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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

LIM, L.S.*, EBI, I., CHOR, W.K., KAWAMURA, G. and SHAPAWI, R.

Borneo Marine Research Institute, Universiti Malaysia Sabah
Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia
**E-mail: leongsen@ums.edu.my*

The aquaculture industry is relying on the fish meal-based compounded feeds for farming carnivorous fish species. However, the price of fish meal is increasing due to its growing demand (Tacon & Metian, 2008). Therefore, alternative protein sources have being exploited to substitute the fish meal in the compounded feeds. The Epinephelinae groupers are one of the carnivorous fish species that are highly targeted by the aquaculture industry especially in the Southeast Asia regions. Alternative protein sources from terrestrial animals and plants have been exploited to substitute fish meal in the compounded feeds for groupers (Lim *et al*., 2014). However, high inclusion level of these protein sources usually deteriorates the feeds palatability that causes reduced fish intake and growth (Luo *et al*., 2004; Usman *et al*., 2007; Shapawi *et al*., 2013). Although the similar problem in other fish species can be solved by supplementing suitable feeding stimulant or attractant into the feed formulation (Dias *et al*., 1997; Papatryphon & Soares, 2000; Xue & Cui, 2001), there is still no feeding stimulant found suitable for groupers (Lim *et al*., 2014).

Betaine is a common additive in aquafeeds. Supplementation of dietary betaine was reported to promote feed intake in many fish species (Fredette *et al*., 2000; Shankar *et al*., 2008; Tiril *et al*., 2008; Trushenski *et al*., 2011; Zakipour *et al*., 2012; Fattahi *et al*., 2013); however, it has never been tried on any grouper species. According to Shapawi *et al*. (2013), 30% of fish meal protein in diet for the juvenile brown-marbled grouper, *Epinephelus fuscoguttatus* can be replaced by soybean meal protein without any adverse effect on the fish intake and weight gain. Replacement of fish meal with soybean meal at 40% was resulted in significantly lower feed intake and growth. Therefore, the present study aimed to find out whether supplementation of dietary betaine can promote intake of the diet with 40% soybean meal in *E. fuscoguttatus*.

Three isoproteic (50%) and isolipidic (11%) experimental diets, namely the Control (fish meal-based diet), SBM40 (diet with 40% fish meal protein replaced by dehulled and defatted soybean meal protein) and BET10 (SBM40 supplemented with 1% betaine) were formulated. The diets formulation and proximate composition are as shown in Table 1. Betaine was supplemented into the diet by pre-dissolving it in the water (in room temperature, 28ºC) that was added into the mixture of ingredients during the mixing process. The feed formulation used in the present study was modified from Shapawi *et al*. (2013).

A total of 108 *E. fuscoguttatus* juveniles (BW 15.62 ± 0.25 g) were obtained from a local fish farmer. The fish were distributed evenly into 9 floating cylinder net cages (cage dimension, 50 cm diameter and 50 cm depth; stocking density 12 fish per cage) placed randomly in two fiberglass tanks (capacity of each tank, 3 tonnes) with constant flow-through sea water and aeration were provided. Each net cage in the tank was maintained at distance as least 50 cm away from the other net cages.

A 4-week feeding trial was conducted to evaluate the performance of betaine in promoting intake of soybean meal-based diet in the *E. fuscoguttatus*. In the trial, each diet was fed to the triplicate groups of fish in the floating net cages. The fish were fed twice daily in the morning and afternoon (approximately 0800h and 1400h) until they were full and showed no interest to the given feed. The flow-through seawater supply and aeration were turned off prior to the feeding so that the uneaten feed will drop at the tank bottom within the zone beneath the floating cages. After feeding, the fish were left for about 15 minutes before the uneaten feed was counted because the fish may keep the feed in mouth for a while and vomited later.

* To whom correspondence should be addressed.

