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Waste excretion of marble goby (*Oxyeleotris marmorata* Bleeker) fed with different diets

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Abstract

Marble goby (*Oxyeleotris marmorata* Bleeker), with its high demand and price, has a great potential as a profitable commercial aquaculture candidate in Malaysia and Southeast Asia region. Efforts are being made to produce this species in a better controlled culture environment like recirculating aquaculture system (RAS) due to poor growth performance and disease problems shown by conventional cage and outdoor pond culture systems. Quantification of waste excreted by fish is critical to RAS design. This study was conducted to characterize the waste excretion rates of marble goby fed with different diets (live food and minced fish). Ammonia-N (TAN), urea-N, nitrite-N (NO_2-N), nitrate-N (NO_3-N), total-N (TN), organic-N (ON), feces-N, 5-day biochemical oxygen demand (BOD_5) and total suspended solid (TSS) produced from marble goby were determined over a 72-h excretion period. Under given experimental conditions, the results showed that feed type had significant influence on the waste excretion rates, with marble goby fed live tilapia (*Oreochromis niloticus*) exhibiting significantly ($P<0.05$) the lowest amount of waste excretion comparable to that of fish fed live common carp (*Cyprinus carpio*) and minced scads (*Decapterus russellii*). This indicates that feeding marble goby with tilapia poses less adverse effects on water quality and is thus a suitable diet for this species. The waste excreted by the fish is composed of nitrogenous excretion (TAN, Urea-N, ON, Feces-N), and productions of dissolved biodegradable organic substances (BOD_5) and TSS ($TSS_{feces}+TSS_{water}$). About 58–71% of the nitrogen consumed in food was excreted and its rate depended mainly on the feed type. TAN was the chief end-product of protein metabolism; about 74–84% of the daily total nitrogenous excretion was TAN. Urea-N accounted for 13–21% of the daily total nitrogenous excretion indicating that urea-N is an important nitrogenous excretory end-product in marble goby. The waste excretion data presented in this study can be served as a pre-requisite for designing a RAS for this species. The overall BOD_5 and TSS production found in this study also point to the need for including bio-filtration unit and suspended solids removal mechanism in the RAS design.

Keywords: Marble goby (*Oxyeleotris marmorata* Bleeker); Waste excretion; Feed type; TAN & Urea-N excretion pattern; Recirculating system design

1. Introduction

Marble goby (*Oxyeleotris marmorata* Bleeker) is a good-eating freshwater fish well known as 'Soon Hock' or 'Ketutu' to millions of Southeast Asians. Its market value is high (US$12/kg) (Luong et al., 2005), and its wholesale price in Malaysia ranges from RM16/kg to RM60/kg. Due to its high commercial value, it does have a great potential as a profitable aquaculture species in Malaysia and vigorous efforts have been made to perfect its culture technique to produce this fish for commercial purpose.

Currently, these fishes are grown in cage and outdoor pond systems where the water is diverted from a nearby lake or river for a single pass through the pond system and then released into the environment. Yet, there are a lot of problems exist in these conventional cage and pond culture of marble goby, namely: serious disease problem caused by *Aeromonas hydrophila* and *Lernaea cyprinacea* (anchor worm), slow growth during juvenile and growout stage, high mortality rate, peculiar feeding behavior, and lack of formulated feeds (Cheah et al., 1994; Lin

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and Kaewpaitoon, 2000). Most of the problems above are much related to the poor water quality and lack of appropriate control of the present conventional culture. Thus, efforts are being made to develop production systems like recirculating aquaculture system (RAS) where the water is at least partly recycled to ensure a better controlled culture condition, which may lead to better yield of marble goby.

The quantity of waste excreted by fish is a major concern when designing systems to reuse water. Quantification of waste excretion, especially the ammonia-N excretion, is important for estimating maximum stocking biomass/density and water flow rates in culture systems, as well as assessing the environmental impact of culture operations (Forsberg and Summerfelt, 1992; Dosdat et al., 1996). Studies on quantifying waste excretion to design a RAS have been reported in other species like lobsters and seed clams (Zhu et al., 1999; Crear and Forteath, 2002). Similar studies have also been reported in fish (Kikuchi, 1995; Leung et al., 1999; Pagand et al., 2000). So far, no similar studies have been reported on marble goby.

Hence, this study was conducted to characterize the waste excretion rates of marble goby fed by several diets that were commonly used by fish farmers in Malaysia. Live foods were used mainly due to lack of suitable artificial feed for the fish. Besides, our preliminary studies had indicated that marble goby fed with live food (tilapia, *Oreochromis niloticus*, with 66% crude protein, 16% crude fat of dry weight) showed better growth (2.00±0.51 g d^{-1}) and feed consumption of 5.26±0.17% body wet weight (BW) d^{-1} compared to fish fed on artificial moist pellet diet (40% crude protein, 10% crude fat balanced by cellulose and αstarch, with addition of vitamin and mineral premix), which only produced the growth of 0.81±0.20 g d^{-1} and feed intake of 2.16±0.24%BW d^{-1} (Table 1). Ammonia-N ($TAN=NH_3+NH_4^+$), urea-N, nitrite-N (NO_2-N), nitrate-N (NO_3-N), total-N (TN), organic-N (ON), feces-N, 5-day biochemical oxygen demand (BOD_5) and total suspended solid (TSS) produced from marble goby were determined over a 72-h excretion period. In addition, temporal patterns of TAN and urea-N excretion after feeding were measured to understand their excretion patterns versus time. Based on the results of the waste excretion study, the information can be used in the design and management of effective culture systems for marble goby. This study was part of the project to develop an effective RAS for commercial production of high quality marble goby in Malaysia aquaculture industry.

