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Short Communication

A preliminary study to determine the potential of a prototype feeding stimulant in improving the weaning of juvenile marble goby (*Oxyeleotris marmoratus*)

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Abstract

This study was conducted to determine the potential of a prototype feeding stimulant (FS) comprised of amino acids and nucleotides to improve the weaning of *Oxyeleotris marmoratus*. Two diets (control and FS) were formulated and fed to 2 groups of fish for 14 days. Six fish were in each group. Each fish was in one aquarium and each fish was a replicate. The body lengths were 7.7-9.3 cm. Each fish was given 5-pellet feedings at 17:00 daily and the ingested feedings were recorded the next day at 12:00. Fish fed the FS diet started ingestion on the1st day and attained a higher ingestion ratio of 0.5 and 50% were successfully weaned. However, on the 8th day, fish fed the control diet had an ingestion ratio of 0.17 and 16.7% were successfully weaned. In conclusion, the FS efficiently promoted feed intake and it can be used to improve the weaning of *O. marmoratus*.

Keywords: sleeper, pellet-training, diet formulation, ingestion, taste preference

1. Introduction

Marble goby *Oxyeleotris marmoratus* is a freshwater fish species found in reservoirs, rivers, swamps, canals, and flooded forests in the Southeast Asian region. It is a highly preferable food fish especially by the people in eastern Asia (Rainboth, 1996). With the increasing market demands for *O. marmoratus*, it has become the targeted species in the freshwater aquaculture industry in many Southeast Asian countries, especially Malaysia, Vietnam, and Thailand (Cheah *et al*., 1994; Luong *et al*., 2005; Hoa & Yi, 2007). However, the seed production of *O. marmoratus* in hatcheries is still inconsistent and most of the supplied seeds are caught from the wild (Amornsakun *et al*., 2003; Loo *et al*., 2013).

One of the factors contributing to the inconsistence in seed production of *O. marmoratus* is related to their feeding at the grow-out stage. This fish rejects formulated feeds (Rojtinnakorn *et al*., 2012) and mass mortality usually occurs during a long weaning period due to starvation (Merican, 2011). The problem is mainly due to the poor palatability of the feeds which can be solved by supplementing a suitable feeding stimulant (FS) into the weaning diets (Kubitza & Lovshin, 1997; Horváth *et al*., 2013; Person-Le Ruyet *et al*., 1983). FS is a taste substance that can promote a high ingestion rate in fish (Kasumyan & Døving, 2003).

In our previous studies, alanine (ALA), glycine (GLY), inosine 5'-monophosphate (IMP), and guanosine 5'-monophosphate (GMP) were identified as potential FSs for *O. marmoratus* through behavioral assays using agar gel pellets (Lim *et al*., 2015, 2016). However, the potential of utilizing these taste substances to improve the weaning of *O. marmoratus* has not yet been determined. A single taste substance is usually less efficient than a mixture of several taste substances to function as a FS for fish (Lim *et al*., 2015; Mackie *et al*., 1980; Ohsugi *et al*., 1978). Therefore, the present study aimed to evaluate the potential of a mixture of ALA, GLY, IMP, and GMP to improve the weaning of

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O. marmoratus. It was hypothesized that the fish in the treatment group fed with the prototype FS-supplemented diet would adapt to feed faster and achieve a higher feed intake than those in the control group fed with diet without the FS. In addition, the number of successfully weaned fish would be higher in the treatment group than the control group at the end of the experiment.

2. Materials and Methods

Table 1 shows the formulation and the proximate composition of the experimental diets. Two fish meal-based diets, one with the FS (treatment group) and one without the FS (control group), were formulated with crude protein and lipid levels at 47 % and 7 %, respectively (modified from Yong *et al*., 2013). The pre-mixed FS powder was dissolved in water and then added into the ingredient mixture during the mixing process. Subsequently, the dough of the mixture was passed through a 3 mm die using a meat mincer and the strands were oven-dried at 40 °C for 2 h. The strands were subsequently hand-broken into small pellets (about 3 × 3 × 3 mm) and stored in a refrigerator at 4 ° C until further use but not more than 3 days before the experiment started. During the weaning, the juvenile *O. marmoratus* may take time to consume the feeds and the feeds may shatter or dissolve in the water after a long period of time. To help the observer count the uneaten feed, the stability of the formulated feeds in water was checked prior to the weaning experiment. Several pellets of the feeds were immersed into the water in an aquarium supplied with aeration and examined every 3-4 h by the naked eye to confirm whether or not the feeds dissolved. It was then confirmed that the feeds could maintain their intact shape in water for approximately 24 h.

