize on heterosis but rather to combine certain traits, such as the fast growth of pure striped bass with the hardiness of their smaller relatives, the white bass. Or, in the historic Taiwanese red tilapia, the red coloration of Mozambique tilapia with the faster growth and superior dress out of Nile tilapia. Hybrids of the carps *Labeo fimbriatus* and *Catla catla* have been produced for decades to combine the relatively small heads of the former with the deep bodies of the latter, resulting in greatly improved meat yield (Lutz., 2021).

Falconer (1981) reported that a cross should be mixed with two or more pure breed lines and continue to improve the good characteristics of the pure breed. By selection method to obtain stable traits first, then crossbreeds were performed to find good traits, which also increased heterozygous in the 1st generation of hybrid populations. There are several methods such as two-way cross, backcross, three-way cross, four-way cross (double cross) and rotational crossing (Figures 3).



Figures 3 Systems for crossbreeding.
Source: Christensen (2003)

3.2 Some recent research

In a recently published study, researchers in Cameroon evaluated reciprocal hybrids between their native catfish *Clarias jaensis* and the non-native *Clarias gariepinus.* Crossing female *C. jaensis* with male *C. gariepinus* resulted in the highest fertilization and hatching rates, as well as the highest larval survival rate and lowest rate of deformities, while the reciprocal cross demonstrated poor performance. Growth of the *C. jaensis* x *C. gariepinus* hybrid (the female parent should always be listed first) was comparable to that of pure *C. gariepinus* from day

4 through day 32 but survival was slightly lower, probably because of greater size variation within the offspring (Tiogué *et al.*, 2020).

Moreover, there were research of catfish, 1) reciprocal backcross hybrid catfish (RCBC), *Pangasianodon gigas* (female) x F_1 hybrid catfish (male) 2) backcross hybrid catfish (BC), *P. gigas* (male) x F_1 hybrid catfish (female) 3) *P. gigas* and 4) F_1 hybrid catfish, *P. gigas* (male) × *P. hypophthalmus* (female) were reared in net cages for 5 months. The results showed that, weight gain (WG) and average daily weight gain (ADG) of *P. gigas* were the highest amount 34.45 g and 0.20 g/fish/day, respectively. The length gain (LG), average daily length gain (ADL) and specific growth rate (SGR) were highest among the RCBC (10.66, 0.062 and 2.34, respectively). While the survival rate of BC, RCBC and F_1 hybrid catfish were higher than *P. gigas* (55, 48.33, 36.67 and 30%, respectively) (Panase and Mengumphan, 2015).

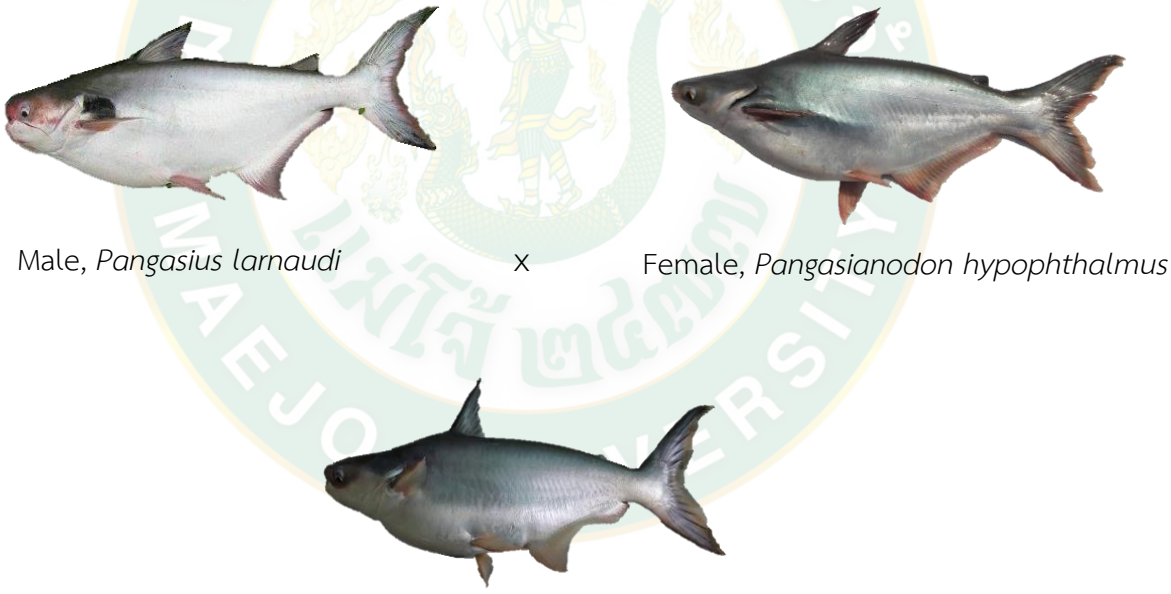
3.3 A cautionary note

One serious problem associated with the use of hybrids in aquaculture involves the potential for genetic impacts on wild populations. In many instances, hybrids are not only fertile, but also fully capable of interbreeding with wild populations of the parental stocks they were derived from. This inadvertent introgression can result in negligible impacts in some instances, but the possibility for pronounced harm to wild populations and their habitats cannot be dismissed and should not be underestimated. Risk analysis should be applied on a case-by-case basis (Lutz., 2021).

4. The performance of hybrid catfish

Hybrid catfish F_1 (Male, *Pangasius larnaudii* x Female, *Pangasianodon hypophthalmus*) was developed for growth performances, survival rate and external characteristics improvement. The results showed that the survival rate, weight gain, length gain, average daily growth (ADG), specific growth rate (SGR) and protein efficiency ratio (PER) of this hybrid were higher than purebred *P. larnaudii*. The survival

rate of this hybrid catfish was lower than *P. larnaudii*, but more than *P. hypophthalmus*. The total length of fish was determined by the external characteristics in 6 inches fingerlings. The external characteristics of this hybrid catfish were similar to the *P. larnaudii* by had black spot on pectoral fin, but the black spot color was lighter than the *P. larnaudii* (Mengumphan *et al*., 2017). The hybrid catfish (*P. larnaudii* x *P. hypophthalmus*) of 1 year old with average weight of 1.54 kg displays high growth, when was fed of 1% fish oil supplement in fish feed, white pink meat color and protein in the fish flesh around 18.95-19.51% dry matter basis (Sattang *et al*., 2018a). Moreover, freshwater fish oil supplementation in fish feed during spawning season supported increased growth and enhanced reproduction indicators of hybrid catfish brood stock in aquaculture (Sattang *et al*., 2021).



Figures 4 Cross breeding of hybrid catfish.

Source: Sattang (2015)

5. Lipids

The lipids are a heterogenous group of substances found in plant and animal tissues, which share the property of being relatively insoluble in water, and soluble in organic solvents, such as ether, chloroform, and benzene. In this respect, dietary lipids may be used to spare the more valuable protein for growth. In particular, free fatty acids derived from triglycerides (fats and oils) are the major aerobic fuel source for energy metabolism of fish muscle.

1) Lipids are essential components of all cellular and subcellular membranes (lipid classes that are involved include the polyunsaturated fatty acid containing phospholipids, and sterol esters).

2) Lipids serve as biological carriers for the absorption of the fat-soluble vitamins A, D, E and K.

3) Lipids are a source of essential fatty acids, which in turn are essential for the maintenance and integrity of cellular membranes, are required for optimal lipid transport (bound to phospholipids as emulsifying agents), and are precursors of the prostaglandin hormones.

4) Lipids are believed to play a role as a mechanical cushion/support for the vital body organs, and aid in the maintenance of neutral buoyancy.

5) Lipids are a source of essential steroids, which in turn perform a wide range of important biological functions (i.e., the sterol cholesterol is involved in the maintenance of membrane systems, for lipid transport, and as a precursor of vitamin D3, the bile acids, and the steroid hormones - androgens, estrogens, adrenal hormones, and corticosteroids).

6) From a feed technology viewpoint, lipids act as lubricants for the passage of feed through pellet dies, as substances which reduce the dustiness of feeds, and play a role in feed palatability (Tacon, 1987).

6. Freshwater fish oil (FFO)

Previous study, Amornlerdpison *et al*. (2010) found that 100 g of crude oil from the hybrid catfish group *Pangasius* sp., contained 44.00 g of saturated fatty acids and 51.09 g of total unsaturated fatty acid consisting of monounsaturated fatty acid (MUFA) 37.60 g, omega-9 fatty acids such as oleic acid in the amount of 34.47 g. Polyunsaturated fatty acid (PUFA) has the total amount of 13.49 g. It is an omega-3 fatty acid, including alpha-lipoic acid (ALA) 0.93 g, eicosapentaenoic acid (EPA) 0.65 g, and docosahexaenoic acid (DHA) 2.72 g and omega-6 fatty acids including linolenic acid (**γ**-Linolenic acid, cis-9,12-Linolenic acid) 8.47 g.

Fish oil from the Maejo Buk Siam freshwater hybrid catfish (*Pangasianodon gigas* x *Pangasianodon hypophthalmus*) contains favorable ratios of fatty acids (saturated: monounsaturated: polyunsaturated as 13.83: 76.64: 9.52 g/100 g, respectively) and contents of omega 3, 6 and 9 fatty acids were reported as 0.73, 8.42 and 42.28 g/100 g, respectively (Sattang *et al*., 2018b). Importance of essential fatty acids in fish diet has been reported (Sargent *et al*., 1999). Unsaturated fatty acids (HUFAs: docosahexaenoic acid, DHA, 22:6 n-3; eicosapentaenoic acid, EPA, 20:5 n-3; and arachidonic acid, ARA, 20:4 n-6), are required for proper growth and reproduction of fish (Sargent *et al*., 1999; Luo *et al*., 2019). Unsaturated fatty acids from fish oil, cod liver oil and squid liver oil contain essential fatty acids (EFA) which serve as precursors for prostaglandins synthesis (Saini and Keum, 2018). Prostaglandins are involved in different regulatory pathways including contractions of smooth muscles in the uterus, ovulation, hormone secretion, and regulation of blood pressure (Takahashi *et al*., 2018).

Sattang *et al*. (2021) reported that the growth performance (WG and ADG) of hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) during spawning season (August) treated with fish diet mixed with 1% freshwater hybrid catfish oil (FFO, *P. gigas* x *P. hypophthalmus*) was higher than control ($p<0.05$). This is in accordance with previous work of Rattanaphot *et al*. (2018b) who reported significant increase in growth of hybrid catfish (*P. gigas* x *P. hypophthalmus*) treated with FFO (*Pangasius* sp.) supplements containing high amount of omega 9 fatty acids

(four times higher than marine fish diet). In addition, recent research report of Tongmee *et al*. (2021) found that FFO (*P. gigas* x *P. hypophthalmus*) treatment inhibited the secretion and mRNA expression of the pro-inflammatory cytokines IL-6, IL-1β, TNF-α. FFO also reduced apoptotic body formation and DNA damage and enhanced the immune response by modulating the cell cycle regulators p53, cyclin D2 and cyclin E_2.

7. Essential fatty acid requirement

In view of the inability of animals to synthesize de novo fatty acids of the n-6 and n-3 series, these fatty acids must be supplied in a ready-made form within the diet. For land animals, the linoleic (n-6) series has been found to have the highest essential fatty acid (EFA) activity, with the linolenic (n-3) series having only partial EFA activity. It follows, therefore, that the predominant fatty acids (PUFA) in the tissues of land animals belong to the linoleic series, namely 18:2 n-6 (linoleic acid) and 20:4 n-6 (Arachidonic acid) (Tacon, 1987).

By contrast, the predominant PUFA in the tissues of fish and shrimp belong to the linolenic (n-3) series, and this applies to freshwater and marine fish alike. The concentration of n-6 PUFA in the tissues of fish is generally low, although higher levels are reported in freshwater fish species. This is perhaps not surprising if one considers that the diet of freshwater fish contains a component derived from terrestrial sources, and consequently rich in n-6 series fatty acids. It is generally believed that the n-3 series fatty acids permit a greater degree of unsaturation a requirement for greater membrane fluidity, flexibility, and permeability at low temperatures. In fact, it is generally believed that the dietary requirement (preferential) of fish for n-3 series EFA, over n-6 series, is fundamentally due to the low water temperature of their aquatic environment (as compared with mammals). In fact, the lower the water temperature, the greater the incorporation of n-3 series PUFA in the tissues. Apart from the differences in n-6 PUFA content of the tissues of

freshwater and marine fish species, freshwater fish also generally have higher tissue concentrations of the shorter chain PUFA n-3 series (Tacon, 1987).

With the exception of strict carnivorous fish species, fish are able to chain elongate and further desaturate 18:2 n-6 or 18:3 n-3 (depending on the fish species) to the corresponding highly unsaturated fatty acid (HUFA): 20:4 n-6 in the case of the n-6 series, and 20:5 n-3 or 22:6 n-3 in the case of the n-3 series. It is generally believed that these HUFA are responsible for the key metabolic functions ascribed to the EFA. In fact, for most fish species, HUFA have greater EFA activity than the corresponding basic unit (18:2 n-6 or 18:3 n-3) (Tacon, 1987). On a general basis, the dietary EFA requirement of fish have been found to increase with increasing in dietary lipid level and/or with decreasing water temperature (Castell *et al*., 1972). Channel catfish is requirement alpha-linolenic acid (18: 3 n-3) less than 1% (Robinson and Lovell, 1978).

8. The fish oil supplement in fish diet

The trend to increase lipid content of fish feeds for fish to optimize growth, feed conversion and protein utilization has caused an increase in demand for fish oil. The global production of fish oil (FO) based on fisheries landing is stable, and it is estimated that by 2020 the fish feed industry will require at least 50% of total world production of fish oil (Barlow, 2000; Montero *et al*., 2005). Aquafeeds are made using fish oil as the main source of lipids, since it has been rapidly available and enjoys a high content of n-3 HUFA (highly unsaturated fatty acids), which is essential fatty acidy for fish. On the other hand, capture fisheries will not be able to meet the increasing demand of fish oil due to the fact that sustainable levels are threatened by overfishing, climate changes and increasing demand from other sectors (Sargent and Tacon, 1999).

Consequently, there is increasing interest in use of alternative oils in both marine and freshwater aquafeeds to partly substitute and decrease the dependence on FO. Fish cannot synthesize the essential fatty acid (EFA), such as linoleic (18:2 n-6) and linolenic acid (18:3 n-3). Substitution of fish oil by alternative

lipid sources seems possible if the EFA requirements are met (Sargent *et al*., 1999). Therefore, substitution of FO using different alternative oils would have a positive impact both on demand for FO and its price (Piedecausa *et al*., 2007).

Hunt *et al*. (2018) determined the effect of fish oil (FO) and cod liver oil (CLO) as the dietary lipid sources on the growth performance, feed utilization and fatty acid (FA) composition of *Oreochromis niloticus*. Results revealed that the source of lipid significantly effects on (p<0.05) final body weight, live body weight and daily growth rate. Fish fed FO diet showed lower lipid deposition and higher protein amount in muscle tissues (p<0.05). In fillet fish fed FO diet, DHA (docosahexaenoic acid), omega 3 and omega 6 PUFA were the higher when compared with fish fed CLO-based diet (p<0.05). However, fish fed CLO contained diet showed significantly higher liver fat (20.20±0.22) than fish fed FO diet (13.88±0.22) (p<0.001). It was also reported that hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) responds to dietary supplementation of freshwater fish oil (1.5%, total fat 7.6%) with increased growth performance (Rattanaphot *et al*., 2018a). Supplementation of catfish brood stock (*Ictalurus punctatus*) diets with 10% fish oil increased spawning success, fecundity, total egg volume (matrix removed), individual egg weight, eggs·spawn^{-1} (volumetric), total egg lipid concentration, hatching success, and fry survival compared to a control diet with 4% fish oil (Sink and Lochmann, 2008).

It is well documented that fish consumption have a positive impact on human health as well as risks related to cardiovascular disease (CVD) and coronary heart disease (CHD) (Mohebi-Nejad and Bikdeli, 2014). Therefore, the relations between fish as food and human health are strongly correlated with the fatty acid composition of the diet (Kris-Etherton *et al*., 2002). The fatty acid content of fish can be changed with diets containing different lipid sources (Naylor *et al*., 2009).

9. Fish reproduction

9.1 Oogenesis and maturation

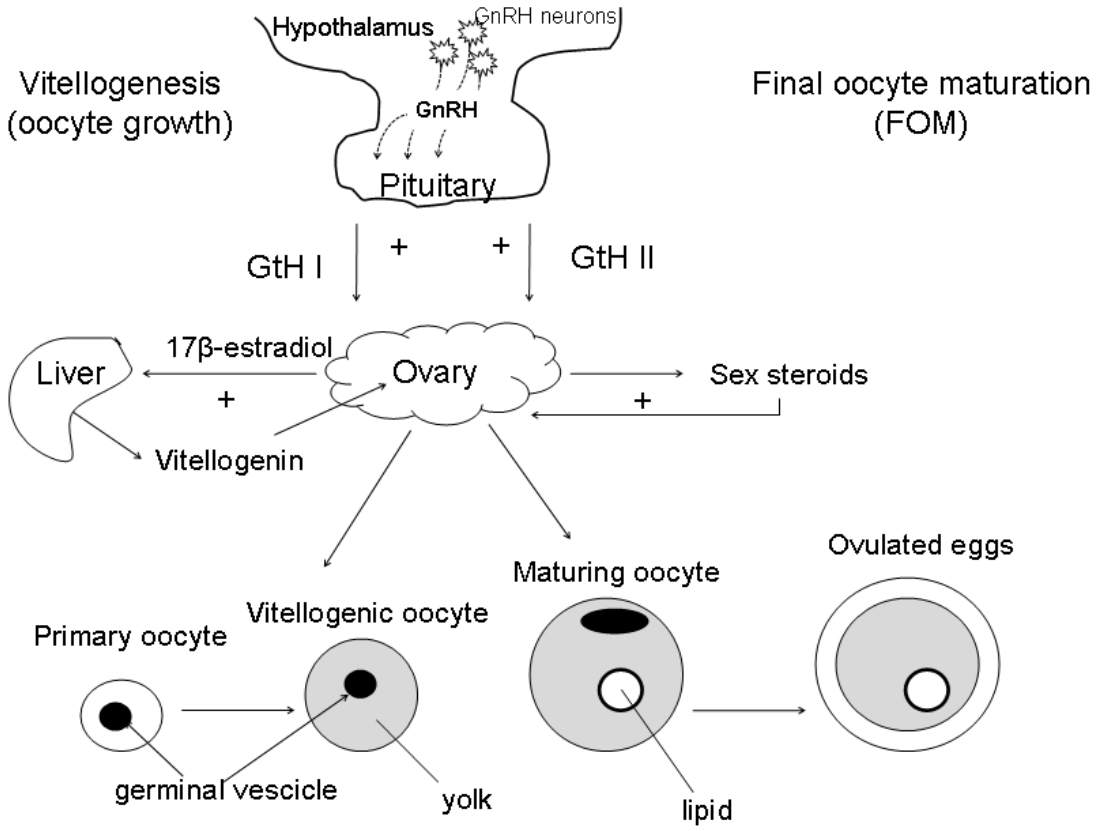
At first, nucleases of primordial germ cells (PGC) are increased, and more organelles are present in the cytoplasm, the cells then continue growing. The granular endoplasmic reticulum is well developed, forming concentric layers and whorls around each mitochondrion. The oogonia multiply rapidly by successive mitosis before the start of meiosis, one oogonium starts a process of successive divisions and formed oogonia remain linked together with a few surrounding somatic, or pre-granulosa cells (Le Menn *et al*., 2007). The first three nucleus stages occur during stage I of primary oocyte development. The nucleolus envelope is almost smooth and nuclear pores are regularly spaced. In stage II, the nucleus exhibits a new feature characteristic of the diplotene stage of the prophase of the first meiotic division. At the end of pre-vitellogenic stage II, the oocytes contain all the molecules and organelles necessary for its subsequent endocytic and exocytic activities during oocyte vitellogenesis. Ovarian follicle enlargement occurs in stage III, while the nucleus remains in the diplotene stage. During this stage, the oocytes accumulate in the yolk containing nutritional reserves (vitellogenesis) from the blood stream needed for embryo development and completes differentiation of its cellular and cellular envelopes. Vitellogenesis is a process whereby yolk eggs are produced; it gives both the synthesis of vitellogenin by the liver and its uptake by growing oocytes, where it is stored as yolk to serve as the food source of the developing embryos (Tyler, 1991). Vitellogenesis is also the incorporation of vitellogenin protein by oocytes and the processing of conversion into yolk proteins (Le Menn *et al*., 2007). During this time, yolk precursors (vitellogenins) are taken up and stored within yolk granules in the oocyte. The yolk serves as a nutrient source for growing embryos and larvae once the eggs have been ovulated and fertilized.

Vitellogenesis starts with production in response to the central nervous system induces a cascade of neurohormones leading to the secretion of the gonadotropin-releasing hormone (GnRH) by specialized neurons. The GnRH acts on

pituitary cells, which secrete the follicle-stimulating hormone (FSH). The FSH signal is mediated by a specific receptor located on thecal and granulose cells. This leads to the synthesis of sexual steroid hormones, such as 17β-estradiol (E_2). E_2 is secreted into the theca blood vessels to reach the blood stream. In response to E_2, specific receptors in hepatocytes mediate the synthesis and the release of vitellogenins (Vtgs) into the blood. These are considered, the main yolk precursors in the plasma (Le Menn *et al*., 2007). The Vtgs reaches the oocyte surface by passing first between the thecal cell, through the basement lamina and between the granulosa cells, and finally, along the oocyte microvilli (Selman and Wallace, 1982; Abraham *et al*., 1984). It is now well found that Vtg is selectively separated by growing ovarian follicles via specific receptors located in the oolemma, which become effective at this early stage in vitellogenesis (Le Menn *et al*., 2007). The mature egg is a metaphase II oocyte released from the ovary after the completion of the ovulatory process. At this stage, the egg is fully formed and contains all the molecules and nutritive reserves needed for embryonic development as previously explained (Brook *et al*., 1997). During maturation, the primary oocyte leaves the diplotene stage and restarts the first miotic division. Maturation processes are induced by gonadotropic hormones and occur in the ooplasm and nucleus of the primary oocytes. The maturation signal consists of a new pulse of gonadotropic hormones, which stimulate ovarian somatic follicle cells, triggering synthesis of the maturation-inducing steroid (MIS). MIS acts on specific receptors located on the oolemma, which in turn, trigger the synthesis and activation of the maturation-promoting factor (MPF) in the ooplasm (Yamashita 1998; Senthilkumaran *et al*., 2004).

In females, during vitellogenesis, GtH I or GtH II stimulates the production of testosterone (T) by the theca cells and its aromatization to estradiol-17 β (E_2) in the granulose cells (Nagahama, 1994). In response to stimulation by E_2, the liver produces vitellogenin, which is sequestered by the oocytes in a receptor-mediated process enhanced by GtH I (Polzonetti-Magni *et al*., 2004). At the completion of vitellogenesis, a surge in plasma GtH II stimulates a drop in plasma E_2, a transient increase in plasma T during germinal vescicle (GV) migration, and a dramatic elevation in the plasma levels of the maturation inducing steroid (MIS),

which acts at the level of the oocyte membrane to induce FOM (Nagahama, 1994; Peter & Yu, 1997). The most common MISs are 17, 20β -dihydroxy-4-pregnen-3-one (17, 20 β-P) and 17, 20 β, 21-trihydroxy-4-pregnen-3-one (17, 20β, 21-P). The elevation of GtH II prior to FOM also induces the maturational competence of the oocytes (Kagawa *et al*., 1998), a process by which the de novo synthesis of MIS receptors enables the oocyte to respond to the MIS and undergo maturation. In addition to GtH II, insulin-like growth factor I (IGF-I) has been shown to induce maturational competence and FOM (Negatu *et al*., 1998) (Figures 5).



Figures 5 Hormonal control of the hypothalamus-pituitary-gonad axis of female fish and morphological changes associated with vitellogenesis and final oocyte maturation.
Source: Cardinaletti *et al*. (2010)

9.2 Spermatogenesis and maturation

Spermatogenesis is the process in which spermatozoa carrying a haploid are produced from male primordial germ cells or diploid spermatogonial stem cells by way of mitosis and meiosis. Germ cells are produced through three major phases: mitotic proliferation (spermatogonia), meiosis (spermatocytes), and spermiogenesis, i.e., the transformation of the haploid spermatids into flagellated spermatozoa (Schulz and Miura, 2002). Spermatogonial stem cells or spermatogonia are committed to miotic proliferation, leading to meiosis and spermiogenesis. Normally, the spermatogonia go through several consecutive cell cycles in order to expand the germ cell so as to provide large number of cells required for fertility. After, mitotic proliferation, the germ cells enter the meiotic phase, which is comprised of two specialized cell cycles. The first one, DNA is duplicated, and the genetic information is recombined in the primary spermatocytes. They divide to form the short-lived secondary spermatocytes, which divide again quickly without DNA duplication, so that the germ cells emerge from meiosis as the haploid spermatids. Spermatogenesis starts with a single primary cell or a single spermatogonium or stem cells.

Based on the different stages of the seminiferous epithelium cycle, it can be classified into three different types (De Rooij and Russell, 2000): type A, intermedia, and type B. Type A spermatogonia gives rise to differentiated type A spermatogonia (two to eight germ cells). Type B spermatogonia involves a progressively decreasing cell and nuclear volume, with a small rim of cytoplasm surrounding an oval to round nucleus that gradually shows more heterochromatin and divides more than type A spermatogonia (these cells occur in cysts with 16 or more germ cells). After the final mitosis, type B spermatogonia differentiate into primary (preleptotene) spermatocytes, from where the ensuing developmental stages are primary spermatocytes (1^{st} meiotic division) to secondary spermatocytes (2^{nd} meiotic division) to spermatids (differentiation without proliferation). The last stage involves spermatozoa. The spermatid undergoes a final differentiation period known as spermiogenesis, during which time they develop a flagellum, wherein the DNA is

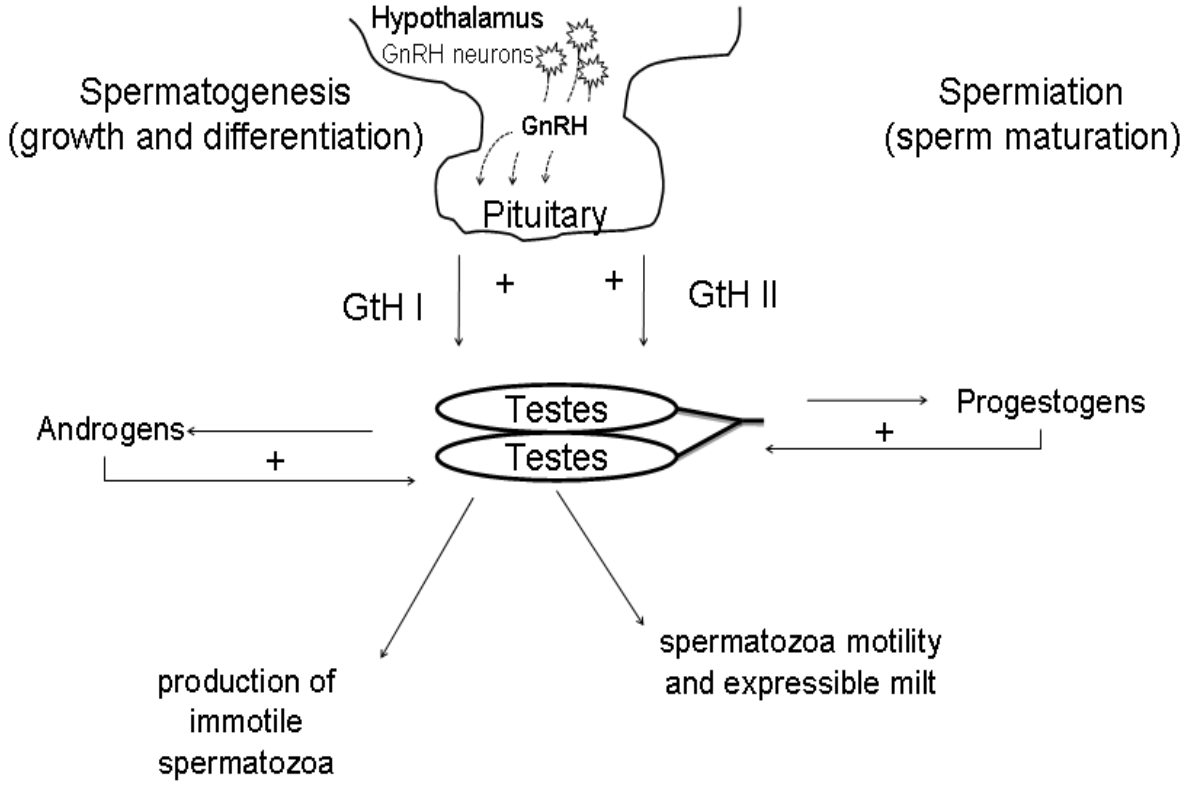
maximally compacted into a small nucleus, and superfluous cellular material is discarded into the so-called residual body (Schulz *et al*., 2010).

Spermiogenesis consists of morphological changes that lead to the differentiation of spermatids into spermatozoa. The changes include nuclear condensation, elimination of organelles and cytoplasm, flagellum formation, and the rearrangement of cellular organelles along the spermatozoon cytoplasm (Jamieson and Leung. 1991). Fish spermiogenesis involves three types; type I is characterized by a perpendicular flagellum in relation to the nucleus with nuclear rotation; in type II, the flagellum develops parallel to the nucleus without nuclear rotation, and in type III, the flagellum is central without nuclear rotation (Quagio-Grassiotto and Oliveira, 2008). Generally, fish spermatozoa have no acrosome, and the impenetrable chorion is pierced by a micropyle that provides entry into the membrane of the oocyte. Moreover, fish spermatozoa can be classified into two forms, aqua sperm and endo sperm, according the external or internal mode of fertilization, respectively (Jamieson, 1991). At the end of spermiogenesis, when intercellular bridges are broken and spermatozoa are individualized, the junctional complex between the cyst-forming sertoli cells undergoes a dynamic remodeling that culminates into the cyst opening and hence the release of spermatozoa into the tubular lumen.

In males, gonadotropins regulate spermatogenesis via testicular production of androgens, mainly 11- ketotestosterone (11-KT) (Borg, 1994). Since T is the precursor of 11-KT, the levels of the two androgens co-vary during most of the reproductive season. Plasma 11-KT levels peak during spermiogenesis and decline just prior to, or during the spermiation period. In vitro studies of the immature testis of the Japanese eel (Anguilla japonica, Anguillidae) have shown that GtH-induced 11-KT production by the Leydig cells stimulates activin B production by the Sertoli cells, which in turn induces spermatogenesis (Miura *et al*., 1991). In a phenomenon parallel to that which occurs in females, an increase in plasma GtH II levels at the onset of the spawning season shifts the testes' steroidogenic production from androgens to MIS (Nagahama, 2002). Luteinizing hormone and the MIS induce increases in expressible milt volume by stimulating production of seminal plasma (Pankhurst,

1994), and the MIS stimulates motility capacitation of the stored spermatozoa via an increase in the pH of the seminal plasma (Ohta *et al*., 1997) (Figures 6).

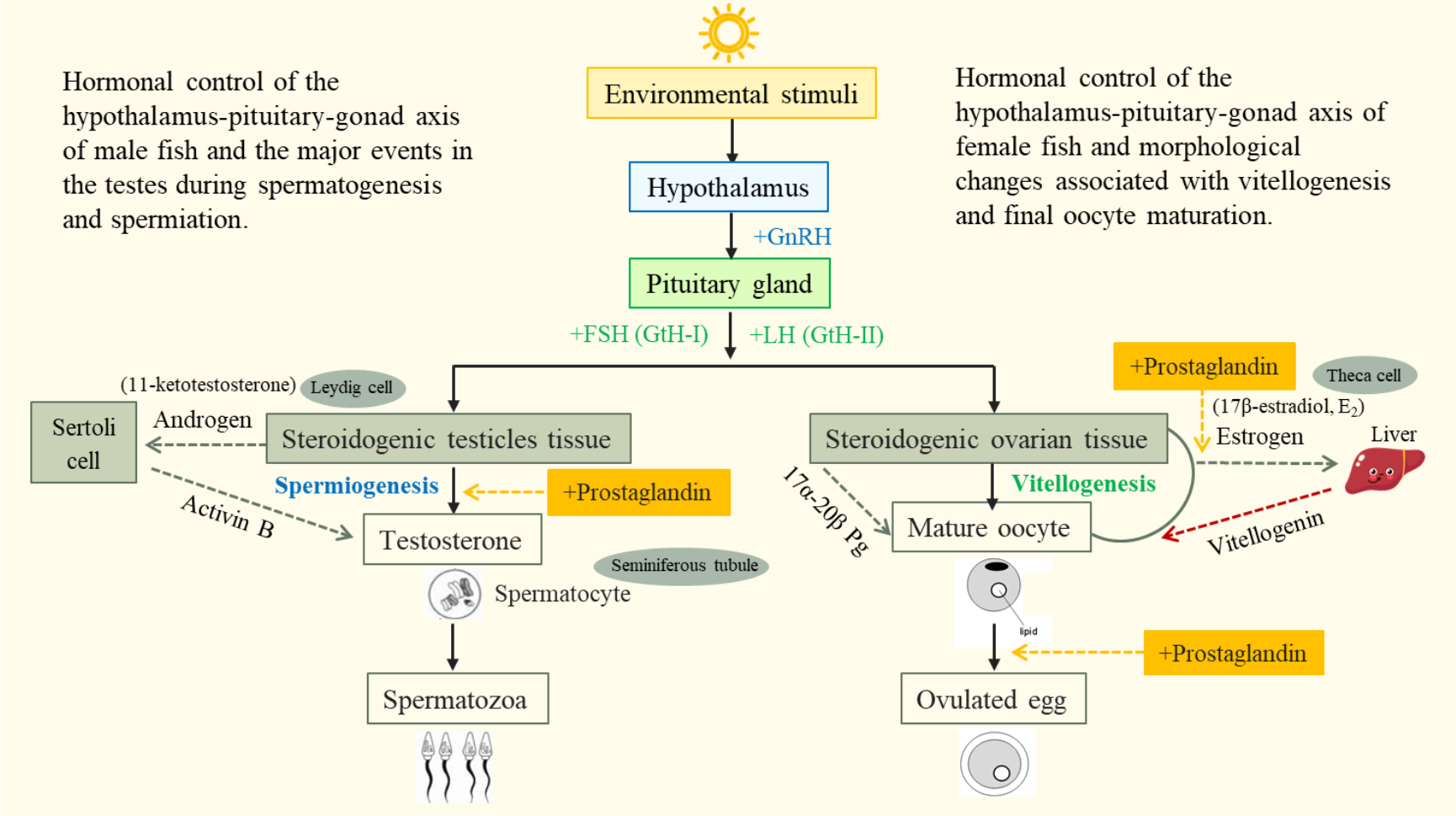
Measurements of gonadal steroid concentrations in the bloodstream provide information about the status of the brain–pituitary–gonadal (BPG) axis, and therefore aid in assessing the reproductive status of the fish (Merson *et al*., 2000).



Figures 6 Hormonal control of the hypothalamus-pituitary-gonad axis of male fish and the major events in the testes during spermatogenesis and spermiation.

Source: Cardinaletti *et al*. (2010)

Summary of egg and spermatozoa production process of fish



9.3 Gonadosomatic index (GSI)

Gonadosomatic Index is one of the parameters used in the reproduction studies of fish. It is the measurement of the relative weight of the gonad with respect to total or somatic weight, and is represented by the formula: Gonadosomatic index (% GSI) = weight of gonad (g) x 100 / body weight (g) (Nikolsky, 1963; Sutthi *et al*., 2014; Al-Deghayem *et al*., 2017). The percentage of body weight of the fish that is used for egg production is determined by the gonadosomatic index. GSI is a common metric of reproductive allocation and reproductive conditions in fishery biology. However, GSI may be an imprecise proxy for the gonadal stage because it assumes isometry between somatic mass and gonad mass as well as a tight correspondence between total gonad mass and the gonad developmental stage (Devlaming *et al*., 1982; Packard and Boardman, 1988).

Nonetheless, if significant gonad growth occurs at distinct developmental stages (e.g. vitellogenesis), GSI cut-off scores (i.e. designating those above the score as 'reproductive' or 'spawning capable') may be useful metrics for sorting fish into gross reproductive categories. Gonadosomatic cut-off scores of 8% (females) and 1%–1.3% (males) are commonly used to select reproductive individuals in round goby invasion studies (Bowley *et al*., 2010; Yavno and Corkum, 2011; Kasurak *et al*., 2012). The increased values of GSI in catfish, such as *Mystus gulio*, indicated development of the gonad from March to July (peak) and droped in December (Sarker *et al*., 2002; Islam *et al*., 2008), Similarly to Brackish water catfish (*Plotosus canius*), the GSI indicated that mature fish become available from April to August, the peak was found in July (Khan *et al*., 2002). Additionally, Kumari (2014) reported that the GSI of *Heteropneustes fossilis* determined that the peaks of the diameter oocytes and testicular lobules occurred during the period of July to August. Kabir, *et al*. (2012) reported that the highest increase GSI values of female *Pangasianodon hypophthalmus* in November (0.77±0.14%) and lowest in June (0.26±0.07%). Similarly, as hybrid catfish F_2 (female), the GSI between May to June was 0.22±0.15 and 3.07±0.19 of male (Sutthi *et al*., 2014). Al-Deghayem *et al*. (2017) revealed that animal-based protein diet and temperature around 28 ℃ were the

critical requirements for the physiological performance and GSI of *Clarias gariepinus*, while research report of Arfah *et al*. (2018), report the pregnant mare serum gonadotropin (PMSG) + Antidopamine and turmeric in feed was able to accelerate the gonadal maturation in spawning season (June-August) of striped catfish (*Pangasionodon hypophthalmus*).

9.4 Sex steroid hormones

In sex steroid hormones, the androgens (testosterone, T; 11-Ketotestosterone, 11-KT) develop biological activity via the testicular somatic cells and increase gradually as spermatogenesis proceeds and decreases at spermiation. Two androgen receptor subtypes (α and β) have been described in fish and they are all predominantly expressed in the gonads (Takeo and Yamashita, 1999; Todo *et al*., 1999; Ikeuchi *et al*., 2001). It has been expressed in sertoli and interstitial cells but not in germ cells (Ikeuchi *et al*., 2001). The effectiveness of androgen is supporting of either the whole process of spermatogenesis, or at least some steps such as spermatogonial multiplication and spermatocyte formation (guppy) or maturation (killifish) (Remacle, 1976; Billard *et al*., 1982; Billard, 1986; Nagahama, 1994; Borg, 1994). They may also participate in the initiation of puberty (Miura *et al*., 1991a, b). In addition, androgens induce spermiation in some species but were clearly less effective than progestins (Ueda *et al*., 1985). The main function of FSH during the initiation of spermatogonial proliferation is to stimulate the production of spermatogenesis-inducing steroids, such as 11-ketotestosterone (Miura *et al*., 1991a), the major androgen of teleost fish. In fact, FSH can induce the production of 11-ketotestosterone in the testis in vitro (Kamei *et al*., 2005; Ohta *et al*., 2007). 11-ketotestosterone was first identified by Idler *et al*. (1961) as a major androgenic steroid in the male sockeye salmon (*Oncorhynchus nerka*). In various teleost fish, this steroid has since shown that it can be synthesized in the testis following GTHs stimulation. High levels were detected in the serum during spermatogenesis (Billard *et al*., 1982) and were involved in the initiation of spermatogonial proliferation toward meiosis.

In the previous study, one-year old specimens of female hybrid catfish F_2 (*Pangasianodon gigas,* male x *Pangasianodon hypophthalmus*, female) cultured in earthen ponds, showed the highest levels of 17 β-estradiol (E_2) in June-October (49.05±8.60 pg/ml), but found that testosterone levels of male 1.33±8.60 ng/ml in May- June (Sutthi *et al*., 2014). Similarly to Manosroi *et al*., 2003, it was reported that 17 β-estradiol levels in female was 47.8 pg/ mL-1 and the testosterone levels was record at 14.23 pg/ mL-1 in male. The highest testosterone (T) value in male *Hemibagrus nemurus* was observed in November with 0.078 ± 0.017 ng/ml and E_2 levels was highest in November (0.728 ±0.016 ng/ml) (Adebiyi *et al*., 2013).

10. Meat quality

Meat quality can be perceived by its sensory attributes (color, texture, juiciness, taste, odor, softness), nutritional composition (fat content, fatty acid profile, protein percentage, minerals, and vitamins), technical parameters (pH, water holding capacity (WHC) and thawing loss) (Hocquette *et al*., 2005; Guerrero *et al*., 2013).

Carcass traits and meat quality, such as tenderness and color, are critical for consumer acceptance (Song and King, 2015). Some aspects of meat quality such as color and amount of fat can be perceived visually, but others such as nutritional value, fatty acid comprosition, vitamin E and the absence of residues are only ensured by means adequate analysis and labeling and certification (dos Santos *et al*., 2019).

