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Taste preference of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) for nucleoside and nucleotides

Leong-Seng Lim*, Rian Freddie Firdaus, Rossita Shapawi and Gunzo Kawamura

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

*Corresponding author: leongsen@ums.edu.my

Abstract

This study was conducted to determine the taste preference of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) for nucleoside (inosine - INO) and nucleotides (inosine-5'-monophosphate – IMP and guanosine-5'-monophosphate - GMP), which are commonly used as feeding stimulants in fish diets. Behavioural assay was conducted by feeding the fish with the agar gel pellets that contained the taste substances and observing their feeding response through video recording. Preference Index (*PrfInd*; minimum = 0, maximum = 1) was calculated to evaluate the fish preference for the taste substances tested. Data so collected suggested that INO, IMP, and GMP were generally preferable by the hybrid grouper. However, INO was the most potential FS for both S and L sizes (19.7 ± 1.4 g and 162.3 ± 50.5 g in BW, mean ± S.D.) hybrid grouper based on its functional dosage. In the S size fish, INO attained high *PrfInd* at both 0.1% and 1% (0.84 and 1) concentration levels, while IMP and GMP attained high *PrfInd* at 1% (both 1) but low *PrfInd* (0.6 and 0.13, respectively) at 0.1%. In the L size fish, INO also attained very high *PrfInd* at 0.01% (0.87) but IMP and GMP were not (0.13 and 0). In addition, improvement in the taste acceptance to INO of the hybrid grouper was evident in this study. The S size fish rejected INO at 0.01% (*PrfInd* = 0) but the L size fish accepted it very well (0.87). These results suggested that the supplementation level of INO in the practical diets for the hybrid grouper can be decreased with the fish growth during the grow-out period.

Keywords: Feeding stimulants, Feed development, Feeding, Behaviour, Growth, Hybrid grouper

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Introduction

Epinephelinae groupers are popular species for mariculture especially in the Southeast Asian countries (Sadovy et al., 2013). In grouper farming, feed usually represents more than 50% of the total of production cost (Pomeroy et al., 2006; Afero et al., 2010). Groupers are carnivorous fish requiring a high dietary protein. Fishmeal (FM) is the most reliable dietary protein source for groupers but its price is increasing (Tacon et al., 2008) and its use is not environment-friendly. Therefore, various alternative protein sources have been tested for their potential to replace the FM used in grouper diets. Nevertheless, diets with alternative protein sources generally come with the palatability issue especially for those with the plant proteins. Feeding stimulant dietary supplementation is, therefore, needed to solve this problem (Lim et al., 2014).

In 2007, Borneo Marine Research Institute (BMRI) of Universiti Malaysia Sabah introduced the hybrid of brown-marbled grouper, *Epinephelus fuscoguttatus* and giant grouper, *Epinephelus lanceolatus* (Ch'ng and Senoo, 2008) to the grouper farming industry. Since then, the culture of this fish has been expanding (Mustafa, 2012) due to its hybrid vigor which results in higher hatching and survival rate, faster growth, greater resilience to culture conditions and relatively more disease resistance than the parental stock (Mustafa et al., 2013; De et al., 2014; Koh et al., 2016; Bunlipatanon and U-taynapun, 2017). Recently, attention has been given to the nutritional requirement of this fish for developing practical diets (Rahimnejad et al., 2015; Jiang et al., 2016). Firdaus et al. (2016) also reported that the soybean meal-based diets are acceptable to this fish. However, there is still no information on the suitable feeding stimulant for this hybrid grouper. Some studies have reported that betaine is a feed enhancer and it can be used to enhance the feed intake of soybean meal-based diet in the *E. fuscoguttatus* (Lim et al., 2015a, b, 2016a).

Nucleotides and nucleosides are among the taste substances that have been commonly used as feeding stimulant in fish feeds (Kasumyan and Døving, 2003; Li and Gatlin III, 2006). However, it is unknown if these substances can be feeding stimulant in groupers. Although Lin et al. (2009) reported that the dietary supplementation of nucleotides can enhance the growth performance and immune response of the malabar grouper (*E. malabaricus*), the potential of nucleotides as feeding stimulant could not be evaluated as the feeding rate in that study was fixed. Taste preference in fish is species-specific and it may change with the fish growth (Kasumyan and Døving, 2003). Therefore, the present study was designed to determine the taste preference of the hybrid grouper belonging to two different grown-out sizes for nucleoside (inosine - INO) and nucleotides (inosine-5'-monophosphate – IMP and guanosine-5'-monophosphate - GMP).

