TEMPEH AS AN ALTERNATIVE PROTEIN SOURCE IN THE DIETS FOR TIGER GROUPER, *Epinephelus fuscoguttatus* JUVENILE

CHOR WEI KANG

BORNEO MARINE RESEARCH INSTITUTE

UNIVERSITI MALAYSIA SABAH

2015

TEMPEH AS AN ALTERNATIVE PROTEIN SOURCE IN THE DIETS FOR TIGER GROUPER, *Epinephelus fuscoguttatus* JUVENILE

CHOR WEI KANG

THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF MASTER SCIENCE

BORNEO MARINE RESEARCH INSTITUTE

UNIVERSITI MALAYSIA SABAH

2015

DECLARATION

I hereby declare the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

13 February 2015

Chor Wei Kang

MY1211017T

CERTIFICATION

NAME : CHOR WEI KANG

MATRIC NO. : MY1211017T

TITLE : TEMPEH AS AN ALTERNATIVE PROTEIN SOURCE IN THE DIETS FOR TIGER GROUPER, *Epinephelus fuscoguttatus* JUVENILE

DEGREE : MASTER OF SCIENCE (AQUACULTURE)

VIVA DATE : 5th August 2015

DECLARED BY

1. SUPERVISOR Signature

Assoc. Prof. Dr. Rossita Shapawi

ACKNOWLEDGEMENT

First of all, I would like to thank God for giving me the opportunity to carry out my Degree of Master Science of Aquaculture in Universiti Malaysia Sabah (UMS) throughout these years. I would also like to thank my family for their understanding, love and continuous support.

Next, I would like to express my deepest gratitude towards my supervisor, Assoc. Prof. Dr. Rossita Shapawi for her guidance, encouragement and helpful advices especially during my hardest time in this study. Besides that, I would also like to thank Datuk Rayner Stuel Galid and Prof. Dr. Shigeharu Senoo for allowing me to conduct both of my feeding trials in Tanjung Badak Fisheries Department of Sabah and UMS hatchery, respectively, as well as providing facilities and technical supports for my experiments.

My sincere gratitude also goes to all of my friends, postgraduate students, laboratory assistants and hatchery staffs of Borneo Marine Research Institute (BMRI) especially Ms. Christine Anthonius, Mr. Lim Leong Seng, Ms. Isabella Ebi, Mr. Rian Freddie Firdaus, Ms. Ooi Shing Yau, Ms. Syafikah, Ms. Lydia, Mr. Norazmi and Mr. Tan Kian Ann who were willing to spend their quality time to help me in completing my study.

The financial supports provided by ScienceFund Program under the Ministry of Science, Technology and Innovation (Grant No. SCF0084-SEA-2012), MyBrain Scholarship and "*Elaun Khas Pembantu Pengajar*" Scheme of UMS in this study are deeply appreciated.