1) Color

Color is one of the most important sensory attributes used to evaluate the quality of products and first criterion consumers use to judge meat quality and acceptability (Karthika *et al*., 2016). The main pigment responsible for meat color is myoglobin, a protein present in the sarcoplasm of the muscle fiber (Aroeira *et al*., 2017). The parameters of evaluation used for color, characterized by luminosity (*L**, *a**, *b**), *L** is the lightness (0 for black and 100 for white), *a** is in the

redness (-100 for green and +100 for red) and *b** is in the yellowness (-100 for blue and +100 for yellow) (Noordin *et al*., 2019).

2) Nutritional composition

Assessment of the major chemical composition in fish meat consisted of determination of moisture, protein, fat, and ash. All this, it was found that meat contains most water, which is about 70-80%. Other components vary depending on several factors, including size, sex, temperature, exercise time, type, formulation and feeding frequency (Shearer, 1994). The three main organic compounds in meat are essential for consumption: moisture, protein, and fat.

The fat content in animals showed the greatest variability. Because the amount of fat depends on the piece of meat. The fat content of fresh fish is related to the amount of nutrients the animal receives. If compared between farmed fish and wild fish. It was found that the fat content of commercial farmed fish was higher because the feed intake and formula were good enough for the fish is needs (Jankowska *et al*., 2003).

In addition, Papoutsoglou *et al*. (1987) reported that the protein and body fat content of fish increases as the fish grows older or as the fish gets bigger. The characteristics of fish meat composition and blood composition (hematological characteristics), or even the total fat content in the liver, can be a good indicator of the growth rate and health of a fish. But the changes of the earlier characteristics usually depend on the size of the fish (Papoutsoglou *et al*, 1978).

3) Carcass quality

The condition factor (CF) is defined as the ratio of the body weight (grams) and body length (cm) cubed, and is commonly used to measure the conformation of fish (Akvaforsk, 2005). This trait was an important economic trait, suitable also for breeding purposes. The CF determines the percentage of flesh present on the fish body, and it coincides with a high fillet yield (Rørå *et al*., 2001).

Fillet yield is the ratio between fillet and carcass weight and is a criterion for the edible part of the body. The carcass percentage was calculated by the following formulas: carcass quality = (carcass weight / whole body weight) x 100 (Hasan *et al*., 2019).

Intarak *et al*. (2015) reported that the carcass quality of Punga fish (*Pangasius bocourti* Sauvage) average body weights of 700, 900 and 1200 g did not changed with slaughter weight and the fillet percentage was 30.4, 31.2 and 30.7%, respectively. The Buk Siam hybrid catfish (*Pangasianodon gigas* X *Pangasianodon hypophthalmus*) average body weight 1.19 kg has carcass quality such as fish fillet, ventral meat, and skeleton average as 37.88, 13.51 and 33.34%, respectively (Sattang *et al*. 2018b).

11. Sensory evaluation

The discipline of hedonic response flourished swiftly in 20th century along with the growth of food processing industries. It encompasses a set of techniques required for the precise measurements of human reactions to foodstuff ultimately persuading the consumer perceptions. According to the Institute of Food Technologists (IFT), sensory evaluation is a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, hearing, touch, smell, and taste (Stone and Sidel, 1993; IFT, 2007). Since its advent in 1940s, sensory assessment has been established as an exciting, dynamic, and continually evolving discipline that is now renowned as a scientific field in its own right. The sensory professionals are regularly challenged with problems which call upon widespread skills derived from array of disciplines, like bio-sciences, psychology, statistics and often required to work with other experts from these areas. Furthermore, working with a human as ‘measuring instrument’ is challenging due to great variability. Today’s lifestyle is entirely different; hypermarkets are offering consumers a great range of food products. The competition between food processing industries is escalating for more space in superstores; hence sensory analysis has become vital part of food production. Sensory evaluation has emerged as an

essential component of food product development and standards for setting up, testing, analyzing, and interpreting sensory results are now at an advanced stage. Moreover, innovations and advancements in electronic devices have further simplified the evaluation process.

11.1 Sensory perception

Sensorics attributes of the food products are perceived by the sensory organs like eyes, tongue, nose, ear etc. by interacting with food components (Kemp *et al*. 2011). The biological mechanisms involved in perception are discussed below:

1) Perception

It is first food attribute which is critical in the selection or rejection of food. The appearance of any product is accessed through the perception. Actually, light waves after striking with food stuff fall on the eye retina which is comprised of rods and cones. Light energy after transforming into neural impulses reaches to the brain through optic nerve. Rods respond to white light and communicate info regarding the lightness of the color. Cones are receptive to diverse wavelengths of light concerning to 'color'. The brain deduces these indicators, and we notice the appearance (shape, size, color, etc.) of the product.

2) Taste

It involves the perception of constituents after being dissolved in saliva, oil, or water by taste receptors in the taste buds found superficially on the tongue and other parts of the mouth or gullet. The consequential discernments can be divided into 5 various taste qualities – sweet, salty, sour, bitter and umami.

- Sweet: sucrose, glucose, fructose, saccharine, aspartame
- Salty: sodium chloride, potassium chloride
- Sour: phosphoric acid, citric acid

- Bitter: quinine, caffeine
- Umami: Chinese salt

3) Smell

The aroma or odor associated with food products is sensed by olfactory receptors present in nasal epithelium. Hence, for the detection of aroma or odor, volatile molecules must be shifted to the nasal cavity. These compounds further move in the nose during inhaling or breathing or during eating through the back of the throat. A specific odor is the outcome of numerous volatile compounds, but sometimes particular volatiles can be associated with a specific smell, e.g. Iso-amyl acetate.

4) Sound

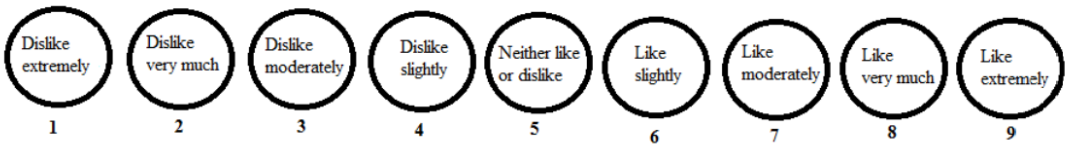
It is detected by tiny hair cells in the ear stimulated by the sound waves. The noise produced by food during eating contributes to the perceived texture of a food, e.g. effervescence of a carbonated drink, crispness of an apple or puffed rice. The sound waves produced during the consumption of food products are conducted by the air and/or bones in the jaw and skull known as intra-oral perception.

5) Touch

Texture is a complicated phenomenon, and it can be divided into categories including mechanical (hardness and chewiness), geometric (graininess and crumbliness) and mouth-feel (oiliness and moistness). Generally, these are professed during biting, chewing after swallowing.

11.2 Affective testing

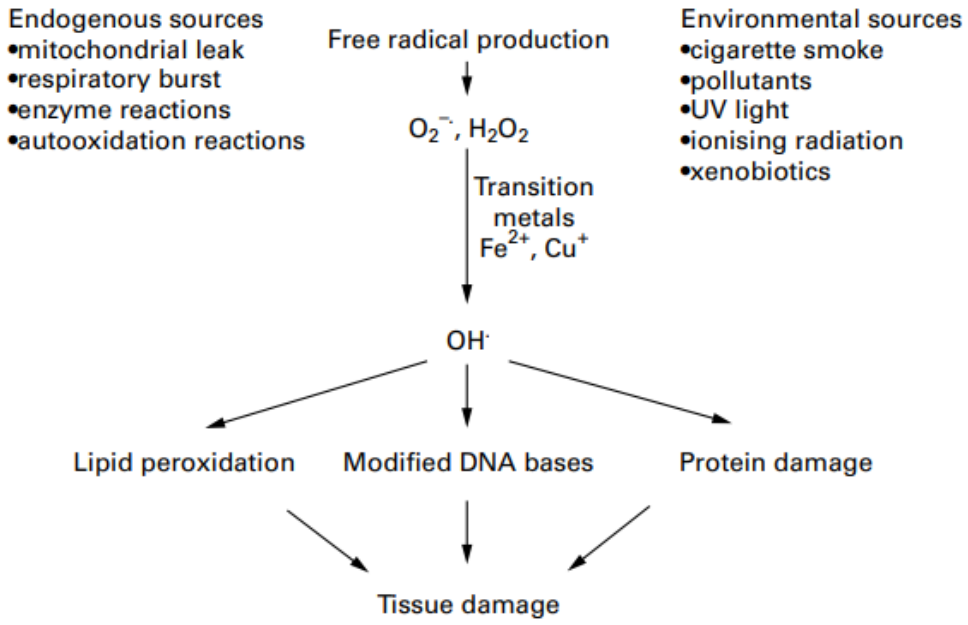
These tests are mostly used to establish the consumer acceptability or preference for a particular product through liking and disliking. Affective tests are employed in the food industry to determine liking and disliking of consumer, preference of one product over another and consumers intention to use a product. Generally, a fresh product is preferred over foodstuff close to end of shelf life. A rusk is expected to lose some of its crispness and slight change in flavor. Consumers are generally enquired whether they still consider these rusks acceptable despite changes in sensorics attributes during the storage. The most commonly used affective methods include paired preferences, ranking for preference and 9-point hedonic scale. In paired preference the assessor is enquired to point out sample of his preference among the two samples. A judge may choose one of the samples but find neither one desirable. This test is quite simple and easy to perform especially when the desirability of one sample is known. In ranking for preference, the assessor is requested to rank 2 or more samples for being favorite. In hedonic scale, degree of liking for a specific product is assessed. The most commonly used hedonic scales are 7-point hedonic scale and 9-point hedonic scale with expressions stretching from dislike extremely to like extremely. By using hedonic scale, the evaluator can compare the acceptability of numerous products (Sharif *et al*., 2017).



Figures 7 The 9-point Hedonic Scale - Society of Sensory Professionals.
Source: Sharif *et al*. (2017)

12. Free radical

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital (Halliwell and Gutteridge, 1989). The presence of an unpaired electron results in certain common properties that are shared by most radicals. Radicals are weakly attracted to a magnetic field and are said to be paramagnetic. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants or reductants. As a result of this high reactivity, most radicals have a very short half-life (10^{-6} seconds or less) in biological systems, although some species may survive for much longer (Halliwell and Gutteridge, 1989). The most important free radicals in many disease states are oxygen derivatives, particularly superoxide and the hydroxyl radical. Radical formation in the body occurs by several mechanisms, involving both endogenous and environmental factors (Figures 8; Young and Woodside, 2001).



Figures 8 Major sources of free radicals in the body and the consequences of free radical damage.

Source: Young and Woodside (2001)

12.1 Oxidative stress

Free radicals and oxidants give rise to a phenomenon known as oxidative stress; this is a harmful process that can negatively affect several cellular structures, such as membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) (Droge, 2002; Willcox *et al*., 2004; Pacher *et al*., 2007; Genestra, 2007; Halliwell, 2007; Young and Woodside, 2001). Oxidative stress emerges when an imbalance exists between free radical formation and the capability of cells to clear them. For instance, an excess of hydroxyl radical and peroxynitrite can cause lipid peroxidation, thus damaging cell membranes and lipoproteins. This in turn will lead to malondialdehyde (MDA) and conjugated diene compound formation, which are known to be cytotoxic as well as mutagenic. Being a radical chain reaction, lipid peroxidation spreads very quickly affecting a large amount of lipidic molecules (Frei, 1997). Proteins may as well be damaged by oxidative stress, undergoing to conformational modifications that could determine a loss, or an impairment, of their enzymatic activity (Halliwell, 2007; Frei, 1997).

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and normally generated as by-products of oxygen metabolism. Therefore, causing the imbalance that leads to cell and tissue damage (oxidative stress) (Pizzino *et al*., 2017). Cells deploy an antioxidant defensive system based mainly on enzymatic components, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), to protect themselves from ROS-induced cellular damage (Deponte, 2013).

A free radical is defined as any species capable of independent existence (hence the term ‘free’) that contains one or more unpaired electrons (Halliwell and Gutteridge 2007). This broad definition encompasses a wide range of species (Tables 1).

Tables 1 Some reactive species

Free radicals	Non-radicals
Oxygen reactive species (ROS)	**Oxygen reactive species (ROS)**
Superoxide, $O_2^{\bullet-}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\bullet}$	Hypobromous acid, HOBr
Hydroperoxyl, $HO_2^{\bullet}$ (protonated superoxide)	Hypochlorous acid, HOCl
Carbonate, $CO_3^{\bullet-}$	Ozone, O_3
Peroxyl, $RO_2^{\bullet}$	Singlet oxygen ($O_2{}^1\Delta g$)
Alkoxyl, $RO^{\bullet}$	Organic peroxides, ROOH
Carbon dioxide radical, $CO_2^{\bullet-}$	Peroxynitrite, $ONOO^-$
Singlet $O_2{}^1\Sigma g^+$	Peroxynitrate, O_2NOO^-
	Peroxynitrous acid, ONOOH
	Peroxomonocarbonate, $HOOCO_2^-$
	Nitrosoperoxycarbonate, $ONOOCO_2^-$
Reactive carbonyl species (RCS)	**Reactive carbonyl species (RCS)**
Atomic chlorine, $Cl^{\bullet}$	Hypochlorous acid, HOCl
	Nitryl chloride, NO_2Cl
	Chloramines
	Chlorine gas (Cl_2)
	Bromine chloride (BrCl)
	Chlorine dioxide (ClO_2)
Reactive bromine species (RBS)	**Reactive bromine species (RBS)**
Atomic bromine, $Br^{\bullet}$	Hypobromous acid (HOBr)
	Bromine gas (Br_2)
	Bromine chloride (BrCl)
Reactive nitrogen species (RNS)	**Reactive nitrogen species (RNS)**
Nitric oxide, $NO^{\bullet}$	Nitrous acid, HNO_2
Nitrogen dioxide, $NO_2^{\bullet}$	Nitrosyl cation, NO^+
Nitrate, $NO_3^{\bullet}$	Nitroxyl anion, NO^-
	Dinitrogen tetroxide, N_2O_4

Dinitrogen trioxide, N_2O_3

Peroxynitrite, $ONOO^-$

Peroxynitrate, O_2NOO^-

Peroxynitrous acid, ONOOH

Nitronium cation, NO_2^+

Alkyl peroxynitrites, ROONO

Alkyl peroxynitrates, RO_2ONO

Nitryl chloride, NO_2Cl

Peroxyacetyl nitrate, CH3C(O)OONO2

Source: Halliwell (2006)

13. Antioxidant

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals (Sies, 1997). Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves (Pal *et al*. 2014).

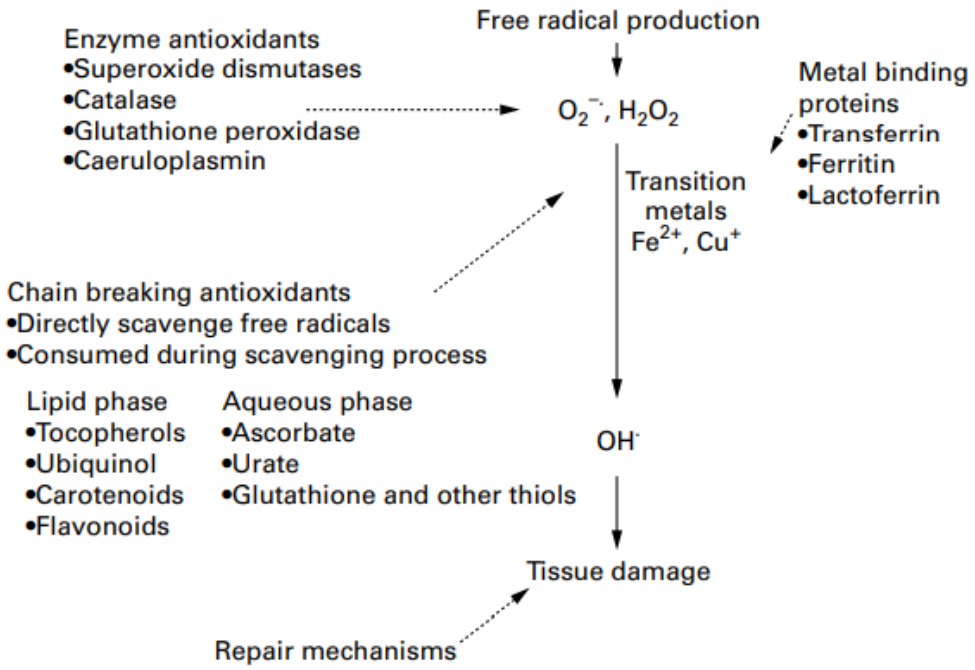
In living cell, two antioxidant defense system are present against free radical damage. The first line of defense includes antioxidant enzymes (such as superoxide dismutase, catalase, GSH peroxidase), whereas the second defence system includes low molecular non-enzymatic antioxidants (thioredoxin, GSH, vitamins A, C, E, lycopene, lutein, quercetin etc.). These antioxidants inhibit the formation of free radicals (FRs) by breaking the chain reaction or can reduce the concentration of FR by donating hydrogen and an electron. They also act as peroxide decomposer (vitamin E), enzyme inhibitor, singlet oxygen quencher (vitamin E),

synergist and metalchelating agents (tranferritin). To provide maximum intracellular protection, antioxidants are strategically compartmentalized throughout the cell. So that FR is produced intracellular and extracellular during metabolism, both enzymatic and non-enzymatic antioxidants are able to detoxify FRs (Singh *et al*., 2014).

13.1 Antioxidant defense systems

Because radicals have the capacity to react in an indiscriminate manner leading to damage to almost any cellular component, an extensive range of antioxidant defenses, both endogenous and exogenous, are present to protect cellular components from free radical induced damage. These can be divided into three main groups: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins (Figure 8; Halliwell and Gutteridge, 1989).

Antioxidant defenses against free radical attack. Antioxidant enzymes catalyze the breakdown of free radical species, usually in the intracellular environment. Transition metal binding proteins prevent the interaction of transition metals such as iron and copper with hydrogen peroxide and superoxide producing highly reactive hydroxyl radicals. Chain breaking antioxidants are powerful electron donors and react preferentially with free radicals before important target molecules are damaged. In doing so, the antioxidant is oxidized and must be regenerated or replaced. By definition, the antioxidant radical is relatively unreactive and unable to attack further molecules (Figures 9; Young and Woodside, 2001).



Figures 9 Antioxidant defenses against free radical attack.

Source: Young and Woodside (2001)

14. Immunity

The immune system is the immune system of fish similar to that of mammals. It consists of several cell types that function together, such as lymphocyte and phagocyte. In normal conditions, these cells are mainly present in tissues, blood, lymph, and bone marrow, with the exception of plasma cells (Sakai, 1999). The immune system of fish which consists of a non-specific immunity and specific immunity. The nonspecific immune system is the first line of defense against the invasion of pathogens. It has a quick response to prevent germ invasion. The specific immune system develops more slowly than the nonspecific immune system. But has a memory system, this gave the fish a quicker response than the initial stimulus (Vadstein, 1997). Immunity can be divided into two types according to the nature of work:

14.1 Cell mediated immunity

The cells involved in the immune system are different types of white blood cells. There are five types of fish white blood cells: monocytes, neutrophils, basophils, eosinophils, and lymphocytes. These white blood cells are responsible for catching foreign substances that enter the body (Pinyowit, 2002). The functions of each type of white blood cell are as follows:

1) Monocytes: Monocytes are diameter approximately 9-12 μm. The nucleus is oval or horseshoe, placed close to either side of the cell. The chromatin in the nucleus is very thin. In the bloodstream, monocytes are approximately 3-5% of all white blood cells. The monocytes are responsible for the removal of antigens by phagocytosis.

2) Neutrophils: In the total number of white blood cells contains 60% of the neutrophils. Mature neutrophils are 12 μm in size, with nucleus 2-5 lobe. Neutrophils act in phagocytosis, where neutrophils leave the bloodstream into the tissues that contain foreign bodies. By inserting between the endothelial cells lining the blood vessels.

3) Basophil: Basophils are diameter approximately 12 μm and the nucleus has 2 lopes. In the cytoplasm there are granules larger than that of neutrophils and eosinophils. The granules are so numerous that they obscure most of the nucleus. The basophils move and catch foreign objects. But the ability to capture foreign matter is much inferior to neutrophils and eosinophils.

4) Eosinophil: Eosinophils are present in the bloodstream, making up about 2-5 percent of all white blood cells. This slightly smaller than neutrophils, about 9 μm in diameter and the nucleus has 2 lopes. Eosinophils are phagocytes that selectively bind to antigenic complexes.

5) Lymphocyte: Lymphocytes in the bloodstream are mostly small cells. which has a diameter of 6-8 μm and lymphocytes can move. In the total white blood cell count, there is about 20 percent lymphocytes (Sarasombat *et al*., 1994).

14.2 Humoral immunity

1) Complement: Complement is a group of serum proteins that play a role in preventing foreign invasion. Serves to help make bacteria and viruses easier to be destroyed. It causes lysis of bacterial cells and enveloped virus, as well as is precursor to opsonization that will enhance cell phagocytosis. It involves covering the foreign body with a piece of complement. These fragments are recognized by receptors on the surface of phagocytic cells and are engulfed and destroyed (Chitmanat, 2002; Wongsawang, 1995).

2) Lysozyme: Lysozyme is produced by cells that absorb foreign substances and release them into the bloodstream, which will be in the blood. It is an enzyme that acts to break the bacterial cell wall until it cannot function. Then it will be easier to be caught and eaten (Dalmo *et al*, 1997; Lim and Webster, 2001).

3) Substances that inhibit the growth of microorganisms such as transferrin and protein C-reaction (CRP) in the serum. Acts in conjunction with the complement to help increase the ability to capture foreign matter (Chitmanat, 2002; Wongsawang, 1995).

4) Cytokine: Cytokine is a group of proteins responsible for signaling to stimulate movement, growth, differentiation of white blood cells and other cells including inhibiting the functioning of immune cells (Chitmanat, 2002; Wongsawang, 1995).

14.3 Analysis of lysozyme and nitroblue tetrazolium (NBT)

Stimulation of lysozyme is often associated with phagocytosis, or ingestion, to destroy germs or foreign matter (Misra *et al*., 2006; Ai *et al*., 2007). The lysozyme is a cationic enzyme which is a component in peptidoglycan bonds of gram-positive cell walls. The lysozyme is specific to the destruction of gram-positive bacteria such as *Micrococcus lysodiekticus*. In addition, lysozyme an important component of the nonspecific immune system, plays an important role in the removal of foreign cells. Through properties that can destroy cancer cells

(anticancer), destroy viruses (antiviral) and opsonin (opsonization properties) (Jolles and Jolles, 1984). Nitroblue tetrazolium reduction (NBT reduction assay) is a method for measuring respiratory burst of phagocytic cells, namely superoxide anions ($O_2^{\bullet -}$). It has a specific reaction and decreases the amount of Nitroblue tetrazolium (NBT), converting to formazan.

CHAPTER 3

MATERIALS AND METHODS

1. Freshwater fish oil, experiment fish and diets

Freshwater fish oil (FFO) obtained from the adipose tissue of freshwater hybrid catfish (*Pangasianodon gigas* x *Pangasianodon hypophthalmus*) which steamed at 90 °C for 30 min. The liquid oil was filtered with a filtering sack to separate the tissue and use of fish oil as supplement in experiment of fish diet. The hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) 1 year old with starting average weight of 1.48-1.57 kg obtained from the Excellent center for social service, Pla Buk and Buk Siam hybrid catfish. The hybrid catfish were reared in cages (4 x 3 x 2 m) on a soil-based pond at density of 15 fish per cage for total of 8 months.

Total of 135 hybrid catfish were distributed to 3 replicate cages per treatment using completely randomized design (CRD) (Steel and Torrie, 1960; Sattang *et al*., 2018b). Three different levels of FFO were supplemented at: 0% (control), 1% and 2% (w/w) in fish diets (Tables 2). Fish were fed at 3% of their body weight for two times per day (09:00 a.m. and 04:00 p.m.) with pelleted feed formulated containing 30% protein, as normal standard (Rattanaphot *et al*., 2018a). Average body weight was calculated monthly based on representative samples from each replicate. Use of fish in the experiment was reviewed and approved by the Maejo University Animal Care and committee. Samples collection and result analysis were divided in two group periods, first during the spawning season (May-August 2017) and second post-spawning season (September-December 2017).

The proximate analysis of the experimental diets included moisture, ash, protein, crude fiber, fat and carbohydrate contents were determined by standard AOAC methods (AOAC, 2000). Fatty acid composition of fish feed was analyzed at the Central Laboratory (Thailand) Co. Ltd., Chiang Mai Branch, following the method based on AOAC 996.06 (AOAC, 2012).

Tables 2 Formulas of pellet fish feed containing freshwater fish oil (FFO) at different level of 0% (control), 1% and 2%.

Ingredients	Formulas		
(per 100 g of feed)	**0% FFO[1]**	**1% FFO**	**2% FFO**
Fish meal	15	15	15
Soybean meal	37	37	37
Broken rice	32	27	26
Rice bran	15	20	20
Soybean oil	1	0	0
Freshwater fish oil	0	1	2
Total (kg)	**100**	**100**	**100**
Energy (kcal/g)	**416.80**	**421.20**	**430.40**
Protein (%)	**30.65**	**30.70**	**30.57**
Cost (baht/kg)	**25.80**	**25.42**	**25.33**

Note. [1]The ingredients of the control diet (0% FFO) unit is adapted from Rattanaphot *et al*. (2018a).

2. Growth performance parameters

Growth performance parameters such as the weight gain (WG), average daily weight gain (ADG), feed conversion ratio (FCR), feed conversion efficiency (FCE), and survival rate (SR) were randomly calculated at 1 month intervals using measured body weight from 5 fish each cage. Parameters were calculated using following formulas (Bagenal, 1978; Panase and Tipdacho, 2018):

WG (g/fish) = final weight (g) - initial weight (g)

ADG (g/fish/day) = [final weight (g) - initial weight (g)] / Days

FCR = total feed fed (g) / weight gain (g)

FCE (%) = [weight gain (g) x 100] / Days

SR (%) = [number of surviving fish / initial number of surviving fish] x 100

3. Indicators of reproductive condition

To determine reproductive capacity and effects of dietary supplements on hybrid catfish, samples were collected for analysis of sex steroid hormones, gonadosomatic index calculation, and histological evaluation of gonadal tissue in August (spawning season) and in December (post-spawning season). Plasma steroid hormone concentrations were determined as per Sutthi *et al.* (2014) with minor modifications. Whole blood samples (1 ml) were collected from caudal vein of 3 females and 3 males per treatment and centrifuged using centrifuge (MIKRO 200R, Hettich, Germany) at 9,500xg for 5 min and the serum was stored at -80 °C until use. 17 β-estradiol (E_2) and testosterone (T) were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits from Cayman Chemical Company by an electro-chemiluminescence immunoassay analyzer (Elecsys 2010, Roche, Germany). For determination of gonadosomatic index, total of 3 male and 3 female fish per treatment were sampled, total body weight and gonad weight of each fish were measured with a digital scale (HT-500, AND, Japan). Gonadosomatic index for ovaries and testis was calculated with following formula (Nikolsky, 1963; Sutthi *et al*., 2014; Al-Deghayem *et al*., 2017):

Gonadosomatic index (% GSI) = weight of gonad (g) x 100 / body weight (g)

For determination of the maturation stages of gonads (Arfah *et al*., 2018; Kabir *et al*., 2012), ovaries and testes were removed from 3 females and 3 males per treatment and fixed in 10% neutrally buffered formalin and stored at -18 °C until use. Fixed ovaries and testes were processed for sectioning and 4 µm thick sections were stained with haematoxylin and eosin. The histological evaluation and staging of oocytes and spermatocytes were determined under light microscope (model SFC-282, brand Motic, Thailand) as per (Coward and Bromage, 1998).

4. Fish fillet qualities

Fish was catch when they attained marketable size (weight 1.5-2 kg/fish) by dividing into 2 periods: in August (spawning season) and in December (post-spawning season). Fish was not fed for 24 hours prior catching. As commonly practice, fish was collected in ice box containg ice until death and recorded fresh weight before being used slaughtering. Afterwards were deheading, cutting off of tails fins and removing viscera. The fish fillet sample was stored in vacuum-packaged form at -20 ℃ for analysis.

4 .1 The color measurement of fillet samples were analyzed using Chroma Meter CR-400 (KONICA MINOLTA, Japan). The meat color was determined using CIE lab system including *L* a** and *b**. *L** is the lightness (0 for black and 100 for white), *a** is in the redness (-100 for green and 100 for red) and *b** is in the yellowness (-100 for blue and +100 for yellow) (Noordin *et al*., 2019).

4 . 2 The proximate composition of fillet samples were analyzed protein, fat, ash, moisture and fiber contents by using the standard of AOAC methods (AOAC, 2000).

4.3 The fatty acid composition of fillet samples were analyzed at the Central Laboratory (Thailand) Co. Ltd., Chiang Mai Branch, following method of TE-CH-208 based on AOAC 996.06 (AOAC, 2012).

4.4 The vitamin E content of fillet samples were also analyzed using the method of Khon Kaen AGR. J. 42 SUPPL.1: 2014 in Chiang Mai, Central Laboratory (Thailand) Co. Ltd.

4.5 The carcass quality was analyzed by cutting off of head, tails and fins and removing viscera followed. Fillets, grated meat, skeleton, visceral, ventral meat, chin meat and gonad were weighed and their proportion of carcass weight was calculated using following formulas (Hasan *et al*., 2019):

Carcass quality = (carcass weight / whole body weight) x 100.

5. Immunity

5.1 Antioxidant

5.1.1 Preparation of plasma and erythrocyte samples

Plasma and erythrocyte samples from fish blood were prepared and measure the amount of antioxidants, including total glutathione, oxidized glutathione and reduced glutathione and measured malondialdehyde (MDA) levels, indicating oxidative stress.

1. Microcentrifuge tube preparations contained anticoagulants such as EDTA.

2. Fish blood (caudal vein) was collected 500-1,000 µl using an anticoagulants in the microcentrifuge tube (return the microcentrifuge tube up-down to mix with the blood).

3. Blood was centrifuged at 1,300xg for 10 minutes at 4 °C.

4. Pipette off the top yellow plasma layer (supernatant) was stored at -80 ° C before analysis of MDA.

5. White buffy layer (leukocyte) was removed as discard.

6. Erythrocytes (red blood cells) were pipette into a new microcentrifuge tube size 1.5 ml (Lot No. 1290428, brand Hycon, Thailand) and added water (HPLC grade) at a ratio of 1: 5 (200: 800 µl) and vortexed for 10-15 second.

7. The samples were centrifuged at 1,300xg for 10 minutes at 4 °C.

8. 200 µl of supernatant in the top section was collected for deproteinated:

8.1 MPA reagent: dissolve metaphosphoric acid (Sigma-Aldrich 239275 or VWR Catalog No. AA33267-22) amount 5 g in 50 ml distilled water (MPA solution is stable at 25 ° C for 4 hours).

8.2 MPA reagent was added into the sample 1 volume (200 µl : 200 µl) and mix by vortex.

8.3 Incubation 5 minutes at room temperature and centrifuged at 3,500xg for 4 minutes.

8.4 Erythrocytes lysate was kept at -20 ℃ for analysis of Glutathione.

5.1.2 Malondialdehyde (MDA)

Malondialdehyde level (MDA) was measured of malondialdehyde in blood to find out the antioxidant activity from lipid peroxidation reaction. The blood was centrifuged for separation of the solution, the supernatant and the plasma were taken to measure the absorbance at the wavelength of 5 4 0 nm using spectrophotometer (model Multiskan GO, brand Thermo fisher, USA) by method using TBARS Assay Kit Item No. 10009055 (Cayman Chemical Company, Ann Abor, Michigan, USA) and measured the concentration from the standard curve and then the values obtained were compared with the protein content obtained by Bradford assay (Rattanaphot *et al*., 2018a).

5.1.2.1 Colorimetric standard preparation

150 µl of the MDA standard (Item No. 10009202) was diluted with 375 µl of water to obtain a stock solution of 125 µM. Taking eight clean glass test tubes and label them A-H. Add the amount of 125 µM MDA stock solution and water to each tube as descried in Tables 3.

Tables 3 MDA colorimetric standards.

Tubes	MDA (µl)	Water (µl)	MDA Concentration (µM)
A	0	125	0
B	2.5	497.5	0.625
C	2.5	247.5	1.25
D	2.5	122.5	2.5
E	5	120	5
F	10	115	10
G	25	100	25
H	50	75	50

5.1.2.2 Performing the assay

1. Label vial caps with standard number or sample identification number.

2. 12.5 µl of sample or standard was added to appropriately labeled 5 ml vial.

3. 12.5 µl of SDS solution was added to vial and swirl to mix.

4. 500 µl of the color reagent forcefully down side of each vial.

5. Cap vials and place vials in foam or some other holder to keep the tubes upright during boiling.

6. Vials to vigorously boiling water was added and boil vials for one hour.

7. After one hour vials, immediately removed, place in ice bath for stop reaction and incubated on ice for 10 minutes.

8. After 10 minutes, the vials were centrifuged for 10 minutes at 1,600 x g at 4 ºC. Vials may appear clear or cloudy. Cloudiness was clear upon warming to room temperature.

9. Vials are stable at room temperature for 30 minutes.

10. 150 µl (in duplicate) was load from each vial to either the clear plate (colorimetric version) or to the black plate (fluorometric version).

11. The absorbance was read at 540 nm using spectrophotometer.

5.1.2.3 Colorimetric calculations

1. The average absorbance was calculated of each standard and sample.

2. The absorbance value of the standard A (0 µM) was subtracted from itself and all other values (both standards and samples). This is the corrected absorbance.

3. The corrected absorbance values (from step 2 above) was ploted of each standard as a function of MDA concentration (see Tables 5).

4. The values of MDA for each sample was calculated from the standard curve.

MDA (µM) = [(corrected absorbance) - (y-intercept) / slope]

5.1.3 Glutathione

The measurement of glutathione in blood, erythrocyte of red blood cells was precipitated protein and supernatant was used to measure total glutathione, oxidized glutathione and reduced glutathione by method using Glutathione Assay Kit Item No. 703002 (Cayman Chemical Company, Ann Abor, Michigan, USA). By using spectrophotometer with microplate reader (model Multiskan GO, brand Thermo fisher, USA) with the absorbance at the wavelength of 405-414 nm using a plate reader at five minutes intervals for 30 minutes, to compare the concentration from the standard curve.

5.1.3.1 TEAM Reagent

1. 4 M solution of triethanolamine (Sigma-Aldrich, Item No. T58300) in water by mixing 531 µl of triethanolamine with 469 µl of water was prepared. The TEAM solution is stable for four hours at 25 °C.

2. 50 µl of TEAM Reagent per ml of the supernatant was added and vortex immediately. TEAM Reagent was adjusted the pH of the sample. The sample was ready for assay of total GSH (*i.e.*, both oxidized and reduced). Any necessary dilutions of the sample was prepared done at this stage with MES Buffer.

By 1,000 µl (1 ml, sample) uses 50 µl

If 100 µl (sample) uses $\frac{100\ \mu l \times 50\ \mu l}{1{,}000\ \mu l}$

Therefore, TEAM Reagent 5 µl (GSH) was added to the sample.

5.1.3.2 Sample preparation for exclusive measurement of GSSG

Quantification of GSSG, exclusive of GSH, is accomplished by first derivatizing GSH with 2-vinylpyridine. This can be achieved as follows:

1. 1 M solution of 2-vinylpyridine (Sigma-Aldrich, Item No. 13229-2) in ethanol was prepared by mixing 108 µl of 2-vinylpyridine and 892 µl of ethanol (total 1,000 µl or 1 ml).

2. 10 µl of the 2-vinylpyridine solution per ml of sample was added and mixed on a vortex mixer (model VTX-3000L, brand LMS, Japan) and incubate at room temperature for about 60 minutes and assay the sample. * This procedure can derivatize up to 1 mM GSH. More concentrated samples were diluted with MES buffer before derivatization.

By sample 1,000 µl uses 2-vinylpyridine 10 µl

If sample 50 µl uses 2-vinylpyridine $\frac{50\ \mu l \times 10\ \mu l}{1{,}000\ \mu l}$

= 0.5 µl

Therefore, if a 100 µl sample is to be prepared, 2-vinylpyridine 1 µl is required. *2-Vinylpyridine inhibits color development in the assay to some extent. Hence, it is essential to prepare the standards also the same way by adding 2-vinylpyridine (*i.e.*, add 5 µl of 2-vinylpyridine solution per Standard tube) and incubating to the same length of time as the sample.

3. TEAM

By sample 50 µl uses TEAM 2.5 µl

If sample 100 µl uses TEAM $\frac{10\ \mu l \times 2.5\ \mu l}{50\ \mu l}$

= 5 µl (Oxidize GSSG)

5.1.3.3 Standard preparation

Eight clean test tube and mark them A-H. Aliquot the GSSG Standard (Item No. 703014) and MES Buffer to each tube as described in Tables 4.

Tables 4 Glutathione standards

Tubes	GSSG Standard (µl)	MES Buffer (µl)	Final Concentration (µM GSSG)	Equivalent Total GSH (µM)
A	0	250	0	0
B	2.5	247.5	0.25	0.5
C	5	245	0.5	1.0
D	10	240	1.0	2.0
E	20	230	2.0	4.0
F	40	210	4.0	8.0
G	60	190	6.0	12.0
H	80	170	8.0	106

*Under the assay condition GSSG is reduced to produce 2 mole equivalents of GSH.

5.1.3.4 Performing the Assay

1. 12.5 µl of standard (tubes A-H) per well was added in the designated wall on the plate.

2. 12.5 µl of sample was added to each of the sample wells.

3. The plate with the plate cover provided.

4. The Assay Cocktail was prepared by mixing the following reagent in a 20 ml vial: MES buffer (11.25 ml), reconstituted cofactor mixture (0.45 ml), reconstituted enzyme mixture (2.1 ml), water (2.3 ml), and reconstituted DTNB (0.45 ml).

Tables 5 Prepare the Assay Cocktail

	Cocktail (ml)	**Cocktail for performing the assay (ml)**
MES buffer	11.25	2.04
cofactor mixture	0.45	0.08
enzyme mixture	2.10	0.38
water	2.30	0.42
DTNB	0.45	0.08
Total	**16.55**	**3.00**

5. The plate cover was removed and added 87.5 µl of the freshly prepared of assay cocktail to each of the well containing standards and samples using a multichannel pipette. The plate cover was replaced and incubated the plate in the dark on an orbital shaker.

6. The absorbance was measured in the wells at 405-414 nm using a microplate reader (model Multiskan GO, brand Thermo fisher, USA) at five minutes intervals for 30 minutes.

5.1.3.5 Calculations

GSH concentration of the sample was determined either by the Kinetic Method.

1. The average absorbance values was plotted as of each standard and sample as a function of time and determine the slope for each curve. This is called i-slope.

2. Plot the i-slope of each standard as a function of the concentration of GSSG or total GSH. The slope of this curve is called f-slope.

3. The values of GSSG or total GSH was calculated for each sample from their respective slopes using the slope *versus* GSSG or GSH standard curve.

$$\text{Total GSH or GSSG} = \frac{[\text{(i-slope for the sample)} - \text{(Y-intercept)}]}{\text{f-Slope}} \times 2^{*} \times \text{Sample Dilution}$$

5.2 Non-specific Immunity

5.2.1 Lysozyme activity assay

Lysozyme activity assay was measured the activity level of lysozyme in blood for the degradation (lysis) of *Micrococcus lysodiekticus* gram-positive bacterial cells by adapted from the method of Parry *et al*., (1965).

5.2.1.1 Sample preparation of serum

Fish blood samples were collected in each of the 3 fish/treatment in the area of the caudal veins volume 1 ml and placed in a 1.5 ml microcentrifuge tube. The sample was kept at room temperature for 20 - 30 minutes for the separation of serum. The top yellow serum layer without disturbing the white buffy layer was pipetted and stored serum at -20 ℃ for wait lysozyme activity assay.

5.2.1.2 Performing the assay

1. Serum 25 µl into 96 well plat 3 replicates/sample (27 well) was pipetted.

2. *Micrococcus lysodeikticus* 100 µl (concentration 3 mg/ml).

- need to prepare 5,000 µl was added

By 1,000 µl uses *Micrococcus lysodeikticus* 3 mg

If 5,000 µl uses $\frac{5000\ \mu l \times 3\ mg}{1{,}000\ \mu l} = 15\ mg$

Therefore, *Micrococcus lysodeikticus* = 15 mg dissolved in PBS (pH 8 concentration 0.05 M) 5 ml. Bank uses PBS pH 8 with a concentration of 0.05 M.

3. The mixed was measured the lysozyme activity with a Microplate reader (model Multiskan GO, brand Thermo fisher, USA) at wavelength of 450 nm, measured every 1 second (for 10 minutes).

5.2.2 Nitroblue tetrazolium dye reduction test (NBT)

Nitroblue tetrazolium dye reduction test (NBT) is a test for the ability of phagocyte cells. The principle is that when a phagocyte undergoes phagocytosis, a respiratory burst occurs, with increased metabolic activity and hydrogen peroxide (H_2O_2) and superoxide free radical ($O_2^{\bullet-}$) production. This is reducing agents to reduce NBT dye which is a type of color changing from yellow to formazan which has blue according to the method of Secombes (1990).

