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Short Communication

Acceptability to betaine as a feed enhancer in the brown-marbled grouper (*Epinephelus fuscoguttatus*) at grow-out stage

Ahmad Zidni Rizqin bin Jamil, Leong-Seng Lim*, Audrey Daning Tuzan, Rossita Shapawi, and Gunzo Kawamura

Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, 88400 Malaysia

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Abstract

This study was conducted to determine the acceptability to betaine as a feed enhancer in the brown-marbled grouper, *Epinephelus fuscoguttatus* at grow-out stage. Different ratios of betaine (BET) + amino acid mixture (AAM) (1:1, 1:2, 1:3, 1:4, 1:5) were delivered to the fish through agar gel pellets in the behavioural assays and the Index of Preference (IndPrf) was calculated. The pure agar gel (PAG) pellet was totally rejected (IndPrf value 0), while the BET and AAM treatments also were not preferred by the fish (0.24 and 0.41, respectively). However, all BET + AAM treatments attained almost perfect IndPrf value (0.83 - 1.0, $P>0.05$), and were significantly higher ($P<0.05$) than the PAG, BET, and AAM treatments. In conclusion, the acceptability to betaine as feed enhancer in the *E. fuscoguttatus* at grow-out stage was very high; small amount of betaine was sufficient to enhance the taste of the AAM.

Keywords: tiger grouper, *Epinephelus fuscoguttatus*, taste preference, betaine, behaviour

1. Introduction

Taste preference in fish is species-specific, and it can change with the fish growth (Kasumyan & Døving, 2003). Through behavioural assays, it was reported that the 2.5 g size of Dover sole *Solea solea* (L.) required the addition of certain L-amino acids in order to accept betaine. However, such requirement was not needed for those with the sizes exceeding 50 g as the fish could accept solely betaine (Mackie, Adron, & Grant, 1980). These results suggested the improvement in the acceptability of *S. solea* (L.) to betaine with growth. Kasumyan (1999) also reported the advancement in the taste acceptability of sturgeons to spectra of amino acids when the fish developed from larval to juvenile stage. Such information is important and it can contribute to develop the aquafeed which is acceptable by the targeted fish species.

*Corresponding author
Email address: leongsen@ums.edu.my

The aquaculture industry of Epinephelinae groupers is expanding especially in the Southeast Asia region (*e.g.* Afero, Miao, & Perez, 2010; Miao & Tang, 2002; Peterson *et al.*, 2013). Epinephelinae groupers are carnivorous fish species; therefore, fish meal (FM) has been used as the main protein source in the formulated compounded diets for grouper farming (Lim, Yong, & Shapawi, 2014; William, 2009). However, the price of FM is hiking due to its increasing demand in global (Tacon & Metian, 2008). Therefore, alternative protein sources such as the terrestrial animals- and plant-based proteins have been exploited to replace the FM in grouper feeds (Lim *et al.*, 2014). Soybean meal (SBM) is one of most potential plant protein to substitute the FM in the diet for groupers due to its high protein content. Shapawi, Ebi, and Yong (2013) reported that 30% of FM protein in diet of the juvenile brown-marbled grouper, *Epinephelus fuscoguttatus* can be replaced with SBM protein without compromising the fish growth. However, high inclusion level of SBM (40%) can reduce the diets palatability, resulting poor feed intake and fish growth. In recent, betaine has been identified as a feed enhancer for juvenile *E. fuscoguttatus* through behavioural assays (Lim,

Chor, Tuzan, Shapawi, & Kawamura, 2016). Indeed, dietary betaine supplementation can significantly improve the feed intake of juvenile *E. fuscoguttatus* on the diets with SBM inclusion level at 40% (Lim, Ebi, Chor, Kawamura, & Shapawi, 2015a), and the optimum dietary betaine supplementation level has been determined at 1% (Lim *et al.*, 2015b). Nonetheless, long-term of betaine supplementation in the grow-out diets for juvenile *E. fuscoguttatus* until they reach to the ideal table size (about 1 kg) can be costly. It is unknown if the dietary betaine supplementation level can be reduced without compromising its effect as a feed enhancer when the fish has grown bigger, and there is also no information on the acceptability to betaine in the *E. fuscoguttatus* at grow-out stage. In the present study, the acceptability to betaine in the *E. fuscoguttatus* at grow-out stage was determined through behavioural assay.

