PROCESSED FISH WASTE AS FISH MEAL REPLACEMENT IN THE DIETS OF CLIMBING PERCH (*Anabas testudineus*) AND RED TILAPIA (*Oreochromis* sp.)

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BORNEO MARINE RESEARCH INSTITUTE

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THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2021

UNIVERSITI MALAYSIA SABAH

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DECLARATION

I hereby declare that acknowledge in this thesis titled "Processed fish waste as fish meal replacement in the diets of climbing perch (*Anabas testudineus*) and red tilapia (*Oreochromis* sp.)" has not been accepted in substance for any degree. Currently submitted to the Universiti Malaysia Sabah. All resources reference and information have been used, they have been certified.

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DEGREE : **MASTER OF SCIENCE**

FIELD : **AQUACULTURE**

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ACKNOWLEDGEMENT

I owe Allah s.w.t my sincere gratitude for guidance and support in completing my studies successfully. I'd like to express my heartfelt gratitude to my supervisor, Assoc. Prof. Dr. Annita Yong Seok Kian, for her guidance, constructive advices to my work, insightful suggestion, constant motivation, and emotional support during the dissertation's completion. My gratitude also goes to my co-supervisors, Assoc. Prof. Dr. Thumronk Amornsakun and Asst. Prof. Dr. Sarawuth Chesoh, who shared their expertise with me during my research.

I wish to thank the Research and Innovation Management Center of University of Malaysia Sabah (UMS) to provide me the research grant UMSGREAT(GUG0175-2/2017) and Discipline of Excellence (DoE), Prince of Songkla University Pattani Campus (PSU), and University of Malaysia Sabah (UMS) for funding my study Dual Master Degree Program (DMDP).

My sincere appreciation is also extended to Borneo Marine Research Institute (BMRI) and the Inland Aquaculture Research and Development Regional Center 12 (Songkhla) for allowing me to use the equipment and facilities during my experiments. I extent my heartfelt thanks to all lecturers especially Assoc. Prof. Dr. Faihana Ching Abdullah and staffs of BMRI and Fish Hatchery especially Ms. Syafikah Usop (BMRI lab's assistant), Mr. Poramat Musikarun (professional fishery officer) and Mr. Pornpanom Promkaew which is the director in Inland Aquaculture Research and Development Regional Center 12, Songkhla.

I'd like to share my heartfelt gratitude to my dear colleagues and friends; Ms. Noorsyarinah Sanudin, Ms. Sajeenuth Srithongthum, Ms. Mok Wai Yee, Ms. Dayang Nur Jazlyn Abang Zamhari, My beloved late Saleda Fitri, Mr. Mohd Faiz Reduan, Mr. Abd Azaharuddin Maidi, Mr. Muhamad Amirrul Ariff Ariffin, Mr. Mohd Syahizwan Laida, Mr. Muhammad Zulfi Al-Umam, Ms. Andrea Joyce Sibarani Maludin, Ms. Shahirah Abd Samad, Mr. Irman, Ms. Rocelene, Ms. Suhailah, Ms. Maria M. and Mr. Mohd Izzat Mahfudz for their sacrificing their precious time and valuable inputs directly or indirectly for the successful completion of this dissertation.

Last but not least, I would like to express my sincere gratefulness to my beloved mom and dad, Sarimah Awang Tuah and Zaihurin Abu Amin and family members for their infinite love, encouragement, patience, financial support, and dua's throughout my difficult period in completing this study.