5.2.2.1 Sample preparation of lymphocyte

1. Fish blood (caudal vein) 3.5 µl was collected using a heparin tube and store blood on ice.

2. RPMI-1640 into heparin tube 1 ml was added.

3. RPMI-1640 1 ml and blood 0.35 ml was carefully pour into HiSep™ LSM 1077 or phecol 1 ml.

4. The mixture was centrifuged using centrifuge (KUBOTA 5100, Japan) at 4,000 rpm for 30 minutes.

5. The leukocytes 1 ml was removed by PBS 1 ml.

6 . The reaction was centrifuged at 1,500 rpm for 10 minutes (Leukocytes stick to the bottom of the tube).

7. Pipette off the top is discarded 1,000 µl and taken to mix well on a vortex mixer.

8. Cells under a microscope (model SFC-282, brand Motic, Thailand) was count with the Hemocytometer (Neubauer, Marienfeld, Germany). To get more than 50 cells / large channel.

5.2.2.2 Performing the assay

1. Leukocytes 175 µl (in duplicate) was load into 96 well plat.

2. RPMI-1640 87 µl + add HiSep™ LSM 1077 87 µl of Bank add Nitro Blue tetrazolium (NBT, Sigma) 25 µl into samples and bank incubate for 2 hours at 25 ℃.

3. Pipette supernatant is removed from the well plat 180 µl drained of the sample.

4. 100% methanol 125 µl was added, leave for 5 minutes and pipette out 125 µl.

5. 70% methanol 125 µl was added, leave for 5 minute and pipette out 125 µl (methanol is used to wash cells).

6. 2KOH 125 µl and DMSO 150 µl were added.

7. 275 µl to new 96 well plat.

8. Nitroblue tetrazolium dye reduction test (NBT) by the Microplate reader (model Multiskan GO, brand Thermo fisher, USA) at wavelength of 655 nm.

Note: Bank = RPMI + HiSep %2 from 175 µl (87.5)

6. Sensory evaluation

Sensory characteristic was evaluated of frozen fish fillets and Sai Aua (Notrhern Thai spicy sausage) from hybrid catfish (*Pangasius larnaudii* x *P. hypophthalmus*). Weight 1.5-2 kg/fish reared with fish diet mixed with freshwater fish oil at different levels of 0, 1 and 2% of were investigated. fish oil supplementation in diet were 0, 1 and 2%. The fillets and Sai Aua were boiled and steam before evaluation. The panelist of 30 person was used for sensory evaluation (Kulawik *et al*., 2016), which were students of Doi Saket Wittayakhom School, Chiang Mai, students, staffs of Faculty of Fisheries Technology and Aquatic Resources and consumers at 2477 market Maejo University, Thailand. The sensory evaluation consisted of seven sensory parameters (color, odor, sweet, spicy, salty, texture and overall acceptability) of both was assessed using a 9-point hedonic scale (Peryam, 1998; Olaniyi *et al*., 2017) following liking score, score 9 was the highest and 1 was the lowest:

9 = Like Extremely
8 = Like Very Much
7 = Like Moderately
6 = Like Slightly
5 = Neither Like nor Dislike
4 = Dislike Slightly
3 = Dislike Moderately
2 = Dislike Very Much
1 = Dislike Extremely

6.1 Preparation of frozen fish fillets

1) 3 groups of frozen fish fillets were used in sensory evaluation, thawed and cut into uniform transverse strip of 1 cm width.

2) The ingredients as shown in Tables 6.

3) Water was boil at 100 °C, then add the prepared ingredients, boil for 5 minutes, and scoop the fillets up into a sieve to drain the water.

4) Afterward, the sample was distributed to the panelists together with water.

Tables 6 Ingredients used for poached frozen fish fillets.

Ingredients	Amount (g)
Frozen fish fillets	200
Kaffir lime leaf	2
Lemon grass	10
Galanga	5

6.2 Preparation of Sai Aua (Northern Thai spicy sausage)

1) 3 groups of frozen fish fillets were used in sensory evaluation, thawed and finely minced (no fish skin). Preparation of the ingredients as shown in Tables 7.

2) All ingredients was pound or finely blended ingredients to make curry paste, such as garlic, coriander root, shallot, lemon grass, turmeric, galangal, dried chili and shrimp paste.

3) The finely minced fish fillets, curry paste, light soy sauce, sugar, salt and Sliced Kaffir lime leaf were mixed until homogeneous.

4) Afterward, the fish fillet has been mixed with the curry paste to be packed in pork intestines and stored in a freezer of -20 ℃ for further sensory evaluation.

Tables 7 Ingredients used in the production of Sai Aua (Northern Thai spicy sausage).

Ingredients:	**Amount (g/kg)**
Finely minced fish fillets	1,000
Salted Pork Intestines	4
Seasoning:	
Light soy sauce	30
Salt	10
Sugar	16
Curry paste:	
Shrimp paste	30
Sliced Kaffir lime leaf	10
Coriander root	30
Shallot	60
Garlic	40
Lemon grass	60
Turmeric	16
Galanga	16
Dried chili	16

7. The fish diet cost

The fish diet cost was analyzed from the market raw material price multiplied by the amount of raw materials were used in the production of fish diet supplemented with 3 levels of freshwater fish oil (0, 1 and 2%). The fish feed cost to produce a kilogram of fish was determined from the feed conversion ratio (FCR). Data were analyzed by taking the fish diet cost (baht/kg) multiplied by the FCR value of each experiment.

8. Statistical analysis

Collected data were analyzed with statistical software (SPSS, version 17.0) using one-way analysis of variance (ANOVA) and are presented as mean ± standard error (SE). Tukey honest significant difference (HSD) post hoc test was applied when statistical differences were observed ($p<0.05$).

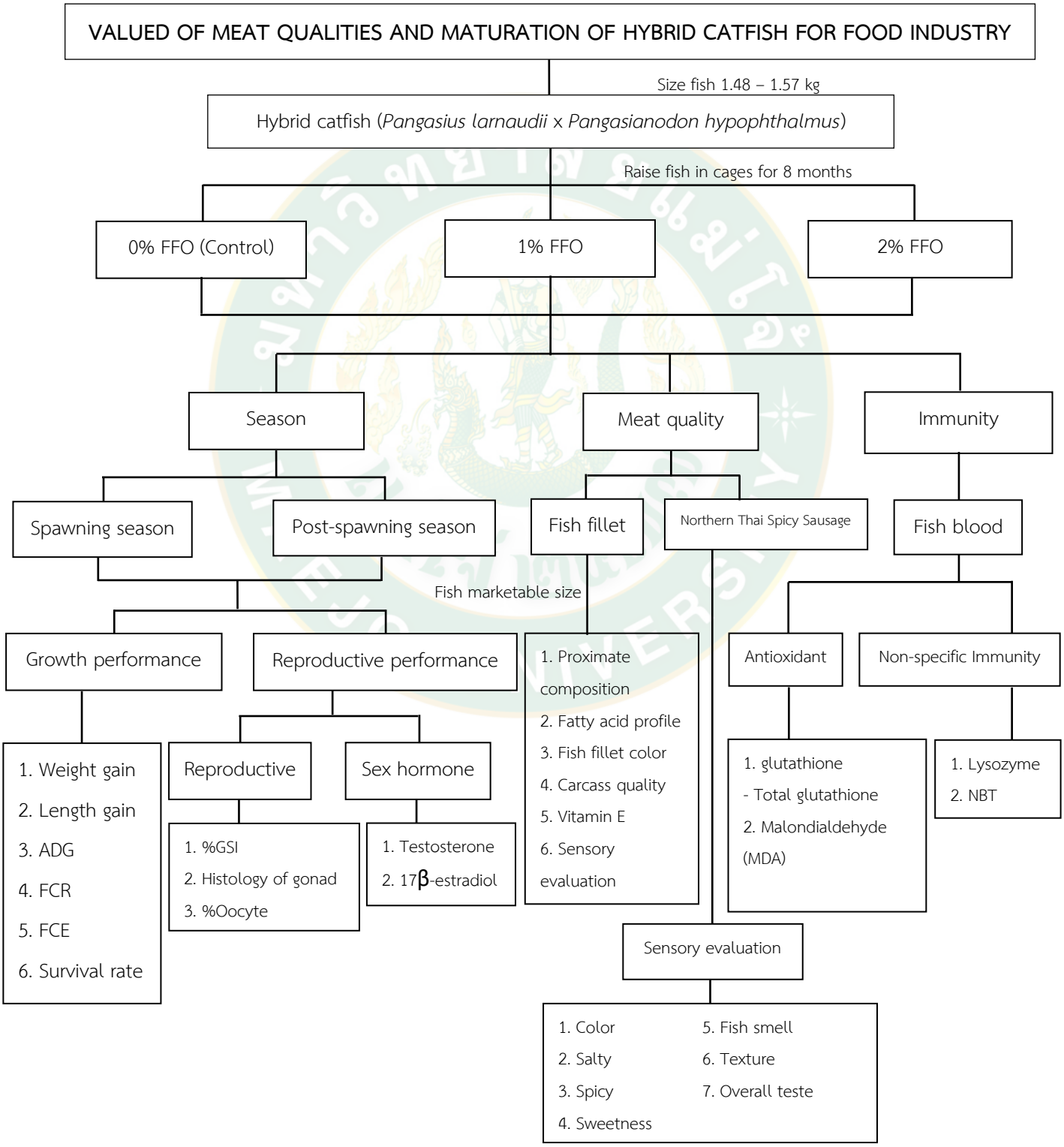
9. Research duration

Activity	Duration (months)					
	1-6	7-12	13-18	19-24	25-30	31-36
Prepare cage, fish and fish diets ingredients for experiment						
Developed fish diets recipes from freshwater fish oil						
Raise fish in cages for 8 months						
Collecting samples of fish growth, reproductive, meat quality and immunity						
Analyze experiment results						
Developed Sai Aua (Northern Thai Spicy Sausage) products from experimental fish						
Sensory evaluation on fish fillet and Sai Aua						
Summary / Manuscript / Writing Complete dissertation						

10. Research location

Excellent center for social service, Pla Buk and Buk Siam hybrid catfish, Faculty of fisheries technology and aquatic resources, Maejo University.

11. Summary of the conceptual framework of the research



CHAPTER 4

RESULTS AND DISCUSSION

1. Proximate analysis, and fatty acid composition in diets

Ingredient and proximate analysis of diets supplemented with freshwater fish oil is presented in Tables 8. There were no significant differences in the feed ingredient composition. Proximate analysis results show similar contents in all three diets, with minor differences in crude protein and fat contents. Fatty acid composition of the experimental diets is presented in Tables 9 where unsaturated and omega-9 fatty acids were found to be higher in the treatment diet containing 2% FFO compared to control (4.44 g/100 g and 2.34 g/100 g, respectively, $p>0.05$). In order to minimize lipid hydrolysis and oxidation, prepared feed was stored in a well-ventilated place under adequate conditions for less than 3 months, and quality raw materials were used to keep the nutritional value in the fish diet (Van't Land *et al*., 2017).

Tables 8 Proximate analysis of the experimental diets containing 0, 1, and 2% freshwater fish oil (FFO).

Proximate compositions	**Formulas**		
(% air dry basis)	**0% FFO**	**1% FFO**	**2% FFO**
Moisture	8.97	8.94	9.45
Ash	6.99	7.14	6.70
Protein	32.53	33.37	31.59
Fiber	8.13	8.74	8.73
Fat	4.48	5.54	6.40
NFE	37.36	35.99	37.01
Energy (Kcal/g)	416.80	421.20	430.40

Note. Nitrogen-free extract (NFE) = 100% - (% protein + % fat + % ash + % fiber).

Fatty acid (FA) composition of the experimental diets showed that total saturated and unsaturated fatty acids specially especially omega-9 fatty acids were higher in diet containing 2% FFO compared to control (Tables 9). Fatty acid composition in fish oil extracted from freshwater hybrid catfish contains high amounts of unsaturated fatty acids (81.17 g/100 g), including Omega 9 fatty acids (42.28 g/100 g). Therefore, fish oil from hybrid catfish is suitable for a dietary supplement to increase fatty acid contents in the complete diets (Sattang *et al*., 2018a). Unsaturated fatty acids from fish oil, cod liver oil and/or squid liver oil contain essential fatty acids (EFA) which serve as precursors for prostaglandins synthesis (Saini and Keum, 2018). Prostaglandins are involved in different regulatory pathways including contractions of smooth muscles in the uterus, ovulation, hormone secretion, and regulation of blood pressure (Takahashi *et al*., 2018).

Deficiency of essential fatty acids in fish diets can cause slow growth, tail fin deformities, liver fat metabolism (fatty livers), excessive fat deposits in coelomic cavity, pale skin, swollen belly, and in severe cases hemolytic anemia and subsequent breathing problems (Usawakesmanee, 2006). Therefore, diets with optimal content of essential fatty acids are critical for achieving economical and sustainable production and reproduction in aquaculture.

Tables 9 Fatty acid composition of the experimental diets containing 0, 1, and 2% freshwater fish oil (FFO, dry weight).

Fatty acid composition (g per 100 g of feed)	**0% FFO**	**1% FFO**	**2% FFO**
Lauric acid (C12:0)	0.0085	0.0120	0.0714
Tridecanoic acid (C13:0)	-	0.0011	0.0021
Myristic acid (C14:0)	0.0804	0.1259	0.2170
Pentadecanoic acid (C15:0)	0.0142	0.0152	0.0176
Palmitic acid (C16:0)	1.1649	1.4439	1.8999
Heptadecanoic acid (C17:0)	0.0254	0.0260	0.0286
Stearic acid (C18:0)	0.2646	0.2980	0.4076

Arachidonic acid (C20:0)	0.0338	0.0354	0.0366
Heneicosanoic acid (C21:0)	0.0031	0.0031	0.0036
Behenic acid (C22:0)	0.0241	0.0227	0.0219
Tricosanoic (C23:0)	0.0038	0.0039	0.0043
Lignoceric acid (C24:0)	0.0234	0.0575	0.0352
Saturated fatty acid (SFA)	1.6462	2.0447	2.7458
Myristoleic acid (C14:1)	0.0024	0.0028	0.0033
cis-10-Pentadecenoic acid (C15:1n10)	-	-	-
Palmitoleic acid (C16:1n7)	0.0788	0.0884	0.1001
cis-10-Heptadecenoic acid (C17:1n10)	-	-	-
Trans-9-Eladic acid (C18:1n9t)	-	-	-
cis-9-Oleic acid (C18:1n9c)	1.6166	1.8290	2.3314
cis-Vaccenic acid (C18:1n7)	0.0729	0.0722	0.0715
cis-11-Eicosenoic acid (C20:1n11)	0.0261	0.0290	0.0480
Erucic acid (C22:1n9)	0.0031	0.0035	0.0052
Lignoceric acid (C24:1)	0.0060	0.0061	0.0068
Monounsaturated fatty acid (MUFA)	1.8059	2.031	2.5663
cis-9,12-Linoleic acid (C18:2n6, LOA)	1.6172	1.3640	1.5132
gamma-Linolenic acid (C18:3n6)	0.0030	0.0066	0.0070
α-Linolenic acid (C18:3n3)	0.1201	0.0877	0.0958
Moroctic acid (C18:4n3)	0.0069	0.0073	0.0070
cis-11,14-Eicosadienoic acid (C20:2n6)	0.0059	0.0097	0.0162
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.0034	0.0077	0.0125
cis-11,14,17-Ecosatrienoic acid (C20:3n3)	0.0017	0.0020	0.0027
Arachidonic acid (C20:4n6, ARA)	0.0321	0.0379	0.0337
Ecosatetraenoic acid (C20:4n3)	0.0041	0.0044	0.0052
cis-13,16-Docosadienoic acid (C22:2)	-	-	-
cis-7,10,13,16-Docosstetraenoic acid (C22:4n6)	0.0088	0.0109	0.0097
Docosapentaenoic acid (C22:5n6)	0.0155	0.0185	0.0162
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3, EPA)	0.0455	0.0449	0.0408

4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3, DHA)	0.1340	0.1343	0.1166
Polyunsaturated fatty acid (PUFA)	1.9982	1.7359	1.8766
Unsaturated fatty acid (UFA)	3.8041	3.7669	4.4429
n-3 fatty acid	0.3123	0.2806	0.2681
n-6 fatty acid	1.6800	1.4456	1.5923
n-9 fatty acid	1.6197	1.8325	2.3366
Unidentified peak	0.2797	0.2691	0.3342
Total	**5.4503**	**5.8116**	**7.1887**

2. Growth performance parameters

During spawning season in August, WG and ADG of caged catfish fed with 1% FFO supplemented diet were significantly increased compared to control (354.90 g and 2.96 g/fish/day, respectively, $p<0.05$), in contrast to post-spawning season (December), no significant difference was observed (Figures 11). Survival rate during spawning and post-spawning season of hybrid catfish in all treatment groups were 100%.

The growth performances (WG and ADG) of hybrid catfish during spawning season (August) fed with 1% FFO supplemented diet were significantly increased ($p<0.05$, Figures 11). This is in accordance with previous work of Rattanaphot *et al*. (2018b) who reported significant increase in growth of hybrid catfish (*P. gigas* x *P. hypophthalmus*) fed with freshwater fish oil supplements containing high amount of omega 9 fatty acids (four times higher than marine fish diet). Similar increase in growth of *P. hypophthalmus broodstock* fed with supplemented essential fish oils was observed prior to the first spawning season (Kabir *et al*., 2012; Arfah *et al*., 2018). Weight gain and average daily gain of F_2 hybrid catfish (*P. gigas* x *P. hypophthalmus*) broodstock was reported higher from June to October, compared to January-June (Sutthi *et al*., 2014). Similarly, Nile tilapia fed with 1 and 1.5% freshwater fish oil supplemented diets and raised in cages for 4

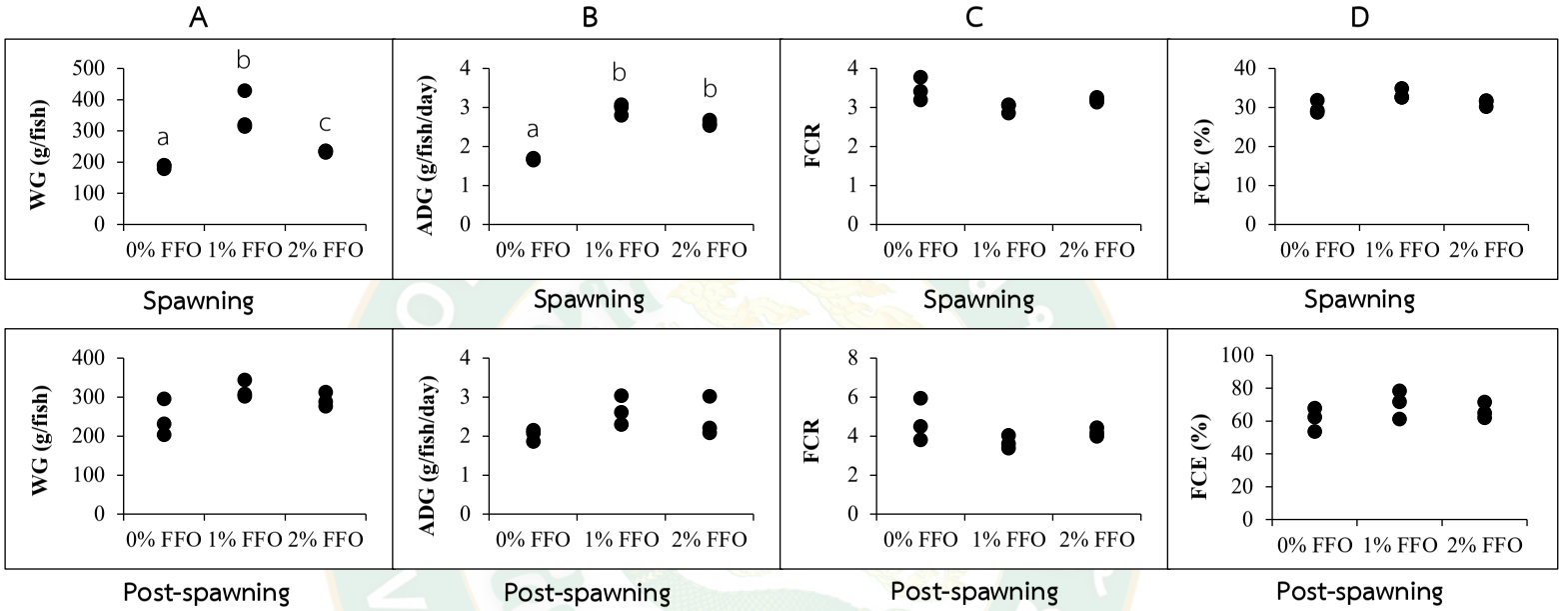
months were significantly increased in WG and ADG while feed conversion rate (FCR) was decreased (Rattanapot *et al*., 2018a).

In another hand, previous studies of reproductive in female C57BL/6 mice found that on a diet supplemented with high fat (45%) reduced number of pups, gestation duration and lead to early labor. Omega-9 fatty acids shifted sex of offspring to females (59.5-60.8%) (Mousavi *et al*., 2016). Sink *et al*. (2010) reported decreased spawning success in catfish fed with plant protein substitute fortified with 10% fish oil mixture. Similarly, Somboon and Semachai (2014) found that growth of Basa catfish (*Pangasius bocourti* Sauvage, 1880) was decreased when fed diet with total lipid content of 12%. Reported discrepancies suggest a possibility that increased FFO levels could interfere with energy balances in fish diets. Such rationale is supported by recent finding that dietary energy content of more than 428.5 kcal/100 g resulted in decreased weight gain and SGR of *P. hypophthalmus* (Ranjan *et al*., 2018). This was also observed in present study where 1% of FFO supplement showed the best growth performance in catfish (Figures 11). Phillips (1972) found that increase in weight gain and growth rate was possible only up to a certain dietary energy level and was followed by a decrease as result of increased efforts to metabolize and store high-energy nutrients.

The prost-spawning season, the supplementation with 1% and 2% FFO did not increase fish growth. One possible explanation is that differences in water temperature (20-25 °C in post-spawning; 25-30 °C in spawning season) could exercise a strong influence on the hybrid catfish biology, including changes in energy metabolism of fatty acids in different spawning phases (Sink and Lochmann, 2008). Water temperature is a major factor that directly affects feed intake, metabolic rates and energy consumption of catfish (Koeypudsa and Jongjareanjai, 2011), and has a direct effect on the growth of fish (Smith, 1989). However, even though significant mobilisation of fatty acids was observed in seabream liver and muscle during pre-spawning period, the fatty acid content remained constant in the gonads (Komilus *et al*., 2008). Thus, it is possible that dietary supplementation with FFO may rescue growth parameters during pre-spawning and spawning season via better availability of

fatty acids, while these mechanisms may not be so important after spawning, when mobilisation of fatty acids is lower (Jerez *et al*., 2006).

Figures 10 Characteristics of hybrid catfish (*P. larnaudii* x *P. hypophthalmus*) fed with different levels of freshwater fish oil (FFO) supplement.



Season		p value		
Spawning	0.004	0.000	0.056	0.050
Post-spawning	0.198	0.200	0.223	0.350

Figures 11 Effects of freshwater fish oil on the A) weight gain (WG), B) weight average daily gain (ADG), C) feed conversion ratio (FCR) and D) feed conversion efficiency (FCE) of hybrid catfish fed with 0% FFO, 1% FFO, and 2% FFO during spawning and post-spawning season. Means in dot plots with different small letters (a, b, c) denote significant difference.

3. Indicators of reproductive condition

3.1 Steroid hormone

During spawning season in August, fish fed with 1% FFO supplement had both 17β-estradiol and testosterone plasma values significantly higher than control and 2% FFO groups (1,577 pg mL^{-1} and 3.28 ng mL^{-1}, $p<0.05$, respectively) as shown in Tables 10. Steroid hormone levels were elevated in plasma of FFO supplemented fish compared to control (Tables 10). Similarly, 17 β-estradiol and testosterone levels of *Hemibagrus nemurus* were shown to be increased during spawning season (May through August) compared to postspawning season (September through December), and their GSI in May (7.04%) and August (7.01%) was higher than in December (1.14%) (Adebiyi *et al*., 2013). Moreover, supplementation of fatty acids in seabass before and during spawning season induced increase in steroid hormones and significantly improved reproductive performance of sea bass (Navas *et al*., 1998). Finally, Mekong giant catfish (*P. gigas*) eight-year old females cultured in earthen ponds similar to our study, showed highest plasma levels of 17β-estradiol in May, prior to their spawning maturation (Manosroi *et al*., 2003).

3.2 Gonad maturity

Gonadosomatic index (GSI) was not significantly different; however, a trend in positive correlation between increasing FFO supplement and GSI was noted. During post-spawning season in December, reproductive indicators in all groups were not significantly different (Tables 10).

Gonadosomatic index has been used as reliable indicator of changes in nutritional and energetic balance condition of fish (Adams *et al*., 1996). In our study, we observed fish in two parts of the growing cycle, fish spawning in August (during rainy season) and post-spawning season in December (entering Thai winter season). Significant differences in GSI were observed in between the two seasons,

with highest difference in the group fed with 2% FO (Tables 10). It was reported recently that critical requirements for optimal reproductive performance including relative gonadal weight in African catfish (*Clarias gariepinus*) may be water temperatures of 28 °C and nutrition (Al-Deghayem *et al*., 2017).

Tables 10 Differences in indicators of reproductive condition during spawning (August) and post-spawning (December) season in catfish fed diets different content of freshwater fish oil (FFO, 0, 1, and 2%).

Month	Fish (n)	Formulas	E_2 (pg mL^{-1})	T (ng mL^{-1})	GSI (%)	MOS	HOS
4 (August)	3	0% FFO	113.12 ± 43.33^{c}	2.56 ± 0.95^{ab}	1.97±0.23	Immature	PG
	3	1% FFO	$1,577.00 \pm 5.26^{a}$	3.28 ± 0.41^{b}	3.34±1.07	Maturing	MGV
	3	2% FFO	694.60 ± 3.10^{b}	1.73 ± 0.34^{a}	4.42 ± 1.28^{y}	Mature	GVBD
8 (December)	3	0% FFO	48.17 ± 6.37	<0.025	1.01 ± 0.65	Immature	PG
	3	1% FFO	51.88 ± 4.29	<0.025	1.92 ± 0.37	Immature	PG
	3	2% FFO	101.92±46.39	<0.025	0.87 ± 0.23^{z}	Immature	PG

Note. HOS stages are listed as: PG (primary growth phase), CGV (central germinal vesicle), MGV (migrating germinal vesicle), PGV (peripheral germinal vesicle), GVBD (germinal vesicle breakdown). Different superscripts (a, b, c, y, z) designate significantly different values in each column ($p<0.05$). Measurements of 17β-estradiol (E_2), testosterone (T), gonadosomatic index (GSI), macroscopic ovary stages (MOS) and histological ovary stages (HOS) of hybrid catfish are presented as mean value ± standard error of means (SEM).

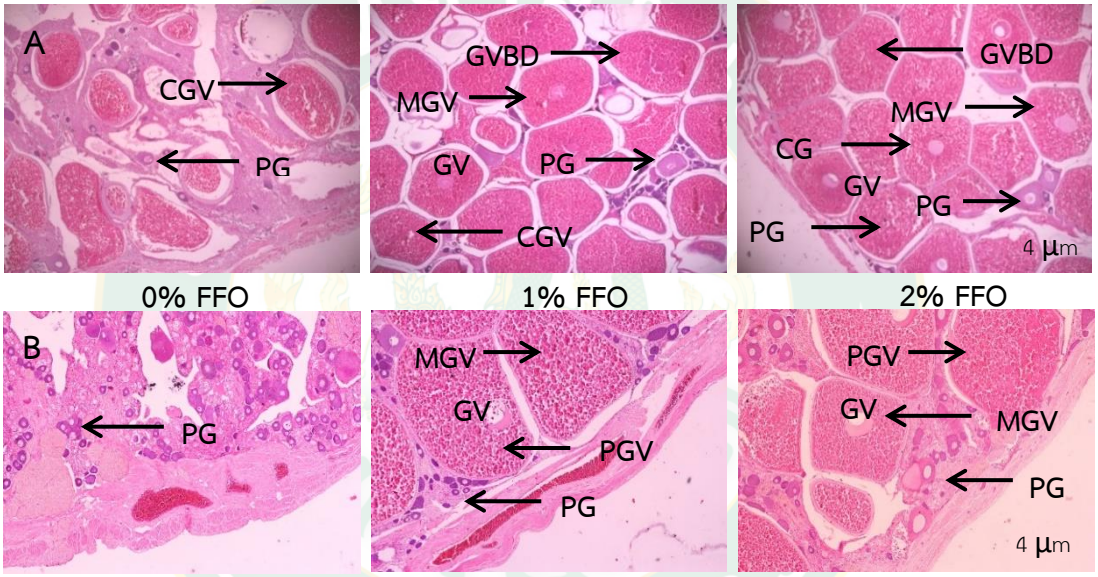
3.3 Histology of gonad

Histologically, fish feed with 1% and 2% FFO had migrating germinal vesicle (MGV), peripheral germinal vesicle (PGV) and germinal vesicle breakdown (GVBD) in more developed stages when compared to fish from control group (Figures 12). Average oocyte histology determined in during spawning season showed that most oocytes were in primary growth (PG) phase, followed by central germinal vesicle (CGV), MGV, PGV and GVBD phases (67.21, 10.38, 20.77, 1.64 and 0.00%; 43.69, 14.34, 30.72, 8.19 and 3.07%; 38.85, 11.51, 35.25, 7.91 and 6.48%) for fish in control, 1% FFO and 2% FFO supplemented groups, respectively (Figures 13). Testicular histology and appearance of spermatocytes showed that spermatid (SD) and spermatozoa (SZ) maturation in fish from 1% and 2% FFO groups were more advanced than in control fish (Figures 14).

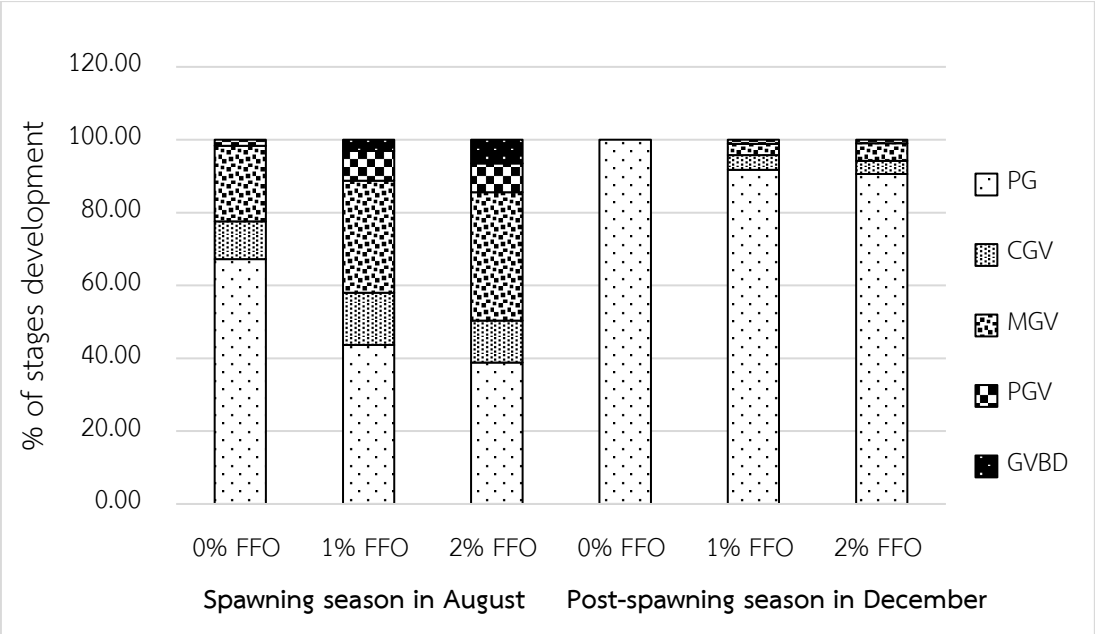
Oocytes from fish fed with FFO supplements showed advanced maturation stages and histological characteristics compared to the control (Figures 12). Rojtinnakorn and Thepnarong reported six oocyte maturation stages of the *Pangasianodon hypophthalmus* as stages 1 and 2 (primary growth phase, PG); stage 3 (central germinal vesicle, CGV); stage 4 (migrating germinal vesicle, MGV); stage 5 (peripheral germinal vesicle, PGV); and stage 6 (germinal vesicle breakdown, GVBD). Our results of histological evaluation of hybrid catfish ovaries in spawning phase (August) confirmed this categorization, and correspond to reported stages (Rojtinnakorn and Thepnarong, 2006; Prat *et al*., 1990). Testicular development in FFO supplemented fish was found to be more advanced than in control fish, and similar results were obtained by Sutthi *et al*. by finding spermatids and spermatozoa during spawning season in male catfish hybrid of F_2 generation (Sutthi *et al*., 2014).

Overall, our results demonstrate clearly that fish oil supplementation at low levels was able to increase plasma concentrations of steroid hormones (likely through increased availability of essential fatty acids used in synthesis of prostaglandin hormone precursor) (Mengumphan, 2010). The fish feed supplemented with 1% and 2% FFO showed increased content of monounsaturated fatty acids and

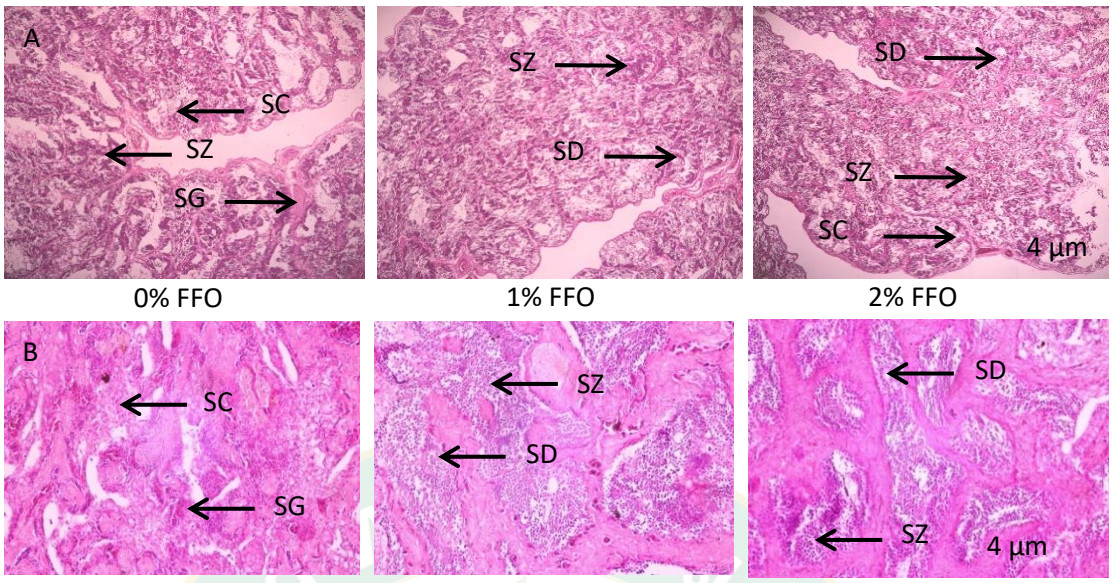
polyunsaturated fatty acids (Tables 9). Essential fatty acids are necessary for the reproductive system and growth of fish, affecting teleost pituitary and gonadal hormone levels, and reproductive performance (Bruce *et al*., 1999). Arachidonic acid deficiency interferes with regulation of the sex hormones, and slows down development of sperm and eggs in sea bass and goldfish (Silva and Dean, 2001). It was also demonstrated that conversion of arachidonic acid to eicosanoids stimulates testosterone production in goldfish ovaries and testis (Mercure and Van Der Kraak, 1996).



Figures 12 Effects of freshwater fish oil on the oocyte stage of hybrid catfish consisting of: A) ovary during spawning season (10x), B) ovary in post-spawning season (40x). Arrows point to: primary growth phase (PG), central germinal vesicle (CGV), peripheral germinal vesicle (PGV), germinal vesicle breakdown (GVBD) and germinal vesicle (GV).



Figures 13 Ovarian oocyte stages development of hybrid catfish fed with 0% FFO, 1% FFO, and 2% FFO during spawning (Aug) and post-spawning (Dec). Labeled as: primary growth phase (PG), central germinal vesicle (CGV), migrating germinal vesicle (MGV), peripheral germinal vesicle (PGV), and germinal vesicle breakdown (GVBD).



Figures 14 Effects of freshwater fish oil on the spermatocyte of hybrid catfish consisting of: A) testis during spawning season (Aug; 10x), B) testis during post-spawning season (Dec; 40x). Labeled as: spermatogonium (SG), spermatocyte (SC), spermatid (SD) and spermatozoa (SZ).

4. Fish fillet qualities

4.1 Color

The colorimetric analyses of during spawning (August) and post-spawning (December) revealed not significant differences ($p>0.05$) in lightness (L^*), redness (a^*) and yellowness (b^*) of fish fillet are shown in Tables 11. Characteristics of fish fillet color fed with different level of freshwater fish oil (FFO) supplement are show in the Figures 15.

In during spawning, L^*, a^* and b^* values of fish fillet fed with FFO of all levels were higher than that of post-spawning. The trend of the L^* values of fish fillet fed with 1 and 2% FFO of both seasons were higher than that of 0% FFO. Post-spawning season (December), which is winter causing the fish to eat diet less. Aquatic animals have adapted by using energy from fat accumulated in the body for their livelihood, affecting the change in meat color (L^*, a^*, b^*) is reduced (Benjakul, 2011).

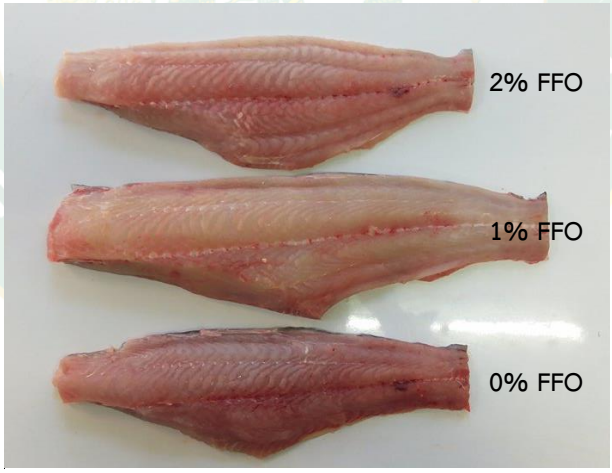
However, fish fillet fed with 1 and 2% FFO of post-spawning gave lower *a** and *b** values than 0% FFO. These results suggested that fish fillet fed with 1 and 2% FFO had a lighter color but lower redness and yellowness than that of 0% FFO (Tables 11). Similarly, lightness (*L**, 40.32-42.48), redness (*a**, 6.83-8.43) and the yellowness (*b**, 7.00-9.45) levels of Maejo Buk-Siam hybrid catfish (*Pangasianodon gigas* x *Pangasianodon hypophthalmus* F_2) fed with three different feed were not significantly different (Kitcharoen *et al*., 2017). While, the color in muscle (lateral line) of giant catfish (*Pangasianodon gigas*) in lightness (*L**), redness (*a**) and the yellowness (*b**) showed the 32.19, 16.92 and 15.99, respectively. The difference in color in muscles might be due to the difference in compositions, especially myoglobin or pigment content (Chaijan *et al*., 2010).

The coloration values found in this study were different from other studies. Kulawik *et al*. (2016) assess the color and sensory evaluation of frozen fillets of Pangasius catfish and Nile tilapia imported to Poland, Germany, and Ukraine. This study showed significant differences between groups in all studied parameters but reported lower level of *a** (-0.21 to -1.42) and high level of *L** (60.44 to 61.31) parameters. Yellowness of fish muscle increased during storage, while redness decreased. This increase in yellowness during storage can be attributed to heme oxidation which results in browning of the bloodline. Moreover, the *a** and *b** (redness and yellowness) values of muscle (*Clarias gariepinus*) color was significantly ($p<0.05$) affected by the diets. In particular, the chicken offal fed catfish appeared to have marked yellowness (*b**) on the muscle was high (7.49) (Noordin *et al*., 2019). According to Qiufen *et al*., (2012) the pigment absorption, transportation and deposition in the fish cells influenced by the fat content in the feed. With bad quality fats, the transportation and absorption of pigment granules in the fish body cannot be done normally.

Tables 11 The effect of freshwater fish oil (FFO) supplemented diets on color of fish fillet.

Month	Fish (n)	Formulas	*L**	*a**	*b**
4 (August)	3	0% FFO	50.77±1.30[a]	24.80±0.86[a]	8.03±0.41[a]
	3	1% FFO	52.36±0.95[a]	24.79±0.66[a]	7.86±0.06[a]
	3	2% FFO	52.17±1.56[a]	24.57±0.47[a]	8.10±0.14[a]
8 (December)	3	0% FFO	45.27±0.96[a]	8.34±0.66[a]	3.44±0.18[a]
	3	1% FFO	47.29±1.00[a]	6.93±0.92[a]	2.72±0.09[a]
	3	2% FFO	48.82±1.91[a]	8.24±1.10[a]	3.12±0.55[a]

Note. Color parameters are given as mean ± S.E. from triplicate determinations. Values in the same columns with the same superscript are not significantly different ($p>0.05$).



