



Short Communication

A preliminary study on the taste preferences of marble goby (*Oxyeleotris marmoratus*) for amino acids

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Abstract

Taste preferences of marble goby (*Oxyeleotris marmoratus*) for a mixture and several single L-isomer amino acids were determined through behavioral tests. Agar gel pellet was used as the medium to deliver the amino acids, and the fish acceptance on these pellets was evaluated by calculating the acceptance index (total pellet ingested/total pellet given; maximum and minimum values are 1 and 0, respectively). *O. marmoratus* were found to moderately accept L-alanine, L-glycine, and L-glutamine (index values 0.38, 0.32, and 0.27, respectively) but reject L-cysteine, L-glutamic acid, L-lysine, L-serine and taurine (index value 0). The amino acids mixture (AAM) showed the highest acceptance (0.91) by the fish. Such results indicated that the fish preferred the amino acids mixture over any of the single amino acids tested. Therefore, the amino acids mixture is potent to function as the feeding stimulant for the *O. marmoratus*.

Keywords: marble goby, *Oxyeleotris marmoratus*, taste preferences, amino acids, behavior

1. Introduction

Fish have different taste preferences as their taste responses and sensitivity to chemical stimulation are species-specific (Kasumyan and Døving, 2003). For aquaculture purpose, knowledge of the *fish's taste*, respectively the preference for taste-elicited chemical substances is therefore critically important for their feed development (Browman, 2005).

Marble goby, *Oxyeleotris marmoratus* is a member of the Eleotridae fish family that can grow to more than 50-60 cm in total length (TL) and 1.8-2 kg in body weight (BW) (Senoo *et al.*, 1994). It is a freshwater species but its natural habitat ranges from freshwater to brackish water environments, including canals, rivers, reservoirs, coves, swamps, and estuarine (Rainboth, 1996). This fish is an aquaculture-

targeted species in many Southeast Asian countries, including Thailand, Malaysia, Singapore, and Vietnam (Tan and Lam, 1973; Tavarutmaneeagul and Lin, 1988; Cheah *et al.*, 1994; Luong *et al.*, 2005). However, there is a major problem in the feeding of *O. marmoratus* culture at grow-out stage. There has been a lack of commercial formulated feed for this species (Cheah *et al.*, 1994; Lin and Kaewpaitoon, 2000). In addition, the fish reject self-formulated feeds (Rojtinnakorn *et al.*, 2012). Such behavior could be mainly due to the poor palatability of the feed. To solve this problem, suitable feeding stimulants can be supplemented into the feeds to promote feed intake (Kubitza *et al.*, 1997). Feeding stimulant is defined as the substance, which has high ingestion rate by the fish (Kasumyan and Døving, 2003). However, there has been no study on the taste preference or identification of feeding stimulant for this species.

Amino acids are among the well-known taste-elicited chemical substances for fish as determined through behavioral or/ and electrophysiological studies (see reviews of Marui and Carpio, 1992; Kasumyan and Døving, 2003).

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A behavioral study is convenient and straight in determining the fish preferable chemical substance through observing the fish responses towards the flavored agar gel or starch pellets (see review of Kasumyan and Døving, 2003). Therefore, the present study aimed to determine the taste preference of *O. marmoratus* for amino acids through a behavioral study, which the results can contribute to the development of feeding stimulant for this species.

2. Materials and Methods

Table 1 shows the groups and composition of agar gel pellets that were used in the present study. For the preparation, agar gel powder (2 % of the seawater volume) and red food dye (0.1 %) were firstly dissolved in filtered freshwater and heated in a microwave until boiled. Amino acids (1 %), either single or mixture, then were dissolved in the boiled mixture. The mixture was poured into a glass petri dish for hardening, cut into uniform size of pellets (approximately 1 x 1 x 0.5 cm) and stored in refrigerator (4°C) until further use. A total of 10 types of agar gel pellets were prepared (one pure agar gel pellet, eight types with single amino acid, and one type with amino acids mixture). To prepare the extract of the commercial pellet (Otohime Brand, EP type, Japan), the powder (10 g) was soaked into a beaker of filtered freshwater (100 ml) and stirred frequently for approximately 45 minutes until the water turned into dark brown, then the homogenate was filtered through a 60 microns mesh net to obtain the liquid. All extracts was freshly prepared just before the preparation of the agar gel pellets. Otohime is well-known for its fine quality which usually used in high commercial value carnivorous marine fish culture, especially groupers.