Twelve juvenile *O. marmoratus* with body lengths of 7.7-9.3 cm were caught from the Sibuga River in Sandakan, Sabah using a hand net and transported to the Wet Laboratory of Borneo Marine Research Institute in Universiti Malaysia Sabah. The fish were acclimatized for 1 week in a 70-liter fiberglass tank supplied with constant aeration and fed with wild-caught freshwater mysids. After the acclimatization, each fish was transferred to a 7-liter aquarium filled with 4 liters of freshwater for the weaning experiment.

A 14-day weaning experiment was conducted to determine the potential of the FS to improve the weaning of the wild-caught *O. marmoratus*. The fish were divided into 2 groups of 6 fish per group. Each fish was considered as a replicate. One group of fish was weaned with the control feed (control group) while the other group was weaned with the FS-supplemented feed (treatment group). After 24 hours of starvation, 5 pellets were given to each fish in the evening at around 17:00 daily. The uneaten pellets in each aquarium were counted the next day at 12:00 to calculate the daily ingestion ratio (IR). Subsequently, the uneaten feeds and fish feces were siphoned from the aquaria and about 20% of the water was exchanged. Along the weaning experiment, the fish that ingested all of the given pellets (n=5) were considered as successfully weaned fish. The number of successfully weaned fish was cumulatively recorded daily to calculate the percentage of successfully weaned fish (%SWF) at the end of the experiment. Along the 14-day experiment, the water temperature, pH, and dissolved oxygen levels at 17:00 were recorded as 29 °C, 7.8, and 5.0 mg/L, respectively. The IR and %SWF were calculated using these formulas.

IR = No. of ingested pellet/no. of pellets given
%SWF = No. of successfully weaned fish/total number of experimental fish × 100

The mean daily IR collected from the 6 replicates was calculated and used as the representative result. The daily IR within each group was analyzed using the non-parametric Wilcoxon rank test to determine if the IR significantly increased with time. Subsequently, the Mann-Whitney U-Test was used to determine if the IR of the FS group was higher than the control in each respective day. Both tests were conducted using the SPSS ver. 17 software. Significant difference was assumed if the P value was less than 0.05. Since the number of successfully weaned fish was recorded as binomial data (0 = not successfully weaned fish, 1 = successfully weaned fish), the binomial probability 95% confidence intervals (CIs) were calculated using the Adjusted Wald Method which is sensitive and suitable for a small sample size (minimum 5) analysis (Sauro & Lewis, 2005). A significant difference (P<0.05) was assumed when the CIs from both fish groups did not overlap.

Table 1. Ingredients and proximate composition (mean, n =3) of the experimental diets.

Ingredients	Control diet per 100 g (dry matter basis)	FS diet per 100 g (dry matter basis)
Fish meal[1]	57.6	57.6
Tapioca starch[2]	25.8	25.8
Alpha-cellulose	0.5	0
Carboxymethyl cellulose[3]	2.0	2.0
Vitamin premix[4]	3.0	3.0
Mineral premix[5]	2.0	2.0
Dicalcium phosphate	1.0	1.0
Wheat gluten	3.8	3.8
Dietary lipid source[6]	4.5	4.5
Feeding stimulant (FS)[7]	0	0.5
Total	100.0	100.0
Proximate Composition (%)		
Moisture Content	17.1±0.7	16.7±0.9
Dry Matter	83.0±0.7	83.4±0.9
Ash Content	11.7±0.1	11.8±0.1
Crude Protein	48.6±0.6	48.1±0.7
Crude Lipid	7.8±0.5	7.8±0.1

[1] Danish fish meal; [2] Tapioca AAA brand. Bake with Me Sdn. Bhd; [3] Carboxymethyl cellulose (CMC), Sigma; [4] Vitamin mixture (g/kg mixture): ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0; thiamine HCl, 0.92; d-calcium panothenate, 3.0; retinyl acetate, 0.60; vitamin D3, 0.083; Menadione, 1.67; DL alpha tocopherol acetate, 8.0; d-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135. All ingredients were diluted with alpha cellulose to 1 kg; [5] Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0; Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium salenite, 0.011; Calcium carbonate, 129.274; [6] Cod liver oil, Seven Seas Brand; [7] L-alanine, L-glycine, Inosine-5'-monophosphate, and Guanosine-5'-monophosphate (all Sigma Brand, each 0.125 g).