Figures 15 Color adderance of fish fillet color fed with different levels of freshwater fish oil (FFO) supplement.

4.2 Proximate composition

Tables 12 show the comparison of the freshwater fish oil (FFO) supplemented diets for proximate composition of fish fillet. All parameters of the proximate composition were not significantly different (p>0.05). However, the tendency of protein and fat were between 18.95-19.51% and 4.37-5.79%, respectively. Different protein values in fish fresh were using samples of different fish, using 3 fish to be replicates per treatment. The high fat in fish fresh fed 1 % freshwater fish oil supplementation was due to the aquatic animals store fat as source of energy for the fasting period. During the maturity of the reproductive organs, fat from the liver and muscles is sent to the reproductive organs. During the spawning period, the fat content is markedly reduced. But after the spawning stage, the fish will eat more diet, resulting in an increase in the amount of fat in the liver and muscles. But the amount of fat in the reproductive organs is reduced (Huss, 1995; Benjakul, 2011).

Similarly, protein (14.36-19.00%) and fat (0.54-8.60) levels of *P. gigas* (Chaijan *et al*., 2010), protein (15.50%) and fat (2.51%) levels of *Rhamdia quelen* (Weber *et al*., 2008), and protein (14.72-18.60%) and fat (3.65-16.10) levels of *Clarias gariepinus* (Noordin *et al*., 2019). Crude protein content in fish flesh varies depending on the species, the nutritional condition, the type of fish, the state of nutrition, and the productive cycle of animal as well as the parts of the organism (Sikorski *et al*., 1990). Puwastien *et al*. (1999) also reported that the protein content ranged from 17 to 20 g/100 g for raw freshwater fish. Generally, the lipid content of cultured fish muscle is higher than that of wild fish which may be caused by lack of exercise, overfeeding and high energy diets (Rodriguez *et al*., 2004).

In addition, Hunt *et al*., (2018) reported the effect of fish oil (FO) and cod liver oil (CLO) as the dietary lipid sources on the muscle tissue proximate composition of Nile Tilapia (*Oreochromis niloticus*). The fish fed with FO (1.70%) diet group contained lower crude lipid in their muscle than that of fish fed with CLO (2.35%) contained diet group, but the fish fed with FO (23.95%) was of crude protein higher than the fish fed with CLO (22.18%) and significantly different (p<0.05). This

could probably be due to quantity of oil used which was not in excess of the fish requirement resulting in better utilization of protein for growth. While the whole-body proximate composition of Siberia sturgeon (*Acipenser baerii*, Brandt) fed diets supplemented with various ratio of DHA/EPA were not significantly different (p>0.05) (Luo *et al*., 2019).

Tables 12 The effect of freshwater fish oil (FFO) supplemented diets on proximate composition of fish fillet in August (spawning season).

Proximate composition (% dry matter basis)	**0% FFO**	**1% FFO**	**2% FFO**
Moisture	76.02±1.86[a]	75.13±1.03[a]	75.89±1.55[a]
Ash	1.23±0.24[a]	1.09±0.27[a]	1.08±0.38[a]
Protein	19.27±2.85[a]	18.95±3.73[a]	19.51±4.45[a]
Fat	4.74±3.17[a]	5.79±3.45[a]	4.37±4.57[a]

Note. Values (means of triplicate ± S.E.) in the same rows with the same superscript are not significantly different (p>0.05).

4.3 Fatty acid composition

The fatty acid composition of hybrid catfish fed the three dietary treatments of freshwater fish oil (FFO) supplemented diets is shown in Tables 13 . The total saturated fatty acid (SFA) content of muscle tissue lipids were 2.61, 1.91 and 1.48 in 0%, 1% and 2% FFO reared fish, respectively. During the spawning period, the fat content and fatty acid is markedly reduced (Huss, 1995; Benjakul, 2011). Regardless of the diets, palmitic acid (C16:0) was the dominant SFA detected in all group of hybrid catfish fillet samples of this study (Tables 13). Among n-9 series of fatty acids, oleic acid (C18:1n9c) was the one of the predominant monounsaturated fatty acid (MUFA) in 0%, 1% and 2% FFO diet in fillet samples of hybrid catfish. However, fish fed with 1% FFO diet, n-3 and n-6 had higher amounts than that of fish fed with 0% and 2% FFO diet in fillet samples (Tables 13).

Similarly, fatty acid profiles of fillets in Nile tilapia (*Oreochromis niloticus*) fed with fish oil (FO) diet had low concentration of palmitic acid (C16:0) and Monounsaturated fatty acid (MUFA), but high levels of polyunsaturated fatty acids (PUFA) when compared to fish fed cod liver oil (CLO) diet (Hunt *et al*., 2018). Inkam (2020) reported, the fatty acid profile of flesh of tilapia (*Oreochromis niloticus*) fed with FO supplementary diet was highest in eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), this were polyunsaturated fatty acids (PUFA). When dietary lipid amount is increase 12 percent dietary lipid on percent yield of the basa catfish (*Pangasius bocourti*). Increasing of the lipid accumulation effect to increasing of essential fatty acids (linoleic (LOA), EPA and DHA) in the fish flesh too, especially unsaturated fatty acids was not significant different (p>0.05). Aquatic feed with 6-9 percent dietary lipid amount in feed formula and 2-4 percent essential fatty acids of total dietary lipid were sufficient for growth performance and lipid accumulation in meat of the basa catfish (Somboon and Semachai, 2014).

Furthermore, it appeared that changing oil source in diet with FO had an increassing effect on the proportion of n-3 (especially EPA and DHA) in muscle tissues of tilapia (Hunt *et al*. 2018). Oleic acid which is the major MUFA in catfish is considered to be of exogenous origin and usually reflects the type of fish diet (Ackman, 1980). The principle acids in the PUFA group was linoleic acid (C18:2n6) shown in the Tables 13. Similar findings were reported by Okonji and Daniel (2013); Noordin *et al*. (2019). EPA was not detected in the fillet from hybrid catfish fed with FFO all levels. This was expected as the EPA contain in the fish diet is low (Tables 9). Feeding on vegetable oils lowered the muscle content of EPA, DHA and arachidonic acid (ARA) and this kind of effect has been documented earlier in Channel catfish (Manning *et al*. 2006), South American catfish (Arslan *et al*., 2008) and Brown Trout (Arslan *et al*., 2012). Synthesis and degradation of fatty acid occurs mainly in the liver, and many enzymes involved in regulating these pathways show varying affinities for the different fatty acids in the organ (Kiessling, and Kiessling, 1993). Therefore, identifying suitable alternatives to FO in aquafeeds or improving efficiency of FO used must meet the issues of balancing lipid storage with lipid burning for

growth and supplying essential fatty acids, while maintaining the health of the fish (Leaver *et al*., 2008).

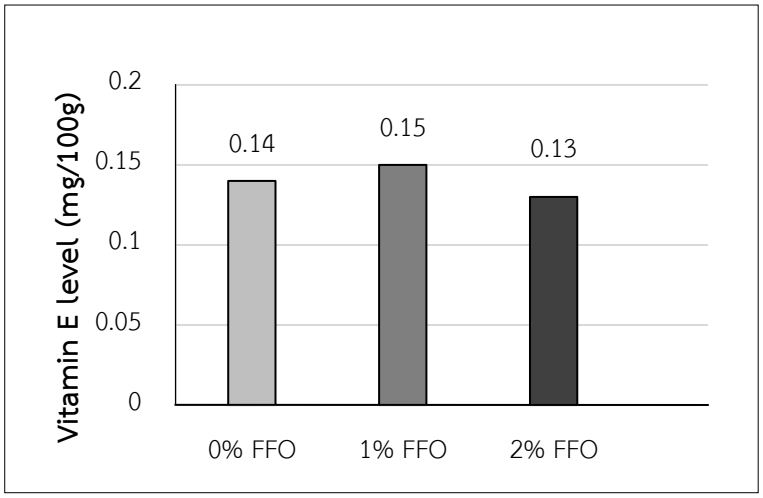
Tables 13 The effect of freshwater fish oil (FFO) supplemented diets on fatty acid composition of fish fillet in August (spawning season).

Fatty acid composition (g per 100 g of fillet)	**0% FFO**	**1% FFO**	**2% FFO**
Myristic acid (C14:0)	0.17	0.09	0.09
Palmitic acid (C16:0)	1.99	1.42	1.11
Heptadecanoic acid (C17:0)	0.11	0.05	0.05
Stearic acid (C18:0)	0.35	0.34	0.23
Saturated fatty acid (SFA)	2.61	1.91	1.48
Palmitoleic acid (C16:1n7)	0.03	0.02	0.02
Trans-9-Eladic acid (C18:1n9t)	0.03	0.03	0.02
cis-9-Oleic acid (C18:1n9c)	2.09	1.77	1.26
cis-11-Eicosenoic acid (C20:1n9)	0.03	0.05	0.02
Monounsaturated fatty acid (MUFA)	2.19	1.88	1.33
cis-9,12-Linoleic acid (C18:2n6, LOA)	0.42	0.50	0.27
cis-11,14-Eicosadienoic acid (C20:2)	-	0.01	-
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.02	0.02	0.01
Arachidonic acid (C20:4n6, ARA)	0.03	0.03	0.02
4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3, DHA)	0.03	0.03	0.02
Polyunsaturated fatty acid (PUFA)	0.51	0.60	0.32
Unsaturated fatty acid (UFA)	2.70	2.48	1.65
n-3 fatty acid	0.02693	0.03517	0.01942
n-6 fatty acid	0.47314	0.55098	0.29605
n-9 fatty acid	2.08858	1.76920	1.26418
Total	**5.31**	**4.39**	**3.13**

4.4 Vitamin E (Alpha-tocopherol)

Vitamin E of the diets supplemented with different level of freshwater fish oil (FFO) shown in Figures 16 where it was observed that addition of FFO did not show significant difference (p>0.05), the tendency of vitamin E in fish fillet with fed supplemented with 1, 0 and 2% FFO were 0.15, 0.14 and 0.13 g/100 g of fillet, respectively.

In another hand, previous studies of *Spirulina* sp. (high vitamin E) supplemented in pellet feed was improve maturation to the broodstock of *Pangasinodon gigas* during spawning season (Meng-umphan and Saengkrachang, 2008). Similarly, Chen *et al*. (2008) reported that the rainbow trout (*Oncorhynchus mykiss*) diet was supplemented with 0, 8.5, or 15 g/100 g of flaxseed oil (FO). The alpha-tocopherol content in fillets was increased with the length of time being fish fed with FO no significant difference (p>0.05). Moreover, striped catfish (*Pangasius hypophthalmus*) were fed with three trial diets prepared by adding different amounts of alpha-tocopheryl acetate (ATA; 0, 90 and 300 mgKg^{-1} diet), alpha- tocopherol level of the fish flesh were increased due to ATA supplementation in diets (Al-Noor *et al*., 2012).



Figures 16 Vitamin E in fish fillet of hybrid catfish fed with freshwater fish oil (FFO) supplemented diets in August (spawning season).

4.5 Carcass quality

Referring to the carcass quality, there were not differences in the proportions of grated meat, skeleton, visceral, fillet, ventral meat, chin meat and gonad between groups during spawning (August) and post-spawning (December) (Tables 14). In the present study, fillet yield was not different, but the post-spawning had a higher fillet (%) than the during spawning, because during spawning season, fish develop and accumulated oocyte and spermatocyte for reproduction. This can be seen from the high percentage of gonads.

Filleting implies removal of bones, fins and ventral meat from the flesh. Filleting and trimming are important for logistics, economics, and addition of value along the marketing chain and for separation of edible part from the inedible ones (Intarak *et al*. 2015). In addition, those who filleting must have experience to achieve a high percentage of fillet. Filleting in fish can be done either by machine or by hand. Hand filleting is labor intensive and time consuming (Rora *et al*. 2001). However, fillet yield depends on the species, on the structural anatomy of the fish and season. Fish with smaller heads and frames relative to their musculature gives a higher fillet yield than fish with larger heads and frames (Intarak *et al*. 2015). The percentage of fillet for Punga fish (*Pangasius bocourti* Sauvage) was found range from 30.4-31.2% weight 700-1200 g (Intarak *et al*. 2015). The percentages of hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) fillet were 30.07-32.79% during spawning and 31.83-35.63% post-spawning.

Tables 14 The effect of freshwater fish oil (FFO) supplemented diets on carcass quality of hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*).

Month	Fish (n)	Formulas	Body weight (kg)	Carcass quality (%)						
				Fillet	Skeleton	Visceral	Grated meat	Ventral meat	Chin meat	Gonad
4 (August)	3	0% FFO	1.83±0.04[a]	32.79±0.45[a]	33.84±0.35[a]	7.71±0.20[a]	5.77±0.14[a]	13.65±0.16[a]	4.26±0.10[a]	1.97±0.23[a]
	3	1% FFO	1.70±0.13[a]	32.71±1.33[a]	32.97±0.15[a]	7.87±0.24[a]	5.67±0.25[a]	12.98±0.26[a]	4.46±0.31[a]	3.34±1.07[a]
	3	2% FFO	1.81±0.13[a]	30.07±0.45[a]	33.69±0.49[a]	7.91±0.62[a]	5.77±0.20[a]	13.76±0.41[a]	4.39±0.21[a]	4.42±1.28[a]
8 (December)	3	0% FFO	1.53±0.04[a]	33.36 ± 1.78[a]	32.89 ± 0.37[a]	9.77 ± 1.25[a]	3.28 ± 0.23[a]	18.42 ± 3.80[a]	1.29 ± 0.30[a]	1.01 ± 0.65[a]
	3	1% FFO	1.82±0.05[a]	31.83 ± 2.85[a]	35.68 ± 2.44[a]	12.62 ± 2.19[a]	3.55 ± 0.26[a]	12.62 ± 2.19[a]	1.46 ± 0.13[a]	1.92 ± 0.37[a]
	3	2% FFO	1.69±0.27[a]	35.63 ± 6.08[a]	31.39 ± 3.80[a]	11.62 ± 1.56[a]	3.60 ± 0.50[a]	15.06 ± 1.45[a]	1.70 ± 0.24[a]	0.87 ± 0.23[a]

Note. Carcass quality are given as mean ± S.E. from six determinations. [a] Mean within the same columns with the same superscript are not significantly different (p>0.05).

REFERENCES

REFERENCES

Abraham, M., Hilge, V., Lison, S. & Tibika, H. 1984. The cellular envelope of oocytes in teleosts. **Cell and Tissue Research**, 235: 403-410.

Ackman, R. G. 1980. Fish lipids. Part I. **Advances in Fish Science and Technology**, 86-103.

Adams, S. M., Ham, K. D., Greeley, M. S., LeHew, R. F., Hinton, D. E., & Saylor, C. F. 1996. Downstream gradients in bioindicator responses: point source contaminant effects on fish health. **Canadian Journal of Fisheries and Aquatic Sciences**, 53(10), 2177-2187.

Adebiyi, F. A., Siraj, S. S., Harmin, S. A., & Christianus, A. 2013. Plasma sex steroid hormonal profile and gonad histology during the annual reproductive cycle of river catfish *Hemibagrus nemurus* (Valenciennes, 1840) in captivity. **Fish Physiology and Biochemistry**, 39(3), 547-557.

Ai, Q., Mai, K., Zhang, L., Tan, B., Zhang, W., Xu, W., & Li, H. 2007. Effects of dietary β-1, 3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*. **Fish and Shellfish Immunology**, 22(4), 394-402.

Aitken, R. J., Wingate, J. K., De Iuliis, G. N., Koppers, A. J., & McLaughlin, E. A. 2006. Cis-unsaturated fatty acids stimulate reactive oxygen species generation and lipid peroxidation in human spermatozoa. **The Journal of Clinical Endocrinology & Metabolism**, 91(10), 4154-4163.

Akvaforsk, Å. 2005. **Selection and breeding programs in aquaculture** (Vol. 2005, p. 360). T. Gjedrem (Ed.). New York: Springer.

Al-Deghayem, W. A., Al-Balawi, H. F., Kandeal, S. A., & Suliman, E. A. M. 2017. Gonadosomatic index and some hematological parameters in African catfish *Clarias gariepinus* (Burchell, 1822) as affected by feed type and temperature level. **Brazilian Archives of Biology and Technology**, 60.

Alhazzaa, R., Nichols, P. D., & Carter, C. G. 2019. Sustainable alternatives to dietary fish oil in tropical fish aquaculture. **Reviews in Aquaculture**, 11(4), 1195-1218.

Al-Noor, S. M., Hossain, M. D., & Islam, M. A. 2012. The study of fillet proximate composition, growth performance and survival rate of striped catfish (*Pangasius hypophthalmus*) fed with diets containing different amounts of alpha-tocopherol (vitamin-e). **Journal of Bio-Science**, 20, 67-74.

Amornlerdpison, D., Mengamphan, K., Lailerd, N., Pongchaidecha A., Supaviriyakorn, W., Srimaroeng, C., & Wangcharoenn, W. 2010. **Complete report; potential of freshwater fish oil as a dietary supplement**. National Research Council of Thailand (NRCT).

Amornlerdpison, D., Rattanaphot, T., Tongsiri, S., Srimaroeng, C., & Mengumphan, K. 2019. Effect of omega-9–rich fish oil on antioxidant enzymes and relative immune gene expressions in Nile tilapia (*Oreochromis niloticus*). **Songklanakarin Journal of Science and Technology**, 41(6), 1287-1293.

Ann Sorbera, L., Francisco Asturiano, J., Carrillo, M., & Zanuy, S. 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). **Biology of Reproduction**, 64(1), 382-389.

AOAC. 2000. **Official Methods of Analysis of AOAC International**. 17th ed., Maryland, USA.

AOAC. 2012. **Official method 996.06 Fat.** (Total, Saturated, and Unstaurated) in Foods.

Arfah, H., Sudrajat, A. O., Supriyadi, M. A., & Zairin, M. 2018. Gonad maturation of female striped catfish *Pangasionodon hypophthalmus* (Sauvage, 1878) using a combination of pregnant mare serum gonadotropin + antidopamine, vitamin E, and curcumin extract mixed feed outside its spawning season. **International Journal of Fisheries and Aquatic Studies**, 6(5), 52-57.

Aroeira, C. N., de Almeida Torres Filho, R., Fontes, P. R., Ramos, A. D. L. S., de Miranda Gomide, L. A., Ladeira, M. M., & Ramos, E. M. 2017. Effect of freezing prior to aging on myoglobin redox forms and CIE color of beef from Nellore and Aberdeen Angus cattle. **Meat Science**, 125, 16-21.

Arslan, M., Rinchard, J., Dabrowski, K., & Portella, M. C. 2008. Effects of different dietary lipid sources on the survival, growth, and fatty acid composition of South American catfish, *Pseudoplatystoma fasciatum*, surubim, juveniles. **Journal of the World Aquaculture Society**, 39(1), 51-61.

Arslan, M., Sirkecioglu, N., Bayir, A., Arslan, H., & Mevlut, A. R. A. S. 2012. The influence of substitution of dietary fish oil with different vegetable oils on performance and fatty acid composition of brown trout, *Salmo trutta*. **Turkish Journal of Fisheries and Aquatic Sciences**, 12(3), 575-583.

Asturiano, J., Sorbera, L., Carrillo, M., Zanuy, S., Ramos, J., Navarro, J., & Bromage, N. 2001. Reproductive performance in male European sea bass (*Dicentrarchus labrax*, L.) fed two PUFA-enriched experimental diets: a comparison with males fed a wet diet. **Aquaculture**, 194(1-2), 173-190.

Bagenal, T. B. 1978. **Methods for assessment of fish production in fresh waters. IBP Handbook No. 3.** Blackwell Science Publication.

Baird, I. G., Inthaphaisy, V., Kisouvannalath, P., Phylavanh, B., & Mounsouphom, B. 1999. **The fishes of southern Lao.** Vientiane (Lao): Ministry of Agriculture and Forestry.

Bardach, J. E. 1958. **Report on fisheries in Cambodia**. USOM.

Barlow, S. 2000. Fishmeal and fish oil: sustainable feed ingredients for aquafeeds. **Global Aquaculture Advocate**, 4, 85-88.

Bartley, D. M., Rana, K., & Immink, A. J. 2000. The use of inter-specific hybrids in aquaculture and fisheries. **Reviews in Fish Biology and Fisheries**, 10(3), 325-337.

Bell, J. G., Ashton, I., Secombes, C. J., Weitzel, B. R., Dick, J. R., & Sargent, J. R. 1996. Dietary lipid affects phospholipid fatty acid compositions, eicosanoid production and immune function in Atlantic salmon (*Salmo salar*). **Prostaglandins, Leukotrienes and Essential Fatty Acids**, 54(3), 173-182.

Benjakul, S. 2011. **Chemistry and aquatic animal quality**. 2nd ed. Bangkok: Odeon Store.

Billard, R., Fostier, A., Weil, C., & Breton, B. 1982. Endocrine control of spermatogenesis in teleost fish. **Canadian Journal of Fisheries and Aquatic Sciences**, 39(1), 65-79.

Billard, R. 1986. Spermatogenesis and spermatology of some teleost fish species. **Reproduction Nutrition Development**, 26: 877-920.

Borg, B. 1994. Androgens in teleost fishes. **Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology**, 109(3), 219-245.

Bowley, L. A., Alam, F., Marentette, J. R., Balshine, S., & Wilson, J. Y. 2010. Characterization of vitellogenin gene expression in round goby (*Neogobius melanostomus*) using a quantitative polymerase chain reaction assay. **Environmental Toxicology and Chemistry**, 29(12), 2751-2760.

Brooks, S., Tyler, C. R., & Sumpter, J. P. 1997. Egg quality in fish: what makes a good egg. **Reviews in Fish Biology and Fisheries**, 7: 387-416.

Bruce, M., Oyen, F., Bell, G., Asturiano, J. F., Farndale, B., Carrillo, M., Zanuy, S., Ramos, J., & Bromage, N. 1999. Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n- 3 and n– 6 highly unsaturated fatty acid to reproductive performance. **Aquaculture**, 177(1-4), 85-97.

Calder, P. C. 2006. Polyunsaturated fatty acids and inflammation. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, 75(3), 197-202.

Cardinaletti, G., Franzoni, M. F., Palermo, F. A., Cottone, E., Mosconi, G., Guastalla, A., Campantico, E., Tibaldi, E., & Polzonetti-Magni, A. M. 2 0 1 0 . Environmental and neuroendocrine control of fish reproduction. **Recent Advances in Fish Reproduction Biology**, 3, 65-87.

Castell, J. D., Sinnhuber, R. O., Wales, J. H., & Lee, D. J. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. **The Journal of Nutrition**, 102(1), 77-85.

Chaijan, M., Jongjareonrak, A., Phatcharat, S., Benjakul, S., & Rawdkuen, S. 2010. Chemical compositions and characteristics of farm raised giant catfish (*Pangasianodon gigas*) muscle. **LWT-Food Science and Technology**, 43(3), 452-457.

Charo-Karisa, H., Opiyo, M. A., Munguti, J. M., Marijani, E., & Nzayisenga, L. 2013. Cost-benefit analysis and growth effects of pelleted and unpelleted on-farm feed on African catfish (*Claries Gariepinus* Burchell 1822) in Earthen Ponds. **African Journal of Food, Agriculture, Nutrition and Development**, 13(4), 8019-8033.

Chattopadhyay, K., Xavier, K. M., Layana, P., Balange, A. K., & Nayak, B. B. 2019. Chitosan hydrogel inclusion in fish mince based emulsion sausages: Effect of gel interaction on functional and physicochemical qualities. **International Journal of Biological Macromolecules**, 134, 1063-1069.

Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S., & Jaczynski, J. 2008. Effects of dietary alpha-tocopheryl acetate on lipid oxidation and alpha-tocopherol content of novel omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets. **LWT-Food Science and Technology**, 41(2), 244-253.

Chitmanat, C. 2002. Aquatic immune booster. **Songklanakarin Journal of Science and Technology**, 24(4), 739-747.

Christensen, K. 2003. **Effect of crossbreeding**. [Online]. Available http://www. ihh.kvl.dk/htm/ kc/popgen/genetics/9/2.htm (March 2021).

Steel, R. G., & Torrie, J. H. 1960. **Principles and procedures of statistics.** McGraw, New York.

Coward, K., & Bromage, N. 1998. Histological classification of oocyte growth and the dynamics of ovarian recrudescence in *Tilapia zillii*. **Journal of Fish Biology**, 53(2), 285-302.

Crawford, M. A., Doyle, W., & Meadows, N. 1987. Gender differences at birth and differences in fetal growth. **Human Reproduction**, 2(6), 517-520.

Dalmo, R. A., Ingebrigtsen, K., & Bøgwald, J. 1997. Non‐specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). **Journal of Fish Diseases**, 20(4), 241-273.

De Rooij, D. G., & Russell, L. D. 2000. All you wanted to know about spermatogonia but were afraid to ask. **Journal of Andrology**, 21(6), 776-798.

Department of Fisheries. 2020. **Thai fishery trade, December 2019**. [Online]. Available https://www.fisheries.go.th/strategy-tradestat/images/pdf/monthly/ 25 62 /dec% 2062.pdf (8 July 2020).

Deponte, M. 2013. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. **Biochimica et Biophysica Acta (BBA)-General Subjects**, 1830(5), 3217-3266.

Devlaming, V., Grossman, G., & Chapman, F. 1982. On the use of the gonosomatic index. **Comparative Biochemistry and Physiology Part A: Physiology**, 73(1), 31-39.

De Vries, P. S., Gielen, M., Rizopoulos, D., Rump, P., Godschalk, R., Hornstra, G., & Zeegers, M. P. 2014. Association between polyunsaturated fatty acid concentrations in maternal plasma phospholipids during pregnancy and offspring adiposity at age 7: the MEFAB cohort. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, 91(3), 81-85.

Dos Santos, T. C., Gates, R. S., de Fátima Souza, C., Tinôco, I. D. F. F., Cândido, M. G. L., & Freitas, L. C. D. S. R. 2019. Meat Quality Parameters and the Effects of Stress: A Review. **Journal of Agricultural Science and Technology B**, 9(5), 305-315.

Droge, W. 2002. Free radicals in the physiological control of cell function. **Physiological Reviews**, 82(1), 47-95.

Falconer, D.S. 1981. **Introduction to quantitative genetics,** 2nd edition. London, New York: Longmans, 340.

FAO. 2017. **Pangasius trade shrinking**. [Online]. Available http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1072503/ (8 July 2020).

FAO. 2020. **Pangasius prices hit bottom as COVID-19 shuts down core markets**. [Online]. Available http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1263847/ (8 July 2020).

Faukner, J., Rawles, S. D., Sink, T. D., Lochmann, R., Proctor, A., Chen, R., & Phillips, H. 2015. The effects of diets containing standard soybean oil, soybean oil enhanced with conjugated linoleic acids, menhaden fish oil, or an algal docosahexaenoic acid supplement on juvenile channel catfish performance, hematology, body composition, and nonspecific immune response. **North American Journal of Aquaculture**, 77(2), 217-229.

Flachs, P., Horakova, O., Brauner, P., Rossmeisl, M., Pecina, P., Franssen-van Hal, N., Ruzickova, J., Sponarova, J., Drahota, Z., Vlcek, C., Keijer, j., Houstek, J., & Kopecky, J. 2005. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat. **Diabetologia**, 48(11), 2365-2375.

Frei, B. Reactive oxygen species and antioxidant vitamins. Linus Pauling Institute. Oregon State University. 1997.

Froese, R., & Pauly, D. 2018. ***Pangasius larnaudii* Bocourt, 1866.** Fish Base. Available: http://www.fishbase.se/summary/Pangasius-larnaudii.html (August 2018).

Genestra, M. 2007. Oxyl radicals, redox-sensitive signaling cascades and antioxidants. **Cellular Signaling**, 19(9), 1807-1819.

Guerrero, A., Velandia Valero, M., Campo, M. M., & Sañudo, C. 2013. Some factors that affect ruminant meat quality: from the farm to the fork. Review. Acta Scientiarum. **Animal Sciences**, 35, 335-347.

Halliwell, B. 2007. Biochemistry of oxidative stress. **Biochemical Society Transactions**, 35(5), 1147-1150.

Halliwell, B., & Gutteridge, J. M. 1989. **Free radicals in biology and medicine**, 2nd ed. Oxford: Clarendon Press.

Halliwell, B. H., & Gutteridge, J. M. C. 2007. **Free radicals in biology and medicine**, 4th edn Oxford University Press.

Halliwell, B. 2006. Oxidative stress and neurodegeneration: where are we now?. **Journal of Neurochemistry**, 97(6), 1634-1658.

Hasan, B., Putra, I., Suharman, I., Iriani, D., & Muchlisin, Z. A. 2019. Growth performance and carcass quality of river catfish *Hemibagrus nemurus* fed salted trash fish meal. **The Egyptian Journal of Aquatic Research**, 45(3), 259-264.

Hocquette, J. F., Richardson, R. I., Prache, S., Medale, F., Duffy, G., & Scollan, N. D. 2005. The future trends for research on quality and safety of animal products. **Italian Journal of Animal Science**, 4(sup3), 49-72.

Hunt, A. Ö., YILMAZ, F. Ö., & Erçen, Z. 2018. Comparing the effects of feeding a fish oil-or a cod liver oil-based diet on growth, feed utilization and muscle fatty acid composition Nile Tilapia (*Oreochromis niloticus*). **Mediterranean Fisheries and Aquaculture Research**, 1(3), 130-142.

Huss, H.H. 1988. **Fresh fish-quality and quality changes**. FAO Fisheries Series, FAO, Rome, Italy.

Idler, D. R., Bitners, I. I., & Schmidt, P. J. 1961. 11-Ketotestosterone: an androgen for sockeye salmon. **Canadian Journal of Biochemistry and Physiology**, 39(11), 1737-1742.

IFT (Institute of food Technologists). 2007. **Sensory evaluation methods**. The Society for the Food Technologists, Chicago, IL.

Ikeuchi, T., Todo, T., Kobayashi, T., & Nagahama, Y. 2001. Two subtypes of androgen and progestogen receptors in fish testes. **Comparative Biochemistry and Physiology-Part B: Biochemistry & Molecular Biology**, 129: 449-455.

Intarak, I., Lhasudta, P., Jathurasitha, S., Wicke, M., & Kreuzer, M. 2015. Effects of slaughter weight on carcass and meat characteristics of Punga fish (*Pangasius bocourti* Sauvage). **Agriculture and Agricultural Science Procedia**, 5, 164-169.

Inkam, Meranee. 2020. **Supplementation of omaga-3 to value added of organic nile tilapia (*Oreochromis niloticus*) in biofloc system**. Master Thesis. Maejo University.

Ish, T. & Doctor, K. 2005. **Tra and basa: Tra (*Pangasius hypophthalmus*) and basa (*Pangasius bocourti*).** Seafood Watch, Seafood Report, Monterey Bay Aqurium, California, USA. 29 pp.

Islam, M. A., Begum, M., Pal, H. K., & Alam, M. J. 2008. Studies on the gonadosomatic index and fecundity of *Mystus gulio* (Ham.). **Progressive Agriculture**, 19(2), 161-166.

Jamieson, B. G., & Leung, L. P. 1991. **Fish evolution and systematics: evidence from spermatozoa: with a survey of lophophorate, echinoderm and protochordate sperm and an account of gamete cryopreservation**. Cambridge University Press.

Jankowska, B., Zakes, Z., Zmijewski, T., & Szczepkowski, M. 2003. Fatty acid profile and meat utility of wild and cultured zander, *Sander lucioperca* (L.). **Electronic Journal of Polish Agricultural Universities**, 6(1).

Jerez, S., Rodriguez, C., Cejas, J. R., Bolanos, A., & Lorenzo, A. 2006. Lipid dynamics and plasma level changes of 17β-estradiol and testosterone during the spawning season of gilthead seabream (*Sparus aurata*) females of different ages. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, 143(2), 180-189.

Jollès, P., & Jollès, J. 1984. What's new in lysozyme research?. **Molecular and Cellular Biochemistry**, 63(2), 165-189.

Kabir, M. A., Ghaedi, A., & Hashim, R. 2012. Ovarian development and sexual maturation of female striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) reared in captivity. **Asian Fisheries Science**, 25(3), 232-244.

Kagawa, H., Tanaka, H., Okuzawa, K., & Kobayashi, M. 1998. GTH II but not GTH I induces final maturation and the development of maturational competence of oocytes of red seabream *in vitro*. **General and Comparative Endocrinology**, 112(1), 80-88.

Kamei, H., Kawazoe, I., Kaneko, T., & Aida, K. 2005. Purification of follicle-stimulating hormone from immature Japanese eel, *Anguilla japonica*, and its biochemical properties and steroidogenic activities. **General and Comparative Endocrinology**, 143(3), 257-266.

Karthika, S., Chandirasekaran, V., & Sureshkumar, S. 2016. Sensory attributes of Namakkal Quail-1 meat. **Journal of Veterinary Science and Technology**, 5, 266-269.

Kasurak, A. V., Zielinski, B. S., & Higgs, D. M. 2012. Reproductive status influences multisensory integration responses in female round gobies, *Neogobius melanostomus*. **Animal Behaviors**, 83(5), 1179-1185.

Kemp, S. E., Hollowood, T., & Hort, J. 2011. **Sensory evaluation: a practical handbook**. John Wiley & Sons.

Khan, M. S. A., Alam, M. J., Rheman, S., Mondal, S., & Rahman, M. M. 2002. Study on the fecundity and GSI of brackish water catfish *Plotosus canius* (Hamilton-Buchanan). **Journal of Biological Sciences**, 2 (4), 232-234.

Khon Kaen AGR. J. 42 Suppl. 1: 2014. **An in-house Method**. Central Laboratory (Thailand). Report No. TRCM60/03590. 2017, p. 1-3.

Kiessling, K. H., & Kiessling, A. 1993. Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. **Canadian Journal of Zoology**, 71(2), 248-251.

Kitcharoen, N., Meekaew, P., Tongsiri, S., & Mengamphan, K. 2017. Preliminary guideline for replacement of fish meal for good aquaculture moving towards organic of Maejo Buk-Siam hybrid catfish. **International Journal of Agricultural Technology**, 13(7.1), 1119-1130.

Koeypudsa, W., & Jongjareanjai, M. 2011. Impact of water temperature and sodium chloride (NaCl) on stress indicators of hybrid catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther). **Songklanakarin Journal of Science & Technology**, 33(4).

Komilus, C. F., Shichi, N., Koshio, S., Ishikawa, M., Yokoyama, S., Michael, F. R., Gao, J. & Makita, C. 2008. Influences of palm oil blended with fish oil on growth performances and lipid profiles of red sea bream Pagrus major. **Aquaculture Science**, 56(3), 317-326.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Circulation**, 106(21), 2747-2757.

Kulawik, P., Migdał, W., Tkaczewska, J., & Özoğul, F. 2016. Assessment of color and sensory evaluation of frozen fillets from *Pangasius catfish* and Nile tilapia imported to European countries. **International Journal of Food Properties**, 19(7), 1439-1446.

Kumari, S. 2014. Study on growth and reproductive behaviour in freshwater catfish (*Heteropneustes fossilis*). **Scholars Academic Journal of Bioscience**, 2(6), 380-383.

Kumar, N., Krishnani, K. K., Kumar, P., Jha, A. K., Gupta, S. K., & Singh, N. P. 2017. Dietary zinc promotes immuno-biochemical plasticity and protects fish against multiple stresses. **Fish and Shellfish Immunology**, 62, 184-194.

Kumar, N., Singh, D. K., Bhushan, S., & Jamwal, A. 2021. Mitigating multiple stresses in *Pangasianodon hypophthalmus* with a novel dietary mixture of selenium nanoparticles and Omega-3-fatty acid. **Scientific Reports**, 11(1), 1-20.

Leaver, M. J., Bautista, J. M., Björnsson, B. T., Jönsson, E., Krey, G., Tocher, D. R., & Torstensen, B. E. 2008. Towards fish lipid nutrigenomics: current state and prospects for fin-fish aquaculture. **Reviews in Fisheries Science**, 16(sup1), 73-94.

Le Menn, F., Cerdà, J., & Babin, P. J. 2007. Ultrastructural aspects of the ontogeny and differentiation of ray-finned fish ovarian follicles. In **The Fish Oocyte** (pp. 1-37). Springer, Dordrecht.

Limbu, S. M. 2020. The effects of on-farm produced feeds on growth, survival, yield and feed cost of juvenile African sharptooth catfish (*Clarias gariepinus*). **Aquaculture and Fisheries**, 5(1), 58-64.

Lim, C., & Webster, C. D. 2001. **Nutrition and fish health**. New York: Food Products Press. 396 p.

Li, X. M., Ma, Y. L., & Liu, X. J. 2007. Effect of the Lycium barbarum polysaccharides on age-related oxidative stress in aged mice. **Journal of Ethnopharmacology**, 111(3), 504-511.

Li, Z., Berk, M., McIntyre, T. M., Gores, G. J., & Feldstein, A. E. 2008. The lysosomal-mitochondrial axis in free fatty acid–induced hepatic lipotoxicity. **Hepatology**, 47(5), 1495-1503.

Luo, L., Ai, L., Liang, X., Xing, W., Yu, H., Zheng, Y., & Xue, M. 2019. Effect of dietary DHA/EPA ratio on the early development, antioxidant response and lipid metabolism in larvae of Siberia sturgeon (*Acipenser baerii*, Brandt). **Aquaculture Nutrition**, 25(1), 239-248.

Lutz, C. G. 2021. **The pros and cons of crossbreeding and hybridization in aquaculture.** [Online]. Available https://thefishsite.com/articles/the-pros-and-cons-of-crossbreeding-and-hybridisation-in-aquaculture (29 March 2021).

Manning, B. B., Li, M. H., Robinson, E. H., & Peterson, B. C. 2006. Enrichment of channel catfish (*Ictalurus punctatus*) fillets with conjugated linoleic acid and omega-3 fatty acids by dietary manipulation. **Aquaculture**, 261(1), 337-342.

Manosroi, A., Meng-Umphan, K., & Manosroi, J. 2003. Annual sex hormone profiles, gonad development and age development determination of the Mekong Giant catfish (*Pangasinodon gigas*, Chevey). **Aquaculture Research**, 34: 1379-1385.

McGee, M. V. 2014. **Pangasius culture in Western Hemisphere**. [Online]. Available https://www.aquaculturealliance.org/advocate/pangasius-culture-in western-hemisphere/ (26 July 2021).

Mengumphan, K., 2010. The Efficiency on the production system of Mekong giant catfish and white meat catfish. In **Final Report. National Research Council of Thailand, Bangkok**, p. 60.

Meng-umphan, K., & Saengkrachang, J. 2008. Production of Generation-2 Mekong giant catfish (*Pangasinodon gigas*) cultured with *Spirulina* sp. **Maejo International Journal of Science and Technology**, 2(03), 559-567.

Mengumphan, K., Sattang, S., & Amornlerdpisan, D. 2017. Growth performances survival rate and external characteristics of *Pangasianodon hypophthalmus*, *Pangasius larnaudii* and their reciprocal hybrids. **Journal of Agricultural Research and Extension**, 34(2), 25-35.

Mercure, F., & Van Der Kraak, G. 1996. Mechanisms of action of free arachidonic acid on ovarian steroid production in the goldfish. **General and Comparative Endocrinology**, 102(1), 130-140.

Merson, R. R., Casey, C. S., Martinez, C., Soffientino, B., Chandlee, M., & Specker, J. L. 2000. Oocyte development in summer flounder: seasonal changes and steroid correlates. **Journal of Fish Biology**, 57(1), 182-196.

Misra, C. K., Das, B. K., Mukherjee, S. C., & Pattnaik, P. 2006. Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. **Aquaculture**, 255(1-4), 82-94.

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. 1991a. Hormonal induction of all stages of spermatogenesis *in vitro* in the male Japanese eel *(Anguilla japonica).* **Proceedings of the National Academy of Sciences**, 88(13), 5774-5778.

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. 1991b. Involvement of steroid hormones in gonadotropin-induced testicular maturation in male eel (*Anguilla japonica*). **Biomedical Research**, 12(4), 241-248.

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. 1991. Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). **Proceedings of the National Academy of Sciences**, 88(13), 5774-5778.

Mohebi-Nejad, A., & Bikdeli, B. 2014. Omega-3 supplements and cardiovascular diseases. **Tanaffos**, 13(1), 6.

Montero, D., Mathlouthi, F., Tort, L., Afonso, J. M., Torrecillas, S., Fernández-Vaquero, A., Negrin, D., & Izquierdo, M. S. 2010. Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream *Sparus aurata*. **Fish & Shellfish Immunology**, 29(6), 1073-1081.

Montero, D., Robaina, L., Caballero, M. J., Gines, R., & Izquierdo, M. S. 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet**. Aquaculture**, 248(1-4), 121-134.