Table 1
Growth and feed consumption of marble goby[1] in relation to different diets

Diet	Absolute growth rate g d^{-1}	Feed consumption %BW d^{-1}
Live food (Tilapia)[2]	2.00±0.51[a,4]	5.26±0.17[a,4]
Artificial food (moist pellet)[3]	0.81±0.20[b]	2.16±0.24[b]

[1]Fish size (100±5 g).
[2]Live tilapia, *Oreochromis niloticus* (66% crude protein, 16% crude fat of dry weight).
[3]Moist pellet (40% crude protein, 10% crude fat balanced by cellulose and αstarch, with addition of vitamin and mineral premix).
[4]Values are means±SD (N=6) and means with the same superscript in the same column are not statistically different (P>0.05).

Table 2
Composition of experimental feeds[1]

	Diet		
	Tilapia[a]	Carp[b]	Scads[c]
Proximate composition (as fed basis)[a]			
Moisture	76.43±0.49[a]	80.20±0.61[b]	79.38±0.64[c]
Crude protein	15.56±0.12[a]	15.09±0.18[b]	14.81±0.09[c]
Crude fat	3.83±0.13[a]	3.17±0.05[b]	4.52±0.04[c]
Ash	3.78±0.07[a]	1.34±0.07[b]	1.00±0.10[c]

[1]Values are means±SD of three replicate analysis (N=3) and means with the same superscript in the same row are not statistically different (P>0.05).

2. Materials and methods

2.1. Fish and acclimation

Marble goby averaging 100 g body weight were obtained from various local sources. They were acclimated individually in 195-L fiberglass rectangular holding tanks supplied with a continuous flow of recirculating well-aerated fresh water (water temperature: 27.5±0.5 °C; pH: 7.5±0.5; dissolved oxygen (DO) > 6 mg L^{-1}). The fresh water would first be disinfected by passing through UV disinfection unit before being supplied to the holding tanks in order to ensure the water is disease free. During acclimation, the fish were fed to satiation once a day with the experimental diet (see below) for 7 d prior to measurements of excretion (Jayaram and Beamish, 1992). They were not deprived of food prior to tests to ensure the conditions mimicked those found in culture of this species (Leung et al., 1999).

A trickling bio-filtration unit was provided to treat the water. Water exchange was timed to ensure the maximum levels of less than 3 mg TAN L^{-1} (<0.6 mg NH_3-N L^{-1}) was maintained (Timmons et al., 2002). To ensure that water quality in the culture tank was suitable for the fish, water samples from each culture tank were collected weekly throughout the experiment and analyzed for TAN and DO according to Parsons et al. (1984).

2.2. Experimental diets

Three experimental diets were given to the fish, namely:

D1: Live tilapia fry (*O. niloticus*) (size per fish: 3±1 cm total length)
D2: Live common carp fry (*Cyprinus carpio*) (size per fish: 3±1 cm total length)
D3: Minced scads (*Decapterus russellii*) (size per slice: thumb-sized in 4±1 g)

Diets were analyzed for moisture, crude protein, crude fat, and ash content according to AOAC (1990) (Table 2). To ensure the live diet (tilapia and carp) is disease free, the live diet was disinfected through UV and pulsed-UV procedures before being fed to the fish to control the possible pathogens entering with the live diet.

2.3. Experimental conditions and measurements

The experiment was conducted in 36-L capacity plastic experimental chambers inside a temperature-controlled room. The volume of the water in each chamber was regulated according to the requirements of the experiment, but generally was in between 20 and 30 L (Crear and Forteath, 2002). Water temperature was maintained at 27.5±0.5 °C and pH at 7.5±0.5 throughout the experimental period. Light was controlled to provide a 12-h light and 12-h dark photoperiod. To ensure that concentrations of DO in the water did not compromise the experiments, the containers were aerated with DO level of about 80% saturation (DO >6 mg L^{-1}). Aeration also acted to mix the water, thus ensuring that water samples were representative of the experimental chamber. The aeration had been gone through CO_2 stripping process to ensure that air free of CO_2 was provided and pH was maintained in the chamber. The volatilization of ammonia from aerated tanks or the effect of unionized ammonia (NH_3) loss due to air diffusion was ignored on TAN concentrations (Forsberg and Summerfelt, 1992) because the ratio of unionized ammonia to TAN was only about 5% for water at 27 °C and pH 7.5 according to Wheaton et al. (1994).