The *O. marmoratus* were trained to accept agar gel pellet before the taste preference experiment can be conducted. For training, one *O. marmoratus* was transferred into an 18 L acrylic-made aquarium with 17 L of water volume, and rigid mesh net was placed on the top of the

aquarium to prevent the fish from escaping. At first, the fish were starved for 24 hours then fed with the FT (for-training) pellets. If the fish ingested the FT pellets, they were subsequently fed with the Otohime commercial pellet *ad libitum* at the end of the daily training. If the fish rejected the FT pellets, they were starved on that day and the training continued on the next day. Only the fish that ingested the FT pellets continuously for three days (one training session per day) were selected for the taste preference experiment. In the present study, 30 individuals of *O. marmoratus* with TL 17.67±2.07 cm and BW 73.64±24.32 g (mean ± SD) fed Otohime commercial pellet were subjected to the agar gel pellet training. However, only three fish (TL 17.50-19.80 cm and BW 65.91-106.95 g) were successfully trained to ingest the FT pellet constantly and they were used for the taste preference experiment.

The method used for the taste preference experiment in the present study was modified from Kasumyan and Morsi (1996). Each fish was firstly fed with two or three FT pellets to trigger their feeding desire and then a TS (test substance) pellet was introduced. Observations were made to confirm whether the TS pellet was ingested or rejected by the fish. Subsequently, the feeding continued with 2 or 3 FT pellets again and then with another TS pellet, until the fish did not show any more interest to any agar gel pellet. Each type of TS pellet was given to each fish at least once and in random sequences. For each fish, each trial generally took about 5 to 10 minutes, with 2 to 4 types of TS pellets were tried. At the end of the experiment, all rejected agar gel pellets were siphoned out, and about 20% of water was exchanged. All the fish were then fed *ad libitum* with the Otohime commercial pellet. On the next day, all feces were siphoned out and another 20% of water was exchanged. This cleaning process was made at least 4 hours before the experiment started to avoid causing stress to the fish. The agar gel pellet training and taste preference experiment were conducted in the indoor wet laboratory. The water temperature in aquarium

Table 1. Type and composition of agar gel pellets prepared and used in the present study.

Treatments	Pure agar gel (PAG)	FT Pellet (PAG + Otohime extract)	TS pellet (PAG + Each free amino acid/ Amino acids mixture)
Ingredients			
Filtered freshwater	10ml	N/A	10ml
Agar gel powder ¹	0.2 g	0.2 g	0.2 g
Red food dye ²	0.01 g	0.01 g	0.01 g
Single amino acid ³ or Amino acids mixture ⁴	N/A	N/A	0.1 g
Extract of Otohime pellet	N/A	10ml	N/A

¹ Mermaid Brand, Thailand; ² Ponceau 4R, Meebo Brand, Malaysia; ³ Including Alanine, Cysteine, Glutamine, Glutamic acid, Glycine, Lysine, Serine (all L-isomers), and Taurine (All Sigma Brand);

⁴ Mixture of Alanine, Arginine, Glutamine, Glycine, Proline, Serine, Tyrosine and Asparagine, each 12.5% (All L-isomers, Sigma Brand).

ranged from 28 to 29°C throughout the experimental period.

To evaluate the fish taste preference, the acceptance index for each type of TS pellet by each fish was calculated using formula “A/ B”, where A is the total number of the ingested pellet, while B is the total number of the given pellet. Therefore, the maximum value of the acceptance index is 1 and minimum is 0. Subsequently, the acceptance index for each type of TS pellet from all fish was summed up to calculate the mean as the representative results.