Mousavi, S. N., Koohdani, F., Shidfar, F., & Shafiei-Neek, L. 2016. Effects of diets enriched in omega-9 or omega-6 fatty acids on reproductive process. **Journal of Family and Reproductive Health**, 10(2), 85.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. **The International Journal of Developmental Biology**, 38: 217– 229.

Nagahama, Y. O. S. H. I. T. A. K. A. 2002. Endocrine regulation of gametogenesis in fish. **International Journal of Developmental Biology**, 38(2), 217-229.

Navas, J. M., Mananos, E., Thrush, M., Ramos, J., Zanuy, S., Carrillo, M., Zohar, Y., & Bromage, N. 1998. Effect of dietary lipid composition on vitellogenin, 17β-estradiol and gonadotropin plasma levels and spawning performance in captive sea bass (*Dicentrarchus labrax* L.). **Aquaculture**, 165(1-2), 65-79.

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., Forster, I., Gatling, D. M., Goldburg, R. J., Hua, K., & Nichols, P. D. 2009. Feeding aquaculture in an era of finite resources. **Proceedings of the National Academy of Sciences**, 106(36), 15103-15110.

Negatu, Z., Hsiao, S. M., & Wallace, R. A. 1998. Effects of insulin-like growth factor-I on final oocyte maturation and steroid production in *Fundulus heteroclitus*. **Fish Physiology and Biochemistry**, 19(1), 13-21.

Nelson, J. S. 1984. **Fishes of the world, 2nd ed**. John Wiley & Sons, New York, 523 pp.

Nikolsky, G. V. 1963. **The Ecology of Fisher**. London: Academic Press.

Noordin, W. N. M., Ibrahim, S., Nawawi, R. A., Sari, M. D., & Huda, N. 2019. Nutritional composition, sensory evaluation and halal perspective African catfish (*Clarias gariepinus*) fed with diets containing najasa (impurities). **Current Research in Nutrition and Food Science**, 7(2), 436-448.

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Iinuma, N., & Hirose, K. 1997. Artificial induction of maturation and fertilization in the Japanese eel, *Anguilla japonica*. **Fish Physiology and Biochemistry**, 17(1), 163-169.

Ohta, T., Miyake, H., Miura, C., Kamei, H., Aida, K., & Miura, T. 2007. Follicle-stimulating hormone induces spermatogenesis mediated by androgen production in Japanese eel, *Anguilla japonica*. **Biology of Reproduction**, 77(6), 970-977.

Okonji, V. A., & Daniel, E. 2013. Fatty acids variation in *Oreochromis niloticus* and *Clarias gariepinus* from semi-intensive fish tank. **World Journal of Fish and Marine Sciences**, 5(2), 169-175.

Olaniyi, W. A., Makinde, O. A., & Omitogun, O. G. 2017. Comparison of proximate composition and sensory attributes of Clariid catfish species of *Clarias gariepinus*, *Heterobranchus bidorsalis*, and their hybrids. **Food Science & Nutrition**, 5(2), 285-291.

Pacher, P., Beckman, J. S., & Liaudet, L. 2007. Nitric oxide and peroxynitrite in health and disease. **Physiological Reviews**, 87(1), 315-424.

Packard, G. C., & Boardman, T. J. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. **Physiological Zoology**, 61(1), 1-9.

Pal, M., Misra, K., Dhillon, G., Kaur Brar, S., & Verma, M. 2014. Antioxidants. **Biotransformation of Waste Biomass into High Value Biochemicals. New York: Springer**, 117-138.

Panase, P., & Mengumphan, K. 2015. Growth performance, length-weight relationship and condition factor of backcross and reciprocal hybrid catfish reared in net cages. **International Journal of Zoological Research**, 11(2), 57-64.

Panase, P., & Tipdacho, P. 2018. Preliminary use of *Polygonum minus* Linn. leaf extract on growth performance, feed utilization, and some hematological indices of *Anabas testudineus* (Bloch, 1792). **Comparative Clinical Pathology**, 27(1), 147-153.

Pankhurst, N. W. 1994. Effects of gonadotropin releasing hormone analogue, human chorionic gonadotropin and gonadal steroids on milt volume in the New Zealand snapper, *Pagrus auratus* (Sparidae). **Aquaculture**, 125(1-2), 185-197.

Papoutsoglou, S. E., Papaparaskeva-Papoutsoglou, E., & Alexis, M. N. 1987. Effect of density on growth rate and production of rainbow trout (*Salmo gairdneri* Rich.) over a full rearing period. **Aquaculture**, 66(1), 9-17.

Parry Jr, R. M., Chandan, R. C., & Shahani, K. M. 1965. A rapid and sensitive assay of muramidase. **Proceedings of the Society for Experimental Biology and Medicine**, 119(2), 384-386.

Pechsiri, J., Chainapong, T., and Panritdam, T. 2021. The efficiency of catfish processing waste in the formulated diets on cage culture of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*). **WICHCHA Journal**, 40(1), 16-30.

Peryam, D. 1998. **The 9-point hedonic scale: Dr. David R. Peryam's Early Papers on the most widely used sensory scale in the world.** Chicago: Peryam & Kroll Research Corporation.

Peter, R. E., & Yu, K. L. 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. **Reviews in Fish Biology and Fisheries**, 7(2), 173-197.

Phillips, A.M., 1972. Calorie and energy requirement. In: **Halver, J.E. (Ed.), Fish Nutrition. Academic, New York,** pp. 2–29.

Pinyowit, A. 2002. **Ichthyology**. Bangkok: Faculty of Agriculture Technology, Rambhai Barni Rajabhat University. 314 p.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. 2017. Oxidative stress: harms and benefits for human health. **Oxidative Medicine and Cellular longevity**, 2017, 8416763.

Polzonetti-Magni, A. M., Mosconi, G., Soverchia, L., Kikuyama, S., & Carnevali, O. 2004. Multihormonal control of vitellogenesis in lower vertebrates. **International Review of Cytology**, 239(Sup C), 1-46.

Poulsen, A., Griffiths, D., Nam, S., & Nguyen, T. T. 2008. Capture-based aquaculture of Pangasiid catfishes and snakeheads in the Mekong River Basin. **Capture-based Aquaculture. Global Overview. FAO Fisheries Technical Paper**, 508, 69-91.

Prat, F., Zanuy, S., Carrillo, M., De Mones, A., & Fostier, A. 1990. Seasonal changes in plasma levels of gonadal steroids of sea bass, *Dicentrarchus labrax* L. **General and Comparative Endocrinology**, 78(3), 361-373.

Puwastien, P., Judprasong, K., Kettwan, E., Vasanachitt, K., Nakngamanong, Y., & Bhattacharjee, L. 1999. Proximate composition of raw and cooked Thai freshwater and marine fish. **Journal of Food Composition and Analysis**, 12(1), 9-16.

Qiufen, D., Yong, Y., & Shi, S. 2012. Nutrition and changes in fish body colouration in catfish. **AQUA Culture Asia Pacific Magazine**, 26-28.

Quagio-Grassiotto, I. & Oliveira, C. 2008. Sperm ultrastructure and a new type of spermiogenesis in two species of Pimelodidae, with a comparative review of sperm ultrastucture in siluriformes (Teleostei: Ostariophysi). **Zoologischer Anzeiger – A Journal of Comparative Zoology**, 247: 55-66.

Rahman, M. A., Arshad, A., Marimuthu, K., Ara, R., & Amin, S. M. N. 2013. Inter-specific hybridization and its potential for aquaculture of fin fishes. **Asian Journal of Animal and Veterinary Advances**, 8(2), 139-153.

Rattanaphot, T., Mengumphan, K., Tongsiri, S., & Amornlerdpison, D. 2018a. Utilization of enriched omega-9 freshwater fish oil as fish feed. **KMUTT Research and Development Journal**, 41(2), 185-198.

Rattanaphot, T., Mengumphan, K., Tongsiri, S., Srimaroeng, C., & Amornlerdpison, D. 2018b. Enhancement of oxidative defence and growth performance of Nile tilapia by omega-9-rich freshwater fish oil. **Maejo International Journal of Science and Technology**, 12(1), 79-88.

Remacle, C. 1976. Actions hormonales sur les cellules germinales males de *Carassius auratus* L. culture organotypique. **General and Comparative Endocrinology**, 29(4), 480-491.

Rice-Evans, C., Halliwell, B., Lunt, G. G., & Davies, K. J. 1995. November). Oxidative stress: the paradox of aerobic life. In **Biochemical Society Symposia** (Vol. 61, pp. 1-31). Portland Press.

Roberts, T. R., & Vidthayanon, C. 1991. Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. **Proceedings of the Academy of Natural Sciences of Philadelphia**, 97-143.

Robinson, E. H., & Lovell, R. T. 1978. Essentiality of biotin for channel catfish *(Ictalurus punctatus*) fed lipid and lipid-free diets. **The Journal of Nutrition**, 108(10), 1600-1605.

Rodríguez, C., Acosta, C., Badía, P., Cejas, J. R., Santamaría, F. J., & Lorenzo, A. 2004. Assessment of lipid and essential fatty acids requirements of black seabream (*Spondyliosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, 139(4), 619-629.

Rojtinnakorn, J., Thepnarong, J., 2006. Techniques of histology and cellularity for study of catfish oocyte. **Proceedings of Fisheries Conference** 355–364. Chiang Mai, Thailand.

Rora, A. M. B., Mørkøre, T., & Einen, O. 2001. Primary processing (evisceration and filleting). **Farmed Fish Quality (SC Kestin and PD Warriss, eds.), Blackwell Science, Oxford**, 249-260.

Saini, R. K., & Keum, Y. S. 2018. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance-A review. **Life Sciences**, 203, 255-267.

Sakai, M. 1999. Current research status of fish immunostimulants. **Aquaculture**, 172(1-2), 63-92.

Sarker, P. K., Pal, H. K., Rahman, M. M., & Rahman, M. M. 2002. Observation on the fecundity and gonado-somatic index of *Mystus gulio* in brackishwater of Bangladesh. **Online Journal of Biological Sciences**, 2(4), 235-237.

Sarasombat, S., Phimonphan, W., Banchuen, N., Sukosol, T., Senavong, S., Tarakul, T., & Songciwilai, S. 1994. **Immunology**. Bangkok: KP Printing Co Ltd. 389 p.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. & Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. **Aquaculture**, 177(1-4), 191-199.

Sargent, J. R., & Tacon, A. G. J. 1999. Development of farmed fish: a nutritionally necessary alternative to meat. **Proceedings of the Nutrition Society**, 58(2), 377-383.

Sattang, S., Amornlerdpison. D., Tongsiri, S., Senphan, T., & Mengumphan, K. 2018a. Growth, fish flesh qualities and reproductive index of hybrid catfish by different level of fish oil supplement in feed. pp. 274-281. In **Proceedings of 2nd national graduate research conference and creative innovation competition**. Chiang Mai, Thailand: Nantakarn Graphic Printing.

Sattang, S., Amornlerdpison, D., Tongsiri, S., Palić, D., & Mengumphan, K. 2021. Effect of freshwater fish oil feed supplementation on the reproductive condition and production parameters of hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*, Sauvage, 1878) broodstock. **Aquaculture Reports**, 20, 100598.

Sattang, S. 2015. **Maturity, nursing, external characteristics of *Pangasianodon hypophthalmus*, *Pangasius larnaudii*, hybrids and sex-reversal.** Master Thesis. Maejo University.

Sattang, S., Tongsiri, S., Amornlerdpison, & Mengumphan, K. 2018b. Effect of fish oil and spirogyra sp. supplement on the flesh quality and maturity of Buksiam hybrid catfish (*Pangasianodon gigas* Chevey, 1930 X *Pangasianodon hypophthalmus* Sauvage, 1878). **Journal of Agricultural Research and Extension**, 35(2), 1-10.

Schulz, R. W., de França, L. R., Lareyre, J. J., LeGac, F., Chiarini-Garcia, H., Nobrega, R. H., & Miura, T. 2010. Spermatogenesis in fish. **General and Comparative Endocrinology**, 165(3), 390-411.

Schulz, R. W., & Miura, T. 2002. Spermatogenesis and its endocrine regulation. **Fish Physiology and Biochemistry**, 26(1), 43-56.

Secombes, C. J. 1990. Isolation of salmonid macrophages and analysis of their killing activity. **Techniques in Fish Immunology**, 1, 137-155.

Selman, K., & Wallace, R. A. 1982. Oocyte growth in the sheepshead minnow: Uptake of exogenous proteins by vitellogenic oocytes. **Tissue and Cell**, 14, 555-571.

Senthilkumaran, B., Yoshikumi, M., & Naghama, Y. 2004. A shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. **Molecular and Cellular Endocrinology**, 215, 11-18.

Sharif, M. K., Butt, M. S., Sharif, H. R., & Nasir, M. 2017. Sensory evaluation and consumer acceptability. **Handbook of Food Science and Technology**, 361-386.

Shearer, K. D. 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. **Aquaculture**, 119(1), 63-88.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. **Experimental Physiology: Translation and Integration**, 82(2), 291-295.

Sikorski, Z. E., Kolakowska, A., & Pan, B. S. 1990. The nutritive composition of the major groups of marine food organisms. In **Z. E. Sikorski (Ed.), Seafood: Resource, nutritional composition, and preservation** (pp. 30–45). New York: CRC Press, Inc.

Silva, J. L. & Dean, S. 2001. **Processed catfish product forms, packaging, yields and product mix.**

Singh, R. L., Sharma, S., & Singh, P. 2014. 16 Antioxidants: Their Health Benefits and Plant Sources. **Phytochemicals of Nutraceutical Importance**, 248-265.

Sink, T. D., & Lochmann, R. T. 2008. Effects of dietary lipid source and concentration on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg and fry production, and egg and fry quality. **Aquaculture**, 283(1-4), 68-76.

Sink, T. D., Lochmann, R. T., Pohlenz, C., Buentello, A., & Gatlin III, D. 2010. Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. **Aquaculture**, 298(3-4), 251-259.

Smith, L.S., 1989. Digestive functions in teleost fishes. In: **Halver, J.E. (Ed.), Fish Nutrition. New York, USA.**

Somboon, S., & Semachai, V. 2014. The effects of dietary lipid amount and fatty acids composition on growth performance of Basa catfish (*Pangasius bocourti* Sauvage, 1880). In **Agricultural sciences: leading Thailand to world class standards. Proceedings of the 52nd Kasetsart University Annual Conference, 4-7 February 2014, Kasetsart University, Thailand. Vol. 3: Fisheries, Agricultural Extension and Home Economics** (pp. 266-274). Kasetsart University.

Song, D. J., & King, A. J. 2015. Effects of heat stress on broiler meat quality. **World's Poultry Science Journal**, 71(4), 701-709.

Stone, H & J. L. Sidel., 1993. **Sensory Evaluation Practices**, 2nd ed. Academic Press, San Diego, CA.

Stoneham, T. R., Kuhn, D. D., Taylor, D. P., Neilson, A. P., Smith, S. A., Gatlin, D. M., Chu, H. S. S., & O'Keefe, S. F. 2018. Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: Improved nutrient value of fillet and offal. **PloS One**, 13(4), e0194241.

Surasani, V. K. R., Raju, C. V., Sofi, F. R., & Shafiq, U. 2021. Utilization of protein isolates from rohu (*Labeo rohita*) processing waste through incorporation into fish sausages; quality evaluation of the resultant paste and end product. **Journal of the Science of Food and Agriculture**, 102(3), 1263-1270.

Sutthi, N., Amornlerdpisan, D., Chitmanat, C., & Mengumphan, K. 2014. Annual growth and reproductive performance in an F_2 catfish hybrid. **Journal of Advanced Agricultural Technologies**, 1(2), 113-118.

Tacon, A. G. 1987. **The nutrition and feeding of farmed fish and shrimp a training manual: the essential nutrients** (No. 639.2085 T32). FAO.

Takahashi, T., Hagiwara, A., Ogiwara, K., 2018. Prostaglandins in teleost ovulation: a review of the roles with a view to comparison with prostaglandins in mammalian ovulation. **Molecular and Cellular Endocrinology**, 461, 236-247.

Takahashi, T., Takahashi, E., Igarashi, H., Tezuka, N., & Kurachi, H. 2003. Impact of oxidative stress in aged mouse oocytes on calcium oscillations at fertilization. **Molecular Reproduction and Development: Incorporating Gamete Research**, 66(2), 143-152.

Takeo, J. & Yamashita, S. 1999. Two distinct isoforms of cDNA encoding rainbow trout androgen receptors. **The Journal of Biological Chemistry**, 247: 5674-5680.

Tiogué, C. T., Nyadjeu, P., Mouokeu, S. R., Tekou, G., & Tchoupou, H. 2020. Evaluation of hybridization in two African catfishes (*Siluriformes, Clariidae*): Exotic (*Clarias gariepinus* Burchell, 1 8 2 2) and native (*Clarias jaensis* Boulenger, 1 9 0 9) species under controlled hatchery conditions in cameroon. **Advances in Agriculture**, 2020, 8985424.

Todo, T., Ikeuchi, T., Kobayashi, T., & Nagahama, Y. 1999. Fish androgen receptor: cDNA cloning, steroid activation of transcript in transfected mammalian cells, and tissue mRNA levels. **Biochemical and Biophysical Research Communications**, 254: 378-383.

Tongmee, B., Ontawong, A., Lailerd, N., Mengamphan, K., & Amornlerdpisan, D. 2021. Anti-inflammatory effects and enhancing immune response of freshwater hybrid catfish oil in RAW264. 7 cells. **Experimental and Therapeutic Medicine**, 22(5), 1-9.

Tyler, C.R. 1991. Vitellogenesis in sahnonids. In Scott, A.E, Sumpter, J.E, Kime, D.E. and Rolfe, J., eds. **Proc. Fourth Int. Symp. Reproductive Physiology of Fish**. Sheffield, UK: Sheffield University Press, pp. 295-9.

Ueda, H., Kambegawa, A., & Nagahama, Y., 1985. Involvement of gonadotrophin and steroid hormones in spermiation in the amago salmon, *Oncorhynchus rhodurus*, and goldfish, *Carassius auratus*. **General and Comparative Endocrinology**, 59: 24-30.

Usawakesmanee, N., 2006. **Food and Fish Feeding.** Faculty of Agricultural Technology, Songkhla Rajabhat University.

Vadstein, O. 1997. The use of immunostimulation in marine larviculture: possibilities and challenges. **Aquaculture**, 155(1-4), 401-417.

Van't Land, M., Vanderperren, E., & Raes, K. 2017. The effect of raw material combination on the nutritional composition and stability of four types of autolyzed fish silage. **Animal Feed Science and Technology**, 234, 284-294.

Wade, M. G., & Van Der Kraak, G. 1993. Arachidonic acid and prostaglandin E_2 stimulate testosterone production by goldfish testis *in vitro*. **General and Comparative Endocrinology**, 90(1), 109-118.

Weber, J., Bochi, V. C., Ribeiro, C. P., Victório, A. D. M., & Emanuelli, T. 2008. Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. **Food Chemistry**, 106(1), 140-146.

Willcox, J. K., Ash, S. L., & Catignani, G. L. 2004. Antioxidants and prevention of chronic disease. **Critical Reviews in Food Science and Nutrition**, 44(4), 275-295.

Wongsawang, S. 1995. **Veterinary immunology**. Bangkok: Department of Pathology, Faculty of Veterinary Medicine, Chulalongkorn University. 114 p.

Yamashita, M. 1998. Molecular mechanism of meiotic maturation and arrest in fish and amphibian oocytes. **Seminars Cell and Development Biology**, 9: 569-579.

Yaqoob, P., & Calder, P. C. 2007. Fatty acids and immune function: new insights into mechanisms. **British Journal of Nutrition**, 98(1), 41-45.

Yavno, S., & Corkum, L. D. 2011. Round goby *Neogobius melanostomus* attraction to conspecific and heterospecific egg odours. **Journal of Fish Biology**, 78(7), 1944-1953.

Yildirim-Aksoy, M., Lim, C., Shelby, R., & Klesius, P. H. 2009. Increasing fish oil levels in commercial diets influences hematological and immunological responses of channel catfish, *Ictalurus punctatus*. **Journal of the World Aquaculture Society**, 40(1), 76-86.

Yildirim-Aksoy, M., Shelby, R., Lim, C., & Klesius, P. H. 2007. Growth performance and proximate and fatty acid compositions of channel catfish, *Ictalurus punctatus*, fed for different duration with a commercial diet supplemented with various levels of menhaden fish oil. **Journal of the World Aquaculture Society**, 38(4), 461-474.

Yoojam, S., Mengumphan, K., Srimaroeng, C., and Amornlerdpison, D. 2015. Effect of freshwater fish oil on growth, oxidative stress and omega-fatty acid content in hybrid catfish. **Journal of Fisheries Technology Research**, 9(1), 11-24.

Young, I. S., & Woodside, J. V. 2001. Antioxidants in health and disease. **Journal of Clinical Pathology**, 54(3), 176-186.

APPENDICES

APPENDICES A

Capital contract of Research and Researchers for Industries (RRI)

สัญญาเลขที่ PHD60I0002
รหัสโครงการ 5912059

สำนักงานกองทุนสนับสนุนการวิจัย

โครงการพัฒนานักวิจัยและงานวิจัยเพื่ออุตสาหกรรม (พวอ.)

สัญญารับทุนผู้ช่วยวิจัยระดับปริญญาเอกเพื่ออุตสาหกรรม

สัญญานี้ทำขึ้น ณ สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) เมื่อวันที่ - 6 มี.ค. 2560 ระหว่างกองทุนสนับสนุนการวิจัย ซึ่งมีสำนักงานใหญ่ชื่อ สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ตั้งอยู่ที่ชั้น 14 อาคาร เอส เอ็ม ทาวเวอร์ เลขที่ 979/17-21 ถนนพหลโยธิน แขวงสามเสนใน เขตพญาไท กรุงเทพมหานคร 10400 โดย นายประเสริฐ ภาสันต์ ตำแหน่ง ผู้อำนวยการโครงการพัฒนานักวิจัยและงานวิจัยเพื่ออุตสาหกรรม ปฏิบัติหน้าที่แทนผู้อำนวยการสำนักงานกองทุนสนับสนุนการวิจัย ตามคำสั่ง สกว. ที่ บค. 21/2559 เรื่อง การมอบให้ลงนามแทนในสัญญารับทุน ซึ่งต่อไปในสัญญานี้เรียกว่า "ผู้ให้ทุน" ฝ่ายที่หนึ่ง กับ บริษัท เอส แอนด์ พี ซินดิเคท จำกัด (มหาชน) ตั้งอยู่ 2034/100-107 อาคารอิตัลไทย ทาวเวอร์ ชั้นที่ 23-24 ถนนเพชรบุรีตัดใหม่ แขวงบางกะปิ เขตห้วยขวาง กรุงเทพมหานคร 10310 โดย นายกำธร ศิลาอ่อน ตำแหน่ง รองผู้จัดการใหญ่อาวุโสสายการผลิตและซับพลายเชน ผู้มีอำนาจกระทำการแทนนิติบุคคลซึ่งต่อไปในสัญญานี้เรียกว่า "ผู้ร่วมให้ทุน" ฝ่ายที่สอง และ มหาวิทยาลัยแม่โจ้ ตั้งอยู่เลขที่ 63 หมู่ที่ 4 ตำบลหนองหาร อำเภอสันทราย จังหวัดเชียงใหม่ 50290 โดย นายพาวิน มะโนชัย ตำแหน่ง รองอธิการบดีฝ่ายวิจัยและเครือข่าย ปฏิบัติหน้าที่แทนอธิการบดี ซึ่งต่อไปในสัญญานี้เรียกว่า "ผู้รับทุน" ฝ่ายที่สาม และ นายเกรียงศักดิ์ เม่งอำพัน สังกัด คณะเทคโนโลยีการประมงและทรัพยากรทางน้ำ มหาวิทยาลัยแม่โจ้ เลขประจำตัวประชาชน 3 9499 00051 33 1 ซึ่งต่อไปในสัญญานี้เรียกว่า "อาจารย์ที่ปรึกษา" ฝ่ายที่สี่ และ นางสาวสุภาพร สัตตัง วุฒิการศึกษา ปริญญาโท อยู่เลขที่ 16 หมู่ที่ 9 ตำบลยางคำ อำเภอโพนทราย จังหวัดร้อยเอ็ด 45240 เลขประจำตัวประชาชน 1 4513 00063 89 5 ซึ่งต่อไปในสัญญานี้เรียกว่า "นักศึกษาผู้ช่วยวิจัย" ฝ่ายที่ห้า

โดยที่ผู้ให้ทุนได้จัดให้มีทุนผู้ช่วยนักวิจัยระดับปริญญาเอกเพื่ออุตสาหกรรมซึ่งเป็นทุนอุดหนุนการศึกษาและการค้นคว้าวิจัยระดับปริญญาเอก ซึ่งต่อไปในสัญญานี้เรียกว่า "ทุนการศึกษาวิจัย"

โดยที่ผู้ร่วมให้ทุนเป็นนิติบุคคลดำเนินธุรกิจประเภทอุตสาหกรรม อาหารและเครื่องดื่ม มีความประสงค์ร่วมให้ทุนกับผู้ให้ทุนและให้การสนับสนุนอื่นๆ เพื่อทำการศึกษาค้นคว้าวิจัยเกี่ยวกับ การเพิ่มมูลค่าคุณภาพเนื้อและการเจริญพันธุ์ปลาลูกผสมเพื่ออุตสาหกรรมอาหาร โดยมีรายละเอียดดังในเอกสารแนบหมายเลข 1 และให้การสนับสนุนอื่นๆ ตามเอกสารแนบหมายเลข 2 ทั้งนี้ให้เป็นไปตามเงื่อนไขและข้อกำหนดของสัญญานี้

โดยที่ผู้รับทุนเป็น สถาบันอุดมศึกษาของรัฐ มีความสนใจขอรับทุนการศึกษาวิจัยจากผู้ให้ทุนและผู้ร่วมให้ทุน เพื่อสนับสนุนการศึกษาค้นคว้าวิจัยร่วมกับผู้ร่วมให้ทุน

ทั้งห้าฝ่ายตกลงทำสัญญากันมีข้อความดังต่อไปนี้

ก. การให้และรับทุน

ข้อ 1. ทุนการศึกษาวิจัยในสัญญานี้เป็นการร่วมให้ทุนระหว่างผู้ให้ทุนและผู้ร่วมให้ทุน เพื่อการอุดหนุนการศึกษาและการค้นคว้าวิจัยระดับปริญญาเอก ในวงเงินไม่เกิน 1,898,000 บาท (หนึ่งล้านแปดแสนเก้าหมื่นแปดพันบาทถ้วน) ซึ่งประกอบด้วยเงินร่วมให้ทุนจาก

(1) ผู้ให้ทุน ร่วมทุนเป็นจำนวนเงิน 1,838,000 บาท (หนึ่งล้านแปดแสนสามหมื่นแปดพันบาทถ้วน)
(2) ผู้ร่วมให้ทุน ร่วมทุนเป็นจำนวนเงิน 60,000 บาท (หกหมื่นบาทถ้วน)

โดยมีรายละเอียดตามเอกสารแนบหมายเลข 3

ข้อ 2. ผู้ให้ทุนและผู้ร่วมให้ทุนตกลงให้ทุน และผู้รับทุนตกลงรับทุนการศึกษาวิจัย เริ่มตั้งแต่ปีการศึกษา 2559 มีกำหนดระยะเวลาไม่เกิน 3 (สาม) ปี นับตั้งแต่วันที่ 1 กุมภาพันธ์ 2560 ถึง 31 มกราคม 2563 ในวงเงินตามข้อ 1. โดยให้แก่นักศึกษาผู้ช่วยวิจัยซึ่งอยู่ภายใต้การดูแลของอาจารย์ที่ปรึกษา เพื่อการทำการศึกษา ค้นคว้า และวิจัยในระดับปริญญาเอกภายใต้หลักสูตรปรัชญาดุษฎีบัณฑิต สาขาเทคโนโลยีการประมงและทรัพยากรทางน้ำ ซึ่งต่อไปในสัญญานี้เรียกว่า "การศึกษาวิจัย" โดยระยะเวลาข้างต้นอาจขยายได้ ทั้งนี้ให้เป็นดุลพินิจเด็ดขาดของผู้ให้ทุน

Figures 1 Presentation of research progress to S&P Syndicate Public Co., Ltd.

Figures 2 Presentation of research progress of Ph.D. student under the program of RRi, North, between 13 - 15 February 2019 at Chiang Mai University.

Figures 3 Organizing an exhibition and presenting research of Ph.D. student under the program of RRi, "Outstanding research" at the TSRI Congress 2019, Siam Paragon center.

Figures 4 The VDO filming of the silver hybrid catfish, pushing stars to export grades under the support of research funding from TSRI, follow at https://www.facebook.com/ ResearchcafeTRF/videos/260037598319569/

APPENDICES B

PETTY PATENT (Fish diet for catfish)

เลขที่อนุสิทธิบัตร 18094

อสป/200 - ข

อนุสิทธิบัตร

อาศัยอำนาจตามความในพระราชบัญญัติสิทธิบัตร พ.ศ. 2522
ซึ่งแก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ. 2542
อธิบดีกรมทรัพย์สินทางปัญญาออกอนุสิทธิบัตรฉบับนี้ให้แก่

มหาวิทยาลัยแม่โจ้

สำหรับการประดิษฐ์ตามรายละเอียดการประดิษฐ์ ข้อถือสิทธิ และรูปเขียน (ถ้ามี) ดังที่ปรากฏในอนุสิทธิบัตรนี้

เลขที่คำขอ	1703001325
วันขอรับอนุสิทธิบัตร	21 กรกฎาคม 2560
ผู้ประดิษฐ์	**รองศาสตราจารย์เกรียงศักดิ์ เม่งอำพัน**
ชื่อที่แสดงถึงการประดิษฐ์	**สูตรอาหารสำหรับปลาหนัง**
เลขที่คำขอ	1703001325
วันขอรับอนุสิทธิบัตร	21 กรกฎาคม 2560
ผู้ประดิษฐ์	**รองศาสตราจารย์เกรียงศักดิ์ เม่งอำพัน**
ชื่อที่แสดงถึงการประดิษฐ์	**สูตรอาหารสำหรับปลาหนัง**

18094 4608

ให้ผู้ทรงอนุสิทธิบัตรนี้มีสิทธิและหน้าที่ตามกฎหมายว่าด้วยสิทธิบัตรทุกประการ

ออกให้ ณ วันที่ 12 เดือน กรกฎาคม พ.ศ. 2564
หมดอายุ ณ วันที่ 20 เดือน กรกฎาคม พ.ศ. 2566

(นายวุฒิไกร สิริระพันธุ์)
อธิบดีกรมทรัพย์สินทางปัญญา
ผู้ออกอนุสิทธิบัตร

พนักงานเจ้าหน้าที่

หมายเหตุ 1. ผู้ทรงอนุสิทธิบัตรต้องชำระค่าธรรมเนียมรายปีเริ่มตั้งแต่ปีที่ 5 ของอายุอนุสิทธิบัตร มิฉะนั้น อนุสิทธิบัตรนี้จะสิ้นอายุ
2. ผู้ทรงอนุสิทธิบัตรจะชำระค่าธรรมเนียมรายปีล่วงหน้าโดยชำระทั้งหมดในคราวเดียวได้
3. ภายใน 90 วันก่อนวันสิ้นอายุอนุสิทธิบัตร ผู้ทรงอนุสิทธิบัตรมีสิทธิขอต่ออายุอนุสิทธิบัตรได้ 2 คราว มีกำหนดคราวละ 2 ปี
ยื่นคำขอต่ออายุ ต่อพนักงานเจ้าหน้าที่
4. การอนุญาตให้ใช้สิทธิตามอนุสิทธิบัตรและการโอนอนุสิทธิบัตรต้องทำเป็นหนังสือและจดทะเบียนต่อพนักงานเจ้าหน้าที่

001328

Ref.256401013090205

APPENDIX C

FOOD AND DRUG ADMINISTRATION

Sai Aua (Notrhern Thai spicy sausage)

แบบ สบ.7

เลขสารบบอาหาร 50-2-10559-6-0144 ให้ไว้ ณ วันที่ 27 มิถุนายน 2562

เพื่อแสดงว่าผลิตภัณฑ์ตามข้อมูลนี้ได้จดทะเบียน/แจ้งรายละเอียดไว้กับสำนักงานคณะกรรมการอาหารและยาหรือจังหวัด ตามระเบียบสำนักงานคณะกรรมการอาหารและยาว่าด้วยการดำเนินการเกี่ยวกับเลขสารบบอาหาร สำนักงานคณะกรรมการอาหารและยาหรือจังหวัด สงวนสิทธิ์ที่จะยกเลิกใบจดทะเบียน/แจ้งรายละเอียดอาหารนี้ รวมทั้งเลขสารบบอาหารที่ได้รับแจ้งตามเอกสาร หากปรากฏว่ามีการกระทำอันเข้าลักษณะอาหารที่ต้องถูกยกเลิกตามระเบียบสำนักงานคณะกรรมการอาหารและยาว่าด้วยการดำเนินการเกี่ยวกับเลขสารบบอาหาร

ใบจดทะเบียน/แจ้งรายละเอียดอาหาร

☑ ผลิต ☐ นำเข้า ☐ ส่งออก (ไม่จำหน่ายในประเทศ)

ชื่ออาหารภาษาไทย	ไส้อั่วปลาลูกผสมซิลเวอร์
ชื่ออาหารภาษาอังกฤษ	
ประเภทอาหาร	อาหารสำเร็จรูปที่พร้อมบริโภคทันที
ชนิดอาหาร	อาหารสำเร็จรูปที่พร้อมบริโภคทันที
กรรมวิธีการผลิตหลัก	แช่เยือกแข็ง
ประกาศกระทรวงสาธารณสุข	(ฉบับที่ 237) พ.ศ.2544

☑ ผู้รับอนุญาตผลิตชื่อ บริษัท เมืองเหนืออุตสาหกรรม168 จำกัด เลขที่ใบอนุญาตผลิต/เลขสถานที่ผลิต 50-2-10559

สถานที่ผลิตชื่อ บริษัทเมืองเหนืออุตสาหกรรม168 จำกัด อยู่เลขที่ 231/1

ตรอก/ซอย - ถนน - หมู่ที่ 3

ตำบล/แขวง สันผักหวาน อำเภอ/เขต หางดง จังหวัด เชียงใหม่

รหัสไปรษณีย์ 50230 โทรศัพท์ 084 5009889 โทรสาร

ไปรษณีย์อิเล็กทรอนิกส์ (E-mail address)

☐ ผู้รับอนุญาตนำเข้าชื่อ เลขที่ใบอนุญาตนำเข้าฯ

สถานที่นำเข้าชื่อ อยู่เลขที่

ตรอก/ซอย ถนน หมู่ที่

ตำบล/แขวง อำเภอ/เขต จังหวัด

รหัสไปรษณีย์ โทรศัพท์ โทรสาร

ไปรษณีย์อิเล็กทรอนิกส์ (E-mail address)

สถานที่ผลิตในต่างประเทศชื่อ

ที่อยู่

จังหวัด ประเทศ รหัสไปรษณีย์

โทรศัพท์ โทรสาร ไปรษณีย์อิเล็กทรอนิกส์ (E-mail address)

แบบ สบ.7

เลขสารบบอาหาร 50-2-10559-6-0144 ให้ไว้ ณ วันที่ 27 มิถุนายน 2562

เพื่อแสดงว่าผลิตภัณฑ์ตามข้อมูลนี้ได้จดทะเบียน/แจ้งรายละเอียดไว้กับสำนักงานคณะกรรมการอาหารและยาหรือจังหวัด
ตามระเบียบสำนักงานคณะกรรมการอาหารและยาว่าด้วยการดำเนินการเกี่ยวกับเลขสารบบอาหาร
สำนักงานคณะกรรมการอาหารและยาหรือจังหวัด สงวนสิทธิ์ที่จะยกเลิกใบจดทะเบียน/แจ้งรายละเอียดอาหารนี้
รวมทั้งเลขสารบบอาหารที่ได้รับแจ้งตามเอกสาร หากปรากฏว่ามีการกระทำอันเข้าลักษณะอาหารที่ต้องถูกยกเลิกตามระเบียบ
สำนักงานคณะกรรมการอาหารและยาว่าด้วยการดำเนินการเกี่ยวกับเลขสารบบอาหาร

ขอรับรองว่า

1. การผลิตอาหารดังกล่าวข้างต้นเป็นไปตามหลักเกณฑ์วิธีการที่ดีในการผลิตอาหารว่าด้วยประกาศกระทรวงสาธารณสุขเรื่อง วิธีการผลิตเครื่องมือเครื่องใช้ในการผลิต และการเก็บรักษาอาหารแปรรูปที่บรรจุในภาชนะพร้อมจำหน่าย(Primary GMP)
2. อาหารที่ผลิตต้องมีลักษณะดังต่อไปนี้
- มีคุณภาพหรือมาตราฐานตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง อาหารสำเร็จรูปที่พร้อมบริโภคทันที
- การใช้วัตถุเจือปนอาหาร ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่องวัตถุเจือปนอาหาร
- ไม่มีการใช้วัตถุที่ห้ามใช้ในอาหาร ตามประกาศกระทรวงสาธารณสุขที่เกี่ยวข้อง
- ไม่เป็นอาหารที่ห้ามผลิต นำเข้า หรือจำหน่าย ตามประกาศกระทรวงสาธารณสุขที่เกี่ยวข้อง
- ไม่มีการบรรจุสิ่งอื่นหรือวัตถุที่มิใช่อาหารในภาชนะบรรจุและหีบห่อตามประกาศกระทรวงสาธารณสุขที่เกี่ยวข้อง
- การใช้ภาชนะบรรจุ ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่องภาชนะบรรจุ
- การแสดงฉลากอาหาร ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่องการแสดงฉลากของอาหารในภาชนะบรรจุและประกาศ
- กระทรวงสาธารณสุขว่าด้วยเรื่อง อาหารสำเร็จรูปที่พร้อมบริโภคทันที
- อื่นๆ

3.ข้าพเจ้าขอรับรองว่าผลิตภัณฑ์ที่แจ้งนี้ เป็นไปตามข้อกำหนดของพระราชบัญญัติอาหาร พ.ศ. 2522

4.ข้าพเจ้าขอรับรองว่า เมื่อมีการเปลี่ยนแปลงรายละเอียดตามที่ได้แจ้งไว้ จะต้องแก้ไขรายละเอียดของอาหารที่จดทะเบียนอาหาร/แจ้งละเอียดอาหาร ตามแบบ สบ.8

5.

ขอรับรองว่ารายละเอียดที่ได้แจ้งในใบจดทะเบียน/แจ้งรายละเอียดอาหารนี้เป็นความจริงและมีเอกสารหลักฐานพิสูจน์ข้อมูลที่แจ้งไว้ข้างต้นแล้ว ทั้งนี้ รวมถึงเอกสารที่เกี่ยวข้องเป็นต้นฉบับจริงหรือสำเนาที่ถูกต้อง และรับทราบว่าจะต้องรับผิดชอบให้ผลิตภัณฑ์อาหารที่ออกสู่ตลาดเป็นไปตามที่แจ้งไว้ต่อพนักงานเจ้าหน้าที่และข้อกำหนดของกฎหมาย รวมถึงไม่หลีกเลี่ยงความรับผิดที่เกิดขึ้น หากผลิตภัณฑ์ไม่เป็นไปตามมาตรฐานหรือข้อกำหนดอื่นๆที่พนักงานเจ้าหน้าที่ได้รับจด/แจ้งไว้

ลงชื่อ นายภัทระ ฉลาดแพทย์ ผู้ดำเนินกิจการ

(นายภัทระ ฉลาดแพทย์)

ปรับปรุงข้อมูลครั้งที่ _____ วันที่ _____

APPENDIX D

THE SATISFACTION QUESTIONNAIRE

Assessment form

Subject: Processing of fish fillets from hybrid catfish (*Pangasianodon hypophthalmus* x *Pangasius larnaudii*) to develop into poached fish products.

Faculty of fisheries technology and aquatic resources, Maejo University.

Explanation: This assessment collects information about consumer opinions and satisfaction with the product. This is to bring the comments and satisfaction that have been summarized for further product development.

Part 1 Information about the respondents' status and background information. Please provide details about yourself by writing a mark ✓ into ( ) actual text page.

Gender ( ) Male ( ) Female

Age ( ) 15-25 years old ( ) 26-35 years old ( ) 36-40 years old
( ) 41-45 year old ( ) 46-50 years old ( ) 51-55 years old
( ) 56-60 years old ( ) 61 years old or older

Occupation .....

Have you ever eaten poached frozen fish fillet or not? ( ) Yes ( ) No

Part 2 Satisfaction level, please put a ✓ that match your actual satisfaction level (Scores between 1 to 9, 1 = dislike extremely and 9 = like extremely).

Formula 1

Sensory parameters	Level of satisfaction								
	1	2	3	4	5	6	7	8	9
1. Color									
2. Odor									
3. Sweet									
4. Texture									
5. Overall acceptability									

Suggestion

.....

.....

.....

Furmula 2

Sensory parameters	Level of satisfaction								
	1	2	3	4	5	6	7	8	9
1. Color									
2. Odor									
3. Sweet									
4. Texture									
5. Overall acceptability									

Suggestion

.....