Materials and Methods

Experimental tank system setup for behavioural examination

A tank–based rearing system to observe the feeding behaviour of the grouper was setup following the design described by Lim et al. (2016a). Figure 1 show the schematic diagram of the experimental tank system. Three square-shaped fiberglass tanks (150 L each) were used in this system. The tanks were placed adjacent to each other, and supplied with flow-through seawater and aeration. A plastic basket with pulley system was setup above the tanks. This basket served as the holder for the video camera (Olympus Brand, TG 2 model, Japan), while the pulley allowed the observer to shift the camera from tank to tank without causing any disturbance to the fish. The tank system was surrounded by a horticultural mesh net (50% light penetration) to protect the fish from being stressed by the movement of the observer during the experimental period.

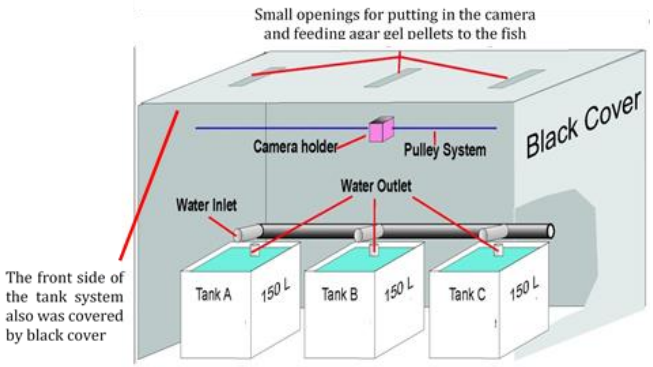

Figure 1. Schematic diagram of the experimental tank rearing system

Preparation of agar gel pellet

Table 1 shows the ingredients and the types of agar gel pellet used in the present study. Three types of agar pellets were prepared: (1) Test Substance - TS pellet, (2) Pure Agar Gel - PAG pellet, and (3) For-Training – FT pellet. The TS pellets contained each nucleotide or nucleoside (INO, IMP or GMP) at different inclusion levels (0.001%, 0.01%, 0.1% or 1.0% of the total volume of filtered seawater added to the agar gel powder) tested. The PAG pellet served as the negative control (without any taste substance), and the FT pellet (which contained the essence of Otohime commercial pellets) was used to condition the fish to accept the agar gel pellet before the experiment started.

The method to prepare the agar gel pellets was adopted from Lim et al. (2016a). In general, all agar gel pellets were prepared by mixing the agar gel powder and red food dye with the filtered seawater then boiled in a microwave. For the TS pellets, each nucleotide or nucleoside (INO, IMP or GMP) was added into the boiled mixture, well-shaken then poured into a glass petri dish to allow it to harden. To obtain the aqueous extract of Otohime for the preparation of FT pellet, 10 g of Otohime commercial pellet was soaked in 100 ml of filtered sea water for at least 45 minutes with occasional stirring until the water colour turned dark brown. The mixture was then filtered through a 60 µm microns net to obtain the aqueous extract. The hardened agar then was cut into approximately 1 cm x 1 cm x 0.5 cm size pellets and kept in a refrigerator at 4 °C until further use. All agar gel pellets were kept in the refrigerator for not more than 4 days to maintain their freshness.

Table 1. Ingredients and formulation of the agar gel pellets used in the present study

Ingredients	*Treatments*		
	Pure agar gel (PAG)	**FT Pellet (PAG + Otohime extract)**	**TS pellet (PAG + Test substance)**
Filter seawater	10 ml	N/A	10 ml
Agar gel powder [1]	0.2 g	0.2 g	0.2 g
Red food dye [2]	0.01 g	0.01 g	0.01 g
Otohime pellet's extract	N/A	10 ml	N/A
Test substances [3]			
Guanosine – 5'–monophosphate (GMP) or Inosine – 5' – monophosphate (IMP) or Inosine (INO) at			
- 0.001%	N/A	N/A	0.0001 g
- 0.01%	N/A	N/A	0.001 g
- 0.1%	N/A	N/A	0.01 g
- 1.0%	N/A	N/A	0.1 g

[1] Mermaid Brand, Thailand
[2] Ponceau 4R, Meebo Brand, Malaysia
[3] All Sigma Brand

Fish specimens

In this study, the taste preference of two size groups (small, S and large, L) of hybrid grouper was determined in two separate experiments. The fish specimens were procured from local fish farmers and acclimatized in the tank system for 2 weeks prior to the conditioning. For the S size fish, 30 specimens were stocked in each tank and for the L size only 10 fish were kept in each tank. During the acclimation period, the fish were fed with the marine finfish commercial pellet (Otohime Brand, EP3 Type, Marubeni Nisshin Feed Ltd. Co., Japan) twice daily. When the conditioning started, body weight and total length of the S and L sizes fish were 19.7 ± 1.4 g and 162.3 ± 50.5 g (mean ± S.D.), and 10.1 ± 0.1 cm and 20.0 ± 1.1 cm, respectively.

