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Growth performance and feed utilization of juvenile marble goby (*Oxyeleotris marmorata*) fed acidified diets

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Abstract

The present study was aimed at evaluating the growth performance and feed utilization of marble goby (*Oxyeleotris marmorata*) juveniles fed with the acidified diets (AD). In feeding trial I, five fish meal-based diets were prepared [control (pH 6.0), AD 5.3, 4.3, 3.2 and 2.5]. Each diet was fed to triplicate batches of wild-caught *O. marmorata* (19 fish/ tank; total length, TL = 4.72±0.46 cm) for 8 weeks. The control, AD 5.3, and AD 4.3 treatments were terminated at week 4, due to drastic decline in total feed intake (TFI=0–0.05 g) and weight loss (weight gain, WG = -15.3 to -16.9%) in the fish. The AD 3.2 and AD 2.5 treatments were continued until week 8. Fish fed with the AD 3.2 showed significantly higher ($P < 0.05$) TFI (0.98 g) compared to those fed with the AD 2.5 (0.73 g) at the end of the trial. Feeding trial II was done to assess the long-term effects of AD for another 7 weeks. Thirty fish specimens were randomly selected from each of AD 3.2 and AD 2.5 treatments and stocked individually in 7 L aquaria to eliminate the territorial behaviour that was observed in the first trial. At the end of the experiment, fish fed AD 3.2 attained significantly higher ($P < 0.05$) WG (34%) than those fed AD 2.5 (13%). However, the growth performance and feed utilization results of the present study were very poor compared to those fed the normal fishmeal-based diet as is evident from synthesis of data from the literature review. Feeding of acidified diets was, therefore, not recommended for an extended period (> 3 weeks). Nevertheless, based on the strong preference of the fish for AD, it is worth trying to develop a weaning protocol using these diets as the starter feed and then slowly replacing them with the normal diet.

Keywords: *Oxyeleotris marmoratus*, acidified diets, weaning, feeding behaviour, growth

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Introduction

Marble goby, *Oxyeleotris marmorata*, is a high-value commercial freshwater fish that is being cultured in many Southeast Asian countries, including Malaysia (Cheah et al., 1994; Lin and Kaewpaitoon, 2000). Aquaculture production of this fish is declining. In Malaysia, *O. marmorata* production decreased 90.6% from 2006 (104.24 tonnes) to 2016 (9.8 tonnes) (Fisheries Department of Malaysia, 2006, 2016). Although many studies have been conducted to improve the farming techniques (Senoo et al., 2008; Darwis et al., 2009; Lam et al., 2014; Loo et al., 2015; Yong et al., 2013, 2015), mass production of *O. marmorata* still remains difficult as heavy mortality usually occurs during their weaning to compounded feeds (Merican, 2011). Indeed, *O. marmorata* has been well known for its peculiar feeding behaviour to reject most of the compounded feeds (Cheah et al., 1994; Lin and Kaewpaitoon, 2000, Rojtinnakorn et al., 2012). Recently, Lim et al. (2015, 2016, 2017) have reported that amino acids, nucleotides and nucleosides are functional feeding stimulants, and these substances can be used to improve the weaning of *O. marmorata* juveniles (Lai et al., 2018). However, these taste substances are expensive and supplementing them to the practical diets may not be cost-effective for the marble goby farmers. Cost-effective alternatives are required.

According to Lim et al. (2017), *O. marmorata* juveniles showed high ingestion ratio of the organic acids-acidified agar gel pellets, and hence the preference for acidic foods was suggested. Teoh et al. (2018) further confirmed the preference of *O. marmorata* for diet of pH = 2.4 – 3.0 through behavioural assays by replacing the organic acids with solely hydrochloric acid. Indeed, the length of weaning period can be shortened and the number of successfully weaned fish increased when the *O. marmorata* juveniles were weaned using the pH 2.5 and pH 3.2 acidified diets, rather than the control diet (pH 6.0) (Lim et al., 2018). It is known that feeding the acidified diets to fish can enhance their growth performance and feed utilization (Lückstädt, 2008; Phromkunthong et al., 2010; Tabrizi et al., 2012; Sherif and Doaa, 2013; Zhu et al., 2014; Shah et al., 2015). However, the growth performance and feed utilization of marble goby offered the acidified diets are still unknown. If the fish fed with the acidified diets can grow and utilize the nutrients efficiently as the control diet, the acidified diets can be proposed to the farmers as the species-specific weaning diet of *O. marmorata*. The present study, therefore, was aimed to evaluate the growth performance and feed utilization of the *O. marmorata* juveniles fed acidified diets.