ABSTRACT

Tiger grouper (TG, *Epinephelus fuscoguttaus*) is among the most commonly cultured grouper species in Asia Pacific region due to high demand and good performance in captivity. However, the fish production is challenged by the high production cost mainly due to the heavy reliance on fish-based feed. Previous study on fish meal (FM) replacement with soybean meal (SBM) reported that at least 30% of the FM protein can be replaced by SBM with supplementation of phytase in the diet of tiger grouper. Tempeh (TMP), another type of processed (fermented) soy product was commonly reported to contain fewer antinutrients, more nutritious and easier to digest for human. It is expected that higher inclusion of soy product in the diet of TG is possible by using TMP with the supplementation of essential amino acids (EAAs) and feeding stimulant (FS). Therefore, in the present study, two experiments were carried out to evaluate 1) the potential of locally available fermented soybean, TMP as an alternative protein source and 2) the effects of EAAs and FS supplementation in TMP-based diets on TG. In Experiment 1, five dietary treatments of isoproteic (50%) and isolipidic (12%) diets were formulated to replace FM with TMP at 0, 30, 40, 50% (namely T0, T30, T40, and T50, respectively), and with SBM at 30% replacement level with phytase (S30) and fed to triplicate groups of juvenile TG (10.09±0.15g) for 56-days. Distal intestine (DI) and feces of experimental fish were sampled after feeding trial for histological observation and determination of apparent digestibility coefficient (ADC) of nutrients, respectively. Fish fed with T0 attained the highest weight gain (WG) and feed intake (FI), followed by S30 than other TMP-based diets ($P< 0.05$). Among all groups fed with TMP-based diets, T40 attained the highest WG (165.55%) but the FI decreased significantly with the increased level on TMP in the diets ($P< 0.05$). Despite the poorer WG and FI, T40 and T50 groups resulted in better feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) ($P< 0.05$) than other diets. In general, fish fed with all dietary treatments showed relatively high dry matter, crude protein and crude lipid apparent ADCs (ranging 81.78-89.87%, 93.07-94.71% and 91.01-99.17%, respectively) except for fish fed T50 in which resulted in poor lipid ADC (78.22%). Interestingly, reduced SBM-induced enteritis occurrence was observed in those fed with T30 compared to S30. In Experiment 2, another 56-day feeding trial was carried out to evaluate the effects of EAAs and FS (predetermined mixture of crystalline amino acids for grouper) supplementation in the diets using T40 as basal diet. T0, T40, T40 supplemented with EAAs (T40AA), T40 supplemented with FS (T40FS), and T40 supplemented with both EAAs and FS (T40MIX) were fed to triplicate of fish (22.93±0.50g) close to apparent satiation. Significantly higher WG were observed in T0 group (144.31%) than the rest of the diets ($P< 0.05$). Among TMP-based diets, better WG and FI were observed in those fed T40MIX with both FS and EAAs supplementation (100.1% and 0.65 g $fish^{-1}$ day^{-1}) than T40AA and T40FS ($P< 0.05$). Histological observation on DI showed that supplementation of either EAAs or FS has the potential to reduce SBM-induced enteritis. In conclusion, the utilization of TMP-based diets can be improved by the supplementation of both EAAs and FS supplementation. The full potential of TMP as an alternative protein source can be further explored by appropriate supplementation level of EAAs and FS in the diet.

ABSRTAK

TEMPEH SEBAGAI SUMBER PROTEIN ALTERNATIF DALAM DIET KERAPU HARIMAU, Epinephelus fuscoguttatus JUVENIL

Kerapu harimau (TG, Epinephelus fuscoguttaus), adalah antara spesies kerapu yang diternak secara meluas di rantau Asia Pasifik disebabkan permintaan yang tinggi dan prestasi yang baik di dalam kurungan. Walaubagaimanapun, pengeluaran ikan tersebut dicabar oleh kos penghasilan yang tinggi akibat daripada pergantungan terhadap makanan berasaskan ikan. Sebelum ini, kajian penggantian tepung ikan (FM) melaporkan FM boleh digantikan oleh 30% SBM dengan penambahan phytase dalam diet TG. Tempeh (TMP), sejenis produk kacang berfermentasi sering dilaporkan dapat menambahbaik nutrisi dan senang untuk dicernakan oleh manusia. Adalah dijangkakan bahawa penggunaan produk kacang soya dapat ditingkatkan dengan menggunakan TMP dengan suplemen asid amino perlu (EAAS) dan perangsang makanan (FS). Oleh itu, dalam kajian ini, dua percubaan makanan telah dilaksanakan untuk menilai 1) potensi TMP sebagai sumber protein alternatif dan 2) kesan-kesan suplemen EAAs dan FS dalam diet berasaskan TMP untuk TG. Dalam Eksperimen 1, lima diet isoproteik (50%) dan isolipidik (12%) diformulasi untuk mengantikan FM dengan menggunakan TMP pada kadar 0, 30, 40, dan 50% (T0, T30, T40 dan T50) dan oleh SBM pada penggunaan 30% dengan suplemen phytase (S30). Diet tersebut diberi makan kepada kumpulan triplikat TG (10.09±0.15g) dua kali sehari selama 56 hari. Usus dan najis ikan disampel selepas percubaan pemakanan masing-masing bertujuan untuk permerhatian histologi dan penentuan koefisyen penghadaman nyata (ADCs). TG yang diberi T0 mencapai pertambahan berat (WG) dan pengambilan makanan (FI) yang paling tinggi, diikuti oleh S30 dan diet lain berasaskan TMP. Diantara kumpulan diet-diet TMP, T40 menunjukkan WG tertinggi (165.55%) tetapi FI semakin berkurang apabila kandungan TMP dalam diet semakin meningkat (P< 0.05). Walaupun mempunyai WG dan FI yang rendah, T40 dan T50 menunjukkan kecekapan permakanan yang lebih baik dari diet lain dalam nisbah penukaran makanan (FCR), kadar kecekapan protein (PER) dan pengunaan protein bersih (NPU) (P< 0.05). Secara umumnya, semua ikan menunjukkan ADC jisim kering, protein dan lipid yang tinggi (masing-masing antara 81.78-89.87%, 93.18-94.83% and 91.36-99.23%) kecuali ADC lipid untuk TMP 50 yang bernilai 78.21%. Menariknya, pengurangan tanda-tanda keradangan yang disebabkan SBM diperhatikan di kalangan T30 berbanding S30. Dalam Eksperimen 2, satu lagi percubaan makanan selama 56 hari telah dijalankan untuk menilai kesan-kesan suplemen EAAs dan FS (formulasi khas untuk kerapu) dalam diet berasaskan T40. T0 (diet kawalan), T40, T40 bersuplemen EAAs (T40AA), T40 bersuplemen FS (T40FS) dan T40 bersuplemen EAAs dan FS (T40MIX) diberikan kepada kumpulan triplikat TG (22.93±0.50g). Ikan yang diberi T0 menunjukkan WG tertinggi (144.31%) berbanding kumpulan lain (P< 0.05). Di kalangan kumpulan yang diberi diet-diet T40, peningkatan WG dan FI yang nyata diperhatikan dalam kumpulan T40MIX (100.1% dan 0.65 g ikan^{-1} hari^{-1}). Dalam pemerhatian histologi, suplemen EAAs dan FS menunjukkan potensi untuk mengurangkan keradangan dalan usus yang disebabkan pengambilan SBM. Kesimpulannya, penggunaan diet berasaskan TMP boleh diperbaiki dengan penambahan suplemen EAAs dan FS. Potensi

