RESPONSE OF MARBLE GOBY (*Oxyeleotris marmorata*) TO TASTE-STIMULANTS FOR THE DEVELOPMENT OF WEANING DIET

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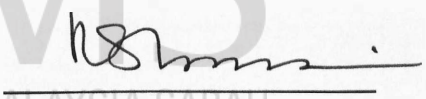

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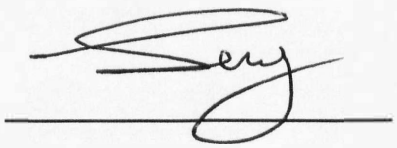

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ABSTRACT

Marble goby, *Oxyeleotris marmorata* is a high commercial value freshwater fish in Southeast Asia. In aquaculture, this fish generally rejects pelleted feed. This problem can be solved by dietary feeding stimulant (FS) or feed enhancer (FE) supplementation. However, there is still no information on the suitable FS and FE for this fish. Therefore, the present study aimed to determine the potential FS and FE, and evaluate the efficiency of selected potential FS to improve pellet-weaning process of juvenile *O. marmorata*. Prior to the behavioral assays, the fish were trained to accept agar gel pellet. Behavioral assays were conducted to determine the taste preference of *O. marmorata* for 19 amino acids, 13 organic acids, 3 nucleotides and nucleoside, 5 sugars and 2 classical taste substances at 0.1 M. In addition, the optimal value of the taste substances identified with the highest total ingestion ratio (TIR) was determined. The role of betaine as FS and FE was also investigated. All taste substances were tested once to each of the 50 fish (hatchery-reared; Total length=6.4 to 8.8 cm) using agar gel pellet. Among the amino acids tested, aspartic acid attained highest TIR (0.94) with optimal level at 0.025 M. Among the nucleoside and nucleotides, inosine was the more potential FS as it attained perfect TIR (1) with optimal value of 0.001 M. All organic acids attained very high TIR (0.96 to 1) at 0.1 M but only citric acid, formic acid, fumaric acid, tartaric acid, acetic acid, maleic acid and malic acid remained with high TIR (0.86 to 1) at the lower concentration (0.01 M). All sugars, classical taste substances were totally rejected by the fish. Betaine was also determined to function neither as a FS or FE. In the next study, nine selected taste substances (aspartic acid, inosine, citric acid, formic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid and malic acid) were individually incorporated into the fish meal-based (FM) diet at 0.1 M, and their efficiency to improve the 19 day-weaning process were evaluated. The FM diet which contained no FS was prepared as the control. Each diet was fed to 20 replicates of juvenile *O. marmorata* (hatchery-reared but naïve-to-pellet; TL=4.9±0.4 cm). Each fish was fed with 5 pieces of the pellet (size=2 mm X 2 mm X 2 mm) at 4 pm daily and the number of pellet eaten by each fish was recorded after 4 hours (8 pm). Among all treatments (were labeled using alphabets as data has been filed for trade secret), "A" diet showed the highest daily mean ingestion ratios (DMIR) from day 5 to 19, with significant higher ($P<0.05$) IR than the other treatments on both day 9 and 19, indicating that the length of weaning period reduced when the fish was weaned to "A" diet. In addition, this treatment also attained highest percentage of successfully weaned fish which was 60%, and was significant ($P<0.05$) compared to other dietary treatment except "B" diet. Another similar weaning experiment was conducted to determine the optimal supplementation level of "A" using 0.01, 0.05, 0.2 M, and the results were compared to those of 0.1 M "A' from the previous weaning experiment. However, the DMIR of all diets showed similar trends to DMIR of control diet of previous weaning experiment, suggesting that 0.1 M could be the optimal level of "A" to function best as a FS. In conclusion, "A" substance with 0.1 M was detected as the best dietary FS and it can be used to improve the weaning process of juvenile *O. marmorata*.

