Apparent digestibility coefficient of black soldier fly (*Hermetia illucens*) larvae in formulated diets for hybrid grouper (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂)

[1]Nor F. N. Mohamad-Zulkifli, [1]Annita S. K. Yong, [1]Gunzo Kawamura, [1]Leong-Seng Lim, [2] Shigeharu Senoo, [3]Emilie Devic, [1]Saleem Mustafa, [1]Rossita Shapawi

[1] Borneo Marine Research Institute, University of Malaysia Sabah, Sabah, Malaysia; [2] Aquaculture Technology and Production Center, Kindai University, Wakayama, Japan; [3] Entofood Sdn Bhd, Kuala Lumpur, Malaysia. Corresponding author: R. Shapawi, rossita@ums.edu.my

Abstract. Insect meal is gaining popularity as an alternative protein source to reduce the dependency on fishmeal in the formulation of aquaculture feed. In the present study, a 12-week feeding trial was carried out to examine the apparent digestibility coefficient (ADC) of dry matter (ADC_{DM}), protein (ADC_{CP}), and lipid (ADC_{CL}) for three different types of black soldier fly (*Hermetia illucens*) larvae (BSFL) meal; spray-dried BSFL (SPR), oven-dried BSFL 1 (OVN1) and oven-dried BSFL 2 (OVN2) in the diets formulated for hybrid grouper (*Epinephelus fuscoguttatus* x *E. lanceolatus*). A reference diet (RD) was formulated using fishmeal and soybean meal. Meanwhile, the test diets were formulated to contain 70% RD and 30% BSFL meal. Chromic oxide was added at 0.5% as digestibility marker. The diets were fed to juvenile hybrid groupers with an initial weight of 11.5±0.9 g, stocked at a density of 15 fish per tank. Faeces were collected from triplicate groups of fish throughout the feeding trial. The ADC values were significantly affected by the types of BSFL meal. The highest ADC_{DM} and ADC_{CP} of the test diets were observed in fish fed with RD (85.88% and 94.32%, respectively), followed by OVN1 (84.20% and 92.56%, respectively), SPR (81.77% and 91.04%, respectively), and OVN2 (78.28% and 90.93%, respectively). The ADC_{CL} of fish fed with OVN2 diet showed the highest digestibility (99.15%) which was not significantly different ($p<0.05$) from the fish fed with RD (98.94%). While, the ADC_{CL} of SPR and OVN1 diets (97.13% and 98.57%, respectively), were significantly lower ($p<0.05$) than the RD. The ADC_{DM}, ADC_{CP}, and ADC_{CL} for the three BSFL ingredients ranged from 61.36-99.30%. The highest ADC_{DM} and ADC_{CP} were observed in OVN1 BSFL meal (80.52% and 88.29%, respectively), followed by SPR (72.46% and 83.24%, respectively), and OVN2 (61.36% and 81.07%, respectively). In contrast, the highest ADC_{CL} was observed in OVN2 BSFL meal (99.30%), followed by OVN1 (98.21%) and SPR (95.24%). All the ingredients showed significantly different ($p<0.05$) results from each other in terms of ADC_{DM} and ADC_{CL}. The ADC_{CP} of SPR and OVN2 BSFL meal were not significantly different ($p<0.05$) from each other, however, both were significantly lower ($p<0.05$) than OVN1 BSFL meal. Generally, the ADC_{DM} and ADC_{CP} of the BSFL meal can be ranked as OVN2<SPR<OVN1. On the contrary, OVN2 yielded the highest ADC_{CL} among all the ingredients. Growth performance, feed efficiency and survival of the fish fed with BSFL meal-based diets exhibited no significant difference from fish fed with RD. The present findings suggested that BSFL meal is highly digestible by the fish and can be used as an excellent alternative protein source in the practical diets to replace fishmeal without affecting growth, feed efficiency and survival of hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*).
Key Words: alternative protein source, insect meal, aquaculture nutrition, fishmeal replacement, growth performance.

