Assessment on the acceptability of hybrid grouper, *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂ to soybean meal-based diets

Rian F. Firdaus, Leong-Seng Lim, Gunzo Kawamura, Rossita Shapawi

Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia. Corresponding author: L.-S. Lim, leongsen@ums.edu.my

Abstract. This study was conducted to evaluate the acceptability of hybrid grouper, *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂ on diets with different inclusion levels of soybean meal. A total of five dietary treatments with 0, 30, 40, 60, and 80% of fish meal protein replaced by soybean meal (SBM) protein (namely SBM0, SBM30, SBM40, SBM60, and SBM80, respectively) were fed to triplicate groups of fish for 14 days, and the feed intake, weight gain, specific growth rate, and survival were evaluated. The overall feed intake (mean ± SD, n = 3) of fish fed all dietary treatments with SBM inclusion (SBM30, 13.6±0.9 g fish^{-1}; SBM40, 14.3±1.6 g fish^{-1}; SBM60, 13.9±1.6 g fish^{-1}) were not significantly different ($p > 0.05$) with that of the SBM0 (15.4±1.2 g fish^{-1}), except SBM80 (9.4±2.9 g fish^{-1}). The fish acceptance to SBM30, SBM40, and SBM60 were also as good as the SBM0 diet in the feed intake calculated once every 2 days. The fish fully accepted SBM30, SBM40, and SBM60 diets after 2 weeks as the feed intake at 14-d were significantly higher ($p < 0.05$) than those at 2-d, similarly to the SBM0. In conclusion, the hybrid grouper can accept diets with maximum SBM inclusion level at 60% without any diets palatability problem. However, the fish fed SBM40 and SBM60 showed significantly lower ($p < 0.05$) weight gain and specific growth rate than the fish fed SBM0, respectively. These results confirmed the poor utilization of SBM40 and SBM60 by the fish. Therefore, the research focus in future to develop practical diets with high SBM inclusion level for this hybrid grouper should be emphasized to improve the feed utilization instead of the diets palatability.

Key Words: hybrid grouper, feeding, growth, plant-based protein, pratical diets.

Introduction. In grouper aquaculture, feed is one of the major expenses in the total production cost (Pomeroy et al 2006; Afero et al 2010). As groupers are carnivorous fish species, the aquafeed industry has heavily relying on fish meal (FM) as the main protein source in their feeds formulation. However, the price of FM is hiking (Tacon & Metian 2008). Therefore, many alternative protein sources have been exploited to substitute the FM in groupers diet, and soybean meal (SBM) has been identified as the most potential plant-based protein for this purpose due to its high protein content (Lim et al 2014). In fact, Shapawi et al (2013) reported that 30% of FM protein in the diet for juvenile *Epinephelus fuscoguttatus* can be replaced by SBM protein without compromising the fish growth. Nonetheless, higher inclusion levels of SBM (> 30%) can reduce the diets palatability and resulted poor feed intake and slow growth in the fish. Therefore, supplementation of suitable dietary taste enhancer, such as betaine (Lim et al 2016) is necessary to solve this problem (Lim et al 2015a, b).

The hybrid grouper (*E. fuscoguttatus* ♀ × *E. lanceolatus* ♂) was first produced at the Borneo Marine Research Institute of Universiti Malaysia Sabah (Ch'ng & Senoo 2008) and now has become a very popular fish for mariculture in the south-east Asia region (Mustafa 2012). This hybrid grouper was reported to attain certain types of hybrid vigor, such as fast growth (De et al 2014), and high tolerances to low water salinity (Liang et al 2013) and low water pH (Mustafa et al 2013). Recently, the nutritional requirements of this hybrid grouper have been reported (Fitriyani et al 2015; Rahimnejad et al 2015). However, there is still no published study on its feeding behaviour. It is unknown if it would possess any hybrid vigor in its feeding behaviour, which can be contributed to improve the feed formulation for its culture.

Food selection and ingestion in fish are generally mediated by their taste sense. By capturing the food into mouth and assess the food palatability by its intra-oral taste buds, fish will ingest the food with its preferred taste and vice versa (Kasumyan & Døving 2003). According to our previous study through behavioural assays, the juvenile *E. fuscoguttatus* × *E. lanceolatus* showed high preference and willing to accept wider range of single amino acids than the *E. fuscoguttatus* (Lim unpublished data). These results suggested that the hybrid grouper may accept wider range of food choice (which also explain for their faster growth) than its maternal species (*E. fuscoguttatus*). For this reason, it is hypothesized that the hybrid grouper may accept diets with higher SBM inclusion levels (> 30%) than the *E. fuscoguttatus*. Such information is crucial to reduce the cost of feed production for the hybrid grouper culture. Therefore, the present study was conducted to evaluate the acceptability of juvenile hybrid grouper (*E. fuscoguttatus* × *E. lanceolatus*) on the formulated diets with different inclusion levels of SBM.