Dg Siti Rahayu binti Zaihurin

16 August 2021

ABSTRACT

Fish waste is one of the fish industry's cost-effective and easily accessible ingredients that has not yet been thoroughly researched and used. This study was carried out to investigate the effect of fish meal (FM) replacement with fish waste (FW) on growth, survival, feed efficiency, body composition of two well-known freshwater fishes climbing perch (*Anabas testudineus*) and red tilapia (*Oreochromis* sp.). In this study, FW was first treated either by heat, hydrolysis using hydrochloric acid or acetic acid then the proximate composition, fatty acids and peptide profiles of the FWs were analyzed. For the hydrolysis, four concentration levels were tested for both type of acids at 2M, 4M, 6M and 8M. The suitable FW was then selected for feeding trials on climbing perch and red tilapia. The first experiment was conducted in Songkla Inland Aquaculture Research and Development Regional Center, Thailand on climbing perch (average initial body, BW 0.89±0.08 g) using experimental diets which contained 0, 25 and 50% of protein from FW (labelled as F0, F25 and F50), respectively. The feeds were fed triplicate groups of fish until satiation (0800 and 1600) for eight weeks. The second experiment was conducted on red tilapia in Universiti Malaysia Sabah, Malaysia. Three different experimental diets were prepared; diet with 25% heat treated FW (diet H), diet with 25% of the selected FW (diet AA) and diet with fish meal as main protein source (diet FM). The feeding trial was performed in duplicate tanks and red tilapia (initial BW 7.01±0.06 g) were fed until satiation (0800 and 1600) for ten weeks. Based on the proximate analysis, the FW treated with 2M concentration level of both acids shown to contain substantially higher protein content compared with other treatments ($P<0.05$) and equivalent to the heat treatment ($P>0.05$). The protein content was inversely proportional to the increasing level of acid concentration. Besides, 2M of hydrochloric and acetic acids did not influence the FW fatty acids ($P>0.05$) and peptides profiles compare to the heat-treated FW. Therefore, FW treated with 2M acetic acid was selected for the feeding trial. Results on climbing perch showed that fish fed diets F25 and F50 obtained lower growth compared with fish fed F0 (control diet) ($P<0.05$). The changes on BW (%) appeared to be inversely proportional to the increasing level of FW inclusion. However, the feed conversion ratio (FCR) from treatment F25 was comparable to F0 and

better than F50 ($P>0.05$). The fish body protein content in F50 was the highest to all treatments ($P<0.05$). As for the fatty acid profiling, FW diets did not appear to affect the body fatty acid content of climbing perch in F25 and F50 and the profiles showed no significant differences with F0 ($P>0.05$). In red tilapia, the growth performance and survival showed no significant differences among the treatments, but the highest BW gain was observed in red tilapia fed AA ($P>0.05$). No significant difference between all treatments was observed in FCR, protein efficiency ratio, and net protein utilization ($P>0.05$). Diet FM diet led to higher viscerosomatic index value compared to fish fed diets with FW. Red tilapia fed with diet H contained highest body protein content, followed by fish fed AA and FM ($P<0.05$) while body lipid content did not vary among the treatments ($P>0.05$). As for the red tilapia body fatty acids, fish fed diets with FW displayed higher levels of saturated fatty acids and monounsaturated fatty acids compared to the control. In conclusion, the heat and hydrolysis treatments changed the nutritional quality of the FW. In climbing perch, replacement of FM with FW affected the growth of the fish, however, a substitute amount of less than 25% could be possible used in climbing perch. On the hand, FW replacement at 25% can be utilized by red tilapia without comprising the growth, survival and feed utilization of the fish.

ABSTRAK

PROSES SISA IKAN SEBAGAI PENGGANTIAN MAKANAN IKAN DALAM DIET IKAN PUYU (Anabas testudineus) DAN TILAPIA MERAH (Oreochromis sp.)