Introduction. Insect meal is emerging as one of the important sources of alternative protein that can replace fishmeal (FM) in aquaculture feeds. The use of insect-based feeds has been progressively discussed among fish-feed producers, scientists and policymakers (Stamer et al 2014). In general, insects contain high protein and can be easily cultured in a short period of time (Taufek et al 2016). Lipid content and fatty acid composition of insects can be controlled by rearing condition and technological processes

(Barroso et al 2014). One of the potential insects that can be used as aquaculture feed ingredient is the black soldier fly (*Hermetia illucens*) larvae (BSFL). This species contains high quality nutrients (about 40% protein and 35% lipid) which make it a promising candidate to replace fish meal in aquaculture feeds (Bondari & Sheppard 1981). In addition, mass rearing methods for the BSFL are already implemented (Henry et al 2015; van Huis et al 2013), leading to commercial production (Kroeckel et al 2012). BSFL were also reported to have a well-balanced essential amino acid profile (Alegbeleye et al 2012; Henry et al 2015) and to be a good source of minerals such as potassium, calcium, iron, magnesium and selenium and also several vitamins (Henry et al 2015).

BSFL feeds on wide range of organic materials such as municipal organic wastes, animal manures (Myers et al 2008) or kitchen wastes (Shakil Rana et al 2015) that can contribute to a circular economy. Thus, they are very sustainable to culture, and hold high potential as a good ingredient for aquaculture feed (Shakil Rana et al 2015). BSFL meal has been included in feed for various fish such as rainbow trout (*Oncorhynchus mykiss*) (St-Hilaire et al 2007), channel catfish (*Ictalurus punctatus*), blue tilapia (*Oreochromis aureus*) (Bondari & Sheppard 1987), turbot (*Scophthalmus maximus*) (Kroeckel et al 2012), gilthead sea bream (*Sparus aurata*) (Karapanagiotidis et al 2014) and European seabass (*Dicentrarchus labrax*) (Magalhães et al 2017). The present study is intended to examine the potential of BSFL meal as the protein source in practical diets for hybrid grouper, (*E. fuscoguttatus* X *E. lanceolatus*). The approach is based on gaining an understanding of the apparent digestibility coefficient (ADC) of different types of BSFL meals. Hybrid grouper is a commercially important fish species in Southeast Asia with high demand due to its appreciated taste, high resistance to diseases and fast growth. Being a strict carnivorous species, the hybrid grouper requires high protein diet. Therefore, finding sustainable alternative ingredients is an important milestone in efforts to develop cost-effective feeds for the species.

Material and Method

Feed formulation and preparation of experimental diets. A reference diet (RD) was developed to meet the nutrient requirement for hybrid grouper (50% crude protein and 12% crude lipid) using Danish fish meal and soy bean meal as protein sources. Danish fishmeal was imported from Denmark, while the defatted soybean meal was purchased from a local soybean meal supplier. The test diets include three different types of *H. illucens* larvae (BSFL) meals; spray-dried BSFL (SPR), oven-dried BSFL 1 (OVN1) and oven-dried BSFL 2 (OVN2). SPR and OVN1 were obtained from a BSF farm located in Kuala Lumpur, Malaysia, while OVN2 was obtained from an individual insect supplier in Likas, Sabah, Malaysia. The SPR and OVN1 were supplied with food industry organic by-products as their foods while for OVN2, they were fed with kitchen wastes. SPR was processed using a spray drier while for OVN1 and OVN2 meals, the larvae were dried using oven at temperature below 100ºC. Each of the BSFL was then grounded into powder form.

The test diets were prepared to contain 70% of the RD mixture and 30% test ingredients. Chromic oxide was added at 0.5% inclusion as the digestibility marker (Table 1). Diets were prepared by mixing all the ingredients into moist dough using a planetary mixer. The dough was then extruded through a 3-mm diameter die plate fitted to a screw mincer attached to a dough mixer to form pellets. The strands of the pellet were dried overnight in the oven at 40ºC. Then, the strands were reduced to pellets of approximately 3 mm length. The pellets were kept at -15ºC before they were used for the feeding trials.