.....

.....

Formula 3

Sensory parameters	Level of satisfaction								
	1	2	3	4	5	6	7	8	9
1. Color									
2. Odor									
3. Sweet									
4. Texture									
5. Overall acceptability									

Suggestion

.....

.....

.....

Which product formulation are you most satisfied with and why?

.....

.....

The price of fish fillets is 300 baht/kg, will you buy it or not? ❒ Yes ❒ No

Thank you very much for your cooperation in answering this questionnaire.

Research team

Assessment form

Subject: Processing of Sai Aua (Northern Thai spicy sausage) from hybrid catfish (*Pangasianodon hypophthalmus* x *Pangasius larnaudii*)

Faculty of fisheries technology and aquatic resources, Maejo University.

Explanation: This assessment collects information about consumer opinions and satisfaction with the product. This is to bring the comments and satisfaction that have been summarized for further product development.

Part 1 Information about the respondents' status and background information. Please provide details about yourself by writing a mark ✓ into ( ) actual text page.

Gender ( ) Male ( ) Female

Age ( ) 15-25 years old ( ) 26-35 years old ( ) 36-40 years old
( ) 41-45 year old ( ) 46-50 years old ( ) 51-55 years old
( ) 56-60 years old ( ) 61 years old or older

Occupation .....

Have you ever eaten boiled frozen fish fillet or not? ( ) Yes ( ) No

Part 2 Satisfaction level, please put a ✓ that match your actual satisfaction level (Scores between 1 to 9, 1 = dislike extremely and 9 = like extremely).

Formula 1

Sensory parameters	Level of satisfaction								
	1	2	3	4	5	6	7	8	9
1. Color									
2. Salty									
3. Spicy									
4. Sweet									
5. Odor									
6. Texture									
7. Overall acceptability									

Suggestion

.....

.....

.....

Formula 2

Sensory parameters	Level of satisfaction								
	1	2	3	4	5	6	7	8	9
1. Color									
2. Salty									
3. Spicy									
4. Sweet									
5. Odor									
6. Texture									
7. Overall acceptability									

Suggestion

.....

.....

.....

Formula 3

Sensory parameters	Level of satisfaction								
	1	2	3	4	5	6	7	8	9
1. Color									
2. Salty									
3. Spicy									
4. Sweet									
5. Odor									
6. Texture									
7. Overall acceptability									

Suggestion

.....

.....

Which product formulation are you most satisfied with and why?

.....

.....

The price of Sai Aua is 400 baht/kg, will you buy it or not? ❒ Yes ❒ No

Thank you very much for your cooperation in answering this questionnaire.

Research team

APPENDIX E

JOURNAL PUBLISHED

1. Aquaculture Reports 2021. 20, 100598.

Aquaculture Reports 20 (2021) 100598

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effect of freshwater fish oil feed supplementation on the reproductive condition and production parameters of hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*, Sauvage, 1878) broodstock

Supaporn Sattang [a], Doungporn Amornlerdpison [a], Sudaporn Tongsiri [a], Dušan Palić [b,*], Kriangsak Mengumphan [a]

[a] Pla Buk and Buk Siam Hybrid Excellent Center, Faculty of Fisheries Technology and Aquatic Resource, Maejo University, Chiang Mai, 50290, Thailand
[b] Chair for Fish Diseases and Fisheries Biology, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, 80539, Germany

ARTICLE INFO

Keywords:
Freshwater fish oil
Polyunsaturated fatty acids
Feed additives
Reproduction parameters
Seasonal cycle
Sustainable management

ABSTRACT

Increased sales of freshwater catfish fillet in global markets is causing high demand for quality fingerlings to be used in aquaculture production. Due to limited freshwater catfish broodstock availability, the gap between fingerling supply and demand drives the continuous need for improvement of production chain, including increased fertility and fecundity of broodstock. Recent technological advances support the possibility to increase hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*) broodstock reproductive parameters by adding small amounts of freshwater fish oil (FFO) to their diet. Catfish broodstock were fed with three different levels of FFO feed supplementation (0%, 1%, and 2% FFO) during the spawning (April-August) and post-spawning (September-December) season. Significant differences in weight gain and average daily growth were observed in fish fed with 1% FFO supplemented diet. Biomarkers of reproductive status in females (17 β-estradiol /E_2; 1577 ± 5.26 pg/mL^{-1}) and males (testosterone /T; 3.28 ± 0.41 ng/mL^{-1}) fed with FFO supplements were significantly higher than the control (113.12 ± 43.33 pg/mL^{-1} and 2.56 ± 0.95 ng/mL^{-1}), respectively). The oocytes and spermatocytes from fish fed with 1% and 2% FFO were in higher histological stages of maturity compared to control. Freshwater fish oil supplementation in fish feed during spawning season supported increased growth and enhanced reproduction indicators of hybrid catfish broodstock in aquaculture.

1. Introduction

Significant increase in demand for freshwater catfish fillet has been recently observed, shifting from U.S. and EU to China, India, and Association of Southeast Asian Nations (ASEAN) markets (FAO, 2017, July 9). Thailand is one of most important ASEAN member countries importer of Pangasius spp. (most frequently *Pangasianodonhypopthalmus* traded as "Pangasius Dory fillet") from Vietnam, reaching over 10,600 tons (Fisheries, 2020, April 7). Significant price increases in freshwater catfish fillet market were observed in period from 2017 to 2019, strongly driving the need for broodstock of high quality and quantity to produce enough fingerlings for catfish aquaculture. Even though COVID-19 pandemic (coronavirus disease 2019) caused an unexpected reduction in the 2020 demands, it is likely that freshwater catfish aquaculture industry will soon recover and continue to grow in longer term (FAO, 2020, July 8). Therefore, providing optimal growth and sexual maturation conditions with adequate diets and husbandry is crucial for achieving higher gamete production to meet both current and future demand of catfish aquaculture industry for quality fingerlings.

It has been well established that fish diets supplementation with fish oils benefits production parameters, including reproductive status in different cultured species, and recent trends in "added value" seafood products are emphasizing the content and benefits of Omega 3/6/9 fatty acids in human diets (Stoneham et al., 2018). For example, tilapia (*O. niloticus*) fed with two different marine fish oil (anchovy and cod liver oil) supplements showed differences in growth parameters (Hunt et al., 2018). Male European sea bass (*Dicentrarchus labrax*) showed better survival rates and reproductive performance when fed two commercial pellet diet enriched with polyunsaturated fatty acid (PUFAs) during the reproductive season compared to wet diet (Asturiano et al.,

* Corresponding author at: Chair for Fish Diseases and Fisheries Biology, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Kaulbachstr. 37, 80539Munich, Germany.
E-mail address: d.palic@lmu.de (D. Palić).

https://doi.org/10.1016/j.aqrep.2021.100598
Received 22 July 2020; Received in revised form 7 January 2021; Accepted 7 January 2021
Available online 24 February 2021

2001). Arachidonic acid is critical precursor for prostaglandin synthesis and plays an important role in regulation of sex hormones involved in development and maturation of sperm and eggs in fish (Ann Sorbera et al., 2001; Wade and Van Der Kraak, 1993). Unsaturated fatty acids (HUFAs: docosahexaenoic acid, DHA, 22:6n-3; eicosapentaenoic acid, EPA, 20:5n-3; and arachidonic acid, ARA, 20:4n-6), are also required for proper growth and reproduction of fish (Sargent et al., 1999; Luo et al., 2019).

Increased demand for marine fish meal and oil increases cost and decreases sustainability of its use in commercial diet formulations (Alhazzaa et al., 2019). Therefore, alternatives are investigated. Industrial processing from whole catfish to frozen fish fillets produces 40–60% of by-products such as bone, fish fat/oil, viscera, and skin. Such offal is not suitable for human consumption and is frequently used as raw material to produce fish meal and oil, or in some cases fertilizer (Silva and Dean, 2001). Using offal from freshwater fish aquaculture to supplement fish diets may present as viable alternative (Rattanapot et al., 2018a).

Freshwater fish oil from the Maejo Buk Siam hybrid catfish contains favourable ratios of fatty acids (saturated : mono-unsaturated : poly-*n*-saturated as 13.83 : 76.64 : 9.52 g/100 g, respectively) and content of omega 3, 6 and 9 fatty acids was reported as 0.73, 8.42 and 42.28 g/100 g, respectively (Sattang et al., 2018). Dietary supplementation of freshwater fish oil (1.5%, total fat average 7.6%) increased growth performance of hybrid catfish (Rattanaphot et al., 2018b). Efficient utilization of fish oil requires determination of optimal, cost-effective, and sustainable levels of supplementation focused on enhancement of reproductive performance in the hybrid catfish broodstock.

There is no available information currently about the effects of freshwater fish oil on reproduction indicators in hybrid catfish. Therefore, the aim of this study is to for the first time determine effects of low level (1 or 2%) freshwater fish oil dietary supplementation on production parameters and reproductive condition of the hybrid catfish (*Pangasius larnaudii* X *P. hypophthalmus*) during and after spawning season in an effort to optimize broodstock husbandry in freshwater hybrid catfish aquaculture industry.

2. Materials and methods

2.1. Freshwater fish oil, experiment fish and diets

Freshwater fish oil from the adipose tissue of freshwater hybrid catfish (*P. gigas* x *P. hypophthalmus*) was steamed at 90 °C for 30 min. The liquid oil was filtered by passing through a filtering sack (300 μM) to remove tissue residues and was used to supplement experimental fish diet. One-year old hybrid catfish (*P. larnaudii* x *P. hypophthalmus*) with starting average weight of 1.54 kg from the Pla Buk and Buk Siam Hybrid Excellence Center was randomly distributed to net pens (4 × 3 × 2 m) and placed in an earthen pond at density of 15 fish per cage for total of 8 months. Total of 135 hybrid catfish were distributed to 3 replicate cages per treatment (Table 1., no supplement control, 1%, and 2% freshwater fish oil) using completely randomized design (Classics Steel and Torrie, 1960; Sattang et al., 2018). Fish were fed pelleted feed with 3% body weight daily (industry standard, 30% protein) divided in two feeding times (Rattanaphot et al., 2018c). Average body weight was calculated monthly based on representative samples from each replicate and feed amount adjusted accordingly. Sample collection and result analysis was divided in two periods: during the spawning (May-August) and post-spawning (September-December) season. The proximate analysis of the experimental diets included moisture, ash, protein, crude fiber, fat, and carbohydrate content as determined by standard AOAC methods (AOAC, 2000). Fatty acid composition of fish feed was analysed at the Central Laboratory (Thailand) Co. Ltd., Chiang Mai Branch, following an in-house method based on AOAC 996.06 (AOAC, 2012). Use of fish in the experiments was reviewed and approved by the Maejo University Animal Care and Use Committee.

Table 1
List of ingredients and proximate analysis of the experimental diets containing 0, 1, and 2% freshwater fish oil (FFO, dry weight).

Ingredient (per 100 g of feed)	Diets		
	0% FFO	1% FFO	2% FFO
Fish meal	15	15	15
Soybean meal	37	37	37
Broken-milled rice	32	27	26
Rice bran	15	20	20
Vegetable oil	1	0	0
Freshwater fish oil	0	1	2
Total	100	100	100
Proximate compositions (% dry weight)			
Moisture	8.97	8.94	9.45
Ash	6.99	7.14	6.70
Crude Protein	32.53	33.37	31.59
Crude Fiber	8.13	8.74	8.73
Crude Fat	6.02	5.82	6.52
NFE	37.36	35.99	37.01
Energy (Kcal/g)	416.80	421.20	430.40

Note. Nitrogen-free extract (NFE) = 100% - (% protein + % fat + % ash + % fiber).

2.2. Growth performance parameters

Growth performance parameters: weight gain (WG), average daily weight gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR), were calculated monthly using body weight from five fish randomly sampled from each cage. Parameters were calculated using following formulae (Bagenal, 1978; Sutthi et al., 2020):

WG (g/fish) = final weight (g) - initial weight (g)

ADG (g/fish/day) = [final weight (g) - initial weight (g)] / Days

SGR = [{*ln* final weight (g) – *ln* initial weight (g)}/ experimental days] × 100

FCR = total feed fed (g) / weight gain (g)

SR (%) = [number of survived fish / initial number of fish] × 100

2.3. Indicators of reproductive condition

To determine effects of freshwater fish oil (FFO) on hybrid catfish broodstock reproductive condition, samples were collected for analysis of sex steroid hormones, gonadosomatic index calculation, and histological evaluation of gonadal tissue in August (spawning season) and in December (post-spawning season). Plasma steroid hormone concentrations were determined as per Sutthi et al. (2014) with minor modifications. Whole blood samples (1 mL) were collected from caudal vein of three females and three males per treatment, centrifuged at 2500 *g* for 5 min and the serum was stored at −80 °C until use. 17β-estradiol (E_2) and testosterone (T) were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits from Cayman Chemical Company by an electro-chemiluminescence immunoassay analyser (Elecsys 2010, Roche, Germany). For determination of gonadosomatic index (GSI) six fish (three of each gender) per treatment were sampled, total body weight and gonad weight of each fish were measured with a digital scale, and GSI calculated with following formula:

% GSI = gonad weight x 100 / body weight (Nikolsky, 1963; Sutthi et al., 2014).

For determination of the maturation stages of gonads (Arfah et al., 2018; Kabir et al., 2012), ovaries and testes were removed from 3 females and 3 males per treatment, fixed in 10% neutrally buffered formalin and stored until use. Fixed ovaries and testes were processed

for sectioning and 4 μm thick sections were stained with haematoxylin and eosin. The histological evaluation and staging of oocytes and spermatocytes were determined under light microscope as per (Coward and Bromage, 1998).

2.4. Statistical analysis

Collected data were analysed with statistical software (SPSS, version 22) using one-way analysis of variance (ANOVA) and are presented as SEM (standard error of means). Tukey honest significant difference (HSD) post hoc test was applied when statistical differences were observed ($p < 0.05$).

3. Results

3.1. Ingredients, proximate analysis, and fatty acid composition in diets

Ingredient and proximate analysis of diets supplemented with freshwater fish oil is presented in Table 1. There were no significant differences in the feed ingredient composition. Proximate analysis results show similar contents in all three diets, with minor (1% < value < 2% dry weight) differences in crude protein and fat contents (Table 1). Fatty acid composition of the experimental diets is presented in Table 2 where unsaturated and omega-9 fatty acids were found to be higher in the treatment diet containing 2% FFO compared to control (4.44 g/100 g and 2.34 g/100 g, respectively, $p > 0.05$).

3.2. Growth performance parameters

During spawning season in August, WG and ADG in cages fed with 1% FFO supplemented diet were significantly increased compared to control (354.90 g and 2.96 g/fish/day, respectively, $p < 0.05$), in contrast to post-spawning season (December), during which no significant difference was observed (Fig. 1). Survival rate during spawning and post-spawning season of hybrid catfish in all treatment groups was 100%.

3.3. Indicators of reproductive condition

During spawning season in August, fish fed with 1% FFO supplement had both 17β-estradiol and testosterone plasma values significantly higher than control and 2% FFO groups (1577 pg mL^{-1} and 3.28 ng mL^{-1}, $p < 0.05$, respectively). Gonadosomatic index (GSI) was not significantly different; however, a trend in positive correlation between increasing FFO supplement and GSI was noted. During post-spawning season in December, reproductive indicators in all groups were not significantly different (Table 3). Histologically, fish feed with 1% and 2% FFO had migrating germinal vesicle (MGV), peripheral germinal vesicle (PGV) and germinal vesicle breakdown (GVBD) in more developed stages when compared to fish from control group (Fig. 2). Average oocyte histology determined in August season showed that most oocytes were in primary growth (PG) phase, followed by central germinal vesicle (CGV), MGV, PGV and GVBD phases (67.21, 10.38, 20.77, 1.64 and 0.00%; 43.69, 14.34, 30.72, 8.19 and 3.07%; 38.85, 11.51, 35.25, 7.91 and 6.48%) for fish in control, 1% FFO and 2% FFO supplemented groups, respectively (Fig. 3). Testicular histology and appearance of spermatocytes showed that spermatid (SD) and spermatozoa (SZ) maturation in fish from 1% and 2% FFO groups were more advanced than in control fish (Fig. 4).

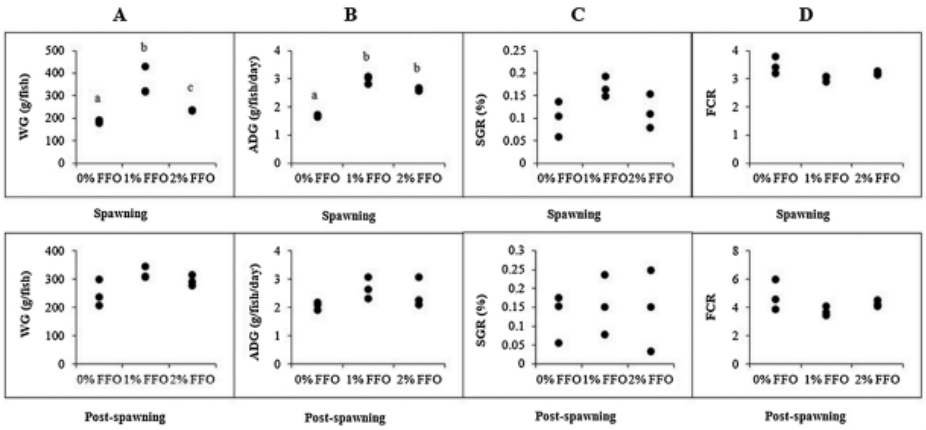
4. Discussion

In order to minimize lipid hydrolysis and oxidation, prepared feed was stored in a well-ventilated place under adequate conditions for less than 3 months, and quality raw materials were used to keep the nutritional value in the fish diet (Van't Land et al., 2017). Fatty acid (FA)

Table 2
Fatty acid composition of the freshwater fish oil and experimental diets containing 0, 1, and 2% freshwater fish oil (FFO, dry weight).

Fatty acid composition (g per 100 g of feed)	FFO	0% FFO	1% FFO	2% FFO
Lauric acid (C12:0)	1.35	0.0085	0.0120	0.0714
Tridecanoic acid (C13:0)	–	–	0.0011	0.0021
Myristic acid (C14:0)	5.84	0.0804	0.1259	0.2170
Pentadecanoic acid (C15:0)	0.10	0.0142	0.0152	0.0176
Palmitic acid (C16:0)	–	1.1649	1.4439	1.8999
Heptadecanoic acid (C17:0)	0.14	0.0254	0.0260	0.0286
Stearic acid (C18:0)	5.97	0.2646	0.2980	0.4076
Arachidonic acid (C20:0)	0.10	0.0338	0.0354	0.0366
Heneicosanoic acid (C21:0)	–	0.0031	0.0031	0.0036
Behenic acid (C22:0)	0.25	0.0241	0.0227	0.0219
Tricosanoic (C23:0)	–	0.0038	0.0039	0.0043
Lignoceric acid (C24:0)	0.08	0.0234	0.0575	0.0352
Saturated fatty acid	13.83	1.6462	2.0447	2.7458
Myristoleic acid (C14:1)	–	0.0024	0.0028	0.0033
cis-10-Pentadecenoic acid (C15:1n10)	30.74	–	–	–
Palmitoleic acid (C16:1n7)	2.29	0.0788	0.0884	0.1001
cis-10-Heptadecenoic acid (C17:1n10)	0.10	–	–	–
Trans-9-Eladic acid (C18:1n9t)	0.25	–	–	–
cis-9-Oleic acid (C18:1n9c)	42.27	1.6166	1.8290	2.3314
cis-Vaccenic acid (C18:1n7)	–	0.0729	0.0722	0.0715
cis-11-Eicosenoic acid (C20:1n11)	0.99	0.0261	0.0290	0.0480
Erucic acid (C22:1n9)	0.02	0.0031	0.0035	0.0052
Lignoceric acid (C24:1)	–	0.0060	0.0061	0.0068
Monounsaturated fatty acid	76.64	1.8059	2.031	2.5663
cis-912-Linoleic acid (C18:2n6)	7.07	1.6172	1.3640	1.5132
gamma-Linolenic acid (C18:3n6)	0.32	0.0030	0.0066	0.0070
α-Linolenic acid (C18:3n3)	0.41	0.1201	0.0877	0.0958
Moroctic acid (C18:4n3)	–	0.0069	0.0073	0.0070
cis-1114-Eicosadienoic acid (C20:2)	0.31	0.0059	0.0097	0.0162
cis-8,1114-Eicosatrienoic acid (C20:3n6)	0.52	0.0034	0.0077	0.0125
cis-1114,17-Ecosatrienoic acid (C20:3n3)	0.01	0.0017	0.0020	0.0027
Arachidonic acid (C20:4n6)	0.51	0.0321	0.0379	0.0337
Ecosatetraenoic acid (C20:4n3)	–	0.0041	0.0044	0.0052
cis-1316-Docosadienoic acid (C22:2)	0.07	–	–	–
cis-7,10,1316-Docosstetraenoic acid (C22:4n6)	–	0.0088	0.0109	0.0097
Docosapentaenoic acid (C22:5n6)	–	0.0155	0.0185	0.0162
cis-5,8,1114,17-Eicosapentaenoic acid (C20:5n3)	0.10	0.0455	0.0449	0.0408
4,7,10,1316,19-Docosahexaenoic acid (C22:6n3)	0.20	0.1340	0.1343	0.1166
Polyunsaturated fatty acid	9.52	1.9982	1.7359	1.8766
Unsaturated fatty acid	81.17	3.8041	3.7669	4.4429
n-3 fatty acid	0.73	0.3123	0.2806	0.2681
n-6 fatty acid	8.42	1.6800	1.4456	1.5923
n-9 fatty acid	42.28	1.6197	1.8325	2.3366
Unidentified peak	–	0.2797	0.2691	0.3342

composition of the experimental diets showed that unsaturated and omega-9 fatty acids were higher in diet containing 2% FFO compared to control (Table 2). Fatty acid composition in fish oil extracted from freshwater hybrid catfish (*P. gigas* x *P. hypophthalmus*) contains high amounts of unsaturated fatty acids (81.17 g/100 g), including Omega 9 fatty acids (42.28 g/100 g). Therefore, fish oil from hybrid catfish is suitable for use as dietary supplement to increase respective fatty acid contents in supplemented complete diets (Sattang et al., 2018). Importance of essential fatty acids in fish diet has been reported before (Sargent et al., 1999). Unsaturated fatty acids from fish oil, cod liver oil and/or squid liver oil contain essential fatty acids (EFA) which serve as precursors for prostaglandins synthesis (Saini and Keum, 2018). Prostaglandins are involved in different regulatory pathways including contractions of smooth muscles in the uterus, ovulation, hormone secretion, and regulation of blood pressure (Takahashi et al., 2018). Deficiency of essential fatty acids in fish diets can cause slow growth, tail fin deformities, liver fat metabolism (fatty livers), excessive fat deposits in coelomic cavity, pale skin, swollen belly, head and in severe cases



Season		p value		
Spawning	0.004	0.000	0.102	0.056
Post-spawning	0.198	0.200	0.910	0.223

Fig. 1. Effects of freshwater fish oil on the A) weight gain (WG), B) weight average daily gain (ADG), C) specific growth rate (SGR) and D) feed conversion ratio (FCR) ofhybrid catfish fed with 0% FFO, 1% FFO, and 2% FFO during spawning and post-spawning season. Means in dot plots with different small letters (a, b, c) denote significant difference.

Table 3
Differences in indicators of reproductive condition during spawning (Aug) and post-spawning (Dec) season in catfish fed diets different content of freshwater fish oil (FFO, 0, 1, and 2%).

Month	Fish (n)	Diets	E_2 (pg mL^{-1})	T (ng mL^{-1})	GSI (%)	MOS	HOS
4 (Aug)	3	0%FFO	113.12 ± 43.33^{c}	2.56 ± 0.95^{ab}	1.97 ± 0.23	Immature	PG
	3	1%FFO	1,577.00 ± 5.26^{a}	3.28 ± 0.41^{b}	3.34 ± 1.07	Maturing	MGV
	3	2%FFO	694.60 ± 3.10^{b}	1.73 ± 0.34^{a}	4.42 ± 1.28^{y}	Mature	GVBD
8 (Dec)	3	0%FFO	48.17 ± 6.37	<0.025	1.01 ± 0.65	Immature	PG
	3	1%FFO	51.88 ± 4.29	<0.025	1.92 ± 0.37	Immature	PG
	3	2%FFO	101.92 ± 46.39	<0.025	0.87 ± 0.23^{z}	Immature	PG

Note. HOS stages are listed as: PG (primary growth phase), CGV (central germinal vesicle), MGV (migrating germinal vesicle), PGV (peripheral germinal vesicle), GVBD (germinal vesicle breakdown). Different superscripts (a, b, c, y, z) designate significantly different values in each column ($p < 0.05$). Measurements of 17β-estradiol (E_2), testosterone (T), gonadosomatic index (GSI), macroscopic ovary stages (MOS) and histological ovary stages (HOS) of hybrid catfish are presented as mean value ± standard error of means (SEM).

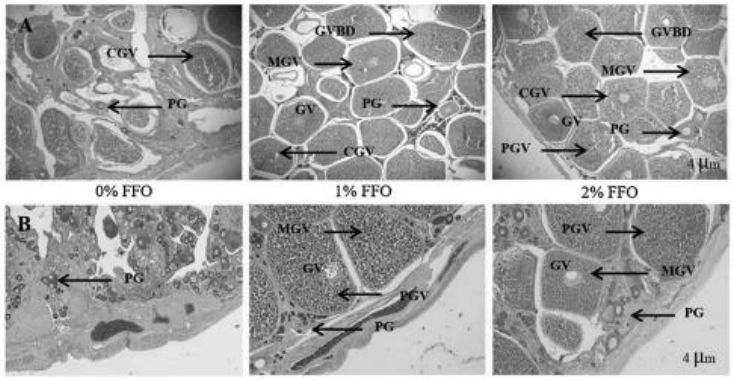


Fig. 2. Effects of freshwater fish oil on the oocyte stage of hybrid catfish consisting of: A) ovary during spawning season (10x), B) ovary in post-spawning season (40x). Arrows point to: primary growth phase (PG), central germinal vesicle (CGV), peripheral germinal vesicle (PGV), germinal vesicle breakdown (GVBD) and germinal vesicle (GV).

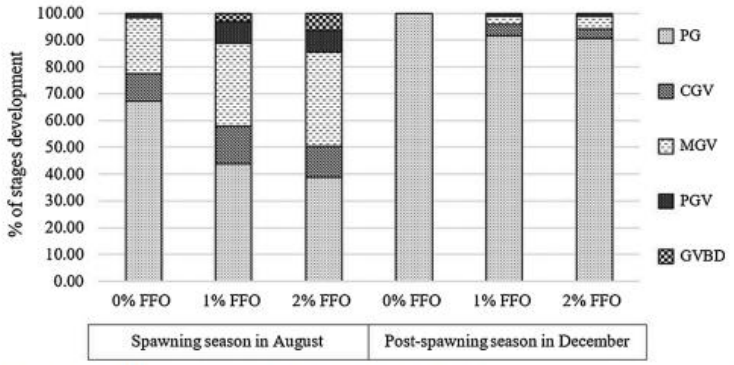


Fig. 3. Ovarian oocyte stages development of hybrid catfish fed with 0% FFO, 1% FFO, and 2% FFO during spawning (Aug) and post-spawning (Dec). Labeled as: primary growth phase (PG), central germinal vesicle (CGV), migrating germinal vesicle (MGV), peripheral germinal vesicle (PGV), and germinal vesicle breakdown (GVBD).

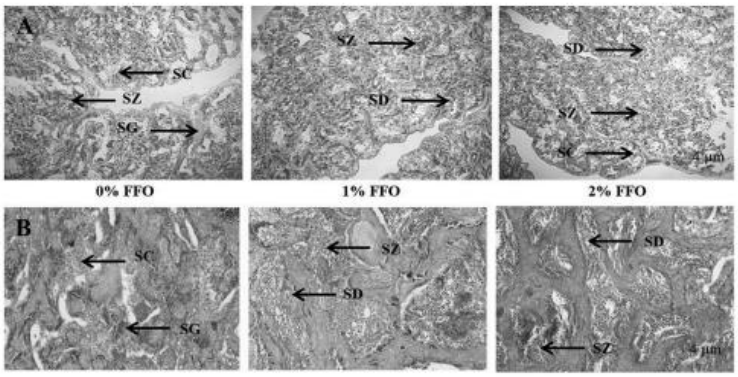


Fig. 4. Effects of freshwater fish oil on the spermatocyte of hybrid catfish consisting of: A) testis during spawning season (Aug; 10x), B) testis during post-spawning season (Dec; 40x). Labeled as: spermatogonium (SG), spermatocyte (SC), spermatid (SD) and spermatozoa (SZ).

hemolytic anemia and subsequent breathing problems (Usawakesmanee, 2006). Therefore, diets with optimal content of essential fatty acids are critical for achieving economical and sustainable production and reproduction in aquaculture.

The growth performance (WG and ADG) of hybrid catfish during spawning season (August) fed with 1% FFO supplemented diet was significantly increased ($p < 0.05$, Fig. 1). This is in accordance with previous work of Rattanaphot et al. (2018b) who reported significant increase in growth of hybrid catfish (*P. gigas* x *P. hypophthalmus*) fed with freshwater fish oil supplements containing high amount of omega 9 fatty acids (four times higher than marine fish diet). Similar increase in growth of *P. hypophthalmus* broodstock fed with supplemented essential fish oils was observed prior to the first spawning season (Kabir et al., 2012; Arfah et al., 2018). Weight gain and average daily gain of F_2 hybrid catfish (*P. gigas* x *P. hypophthalmus*) broodstock was reported higher in period from June to October, compared to January-June (Sutthi et al., 2014). As reported in Nile tilapia, feed supplemented with 1 and 1.5% freshwater fish oil in cages for 4 months can significantly increase WG and ADG and decrease feed conversion rate (FCR) (Rattanapot et al., 2018a). On the contrary, Sink et al. (2010) report decreased spawning success in catfish fed with plant protein substitute fortified with 10% fish oil mixture. Similarly, Somboon and Semachai (2014) found that growth of Basa catfish (*Pangasius bocourti* Sauvage, 1880) was decreased when fed diet with total lipid content of 12%. Reported discrepancies suggest a possibility that increased FFO levels could interfere with energy balances in fish diets. Such rationale is supported by recent finding that dietary energy content of more than 428.5 kcal/100 g resulted in decreased weight gain and SGR of *P. hypophthalmus* (Ranjan et al., 2018), and is also observed in present study where 1% of FFO supplement show best growth performance (Fig. 1). Phillips (1972) found that increase in weight gain and growth rate is possible only up to a certain dietary energy level, and is followed by a decrease as result of increased efforts to metabolize and store high-energy nutrients.

Contrary to the pre- and spawning season, the supplementation with 1% and 2% FFO did not increase fish growth during post-spawning season. One possible explanation is that differences in water temperature (20–25 °C in post-spawning; 25–30 °C in spawning season) could exercise a strong influence on the hybrid catfish biology, including changes in energy metabolism of fatty acids in different spawning phases (Sink and Lochmann, 2008). Water temperature is a major factor that directly affects feed intake, metabolic rates and energy consumption of

catfish (Koeypudsa and Jongjareanjai, 2011), and has a direct effect on the growth of fish (Smith, 1989). However, even though significant mobilisation of fatty acids was observed in seabream liver and muscle during pre-spawning period, the fatty acid content remained constant in the gonads (Komilus et al., 2008). Thus, it is possible that dietary supplementation with FFO may rescue growth parameters during pre-spawning and spawning season via better availability of fatty acids, while these mechanisms may not be so important after spawning, when mobilisation of fatty acids is lower (Jerez et al., 2006).

Steroid hormone levels were elevated in plasma of FFO supplemented fish compared to control (Table 3). Similarly, 17 β-estradiol and testosterone levels of *Hemibagrus nemurus* were shown to be increased during spawning season (May through August) compared to post-spawning season (September through December), and their GSI in May (7.04%) and August (7.01%) was higher than in December (1.14%) (Adebiyi et al., 2013). Moreover, supplementation of fatty acids in seabass before and during spawning season induced increase in steroid hormones and significantly improved reproductive performance of sea bass (Navas et al., 1998). Finally, Mekong giant catfish (*P. gigas*) eight-year old females cultured in earthen ponds similar to our study, showed highest plasma levels of 17β-estradiol in May, prior to their spawning maturation (Manosroi et al., 2003).

Oocytes from fish fed with FFO supplements showed advanced maturation stages and histological characteristics compared to the control (Fig. 2). Rojtinnakorn and Thepnarong reported six oocyte maturation stages of the *Pangasianodon hypophthalmus* as stages 1 and 2 (primary growth phase, PG); stage 3 (central germinal vesicle, CGV); stage 4 (migrating germinal vesicle, MGV); stage 5 (peripheral germinal vesicle, PGV); and stage 6 (germinal vesicle breakdown, GVBD). Our results of histological evaluation of hybrid catfish ovaries in spawning phase (August) confirmed this categorization, and correspond to reported stages (Rojtinnakorn and Thepnarong, 2006; Prat et al., 1990). Testicular development in FFO supplemented fish was found to be more advanced than in control fish, and similar results were obtained by Sutthi et al. by finding spermatids and spermatozoa during spawning season in male catfish hybrid of F_2 generation (Sutthi et al., 2014).

Overall, our results demonstrate clearly that fish oil supplementation at low levels was able to increase plasma concentrations of steroid hormones (likely through increased availability of essential fatty acids used in synthesis of prostaglandin hormone precursor) (Mengumphan, 2010). The fish feed supplemented with 1% and 2% FFO showed increased content of mono- and poly-unsaturated fatty acids (Table 2). Essential fatty acids are necessary for the reproductive system and growth of fish, affecting teleost pituitary and gonadal hormone levels, and reproductive performance (Bruce et al., 1999). Arachidonic acid deficiency interferes with regulation of the sex hormones, and slows down development of sperm and eggs in sea bass and goldfish (Silva and Dean, 2001). It was also demonstrated that conversion of arachidonic acid to eicosanoids stimulates testosterone production in goldfish ovaries and testis (Mercure and Van Der Kraak, 1996).

Gonadosomatic index has been used as reliable indicator of changes in nutritional and energetic balance condition of fish (Adams et al., 1996). In our study, we observed fish in two parts of the growing cycle, fish spawning in August (during rainy season) and post-spawning season in December (entering Thai winter season). Significant differences in GSI were observed in between the two seasons, with highest difference in the group fed with 2% FO (Table 3). It was reported recently that critical requirements for optimal reproductive performance including relative gonadal weight in African catfish (*Clarias gariepinus*) may be water temperatures of 28 °C and nutrition (Al-Deghayem et al., 2017).

5. Conclusions

Hybrid catfish was fed with 1% and 2% freshwater fish oil supplemented diets. Increased growth and sex hormone levels were observed during spawning season in fish treated with 1% freshwater fish oil, and advanced development of ovaries and testes was observed in fish fed with freshwater fish oil supplements. Dietary supplementation with low levels of freshwater fish oil to hybrid catfish broodstock before and during spawning season improves overall condition and increases reproductive indicators. Freshwater fish oil supplementation at low level can be considered as effective approach in management of hybrid catfish broodstock reproductive cycle.

CRediT authorship contribution statement

Supaporn Sattang: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Visualization. **Doungporn Amornlerdpison:** Methodology, Investigation, Formal analysis. **Sudaporn Tongsiri:** Methodology, Investigation, Formal analysis. **Dušan Palić:** Conceptualization, Formal analysis, Writing - review & editing, Validation, Supervision, Resources. **Kriangsak Mengumphan:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Resources.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This study was financially supported by the Research and Researchers for Industries (RRI) fund, Thailand, Grant/Award Number: PHD60I0002.

References

Adams, S., Ham, K., Greeley, M., LeHew, R., Hinton, D., Saylor, C., 1996. Downstream gradients in bioindicator responses: point source contaminant effects on fish health. Can. J. Fish. Aquat. Sci. 53 (10), 2177–2187. https://doi.org/10.1139/f96-191.

Adebiyi, F.A., Siraj, S.S., Harmin, S.A., Christianus, A., 2013. Plasma sex steroid hormonal profile and gonad histology during the annual reproductive cycle of river catfish *Hemibagrus nemurus* (Valenciennes, 1840) in captivity. Fish Physiol. Biochem. 39 (3), 547–557. https://doi.org/10.1007/s10695-012-9718-x.

Al-Deghayem, W.A., Al-Balawi, H.F., Kandeal, S.A., Suliman, E.A.M., 2017. Gonadosomatic index and some hematological parameters in African catfish *Clarias gariepinus* (Burchell, 1822) as affected by feed type and temperature level. Braz. Arch. Biol. Technol. 60 https://doi.org/10.1590/1678-4324-2017160157.

Alhazzaa, R., Nichols, P.D., Carter, C.G., 2019. Sustainable alternatives to dietary fish oil in tropical fish aquaculture. Rev. Aquac. 11 (4), 1195–1218. https://doi.org/10.1111/raq.12287.

Ann Sorbera, L., Francisco Asturiano, J., Carrillo, M., Zanuy, S., 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biol. Reprod. 64 (1), 382–389. https://doi.org/10.1095/biolreprod64.1.382.

AOAC, 2000. Official Methods of Analysis of AOAC International, 17th ed. Maryland, USA.

AOAC, 2012. Official method 996.06. Fat (total, saturated, and unsaturated) in foods. Hydrolytic extraction gas chromatographic method. In: Official Methods of Analysis of AOAC Internationnal. Maryland, USA, 19th ed.

Arfah, H., Sudrajat, A.O., Supriyadi, M.A., Zairin, M., 2018. Gonad maturation of female striped catfish *Pangasionodon hypophthalmus* (Sauvage, 1878) using a combination of pregnant mare serum gonadotropin+ antidopamine, vitamin E, and curcumin extract mixed feed outside its spawning season. Int. J. Fish. Aquat. Stud. 6 (5), 52–57.

Asturiano, J., Sorbera, L., Carrillo, M., Zanuy, S., Ramos, J., Navarro, J., Bromage, N., 2001. Reproductive performance in male European sea bass (*Dicentrarchus labrax*, L.) fed two PUFA-enriched experimental diets: a comparison with males fed a wet diet. Aquaculture 194 (1-2), 173–190. https://doi.org/10.1016/S0044-8486(00)00515-9.

Bagenal, T., 1978. Methods for Assessment of Fish Production in Fresh Waters, 3rd ed. Blackwell Scientific Publication, London.

Bruce, M., Oyen, F., Bell, G., Asturiano, J.F., Farndale, B., Carrillo, M., Bromage, N., 1999. Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n− 3 and n− 6 highly unsaturated fatty acid to reproductive performance. Aquaculture 177 (1-4), 85–97. https://doi.org/10.1016/S0044-8486 (99)00071-X.

Classics Steel, R.G., Torrie, J.H., 1960. Principles and Procedures of Statistics. McGraw, New York.

Coward, K., Bromage, N., 1998. Histological classification of oocyte growth and the dynamics of ovarian recrudescence in Tilapia zillii. J. Fish Biol. 53 (2), 285–302. https://doi.org/10.1111/j.1095-8649.1998.tb00981.x.

FAO, 2020, July 8. Pangasius Prices Hit Bottom As COVID-19 Shuts Down Core Markets. Retrieved from. http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1296664/.

FAO, 2017, July 9. Pangasius Trade Shrinking. Retrieved from. http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1072503/.

Fisheries, D.o., 2020, April 7. Thai Fishery Trade, December 2019. Retrieved from. https://www.fisheries.go.th/strategy-tradestat/images/pdf/monthly/2562/dec%2062.pdf.

Hunt, A.Ö., Yilmaz, F.Ö., Erçen, Z., 2018. Comparing the effects of feeding a fish oil-or a cod liver oil-based diet on growth, feed utilization and muscle fatty acid composition Nile Tilapia (*Oreochromis niloticus*). Mediterr. Fish. Aquacult. Res. 1 (3), 130–142.

Jerez, S., Rodriguez, C., Cejas, J., Bolanos, A., Lorenzo, A., 2006.). Lipid dynamics and plasma level changes of 17β-estradiol and testosterone during the spawning season of gilthead seabream (*Sparus aurata*) females of different ages. Comp. Biochem. Physiol. B 143 (2), 180–189. https://doi.org/10.1016/j.cbpb.2005.11.002.

Kabir, M.A., GHAEDI, A., HASHIM, R., 2012. Ovarian development and sexual maturation of female striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) reared in captivity. Asian Fish. Sci. 25 (3), 232–244.

Koeypudsa, W., Jongjareanjai, M., 2011. Impact of water temperature and sodium chloride (NaCl) on stress indicators of hybrid catfish (*Clarias gariepinus* Burchell x *C. Macrocephalus* Gunther). Songklanakarin J. Sci. Technol. 33 (4).

Komilus, C.F., Shichi, N., Koshio, S., Ishikawa, M., Yokoyama, S., Michael, F.R., Makita, C., 2008. Influences of palm oil blended with fish oil on growth performances and lipid profiles of red sea bream Pagrus major. J. Aquac. Feed. Sci. Nutr. 56 (3), 317–326.