menyeluruh TMP sebagai sumber protein alternatif boleh di terokai lebih lanjut dengan menentukan kadar suplemen EAAS dan FS bersesuaian di dalam diet.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADC	Apparent digestibility coeeficient
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BW	Body weight
CF	Condition factor
CL	Crude lipid
CP	Crude protein
DI	Distal intestine
DM	Dry matter
EAAs	Essential amino acids
FAO	Food and Agriculture of United Nations
FCR	Feed conversion ratio
FI	Feed intake
FM	Fishmeal
FS	Feeding stimulant
FSBM	Fermented soybean
HSI	Hepatosomatic index
Lys	Lysine
Met	Methionine
NPU	Net protein utilization
P	Phosphorus
P:E	Protein energy ratio
PER	Protein efficiency ratio

SBM	Soybean meal
SGR	Specific growth rate
SPSS	Statistical Package for the Social Science
TG	Tiger grouper
TMP	Tempeh
VSI	Visceral somatic index
WG	Weight gain

LIST OF SYMBOLS

%	Percentage
°C	Degree celcius
cm	Centimeter
et al.	And others
g	Gram
L	Liter
KJ	Kilo Joule

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of Study

For centuries, fish has been considered as a cheap protein source and it can be found in the menu of most household. Supplies of fish relied very much on artisanal fisheries before the emerging of commercial fishing which use technologies in detecting, fishing, preserving and transporting the fish in more efficient ways. The rise of commercial fishing can be closely related to the increasing buying power of consumer as the economic improved, and subsequently demanding for higher value fish on their menu list. In Asia, grouper is among the most sought after reef fish especially in Hong Kong market for their taste and texture. In certain cases such as giant grouper and humpback wrasses, consuming the fish is also regards as a symbol of status due to rarity and high price (Lee and Sadovy, 1998; Lau and Parry-Jones, 1999). Most specimens found in the market were sexually immature because of the preference for smaller fish. In addition, increase in supply of certain grouper during spawning season showed that the fish were targeted during spawning aggregations and can be destructive to the wild stock population. Responsible measure has to be taken to prevent over-fishing and sustain the stock of these previous marine species in the wild. One of the solutions is through aquaculture.