Material and Method

Preparation of diets. Table 1 shows the formulation and proximate of the experiment diets prepared in the present study. Five isoproteic and isolipidic (crude protein and lipid levels at 47% and 12%, respectively) experimental diets with different inclusion levels of dehulled and defatted SBM were formulated, namely SBM0 (FM protein diet), SBM30, SBM40, SBM60, and SBM80 (diets with SBM protein inclusion levels at 30%, 40%, 60%, and 80%, respectively). The diets formulation was modified from the studies conducted on juvenile *E. fuscoguttatus* (Shapawi et al 2013; Lim et al 2015a, b).

Table 1

Ingredients and composition of the prepared experimental diets

Ingredients (dry matter basis) g/100 g	*Control*	*SBM30*	*SBM40*	*SBM60*	*SBM80*
Fish meal[a]	62.9	44.0	37.8	25.2	12.6
Soybean meal	0.0	26.9	35.9	53.9	71.8
Tapioca starch[b]	26.2	16.6	13.4	7.0	0.5
Alfa-cellulose	0.9	0.5	0.4	0.2	0.0
CMC[c]	1.5	1.5	1.5	1.5	1.5
Vitamin Premix[d]	2.0	2.0	2.0	2.0	2.0
Mineral Premix[e]	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Fish oil[f]	4.5	6.4	7.0	8.3	9.5
Total	100.0	100.0	100.0	100.0	100.0
Proximate composition (%)					
Crude protein	47.0	47.5	47.5	47.2	47.1
Crude lipid	9.4	10.9	10.2	11.2	10.5
Moisture	8.7	7.7	8.0	8.4	9.5
Ash	8.9	11.1	10.3	9.8	9.0
Gross energy (Kcal/100g)	400.8	400.8	400.8	400.8	400.8

[a] Danish fish meal; [b] Tapioca AAA brand. Bake with Me Sdn. Bhd.; [c] Carboxymethyl cellulose, Sigma Brand; [d] Vitamin mixture (g kg^{-1} 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^{-1} 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.

Experimental conditions and procedures. This experiment was conducted in the hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah from April to May 2015. A total of 180 juvenile hybrid groupers were purchased from a local fish farmer. After acclimatization, the fish (body weight, 23.3±4.1 g, mean±SD) were distributed randomly into 15 fiberglass tanks (tank capacity 150 L, 12 fish per tank), provided with aeration and flow-through filtered seawater. A 16-day feeding experiment was conducted to evaluate the acceptance of the juvenile hybrid grouper on the diets with different inclusion levels of SBM. The experimental procedures were similar with those described in Lim et al (2015a). Each experimental diet was hand-fed to triplicate groups of fish twice daily (about 09:00 and 15:00) until apparent satiation level. The uneaten feeds in each tank were counted only after the feeding has ended for about 20 minutes to ensure there was no feed kept in mouth by the fish (Lim et al 2015a). The uneaten feed was then scooped out from the tank using a hand net. Fish mortality was observed daily. At the end of the experiment, all fish were weight individually. The collected data were then used to calculate the overall feed intake, weight gain, specific growth rate and survival of the fish using the formula as following:

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 (% day^{-1}) = [(ln final weight – ln initial weight)/ days] × 100;
Survival (%) = Final fish number/Initial fish number × 100.

In addition, the feed intake for each dietary treatment were also calculated once every 2 days (cumulative of every 2 days) to evaluate the time period required by the fish to fully accept these diets. These parameters were analyzed using the One-way ANOVA and the Tukey's multiple range tests in the SPSS computer software. Significant differences were assumed when $p < 0.05$.