Six pieces of marble goby (same fish size in 100±5 g; N=6) were individually assigned to each of six replicates of the 3 diet treatments (Leung et al., 1999). At the beginning stage, 6 pieces of fish from the same population were used as the positive control in this experiment, where they were starved for 72 h before the measurement day in order to measure their endogenous or fasting TAN and urea-N excretion rates. On the measurement day, each experimental fish, excluding the fish used for positive control, was fed once a day to satiation (approximately at feeding rate ranging from 2 to 6%BW d^{-1}) with the experimental diets (around 9 am) and the amount of feed consumed by each individual was recorded and expressed as %BW d^{-1}. The actual feed rate of each individual was calculated on the basis of the ration level on the measurement day and the body weight of the fish. One hour after the completion of feeding (10 am), the fish were transferred from the holding tank and placed individually into the experimental chambers (Kikuchi, 1995; Leung et al., 1999). The fish were then allowed to settle for 1 h before measurements commenced.

After 1 h of adaptation, water samples for full analysis were taken from each chamber to determine initial background concentrations. Then, water samples for TAN (NH_3+NH_4^+) and urea-N determination were collected from each chamber at 2 h interval for the first 12 h, and then at 4 h interval from 12 to 24 h. Since culture fish are normally fed daily, so intensive measurements for TAN and urea-N were carried out for the first 24 h to simulate the culture situation (Leung et al., 1999). While triplicate measurements were carried out on the full analysis of TAN, urea-N, NO_2-N, NO_3-N, TN, BOD_5, and TSS at 24 h interval for 3 d. ON was calculated based on the difference between TN, and TAN, urea-N, NO_2-N, NO_3-N ON, organic nitrogen [ON=TN−(TAN+Urea-N+NO_2-N+NO_3-N)]. Fish were monitored on their waste excretions for 3 d.

At the end of the 3-d experiment, the fish were removed from the experimental chamber, weighed and transferred back to holding tank. The whole volume of water in the experimental chamber was then drained and filtered through Whatman GF/C filters (1.2-μm filter) to collect feces. The feces (residue on the filter) were dried at 60 °C for 48 h, weighed and analyzed for TSS_{feces} (APHA, 1995) and feces-N (AOAC, 1990) using Kjeldahl method (Kjeldahl, selenium catalyst; N×6.25), where sample was decomposed in hydrogen peroxide (H_2O_2) and sulfuric acid, then distilled to determine nitrogen.

A chamber without fish was served as the negative control and the changes in waste excretion rates were calculated based on the difference between the concentrations obtained for two consecutive (timed) samples, using the equation like below:

$$\text{Waste excretion rate} = [(C_o - C_I) - C_c] \times V/(W \times t) \times (\text{mg kg}^{-1}\text{d}^{-1} \text{ or mg kg}^{-1}\text{h}^{-1}) \quad (1)$$

where:

- C_I is the related waste concentration (mg L^{-1}) of the experimental chamber at time 1
- C_o is the related waste concentration (mg L^{-1}) of the experimental chamber at time 2
- C_c is the related waste concentration (mg L^{-1}) of the control chamber at time 2
- V is the water volume (L) of the chamber
- W is the wet body weight (kg) of the fish
- t time lapse (day or hour)

By plotting TAN & urea-N excretion against time (mg N kg^{-1} h^{-1}), ammonia and urea excreted over the 24 h period (mg N kg^{-1} d^{-1}) were estimated by integrating the area under the curve. Besides, mean daily waste excretion rates were also calculated for each treatment. All waste excretion parameters were analyzed using standard method adapted by Parsons et al. (1984), namely: TAN; urea-N; NO_2-N; NO_3-N; TN. While TSS and BOD_5 concentrations were performed in accordance with the standard methods (APHA, 1995). When the water samples could not be analyzed immediately they were frozen at −20 °C for a maximum of 1 week (Parsons et al., 1984).

2.4. Statistical analyses

Data analyses were performed on the SPSS ver 13.0 statistical package (SPSS Inc. USA) with the α set at 0.05 (significance at P<0.05). All the data were presented as means±SD throughout the text and were subjected to one-way ANOVA. If significant differences were indicated at the 0.05 levels, then