Sisa ikan adalah salah satu ramuan industri ikan yang jimat dan mudah diakses yang belum diteliti dan digunakan secara menyeluruh. Kajian ini dilakukan untuk meneliti kesan penggantian tepung ikan dengan produk sampingan sisa ikan (FW) terhadap pertumbuhan, kelangsungan hidup, kecekapan pakan, komposisi tubuh dua ikan air tawar terkenal iaitu ikan puyu (*Anabas testudineus*) dan tilapia merah (*Oreochromis* sp .). Dalam kajian ini, FW pertama kali dirawat sama ada dengan haba, hidrolisis menggunakan asid hidroklorik atau asid asetik kemudian komposisi dekat, asid lemak dan profil peptida FW dianalisis. Untuk hidrolisis, empat tahap kepekatan diuji untuk kedua-dua jenis asid pada 2M, 4M, 6M dan 8M. FW yang sesuai kemudian dipilih untuk memberi makan percubaan pada ikan puyu dan ikan tilapia merah. Eksperimen pertama dijalankan di Pusat Penyelidikan dan Pembangunan Akuakultur Darat Songkla, Thailand pada ikan puyu (purata berat badan awal, BW 0.89±0.08 g) menggunakan diet eksperimen yang mengandungi 0, 25 dan 50% protein dari FW (dilabel sebagai F0, F25 dan F50) masing-masing. Makanan itu diberi makan tiga ulangan untuk setiap kumpulan ikan hingga kenyang (0800 dan 1600) selama lapan minggu. Eksperimen kedua dilakukan pada ikan tilapia merah di Universiti Malaysia Sabah, Malaysia. Tiga diet eksperimen berbeza disediakan; diet dengan 25% FW yang dirawat oleh haba (diet H), diet dengan 25% FW terpilih (diet AA) dan diet dengan tepung ikan sebagai sumber protein utama (diet FM). Percubaan makan dilakukan dalam tangki pendua dan tilapia merah (BW awal 7.01 ± 0.06 g) diberi makan sehingga kenyang (0800 dan 1600) selama sepuluh minggu. Berdasarkan analisis komposisi, FW dirawat dengan tahap kepekatan 2M kedua-dua asid yang terbukti mengandungi kandungan protein yang jauh lebih tinggi berbanding dengan rawatan lain ($P < 0.05$) dan setara dengan rawatan haba ($P > 0.05$). Kandungan protein berbanding terbalik dengan peningkatan kadar kepekatan asid. Selain itu, asid hidroklorik dan asetik 2M tidak mempengaruhi asid lemak FW ($P > 0.05$) dan profil peptida dibandingkan dengan FW yang dirawat dengan haba. Oleh itu, FW yang dirawat dengan asid asetik 2M dipilih untuk percubaan makan. Hasil percubaan

makan untuk ikan puyu menunjukkan bahawa makanan yang diberi makan ikan F25 dan F50 memperoleh pertumbuhan yang lebih rendah dibandingkan dengan ikan yang diberi makan F0 (diet kontrol) ($P<0.05$). Perubahan pada BW (%) nampaknya berbanding terbalik dengan peningkatan tahap kemasukan FW. Walau bagaimanapun, penukaran makanan (FCR) dari rawatan F25 setanding dengan F0 dan lebih baik daripada F50 ($P> 0.05$). Kandungan protein badan ikan di F50 adalah yang tertinggi untuk semua rawatan ($P<0.05$). Mengenai profil asid lemak, diet FW nampaknya tidak mempengaruhi kandungan asid lemak badan ikan puyu pada F25 dan F50 dan profil tidak menunjukkan perbezaan yang signifikan dengan F0 ($P>0.05$). Pada tilapia merah, prestasi pertumbuhan dan kelangsungan hidup tidak menunjukkan perbezaan yang signifikan antara rawatan, tetapi kenaikan BW tertinggi diperhatikan pada ikan tilapia yang diberi makan AA ($P> 0.05$). Tidak ada perbezaan yang signifikan dalam FCR, nisbah kecekapan protein, dan penggunaan protein bersih ($P>0.05$). Diet FM diet menyebabkan nilai indeks viserosomatik lebih tinggi berbanding dengan diet ikan yang diberi FW. Tilapia merah yang diberi makanan H mengandungi kandungan protein tubuh tertinggi, diikuti ikan yang diberi makan AA dan FM ($P<0.05$) sementara kandungan lipid badan tidak berbeza antara rawatan ($P>0.05$). Bagi kandungan asid lemak badan ikan tilapia, makanan yang diberi makan ikan dengan FWP menunjukkan kadar asid lemak tepu dan asid lemak tak yang lebih tinggi dibandingkan dengan ikan diberi makan FM. Kesimpulannya, rawatan haba dan hidrolisis mengubah kualiti pemakanan FW. Dalam ikan puyu, penggantian tepung ikan dengan FW mempengaruhi pertumbuhan ikan, namun, jumlah pengganti kurang dari 25% dapat digunakan untuk ikan puyu. Sebaliknya, penggantian FW pada 25% dapat dimanfaatkan oleh ikan tilapia merah tanpa mengganggu pertumbuhan, kelangsungan hidup dan penggunaan makanan ikan.