Table 1. Formulation of the experimental diets and its proximate composition

Ingredients (Dry matter basis) g/ 100 g	Control	SBM40	BET10
Fish meal[a]	62.2	37.3	37.3
Soybean meal	0.0	37.2	37.2
Tapioca starch[b]	8.6	7.9	7.9
Alfa-cellulose	15.0	1.0	0.0
CMC[c]	1.5	1.5	1.5
Vitamin Premix[d]	3.0	3.0	3.0
Mineral Premix[e]	2.0	2.0	2.0
Dicalcium phosphate	1.0	1.0	1.0
Wheat gluten	3.8	3.8	3.8
Fish oil[f]	2.9	5.4	5.4
Betaine[g]	0.0	0.0	1.0
Total			
Proximate composition (%)			
Crude protein	48.9	50.0	49.4
Crude lipid	11.0	10.0	11.0
Moisture	16.2	11.8	13.7
Ash	10.0	9.8	9.6
Gross energy (Kcal /100g)	340.0	340.0	340.0

[a] Danish fish meal.
[b] Tapioca AAA brand. Bake with Me Sdn. Bhd.
[c] Carboxymethyl cellulose, Sigma Brand.
[d] Vitamin mixture (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 1 kg.
[e] 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 salenite, 0.011; Calcium carbonate, 129.274.
[f] Cod liver oil, Seven Seas Brand.
[g] Sigma Brand.

After the counting process, all the uneaten feed was siphoned out, and the flow-through seawater supply and aeration were resumed. About 20% of water was exchanged and the fish mortality was monitored daily. At the end of the trial, the fish weight was measured. The feeding intake, weight gain, specific growth rate, and survival rate of fish were calculated with the formulae listed below. All data were analysed using One-way ANOVA with Tukey's multiple range test in computer software SPSS version 17.0. Significant differences were assumed when $P< 0.05$.

Feed intake (dry matter, g fish^{-1}) = (Total feed given – Total uneaten feed)/ Fish number

Weight gain (%) = (Final fish weight – Initial fish weight)/ Initial fish weight × 100

Specific growth rate (%) = [(ln final weight – ln initial weight)/ days] × 100

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

The feed intake, weight gain, specific growth rate, and survival rate of fish fed with the experimental diets are shown as in Figure 1. The intake of BET10 (19.5 ± 0.6 g fish^{-1}) was significantly higher than that of SBM40 (15.2 ± 0.4 g fish^{-1}) and comparable to that of Control (22.3 ± 1.9 g fish^{-1}). These results evidenced that supplementation of dietary betaine can be used to promote the intake of soybean meal-based diet in juvenile grouper, similar as in the rainbow trout (*Oncorhynchus mykiss*) and juvenile cobia (*Rachycentron canadum*) reported by Tiril *et al.* (2008) and Trushenski *et al.* (2011), respectively. These results also confirmed the hypothesis in our previous study (Shapawi *et al*., 2013) that the poor diets palatability is one of the factors that caused the poor feed intake and growth in the juvenile *E. fuscoguttatus* fed SBM40. In the present study, the weight gain, specific growth rate, and survival rate of fish fed BET10 were higher (65.3 ± 1.9%, 1.80 ± 0.04% day^{-1}, and 100%) than those of Control (63.5 ± 13.2%, 1.75 ± 0.29% day^{-1}, and 94.4 ± 9.6%) and SBM40 (50.4 ± 6.1%, 1.46 ±

