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RESEARCH ARTICLE

Feeding performance of juvenile marble goby (*Oxyeleotris marmorata* Bleeker, 1852) fed acidified diets

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Abstract. The present study aimed to evaluate the feeding performance of juvenile marble goby (*Oxyeleotris marmorata*) on acidified diets. Five fish meal-based diets at various pH levels (2.5, 3.2, 4.3, 5.3, and 6.0 (control)) were prepared, and each of them was fed to 10 wild-caught *O. marmorata* juveniles (body length 7.7–9.3 cm; each fish was placed in an aquarium, hence each dietary treatment was in 10 replicates) for 20 days. Throughout the feeding trial, *O. marmorata* juveniles showed clear increments in the daily ingestion ratio (IR) values of AD 2.5, AD 3.2, and AD 4.3. The control diet and AD 5.3 were almost totally rejected by the fish. On day 20, the IR of AD 2.5 (0.46) and AD 3.2 (0.36) were significantly higher ($P < 0.05$) than those of AD 5.3 (0.1) and the control diet (0.02). In addition, 70% of fish fed AD 2.5 achieved a high IR value (0.6 – 1.0), and this was the best result among all the treatments (AD 3.2, 60%; AD 4.3 50%; AD 5.3 and control, 10%). These results suggested that acidified diets can be used to wean *O. marmorata* juveniles, and the recommended pH level was 2.5.

Keywords: Weaning, *Oxyeleotris marmorata*, dietary pH preference, taste, hydrochloric acid

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Introduction

Marble goby, *Oxyeleotris marmorata*, is a highly commercially valuable freshwater fish in Southeast Asia (Asia-Pacific Fishery Commission 2014). It is a popular candidate for aquaculture; however, this fish generally rejects formulated feeds and mass mortality usually occurs during the weaning period (Merican 2011, Rojtinnakorn et al. 2012, Lai et al. 2018). To solve this problem, various types of taste substances including nucleotides and nucleosides, amino acids, and organic acids have been tested on the fish through behavioral assays to identify functional feeding stimulants (Lim et al. 2015, 2016, 2017). Indeed, dietary supplementation with selected amino acids and nucleotides can be practiced to improve *O. marmorata* weaning (Lai et al. 2018), but these taste substances are expensive. On the other hand, Lim et al. (2017) reported that dietary pH may be a key factor in the food selection of the *O. marmorata* as it showed very high preference for the acidic agar gel pellets during behavioral assays. The high preference of *O. marmorata* for acidic food was further confirmed when the fish exhibited high ingestion ratios for strongly acidic agar gel pellets (pH 2.4) with merely hydrochloric acid (HCl), which is a non-taste-elicited substance (Teoh et al. 2018).

These outcomes suggest the potential of weaning *O. marmorata* by using acidified diets, although, to date, no similar study has reported on other fish species. Therefore, the present study was conducted to examine the feeding performance of *O. marmorata* juveniles with acidified diets.

Materials and methods

Five dietary treatments were prepared for this study. Table 1 shows the formulation and proximate composition of the experimental diets. All diets were based on fish meal. Four of them were included HCl, and their pH levels were adjusted to 5.3, 4.3, 3.2, and 2.5 (namely Acidified Diets: AD 5.3, AD 4.3, AD 3.2, and AD 2.5); the control diet (without adding HCl) pH level was 6. Fish meal is a strong pH buffer. Therefore, the volume of HCl needed for the preparation of each dietary treatment was determined preliminarily and was slowly added into the ingredient mixtures during mixing. The mixture dough was passed through a 3 mm die using a meat mincer, and the strands were oven-dried at 40°C for 2 hours. The strands were then broken into small pellet sizes and stored in a refrigerator at 4°C until further use. The proximate composition of the diets was determined following the protocols by AOAC (1997). Prior to the

Table 1
Ingredients and proximate compositions of the experimental diets used in the present study