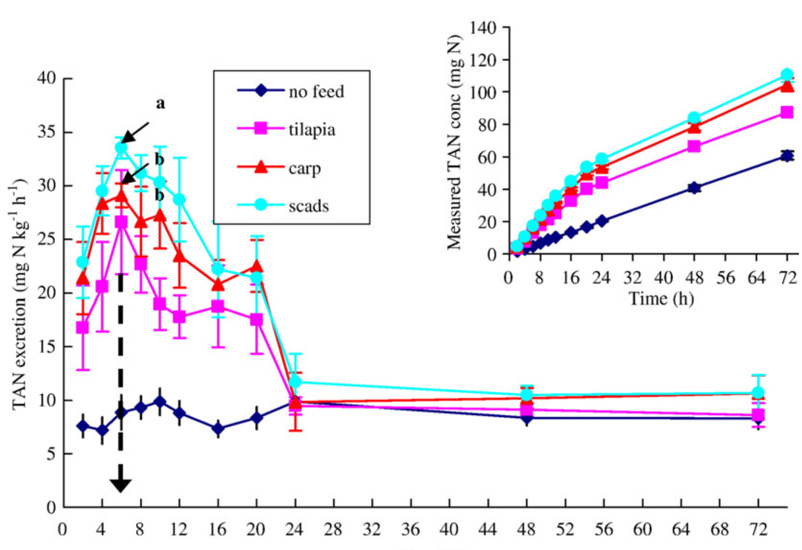

Fig. 1. Total ammonia nitrogen (TAN) (mg N kg^{-1} h^{-1}) excretion pattern of marble goby in relation to diets (N=6, means±SD). Peaks of TAN excretion are indicated by arrows. The peaks with the same superscript are not statistically different (P>0.05). The small line chart shows the measured concentration of TAN in the chamber over the 72-h experimental period.

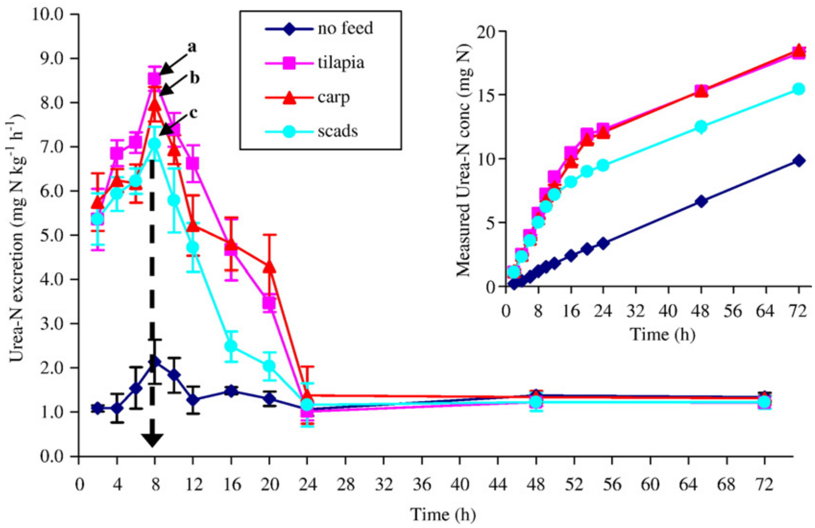

Fig. 2. Urea nitrogen (Urea-N) (mg N kg^{-1} h^{-1}) excretion pattern of marble goby in relation to diets (N=6, means±SD). Peaks of urea-N excretion are indicated by arrows. The peaks with the same superscript are not statistically different (P>0.05). The small line chart shows the measured concentration of Urea-N in the chamber over the 72-h experimental period.

Duncan's Multiple Range test was used to identify significant difference among treatments.

3. Results

3.1. Diurnal TAN and urea-N excretion patterns

All diet treatments showed an increase in TAN excretion after feeding (Fig. 1). Shortly after feeding, the hourly TAN excretion rates of fed marble goby were 2 to 3 times higher than those of starved or unfed individuals (Fig. 1). The peak rates of hourly TAN excretion of marble goby were observed at 6 h after feeding for all diet treatments, and they were about 3 to 4 times higher than the rates of unfed fish (Fig. 1). The peak rates were maintained for a short period of time (≤2 h), then the rates slowly declined and were not significantly (P>0.05) different to the rates of unfed fish (endogenous-N excretion) after 24 h, which were ranged from 9.48 to 11.74 mg N kg^{-1} h^{-1} (Fig. 1). Results of ANOVA analysis have shown that the peak rates of TAN shown by fish fed scads were significantly (P<0.05) higher than the peak rates shown by those of fish fed tilapia and carps, thus indicating that the highest hourly TAN excretion rate was shown in marble goby fed by minced scads. Yet, all diet treatments showed a nearly similar excretion pattern. In contrast to TAN excretion, the peak rates of urea-N excretion occurred at 8 h after feeding in all diet treatments, which were about 3 to 4 times higher than the rates of unfed fish (Fig. 2). However, the peak rates declined to the rates similar (P>0.05) to the rates of unfed fish at 24 h as well (Fig. 2). The highest hourly Urea-N excretion rate was significantly (P<0.05) shown in minced scads fed group (Fig. 2).

3.2. Waste excretion rates

Daily rates of nitrogenous waste excretion are shown in Table 3. It is revealed that marble goby fed with minced scads gave the highest TAN excretion (570.86±42.65 mg TAN kg^{-1} d^{-1}) compared to those of fish fed live carp and tilapia, which was 527.90±28.42 mg TAN kg^{-1} d^{-1}

Table 3
Mean daily rates of nitrogenous waste excretion[1] by the marble goby in relation to different diets