ABSTRAK

RESPON PEMAKANAN KETUTU OXYELEOTRIS MARMORATA TERHADAP BAHAN PERANGSANG DERIA RASA BUKAN CONVENSIONAL DAN PENAMBAH MAKANAN DALAM DIET PEMBIASAAN

Ketutu, Oxyeleotris marmorata ialah ikan air tawar yang tinggi nilai komersialnya di Asia Tenggara. Dalam pengkulturannya, ikan ini tidak menerima makanan pelet. Masalah ini boleh diatasi dengan suplemen perangsang makanan (FS). Namun, tiada maklumat mengenai FS untuk ikan ini. Justeru, kajian ini dijalankan untuk mengenalpasti FS berpotensi dan menilai keberkesanan FS terpilih untuk menambahbaik proses pembiasaan O. marmorata terhadap makanan pelet. Sebelum penilaian perlakuan dijalankan, ikan telah dilatih untuk menerima pelet gel agar-agar. Penilaian perlakuan ikan dijalankan bagi mengenalpasti rasa kegemaran O. marmorata pada 19 asid amino, 13 asid organik, 3 nukleotida dan nukleosida, 5 gula dan 2 bahan rasa klasikal pada kepekatan 0.1 M. Tambahan, semua bahan yang memperolehi jumlah nisbah pengambilan (TIR) yang tertinggi dipilih untuk pengenalpastian nilai optima. Fungsi betain sebagai FS dan FE juga diselidik. Semua bahan rasa diberi makan sekali saja kepada 50 ekor ikan (ternakan hatcheri; panjang keseluruhan (TL)=6.4-8.8 cm) menggunakan pelet gel agar-agar. Asid aspartik memperoleh TIR tertinggi (0.94) di antara asid amino dengan nilai optima 0.025 M. Antara nukleotida dan nukleosida, inosin ialah FS yang lebih berpotensi dengan TIR yang sempurna (1) dan nilai optima 0.001 M. Semua asid organik mendapat TIR yang tinggi (0.96-1) pada 0.1 M, tetapi hanya asid sitrik, asid formik, asid fumarik, asid osalik, asid tartarik, asid maleik, dan asid malik kekal dengan IR yang tinggi (0.86 hingga 1) pada 0.01 M. Semua gula dan bahan rasa klasikal ditolak sepenuhnya. Betain dikenalpasti berfungsi sebagai bukan FS dan bukan penambah perisa. Dalam kajian seterusnya, 9 bahan rasa terpilih (asid aspartik, inosin, asid sitrik, asid formik, asid fumarik, asid tartarik, asid osalik, asid maleik, dan asid malik) ditambah secara berasingan ke dalam diet yang berasaskan tepung ikan (FM) pada kepekatan 0.1 M, dan kecekapan mereka menambahbaik proses pembiasaan ikan terhadap pelet selama 19 hari telah dinilai. Diet FM tanpa bahan rasa disediakan sebagai kawalan. Setiap diet diberi makan kepada 20 ikan juvana (ternakan hatcheri tetapi tidak pernah makan pelet; TL=4.9±0.4 cm). Setiap ikan diberi 5 biji pelet (saiz=2 mm X 2 mm X 2 mm) pada 4 pm setiap hari, dan jumlah pelet yang dimakan oleh setiap ikan telah direkodkan selepas 4 jam (8 pm). Antara semua diet (dilabel dengan huruf demi rahsia perdagangan), diet A mempamerkan purata nisbah pengambilan harian (DMIR) tertinggi sepanjang proses pembiasaan, dan dengan signifikasinya lebih tinggi ($P<0.05$) berbanding diet lain pada hari 9 dan 19, bererti tempoh pembiasaan pelet disingkatkan. Tambahan, diet A juga memperolehi peratusan ikan yang berjaya dibiasakan yang tertinggi, iaitu 60%, dan bererti ($P<0.05$) berbanding dengan semua diet kecuali diet B. Satu lagi eksperimen pembiasaan yang sama dijalankan untuk mengenalpasti nilai optima suplemen A, dengan menggunakan 0.01, 0.05 dan 0.2 M, dan keputusan dibandingkan dengan keputusan 0.1 M dari eksperimen sebelum ini. Namun, semua diet menunjukkan pola DMIR yang sama dengan diet kawalan yang ada dalam ekperimen pembiasaan sebelum ini, mencadangkan 0.1 M ialah suplemen yang optima untuk berfungsi cemerlang sebagai FS. Konklusinya, bahan A dengan

kepekatan 0.1 M telah dikenalpasti sebagai FS yang terbaik dan ia berkesan untuk menambahbaik proses pembiasaan O. marmorata juvana terhadap makanan pelet.