Materials and Methods

Experimental fish

Three hundred *O. marmorata* juveniles (total length, TL = 3.0 to 4.0 cm) caught from the Kimanis River in Papar, Sabah were used for the experiment. The fish were transported to a wet laboratory of Borneo Marine Research Institute (BMRI) in Universiti Malaysia Sabah, and treated with 30 ppt salinity seawater for 10 minutes to eliminate the monogenean parasites on the fish body surface (Reed et al., 1996). The fish were separately acclimatized in 12 rectangular fiberglass tanks (150 L) with continuous aeration for 6 weeks before the experimental trials. During this acclimatization period, the fish were fed with minced chicken flesh twice daily at 09:00 am and 04:00 pm until satiation. Tank cleaning by syphoning out of the solid wastes and about 50% of water volume exchange were done daily in order to maintain the water quality.

Table 1. Formulation and proximate analysis of experimental diets (g/100g dry weight basis).

Ingredients (g/100 g)	Dietary Treatments (% Dry Matter Basis)				
	Control	AD 5.3	AD 4.3	AD 3.2	AD 2.5
Fishmeal[1]	62.95	62.95	62.95	62.95	62.95
Tapioca starch[2]	20.48	20.48	20.48	20.48	20.48
Wheat gluten	3.00	3.00	3.00	3.00	3.00
Alpha-cellulose	2.14	2.14	2.14	2.14	2.14
CMC[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
Fish oil	3.43	3.43	3.43	3.43	3.43
HCl[6] (ml)	0.00	2.20	6.80	14.30	25.10
Proximate analysis (%)					
Crude Protein	48.2±0.3	47.4±0.2	47.1±0.1	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
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

[1] Danish fish meal
[2] Tapioca Golden Fish Brand
[3] Carboxymethyl cellulose (CMC), Sigma Brand
[4] Vitamin premix (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 1kg
[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 selenite, 0.011; Calcium carbonate, 129.274
[6] 37% Hydrochloric acid, 16 M, Merck Brand, United States

Preparation of the experimental diets

Table 1 shows the ingredients and composition of the formulated diets prepared in this study. Five fishmeal-based diets with 48% crude protein and 10% crude lipid were prepared (Yong et al., 2013). Their formulation was similar but 4 of them were then added with various amounts of hydrochloric acid (HCl) to adjust the pH levels. The pH level of the control diet was 6.0 (standard pH for most of the commercial diets), while values for the acidified diets (AD) were 5.3, 4.3, 3.2, and 2.4. To prepare the acidified diets, the dry ingredients were mixed thoroughly then the HCl and water were slowly added into the mixture during the mixing process. Subsequently, the dough was screw-pressed through a 3-mm die with a meat mincer. The strands so formed were fan-dried and crushed into smaller pellet size (approximately 3mm x 2mm x 1mm), and stored in a refrigerator at 4 °C until further use. The moisture, crude protein, crude lipid, and ash contents of these diets were determined following the AOAC methods (AOAC International, 2002), and their composition is shown in Table 2. Throughout the experimental period, the pH levels of all the experimental diets were checked to confirm that they remained unchanged, following the method by Sathe (1999). In brief, several pellets of each dietary treatment were immersed in a 50 ml beaker with distilled water. The pellets were then crushed in the smaller pieces using a spatula, and the mixture was stirred for about 3 minutes. Subsequently, the stirred mixture was measured for its pH using a pH meter (Oakton EcoTestr® pH 2, Eutech Instruments, Singapore).

Table 2. Growth parameters of *O. marmorata* juveniles fed with the experimental diets for 8 weeks in the Feeding Trial I. Different superscripted alphabets indicate significant difference at $P < 0.05$ (compared in row).