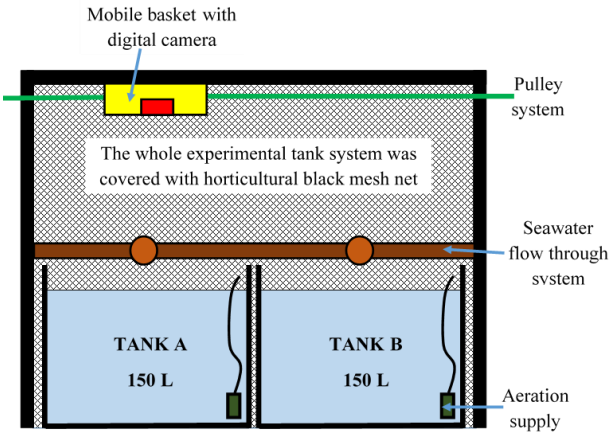

Figure 1. Schematic diagram of the experimental tank system.

2. Materials and Methods

In this study, 28 *E. fuscoguttatus* were used. The total length and body weight of the fish were 15.9 ± 0.8 cm and 65.6 ± 10.0 g (mean ± SD). The fish were obtained from a local fish farmer and distributed evenly in the two experimental fiberglass tanks (tank capacity 150 L; 14 fish per tank). The tank system designed for the behavioural assays in the present study was adopted from the work by Lim *et al.* (2016). Figure 1 shows the schematic diagram of the experimental tank system setup in this study. Briefly, the two fiberglass tanks (150 L each) were equipped with sea water flow-through system and provided with aeration. Each tank was considered as a replicate. Surrounding of the tanks was curtained with the horticultural black mesh net to avoid disturbance from the observer's movement. A mobile basket with a horizontal pulley system was installed above the tanks, and a digital camera (Olympus brand, TG2 model, Japan) was placed inside the basket to video - record the fish feeding response.

The types of agar gel pellet and its preparation method were also adopted from the work reported by Lim *et al.* (2016). Table 1 shows the types of agar gel pellet used in the present study, together with its composition. In brief, the agar gel pellets were prepared by dissolving the agar gel powder (2% of the seawater volume) and red dye (0.1%) in the filtered seawater, and the mixture was heated in a microwave until boiled. The chemical test substance (betaine, amino acids mixture or betaine + amino acids mixture, 1%) then was dissolved in the boiled mixture. Subsequently, the mixture was poured into a glass petri dish for hardening and cut into uniform size of pellets (about 1 cm x 1 cm x 0.5 cm). The agar gel pellets were stored in a refrigerator (4 °C) until further use. For the production of the Feed Extract (FE) pellet (Table 1), the liquid extract of the commercial feed (Otohime brand, EP type, Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan) was prepared by soaking the pellet powder in filtered seawater until the water color turned into dark brown (approximately 20 min) and then filtered away the homogenate through a 60 microns mesh net. In order to maintain the freshness, all agar gel pellets were in refrigerator for not more than 4 days.

The fish were trained to recognize the agar gel pellet as food prior to the behavioural experiment. The procedure of fish training was as described by Lim *et al.* (2016). Firstly, the fish in each tank were starved for 24 hours, and then fed with

Table 1. Ingredients and types of agar gel pellet used in the present study (adopted from Lim *et al.*, 2016).

Treatments	Pure agar gel (PAG)	Feed extract (FE)	Betaine (BET)	Amino acids mixture (AAM)	BET+AAM (ratio 1:1)	BET+AAM (ratio 1:2)	BET+AAM (ratio 1:3)	BET+AAM (ratio 1:4)	BET+AAM (ratio 1:5)
Ingredients (in g or ml)									
Filtered seawater	10 ml	-	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Agar gel powder [1]	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Red food dye [2]	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g
Liquid extract of pellet feed [3]	-	10 ml	-	-	-	-	-	-	-
Betaine [4]	-	-	0.1 g	-	5.0×10^{-2} g	3.0×10^{-2} g	2.5×10^{-2} g	2.0×10^{-2} g	1.5×10^{-2} g
Amino acids mixture [5]	-	-	-	0.1 g	5.0×10^{-2} g	7.0×10^{-2} g	7.5×10^{-2} g	8.0×10^{-2} g	8.5×10^{-2} g

[1] Mermaid Brand, Thailand
[2] Ponceau 4R, Meebo Brand, Malaysia
[3] Otohime Brand, EP type, Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan
[4] Sigma Brand
[5] Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, and Valine (all L-isomer, Sigma Brand, each 12.5%)

10 pieces of FT pellet daily at afternoon 1600. The fish were rewarded with Otohime commercial pellet until satiation if they consumed all the FT pellets given, and vice versa. The training was completed when the fish ingested all FT pellet given continuously for 3 days, then the behavioural experiment started.