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cm	-	centimeter
cm^2	-	square centimeter
°C	-	degree celcius
g	-	gram
kg	-	kilogram
L	-	liter
M	-	molar
m	-	milliliter
mm	-	millimeter
mg/L	-	milligram per liter
RM	-	Malaysian ringgit
%	-	percentage

LIST OF ABBREVIATIONS

AAM	-	amino acid mixture
ASP	-	aspartic acid
BET	-	betaine
BW	-	body weight
CA	-	citric acid
CI	-	confidence interval
CMFF	-	commercial marine finfish
DMIR	-	daily mean ingestion ratio
DO	-	dissolved oxygen
FUM	-	fumaric acid
FE	-	feed enhancer
FC	-	For-Conditioning
FM	-	fish meal-based
FO	-	formic acid
FS	-	feeding stimulant
GMP	-	guanosine-5′-monophosphate
IMP	-	inosine-5′-monophosphate
INO	-	inosine
IR	-	ingestion ratio
MA	-	maleic acid
MALEIC-		maleic acid
OXA	-	oxalic acid
pH	-	potential hydrogen
PSWF	-	percentage of successfully weaned fish
SE	-	standard error
TAR	-	tartaric acid
TIR	-	total ingestion ratio
TS	-	taste substance

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Marble Goby (*Oxyeleotris marmorata*) Culture in Malaysia

Marble goby (*Oxyeleotris marmorata*) is a freshwater fish that widely distributed in the Southeast Asian countries, such as Malaysia, Thailand, Vietnam, Laos, Singapore and Indonesia (Mohsin and Ambak, 1983; Inger and Chin, 2002). In Malaysia, *O. marmorata* is commonly known as *ketutu* in Malay, and *soon hock* in Mandarin. *O. marmorata* is a popular and highly preferable delicacy among the Chinese people due to its tasty, soft, lean and boneless flesh. In the local restaurants, the price of *O. marmorata* can fetch RM120 to RM150 per kg, and it has been well-known as one of the most expensive freshwater food fish in Malaysia. *O. marmorata* is also exported from Malaysia to many countries, especially those populated with majority Chinese, such as China, Hong Kong and Taiwan (Loo *et al.*, 2013).

The high demand and lucrative market price of *O. marmorata* has encouraged farmers from Malaysia as well as other Southeast Asia countries including Singapore, Thailand and Vietnam to breed and culture this fish (Tan and Lam, 1973; Tavarutmaneegul and Lin, 1988; Cheah *et al.*, 1994; Luong *et al.*, 2005; Wang *et al.*, 2011). Many studies have been conducted to improve the *O. marmorata* culture, including the seed production and larval rearing techniques (Lam *et al.*, 1990; Cheah *et al.*, 1991, 1994, Senoo *et al.*, 1994, 2008; Amornsakun *et al.*, 2002, 2003a, b; Loo *et al.*, 2012, 2013), disease control (Idris and Amba, 2011), nutritional requirement and feeding (Bundit, 2007; Yong *et al.*, 2013, 2015). However, there is still a major constraint remaining unsolved up-to-date, which is the feeding management of *O. marmorata* at grow-out stage.