Diet	Consumed-N	Excreted-N						
	CN	TAN	NO_2-N	NO_3-N	Urea-N	ON	TN	Feces-N
	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}	mg N kg^{-1} d^{-1}
No feed	0.00[a]	205.63[a]±4.53	n.p.[4]	n.p.	33.32[a]±0.77	4.61[a]±0.54	243.57[a]±0.60	n.d.[5]
Tilapia 4.78%BW d^{-1} [2a]	1168.61[b]± 44.04 (100%)[3b]	430.02[b]±17.39 (36.80%)[b]	n.p.	n.p.	119.96[b]±3.22 (10.27%)[b]	28.44[b]±4.52 (2.43%)[b]	578.41[b]±22.70 (49.50%)[b]	101.87[a]±5.31 (8.72%)[a]
Carp 5.17%BW d^{-1} [a]	1227.07[b]±58.72 (100%)[b]	527.90[c]±28.42 (43.01%)[c]	n.p	n.p.	118.24[b]±7.50 (9.63%)[b]	25.77[b]±1.62 (2.11%)[b]	671.92[c]±35.12 (54.75%)[c]	131.82[b]±8.35 (10.74%)[b]
Scads 5.00%BW d^{-1} [a]	1170.75[b]±115.06 (100%)[b]	570.86[d]±42.65 (48.88%)[d]	n.p.	n.p.	92.87[c]±6.81 (7.96%)[c]	18.42[c]±4.91 (1.58%)[c]	682.15[c]± 49.94 (58.41%)[d]	144.94[b]±16.59 (12.37%)[c]

TAN, total ammonia nitrogen (NH_3+NH_4^+); NO_2-N, nitrite nitrogen; NO_3-N, nitrate nitrogen; TN, total nitrogen; ON, organic nitrogen [TN−(TAN+Urea-N+NO_2-N+NO_3-N)].

[1]Values are means±SD (N=6) and means with the same superscript in the same column are not statistically different (P>0.05).

[2]Feeding rate as percentage of body weight per day (%BW d^{-1}).

[3]Percentage of consumed nitrogen (CN).

[4]n.p. — no production as concentrations was found not significantly different (P>0.05) with control.

[5]n.d. — not detectable.

and 430.02±17.39 mg TAN kg^{-1} d^{-1}, respectively (Table 3). The TAN rates excreted by fed marble goby in this study were 2 to 3 times higher than the rates of unfed individuals. Daily TAN excretion as a percentage of consumed-N also exhibited similar results by following the sequence of scads > carp > tilapia, which represented 36.8–48.9% of CN (Table 3).

Low NO_2-N and NO_3-N levels were found in the experimental chamber and their concentrations were found not significantly different (P>0.05) to their respective concentrations in control chamber throughout the 3-d experiment period, thus indicating that there were no conversion of TAN to NO_2-N and NO_3-N by any existing nitrifying bacteria in the water, which in other words there were no productions of NO_2-N and NO_3-N during the experimental period.

Urea-N and ON excretion rates did not differ significantly (P>0.05) between marble goby fed tilapia and carp, while significant (P<0.05) lower excretion rates were shown by marble goby fed scads (Table 3). Generally, urea-N occupied 8.0–10.3% and ON accounted for 1.6–2.4% of CN (Table 3). The lowest TN excretion was significantly (P<0.05) observed in marble goby fed tilapia, whereas no significant difference (P>0.05) was shown between marble goby fed carp and scads (Table 3). In general, TN excretion accounted for 49.5–58.4% of CN (Table 3). On the other hand, the feces-N produced by marble goby fed tilapia was significantly (P<0.05) lower than other diet treatments, which represented 8.7–12.4% of CN (Table 3). On the whole, total nitrogenous excretion (TN + Feces-N) of marble goby represented 58.2–70.8% of CN in this study.

Results of BOD_5 and TSS analyses for the marble goby in diet treatment are also presented in Table 4. It appeared that fish in the scads fed group exerted the highest oxygen demand (Table 4). The daily BOD_5 for marble goby in this study ranged from 1502 to 2362 mg DO kg^{-1} d^{-1} (8.4–13.2 mg DO L^{-1}) (Table 4). For the purpose of comparison, BOD_5 excretion can also be related to TAN (Fig. 3). Linear regression of the experimental data resulted in the equation below:

$$R_{BOD_5} = 3.43\ R_{TAN}\left(r^2 = 0.90,\ N = 18,\ P < 0.001\right) \quad (2)$$

The number of samples for BOD_5 analyses was limited (N=18), but the linear relationship between BOD_5 and TAN excretion rates was proved significant (P<0.001) and strong (r^2=0.90, excluding data from fish fed with scads diet with BOD_5 increasing with TAN). Because of the extremely high rates of both TAN and BOD_5 production, data produced by fish in scads fed group were not taken into account for the regression.