Non-parametric Kruskal-Wallis statistical analysis was used as the data were not normally distributed. If the Kruskal-Wallis Test results showed significant difference among the treatments ($P < 0.05$), the non-parametric Conover-Inman Test was used for pairwise comparisons. Significant differences were assumed if the P -values in the Conover-Inman Test were less than 0.05. Both tests were conducted using the software StatsDirect (StatsDirect Ltd., England).

3. Results and Discussion

This is the first report on the taste preference of *O. marmoratus* for amino acids and a total number of 159 TS pellets have been tried. The P -value from the Kruskal-Wallis Test was 0.02, showing that there were significant differences among the treatments. The Conover-Inman Test hence was performed and the results are shown in Table 2.

In the present study, the pure agar gel pellet was totally rejected by the fish (acceptance index value 0). Therefore, any amino acids that made the agar gel pellet acceptable by the fish can be the potential feeding stimulant. Among all the TS pellets, L-alanine, L-glutamine, and L-glycine treatments attained the moderate acceptance index values, with 0.38 ± 0.11 , 0.32 ± 0.32 , and 0.27 ± 0.16 , respectively. The other treatments with L-cysteine, L-glutamic acid, L-lysine, L-serine, and taurine were totally rejected by the fish. According to Kasumyan and Døving (2003), L-alanine, L-cysteine and L-serine were reported to act most frequent as

the feeding stimulants for fishes. Through similar behavioral experiments, L-alanine and L-serine were found effective as feeding stimulants for 12 and 8 out of 21 species of the fishes examined, respectively. L-cysteine was identified as the active feeding stimulant for 9 out of 20 fish species. For L-glutamine, L-glycine, L-glutamic acid, and L-lysine, they were operative feeding stimulants for 7, 7, 7, and 3 out of 21 fish species examined, respectively. In the present study, L-alanine was acceptable by the *O. marmoratus*, but L-cysteine and L-serine were not. *O. marmoratus* also accepted L-glutamine and L-glycine, but not L-glutamic acid and L-lysine. Although taurine was reported to promote the feed intake of fish on different types of diet (e.g. Chatzifotis *et al.*, 2008; Qi *et al.*, 2012), it was not accepted by the *O. marmoratus* in the present study. Such results further supported the fact that fish taste preference is species-specific; common feeding stimulants can work in most but not all of the fish species.

The TS pellets with AAM attained the highest acceptance index value (0.91 ± 0.1) among all treatments, although no significant difference was found with the L-alanine, L-glycine, and L-glutamine treatments. Apparently, the *O. marmoratus* preferred the amino acids mixture over any of the single amino acids tested. This result is in agreement with Carr (1976), Carr *et al.* (1976), Carr *et al.* (1977), Oshugi *et al.* (1978) and Mackie *et al.* (1980) that the mixture of chemical substances is generally more potent than any single chemical substance to be preferred by the fish.

4. Conclusions

It can be concluded that *O. marmoratus* preferred a mixture of amino acids over any of the single amino acid tested. Therefore, the amino acids mixture is potent to function as the feeding stimulant for the *O. marmoratus*.

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Table 2. Acceptance index of *O. marmoratus* on each type of TS pellet.

Test Substances	Total Number of Trial	Acceptance Index (Mean \pm S.D.)
Amino acids mixture	24	0.91 ± 0.10^a
L-Alanine	41	0.38 ± 0.12^a
L-Glycine	33	0.27 ± 0.16^a
L-Glutamine	16	0.32 ± 0.32^{ab}
L-Glutamic acid	10	0^b
Taurine	6	0^b
L-Cysteine	5	0^b
L-Serine	4	0^b
L-Lysine	4	0^b
Control (Pure agar)	16	0^b

Different superscript indicates significant level at 0.05

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