Parameters	4 weeks			8 weeks	
	Control*	AD 5.3*	AD 4.3*	AD 3.2	AD 2.5
TFI [1]	0.02±0.0	0.00±0.0	0.05±0.0	0.98±0.0[a]	0.73±0.0[b]
WG [2]	-16.9±3.1	-16.7±1.9	-15.3±0.8	23.4±4.7[a]	3.58±5.2[b]
SGR [3]	-0.71±0.1	-0.7±0.1	-0.64±0.0	0.36±0.1[a]	0.18±0.1[a]
SR [4]	96.5±6.1	96.5±3.0	96.5±6.1	98.3±3.0[a]	96.5±6.1[a]
FCR [5]	-0.11±0.2	-0.02±0.0	-0.27±0.2	3.55±0.6[a]	82.7±87.9[b]
CF [6]	0.94±0.0	0.90±0.0	0.91±0.0	0.97±0.1[a]	0.97±0.0[a]

* Terminated after 4-week.
All values are mean ± S.D. (n = 3).
[1] Total Feed Intake (g); [2] Weight Gain (%); [3] Specific Growth Rate (%/d); [4] Survival Rate (%); [5] Feed Conversion Ratio; [6] Condition Factor

Experimental protocols

1. Feeding trial I

A total of 285 specimens of *O. marmorata* juveniles (TL 4.72 ± 0.46 cm) were randomly selected from the fish stock for the 8-week feeding trial. The fish were equally distributed into 15 fiberglass tanks (150 L each) with the stocking density at 19 fish/ tank. Each tank contained approximately 38 L of de-chlorinated tap water and was continually aerated. Each dietary treatment involved hand-feeding to triplicate groups of fish until apparent satiation twice daily at 0900 and 1600 hours. The uneaten feed remaining at the tank bottom was measured and siphoned out from the tanks at 2 hours after feeding. Approximately 50% of water was exchanged daily during the tank cleaning. The water temperature, dissolved oxygen and pH levels were recorded at 29 °C, 5.0 mg/l, and

7.8, respectively. At week-4, feeding trials for the fish fed control, AD 5.3, or AD 4.3 diet were terminated as the fish rejected these diets and showed significant decline in their body weight. Nevertheless, feeding trials for the fish fed AD 3.2 and AD 2.5 were continued until week-8. At the end of the feeding trial, the TL and body weight were measured individually. In this feeding trial, the authors noticed the territorial behaviour of *O. marmorata* juveniles. Another feeding trial was designed to avoid the behavioural influence, and this was conducted to assess the effect of long-term feeding with acidified diets on the growth and feed utilization of *O. marmorata* juveniles.

2. Feeding trial II

In this experiment, the fish fed AD 3.2 or AD 2.5 (feeding trial I) were used. From each of these dietary treatments, the triplicate groups of fish were pooled and thirty of them (TL = 5.35 to 5.64 cm) were randomly selected for a 7-week feeding trial. Each fish was maintained in a 7-L acrylic aquaria with 4-L of dechlorinated tap water, and supplied with aeration. Each fish represented as a replicate and each dietary treatment has 30 replicates. After 24 hours of acclimatization, the fish were hand-fed until apparent satiation twice daily at 0900 and 1600 hours. The uneaten feed was counted and syphoned out from the aquaria 2 hours after feeding. About 50% of water volume was exchanged during the tank cleaning. On the next day, the fish faeces were syphoned out and another 50% of water volume was exchanged before the feeding commenced. The water quality was recorded in the same way as in the previous trial (feeding trial I). All the fish specimens were individually measured for their total length and body weight at the end of the trial. The fish from each dietary treatment then were pooled and deprived of food for 24 hours for emptying their digestive tract. Subsequently, twenty specimens were randomly sampled from each group and kept in -20 °C for the whole body proximate composition analysis.

The fish whole body proximate composition analysis was conducted following the standard methods by Association of Analytic Chemists (AOAC, 2002). Carcass of the sampled fish was dried in oven and powdered prior to analyses. To determine the ash content, the samples was incinerated in a muffle furnace at 550 ˚C for six hours, and weight of the residue was recorded. Due to small sample weight, wet samples were used to determine the fish whole body crude protein and lipid levels. Crude protein level was determined using the Kjeldahl automatic system (Kjeltec™ 2300 Analyzer Unit, FOSS Analytical, Denmark), while the crude lipid level was determined using a soxhlet extraction unit (Soxtec™ 2043 Fat Extraction System, FOSS Analytical, Denmark).