Table 4
Mean daily rates[1] of TSS_{feces}, TSS_{water} and BOD_5 by the marble goby in relation to different diets

Diet	TSS_{feces}	TSS_{water}	BOD_5
	mg TSS kg^{-1} d^{-1}	mg TSS kg^{-1} d^{-1}	mg DO kg^{-1} d^{-1}
No feed	n.d.[3]	n.d.	89.39±4.87[a] (0.49 mg/L)[a]
Tilapia 4.78%BW d^{-1} [2,a]	4159.52±245.04[a] (8.86%)[4a]	168.26±24.20[a] (0.94 mg TSS/L)[a]	1502.15±81.91[b] (8.43 mg DO/L)[b]
Carp 5.17%BW d^{-1} [a]	5469.54±346.51[b] (10.74%)[b]	178.94±38.61[a] (1.00 mg TSS/L)[a]	1789.17±53.76[c] (10.01 mg DO/L)[c]
Scads 5.00%BW d^{-1} [a]	6115.33±700.13[c] (12.36%)[c]	357.10±0.97[b] (2.00 mg TSS/L)[b]	2361.23±73.57[d] (13.22 mg DO/L)[d]

TSS_{feces}, total suspended solid measured as fish feces; TSS_{water}, total suspended solid measured from water sample; BOD_5, 5-day biochemical oxygen demand.
[1]Values are means±SD (N=6) and means with the same superscript in the same column are not statistically different (P>0.05).
[2]Feeding rate as percentage of body weight per day (%BW d^{-1}).
[3]n.d. — not detectable.
[4]Percentage of daily feeding rate.

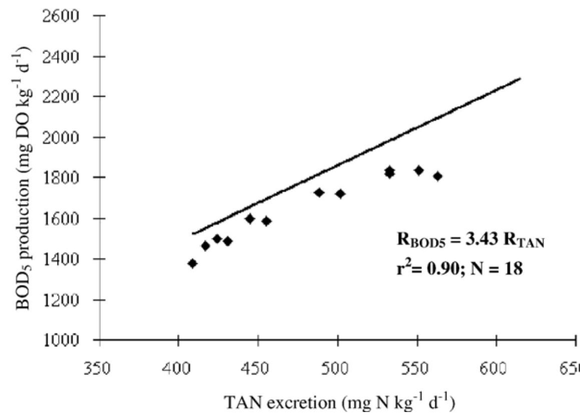

Fig. 3. Relationship between BOD_5 (R_{BOD5}) and TAN excretion rates (R_{TAN}) of marble goby.

For TSS measured from water (TSS_{water}), a significant (P<0.05) higher TSS_{water} concentration of 357.10±0.97 mg TSS kg^{-1} d^{-1} was also observed for marble goby fed scads diet (Table 4). In this study, daily TSS_{water} concentration ranged from 168 to 357 mg TSS kg^{-1} d^{-1} (0.9–2.0 mg TSS L^{-1}) (Table 4). While for TSS measured as fish feces (TSS_{feces}), it ranged from 4159 to 6115 mg TSS kg^{-1} d^{-1}, and they represented 8.9–12.4% of the daily feeding rate (Table 4). This fraction may be a low estimate since the feces were allowed to remain in the water column for 3 d before being collected for analysis. The highest (P<0.05) amount of TSS_{feces} was also shown in scads group (Table 4). For the purpose of comparison, the production of TSS_{total} (TSS_{water} + TSS_{feces}) can also be related to BOD_5 (Fig. 4). Linear regression of the experimental data resulted in the equation below:

$$R_{BOD_5} = 0.34\ R_{TSS}\left(r^2 = 0.80,\ N = 18,\ P<0.001\right) \quad (3)$$

The linear relationship between BOD_5 and TSS_{total} production rates was also proved significant (P<0.001) with BOD_5 increasing with TSS_{total}.

There were no measurable changes of TN, BOD_5 and TSS in the experimental chamber on the 2nd and 3rd day of experiment as their concentrations on the 2nd and 3rd day were found not significantly different (P>0.05) to their respective concentration on 1st day, thus indicating that there were no further excretion/production of these waste parameters on day 2 and day 3 of the experiment.

In terms of TN excretion following feeding, TAN was the greatest part of daily nitrogenous excretion, which accounted for 74–85% of the

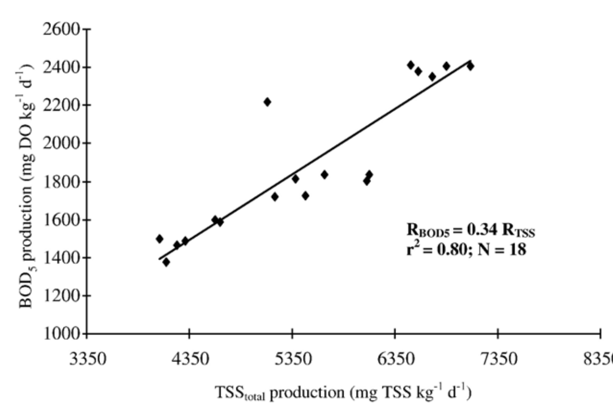

Fig. 4. Relationship between BOD_5 (R_{BOD5}) and TSS_{total} (TSS_{feces} + TSS_{water}) production rates (R_{TSS}) of marble goby.