Ingredients per 100 g diet (dry matter basis)	Control diet (pH6.0)	Acidified diets (AD) at different pH levels			
		AD 5.3	AD 4.3	AD 3.2	AD 2.5
Fish meal[1]	62.95	62.95	62.95	62.95	62.95
Tapioca starch[2]	20.48	20.48	20.48	20.48	20.48
Alpha-cellulose	2.14	2.14	2.14	2.14	2.14
Carboxymethyl cellulose[3]	2.00	2.00	2.00	2.00	2.00
Vitamin premix[4]	3.00	3.00	3.00	3.00	3.00
Mineral premix[5]	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Wheat gluten	3.00	3.00	3.00	3.00	3.00
Dietary lipid source[6]	3.43	3.43	3.43	3.43	3.43
Total	100.00	100.00	100.00	100.00	100.00
HCl (ml)	N/A	2.2	6.8	14.3	25.1
Proximate Composition (%, mean ± SD)					
Moisture	16.4 ± 0.3	20.1 ± 0.3	20.2 ± 0.4	17.6 ± 0.4	19.3 ± 0.1
Ash	14.9 ± 0.3	14.7 ± 0.3	14.8 ± 0.3	14.7 ± 0.2	14.8 ± 0.1
Crude protein	48.2 ± 0.3	47.4 ± 0.2	47.1 ± 0.2	46.0 ± 0.3	45.2 ± 0.4
Crude lipid	9.9 ± 0.1	10.1 ± 0.1	10.1 ± 0.5	8.7 ± 0.0	6.1 ± 0.5

[1]Danish fish meal, [2]Tapioca AAA brand. Bake with Me Sdn. Bhd, [3]Carboxymethyl cellulose (CMC), Sigma Brand, [4]Vitamin mixture (g × kg^{-1} 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^{-1} 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

feeding experiment, the pellets were further broken down into a granular size of 1 mm to suit the fish mouth size. The stability of the experimental diets in water was also tested since *O. marmorata* may take time to approach and ingest the feeds during the experiment. Simple water stability tests were conducted by soaking several pellets from each dietary treatment in separate beakers of water with aeration, and visual observations were recorded at 3-4 hour intervals. The pellets of all the diets maintained their intact shape in water for approximately 24 hours. In order to confirm that the dietary pH level did not change along the experimental period, all dietary treatments were checked for pH level weekly following the method by Sathe (1999). In brief, a few pellets were immersed into a 50 ml beaker of distilled water, crushed using a spatula, stirred, and then the pH level was measured using a pH meter (Oakton EcoTestr® pH 2, Eutech Instruments, Singapore).

Fifty wild-caught *O. marmorata* (body length 7.7-9.3 cm) were purchased from a fisher in the local wet market, and transported to the Wet Laboratory of Borneo Marine Research Institute at the Universiti Malaysia Sabah. The fish were acclimatized for one week in a rectangular plastic tank (about 100 L) supplied with constant aeration and fed with minced chicken flesh. Following acclimatization, each fish was transferred to a 7 L aquarium (filled with 4 L of fresh water) for the feeding experiment. The fish were then divided into five groups (each group consisted 10 fish or replicates), and each group was fed with one of the dietary treatments continuously for 20 days. The feeding protocol in this study was adopted from Lai et al. (2018) with slight modifications. In the daily feeding trial, five pellets were given to each fish in the evening around 16:00 h. Subsequently, uneaten pellets were counted at 20:00 h, siphoned out from the aquaria, and about 20% of the water was exchanged. Throughout the 20-day experiment, the water temperature and dissolved oxygen levels at 16:00 h were 29°C and 5.0 mg L^{-1}, respectively. The water pH level did not change after feeding, and it was maintained at pH 7.8, which was the same as the level before feeding.