Results and Discussion. This is the first report on the acceptability of hybrid grouper on the formulated diets with different inclusion levels of SBM. Figure 1(i) shows the overall feed intake of the experimental diets of the hybrid grouper. Except for SBM80 (9.4±2.9 g fish^{-1}), the overall feed intake of SBM30 (13.6±0.9 g fish^{-1}, mean±SD), SBM40 (14.3±1.6 g fish^{-1}), and SBM60 (13.9±1.6 g fish^{-1}) were not significantly different ($p > 0.05$) with that of the SBM 0 (control) diet (15.4±1.2 g fish^{-1}). These results indicated that the hybrid grouper can accept diets with the maximum SBM inclusion level of 60% without any palatability issue, and this level is 2 times higher than that of its maternal species *E. fuscoguttatus* (30%). In addition to that, the acceptability of the hybrid grouper to SBM30, SBM40, and SBM60 was as good as to the SBM0 (control) diet. Figure 2 shows the feed intake of the experimental diets by the hybrid grouper calculated once every 2 days. The feed intake of all diets (except SBM80) at 14-day (mean 28.2, 27.0, 29.0, and 27.9 g fish^{-1}, respectively) were significantly higher ($p < 0.05$) than those at 2-day (mean 16.8, 15.4, 14.8, and 13.6 g fish^{-1}, respectively). Moreover, the feed intake was increasing steadily, and the trend of increment was similar to each other throughout the experiment (Figure 2(vi)). Therefore, it was proven that the *E. fuscoguttatus* × *E. lanceolatus* has acceptance to wider range of food choice, and this hybrid grouper can accept diets with higher SBM inclusion level than its maternal species *E. fuscoguttatus* without the common diets palatability problem, which can be considered as a new hybrid vigor known to this hybrid grouper.

Although the *E. fuscoguttatus* × *E. lanceolatus* can accept diets with high SBM inclusion level, formulating the practical diet for this hybrid grouper with high SBM inclusion level (40-60%) for long term usage might be hindered by the interior nutritional content. Figures 1(ii) and 1(iii) show the weight gain and specific growth rate of fish fed with the experimental diets. The weight gain of fish fed SBM40 (102.7%) and the specific growth rate of fish fed SBM60 (3.16% day^{-1}) were significantly lower ($p < 0.05$) than those fed SBM0 (148.5% and 5.69% day^{-1}, respectively). However, no significant difference ($p > 0.05$) was found between both the weight gain (121.2%) and specific growth rate (4.96% day^{-1}) of fish fed SBM30 with those fed SBM0, which were similar to the results reported by Shapawi et al (2013) on juvenile *E. fuscoguttatus*. These results clearly suggested that the hybrid grouper had poor feed utilization on high SBM inclusion level in the diets. Therefore, the optimum SBM inclusion level in the practical diet for the hybrid grouper is still recommended at 30%, similar to that of its maternal species *E.*

fuscoguttatus. For the development of practical diet with high SBM inclusion levels (40-60%) for the hybrid grouper, more research should be done on improving the utilization of the diets instead of its palatability.