Luo, L., Ai, L., Liang, X., Xing, W., Yu, H., Zheng, Y., Xue, M., 2019. Effect of dietary DHA/EPA ratio on the early development, antioxidant response and lipid metabolism in larvae of Siberia sturgeon (*Acipenser baerii*, Brandt). Aquac. Nutr. 25 (1), 239–248. https://doi.org/10.1111/anu.12848.

Manosroi, A., Meng-Umphan, K., Manosroi, J., 2003. Annual sex hormonal profiles, gonad development and age determination of the Mekong giant catfish (*Pangasianodon gigas*, Chevey). Aquac. Res. 34 (15), 1379–1385. https://doi.org/10.1111/j.1365-2109.2003.00955.x.

Mengumphan, K., 2010. The Efficiency on the Production System of Mekong Giant Catfish and White Meat Catfish. In Final Report. National Research Council of Thailand, Bangkok, p. 60.

Mercure, F., Van Der Kraak, G., 1996. Mechanisms of action of free arachidonic acid on ovarian steroid production in the goldfish. Gen. Comp. Endocrinol. 102 (1), 130–140. https://doi.org/10.1006/gcen.1996.0054.

Navas, J.M., Mananos, E., Thrush, M., Ramos, J., Zanuy, S., Carrillo, M., Bromage, N., 1998. Effect of dietary lipid composition on vitellogenin, 17β-estradiol and gonadotropin plasma levels and spawning performance in captive sea bass (*Dicentrarchus labrax* L.). Aquaculture 165 (1-2), 65–79. https://doi.org/10.1016/S00 44-8486(98)00246-4.

Nikolsky, G.V., 1963. The Ecology of Fisher. Academic Press, London.

Phillips, A.M., 1972. Calorie and energy requirement. In: Halver, J.E. (Ed.), Fish Nutrition. Academic, New York, pp. 2–29.

Prat, F., Zanuy, S., Carrillo, M., De Mones, A., Fostier, A., 1990. Seasonal changes in plasma levels of gonadal steroids of sea bass, *Dicentrarchus labrax* L. Gen. Comp. Endocrinol. 78 (3), 361–373. https://doi.org/10.1016/0016-6480(90)90026-I.

Ranjan, A., Jain, K.K., Srivastava, P.P., Muralidhar, P.A., 2018.). Dietary energy requirement of *Pangasianodon hypophthalmus* (Sauvage,1878) juveniles reared at two temperatures. Turk. J. Fish. Aquat. Sci. 18 (1) https://doi.org/10.4194/1303-2712-v18_1_11.

Rattanaphot, T., Mengumphan, K., Tongsiri, S., Amornleardpison, D., 2018b. Utilization of enriched omega-9 freshwater fish oil. KMUTT Res. Dev. J. 41, 185–198.

Rattanaphot, T., Mengumphan, K., Tongsiri, S., Srimaroeng, C., Amornlerdpison, D., 2018c. Enhancement of oxidative defence and growth performance of Nile tilapia by omega-9-rich freshwater fish oil. Maejo Int. J. Sci. Technol. 12 (1), 79–88.

Rattanapot, T., Mengumphan, K., Tongsiri, S., Amornlerdpison, D., 2018a. Fatty acid composition of freshwater fish oil and its efficiency on growth performance in Nile tilapia. J. Agric. Res. Ext. 35 (2 Suppl), 11–20.

Rojtinnakorn, J., Thepnarong, J., 2006. Techniques of histology and cellularity for study of catfish oocyte. Proceedings of Fisheries Conference 355–364. Chiang Mai, Thailand.

Saini, R.K., Keum, Y.-S., 2018. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance-A review. Life Sci. 203, 255–267. https://doi.org/10.1016/j.lfs.2018.04.049.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177 (1-4), 191–199. https://doi.org/10.1016/S0044-8486(99)00083-00086.

Sattang, S., Amornlerdpison, D., Tongsiri, S., Senphan, T., Mengumphan, K., 2018. Growth, fish flesh qualities and reproductive index of hybrid catfish by different level of fish oil supplement in feed. Proceedings of 2nd NationalGraduateResearchConference and CreativeInnovationCompetition. Nantakarn Graphic Printing, Chiang Mai, Thailand, pp. 274–281.

Silva, J.L., Dean, S., 2001. Processed Catfish Product Forms, Packaging, Yields and Product Mix (184). Retrieved from Southern Regional Aquaculture Center Publication.

Sink, T.D., Lochmann, R.T., 2008. Effects of dietary lipid source and concentration on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg and fry production, and egg and fry quality. Aquaculture 283 (1-4), 68–76.

Sink, T.D., Lochmann, R.T., Pohlenz, C., Buentello, A., Gatlin, I.I.I.D., 2010. Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. Aquaculture 298 (3-4), 251–259. https://doi.org/10.1016/j aquaculture. 2008.07.024.

Smith, L.S., 1989. Digestive functions in teleost fishes. In: Halver, J.E. (Ed.), Fish Nutrition. New York, USA.

Somboon, S., Semachai, V., 2014. The Effects of Dietary Lipid Amount and Fatty Acids Composition on Growth Performance of Basa Catfish (Pangasius Bocourti Sauvage, 1880). Kasetsart University, Bangkok.

Stoneham, T.R., Kuhn, D.D., Taylor, D.P., Neilson, A.P., Smith, S.A., Gatlin, D.M., O'Keefe, S.F., 2018. Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: Improved nutrient value of fillet and offal. PLoS One 13 (4), e0194241. https://doi.org/10.1371/journal.pone.0194241.

Sutthi, N., Amornlerdpisan, D., Chitmanat, C., Mengumphan, K., 2014. Annual growth and reproductive performance in an F_2 catfish hybrid. J. Adv. Agric. Technol. 1 (2) https://doi.org/10.12720/joaat.1.2.113-118.

Sutthi, N., Panase, A., Chitmanat, C., Sookying, S., Ratworawong, K., Panase, P., 2020. Effects of dietary leaf ethanolic extract of *Apium graveolens* L. On growth performance, serum biochemical indices, bacterial resistance and lysozyme activity in *Labeo chrysophekadion* (Bleeker, 1849). Aquac. Rep. 18, 100551 https://doi.org/10.1016/j.aqrep.2020.100551.

Takahashi, T., Hagiwara, A., Ogiwara, K., 2018. Prostaglandins in teleost ovulation: a review of the roles with a view to comparison with prostaglandins in mammalian ovulation. Mol. Cell. Endocrinol. 461, 236–247. https://doi.org/10.1016/j.mce.2017.09.019.

Usawakesmanee, N., 2006. Food and Fish Feeding. Faculty of Agricultural Technology, Songkhla Rajabhat University.

Van't Land, M., Vanderperren, E., Raes, K., 2017. The effect of raw material combination on the nutritional composition and stability of four types of autolyzed fish silage. Anim. Feed Sci. Technol. 234, 284–294. https://doi.org/10.1016/j.anifeedsci.2017.10.009.

Wade, M.G., Van Der Kraak, G., 1993. Arachidonic acid and prostaglandin E_2 stimulate testosterone production by goldfish testis in vitro. Gen. Comp. Endocrinol. 90 (1), 109–118. https://doi.org/10.1006/gcen.1993.1065.

2. Journal of Agricultural Research and Extension 2018. 35(2) (Special): 1-10.

Journal of Agri. Research & Extension 35(2) (Suppl.): 1-10

ผลของน้ำมันปลาและสาหร่ายเตาต่อคุณภาพเนื้อและการเจริญพันธุ์ของปลาลูกผสมบึกสยามแม่โจ้ (ปลาบึก X ปลาสวาย)

Effect of Fish Oil and *Spirogyra* sp. Supplement on the Flesh Quality and Maturity of Buksiam Hybrid Catfish (*Pangasianodon gigas* Chevey, 1930 X *Pangasianodon hypophthalmus* Sauvage, 1878)

สุภาพร สัตตัง สุดาพร ตงศิริ ดวงพร อมรเลิศพิศาล และเกรียงศักดิ์ เม่งอำพัน*

Supaporn Sattang, Sudaporn Tongsiri, Doungporn Amornlerdpisan and Kriangsak Meng-umphan*

คณะเทคโนโลยีการประมงและทรัพยากรทางน้ำ มหาวิทยาลัยแม่โจ้ เชียงใหม่ 50290

Faculty of Fisheries Technology and Aquatic Resource, Maejo University, Chiang Mai, Thailand 50290

*Corresponding author: kriang1122sak@gmail.com

Abstract

The effect of fish oil and *Spirogyra* sp. supplemented on the meat quality and maturity of Buksiam hybrid catfish (*P. gigas* X *P. hypophthalmus*, F_2) in the 4 different diets such as 1) supplemented fish-feed (control), 2) 10% *Spirogyra* sp., 3) 1.5% fish oil and 4) 10% *Spirogyra* sp. + 1.5% fish oil were tested in Buksiam hybrid catfish for 4 months. The results of growth and survival rate were not significantly different ($p>0.05$). The meat quality in group fish-feed supplemented with 1.5% fish oil, protein value in fish flesh was 18.35±0.14% which higher than the group fish-feed supplemented with 10% *Spirogyra* sp. ($p<0.05$). The fat value in fish flesh supplemented with 10% *Spirogyra* sp. (1.49±0.02%) was demonstrated as the highest groups ($p<0.05$). Increasing of omega 3, 6 and 9 fatty acids accumulation were found in fish flesh. The highest estradiol hormone of fish was revealed in the group fed with 10% *Spirogyra* sp. ($p<0.05$). The results of this study could be concluded that *Spirogyra* sp. and fish oil have the potential to supplement in fish diets and can increase maturity and omega 3, 6 and 9 in fish flesh.

Keywords: growth performance, maturity, flesh quality, Buksiam hybrid catfish, fish oil

บทคัดย่อ

ผลของน้ำมันปลาและสาหร่ายเตาต่อคุณภาพเนื้อและการเจริญพันธุ์ของปลาลูกผสมบึกสยามแม่โจ้รุ่นที่ 2 (ปลาบึก X ปลาสวาย) เลี้ยงด้วยอาหาร 4 สูตร คือ 1) อาหารปกติ (ชุดควบคุม) 2) อาหารเม็ดเสริมน้ำมันปลาหนังน้ำจืด 1.5% 3) อาหารเม็ดเสริมสาหร่ายเตา 10% และ 4) อาหารเม็ดเสริมน้ำมันปลาหนังน้ำจืด 1.5% +

วารสารวิจัยและส่งเสริมวิชาการเกษตร 35(2) (พิเศษ): 1-10

สาหร่ายเตา 10% ทดสอบในปลาลูกผสมบึกสยามฯ นาน 4 เดือน พบว่าคุณภาพเนื้อของกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมน้ำมันปลาหนังน้ำจืด 1.5% มีโปรตีน 18.35±0.14% สูงกว่ากลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10% และมีความแตกต่างทางสถิติ ($p<0.05$) กลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10% มีไขมันในเนื้อสูงกว่าทุกกลุ่มการทดลอง และมีค่า 1.49±0.02% ($p<0.05$) นอกจากนี้มีการสะสมปริมาณกรดไขมันชนิดโอเมก้า 3, 6 และ 9 ในเนื้อปลาเพิ่มสูงขึ้น ส่วนฮอร์โมน Estradiol ของปลาสูงที่สุดในกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10% ($p<0.05$) จากผลการศึกษาครั้งนี้ สาหร่ายเตาและน้ำมันปลามีศักยภาพในการเสริมอาหารปลาเพื่อเพิ่มการเจริญพันธุ์ และโอเมก้า 3, 6 และ 9 ในเนื้อปลา

คำสำคัญ: การเจริญเติบโต การเจริญพันธุ์ คุณภาพเนื้อ ปลาลูกผสมบึกสยามแม่โจ้ น้ำมันปลา

คำนำ

ปัจจุบันกลุ่มปลาหนังลูกผสมเนื้อขาวได้รับความนิยมจากผู้บริโภค เนื่องจากเป็นอาหารสุขภาพที่มีคุณค่าทางโภชนาการและกรดไขมันที่ดี เช่น ไขมันกลุ่มโอเมก้า 3, 6 และ 9 ทำให้มีการเพาะเลี้ยงปลาหนังลูกผสมเนื้อขาวมากขึ้นในประเทศไทย (ดวงพร และคณะ, 2553) และในปี พ.ศ. 2557 ปริมาณสัตว์น้ำจืดจากการเพาะเลี้ยงเพิ่มขึ้นเป็น 394.9 พันตัน คิดเป็นกลุ่มปลาสวาย 22.5 พันตัน (กรมประมง, 2559) ซึ่งปัจจัยเรื่องคุณภาพของอาหารปลาที่ใช้ในการเลี้ยงมีความสำคัญต่อการเจริญเติบโต การเจริญพันธุ์ และคุณภาพเนื้อของปลา ส่งผลกระทบโดยตรงต่อความต้องการของผู้บริโภคที่ต้องการเนื้อปลาที่อุดมด้วยคุณค่าทางโภชนาการ ปลอดภัย และได้จากการเลี้ยงด้วยอาหารที่มีคุณภาพ ทำให้ต้นทุนค่าอาหารที่ใช้ในการเลี้ยงสูงขึ้นด้วย

จากปัจจัยด้านวัตถุดิบที่เป็นส่วนผสมของอาหารสัตว์มีแนวโน้มที่สูงขึ้นอย่างเห็นได้ชัด จึงได้มีความพยายามที่จะนำผลพลอยได้จากการแปรรูปสัตว์น้ำและวัสดุในท้องถิ่นมาเป็นส่วนผสมในสูตรอาหารปลาเพิ่มมากขึ้น เช่น น้ำมันปลาและสาหร่ายเตา ซึ่งจากการวิเคราะห์ส่วนประกอบของน้ำมันดิบในปลาลูกผสมบึกสยามแม่โจ้รุ่นที่ 2 (พ่อปลาบึก x แม่ปลาสวาย, F_2) เบื้องต้นพบว่ามีส่วนประกอบของไขมันอิ่มตัวเท่ากับ 13.83 กรัม และไขมันไม่อิ่มตัวเท่ากับ 81.17 กรัม (กรดไขมันไม่อิ่มตัวเชิงเดี่ยว 76.64 กรัม และกรดไขมันไม่อิ่มตัวเชิงซ้อน 9.52 กรัม) โดยกรดไขมันชนิดโอเมก้า 3, 6 และ 9 เท่ากับ 0.73, 8.42 และ 42.28 กรัม ตามลำดับ มีรายงานการศึกษาเกี่ยวกับการเจริญเติบโตของปลาหนังที่ให้อาหารผสมน้ำมันปลาในระดับ 1.5% เป็นเวลา 5 เดือน พบว่ามีการเจริญเติบโตดีที่สุด และยังช่วยเพิ่มปริมาณกรดไขมันชนิดโอเมก้า 3, 6 และ 9 ในเนื้อปลาได้อีกด้วย (ดวงพร และคณะ, 2556) ขณะที่ Manning *et al.* (2006) ศึกษาการเลี้ยงลูกปลา Channel catfish น้ำหนักประมาณ 50-60 กรัม ด้วยอาหารเสริมน้ำมันปลา โดยมีไขมันรวมในสูตรอาหารระดับ 3% เพาะเลี้ยงเป็นเวลา 6 สัปดาห์ พบว่าปลาที่เลี้ยงด้วยอาหารเสริมน้ำมันปลาระดับ 1.5% มีการสะสมที่เลี้ยงด้วยอาหารเสริมน้ำมันปลาระดับ 1.5% มีการสะสมของกรดไขมัน Omega 3 เพิ่มขึ้นในกล้ามเนื้อปลาอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ยังมีการนำวัตถุดิบท้องถิ่นมาพัฒนาเป็นส่วนผสมของสูตรอาหารปลา เช่น สาหร่ายเตา (*Spirogyra* sp.) ที่มีคุณค่าทางโภชนาการสูงประกอบด้วยโปรตีน (23%) ไขมัน (5%) คาร์โบไฮเดรต (55%) เยื่อใย (8%) แร่ธาตุ เช่น แคลเซียม (26.88%) เหล็ก (33.85%) แมกนีเซียม (241.10%) ฯลฯ และวิตามินเอ (0.25%) วิตามินบี 1 (0.04%) วิตามินบี 2 (0.55%) และวิตามินบี 6 (0.84%) ซึ่งเป็นสารตั้งต้นในการสร้างไนอะซิน (3.65%) จากทริปโตแฟน โดยไนอะซินมีส่วนสำคัญในการสังเคราะห์ฮอร์โมนเพศ (เอสโทรเจน โพรเจสเทอโรน เทสทอสเทอโรน) มีรงควัตถุหลายชนิด เช่น คลอโรฟิลล์ เอ และบี เบต้าแคโรทีน และเซนโทฟิล (ยุวดี, 2549) ที่ผ่านมาได้มีการนำสาหร่าย *Spirulina* sp. มาเสริมในอาหารเลี้ยงพ่อแม่พันธุ์ปลาชนิดต่างๆ เช่น ปลาบึกในอัตราส่วน 0 และ 10% ปลาเผาะในอัตราส่วน 0, 3 และ 6% และปลาสวายในอัตราส่วน 3%

Journal of Agri. Research & Extension 35(2) (Suppl.): 1-10

พบว่าปลาทั้งสามชนิดมีการเจริญพันธุ์ มีความสมบูรณ์เพศและสามารถเพาะผสมเทียมได้ในหน่วยการทดลองที่เลี้ยงด้วยอาหารผสมสาหร่ายสไปรูลินา 3, 6 และ 10% มากกว่าพ่อแม่พันธุ์ปลาที่เลี้ยงด้วยอาหารไม่ผสมสาหร่ายสไปรูลินา

จากปัญหาคุณภาพเนื้อของกลุ่มปลาหนังที่เลี้ยงด้วยอาหารปลาคุณภาพต่ำและการเลี้ยงไม่ได้มาตรฐานตลอดจนการเจริญเติบโตและการเจริญพันธุ์ช้า ดังนั้นการนำน้ำมันปลาหนังน้ำจืดและสาหร่ายเตามาเสริมในอาหารปลา เพื่อศึกษาการเจริญเติบโต การเจริญพันธุ์และคุณภาพเนื้อของปลาลูกผสมบึกสยามแม่โจ้ (ปลาบึก X ปลาสวาย, F_2) ให้มีคุณค่าทางโภชนาการดีขึ้น (กรดไขมัน) สามารถพัฒนาต่อยอดในเชิงอาชีพและพาณิชย์ได้อย่างดีในอนาคต

วิธีดำเนินการวิจัย

กิจกรรมที่ 1 การเลี้ยงปลาในกระชังให้ได้ขนาดตลาดและพ่อแม่พันธุ์

เตรียมปลาลูกผสมบึกสยามฯ (พ่อปลาบึก x แม่ปลาสวาย, F_2) อายุ 1 ปี น้ำหนักเฉลี่ย 494.17±20.59 กรัม/ตัว เลี้ยงในกระชังขนาด 4x3x2 เมตร จำนวน 12 กระชัง โดยอัตราการปล่อย 15 ตัว/กระชัง ให้อาหารเม็ดที่มีระดับโปรตีน 30% โดยวิเคราะห์คุณค่าทางโภชนาการของอาหารปลา ได้แก่ โปรตีน ไขมัน เถ้า เยื่อใย ตามวิธีของ AOAC (2000) อัตราอาหารที่ให้ 3%ของน้ำหนักตัว/วันๆ ละ 2 ครั้ง (9.00 และ 16.00 น.) ระยะเวลาเพาะเลี้ยงนาน 4 เดือน โดยแบ่งการทดลองเป็น 4 ชุดการทดลอง แบ่งเป็น 3 ซ้ำๆ ละ 15 ตัว ดังนี้

ชุดการทดลองที่ 1 อาหารปกติ (ชุดควบคุม)

ชุดการทดลองที่ 2 อาหารเม็ดเสริมน้ำมันปลา 1.5%

ชุดการทดลองที่ 3 อาหารเม็ดเสริมสาหร่ายเตา 10%

ชุดการทดลองที่ 4 อาหารเม็ดเสริมน้ำมันปลา 1.5% + สาหร่ายเตา 10%

เก็บข้อมูลการเจริญเติบโตโดยการชั่งน้ำหนักและวัดความยาวของปลาเดือนละ 1 ครั้ง และวิเคราะห์ข้อมูลทางด้านการเจริญเติบโตตามสูตร ดังนี้ (Bagenal 1978; Panase and Tipdacho, 2018) น้ำหนักที่เพิ่มขึ้น (Weight Gain) = น้ำหนักสุดท้าย (g) - น้ำหนักเริ่มต้น (g); อัตราการเจริญเติบโตต่อวัน (Average Daily Growth; ADG) = (น้ำหนักเมื่อสิ้นสุดการทดลอง (g) - น้ำหนักเมื่อเริ่มทดลอง (g))/จำนวนวันที่ทดลอง; อัตราการเปลี่ยนอาหารเป็นเนื้อ (Feed Conversion Ratio; FCR) = น้ำหนักอาหารที่กิน (g)/น้ำหนักที่เพิ่มขึ้น (g); ประสิทธิภาพการเปลี่ยนอาหารเป็นเนื้อ (Food Efficiency; FE) = น้ำหนักที่เพิ่มขึ้น (g)/อาหารที่กิน (g); อัตราการรอดตาย (Survival Rate) = (จำนวนปลาที่เหลือ) x 100/จำนวนปลาเริ่มต้น

กิจกรรมที่ 2 ศึกษาการเจริญพันธุ์

2.1 เมื่อสิ้นสุดการทดลองวันที่ 28 กรกฎาคม พ.ศ. 2559 ศึกษาดัชนีการเจริญพันธุ์ (Gonadosomatic Index, GSI) ตามสูตร น้ำหนักอวัยวะสืบพันธุ์ x 100/น้ำหนักตัว ใช้ปลาจำนวน 6 ตัว/ชุดการทดลอง และตรวจเนื้อเยื่อเซลล์สืบพันธุ์ (Gonad Histology) ในรังไข่ของปลาเพศเมีย ด้วยวิธีวิเคราะห์เนื้อเยื่อ (Histological analysis) ใช้ปลาจำนวน 1 ตัว/ชุดการทดลอง (Coward and Bromage, 1998) ส่วนในปลาเพศผู้วิเคราะห์เป็นเปอร์เซ็นต์ของปลาที่สามารถรีดน้ำเชื้อได้ ตามสูตรดังนี้ จำนวนปลาที่มีน้ำเชื้อ (ตัว) x 100/จำนวนปลาทั้งหมด (ตัว)

2.2 เจาะเลือดบริเวณโคนหางของปลา ประมาณ 1 mL นำไปปั่นเหวี่ยงที่ 10,000 รอบต่อนาที นาน 5 นาที เพื่อแยกเอา Serum เก็บไว้ที่อุณหภูมิ -800°ซ.และตรวจวัดระดับของฮอร์โมน 17β-estradiol ในตัวเมีย และ Testosterone ในตัวผู้ ด้วยเครื่อง Electrochemilumin Escence Immunoassay (Elecsys 2010, Roche, Germany) ใช้ปลาจำนวน 6 ตัว/ชุดการทดลอง

กิจกรรมที่ 3 การศึกษาคุณภาพเนื้อและคุณภาพซาก (ปลาขนาดตลาดเฉลี่ย 1.19 กก./ตัว)

3.1 วิเคราะห์คุณค่าทางโภชนาการของเนื้อปลาลูกผสมฯ โดยวิธี Proximate analysis ได้แก่ โปรตีน ไขมัน เถ้า ความชื้น และเยื่อใย ตามวิธีของ AOAC (2000) ใช้ปลาจำนวน 3 ตัว/หน่วยการทดลอง

3.2 วิเคราะห์ชนิดและปริมาณของกรดไขมัน (Fatty acid profile) โดยการทำ GC (Gas-Chromato graphy) ด้วยวิธีการ In House Method TE-CH-208 (AOAC, 2012) ใช้ปลาจำนวน 1 ตัว/ชุดการทดลอง

3.3 วิเคราะห์คุณภาพซาก นำเนื้อปลาลูกผสมฯ จำนวน 10 ตัว แยกชิ้นส่วนต่างๆ ได้แก่ เนื้อข้างลำตัว เนื้อท้อง เครื่องใน เนื้อขูด และกระดูก ทำการชั่งน้ำหนักและคำนวณเป็นเปอร์เซ็นต์

สถิติที่ใช้ในการทดลอง

วางแผนการทดลองแบบ Completely Randomized Design (CRD) ข้อมูลแสดงในรูปของ mean±SE เปรียบเทียบความแตกต่างของค่าเฉลี่ยระหว่างกลุ่มทดลองโดยวิเคราะห์แบบ One way ANOVA และเปรียบเทียบค่าเฉลี่ยเป็นรายคู่ด้วยวิธีของ Turkey ที่ระดับนัยสำคัญทางสถิติ $p<0.05$ โดยใช้โปรแกรม SPSS เวอร์ชั่น 17

ผลการวิจัย

คุณค่าทางโภชนาการของอาหารปลา พบว่าโปรตีนในอาหารชุดควบคุมมีค่าสูงกว่าสูตรอาหารอื่นๆ ($p<0.05$) ส่วนไขมันที่เพิ่มขึ้นในอาหารเม็ดเสริมสาหร่ายเตา 10% + น้ำมันปลา 1.5% สูงที่สุด (6.62±0.04%) รองลงมาคือ น้ำมันปลา 1.5% (5.34±0.54%) สาหร่ายเตา 10% (3.70±0.05%) และ ชุดควบคุม (3.42±0.46%) ตามลำดับ ($p<0.05$) ดังแสดงค่าใน Table 1

Table 1 Chemical composition of the 4 fish feed groups for Buksiam hybrid catfish

Chemical composition	Control	Crude oil 1.5%	*Spirogyra* sp. 10%	10% *Spirogyra* sp. + 1.5% Crude oil
Moisture (%)	10.10^{b}±0.01	7.19^{a}±0.02	7.19^{a}±0.03	10.07^{b}±0.04
Ash (%)	6.27^{a}±0.04	6.93^{b}±0.02	10.91^{c}±0.07	9.89^{b}±0.03
Protein (%)	31.91^{c}±0.01	30.34^{a}±0.04	30.36^{a}±0.02	30.43^{b}±0.52
Fat (%)	3.42^{a}±0.46	5.34^{c}±0.54	3.70^{b}±0.05	6.62^{d}±0.04
Crude fiber (%)	9.73^{a}±1.81	6.23^{a}±1.03	8.14^{a}±0.19	10.29^{a}±0.63
Carbohydrate (%)	38.57^{ab}±1.88	43.97^{c}±1.03	39.71^{bc}±0.17	34.04^{a}±0.67

Display value is Mean ± Standard Error (SE), n = 12

$^{a, b, c, d}$ Means with different superscripts in a rows were significantly different ($p<0.05$).

การเจริญเติบโต

การเลี้ยงปลาลูกผสมบึกสยามฯ ด้วยอาหารปลาเสริมสาหร่ายเตา 10% + น้ำมันปลา 1.5% มีแนวโน้มของน้ำหนักที่เพิ่มขึ้น อัตราการการเจริญเติบโตต่อวันสูงกว่าชุดการทดลองอื่นๆ และมีอัตราการรอดตาย 100% แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ดังแสดงใน Table 2

Journal of Agri. Research & Extension 35(2) (Suppl.): 1-10

Table 2 Growth performance of Buksiam hybrid catfish reared in net cages for 4 months.

Growth performance	Control	Crude oil 1.5%	*Spirogyra* sp. 10%	10% *Spirogyra* sp. + 1.5% Crude oil
Initial weight (g)	518.00[a]±17.01	456.00[a]±11.14	488.67[a]±26.41	514.00[a]±27.78
Weight gain (g)	513.85[a]±68.69	546.40[a]±37.60	483.40[a]±95.40	573.73[a]±74.26
ADG (g/fish/day)	4.28[a]±0.57	4.55[a]±0.31	4.03[a]±0.80	4.78[a]±0.62
Survival rate (%)	100.00[a]±0.00	100.00[a]±0.00	100.00[a]±0.00	100.00[a]±0.00
FCR	2.15[a]±0.44	2.34[a]±0.61	1.77[a]±0.08	1.97[a]±0.27
FCE	50.72[a]±10.37	48.37[a]±10.96	56.78[a]±2.54	52.54[a]±6.70

Display value is mean ± Standard error (SE), n = 60

[a] no means with different superscripts in a rows were significantly different (p>0.05).

คุณภาพเนื้อปลา

จาก Table 3 คุณภาพเนื้อปลาลูกผสมบึกสยามที่เลี้ยงด้วยอาหารเม็ดเสริมน้ำมันปลาหนังน้ำจืด 1.5% มีโปรตีนสูงที่สุด เท่ากับ 18.35±0.14% ส่วนในเนื้อปลาที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตา 10% พบว่าโปรตีนในเนื้อต่ำกว่าชุดควบคุม แต่มีไขมันในเนื้อปลา เท่ากับ 1.49±0.02% สูงกว่าชุดการทดลองอื่นๆ (p<0.05)

คุณภาพซากในปลาลูกผสมบึกสยามฯ น้ำหนักเฉลี่ย 1.19 กิโลกรัม/ตัว ทั้ง 4 กลุ่มการทดลอง มีปริมาณเนื้อข้างลำตัว เนื้อท้อง และโครงกระดูก เฉลี่ยเท่ากับ 37.88, 13.51 และ 33.34% ตามลำดับ โดยไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p>0.05) ขณะที่อวัยวะภายในของปลาที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตา 10% มีค่าสูงกว่ากลุ่มอื่นๆ เท่ากับ 9.92% ส่วนเนื้อชุดของปลาที่เลี้ยงด้วยอาหารเสริมน้ำมันปลา 1.5% มีค่า 5.49% ซึ่งสูงกว่าชุดควบคุม (4.53%) โดยมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p<0.05)

Table 3 Proximate composition in fish flesh of Buksiam hybrid catfish fed with the 4 different diets for 4 months

Proximate composition	Control	Crude oil 1.5%	*Spirogyra* sp. 10%	10% *Spirogyra* sp. + 1.5% Crude oil
Moisture (%)	22.48[a]±0.020	23.46[b]±0.100	23.93[c]±0.030	23.83[c]±0.100
Ash (%)	1.12[b]±0.230	1.02[a]±0.003	1.01[a]±0.008	1.04[a]±0.005
Protein (%)	18.27[b]±0.150	18.35[b]±0.140	17.25[a]±0.140	18.10[b]±0.050
Fat (%)	0.71[b]±0.020	0.22[a]±0.040	1.49[c]±0.020	.21[a]±0.040
Energy (Cal/g)	1,354.93[a]±16.68	1,455.00[c]±3.000	1,358.00[a]±29.89	1,418.00[ab]±9.270

Display value is mean ± Standard error (SE), n = 12

[a, b, c, d] means with different superscripts in a rows are significantly different (p>0.05).

องค์ประกอบของกรดไขมันในเนื้อปลาที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตา 10% มีกรดไขมันชนิดอิ่มตัว (Saturated fatty acid) และไม่อิ่มตัว (Unsaturated fatty acid) สูงที่สุด รองลงมา คือ กลุ่มที่เสริมน้ำมันปลา 1.5%, สาหร่ายเตา 10% + น้ำมันปลา 1.5% และชุดควบคุม ตามลำดับ และมีการสะสมกรดไขมันชนิดโอเมก้า 3, 6 และ 9 ในกลุ่มที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตาและน้ำมันปลา นอกจากนี้ยังพบว่ามีการสะสมกรดไขมันชนิดโอเมก้า 9 เพิ่มขึ้นในเนื้อปลาที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตา 10% สูงที่สุด เท่ากับ 1.43 กรัม ดังแสดงค่าใน Table 4

Table 4 Fatty acid composition in fish flesh of Buksiam hybrid catfish fed with the 4 diets for 4 months

Fatty acid composition (g/100g)	Control	Crude oil 1.5%	*Spirogyra* sp. 10%	10% *Spirogyra* sp. + 1.5% Crude oil
Myristic acid (C14:0)	0.03	-	-	0.04
Palmitic acid (C16:0)	0.31	0.52	1.19	0.40
Stearic acid (C18:0)	0.09	0.15	0.35	0.11
Arachidonic acid (C20:0)	-	-	0.01	-
Saturated fatty acid	0.44	0.69	1.58	0.57
Palmitoleic acid (C16:1n7)	-	0.02	0.06	0.01
cis-9-Oleic acid (C18:1n9c)	0.28	0.57	1.43	0.36
cis-11-Eicosenoic acid (C20:1n11)	-	0.02	0.05	0.01
Monounsaturated fatty acid	0.30	0.62	1.54	0.39
cis-9,12-Linoleic acid (C18:2n6)	0.05	0.13	0.29	0.08
α-Linolenic acid (C18:3n3)	-	-	0.02	-
cis-11,14-Eicosadienoic acid (C20:2)	-	-	0.01	-
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	-	-	0.01	-
Arachidonic acid (C20:4n6)	0.01	0.01	0.02	0.02
4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	0.01	-	0.03	0.02
Polyunsaturated fatty acid	0.09	0.18	0.41	0.14
Unsaturated fatty acid	0.39	0.79	1.95	0.53
n-3 fatty acid	0.01512	0.01597	0.06091	0.02827
n-6 fatty acid	0.06993	0.01520	0.33279	0.11059
n-9 fatty acid	0.28509	0.56975	1.43363	0.36360

Journal of Agri. Research & Extension 35(2) (Suppl.): 1-10

จาก Table 5 การเจริญพันธุ์ในปลาเพศเมียดีที่สุด โดยเฉพาะในกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10% มีปริมาณฮอร์โมน Estradiol สูงกว่ากลุ่มการทดลองอื่นๆ เท่ากับ 143.47±50.62 pg/ml ($p<0.05$) และพบเซลล์สืบพันธุ์ในระยะก่อนไข่สุก (Cortical alveolar oocyte, *IV*) มากกว่าปลาในกลุ่มอื่นๆ (Figure 1) ส่วนเพศผู้ที่มีน้ำเชื้อ 35.71±2.14% สูงกว่าชุดควบคุม (21.43±1.02%) และมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

Table 5 Valuation of gonadosomatic index (GSI) and estradiol hormone in the experimental fish fed with the 4 diets for 4 months.

Sex	Maturity	Control	Crude oil 1.5%	*Spirogyra* sp. 10%	10% *Spirogyra* sp. + 1.5% Crude oil
Female	GSI (%)	0.34^{a}±0.050	0.37^{a}±0.020	0.33^{a}±0.030	0.33^{a}±0.010
	Estradiol (pg/ml)	72.60^{b}±15.80	44.93^{a}±7.950	143.47^{c}±50.62	36.13^{a}±7.710
Male	GSI (%)	5.16^{a}±0.000	6.17^{a}±0.000	4.35^{a}±0.970	5.81^{a}±0.850
	Sperms (%)	21.43^{a}±1.020	20.00^{a}±0.970	35.71^{b}±2.140	33.33^{b}±2.080

Display value is mean ± Standard error (SE), n = 24

$^{a, b, c, d}$ means with different superscripts in a rows were significantly different ($p>0.05$).

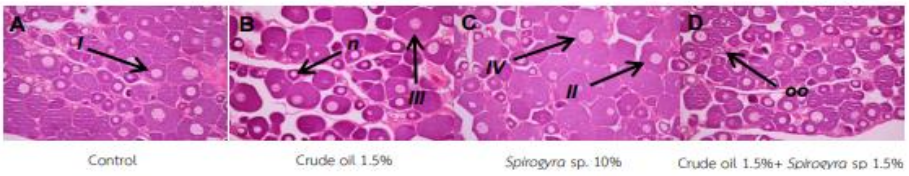


Figure 1 The oocyte stage of Buksiam hybrid catfish has identified in to 5 stages (In July), (40 x) *n* (nucleus), *oo* (oogonium), *I* (chromatin nucleolar oocyte), *II* (early perinucleolar oocyte), *III* (late perinucleolar oocyte), *IV* (cortical alveolar oocyte), *VI* (mature oocyte).

วิจารณ์ผลการวิจัย

การเจริญเติบโตและอัตราการรอดตายไม่แตกต่างกัน แต่ในกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10% + น้ำมันปลา 1.5% มีแนวโน้มของน้ำหนักที่เพิ่มขึ้นมากกว่ากลุ่มการทดลองอื่นๆ ซึ่งผลการทดลองในครั้งนี้สอดคล้องกับการศึกษาผลการเจริญเติบโตของปลาหนังที่ได้รับอาหารเม็ดเสริมน้ำมันปลาในระดับ 1.5% เป็นเวลา 5 เดือน พบว่ามีการเจริญเติบโตดีที่สุด และยังช่วยเพิ่มปริมาณไขมันโอเมก้า 3, 6 และ 9 ในเนื้อปลา (ดวงพร และคณะ, 2556) สาหร่ายเตามีคุณสมบัติในการต้านอนุมูลอิสระ โดยมีกลุ่มสารฟีโนลิกที่เป็นกลุ่มสาระสำคัญในการต้านออกซิเดชัน ป้องกันการเกิดภาวะเครียด (Oxidative stress) ที่เป็นสาเหตุทำให้ปลาเป็นโรคง่ายและเกิดการตาย ซึ่งมีรายงานการวิจัยการเลี้ยงปลานิล (*Oreochromis niloticus*) ด้วยอาหารผสมสาหร่ายเตา 4 ระดับ คือ 0, 2.5, 5, 10% เลี้ยงนาน 4 เดือน พบว่าปลานิลที่เลี้ยงด้วยอาหารผสมสาหร่ายเตาทุกระดับมี

แนวโน้มของน้ำหนักที่เพิ่มขึ้นและอัตราการรอดตายสูงกว่ากลุ่มที่เลี้ยงด้วยอาหารไม่ผสมสาหร่ายเตาอย่างมีนัยสำคัญทางสถิติ (ธีระวัฒน์ และคณะ, 2555) การสกัดน้ำมันปลาจากก้อนไขมันในช่องท้องของปลาลูกผสมบึกสยามแม่โจ้ รุ่นที่ 2 (พ่อปลาบึก x แม่ปลาสวาย) พบว่าในน้ำมันปลาปริมาณ 100 กรัม มีปริมาณกรดไขมัน omega 3 ได้แก่ ALA 0.41 กรัม EPA 0.10 กรัม และ DHA 0.20 กรัม มีปริมาณกรดไขมัน Omega 6 ได้แก่ กรดไลโนเลนิก (γ-linolenic acid, cis-9,12-linolenic acid) 7.07 กรัม ซึ่งเป็นกรดไขมันที่มีความสำคัญต่อการเจริญเติบโตของปลา โดยอาหารปลาส่วนใหญ่จำเป็นต้องผสมน้ำมันปลาเพื่อให้ได้ปริมาณกรดไขมันที่จำเป็นในอาหารเพียงพอต่อความต้องการ (อุธร, 2550) มีรายงานการทดลองเลี้ยงปลา *Pseudoplatystoma faciatum* วัยอ่อน ด้วยอาหารเสริมกรดไขมัน ได้แก่ Oleic acid, Linoleic acid อาหารผสมน้ำมันตับปลาจากปลาค็อด (Cod) และอาหารผสม Lecithin จากถั่วเหลือง เลี้ยงนาน 2 เดือน พบว่ามีการเจริญเติบโตที่ดี (Murat *et al.*, 2008)

ในส่วนคุณภาพเนื้อปลาลูกผสมบึกสยามฯ ที่เลี้ยงด้วยอาหารเม็ดเสริมน้ำมันปลาหนังน้ำจืด 1.5% และปลาที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตา 10% มีโปรตีนในเนื้อต่ำแต่มีไขมันสูงที่สุด เนื่องจากปลาที่มีขนาดใหญ่มีการใช้พลังงานในกระบวนการเมแทบอลิซึม จึงทำให้เกิดการสะสมไขมันมากกว่า ส่งผลให้องค์ประกอบโปรตีนในตัวปลามีสัดส่วนลดลง (NRC,1997) และมีการสะสมโอเมก้า 3, 6 และ 9 เพิ่มขึ้นในเนื้อปลาที่เลี้ยงด้วยอาหารเสริมสาหร่ายเตาและน้ำมันปลา เช่นเดียวกับ Chen *et al.* (2008) พบว่าในปลา *Oncorhynchus mykiss* น้ำหนัก 240 กรัม ที่เลี้ยงด้วยอาหารเสริมไขมัน omega 3 จาก Flaxseed oil ในระดับ 8.5 และ 15% เลี้ยงเป็นเวลา 120 วัน พบว่ามีการสะสมของกรดไขมันชนิด Omega 3 เพิ่มขึ้นในกล้ามเนื้ออย่างมีนัยสำคัญทางสถิติ

การเจริญพันธุ์ของปลาลูกผสมบึกสยามฯ ในเดือนกรกฎาคมซึ่งเป็นช่วงฤดูการผสมพันธุ์ พบว่า ปลาเพศเมียในกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10% มีการเจริญพันธุ์ดีที่สุด โดยพิจารณาจากระดับฮอร์โมน Estradiol ที่มีค่าสูงถึง 143.47±50.62 pg/ml และจำนวนปลาตัวผู้ที่น้ำเชื้อคิดเป็น 35.71% มากสุดและแตกต่างทางสถิติ ซึ่งผลการศึกษาครั้งนี้สอดคล้องกับการรายงานของ Mengumphan (2009) ในปลาเผาะอายุ 3 ปี ที่เลี้ยงด้วยอาหารผสมสาหร่ายสไปรูลินา 3% มีระดับฮอร์โมนเพศ Estradiol และ Testosterone เฉลี่ยสูงกว่าปลาเผาะที่เลี้ยงด้วยอาหารไม่ผสมสาหร่าย และมีระดับฮอร์โมนเพศสูงสุดในเดือนมีนาคมและเดือนกรกฎาคม ส่วนการเลี้ยงปลาบึกในบ่อดินด้วยอาหารที่มีการผสมสาหร่ายสไปรูลินา 5 และ 10% พบว่ามีปริมาณวิตามินอีมากที่สุดในอาหารผสมสาหร่าย 10% และพบกรดไขมันชนิด Gamma linolenic acid (GLA), Arachidonic acid (AA), Eicosa pentanoic acid (EPA) และ Docosa hexanoic acid (DHA) ซึ่งช่วยให้มีการพัฒนาของระบบสืบพันธุ์และส่งผลให้พ่อแม่พันธุ์ปลาบึกสามารถเพาะผสมเทียมได้ (Mengumphan, 2010)

สรุปผลการวิจัย

จากผลการศึกษาในครั้งนี้ การเสริมสาหร่ายเตา 10% ในสูตรอาหารมีผลให้คุณภาพเนื้อปลาลูกผสมบึกสยามฯ มีระดับไขมันในเนื้อสูงกว่ากลุ่มการทดลองอื่นๆ เท่ากับ 1.49±0.02% ($p<0.05$) และมีการสะสมปริมาณกรดไขมันชนิดโอเมก้า 3, 6 และ 9 ในเนื้อปลาเพิ่มมากขึ้น และสามารถช่วยให้มีการเจริญพันธุ์ของพ่อแม่พันธุ์ปลาได้ดีที่สุด โดยปลาเพศเมียมีระดับฮอร์โมน Estradiol 143.47±50.62 pg/ml และจำนวนของปลาตัวผู้ที่พบว่ามีน้ำเชื้อเท่ากับ 35.71% ซึ่งสามารถนำผลการศึกษาไปประยุกต์ใช้กับการเลี้ยงพ่อแม่พันธุ์ปลาในกลุ่มปลาหนังน้ำจืดได้ ตลอดจนสามารถแก้ปัญหาการขาดแคลนพ่อแม่พันธุ์ปลาได้

Journal of Agri. Research & Extension 35(2) (Suppl.): 1-10

กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณ รองศาสตราจารย์ ดร. เกรียงศักดิ์ เม่งอำพัน ในการตรวจแก้ไขและให้คำปรึกษา สำนักวิจัยและส่งเสริมวิชาการการเกษตร มหาวิทยาลัยแม่โจ้ และสำนักงานคณะกรรมการวิจัยแห่งชาติ ที่ให้การสนับสนุนทุนอุดหนุนการวิจัยในครั้งนี้ คณะเทคโนโลยีการประมงและทรัพยากรทางน้ำที่อนุเคราะห์สถานที่และอำนวยความสะดวกในการทำงานวิจัยในครั้งนี้จนเสร็จสิ้นอย่างสมบูรณ์

เอกสารอ้างอิง

กรมประมง. 2559. **สถิติการประมงแห่งประเทศไทย พ.ศ. 2557**. เอกสารฉบับที่ 11/2559. กรุงเทพฯ: กลุ่มวิจัยและวิเคราะห์สถิติการประมง ศูนย์เทคโนโลยีสารสนเทศและการสื่อสาร.

