DIETARY PROTEIN AND LIPID NUTRITION OF MARBLE GOBY, *Oxyeleotris marmoratus* JUVENILE

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BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
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CERTIFICATION

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DEGREE	:	**MASTER OF SCIENCE (AQUACULTURE)**
VIVA DATE	:	**4th March 2014**

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ABSTRACT

Three feeding experiments were conducted to investigate the dietary protein and lipid and the utilization of soybean meal in the diet by marble goby, *Oxyeleotris marmoratus* juvenile. Three isolipidic (10%) diets were formulated in Experiment I to contain 40, 45 and 50% of dietary protein (as P40, P45 and P50, respectively) and fed to juveniles of initial body weight (BW) 1.93 ± 0.01g. In Experiment II, four isonitrogenous (48% protein) diets containing different levels dietary lipid at 10, 14, 18 and 22% (as L10, L14, L18 and L22, respectively) was fed to groups of juveniles with initial BW:2.76 ± 0.02g. In Experiment III, three isonitrogenous (48% protein) and isolipidic (10% lipid) diets were formulated by replacing 0, 20% of the fishmeal protein with defatted soybean meal protein and 20% soybean meal with 2000 FTU/kg of phytase supplementation (as SB0, SB20 and SB20+PT, respectively) and fed to juveniles with initial BW 0.28 ± 0.01g. Experiment duration for Experiment I and II was 15 weeks while Experiment III was 40 days. In each of the feeding trials, a recirculation culture system was used and water quality was monitored periodically. Before commencing the feeding trials, all juveniles were weaned to formulated feed. During experiments, juveniles were fed to apparent satiation twice times daily. At the end of the feeding trial, some juveniles from each treatment were sacrificed for body indices and whole body proximate analysis. The remaining juveniles in Experiment I and II were used to collect faeces for apparent digestibility test in each respective experiment. The results of Experiment I showed that diet P45 which contained 48.6% protein supported highest growth, final BW, specific growth rate (SGR), protein efficient ratio (PER), nitrogen retention efficiency (NRE), apparent protein and lipid digestibility coefficient; and significant better feed conversion ratio (FCR) (P<0.05) than other treatments. Results of Experiment II showed that L10 that contained 10% of dietary lipid led to highest growth, SGR, final BW, PER; and significant lower hepatosomatic index, viscerosomatic index and intraperitoneal fat (P<0.05) than other treatments. Experiment III showed that 20% of soybean meal protein replacement was too high for small size of marble goby juvenile in term of growth, PER and FCR. However, juveniles fed SB20+PT achieved higher growth, FCR and PER compared with fish fed SB20. This present study showed that juveniles fed dietary protein and lipid of 48.6% and 10% achieved highest growth and the increase of protein or lipid higher than this level did not supported higher growth and the supplementation of phytase had improved the soybean meal utilization by young marble goby juvenile.