Conditioning of fish for accepting agar gel pellet

The fish were conditioned to accept agar gel pellet using the FT pellet. The conditioning was conducted in the afternoon at about 4 pm daily. At first, the fish were deprived of food for 24 hours then fed with 10 pieces of FT pellets in each tank. If the fish consumed all the FT pellets given, they were offered Otohime commercial pellet until satiation and vice versa. The conditioning was completed when the fish ingested all FT pellets given continuously for 3 days, and the behavioural experiment started (Lim et al., 2016a).

Experimental procedures and data collection

1. Video recording

The video camera was placed in the movable holder and then operated for recording the behavior. The experiment was started by giving several FT pellets to the fish in a tank to induce their feeding state. Then, a TS pellet was given to the fish in the midst of providing them the FT pellets. The video recording continued until the fish showed no interest in any of the agar gel pellets given (usually about 30 minutes). Subsequently, the camera was shifted to the other tank and the same procedure was repeated. The video recording was completed when the feeding behaviour of fish in all tanks (3 tanks, each tank represented a replicate) was recorded. After the video recording, all the fish were offered (as a reward!) Otohime commercial pellet until satiation. In total, each type of TS pellet treatment was repeatedly tested for 15 times (5 times in each replicate tank of fish) in a random sequence. Throughout the video recording, water temperature, salinity and pH were 28 – 30°C, 29 – 31 ppt, and 7.6 – 7.8, respectively.

2. Video and data analysis

From the recorded videos, two behavioural parameters were evident: (A) – ingestion or rejection of the TS pellet, and (B) – frequency of capturing the TS pellet before final ingestion/ rejection. These parameters were then used to evaluate the taste preference of the fish for the particular TS pellet to calculate the Preference Index (*PrfInd*) by (A)/ (B). For parameter (A), 1.0 was recorded if the TS pellet offered to the fish were ingested. The value '0' was recorded if the TS pellet were rejected. Therefore, the maximum score for the *PrfInd* was 1.0, indicating that the fish ingested the TS pellet in just a single capture attempt without any sort of manipulation of the feed. If the frequency of capturing increased, value of the *PrfInd* decreased, indicating that the fish finally ingested the TS pellet after manipulating it which also indicated their lesser preference for the TS pellet. On the other hand, the minimum value (0) indicated total rejection of the TS pellet irrespective of the number of attempts for probing or procuring it (Lim et al., 2016a).

Statistical analysis

Statistical analysis was conducted using Systat13 computer software. Kruskal Wallis Test was used to determine the significance of difference (P<0.05) among the data, and Conover-Inman Test (post-hoc test) was performed to evaluate the significance of difference in each set of treatment data (Lim et al., 2016a).

Results and Discussion

This is the first report on the taste preference of the hybrid grouper (*E. fuscoguttatus* × *E. lanceolatus*) for nucleotides – GMP and IMP, and nucleoside – INO. The *PrfInd* of S and L size groups of the fish for different concentrations of the tested substances are shown in Figure 2. In general, all GMP, IMP, and INO at 1% were completely accepted (*PrfInd* = 1), hence they were all potential feeding stimulant for the hybrid grouper. Nevertheless, the *PrfInd* for GMP, IMP, and INO decreased generally when they were tested at lower concentrations. For GMP and IMP at 0.1%, both S and L size groups of the fish showed significantly lower (P< 0.05) *PrfInd* (S size group: 0.13 and 0.6; L size group: 0.5 and 0.6) than those at 1%. At 0.01%, the S size group fish completely rejected (*PrfInd* = 0) both GMP and IMP; the L size group fish rejected GMP but achieved very low *PrfInd* for IMP (0.13). Interesting results were found in the INO treatment. Although the *PrfInd* for INO in both sizes of fish significantly decreased when the concentration levels tested were reduced, the *PrfInd* still remained high (0.84 – 1). In the S size fish, the *PrfInd* for INO at 0.1% was 0.84 while in the L size fish, the *PrfInd* for INO at 0.1% and 0.01% were 1 and 0.87, respectively. Based on these functional dosage results, INO is therefore recommended as the most potential feeding stimulant for the hybrid grouper. In fact, INO has also been reported as effective feeding stimulant for many other fish species in previous studies, including turbot, *Scophthalmus maximus* (Mackie and Adron, 1978), brill, *Scophthalmus rhombus* (Mackie and Mitchell, 1985), rainbow trout, *Salmo gairdneri* (Mearns et al., 1987) and marble goby, *Oxyeleotris marmorata* (Lim et al., 2016b) but not for yellowtail, *Seriola quinqueradiata* (Takeda, 1980), jack mackerel, *Trachurus japonicus* (Ikeda et al., 1988; 1991) and largemouth bass, *Micropterus salmoides* (Kubitza et al., 1997), although all these fish are carnivorous. Apparently, the taste preference of fish for INO is species-specific as suggested by Li and Gatlin III (2006) and it is not due to their feeding habit.