Table 5
Mean percentage (%) of daily rates of nitrogenous excretion[1] by the marble goby in relation to different diets

Diet	Excreted-N (%)					
	TN	TAN	Urea-N	ON	NO_2-N	NO_3-N
No feed	100.00[a]	84.43[a]±0.27	13.68[a]±0.18 (16.21%TAN)[a]	1.89[a]±0.19	n.p.[2]	n.p.
Tilapia	100.00[a]	74.34[b]±0.60	20.75[b]±0.38 (27.91%TAN)[b]	4.91[b]±0.66	n.p.	n.p.
Carp	100.00[a]	78.56[c]±0.42	17.59[c]±0.39 (22.40%TAN)[c]	3.85[c]±0.33	n.p.	n.p.
Scads	100.00[a]	83.68[d]±0.40	13.62[a]±0.38 (16.28%TAN)[a]	2.70[d]±0.71	n.p.	n.p.

TN, total nitrogen; TAN, total ammonia nitrogen ($NH_3+NH_4^+$); ON, organic nitrogen [TN−(TAN+Urea-N+NO_2-N+NO_3-N)]; NO_2-N, nitrite nitrogen; NO_3-N, nitrate nitrogen.
[1]Values are means±SD (N=6) and means with the same superscript in the same column are not statistically different (P>0.05).
[2]n.p. — no production as concentrations was found not significantly different (P>0.05) with control.

TN excretion, while the second largest nitrogenous excretion product was urea-N, which represented 13–21% of the TN excretion. ON only contributed 2–5% of the TN excretion (Table 5).

4. Discussion

The results showed a pattern of immediate rise in TAN excretion rate after feeding followed by a more prolonged and gradual decrease. This is in agreement with the findings of other workers (Jobling, 1981; Engin and Carter, 2001). Brett and Zala (1975) also found that following the single morning feed, the excretion rate in sockeye salmon rose sharply to a peak, falling rapidly thereafter in an almost exponential decrease to the early morning base level. The peak rates occurred 6 h after feeding in all the treatments in this study, with peak rates of excretion being 3 to 4 times higher than fasting rates. This is within the range found for other fish in previous findings (Kikuchi, 1995; Leung et al., 1999; Engin and Carter, 2001). Dosdat et al. (1996) also reported that only one peak of TAN excretion appeared for 100 g fish which were fed once daily and it appeared 5–8 h after feeding. Despite the variation in the magnitude of peak TAN excretion rate, the daily patterns were similar among the diet treatment. This indicates that the same metabolic processes governing ammonia production from deamination occur at the same time irrespective of diet under the experimental conditions employed. This finding is in accordance with results reported on other teleost genera and species: sockeye salmon (Brett and Zala, 1975), and Japanese flounder (Kikuchi et al., 1992). The differences in peak TAN amplitudes observed in this study indicate that feed type may have effect on the metabolic activities of marble goby, since the amplitude and time of appearance of peak TAN excretion rates are dependent upon fish size, water temperature and nitrogen intake (Jobling, 1981; Kaushik and Cowey, 1990). To be more precise, TAN excretion rates found in this study are influenced by the quality and quantity of nitrogen intake from the feed, or in other words, the crude protein content of feed may explain the variation of the TAN excretion rates. However, this only applies provided that same fish size was used and water temperature was maintained throughout the experiment and in all treatments. Various studies on fish have reported that TAN excretion after feeding represented 30–49% of N-intake or CN, and are linked to feed type and its dietary nitrogen, ingested protein and protein intake quality of the diet (Dosdat et al., 1996; Leung et al., 1999). Our findings of TAN excretion representing 36–49% of the CN in 100 g marble goby are in agreement with these values. Lanari et al. (1993) also found 49% in 100 g rainbow trout.

The daily excretion patterns of urea-N were similar among the diet treatment. Yet, maximum urea-N hourly excretion rates found in this study were 3 times higher than what was found by Dosdat et al. (1996) on turbot. The high excretion rates of urea-N shown by marble goby in this study were also reported by Kikuchi (1995) on Japanese flounder and Engin and Carter (2001) on *Anguilla australis australis*. This indicates that urea-N can be an important additional component of nitrogenous excretion in some species. Further study is needed as there is still little known about the urea-N excretion rates in marble goby.

In this study, marble goby fed live tilapia exhibited significantly (P<0.05) the lowest amount of nitrogenous excretion (Table 3). When protein retention is increased, it is logical that the TAN excretion produced from catabolized protein would be reduced (Webb and Gatlin, 2003). In the case of the dead scads fed groups, which exhibited the highest excretion and loss, this was most likely due to the deterioration of scads diet during storage. Fish will not fully utilize the feed if it is deficient in nutrients due to break down over time which will increase the amount of waste produced by the fish. The results are in agreement with those studied in different carnivorous species fish such as red drum and Atlantic salmon (Reigh and Ellis, 1992; Carter et al., 1994). Tantikitti et al. (2005) also indicated that Asian seabass fed minced trash fish that were low in protein quality would resulted in poor nutrient utilization and high ammonia excretion. Unfortunately, the amino acid composition of the experimental diets was not analyzed and it thus needs further investigation. Although minced or trash fish is not recommended for use as feed for aquatic animals, most fish farmers in Malaysia are still using it because of its low price and convenience. On the whole, it was shown in this study that feed type had significantly affected the nitrogenous excretion of marble goby. Similar results were obtained by Sun et al. (2006) showing that feed type also had an obvious influence on nitrogenous excretion of juvenile cobia and the dietary protein content was one of the determinants of nitrogenous excretion for juvenile cobia.