Early grouper aquaculture was restricted to the on-going of wild-caught juveniles as seed production techniques were not established during that time. As a result, the commonly cultured groupers were the low-value estuarine species *Epinephelus coioides* and *E. malabaricus* (Williams, 2009). These fish were normally fed with chopped-up fishery bycatch (trash fish) during the on-growing period until they reach marketable size. Success of artificial propagation coupled with improvement of hatchery technologies and techniques have enabled the production

of more valuable grouper such as giant grouper *E. lanceolatus*, tiger grouper *E. fuscoggutatus*, humpback grouper *Cromileptes altivelis*, and leopard coral trout *Plectropomus leopardus* (Giri *et al*., 2004; Sim *et al*., 2005, Pierre *et al*., 2007). Besides that, the development also ensures a more sustainable seed supply, leading to drastically increase of world grouper aquaculture production. China, Taiwan and Indonesia are the leading countries in world grouper production, together providing more than 90% of total production (Pierre *et al*., 2007).

Commercial grouper aquaculture in Asia relies heavily on the feeding of low-value fish and fishery by-product, commonly known as trash fish. These trash fish are cheap and readily accepted by the cultured grouper. However as the industry expanded, the use of trash fish as fish feed were proven to be restricted in most cases due to increasing price, limited supply, poor feed conversion ratio, polluting adjacent water, transmitting diseases and competition with other consumer (Boonyaratpalin, 1997; Sim *et al*., 2005). Over the last decades, researches have been carrying out to develop formulated feed that better meet the nutritional requirement of the fish. Fishmeal is the major protein source use in the manufacturing of the feeds. Growth rates and feed conversions have usually better in fish fed with formulated feed when directly compared (expressed on a similar dry matter basis) to fish fed with fishery bycatch (Ahmad *et al.,* 1991; Tacon *et al*., 1991).

Although resulted in better growth, continuously reliance on fish-based protein source such as trash fish and fishmeal as fish feed has led to significant pressure to the wild fish stock. Faced with the feed supply problem for cultured fish, ongoing intensive research has been done for decades on the potential alternative protein source to replace fishmeal. By understanding the exact nutritional requirements of the fish, fishmeal was able to be partially or totally replaced by alternative protein sources in the fish diets. Alternative protein source can be derived from plant or animal based, including soy meal, rapeseed meal, poultry byproducts, feather meal, blood flour, fish silage and single cell protein (Spinelli, 1980). These protein sources are promising candidates to replace fishmeal in the feed as they are cheaper agriculture by-products and containing high protein level.

Soybean based-protein sources are among the most utilized plant protein found in the manufacturing of aquaculture feed. With regard to its composition, soybean has been long considered as low cost protein, containing 47-50% protein content. With the exception of weather-induced yield losses in 2011/12, the supply is considered fairly stable, as it is commercially produced for soybean oil extraction (FAO, 2014a). Although soybean-based protein has successfully replaced fish meal in the diet of certain fish species such as rainbow trout, higher inclusion of soybean in aquaculture feed however remain difficult especially in carnivorous species (Lim *et al*., 2014). The utilization of soybean-based protein is limited due to presence of antinutrient factors, deficiency in certain amino acids, low palatability, and poor digestibility by monogastric species (Hardy, 2010; Kader *et al*., 2012). Several attempts have been taken to improve the nutritional value of soybean and feed utilization in fish feed. These include heat treatment, aqueous alcohol extraction, and fermentation, or supplementation with amino acids, enzyme and feeding stimulants (Berk, 1992; Shapawi *et al*., 2013b; Lim *et al*., in press).

Fermentation is a traditional and cheap method to preserve and improve the nutrient availability of food. Technically, the fermentation process involves enzymatic action by a considerable amount of unicellular biomass such as microbial or single cell protein and resulted in chemical composition changes (Odugawa *et al*., 2008). In Asia, a variety of traditional fermented soy food can be easily found in many countries, such as tempeh, miso, natto and meju. Tempeh (TMP) is fermented soybean by using *Rhizopus* spp. and originated from Indonesia. In Malaysia, TMP is among the common fermented soy food found in the market and regarded as delicacies which can be found in most Malay restaurant. The benefits of TMP consumption for human have been reported in several articles (Astuti *et al*., 2000; Amadou *et al*., 2009).