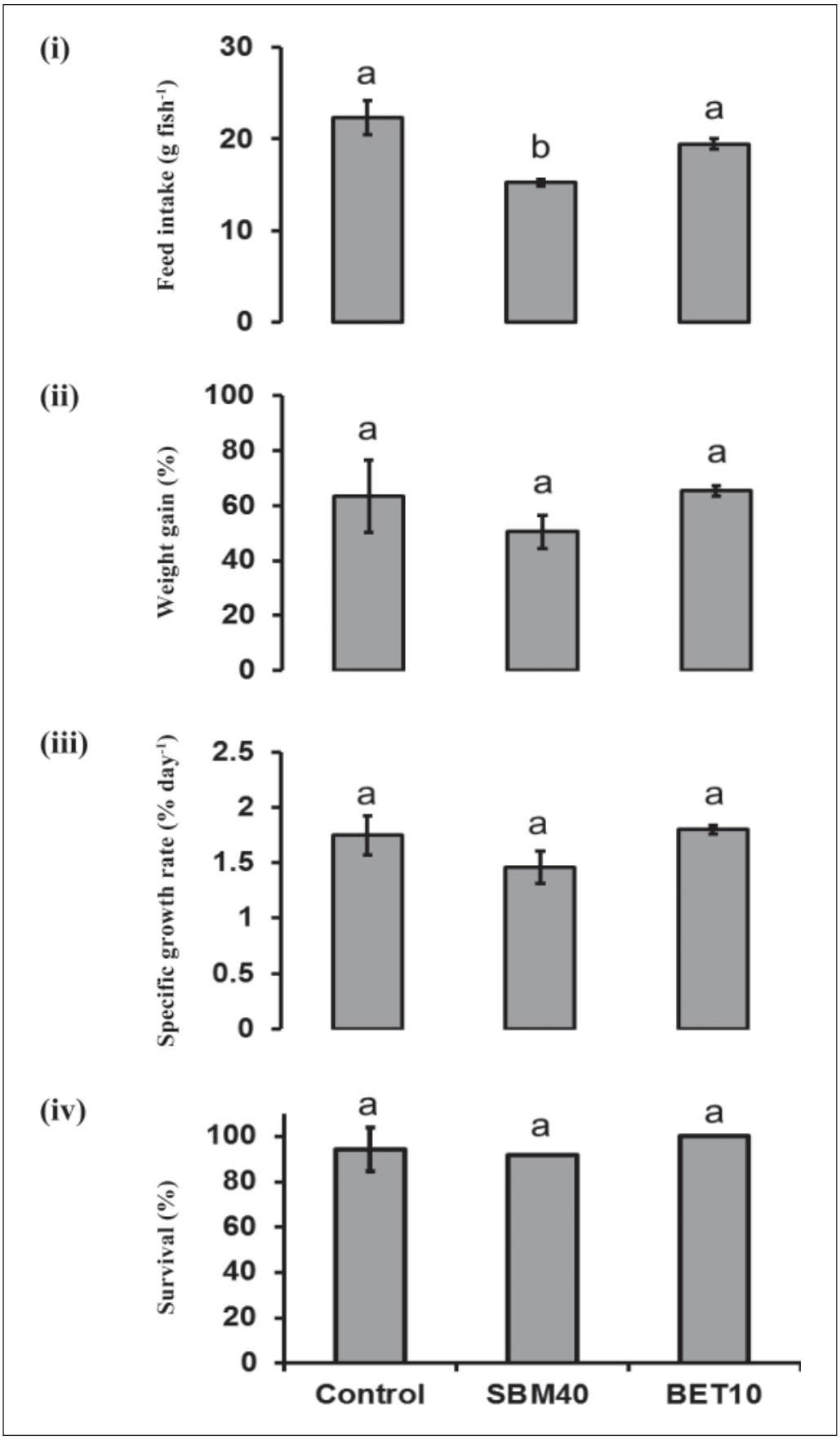

Fig. 1. Feed intake (i), weight gain (ii), specific growth rate (iii), and survival rate (iv) of juvenile groupers fed with different types of experimental diet.

0.15% day^{-1}, and 91.7 ± 1.7%) but no significant difference was found among them. Interestingly, the growth of fish fed BET10 was comparable to that of the control, suggesting that the dietary betaine supplementation may contribute other advantageous factors to the fish performance, other than the promotion in feed intake. In fact, betaine is reported as a methyl-donor which plays important roles in growth and stress mitigation in fish (Kumar *et al*., 2012a, b). It is also known as the osmo-protectant which can be used to mitigate the fish from osmotic stress (e.g. Clarke *et al*., 1994; Castro *et al*., 1998). However, duration of the present study was too short hence longer period of feeding trial (at least 8 weeks) should be conducted in future to fully evaluate the growth performance and feed utilization of the fish. In addition, it would be beneficial to determine the optimum supplementation level of betaine in the soybean meal-based diet for groupers in future.

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