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LIST OF ABBREVIATIONS

ADC	-	Apparent digestibility coefficient
ADMD	-	Apparent dry matter digestibility
AED	-	Apparent energy digestibility
ANFs	-	Anti-nutritional factors
ANOVA	-	Analysis of Variance
APD	-	Apparent protein digestibility
BMRI	-	Borneo Marine Research Institute
BWG	-	Body weight gain
CF	-	Condition factor
CFU	-	Colony-forming unit
cm	-	Centimeter
DHA	-	Docosahexaenoic acid
DOC	-	Deoxycholate
EAA	-	Essential amino acid
EFA	-	Essential fatty acid
EPA	-	Eicosapentaenoic acid
FAO	-	Food and Agriculture Organization
FASTA	-	FAST-AII
FCR	-	Feed conversion ratio
FI	-	Feed intake
FM	-	Fish meal
FPH	-	Fish protein hydrolysates
FW	-	Fish waste
g	-	Grams
GC	-	Gas chromatography
h	-	Height
HCl	-	Hydrochloric acid
HSI	-	Hepatosomatic index

HUFA	-	Highly unsaturated fatty acid
LCMS	-	Liquid chromatography-mass spectrometry
m	-	Metre
mg	-	Milligram
min	-	Minute
mt	-	Million tonnes
MUFA	-	Monounsaturated fatty acid
mm	-	Millimeter
n	-	Number
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NCBI	-	National Center For Biotechnology Information
NEAA	-	Non-essential amino acid
NPU	-	Net protein utilization
PER	-	Protein efficiency ratio
ppt	-	Part-per thousand
PUFA	-	Polyunsaturated fatty acid
RPM	-	Revolutions per minute
SFA	-	Saturated fatty acid
SGR	-	Standard growth rate
Sp.	--	Species
SPSS	-	Statistical Program for the Social Sciences
TSE	-	Transmissible spongiform encephalopathies
V	-	Volume
VSI	-	Viscerosomatic index
WG	-	Weight gain

LIST OF SYMBOLS

ºC	-	Degree Celcius
%	-	Percent
<	-	Less than
>	-	Greater than

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CHAPTER 1

INTRODUCTION

1.1 The Significant of Aquaculture and Fish Meal

Aquaculture was defined by the Food and Agriculture Organization of United Nations (FAO, 1990) as;

> "cultivating of oceanic life forms, counting fish, mollusks, shellfish and oceanic plants. Other than that, it is also suggests a few frames of mediation within the raising handle in order to upgrade generation, such as standard stocking, nourishing and also assurance from predators. Cultivating moreover infers person or corporate proprietorship of the stock being developed for subsequent purpose"

Annually, the world is facing with enormous challenge from the increasing number of human populations that raising the urge in providing demand of protein sources. Hence, in order to combat malnutrition among society, the fisheries industry including aquaculture, plays an important role in obtaining this important source of nutrition by increasing their number of activities and production. Aquaculture has become one of the globally predominant sectors that meets the daily demand in providing fish protein for local consumption (Chotipuntu & Avakul, 2010). Other than available protein source such as livestock and poultry, fish recently has become a staple diet in human consumption. In the meantime, the expanded global demand of fish protein has raised the number of marine and aquaculture activities in most developing countries with huge land and water resources (Hamdan *et al.*, 2015). It has been reported that, the number of aquaculture activities has been increasing since over a

decade (Figure 1.1). According to FAO (2020), global fish production including both capture and aquaculture activities approximately about 179 million tonnes in 2018, of where aquaculture produce 82 mt. (46% of total production). Fish was accounted about 52% for human consumption from aquaculture production. Excluding world main producer China, recently Asia has become one of the share of fish production contributing 34%, where Malaysia and Thailand, both contribute 2% each (FAO, 2020). From overall total of both marine and aquaculture production, only 22 million tonnes were destined for non-food uses, primarily for fish meal and fish oil purpose. However, owing to its huge demand in the animal and fish feed industries, fish meal (FM) has become both costly and scarce (Akegbejo-Samsons & Fasakin, 2008).