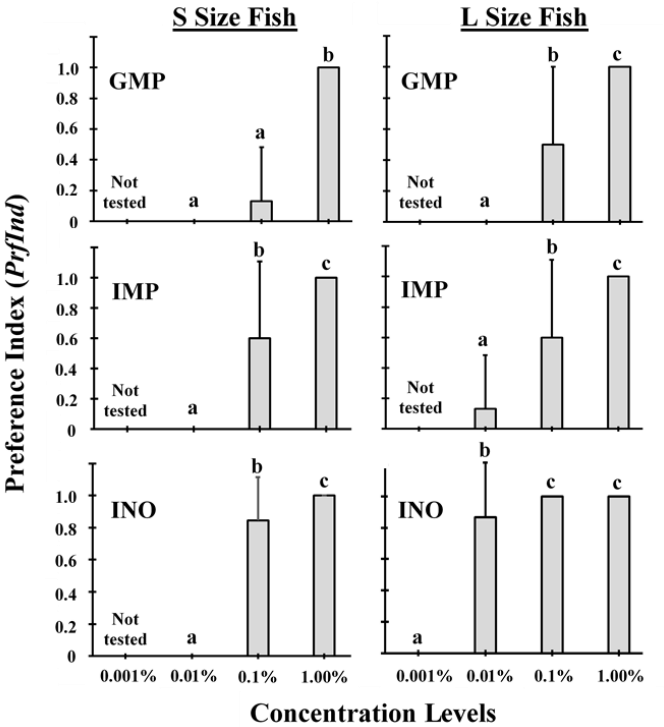

Figure 2. Preference Index (*PrfInd*) for the agar gel pellets with different concentrations of the taste substances tested on the S and L size groups of the hybrid grouper

In the present study, the changes in taste preference for/ acceptance to GMP, IMP, and INO in the hybrid grouper with growth were also evident. In the S size fish, the threshold for accepting all the taste substances tested was at 0.1%; however, the L size fish could accept IMP (but poorly) and INO at 0.01%. Although the L size fish rejected GMP at 0.01%, the *PrfInd* for GMP at 0.1% was higher than that for the S size fish. These results suggested that the taste preference or acceptance for GMP, IMP, and INO in the hybrid grouper has improved with growth, probably due to the development of the functional taste organs. Indeed, *E. fuscoguttatus* can accept lower concentration of betaine as the functional feed enhancer with growth (Jamil et al., In Press). Mackie et al. (1980) also reported the improvement in taste acceptability to betaine in the Dover sole, *Solea solea* (L.) when the fish grows from 2.5 g to 50 g in body weight. The improvement in the sense of taste and acceptability to the spectra of amino acids in sturgeons when the fish develops from larval to juvenile stage was also reported by Kasumyan (1999). Such information is useful in developing practical feed for the hybrid grouper as the dietary supplementation of INO can decrease with the fish growth during the grow-out period.

In conclusion, INO, IMP, and GMP are all acceptable to the hybrid grouper. However, INO is the most potential feeding stimulant based on its functional dosage, while IMP and GMP are functional feeding stimulant only at 1% concentration. Taste preference of the hybrid grouper for INO, GMP, and IMP generally improved with the fish growth. In the development of practical diets for the hybrid grouper, dietary supplementation of INO can be decreased with the growth of the fish. This study opens the way for worthwhile studies of practical importance in aquaculture nutrition.

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