Data collection and statistical analysis

1. Feeding trial I

In this trial, the weekly and total feed intake, fish weight gain (WG), specific growth rate (SGR), survival (SR), feed conversion ratio (FCR) and condition factor (CF) were estimated using the following formulas:

Weekly feed intake (WFI, g/fish) = Total feed intake in a week/ total number of fish

Total feed intake (TFI, g/fish) = Total feed intake in 7 weeks/ total number of fish

WG (%) = (Final fish weight, g – Initial fish weight, g)/ Initial fish weight, g × 100%

SGR (%/d) = [ln (final weight, g) – ln (initial weight, g)] × 100% / 49 days

SR (%) = (Final fish number/ Initial fish number) × 100%

FCR = Total feed consumed (in dry weight, g)/ fish wet weight gain, g

CF = (Fish weight, g/ Fish total length, cm)

Data collected from the control, AD 5.3 and AD 4.3 treatments were not analysed statistically as the trials were terminated during the course of the experiment. The WFI at week-3 was analysed by the One-way ANOVA with Tukey's Test while the TFI, WG, SGR, SR, FCR and CF of fish fed AD 3.2 or AD 2.5 diet were subjected to Independence-Samples Kruskal-Wallis Test. All the statistical tests were conducted using the SPSS ver. 20 computer software. Significant different was assumed when $P < 0.05$.

2. Feeding trial II

WFI, TFI, WG, SGR, FCR, and CF of the fish were estimated using the formula as mentioned earlier for the feeding trial I. In addition, the net protein utilization (NPU), protein efficiency ratio (PER), hepato-somatic index (HSI), and viscero-somatic index (VSI) of the fish were also determined by help of the formula as described below:

NPU = (Final fish body protein – Initial fish body protein)/ Total protein intake

PER = Wet weight gain, g/ Total protein intake

HSI = Liver weight, g/ Fish weight, g

VSI = Viscera weight, g/ Fish weight, g

All parameters (for WFI, only data at week-3) were analysed by the Independence-Samples Kruskal-Wallis Test using the SPSS ver. 20 computer software, and significant difference was assumed when $P < 0.05$.

For analysing the data for the fish whole body proximate composition, the One-Way ANOVA and Tukey's tests in the SPSS ver. 20 computer software were used, and the level of significance was set at 0.05.

Results

Feeding trial I

1. Growth performance and feed utilization

Table 2 shows the growth performance and feed utilization of *O. marmorata* fed with the experimental diets. The control, AD 5.3, and AD 4.3 treatments were terminated after week-4 due to poor feed intake and weight loss of the fish. The change of weight of the fish in these treatments was in the range of -16.9 to -15.3%, while the TFI was only in the range of 0.00 to 0.05 g. For the AD 3.2 and AD 2.5 treatments, positive TFI and WG were observed. Therefore, these two treatments were continued for the trial until week-8. At the end of this feeding trial, TFI (0.98 g), WG (23.4%), and FCR (3.55) of fish fed with AD 3.2 were significantly higher (P < 0.05) than those fed AD 2.5 (0.73 g, 3.58%, and 82.7, respectively). The SGR (0.36%) of fish fed AD 3.2 was higher than that of AD 2.5 (0.18%) but no significant difference (P > 0.05) was observed. SR of fish in the AD 3.2 and AD 2.5 treatments was 98.3% and 96.5%, respectively. There was no significant differences (P > 0.05) detected in the CF between the fish from AD 3.2 (0.97) and AD 2.5 (0.97).