1.2 Feeding Challenges in *O. marmorata* Culture

During the grow-out stage, *O. marmorata* is primarily fed with the chopped or minced low-value fish, and sometimes the farm-made feed produced using low

value fish as the main ingredient (Bundit, 2007). Although the nutritional (protein and lipid) requirement of *O. marmorata* has been established recently (Yong *et al.*, 2013, 2015), but there is still no attempt in producing formulated diet specifically for this fish (Cheah *et al.*, 1994; Lin and Kaewpaitoon, 2000; Lim *et al.*, 2015). This is critical as the juvenile *O. marmorata* generally rejected the other formulated feed during the weaning period (Darwis *et al.*, 2009; Rojtinnakorn *et al.*, 2012), which subsequently caused mass mortality due to starvation. Such behavior can be due to the poor palatability of the formulated feeds. Suitable taste-stimulant can be supplemented into the formulated diet to improve the feed palatability and feed intake. However, there is still no information available on the suitable taste-stimulant for *O. marmorata*.

1.3 Taste-Stimulant (Feeding Stimulant and Feed Enhancer) in Fish Culture

In present study, taste-stimulant is referred to the substance that can be detected by the gustatory (taste) sensory system of fish. Feeding stimulant (FS) and feed enhancer (FE) are the common types of taste-stimulant applied in formulated fish feed.

FS are the substances which can trigger high ingestion in fish (Kasumyan and Døving, 2003). FS are detected by the fish through their gustatory sense (taste sense). For decades, a lot of studies had been done to identify the suitable FS for various fish species through behavioral assays, and these studies have proven that amino acids, organic acids, nucleotides and nucleosides, sugars and classical taste substances are potential FS for fish (Kasumyan and Døving, 2003). However, the FS identified for a fish species may not work the same in other species due to the species-specificity in their taste preferences. For example, L-glutamic acid and L-lysine can function as the good FS for the guppy, (*Poecilia reticulata*) but these amino acids are totally rejected by the Navaga, (*Eleginus navaga*) (Kasumyan and Døving, 2003). Therefore, the suitable FS for the targeted fish species should be identified before it can be supplemented into the formulated feed. Nonetheless, there is still no information on the suitable FS for *O. marmorata*.

FE are the substances which are indifferent in taste for fish, but it able to enhance the palatability of other taste substances such as amino acids through synergistic reaction. Betaine is one of the most common feed enhancer, which works well in many fishes (*eg.* Carr and Chaney, 1976; Carr *et al.*, 1977; Ohsugi *et al.*, 1978; Goh and Tamura, 1980; Mackie *et al.*, 1980; Carr, 1982; Mackie, 1982; Lim *et al.*, 2016). Besides as a feed enhancer, betaine can function as FS, taste-indifferent substance or deterrent for different fish species due to the species-specificity in their taste preference. For *O. marmorata*, the effect of betaine on its feeding response has not yet been determined. In favor of searching for the suitable substance to enhance the palatability of diets for *O. marmorata*, information on the feeding response of *O. marmorata* to betaine is needed.

1.4 Improve Pellet-Weaning Process through Dietary Supplementation of FS and FE (Betaine)

Rejection of formulated diets by juvenile fish during weaning is common. Such problem can be solved through dietary supplementation of the suitable FS or FE into the weaning diet. In previous study, the dietary supplementation of FS were shown to effectively improve the number of successfully weaned fish in the pellet-weaning process of the pike perch (*Sander Lucioperca*) juveniles (Horvath *et al.*, 2013) and largemouth bass (*Mycropterus salmoides*) juveniles (Kubitza and Lovshin, 1997). The dietary supplementation of betaine alone (as a FS) or mixed together with amino acid mixture (as a FE) had been demonstrated to improve feed intake in several species, such as Chinook salmon (*Oncorhynchus tshawytscha*) (Clarke *et al.*, 1994), coho salmon (*Oncorhynchus kisutch*) juveniles (Castro *et al.*, 1998), winter flounder *(Pleuronestes americanus*) juveniles (Fredette *et al.*, 2000), gibel carp (*Carassius auratus*) juveniles (Xue and Cui, 2001), Indian major carp (*Labeo rohita*) fingerlings (Shankar *et al.*, 2008) and pike perch (*Sander lucioperca*) fingerlings (Zakipour *et al.,* 2012). Therefore, in *juvenile O. marmorata*, dietary supplementation of suitable FS or FE could also be applied to overcome the rejection of formulated diet.