ABSTRAK

NUTRISI PROTEIN DAN LIPID UNTUK PEMAKANAN JUVENIL KETUTU, Oxyeleotris marmoratus

Tiga eksperimen pemakanan telah dijalankan untuk mengkaji keperluan protein dan lipid, dan penggunaan tepung kacang soya dalam diet untuk juvenil ikan ketutu, Oxyeleotris marmoratus. Untuk menentukan keperluan protein ikan ketutu, tiga diet isolipidik (10% lipid) dirumuskan dalam Eksperimen I mengandungi 40, 45 dan 50% protein (sebagai P40, P45 dan P50, masing-masing) dengan menggunakan ikan yang mempunyai purata berat badan (BW) 1.93 ± 0.01g. Dalam Eksperimen II, keperluan lipid ditentukan dengan memberi ikan (BW:2.76 ± 0.02g) empat diet isoproteic (48%) yang mengandungi kandungan lipid yang berbeza pada 10, 14, 18 dan 22% (sebagai L10, L14, L18 dan L22, masing-masing). Dalam Eksperimen III , empat diet isoproteic (48% protein) dan isolipidic (10% lipid) dirumuskan untuk menggantikan 0, 20% protein tepung ikan dengan protein tepung kacang soya dan 20% tepung kacang soya dengan penambahan 2000 FTU/kg phytase (sebagai SB0, SB20 dan SB20+PT, masing-masing). Dalam eksperimen ini, BW ikan pada permulaan ialah 0.28 ± 0.01g. Tempoh Eksperimen I dan II ialah 15 minggu manakala Eksperimen III ialah 40 hari. Sistem pengkulturan kitar semula digunakan dalam semua eksperimen dan kualiti air dipantau secara berkala. Sebelum eksperimen dimulakan, semua ikan didedahkan kepada makanan rumusan. Dalam semua eksperimen, ikan diberi makan sehingga menghampiri tahap kepuasan sebanyak dua kali sehari. Pada akhir eksperimen, beberapa ekor ikan dari setiap rawatan telah dikorbankan untuk analisis indeks badan dan komposisi proksimat badan. Ikan yang selebihnya dalam Eksperimen I dan II digunakan untuk ujian kebolehcernaan ketara. Keputusan dalam Eksperimen I mencadangkan bahawa P45 yang mengandungi 48.6% protein adalah mencukupi untuk ikan ketutu berdasarkan tumbesaran, BW terakhir, kadar tumbesaran tertentu (SGR), kadar kecekapan protein (PER), kecekapan pengekalan nitrogen (NRE), kebolehcernaan ketara protein dan lipid yang lebih tinggi; serta kadar penukaran makanan (FCR) yang lebih baik ($P<0.05$) berbanding dengan rawatan lain. Keputusan Eksperimen II menunjukkan bahawa L10 yang mengandungi 10% lipid dalam makanan adalah optimum untuk ikan ketutu berdasarkan tumbesaran, SGR, BW akhir, PER, yang lebih tinggi; serta indeks hepatosomatik, indeks viserosomatik dan lemak intraperitoneum ikan yang lebih rendah ($P<0.05$) berbanding dengan rawatan lain. Keputusan Eksperimen III menunjukkan bahawa penggantian tepung ikan dengan 20% tepung kacang soya adalah terlalu tinggi untuk ikan ketutu yang bersaiz lebih kecil pada tumbesaran, PER dan FCR ikan tersebut. Namun demikian, ikan yang diberi makanan SB20+PT mencapai tumbesaran, FCR dan PER yang lebih baik berbanding dengan ikan yang makan SB20. Kesimpulannya, 48.6% protein dan 10% lipid adalah optimum untuk juvenil ikan ketutu. Penambahan phytase dapat menambahbaikan kegunaan tepung kacang soya dalam makanan juvenil ikan ketutu.

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LIST OF ABBREVIATIONS

µm	micrometre
µmol	micromole
ADC	apparent digestibility coefficient
ANOVA	One-way Analysis of Variance
AOAC	*Association of Official Analytical Chemists*
BMRI	Borneo Marine Research Institute
BL	body length
BW	body weight
CF	condition factor
cm	centimetre
Co.	corporation
Cr_2O_3	chromium oxide (III)
Cu_2SO_4	copper sulfate
eds.	edited, edition, editor
DFI	daily feed intake
et al.	and others, and the rest
FAO	*Food and Agriculture Organisation*
FCR	feed conversion ratio
FTU	phytase activity units
g	gram
GE	gross energy
HDPE	high density polyethylene
HSI	hepatosomatic index
IPF	intraperitoneal fat
K_2SO_4	potassium sulfate
kg	kilogram
kJ	kilojoule
L	litre
ln	natural logarithm
LP	large propria
LV	large vacuole
M	molar
mg	milligram
mL	mililitre
mm	milimeter
Mol	mole
n	number
N	nitrogen
$Na_2S_2O_3$	sodium thiosulfate
NFE	nitrogen-free extract
nm	nanometre
NRC	National Research Council
NRE	nitrogen retention efficiency
PT	phytase
PER	protein efficiency ratio
pH	potantra of hydogeni (power of hydrogen)
ppm	part per million