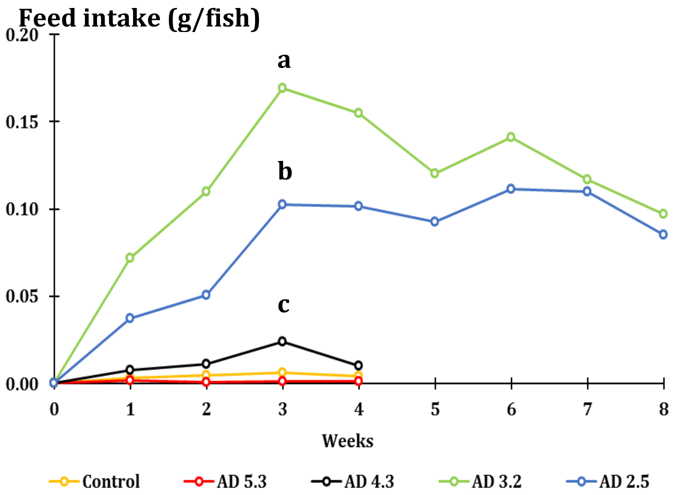

Figure 1. Weekly feed intake of *O. marmorata* juveniles in group culture provided with control (pH 6.0), AD 5.3, AD 4.3, AD 3.2 or AD 2.5 in the Feeding Trial I. Each point represented the mean of feed intake from triplicate groups of fish. Different superscripted alphabets indicate significant difference at P < 0.05.

2. Weekly feed intake

Figure 1 shows the WFI by *O. marmorata* fed with the experimental diets. At the beginning of the feeding trial, all the specimens fed AD 3.2 and AD 2.5 showed higher ingestion ratio than those in the other treatments. Fish fed AD 3.2 began to ingest at day-1. The feed intake increased, reaching peak at week-3 (0.17 g). After that, it started to drop. Similar trend was also observed for the feed intake of AD 2.5.

For fish fed with the AD 2.5, the TFI trend during the 8-week period was similar with AD 3.2. The TFI of AD 2.5 increased gradually with less fluctuations than those seen with AD 3.2. The feed intake of fish fed AD 2.5 treatment peaked at week 6 with 0.11 g. At week 3, significant differences (P < 0.05) were observed in the feed intake of AD 2.5, AD 3.2, and AD 4.3.

Feeding trial II

1. Growth performance and feed utilization

Growth performance and feed utilization of *O. marmorata* fed AD 3.2 or AD 2.5 for 7-week trials are summarized in Table 3. There was no significant difference (P > 0.05) between the TFI of fish fed with AD 3.2 or AD 2.5. The highest TFI was observed in the fish fed AD 3.2 (1.22 g). The WG and SGR of fish fed AD 3.2 (34.99%, 0.6%) were significantly higher (P < 0.05) than those fed AD 2.5 (13.02%, 0.26%). The FCR of fish fed AD 3.2 (4.30 ± 5.36) was also better than that of AD 2.5 (10.38 ± 8.86). NPU (0.21 ± 0.06) and CF (0.88 ± 0.07) were significantly lower (P < 0.05) in fish fed AD 3.2. PER (1.02 ± 0.63) was significantly higher (P < 0.05) for fish fed AD 3.2. The HSI (4.51) and VSI (8.26) of fish fed AD 3.2 were significant higher (P < 0.05) than those of AD 2.5 (2.07, 7.42). No significant difference (P > 0.05) was observed in the SR of fish fed with AD 3.2 (96.67%) and AD 2.5 treatment (100%).

Table 3. Parameters of growth performance and feed utilization of *O. marmorata* juveniles fed with the acidified diets for 7 weeks in the Feeding Trial II. Different superscripted alphabets indicate significant difference at P < 0.05 (compared in row).

Parameters	AD 3.2	AD 2.5
TFI [1]	1.22 ± 0.4	1.08 ± 0.2
WG [2]	34.99 ± 21.3 [a]	13.02 ± 7.6 [b]
SGR [3]	0.60 ± 0.3 [a]	0.26 ± 0.1 [b]
SR [4]	96.67	100
FCR [5]	4.30 ± 5.4 [a]	10.38 ± 8.9 [b]
NPU [6]	0.21 ± 0.7 [a]	2.20 ± 0.4 [b]
PER [7]	1.02 ± 0.6 [a]	0.55 ± 0.3 [b]
CF [8]	0.88 ± 0.1 [a]	0.93 ± 0.1 [b]
HSI [9]	4.51 ± 0.8 [a]	2.07 ± 0.7 [b]
VSI [10]	8.26 ± 0.9 [a]	7.42 ± 1.1 [b]