During the experiment, the FE-Test-FE (pellets) feeding protocol was applied. The fish in a tank was continuously fed with the FE pellet. In the midst of giving out the FE pellet, a piece of the test agar gel pellet [pure agar gel (PAG), betaine (BET), amino acids mixture (AAM) or betaine + amino acids mixture (BET+AAM) at different ratios] was given to the fish, and their feeding response was video-recorded. Until the fish showed no interest to the agar gel pellet given, the video recording was stopped and the trial ended. Each trial usually took about 10 minutes. Subsequently, the camera was moved to the next tank and the same experimental procedure was repeated. Prior to every trial, the flow-through seawater and aeration supplies were off for the clear picture of video recording. After the trials were completed, all the remaining agar gel pellets in tanks were removed using a hand net; the flow seawater and aeration and supplies were resumed, and all fish were rewarded with the commercial feed until apparent satiation level. In a day, only one trial was conducted on each tank of fish, and only one type of test agar gel pellet was tried on them. In total, each type of test agar gel pellet was repeatedly tested for 10 times (5 times on each tank of fish) in random sequence. Throughout the video recording sessions, water temperature, salinity and pH were 28 – 30°C, 29 – 31 ppt, and 7.6 – 7.8, respectively.

From the recorded videos, two parameters were observed to analyze the fish preference for the test agar gel pellets: (I) the test pellet was ingested or rejected [*A*] - if ingested, recorded 1, if rejected, recorded 0, (II) frequency of the pellet been captured before it was ingested or finally rejected [*B*]. Subsequently, the Index of Preference (IndPrf) was calculated through dividing [*A*] by [*B*]. Mean of the preference index for each type of agar gel pellet then was calculated and expressed as the representative result (Lim *et al.* 2016).

Kruskal-Wallis Test (non-parametric statistical analysis method) was used to analyze the data as the data was not normally distributed. Conover-Inman Test was performed as the post hoc test for pairwise comparisons when Kruskal-Wallis Test showed significant difference (P<0.05) among treatments. Both tests were conducted using the computer statistical analysis software SYSTAT 13 (Systat Software, Inc.).

3. Results and Discussion

Table 2 shows the IndPrf of the *E. fuscoguttatus* for each type of agar gel pellet. In general, the PAG pellet was totally rejected by the fish and the IndPrf value was zero. The pellets with BET or AAM both were poorly accepted by the fish (index value 0.24 and 0.41, respectively). However, all BET+AAM treatments were highly accepted (0.83 – 1.00) by the fish and these results were significantly higher (P<0.05) than those of the PAG, BET, and AAM treatments. Such outcome was in parallel with that reported earlier by Lim *et al.* (2016) on *E. fuscoguttatus* at the younger stage (BW 13.8 g – 15.6 g) (Table 2). Although the IndPrf for BET in the present study was significantly higher (P<0.05) than the PAG treatment (0), its value (0.24) was still very low and similar with that reported in the previous study by Lim *et al.* (2016) (0.17), indicating that the function of betaine may not change from being a feed enhancer to a feeding stimulant for *E. fuscoguttatus* when it grows. Such outcome was opposite with that reported by Mackie *et al.* (1980) on *S. solea* (L.), and further support the fact that fish taste preference for chemical substances is species-specific (Kasumyan & Døving, 2003).

In the present study, it was interesting to find that the IndPrf for the BET+AAM (1:5) (0.83) was not significantly different (P>0.05) from that of the BET+AAM (1: 1) (0.95). This result was different with that reported by Lim *et al.* (2016) on *E. fuscoguttatus* at the younger stage, in which the IndPrf for BET+AAM (1:5) (0.60) was significantly lower (P<0.05) than that of BET+AAM (1:1) (0.90). These contradict results clearly demonstrated that the acceptability to betaine as feed enhancer in the *E. fuscoguttatus* at grow-out stage has been improved. Therefore, there is a possibility to reduce the dietary betaine supplementation level but without compromising the effect of betaine as a functional feed enhancer for the *E. fuscoguttatus* at grow-out stage. Lim *et al.* (2015b) reported that the optimum dietary betaine supplementation level for the 15.6 g size of *E. fuscoguttatus* was 1%. Therefore, the betaine supplementation level in the grow-out diets for *E. fuscoguttatus* (> 65 g) can be reduced to less than 1%, yet the threshold level remained to be further determined.

Table 2. Preference indexes of *E. fuscoguttatus* for different types of agar gel pellets from the present and the previous studies.