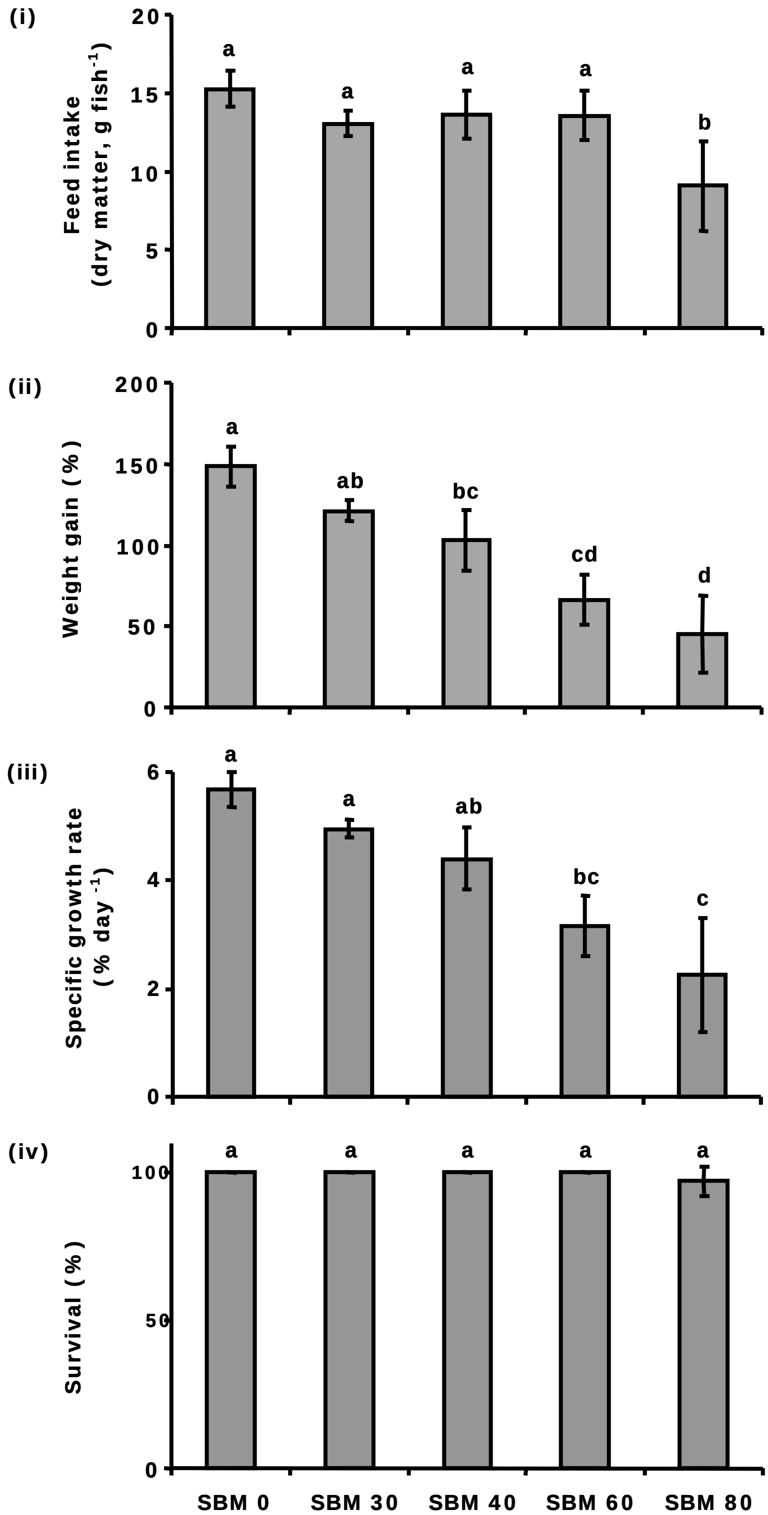

Figure 1. Overall feed intake (i), weight gain (ii), specific growth rate (iii) and survival (iv) of fish fed different treatment of experimental diets.