For data collection, the daily ingestion ratio (IR) for each fish was calculated by dividing the number of the ingested pellets by the total number of pellets (5) given, and the mean was expressed as the result. The daily IR on days 9 and 20 were compared among treatments and analyzed statistically using the non-parametric Kruskal-Wallis test. If significant differences ($P < 0.05$) were found among treatments, the Conover-Inman post-hoc comparison test was conducted. Both of these tests were conducted using Systat 13 computer software. At the end of the experiment, the fish in each dietary treatment were categorized into two groups of feeding performance based on the highest IR (0.0, 0.2, 0.4, 0.6, 0.8, or 1.0) that they achieved throughout the feeding trial. The feeding performance groups were the "poor" group with IR of 0.0, 0.2, and 0.4, and the "good" group with IR of 0.6, 0.8 and 1.0. The number of fish in these feeding performance groups in each dietary treatment were then analyzed statistically using the Chi-square test in SPSS v.17 computer software. Differences were significant at $P < 0.05$.

Results

Figure 1 shows the daily IR of the experimental diets achieved by *O. marmorata* juveniles throughout the 20-day feeding trial. In general, fish fed AD 2.5, AD 3.2, or AD 4.3 showed an increasing trend in their daily IR. Fish fed AD 2.5 started to accept pellets on day 1 (IR = 0.08); the IR increased to 0.3 on day 9, and the highest IR (0.46, highest IR in the treatment and among all treatments) was achieved on day 20. Fish fed AD 3.2 only started to accept pellets on day 6 (IR = 0.1), and the highest IR in this treatment (0.36) was achieved on day 20. For those fed AD 4.3, they started to accept pellets on day 2 (IR = 0.1), while the highest IR during the treatment (0.28) was achieved on day 15. On the other hand, fish fed AD 5.3 or control (pH 6.0) diet attained very low IR throughout the feeding trial (IR ranged from 0.0-0.06), and showed only a slight increase in IR from days 15 and 19, respectively. The IR for AD 5.3