3. Results and Discussion

Figure 1 shows the daily IR of the experimental diets by the fish along the weaning period. In the control group, the fish started to ingest only on the 8th day and the IR was maintained at 0.17 until the end of the weaning experiment, except for a slight drop to 0.13 on the 11th day. On the other hand, the fish in the treatment group started ingestion on the first day with an IR of 0.03. The IR then increased to 0.20 on the 7th day and to 0.53 on the 9th day and then maintained at 0.50 until the end of the weaning experiment. Although the daily IR showed an increasing trend, no significant difference was found among the daily IRs within the group (Wilcoxon rank test, P>0.05). There was also no significant difference between the respective daily IR from both groups of fish (Mann-Whitney U-Test, P>0.05). Nevertheless, the fish in the treatment group adapted faster to feeding which was clearly observed at day 3 compared to day 8 in the control group. The IRs on day 1 and day 2 in the treatment group were very poor as the fish were possibly still undergoing adaptation but achieved higher IRs than the control group along the weaning period. In addition, the %SWF from the FS treatment (50%, *n*=3) was higher than the control treatment (16.7%, *n*=1), although they were not significantly different (P>0.05) (Figure 2). Apparently, the prototype FS successfully improved the palatability of the diet and made it more palatable to the fish, which contributed to the improvement of weaning (Métailler *et al*., 1983; Person Le-Ruyet *et al*., 1983). However, the underlying reason for the question on why ALA and GLY can stimulate feed intake in the *O. marmoratus* is unknown (Li *et al*., 2009). IMP and GMP are commonly known to produce the "umami" taste which is generally associated with foods that are rich in protein, especially shrimp and fish (Maga, 1983; Wifall *et al*., 2007). Since the *O. marmoratus* is a carnivorous species and well known to be a predator for shrimp and fish (Hoa & Yi, 2007; Jiwyam, 2008), this probably explains why IMP and GMP dietary supplementation can improve the intake of a formulated feed in the *O. marmoratus*.

In the study by Kubitza and Lovshin (1997), the largemouth bass *Micropterus salmoides* was successfully weaned using freeze-dried krill and krill meal as the FS. Horváth *et al*. (2013) also reported on their success in weaning in the pikeperch *Sander lucioperca* through dietary supplementation of the commercial aroma flavored substances. However, contradictory results were reported by Moura *et al*. (2000) in which the addition of cod liver oil as the feed enhancer failed to improve the weaning of peacock bass (*Cichla* spp.). Cyrino and Kubitza (2003) also reported failure in weaning *Cichla* spp. with meat- and fish-flavored dry pellets as the weaning diets. Apparently, identification of a suitable FS before dietary supplementation plays an important role in successful weaning. The present study confirmed that the prototype FS dietary supplementation of amino acids with nucleotides can accelerate the *O. marmoratus* adaptation to the pelleted feed, enhance their feed intake, and eventually increase the number of successfully-weaned fish. Nevertheless, the number of specimens used in the present study was low. Three fish were successfully weaned and poor ingestion was observed in the other three specimens which resulted in a large standard error in the data (Figure1). A larger number of specimens should be used in a future study to further confirm the efficiency of the FS to improve the weaning of *O. marmoratus*. Determination for the optimum dietary supplementation level and the retention period of the FS in diets are also highly recommended for development of weaning diets of *O. marmoratus*.

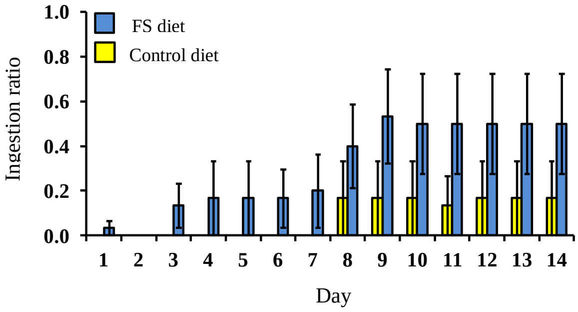

Figure 1. Daily ingestion ratio of the *Oxyeleotris marmoratus* fed with the control and FS diets along the experimental weaning period. Vertical bars indicate the standard errors.

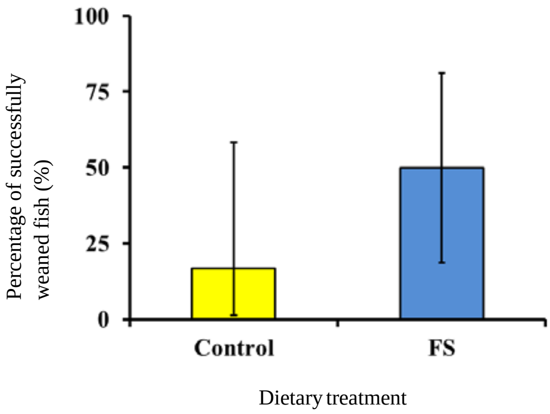

Figure 2. Percentage of successfully weaned *Oxyeleotris marmoratus* fed with the control and FS diets. Vertical bars indicate the 95% confidence interval.

4. Conclusion

In conclusion, the prototype FS formulated with ALA, GLY, IMP, and GMP can be used to improve the weaning of *O. marmoratus* by accelerating the fish adaptation to the formulated feed, promoting feed intake, and increasing the percentage of successfully weaned fish.

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