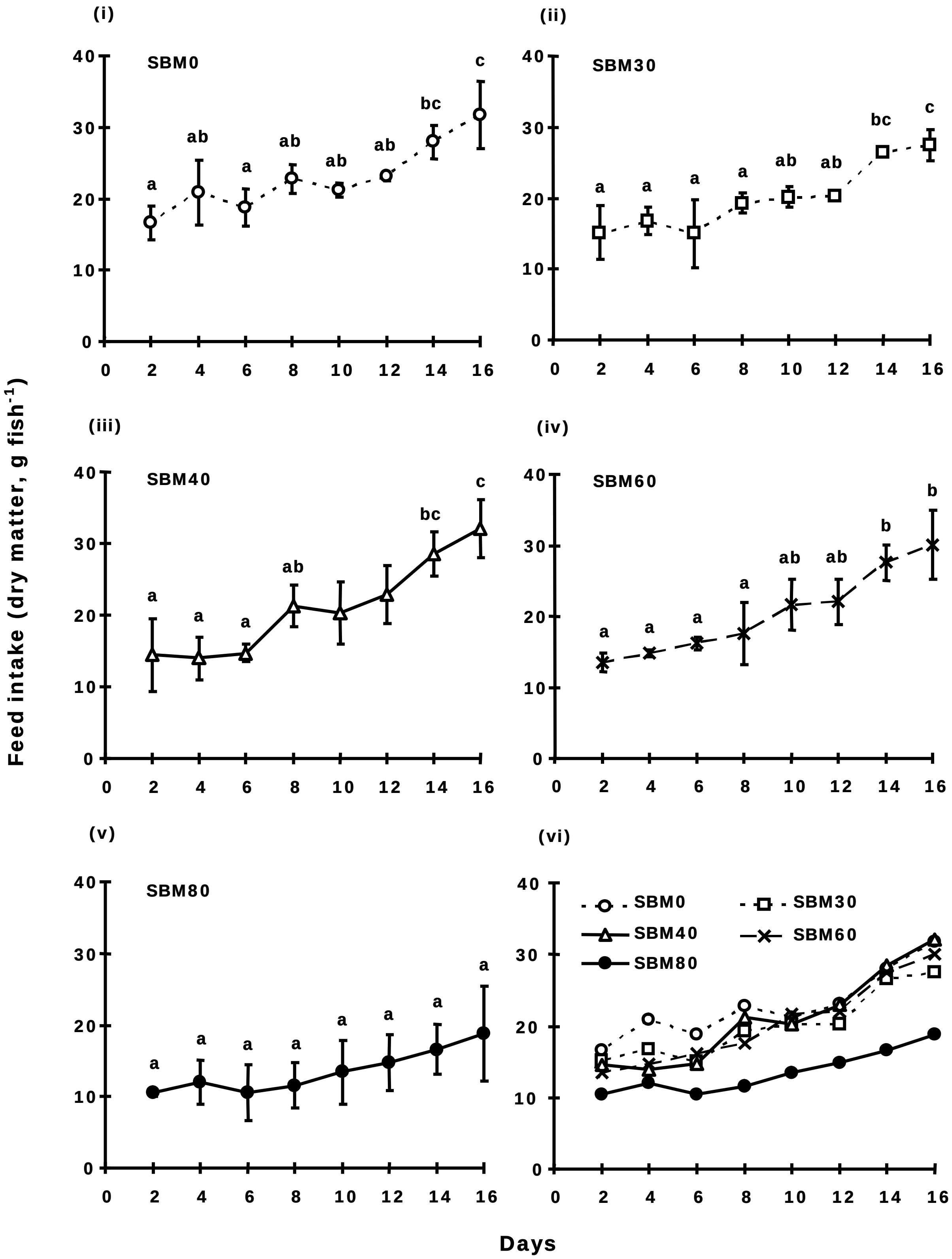

Figure 2. Feed intake of the experimental diets of the hybrid grouper calculated once every 2 days (cumulative of every 2 days).

Conclusions. The juvenile hybrid grouper (*E. fuscoguttatus* × *E. lanceolatus*) can accept diets with 30–60% inclusion levels of soybean meal without the palatability problem. However, the weight gain and specific growth rate of fish fed diets with 40–80% inclusion levels of soybean meal were poor, indicating the poor feed utilization by the fish.

Therefore in overall, the optimum inclusion level of soybean meal in the diets for the hybrid grouper was 30%.

Acknowledgements. This study was supported by the E-Science fund from the Ministry of Science, Technology, and Innovative of Malaysia (MOSTI) (project code: SCF0084-SEA-2012).

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 18:725-739.

Ch'ng C. L., Senoo S., 2008 Egg and larval development of a new hybrid grouper, tiger grouper *Epinephelus fuscoguttatus* × giant grouper *E. lanceolatus*. Aquaculture Science 56:505-512.

De M., Ghaffar M. A., Das S. K., 2014 Temperature effect on gastric emptying time of hybrid grouper (*Epinephelus* spp.). AIP Conference Proceedings 1614:616-618.

Fitriyani, Kusdianto H., Sukarti K., 2015 Effect of different dietary lipid sources on feed efficiency and feed conversion ratio of cantang grouper (*Epinephelus* sp.). Tropical Fishery Science Journal 20:8-14.

Kasumyan A. O., Døving K. B., 2003 Taste preferences in fish. Fish and Fisheries 4:289-347.

Liang H. F., Huang D. K., Wu Y. H., Wang C. Q., Zhong W. J., 2013 Effects of temperature and salinity on survival and food intake of grouper hybrid (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀). Journal of Guangdong Ocean University 33:22-26.

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 4:3071-3086.

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 44:137-141.

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(7):12-17.

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 44:415-418.

Mustafa S., 2012 Climate change adaptation in aquaculture in Sabah: strategic choices and imperatives. CCIR News 1:12.

Mustafa S., Senoo S., Luin M., 2013 Response of pure stock of coral reef tiger grouper and hybrid grouper to simulated ocean acidification. International Journal of Climate Change: Impact and Responses 5:47-54.

Pomeroy R. S., Parks J. E., Balboa C. M., 2006 Farming the reef: is aquaculture a solution for reducing fishing pressure on coral reef? Marine Policy 30:111-130.

Rahimnejad S., Bang I. C., Park J. Y., Sade A., Choi J., Lee S. M., 2015 Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile hybrid grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus*. Aquaculture 446:283-289.

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 Sciences 4:317-323.

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 285:146-158.

Received: 13 February 2016. Accepted: 14 March 2016. Published online: 05 April 2016.
Authors:
Rian Freddie Firdaus, Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia, e-mail: kenshin_emp@hotmail.com
Leong-Seng Lim, Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia, e-mail: leongsen@ums.edu.my
Gunzo Kawamura, Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia, e-mail: prof.gunzo@gmail.com
Rossita Shapawi, Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia, e-mail: rossita@ums.edu.my

How to cite this article:
Firdaus R. F., Lim L. S., Kawamura G., Shapawi R., 2016 Assessment on the acceptability of hybrid grouper, *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂ to soybean meal-based diets. AACL Bioflux 9(2):284-290.