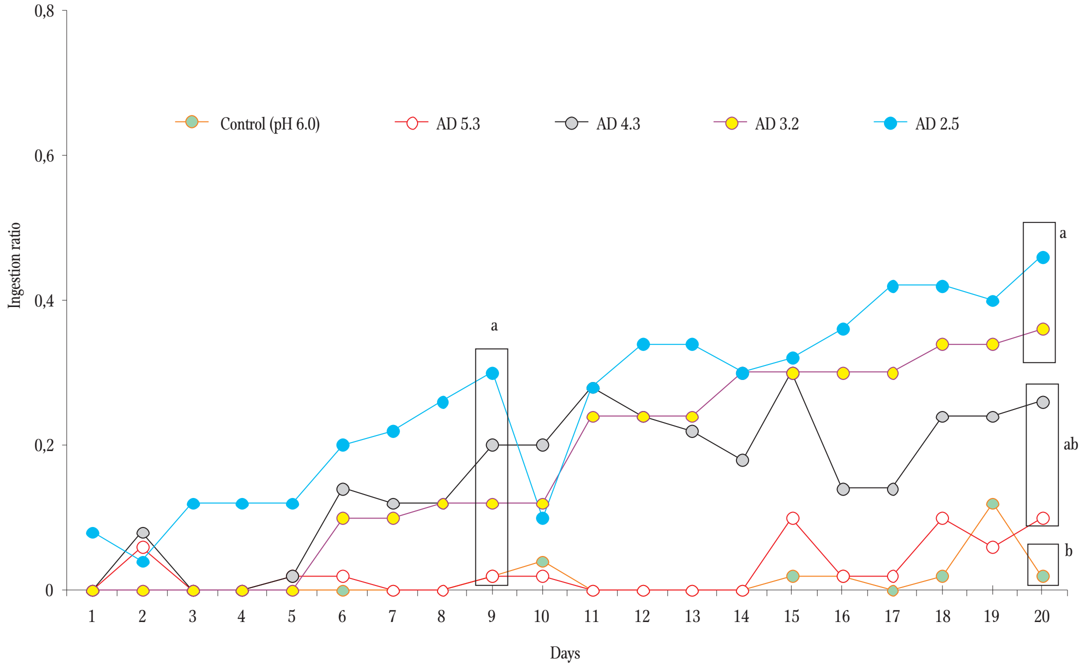

Figure 1. Daily ingestion ratio of the experimental diets by the juvenile *O. marmorata*. Each point represents the mean ingestion ratio of 10 replicates. The frame marks groups of means that are not significantly different from each other. Different letter indexes indicate significant differences ($P < 0.05$) among the data.

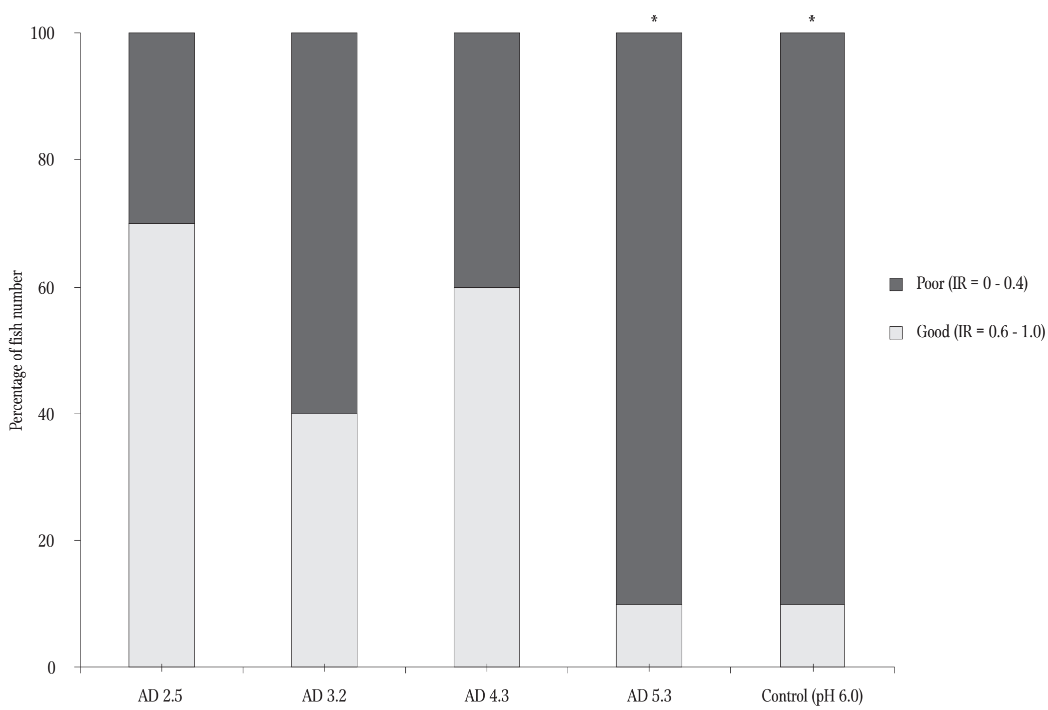

Figure 2. Percentage of fish with the “good” and “poor” feeding performance in each dietary treatment. Asterisks (*) indicate significant differences ($P < 0.05$) within treatments.

and the control diet from day 15 was in the range of 0.0-0.12. On day 9, no significant differences ($P > 0.05$) were detected among the IR of any of the dietary treatments. However on day 20, the IR of AD 2.5 and AD 3.2 was significantly higher ($P < 0.05$) than that of the control diet.