ธีระวัฒน์ รัตนพจน์ เกรียงศักดิ์ เม่งอำพัน ชุติมา ศรีมะเริง รัตนาภรณ์ จันทร์ทิพย์ และดวงพร อมรเลิศพิศาล. 2555. ฤทธิ์ต้านอนุมูลอิสระและผลการเสริมสาหร่ายเตาต่อการเจริญเติบโตของปลานิลในกระชัง. **ว. วิจัยเทคโนโลยีการประมง** 6(2): 23-34.

ดวงพร อมรเลิศพิศาล เกรียงศักดิ์ เม่งอำพัน นริศรา ไล้เลิศ อัญชลี พงศ์ชัยเดชา วุฒิพจน์ ศุภวิริยากร ชุติมา ศรีมะเริง และวิวัฒน์ หวังเจริญ. 2553. **ศักยภาพของน้ำมันปลาหนังน้ำจืดในการเป็นผลิตภัณฑ์เสริมอาหาร**. *ใน* รายงานผลการวิจัย. กรุงเทพฯ: สำนักงานคณะกรรมการวิจัยแห่งชาติ.

ดวงพร อมรเลิศพิศาล เกรียงศักดิ์ เม่งอำพัน นริศรา ไล้เลิศ ชุติมา ศรีมะเริง และธนนันท์ ศุภกิจจานนท์. 2556. **การพัฒนาและเพิ่มมูลค่าปลาหนังลูกผสม (ปลาเทโพ X ปลาสวาย) เพื่อการเพิ่มผลผลิตเชิงพาณิชย์และเพิ่มศักยภาพในการแข่งขัน**. *ใน* รายงานผลการวิจัย. กรุงเทพฯ: สำนักงานคณะกรรมการวิจัยแห่งชาติ.

ยุวดี พีรพรพิศาล. 2549. **สาหร่ายน้ำจืดในภาคเหนือของประเทศไทย.** เชียงใหม่: ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่. 361 น.

อุธร ฤทธิลึก. 2550. **การเลี้ยงปลาเพื่อการค้า.** พิมพ์ครั้งที่ 1. กรุงเทพฯ: สำนักพิมพ์โอเดียนสโตร์. 248 น.

AOAC. 2012. **Official method 996.06 Fat.** (Total, Saturated, and Unstaurated) in Foods. Maryland: AOAC International. 5 p.

AOAC. 2000. **Official Methods of Analysis of AOAC International.** 17th ed. Maryland: AOAC International. 2200 p.

Bagenal, T. 1978. **Methods for the Assessment of Fish Production in Fresh Waters, 3rd edn**. London: Blackwell Scientific Publication.

Chen, Yi-C., J. Nguyen, K. Semmens, S. Beamer, and J. Jaczynski. 2008. Effects of dietary alpha-tocopheryl acetate on lipid oxidation and alpha-tocopherol content of novel omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets. **LWT - Food. Sci. Technol** 41(2) : 244-253.

Coward, K. and N.R. Bromage. 1998. Histological classification of ovarian growth and the dynamics of ovarian recrudescence in tilapia zillii (gervais). **J. Fish Biol**. 53: 285-302.

Manning, B.B., M.H. Li, E.H. Robinson and B.C. Peterson. 2006. Enrichment of channel catfish (*Ictalurus punctatus*) fillets with conjugated linoleic acid and omega-3 fatty acids by dietary manipulation **Aquaculture**. 261: 337-342.

Murat, A., J. Rinchard. K. Dabrowski. and M.C. Portella. 2008. Effects of Different Dietary Lipid Sources on the Survival, Growth, and Fatty Acid Composition of South American Catfish, Pseudoplatystoma faciatum, Surubim Juviniles. **Journal of World Aquaculture Society** 39(12): 51-61.

Mengumphan, K. 2009. Growth performance, sex hormone levels and maturation ability of Pla Pho (*Pangasius bocourti*) fed with *Spirulina* sp. supplementary pellet and hormone application. **Int J. Agric. Biol** 11: 458-462.

Mengumphan, K. 2010. **The Efficiency on the Production System of Mekong Giant Catfish and White Meat Catfish.** Final Report. Bangkok: National Research Council of Thailand. 60 p. [in Thai].

NRC. 1997. **Nutrient requirement of warm water fish**. Washington, D.C: National Acadamy Press. 87 p.

Panase, P. and P. Tipdacho. 2018. Preliminary use of *Polygonum minus* Linn. leaf extract on growth performance, feed utilization, and some hematological indices of *Anabas testudineus* (Bloch, 1792). **Comp Clin Pathol** 27: 147-153.

APPENDIX F

PROCEEDINGS

1. The 1st National Graduate Research Conference and Creative Innovation Competition. 17-18 August 2017. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand. pp. 5.

การประชุมวิชาการและประกวดนวัตกรรมบัณฑิตศึกษาแห่งชาติ ครั้งที่ 1
"เทิดพระเกียรติวันแม่แห่งชาติ สู่ความมั่นคง มั่งคั่ง ยั่งยืน"
วันที่ 17–18 สิงหาคม 2560 ณ ศูนย์ประชุมนานาชาติดิเอ็มเพรส โรงแรมดิเอ็มเพรส เชียงใหม่

การเจริญเติบโต การเจริญพันธุ์ และคุณภาพเนื้อของปลาลูกผสมบึกสยามแม่โจ้ (ปลาบึก X ปลาสวาย)

Growth performance, maturity and meat quality of Buksiam hybrid catfish (*P. gigas* X *P. hypophthalmus*)

สุภาพร สัตตัง [1] ดวงพร อมรเลิศพิศาล[1] และเกรียงศักดิ์ เม่งอำพัน[2*]
Sattang, Sattang[1], Doungporn Amornlerdpisan[1] and Kriangsak Mengumphan[2*]

[1]คณะเทคโนโลยีการประมงและทรัพยากรทางน้ำ [2]บัณฑิตวิทยาลัย มหาวิทยาลัยแม่โจ้ เชียงใหม่ 50290
[1]Faculty of Fisheries Technology and Aquatic Resource, [2]Graduate School, Maeja University, Chiang Mai, Thailand 50290
*Corresponding author: kriang1122sak@gmail.com

บทคัดย่อ

ผลการวิเคราะห์การเจริญเติบโต การเจริญพันธุ์ และคุณภาพเนื้อของปลาลูกผสมบึกสยามแม่โจ้ (ปลาบึก X ปลาสวาย) เลี้ยงด้วยอาหาร 4 สูตรคือ อาหารปกติ (ชุดควบคุม) อาหารเม็ดเสริมน้ำมันปลาหนังน้ำซึด 1.5 เปอร์เซ็นต์ อาหารเม็ดเสริมสาหร่ายเตา 10 เปอร์เซ็นต์ และอาหารเม็ดเสริมน้ำมันปลาหนังน้ำซึด 1.5 + สาหร่ายเตา 10 เปอร์เซ็นต์ นาน 4 เดือน พบว่าปลาลูกผสมบึกสยามฯ มีน้ำหนักที่เพิ่มขึ้น อัตราการเจริญเติบโตต่อวัน อัตราการรอดตาย และอัตราแลกเนื้อ มีค่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p>0.05) คุณภาพเนื้อของกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมน้ำมันปลาหนังน้ำซึด 1.5 เปอร์เซ็นต์ มีโปรตีนสูงที่สุด (18.35±0.14 เปอร์เซ็นต์) แต่ไม่มีความแตกต่างทางสถิติ (p>0.05) กับชุดควบคุม สีของเนื้อปลาที่เลี้ยงด้วยอาหารเม็ดเสริมน้ำมันปลาและสาหร่ายเตามีค่าสีเหลืองของเนื้อต่ำกว่าชุดควบคุม (p>0.05) และมีการสะสมโอเมก้า 3, 6 และ 9 เพิ่มขึ้น การเจริญพันธุ์ในเพศเมียดีที่สุดในกลุ่มที่เลี้ยงด้วยอาหารเม็ดเสริมสาหร่ายเตา 10 เปอร์เซ็นต์ และมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p<0.05) กับกลุ่มการทดลองอื่นๆ ในขณะที่เพศผู้ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p>0.05) ผลการศึกษาครั้งนี้สรุปได้ว่า สาหร่ายเตาและน้ำมันปลามีศักยภาพในการเสริมอาหารปลาเพื่อการเพาะเลี้ยงสัตว์น้ำได้

คำสำคัญ: การเจริญเติบโต การเจริญพันธุ์ คุณภาพเนื้อ ปลาลูกผสมบึกสยามแม่โจ้

Abstract

The analysis results of growth performance, maturity and meat quality of Buksiam hybrid catfish (*P. gigas X P. hypophthalmus*). The culture with four different of fish–feed supplemented (normal fish–feed (control), *Spirogyra* sp. 10%, Crude oil 1.5% and *Spirogyra* sp. 10% + Crude oil 1.5%) for 4 months were analyzed in this study. The result of this research indicates that the weight gain, average daily growth, survival rate and feed conversion ratio were not significantly different (p>0.05). The meat quality in group fish–feed supplemented with crude oil 1.5% had highest incline level of protein (18.35±0.14%), but not significantly different with control (p>0.05). The meat color in all group at the fish–feed supplemented with crude oil and *Spirogyra* sp. of have the meat were less yellow lower than control (p>0.05). In addition, and omega 3, 6 and 9 accumulation was

การประชุมวิชาการและประกวดนวัตกรรมบัณฑิตศึกษาแห่งชาติ ครั้งที่ 1
"เทิดพระเกียรติวันแม่แห่งชาติ สู่ความมั่นคง มั่งคั่ง ยั่งยืน"
วันที่ 17–18 สิงหาคม 2560 ณ ศูนย์ประชุมนานาชาติดิเอ็มเพรส โรงแรมดิเอ็มเพรส เชียงใหม่

increased. The female highest best maturity was revealed in the group *Spirogyra* sp. 10% and significantly different (p>0.05) with other groups. Moreover, the maturity of male not significantly different (p>0.05) This study can be summarized that *Spirogyra* alga and crude oil were potentially used in fish feed supplemented in all the above findings lead to the conclusion that the use of *Spirogyra* alga and crude oil as a feed supplement in aquaculture is potentially possible.

Keywords: Growth performance, Maturity, Meat quality, Buksiam hybrid

2. The 2nd GCIC, 46th National and 9th International Graduate Research Conference. May 17th -18th, 2018. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand. pp. 25.

การประชุมวิชาการ และการประกวดนวัตกรรมบัณฑิตศึกษาระดับชาติและนานาชาติ
ระหว่างวันที่ 17–18 พฤษภาคม 2561
ณ ศูนย์ประชุมนานาชาติดิเอ็มเพรส โรงแรมดิเอ็มเพรส เชียงใหม่

iGRC02010223

Growth, fish flesh qualities and reproductive index of hybrid catfish by different level of fish oil supplement in feed

Supaporn Sattang[1], Doungporn Amornlerdpison[1], Sudaporn Tongsiri[1], Theeraphol Senphan[2] and Kriangsak Mengumphan[1],*

[1] Faculty of Fisheries Technology and Aquatic Resource, Maejo University, Chiang Mai, Thailand 50290
[2] Faculty of Engineering and Agroindustry, Maejo University, Chiang Mai, Thailand 50290

**Corresponding author,* e-mail: kriang1122sak@gmail.com

Abstract

The culture hybrid catfish (*Pangasius larnaudii X Pangasianodon hypophthalmus*) were fed by three levels of fish oil (FO) supplement in fish feed (control, 1% FO, and 2% FO) in 4 months. The best result of growth in terms of weight gained, average daily growth (ADG), feed conversion ratio (FCR) were obtained from fish feed supplemented with 1% FO significantly different (p<0.05). Moreover, reproductive index such as 17 β-estradiol (female) 1,577±5.26 pg/ml and testosterone (male) 3.28±0.41 ng/ml were higher than the control (1.97±0.23, 113.12±43.33 pg/ml and 2.56±0.95 ng/ml, respectively (p<0.05). The oocyte and spermatocyte stage of fish fed with 1% FO and 2% FO were developed better than control. However, fish flesh qualities (nutrition composition and color) of all groups were not significantly different (p>0.05). This study could be summarized that fish oil supplemented in fish feed increased growth and reproductive index abilities of hybrid catfish. Therefore, this result could be supported the demand of hybrid catfish aquaculture.

Keywords: Hybrid catfish, Fish oil, Growth, Fish flesh qualities, Reproductive index

APPENDIX G

POSTER PRESENTATION

1. The 12th International Conference and Exhibition on Nutraceuticals and Functional Foods (ISNFF2019) on December 1 - 5, 2019, Kobe, Japan.

Freshwater fish oil from catfish on anti-hyperglycemic and anti-hyperlipidemic effects in obese rats

Kriangsak Mengumphan[1,2] Supaporn Sattang[1] Narissara Lailerd[3] and Doungporn Amornlerdpison[1,2]

[1] Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand
[2] Center of Excellence in Agricultural Innovation for Graduate Entrepreneur, Maejo University, Chiang Mai, Thailand
[3] Department of Physiology Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

*Correspondence: kriang1122sak@gmail.com

Objectives

- To extract the freshwater fish oil (FFO) from visceral fat of Mekong Giant Catfish (MGC), Striped Catfish (SC) and Hybrid Catfish (HC)
- To evaluate three freshwater oil on blood glucose and lipid profile in obese rats

Mekong Giant Catfish (MGC, *Pangasinodon gigas*)

Striped Catfish (SC, *Pangasius hypophthalmus*)

Hybrid Catfish (HC, *Pangasianodon hypophthalmus* x *Pangasius larnaudii*)

Figure 1 Three species of freshwater catfish

Methods

Extraction of fish oil (Figure 2)

Adipose tissue from visceral fat of MGC, SC and HC were steamed, the obtained liquid was centrifuged at 4,500 rpm at 25 °C for 10 min. Characterization of the fatty acids composition of these FFO were analyzed using GC-MS.

Animal study : Effect of fish oil in obese rats (Figure 3)

Group 1: normal control: ND (n=10/group)
Group 2: High fat diet control: HFC
Group 3: High fat diet+ Commercial fish oil (CFO) 1 ml/kg/day
Group 4: High fat diet+ MGC oil 1 ml/kg/day
Group 5: High fat diet+ SC oil 1 ml/kg/day
Group 6: High fat diet+ HC oil 1 ml/kg/day

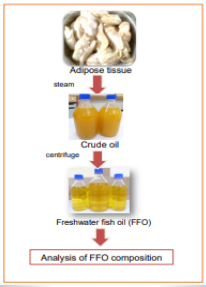


Figure 2 Extraction of freshwater catfish

Figure 3 Evaluation of FFO on blood glucose and lipid profile in obese rats

Table 1 Characterization of fatty acid composition of FFO

Fatty acid composition (g per 100g)	Mekong giant catfish	Striped catfish	Hybrid catfish
Saturated fatty acid	47.43	45.06	44.00
Unsaturated fatty acid	47.69	50.02	51.09
Monounsaturated fatty acid	**25.15**	**39.51**	**37.60**
Polyunsaturated fatty acid	22.54	10.51	13.49
n-3 fatty acid	15.58	0.83	4.36
α-Linolenic acid	7.84	0.59	0.93
EPA	3.23	0.07	0.65
DHA	4.24	0.13	2.72
n-6 fatty acid	6.36	9.19	8.77

Results

Table 1 shows the fatty acid compositions contained high amount of monounsaturated and polyunsaturated fatty acids which possess cardiovascular benefit. The high-fat diet increased visceral fat, body weight and displayed obese rats at period of 4-12 weeks. At the end of experiment, obese rats were fed with FFO of MGC, SC and HC at the dose of 1ml/kg significantly exhibited anti-hyperglycemia and anti-hyperlipidemia which the effects similar to commercial fish oil (Figure 4).

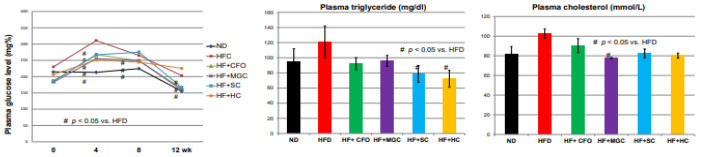


Figure 4 Effect of freshwater catfish on anti-hyperglycemia and anti-hyperlipidemia in obese rats

Conclusion

The findings provide the evidence to substantiate the potential of the freshwater fish oil from catfish to be developed as a dietary supplement for metabolic syndrome and diabetes treatment in the future.

Acknowledgements

The authors are grateful to Thailand Science Research and Innovation (TSRI) for awarding them a scholarship under the program me of Research and Researcher for Industry (RRI) as part of a Ph.D. program me (Project ID: PHD60I0002). The Maejo University is also acknowledged for kindly support.

2. Academic conference: Animal Genetic Improvement and Biotechnology: Moving towards Creative Economy, 13-14 July 2017. At the Grand Ballroom, Rama Gardens Hotel, Bangkok. pp. 40.

ลักษณะภายนอกและการเจริญเติบโตของปลาสวาย ปลาเทโพ และปลาลูกผสมแบบสลับเพศ

External characteristics and growth performance of *Pangasianodon hypophthalmus*, *Pangasius larnaudii* and their reciprocal hybrids

เกรียงศักดิ์ เม่งอำพัน นิสรา กิจเจริญ และ สุภาพร สัตตัง*

Kriangsak Mengumphan, Nissara Kitcharoen and Supaporn Sattang*

คณะเทคโนโลยีการประมงและทรัพยากรทางน้ำ มหาวิทยาลัยแม่โจ้ เชียงใหม่ 50290

Faculty of Fisheries Technology and Aquatic Resource, Maejo University, Chiang Mai, Thailand 50290

*Corresponding author: dream_nid@hotmail.com

Abstract

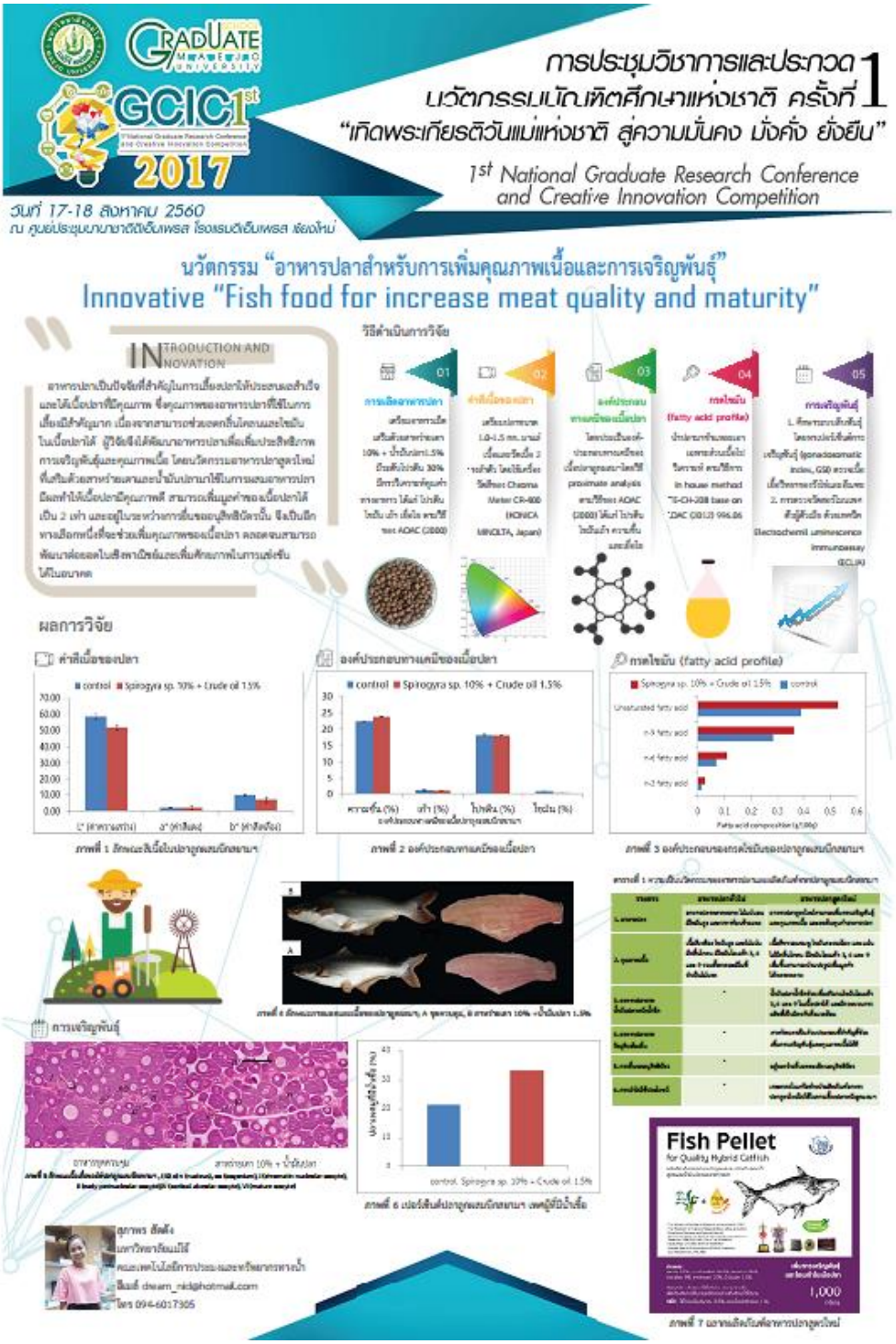
The study of the external characteristics of PH *(Pangasianodon hypohthalmus)*, PL *(Pangasius larnaudii)*, hybrid PHPL (Male *P. hypohthalmus* x *P. larnaudii*, Female) and their reciprocal hybrid PLPH (Male *P. larnaudii* x *P. hypohthalmus*, Female) in 6 inches. Found that the anal fin length of PH and their reciprocal hybrids were longer than PL. Length of Maxillary Barbell (MBL) of PH was the longest compared to others. Their head of reciprocal hybrids and PL were bigger than PH. Eye diameter of PL was larger than PH. Moreover, PL and their reciprocal hybrids represented black spot on pectoral fin. Consequently, the results of this study demonstrated that the hybrids species can increase productivity and easily identified by external characteristics of fish species. The weight gain of PHPL was the highest (21.04 g) followed by PH, PLPH and PL that were 20.34, 10.65 and 6.51 g, respectively. However, there are no significant difference growth rate between PHPL and PLPH. While, the survival rate of PL was the highest 97.78% and feed conversion rate of PHPL was the lowest (2.40). Therefore, the development of the species by crossbreeding, should choose the male as a *Pangasius larnaudii* because of the good quantity sperm. The female as a *Pangasianodon hypohthalmus* because of the high maturity/fecundity and the average daily growth was higher than *Pangasius larnaudii*.

Keywords: *P. hypohthalmus*, *P. larnaudii*, hybrid catfish, growth performance, external characteristics

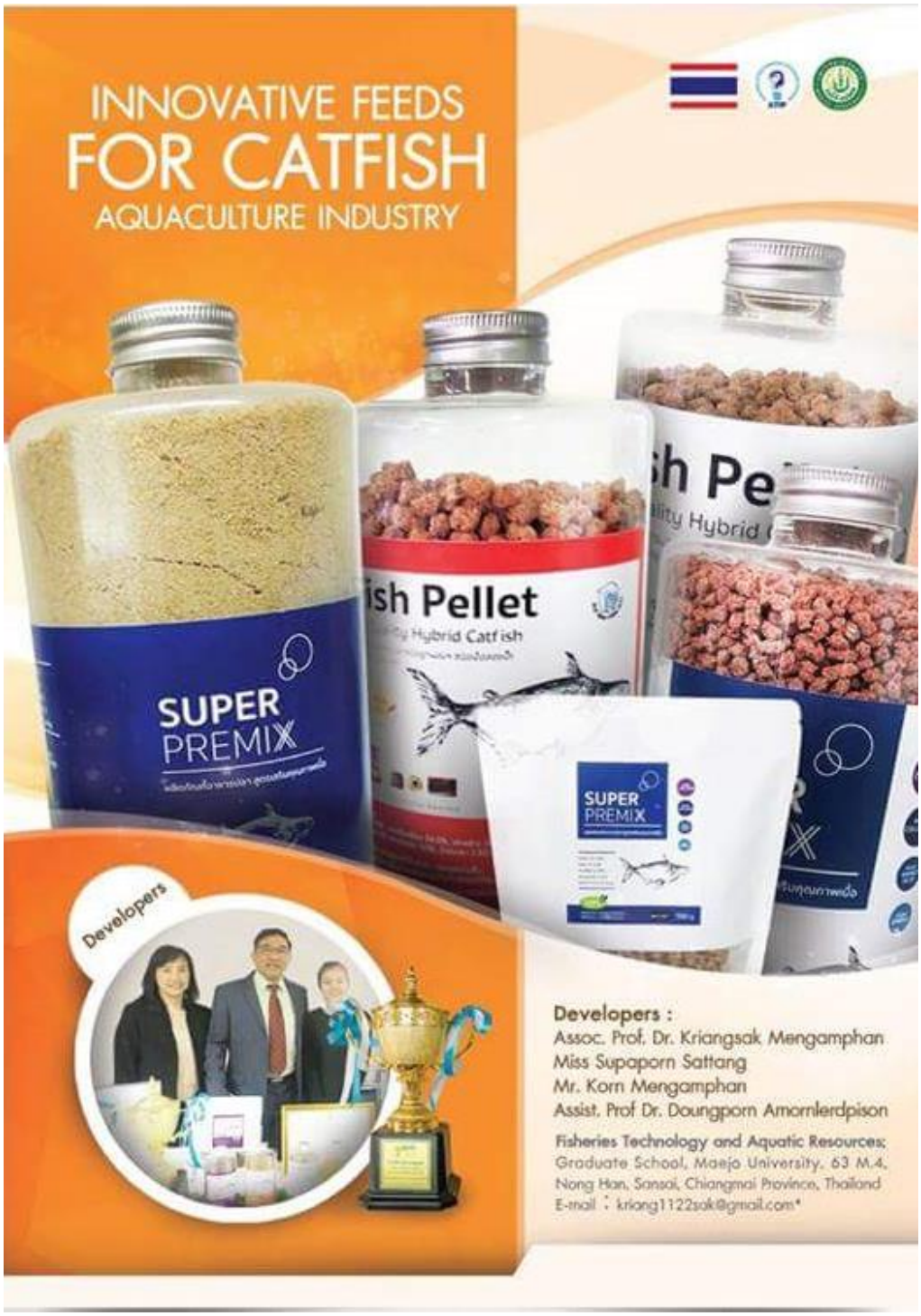
APPENDIX H

CREATIVE INNOVATION COMPETITION

1. The 1st National Graduate Research Conference and Creative Innovation Competition. 17-18 August 2017. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand.



2. Innovative feeds for catfish aquaculture industry. Award gold medal, special prize and special awards. In XXI Moscow International Salon of Inventions and Innovation Technologies «ARCHIMEDES-2018». At Moscow, Soviet Union, Russia (5-8 April 2018).



CATFISH
AQUACULTURE INDUSTRY

To optimize growth of the catfish at each stage it requires different feeds for maximizing production and profit. Therefore the innovative feeds from fish by products and fresh water algae are developed for catfish aquaculture industry. The feeds comprise of 3 formulae for different growth stage (Formula 1: super premix, formula 2: 1.5% freshwater fish oil and formula 3: 10% fresh water algae). At present, these fish feed products has been used in fish farming. licensing by private company and patty patent in Thailand

Figure 1 The growth of fingerlings receiving super premix (F1)

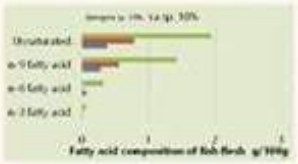


Figure 2 Fatty acid compositions of fish flesh receiving crude oil 1.5% (F2) and *Spirogyra* sp. 10% (F3)

Figure 4 Catfish aquaculture in cage and pond of private sector in Chiang Mai, Thailand

Figure 3 Histology of the ovary catfish from 3 formulae of fish feed

Fingerling and juvenile 1

Formula 1. For fingerling and juvenile fish
- Active ingredient: 10 % fish super premix, containing of essential amino acid, omega, and calcium
- Enhancement of growth and survival rate
- Petty patent application: in process

Maketable Fish 2

Formula 2. For marketable fish
- Active ingredient: 1.5% freshwater fish oil, containing omega 3, 6 and 9
- Accelerated growth and health of catfish
- High quality of fish flesh enriched omega 3,6, 9
- Petty patent application: - in process

Broodstock 3

Formula 3. For broodstock fish
- Active ingredient: 10% freshwater algae (*Spirogyra* sp.) containing phenolic and phytoestrogen
- Increase of Gonadosomatic index (GSI), sex hormone and maturation
- No. of petty patent application: 1703001325

APPENDIX I

Study and research visits in Fish Diseases and Fisheries Biology, the Faculty of Veterinary Medicine, Ludwig Maximilian University of Munich, Germany.

Figures 5 University visit and work planning.

Figures 6 The atmosphere of the immune system lab.

Figures 7 Presentation and summary of research studies before returning to Thailand.

APPENDIX J

CURRICULUM VITAE

CURRICULUM VITAE

NAME Miss Supaporn Sattang

DATE OF BIRTH 16 May 1990

EDUCATION 2007: High school education: Tharua "Nittayanukul" School, Phra Nakhon Si Ayutthaya, Thailand.

2010: Diploma/High Vocational Certificate (Dip. /High Voc. Cert., Aquaculture): Chiang Mai College of Agriculture and technology, Chiang Mai, Thailand.

2012: Bachelor Degrees (Bachelor of Science, B. Sc.): Maejo University, Chiang Mai, Thailand.

2015: Master Degrees (Master of Science, M.S.): Maejo University, Chiang Mai, Thailand.

WORK EXPERIENCE 2010: Trainee at the Inland Fisheries Research and Development, Chiang Rai, Thailand (2 Months).

2012: Trainee at the Chamnong Farm, Phan, Chiang Rai, Thailand (2 Months).

2013-2019: Teaching Assistant in subject Principles of Aquaculture, Aquaculture of Giant Catfish, Aquaculture Breeding and Agriculture for Life, Faculty of Fisheries Technology and Aquatic Resources, Maejo University.

2019: Study and research visits on the immune system, Distinguish Fish Blood Leukocytes, Striped cell culture and gene expression in fish, Fish Diseases and Fisheries Biology, the

Faculty of Veterinary Medicine, Ludwig Maximilian University of Munich, Germany on 26 August 2019 to 21 November 2019 (3 months).

2020: Research assistant for project: Adding value of waste from coconut pulp and fermented fish as protein supplemented fish diet (1 year).

2021: Research assistant for project: Production process of pickled fish from Buk Siam hybrid catfish with pure microorganisms, product development, probiotic production, testing for food pathogens and product processing (1 year).

Proceedings presentation:

1	**Sattang, S**., Amornlerdpisan, D. and Mengumphan, K. 2014. Growth Performances and External Characteristics of *Pangasianodon hypophthalmus* and Their Hybrid (*Pangasianodon hypophthalmus*, Male x *Pangasius larnaudii*, Female). pp. 207-210. *In* Thai Journal of Animal Science. The 3rd National Animal Science Conterence of Thailand, 2014 (NASCoT 2014). 8-10 April 2014. Hotel Centara Duangtawan, Chiang Mai.
2	**Sattang, S**., Amornlerdpisan, D., Tongsiri, S. and Mengumphan, K. 2015. Growth performance of hybrid catfish (hybrid, male x *P. hypohthalmus*, female) sex-reversed by 17 β - estradiol and 17 α - methyltestosterone. pp. 22-23. *In* The 9rd Fisheries Conference, 2015. 26-27 February 2015. Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai.
3	**Sattang, S**., Amornlerdpison, D., and Mengumphan, K. Growth performance, maturity and meat quality of Buksiam hybrid catfish (*P. gigas* X *P. hypophthalmus*). *In* 1st National Graduate Research Conference and Creative Innovation Competition. 17-18 August 2017. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand. pp. 5.

4	**Sattang, S**., Amornlerdpison, D., Tongsiri, S., Senphan, T. and Mengumphan, K. Growth, fish flesh qualities and reproductive index of hybrid catfish by different level of fish oil supplement in feed. *In* 2nd GCIC, 46th National and 9th International Graduate Research Conference. May 17th - 18th, 2018. At The Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand. pp. 25.
5	Suksri, T., Senphan, T., Tongsiri, S., **Sattang S**., and Mengumphan, K.. 2018. *In* 2nd GCIC, 46th National and 9th International Graduate Research Conference. May 17th -18th, 2018. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand. pp. 38.

Poster presentation:

1	**Sattang, S**., Mengumphan, K., and Kitcharoen, N. 2017. External characteristics and growth performance of *Pangasianodon hypophthalmus*, *Pangasius larnaudii* and their reciprocal hybrids. *In* Academic conference: Animal Genetic Improvement and Biotechnology: Moving towards Creative Economy, 13-14 July 2017. At the Grand Ballroom, Rama Gardens Hotel, Bangkok. pp. 40.
2	Mengumphan, K., **Sattang, S**., Lailerd, N., and Amornlerdpison, D. 2019. Freshwater fish oil from catfish on anti-hyperglycemic and anti-hyperlipidemic effects in obese rats. *In* The 12th International Conference and Exhibition on Nutraceuticals and Functional Foods (ISNFF2019) on December 1 - 5, 2019, Kobe, Japan.

Journal Published:

1	Mengumphan, K., **Sattang, S**., and Amornlerdpison, D. 2017. Growth Performances Survival Rate and External Characteristics of *Pangasianodon hypophthalmus*, *Pangasius larnaudii* and Their Reciprocal Hybrids. **Journal of Agricultural Research and Extension** 34(1) : 25-35.

2	**Sattang, S**., Tongsiri, S., Amornlerdpison, D., and Mengumphan, K. 2018. Effect of Fish Oil and *Spirogyra* sp. Supplement on the Flesh Quality and Maturity of Buksiam Hybrid Catfish (*Pangasianodon gigas* Chevey, 1 9 3 0 X *Pangasianodon hypophthalmus* Sauvage, 1 8 7 8). Journal of Agricultural Research and Extension 35(2) (Special): 1-10.
3	Mengumphan, K., Suksri, T., **Sattang, S**., and Tongsiri, S. 2022. Effect of fish meal replacement with filleting waste in fish feed for Maejo Buk Siam hybrid catfish on growth performance**.** Journal of Agricultural Research and Extension, 39(2).
4	**Sattang, S**., Amornlerdpison, D., Tongsiri, S., Palic, D. and Mengumphan, K. 2021. Effect of freshwater fish oil feed supplementation on the reproductive condition and production parameters of hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*, Sauvage, 1878) broodstock. Aquaculture Reports. 20, 100598.

Awards:

1	**Sattang, S.** 2017. Growth performance, maturity and meat quality of Buksiam hybrid catfish (*P. gigas* x *P. hypophthalmus*). Best presentation award, 3rd place (Proceedings). *In* 1st National Graduate Research Conference and Creative Innovation Competition. 17-18 August 2017. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand.
2	**Sattang, S.** 2017. Fish food for increase meat quality and maturity. Gold award. *In* 1st National Graduate Research Conference and Creative Innovation Competition. 17-18 August 2017. At the Empress International Convention Center, The Empress Hotel, Chiang Mai, Thailand.
3	Mengumphan, K., **Sattang S.**, Mengumphan, K., Suksri, T., and Amornlerdpison, D. 2018. Innovative feeds for catfish aquaculture industry. Award gold medal, special prize and special awards. *In* XXI Moscow International Salon of Inventions and Innovation Technologies «ARCHIMEDES-2018». At Moscow, Soviet Union, Russia (5-8 April 2018).

Petty Patent:

1	Patent in title “Fish diet formula for hybrid catfish”. No. 1703001325 (21/07/2017)

Food and Drug Administration:

1	Food and Drug Administration for Sai Aua (Notrhern Thai spicy sausage) of Silver hybrid catfish (*Pangasius larnaudii* x *Pangasianodon hypophthalmus*). No. 50-2-10559-6-0144 (27/06/2019).

CURRICULUM VITAE

NAME Miss Supaporn Sattang

DATE OF BIRTH 16 May 1990

EDUCATION

- 2007: High school education, Tharua “Nittayanukul” School, Phra Nakhon Si Ayutthaya, Thailand.
- 2010: Diploma/High Vocational Certificate (Dip./High Voc. Cert., Aquaculture), Chiang Mai College of Agriculture and technology, Chiang Mai, Thailand.
- 2012: Bachelor Degrees (Bachelor of Science, B. Sc.), Maejo University, Chiang Mai, Thailand.
- 2015: Master Degrees (Master of Science, M.S.), Maejo University, Chiang Mai, Thailand.

WORK EXPERIENCE

- 2013-2019: Teaching Assistant in subject Principles of Aquaculture, Aquaculture of Mekong Giant Catfish, Aquaculture Breeding and Agriculture for Life, Faculty of Fisheries Technology and Aquatic Resources, Maejo University.
- 2019: The 12th International Conference and Exhibition on Nutraceuticals and Functional Foods (ISNFF2019) on December 1 - 5, 2019, Kobe, Japan.
- 2019: Study visits on the immune system, Distinguish Fish Blood Leukocytes, Striped cell culture and gene expression in fish, Lehrstuhl für Fischkrankheiten und Fischereibiologie, Tierärztliche Fakultät, Ludwig-Maximilians-Universität München on 26 August 2019 to 21 November 2019 (3 months), Munich, Germany.
- 2020: Research assistant for project: Adding value of waste from coconut pulp and fermented fish as protein supplemented fish diet (1 year).

- 2021: Research assistant for project: Production process of pickled fish from Buk Siam hybrid catfish with pure microorganisms, product development, probiotic production, testing for food pathogens and product processing (1 year).