At present, information of the use of fermented soybean-based protein in fish feed is limited. Yamamoto *et al*., (2010) reported that most of the researches done showed that fish can utilize fermented soybean better than unfermented soybean in the diet at the same inclusion level, although improvement in growth performance was limited. Besides fermentation, supplementation of essential amino

acids and/or feeding stimulants were also reported to be able to improve the nutritional value of soybean and other plant-based diets for other fish species. The potential of fermented soybean as a new alternative protein source in fish feed should be further investigated in order to improve feed utilization of this cheap plant protein.

1.2 Significant of Study

Growth of aquaculture feed industry has increased linearly with the expansion of aquaculture sector. Feed alone constitute up to 60-70% of the aquaculture management cost. The price of aquaculture feed is mainly affected by the cost of protein source, which is commonly the expensive fishmeal. In order to reduce the feed cost and increase revenue, fish meal has to be substituted with cheaper alternative protein source. Previous study (Shapawi *et al*., 2013a) showed that soybean meal was able to replace up to 30% of fishmeal protein with the supplementation phytase in the diet of juvenile tiger grouper. It is in our interest to investigate the potential of nutritional improved soybean (TMP) to replace fishmeal at higher inclusion level compare to soybean meal. Current study was carried out to determine the potential of TMP as fishmeal substitute as well as to study the effects of supplementation of amino acids and feeding stimulant TMP-based diets for juvenile tiger grouper.

1.3Hypothesis

With the nutritional value improvement through fermentation, TMP is hypothesized to be a better alternative fishmeal protein source than unfermented soybean. In addition, supplementation of essential amino acids (EAAs) and/ or feeding stimulant (FS) can improve growth performances and feed utilization of tiger grouper by preventing EAAs deficiencies in plant-based diet and improving feed intake.

1.4 Objectives

In the present studies, there are three objectives to be achieved by the end of the experiment as listed below.

a) To determine the effects on growth performances and feed utilization of juvenile tiger grouper fed with diets containing different TMP inclusion level.

b) To determine the effects of supplementing essential amino acids (EAAs) in TMP-based diet on growth performance and feed utilization of juvenile tiger grouper.
c) To determine the effects of supplementing feeding stimulant (FS) in TMP-based diet on growth performance and feed utilization of juvenile tiger grouper.

CHAPTER 2

LITERATURE REVIEW

2.1 Groupers

2.1.1 Biology and Distribution of Groupers

Groupers are a diverse group of fish classified under 22 genera of the subfamily *Epinephelinae*, which comprise at least 115 species in the family of *Serranidae* (Baldwin and Johnson, 1993; Williams *et al*., 2004a). The fish species is widely distributed throughout the tropical and subtropical seas of the world and particularly those of the Indo-Pacific region (Williams, 2009). Equipped with euryhaline characteristic, groupers can tolerate salinity range from 10 to 50 ppt. Some are small with the maximum size of 12 cm for the Pacific creole-fish (*Paranthias colonus*) while largest species can grow up to 4 m long giant, *Epinephelus lanceolatus*, which was reported to weigh more than 440kg. Groupers are carnivorous species feeding on fish, shrimp, mantis shrimp, lobsters and mollusks in the wild. Most grouper species that have been studied so far mature within 2 to 6 years. Many serranids are protogynous hermaphrodite, maturing first as female and developed to male afterward as they grow. In other cases, the fish might only change sex if there is a shortage of male (Tucker, 1999).

2.1.2 Grouper Productions and Markets

Groupers fishery, is one of the largest live reef food-fishes trade in many Asia-Pacific region involving more than 10 countries in Southeast Asia and the Indian and Pacific Oceans as it has high market demand and yielding high prices in Hong Kong and China live fish trade (Sadovy, 2013). Popular trade grouper species belong to the genera *Epinephelus*, *Cromileptes* and *Plectropomus* of *Epinephelinae*. These included humpback grouper (*Cromileptes altivelis*), red grouper (*Epinephelus akaara*), giant grouper (*Epinephelus lanceolatus*) and coral trouts (*Plectropomus spp*) (Petersen *et al*., 2006; Johnston and Yeeting, 2007). The prices range from