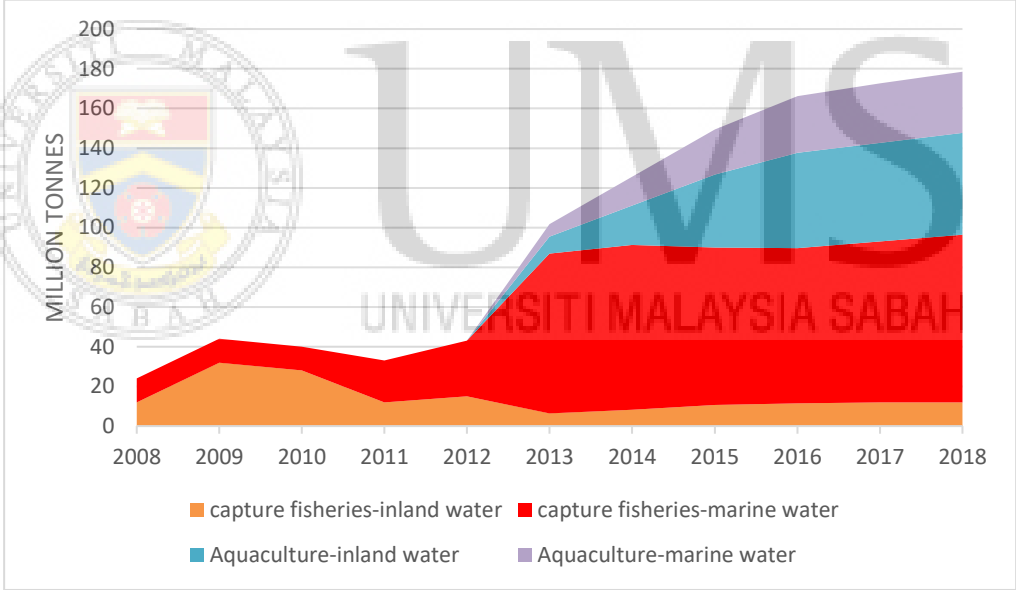

Figure 1.1 : World Capture Fisheries and Aquaculture Production From 2008 Until 2018

Source : Food and Agriculture Organization, FAO, 2020

Like any other animal-based production system, aquaculture has become as a highly globalized trade-dependent industry where the major part of its technology

requires fish meal as pillar of ingredient feed for farmed species. As a result, the production cost predominantly come from the feed and feeding management approximately 50 to 72% (Ng *et al.,* 2013). Fish meal (FM) known as globally conventional sources which is primarily used to provide protein in fish diets (Sogbesan & Ugwumba, 2008). Basically, FM can be produced using any types of seafood however the most generally manufactured were from less valuable wild-caught such as, small marine fishes. The less valuable fish were those that considered not good enough for human consumptions (Schipp, 2008). Other than that, this less valuable fish were defined as a group of fish that is juveniles of well-commercial species, juveniles and adults of less demand food species, minority species for its importance and also the fish that spoilt fishes of high value species (Malvas, 2005). FM is obtained from various method of processing; cooking (heat is applied), pressing, drying and milling freshly caught or trash fish and fish trimmings.

1.2 Fish Waste

More than 60% of the by-products generated from the fish processing industry are waste, including head, skin, trim, fins, frames, viscera and roe and only 40% are fish products for human consumption (Dekkers *et al.,* 2011). Fish waste (FW) is the secondary products or unutilized parts of fish source of protein (Ishak & Sarbon, 2017). These large volumes of FW from fisheries will cause serious pollution and disposal problems in both developed and developing countries in terms of contamination and disposal. Apart from being one of the more cost-efficient and easily obtained ingredient, the utilization of FW helps to reduce environmental pollution (Harnedy & Fitzgerald, 2012). These FW contains a significant amount of protein rich content that is usually converted into low market-value products, such as animal feed, FM and fertilizer (Hsu, 2010). Many biotechnologies have been developed to recover essential nutrients and bioactive compounds to help enhance human health by defending against several diseases and supplying essential nutrients to sustain good health, as well to solve contamination and disposal problems through the use of these protein-rich FW (Chalamaiah *et al.,* 2012).