The percentage of fish with "good" and "poor" feeding performances in each dietary treatment is shown in Figure 2. The highest percentage of fish with "Good" feeding performance (70%) was in the AD 2.5 treatment, followed by the AD 4.3 (60%) and AD 3.2 (40%) treatments. Nevertheless, no significant differences ($P > 0.05$) were found between the percentages of fish exhibiting "good" and "poor" feeding performances in these dietary treatments. On the other hand, only 10% of "good" feeding performance fish were in the AD 5.3 and control (pH 6.0) treatments; these percentages were significantly lower ($P < 0.05$) than those of the "poor" feeding performance fish in these treatments.

Discussion

This is the first report on the feeding response of *O. marmorata* to acidified diets. In this study, *O. marmorata* juveniles clearly showed their preference for the AD 2.5, AD 3.2, and AD 4.3 (strongly acidified) over the the AD 5.3 and control diets. The daily IR (mean) in the former dietary group (0.3-0.42) was at least five times higher than that in the latter group (0.06-0.08) on the final day of the feeding trial (day 23). These results were in agreement with our previous findings on the strong preference of *O. marmorata* for acidic food (Lim et al. 2017, Teoh et al. 2018). These results also were similar to those reported for several species of tilapia (e.g., Adam et al. 1988, Xie et al. 2003, Sherif and Doaa 2013) fed diets supplemented with different types of organic acid. However, the opposite results were reported for the African catfish, *Clarias gariepinus* (Burchell), in which feed intake decreased significantly when the dietary supplementation level of fumaric acid increased (Omosowone et al. 2015). Erteken and Nezaki (2002) reported that the feed intake by Black Sea turbot increased when the dietary pH level increased. Goldfish, *Carassius auratus* (L.), was also reported to reduce feed intake when it was fed a diet supplemented with 5%, but not 2%, citric acid (Sugiuraa et al. 1998). As suggested by Kasumyan and Døving (2003), apparently fish taste preferences for dietary pH is species specific.

In this study, the daily IR of the fish for AD 2.5, AD 3.2, and AD 4.3 generally increased, and similarly high IR (0.3-0.42) was achieved at the end of the feeding trial. On the other hand, the daily IR of fish fed AD 5.3 and the control (pH 6.0) diet were mostly zero (IR = 0.0-0.06 almost rejected by the fish) from days 1 to 14 with only a slight increase from days 15 to 23 (highest IR 0.12). These results clearly indicate that *O. marmorata* indeed can be weaned to acidified diets, and that this is similar to the Lai et al. (2018) report on the feeding response of *O. marmorata* fed a diet supplemented with a feeding stimulant. Nevertheless, with *O. marmorata* AD 2.5 appeared to be more favorable than either AD 3.2 or AD 4.3. The fish accepted AD 2.5 from day one. Subsequently, IR generally increased (except for the sharp drop on day 10), and it was usually the highest IR recorded daily among all dietary treatments until day 20 (see Fig. 1). On the other hand, the fish fed AD 3.2 or AD 4.3 only started ingestion on days 5 and 2, respectively. Although the IR increased daily, the values were generally lower than in the fish fed AD 2.5. These results indicated that the fish fed AD 2.5 adapted faster to the feed than those fed AD 3.2 or AD 4.3. Additionally, the percentage of fish with a "Good" feeding performance in the AD 2.5 treatment was the highest among all dietary treatments (70%), although no significant difference ($P > 0.05$) was detected in this result. Based on these outcomes, AD 2.5 is recommended as the weaning diet for *O. marmorata* rather than AD 3.2 or AD 4.3. Further studies should be conducted to evaluate the growth performance and feed utilization of *O. marmorata* fed with AD 2.5. In this study, the color and texture of AD 2.5 were slightly different from the other diets; it was darker, softer, and stickier than the others. Since the experimental fish were observed to capture and manipulate all dietary treatments in their mouths, it was confirmed that the darker coloration did not contribute to the higher preference of the fish for the AD 2.5 diet. However, it was unclear if the fish preferred the soft, sticky texture of the AD 2.5 diet. Further studies on the dietary texture preferences of *O. marmorata* juveniles is recommended.

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