Types of Agar Gel Pellet	Total Trial (present study)	Preference Index (Mean ± SD)	
		Present study	Lim *et al.* (2016)
Pure agar gel (PAG)	10	0.00^{a}	0.00^{a}
Betaine (BET)	10	0.24 ± 0.41^{b}	0.17 ± 0.39^{a}
Amino acids mixture (AAM)	10	0.41 ± 0.35^{b}	0.19 ± 0.31^{a}
BET+AAM 1: 1	10	0.95 ± 0.16^{c}	0.90 ± 0.23^{b}
BET+AAM 1: 2	10	1.00 ± 0^{c}	0.88 ± 0.23^{bc}
BET+AAM 1: 3	10	1.00 ± 0^{c}	0.75 ± 0.39^{bc}
BET+AAM 1: 4	10	1.00 ± 0^{c}	0.79 ± 0.40^{bc}
BET+AAM 1: 5	10	0.83 ± 0.29^{c}	0.60 ± 0.45^{cd}

- Different superscripted alphabets indicate significant difference at level 0.05
- Comparison was made among the treatments within each study

4. Conclusions

In conclusion, the acceptability to betaine in the *E. fuscoguttatus* at grow-out stage was high. Small amount of betaine was sufficient to enhance the taste of the amino acid mixture

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References

Afero, F., Miao, S., & Perez, A. A. (2010). Economic analysis of tiger grouper *Epinephelus fuscoguttatus* and humpback grouper *Cromileptes altivelis* commercial cage culture in Indonesia. *Aquaculture International*, *18*(5), 725-739. doi:10.1007/s10499-009-9295-x

Kasumyan, A. O. (1999). Olfaction and taste senses in sturgeon behaviour. *Journal of Applied Ichthyology*, *15*(4-5), 228-232. doi:10.1111/j.1439-0426.1999.tb00240.x

Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fish. *Fish and Fisheries*, *4*(4), 289-347. doi:10.1046/j.1467-2979.2003.00121.x

Lim, L. S., Chor, W. K., Tuzan, A. D., Shapawi, R., & Kawamura, G. (2016). Betaine is a feed enhancer for juvenile grouper (*Epinephelus fuscoguttatus*) as determined behaviourally. *Journal of Applied Animal Research*, *44*(1), 415-418. doi:10.1080/09712119.2015.1091329

Lim, L. S., Ebi, I., Chor, W. K., Kawamura, G., & Shapawi, R. (2015a). Determination on the possibility of dietary betaine supplementation to improve feed intake of soybean meal-based diet in the juvenile grouper (*Epinephelus fuscoguttatus*): A pilot study. *Malaysian Applied Biology*, *44*(2), 137-141.

Lim, L. S., Ebi, I., Chor, W. K., Lu, K. C., Chong, M., Sade, A., & Shapawi, R. (2015b). Optimizing betaine supplementation level in soybean meal-based diets to enhance feed intake and growth performance of juvenile grouper, *Epinephelus fuscoguttatus*. *Advances in Environmental Biology*, *9*(17), 12-17.

Lim, L. S., Yong, A. S. K., & Shapawi, R. (2014). Terrestrial animal- and plant-based ingredients as alternative protein and lipid sources in the diets for juvenile groupers: Current status and future perspectives. *Annual Research and Review in Biology*, *4*(20), 3071-3086. doi:10.9734/ARRB/2014/10963

Mackie, A. M., Adron, J. W., & Grant, P. T. (1980). Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). *Journal of Fish Biology*, *16*(6), 701-708. doi:10.1111/j.1095-8649.1980.tb03749.x

Miao, S., & Tang, H. C. (2002). Bioeconomic analysis of improving management productivity regarding grouper *Epinephelus malabaricus* farming in Taiwan. *Aquaculture*, *211*, 151-169. doi:10.1016/S0044-8486(02)00190-4

Petersen, E. H., Chinh, D. T. M., Diu, N. T., Phuoc, V. V., Phuong, T. H., Dung, N. V., . . . Glencross, B. D. (2013). Bioeconomics of grouper, Serranidae: Epinephelinae, culture in Vietnam. *Reviews in Fisheries Science*, *21*(1), 49-57. doi:10.1080/10641262.2012.753403

Shapawi, R., Ebi, I., & Yong, A. (2013). Soybean meal as a source of protein in formulated diets for tiger grouper, *Epinephelus fuscoguttatus* juvenile. Part I: Effects on growth, survival, feed utilization and body compositions. *Agricultural Science*, *4*(7), 317-323. doi:10.4236/as.2013.47045

Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, *285*(1-4), 146-158. doi:10.1016/j.aquaculture.2008.08.015

William, K. C. (2009). A review of feeding practices and nutritional requirements of postlarval groupers. *Aquaculture*, *292*(3-4), 141-152. doi:10.1016/j.aquaculture.2009.04.026