RM	Ringgit Malaysia
SB	soybean
SD	standard deviation
SGR	specific growth rate
SNV	supranuclear vacuoles
sp.	species (singular)
Spp.	several species (plural)
SPSS	*Statistical Package for Social Science*
t	tonnes
USDA	U.S. Department of Agriculture
VSI	viserosomatic index
W	weight

LIST OF SYMBOLS

%	percentage
<	less than
>	more than
°C	degree Celsius
®	original
RM	Ringgit Malaysia
™	trademark
USD$	Dollar United State of America
III	three

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Marble Goby, *Oxyeleotris marmoratus*

Marble goby, scientifically known as *Oxyeleotris marmoratus*, is a freshwater carnivorous fish that distributed mostly in Southeast Asia countries such as Thailand, Indonesia, Malaysia, Singapore, Vietnam and other countries. It is a delicacy fish that has a lucrative market in Asian region especially in countries where most of the ethnic Chinese residing such as China, Hong Kong, Taiwan, Malaysia and Singapore (Sreevatana, 1993; Masagca and Sumantadinata, 1994; Sverdrup-Jensen, 2002).

In Malaysia, the market value of marble goby is getting higher from RM 28/kg in year 2000 to RM 53/kg in year 2011 (Department of Fisheries Malaysia, 2012). High market value and demand of marble goby had attracted a lot of aquaculturist to start culture and study on this fish since the early 1980's (Tan and Lam, 1973; Tavarutmaneegul and Lin, 1988; Cheah *et al*., 1994). However, total aquaculture production of marble goby was reported to decline in Malaysia mainly Amornsakun *et al*., 2002; Van *et al*., 2005). Study on genetic of marble goby has also been conducted to improve their growth, adaptability to farming system and disease resistant (FAO, 1995). Despite of the successful breeding and culture techniques of marble goby larvae, the seed production still remained low and the feeding is one of the constraints to expand the marble goby aquaculture (Bundit, 2007).

Using formulated feed is needed in a sustainable fish production (D'Abramo, 2002). However, the feeding practices of marble goby in most aquaculture farms still predominantly relying on the use of trash fish solely or combined with farm-made feed which made up from trash fish, rice bran and other ingredients (Edwards and Allan, 2004; Suchart *et al*., 2005). These feeds are difficult to store,

contain variable nutrient quality and easily resulted in water pollution (Zhang *et al.*, 2010). Without proper storage facilities, these trash fish or farm-made feed can easily oxidize or became rancid. Bundit (2007) reported mortalities of adult marble goby after prolong feeding of spoilt feed. Therefore, formulated feed for marble goby is very necessary to be developed as the practical feed for sustainable marble goby aquaculture production.

Formulated feed is a balanced mixture of ingredients to supply all essential nutrients and energy such as protein, lipid, carbohydrate, vitamin and mineral (Kaushik, 2000). These balanced rations of nutrients are formulated according to the animal's needs for the maintenance of vital physiological functions such as growth, reproduction and health (Kaushik, 2000). Furthermore, a practical formulated feed should be easily accepted by the cultured species which can enhance their growth and have little adverse effect on environment. In order to formulate such feed, it is important to gather information on the dietary nutrient requirement of the animal such as protein, lipid, vitamin and other. However, the fundamental nutrition study on dietary protein and lipid of marble goby still remains unknown (Cheah *et al.*, 1994; Bundit, 2007). These fundamental studies are very necessary to offer the basic knowledge for developing a nutrient balanced formulated feed for marble goby.