All values are mean ± S.D. (n = 30)
[1] Total Feed Intake (g/fish); [2] Weight Gain (%); [3] Specific Growth Rate (%/d); [4] Survival Rate (%); [5] Feed Conversion Ratio; [6] Net Protein Utilization; [7] Protein Efficiency Ratio; [8] Condition Factor; [9] Hepato-somatic Index; [10] Viscero-somatic Index

2. Weekly feed intake

The WFI of AD 3.2 and AD 2.5 diets by the *O. marmorata* are presented in Figure 2. At the beginning of this feeding trial, the trend was similar to that observed in the Feeding trial I (Figure 1). Fish started to ingest AD 3.2 and AD 2.5 diets from day 1 and the feed intake peaked at week 3 (0.29 g for AD 3.2, 0.22 g for AD 2.5). Thereafter, the feed intake generally decreased until the end of the trial. Significant differences (P < 0.05) were found between the feed intakes at week 3 between these 2 dietary treatments.

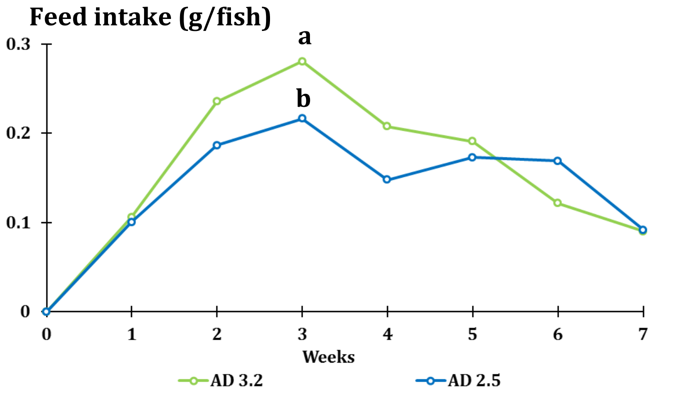

Figure 2. Weekly feed intake of *O. marmorata* fed with AD 3.2 and AD 2.5 diets during the 7-week of Feeding Trial II. Each point represented the mean of feed intake from 30 replicates of fish. Different superscripted alphabets indicate significant difference at $P < 0.05$.

3. Fish whole body proximate composition

Table 4 shows the whole-body proximate composition of the experimental fish. The crude protein content (17.4%) of fish fed AD 2.5 was higher ($P < 0.05$) than that of AD 3.2 (16.6%) but no significant difference ($P > 0.05$) was detected. The crude lipid (0.9%) and moisture content (74.3%) of fish fed AD 3.2 were higher than those fed AD 2.5 (0.8%, 74.0%) but no significant differences ($P > 0.05$) was found. The moisture content of fish fed with acidified diets was significantly lower ($P < 0.05$) than in the initial stage (76.4%). No significant differences ($P > 0.05$) were observed in crude lipid of initial fish and fish fed AD 3.2. The crude lipid and moisture contents of fish fed with the acidified diets decreased as the dietary pH level decreased. Ash contents of fish fed acidified diets (AD 3.2 and AD 2.5) were significantly changed ($P < 0.05$) following the dietary treatments. The ash content of fish fed with AD 3.2 (19.4%) was higher than those fed AD 2.5 (17.1%) but no significant difference was detected ($P > 0.05$).

Table 4. Whole body proximate composition of the fish-initial values, and after feeding with the AD 3.2 and AD 2.5 from the Feeding Trial II. Different superscripted alphabets indicate significant difference at $P < 0.05$.

Dietary Treatment	**Whole fish body proximate analysis (% wet weight basis)**		
	Initial	**AD 3.2**	**AD 2.5**
Crude protein	16.5 ± 1.0 ^{a}	16.6 ± 0.9 ^{a}	17.4 ± 0.2 ^{a}
Crude lipid	0.5 ± 0.1 ^{a}	0.9 ± 0.2 ^{b}	0.8 ± 0.1 ^{b}
Moisture	76.4 ± 0.1 ^{a}	74.3 ± 0.6 ^{b}	74.0 ± 0.3 ^{b}
Ash	13.2 ± 1.3 ^{a}	19.4 ± 0.7 ^{b}	17.1 ± 0.9 ^{b}