It appeared that fish in the scads fed group exerted the highest oxygen demand. It may well be that these higher values were not only originated from the fish metabolism, but also due to the increase of dissolved biodegradable organic substances and oxygen demand associated with the decomposition of the uneaten scads diet. It was visually observed that higher feces were yielded and more uneaten feed was spitted out from the mouth of marble goby fed scads diet during the experimental period, which would bring about the increase of the amount of dissolved biodegradable organic substances in the water. Thus, this may potentially account for the higher oxygen demand or BOD_5 values. In the design of nitrification biofilters for recirculating aquaculture systems, the TAN excretion rate is usually estimated as 3% of the daily feeding rate (Wheaton et al., 1994). The BOD_5 production rate for finfish is often estimated to be 10–50% of the daily feed rate (Chen et al., 1997). If 10% is used for the purpose of discussion, the BOD_5 excretion rate would be about 3.3 times that of the TAN excretion rate. This ratio matches quite well the BOD_5 and TAN excretion relationship, as demonstrated by Eq. (2). Besides, the daily BOD_5 for marble goby in this study had exceeded the acceptable 5 mg L^{-1} BOD_5 limit for aquaculture by Law (1987). This indicates that a biofiltration unit must be provided in the RAS for the removal of dissolved biodegradable organic substances in order to reduce the BOD_5 or oxygen demand in the water.

The water used in the study had an undetectable initial TSS_{water} concentration value. It is assumed the TSS_{water} materials measured were comprised of small fraction of uneaten feed and also the excreted fecal material produced by the fish even though characterization of the measured TSS_{water} material was not conducted. The TSS measurements of the excretion study for the marble goby fed scads diet were significantly higher than those of fed live diet (tilapia and carp). No clear explanation can be provided for this result. However, it was visually observed that higher feces were yielded and more uneaten feed was spitted out from the mouth of marble goby fed scads diet during the experimental period. This may potentially account for the higher TSS values. Generally, low TSS concentration values were found in this study. However, if the amount of TSS contributed by the fish feces was considered, then the overall TSS excretion (Feces+TSS) in this study would be ranged from 4328 to 6472 mg TSS kg^{-1} d^{-1} (24–36 mg TSS L^{-1}), and they represented approximately 9–13% of the daily feeding rate (Table 4). This fraction may be a low estimate since the uneaten feed (spitted out from the fish's mouth during experiment) was difficult to measure precisely, the feces were allowed to remain in the water column for 3 d before being collected for analysis, and also the lower stocking density or the use of comparative larger tanks (only one fish in each experimental chamber) may also have impacted on the overall TSS excretion value. Nevertheless, the overall TSS excretion concentrations (24–36 mg TSS L^{-1}, Table 4) had exceeded the recommended limit of 15 mg TSS L^{-1} for warm water species in recirculating systems, and also the tentative upper limit of 25 mg TSS L^{-1} for freshwater fish (FIFAC, 1980; Timmons et al., 2002), thus this may threaten the fish by damaging the fish gills and harboring pathogens detrimental to fish health. This implies a need to include a suspended solids removal mechanism in the recirculating aquaculture system design for marble goby culture, which would lead to reducing the TSS excretion and BOD_5 loading rate, maintaining water clarity, and provide a good water quality environment for the culture organism.

The excretion values obtained reflect the conditions of the study and most likely will result in different values with respect to the environmental and physical conditions and species stated. The measurement values obtained from this study can serve as a general basis or pre-requisite for designing a RAS for marble goby culture.

5. Conclusion

Feed type had significant influence on the waste excretion rates, with marble goby fed live tilapia exhibiting significantly ($P<0.05$) the lowest amount of waste excretion comparable to that of fish fed carp and scads. This indicates further that tilapia is a suitable diet for marble goby as its lowest amount of waste excretion would cause less adverse effects on the cultured water quality, which would lead to the potential of higher survival and faster growth of the fish. In contrast, high waste excretions were significantly observed in marble goby fed with mince scads, thus indicating that it is an inappropriate diet with respect to its highest amount of nitrogen loss and poor nutrient utilization.

Waste excretion rate of marble goby after feeding is composed of nitrogenous excretion (TAN, Urea-N, ON, Feces-N), and productions of dissolved biodegradable organic substances (BOD_5) and TSS ($TSS_{feces}+TSS_{water}$). The mean rates of nitrogenous excretion were in the range of 430–571, 93–120, 18–29, 102–145 mg N kg^{-1} d^{-1}, respectively, while 1502–2361 mg DO kg^{-1} d^{-1} (8–13 mg L^{-1}) for BOD_5 and 4328–6472 mg TSS kg^{-1} d^{-1} (24–37 mg L^{-1}) for TSS productions. The measurement values obtained from this study can serve as a general basis or pre-requisite for designing a RAS for marble goby culture. The overall BOD_5 and TSS production found in this study also point to the need for including bio-filtration unit and suspended solids removal mechanism in the RAS design.

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