Protein is the major nutrient in the formulated diet (Miller *et al.*, 2005; Cho *et al.*, 2005). Studies have proven that the level of protein in diet can directly influence the growth and feed conversion ratio (FCR) of fish (Lee *et al.*, 2000; Giri *et al.*, 2011). In most formulated feed, fishmeal is used to provide the major protein source. Marble goby might be similar to other carnivorous fish, require high protein level in their diet compared to omnivorous and herbivorous fish for optimum growth performances (Schulz *et al.*, 2008; Aliyu-Paiko *et al.*, 2010). Hence, investigating the protein level that meet but not exceed the requirement of the fish is very crucial to ensure this expensive protein source is utilized for growth (Williams *et al.*, 2003; Ozorio *et al.*, 2006).

Dietary lipid is one of the main non-protein energy sources in the formulated diet and provides essential fatty acids to the culture animals. In most

formulated feed for fish and invertebrate in aquaculture, this lipid source is provided by fish oil. The lipid requirement of fish is species – specific, as the requirement changes with their feeding habit, stage of life and habitat (Biswas *et al.*, 2009). It is very important to determine a balanced ration of non-protein energy to ensure good growth, nutrient utilization and body lipid deposition of fish (Garling and Wilson, 1976). Kim and Lee (2005) had reported the importance to balance the dietary protein and energy ratio in order to increase the growth performance and protein utilization efficiency in bagrid catfish, *Pseudobagrus fulvidraco*.

Fishmeal processed from trash fish is widely used as the main protein and lipid source in the formulated diet for fish. It is one of the excellent protein sources due to its balanced amino acid profile and high nutrient digestibility (Jackson, 2009). However, unstable supply of trash fish due to overfishing had dramatically increased the price of fishmeal and fish oil recently (Chamberlain, 2011). Therefore, given the increasing global needs of fishmeal for aquaculture with its unstable supplies, there is an increasing demand searching for alternative protein sources (Kaushik *et al.*, 2004) such as soybean meal.

Soybean meal, scientifically known as *Glycine max* is one of the most promising alternative plant protein source due to its steady supplies, economical protein source, higher digestible protein and relatively well-balanced of amino acid profile comparable to fishmeal (Hardy, 1999; Swick, 2002; Biswas *et al.*, 2007; Chamberlain, 2011). Many studies showed that soybean meal can be a viable protein source for carnivorous fish such as Japanese flounder, *Paralichthys olivaceus* (Kikuchi *et al.*, 1994; Kikuchi, 1999), tiger puffer, *Takifugu rubripes* (Kikuchi and Furata, 2009; Lim *et al.*, 2011) and rainbow trout, *Oncorhynchus mykiss* (Tacon *et al.*, 1983; Kaushik *et al.*, 1995; Refstie *et al.*, 1997). Recent improved technology on soybean meal processing has increased the acceptability of soybean meal by aquaculture fish (Gabriel *et al.*, 2007). Some of the omnivorous and carnivorous fish species were able to utilize soybean meal in their diet up to 98% without compromising their growth with the supplementation of some amino acids, attractant or enzyme (Webster *et al.*, 1995; Peres *et al.*, 2003; Jahan *et al.*, 2003; Kaushik *et al.*, 2004). Therefore, study on the utilization of the soybean meal

in the diet for marble goby is very necessary to find an alternative ingredient that can replace fishmeal for future production of this fish.

The utilization of dietary protein by marble goby as a carnivorous fish (Larson and Murdy, 2001) may be similar with other carnivorous fish such as European eel, *Anguilla anguilla* and snakehead fry, *Channa striatus*. Both species required high protein at minimum of 45% in their diet for optimum growth (Degani *et al*., 1985; Aliyu-Paiko *et al*., 2010). However, marble goby is considered as a lean fish where the body fat of the wild caught fish contained approximately 0.95% of lipid (Ackman, 1980; Bundit, 2007). Marble goby juvenile may also possible to utilize certain level of soybean meal in their diet as shown in the studies of other carnivorous fish (Olli *et al*., 1995; Tantikitti *et al*., 2005; Yigit *et al*., 2010) and might yield better result with the supplementation of phytase in the diet.