Discussion

According to Yong et al. (2015), the specimens of *O. marmorata* (TL =5.22 cm) which were already successfully weaned, and fed with the normal fishmeal-based diet (crude protein and lipid levels 50.3% and 9.5%, respectively) achieved approximately 395% in WG with the FCR value at about 1.04; PER at 1.85; HSI at 3 and VSI at 6.4, respectively; after a 15-week feeding trial. In the present study, the marble goby WG was generally less than 35%, and the FCR values were higher than 3. From Feeding Trial II (7-week), the fish whole-body crude protein level did not differ significantly from the initial value (16.5%). The PERs were relatively lower while the HSI and VSI were generally higher (PER, 1.85; HSI, 3; VSI, 6.3) than those reported by Yong et al. (2015), indicating that the *O. marmorata* could not utilize the dietary protein efficiently for their optimum growth, and might even encounter liver enlargement. In vertebrates, it is commonly known that food digestion initiates in stomach through the hydrochloric acid and pepsin secretion, and is completed in the intestine by the action of other protease enzymes including trypsin and chymotrypsin where their functions are optimized under the alkaline pH (Márquez et al., 2012). Fish stomach is known to develop less acidity during digestive hydrolysis than in the mammals (Sugiura et al., 2006; Márquez et al., 2012). While fish meal's pH buffering capacity is strong, supplementing feed acidifiers, especially organic acids, to fishmeal-based diet can improve its digestibility, and subsequently enhance the feed utilization and growth performance in the fish (reviewed by Ng and Koh, 2017). However, contradictory results can happen if the diet is strongly acidified. Rungruangsak and Utne (1981) reported that the growth and feed utilization in rainbow trout, *Salmo gairdneri,* were impeded when the fish were fed with strongly acidified diets (pH 3.3 – 5.3), compared to those fed the normal pH diet. The adverse effects of strong dietary acidification on fish growth and feed conversion were confirmed by Hardy et al. (1983) when they found that the growth and feed conversion in the rainbow trout were restored, after the acidified diet was neutralized using calcium hydroxide. The physiological response of fish to dietary acid intake is very limited (Sugiura et al., 2006), and there is a paucity of information on the digestive physiology of *O. marmorata*. Therefore, it is difficult to speculate why *O. marmorata* could not efficiently digest the acidified diets. Further study on the digestive physiology of *O. marmorata* is recommended to answer this question.

Although the acidified diet intake did not support the fish growth, *O marmorata* showed higher intake of AD 2.5 and AD 3.2 than the other diets (Control, AD 5.3, and AD 4.3) in this study. This result was in agreement with the findings by Teoh et al. (2018) and Lim et al. (2018) indicating that *O. marmorata* has strong preference for acidic food. Nevertheless, the present study shows that *O. marmorata* only accepted the acidified diets for 3 weeks and the intake decreased after the 3rd week of the feeding trials. In the behavioural study by Lim et al. (2018), the experiment was designed only for 3 weeks, and the good feeding performance of *O. marmorata* on AD 2.5 and AD 3.2 was reported only up to that period. Therefore, in light of the results of the present study, feeding *O. marmorata* with the acidified diets for an extended period (> 3 weeks) is not recommended. Similar finding was also reported by Rungruangsak and Utne (1981) who observed that the rainbow trout was not favourably disposed to consuming the acidified diet at pH 3.3 to pH 4.4 after about 1 month of feeding. Indeed, feeding fish with acidified diets for an extended period can cause stress to them. This could be due to the alteration of enzyme or hormone concentrations in their circulatory or digestive systems. These changes may further influence the feeding behaviour, and efficiency of digestion and absorption of dietary nutrients (Sugiura et al., 1998). Nevertheless, considering that *O. marmorata* has strong preference for the AD 2.5 and AD 3.2, it is worth trying to develop a pellet weaning protocol for the fish by using these diets as the starter feed and then slowly replacing them with the normal diet.

Conclusion

The present study confirmed that feeding acidified diets (AD 2.5 or AD 3.2) to *O. marmorata* juveniles for an extended period (> 3 weeks) did not yield any growth advantage. Nevertheless, it is worth trying to develop a weaning protocol using AD 2.5 and AD 3.2 as the starter feed for *O. marmorata* juveniles, based on their strong preference for these acidified diets. Further study should be conducted to confirm this possibility.

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