1.2 Objectives

In this present study, the effect of feeding marble goby juveniles with different level of protein and lipid and the possibility of using soybean meal in their diet were evaluated. The objectives are:-

i. To investigate the effect of different level of dietary protein on growth, nutrient utilization, digestibility and whole body composition of marble goby juvenile.
ii. To investigate the effect of different level of dietary lipid on growth, nutrient utilization, digestibility and whole body composition of marble goby juvenile.
iii. To investigate the possibility of utilization of soybean meal and phytase supplementation in the diet of marble goby juvenile.

Chapter 2

LITERATURE REVIEW

2.1 Biology Characteristic of Marble Goby

Marble goby or sand goby is locally known as "*ketutu*" in Malay (Ambak *et al.*, 2010) and "*soon hock*" in Chinese. It is classified under the Phylum Chordate, Class Actinopterygii, Division Teleostei, Superorder Acanthopterygii, Order Perciformes, Suborder Gobiodei, Family Eleotridae, Genus *Oxyeleotris* and Species *marmoratus*. It is widely distributed in Mekong and Chao Phraya basins, Malay Peninsula, Indochina, Philippines and Indonesia (Kottlelat *et al.*, 1993). Marble goby can be found in freshwater or brackish water such as rivers, swamp, reservoirs and canals (Roberts, 1993; Rainboth, 1996; Randall and Lim, 2000).

Marble goby can reach up to approximately maximum length of 65 cm and body weight up to 2 kg (Kechik, 1995; Kottelat, 2001). It has an elongated body, a blunt snout and its teeth usually small, sharp and conical. Marble goby can be differentiated from true gobies with their separated pelvic fin that do not form a cup-shape sucker as true gobies. Dorsal side of its body is in dark brown colour while ventral side is in pale colour; with the long dark blotches along the body (Figure 2.1). The body colour is variable according to the environment of habitat bottom and lighting. Their scales are from small to large in either cycloid or ctenoid shape and no lateral line is available on the body of this fish (Larson and Murdy, 2001).

They have two separated dorsal fins where the first fin has 5 spines while the second fin has 1 spine and 6 to 15 soft rays. Its anal fins have 1 spine and 7 soft rays while pectoral fins are broad and have 14 to 25 rays. Whereas, its pelvic fin have 1 spines and 5 soft rays while caudal fin which is round and broad has 15 to 17 segmented rays (Larson and Murdy, 2001). Female marble goby have longer urogenital length while male marble goby have longer caudal fin length and caudal peduncle length (Idris *et al.*, 2012).

Figure 2.1: Wild marble goby, *Oxyeleotris marmoratus* obtained from local market, Sabah, Malaysia. (Photo by Ooi, 2012)

According to Tavarutmaneegul and Lin (1988); marble goby is multiple spawner and able to spawn throughout the year under suitable condition. In addition, this fish has high fecundity where the matured female is able to produce about 20000 – 30000 eggs in a natural spawning (Tavarutmaneegul and Lin, 1988; Senoo *et al.*, 1993). The hatching rate of the eggs was considered high with 80% of hatching in hatchery condition (Tavarutmaneegul and Lin, 1988).

Marble goby can tolerate to a wide range of salinity. It can be found in freshwater and brackish water habitats such as canals, rivers, reservoirs, swamps and estuaries (Rainboth, 1996; Randall and Lim, 2000). In addition, marble goby juveniles were reported having an ability to tolerate 6 days of progressive increases in salinity from freshwater to seawater and even able to survive in seawater for at least 14 days without mortality (Chew *et al.*, 2009). This enable fish farmer to culture this fish in a wide range of location.

Besides, marble goby was found to be facultative air-breather fish which capable of surviving under terrestrial condition with some extent of moisture for up to 7 days (Choo, 1974) as this fish able to activate the hepatic glutamine synthetase when exposed to air in order to detoxify internally produced ammonia (Jow *et al.*, 1999). This capability may due to the voluntary emergence during the habitat desiccation in their natural habitat (Choo, 1974). These benefits enable the intensive culture of marble goby in high stocking density and also ease the transportation of marble goby in high stocking density with very little of water.

2.2 Marble Goby in Malaysia

Over the last decade from year 2000 to 2011, total aquaculture production of marble goby in Malaysia (Figure 2.2) declined nearly 9 times from 180 tonnes (t) to 21 t (Department of Fisheries Malaysia, 2012). However, the retail price of marble goby (Figure 2.3) increased nearly double from RM 28/kilogram (kg) in year 2000 to RM 53/kg in year 2011 (Department of Fisheries Malaysia, 2012). Marble goby is currently commands a high price of RM 210 – RM 240/kg selling at seafood restaurant (Chen *et al*., 2013). This high demand fish is also reported as one of the highest market valued fish among the freshwater fish cultured in Malaysia. Hasan (2012) reported that the retail value for marble goby was higher at RM 42/kg in year 2010 compared to the other freshwater species such as *Tilapia* sp., common carp, grass carp and *Pangas* sp. catfish which are sold at lower price of RM 5 – RM 8/kg. However, the production of marble goby seed from hatchery is very low and most of the fish farmers have to obtain the seed from wild for grow out purpose (Tan and Lam, 1973; Sverdrup-Jensen, 2002; Budhiman, 2007; Chew *et al*., 2009). Looking into the high market value and demand of marble goby, and also its excellent adaptation to the aquaculture farm condition, this species can be a good aquaculture candidate.

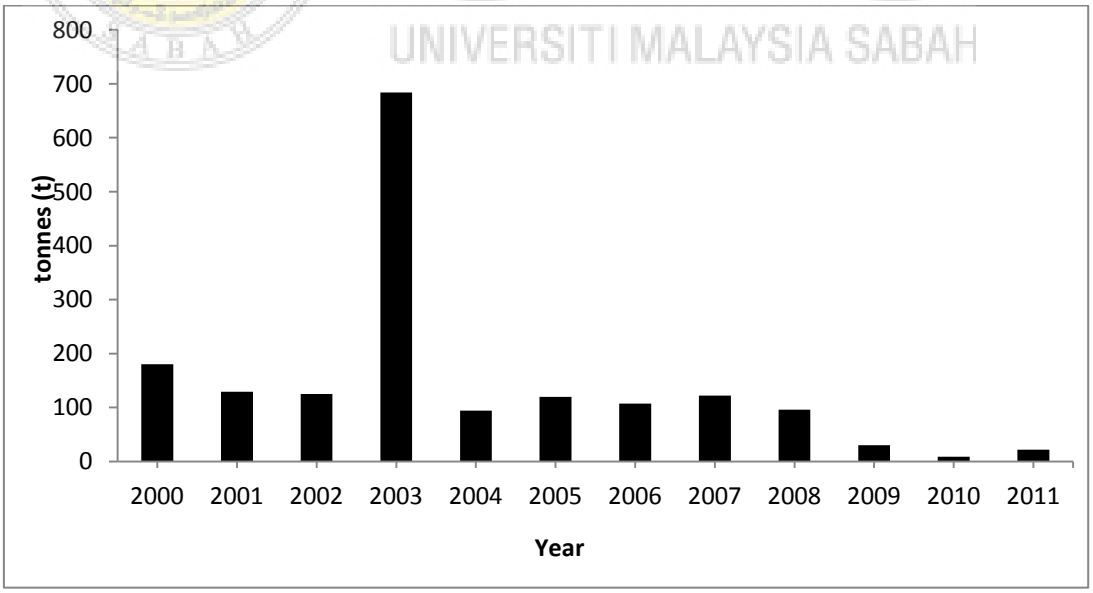

Figure 2.2: Total aquaculture productions of marble goby in Malaysia from year 2000 till 2011.
Source: Department of Fisheries Malaysia (2012), Hasan (2012)