Table 1

Ingredients and proximate compositions of experimental diets

Ingredients (%)	*RD*	*SPR*	*OVN1*	*OVN2*
Fish meal	51.7	36.2	36.2	36.2
Soybean meal	22.1	15.5	15.5	15.5
Fish oil	1.7	1.2	1.2	1.2
Vitamin premix[a]	3.0	2.1	2.1	2.1
Mineral premix[b]	2.0	1.4	1.4	1.4
Tapioca starch	17.0	11.1	11.1	11.1
Dicalcium phosphate	1.0	1.0	1.0	1.0
Chromic oxide	0.5	0.5	0.5	0.5
CMC	1.0	1.0	1.0	1.0
BSFL meal	-	30.0	30.0	30.0
Proximate composition (%, mean±SE)				
Moisture	9.77±0.07	10.11±0.02	10.40±0.10	10.38±0.12
Ash	11.72±0.18	11.14±0.09	10.96±0.07	10.82±0.01
Crude protein	49.24±0.16	48.19±0.07	47.15±0.09	45.04±0.14
Crude lipid	11.86±0.51	13.76±0.32	16.07±0.15	17.45±0.06

[a]Vitamin premix (contains (g/kg dry weight): ascorbic acid 45 g; inositol 5 g; choline chloride 75 g; niacin 4.5 g; riboflavin 1 g; pyridoxine HCl 1 g; thiamine HCl 0.92 g; dicalcium pantothenate 3 g; retinyl acetate 0.6 g; vitamin D3 0.08 g; menadione 1.67 g; dialpha tocopherol acetate 8 g; d-Biotin 0.02 g; folic acid 0.09 g; vitamin B12 0.001 g; cellulose. [b]Mineral premix contains (g/kg dry weight): calcium phosphate monobasic 270.98 g; calcium lactate 327 g; ferrous sulphate 25 g; magnesium sulphate 132 g; potassium chloride 50 g; potassium iodide 0.15 g; copper sulphate 0.785 g; manganese oxide 0.8 g; cobalt carbonate 1 g; zinc oxide 3 g; sodium selenite 0.011 g; calcium carbonate 129.27 g.

Fish rearing and commencement of feeding trial. This experiment was conducted in fish hatchery of Borneo Marine Research Institute, Malaysia Sabah University, Malaysia from August 2017 until November 2017. A total of 180 juvenile specimens of the hybrid grouper were purchased from a local farmer. The fish (body weight, 11.5±0.9 g) were acclimatized to the experimental conditions for a week and were fed with RD before commencing the feeding trial. The fish were then randomly allocated to 12 fiberglass tanks (tank capacity: 150 L) at stocking number of 15 fish per tank supplied with aeration, and held in a flow-through seawater system. The tanks were randomly arranged for each treatment.

During the 12-weeks feeding trial, each experimental feed was fed to triplicate group of fish twice a day at 07:00 and 16:00 until apparent satiation level. The amount of feed consumed was recorded daily and uneaten feed was scooped out from the tank using hand net, and dried before weighing. The daily feed intake was calculated by subtracting the weight of the uneaten feed from the weight of the given feed. Initial and final body weight and length of the fish were measured individually. While for every two weeks, bulk weight was determined in order to observe the growth performance of the fish.

Calculation of growth indices. The following variables were evaluated:

Weight gain (WG, %) = (final weight – initial weight) × 100 / initial weight

Specific growth rate (SGR, % day^{-1}) = {Ln (final weight) – Ln (initial weight) / duration in days} × 100

Survival (%) = 100 × (final no. of fish / initial no. of fish)

Feed intake (g fish^{-1} days^{-1}) = (dry diet given – dry remaining diet recovered) / no. of fish

Feed conversion ratio (FCR) = total dry feed fed (g) /wet weight gain (g)

$$\text{Condition factor (CF, \%)} = \text{weight of fish} / (\text{length of fish})^3 \times 100$$

Proximate composition. The experimental diets, ingredients and faeces were analyzed for proximate composition according to the Association of Official Analytical Chemist methods (AOAC 1999). The crude protein was analyzed using Kjeldahl method by a Kjeltec TM 2300 machine. Moisture and dry matter were estimated by drying the samples in the oven at 105°C until constant weight was obtained. Ash was analyzed by combustion of the samples in a muffle furnace at 550°C. Crude lipid content was measured using the Soxtec TM 2043 machine with petroleum ether extraction.

Amino acid analysis. Each BSFL meal was sent for amino acid analysis at Laboratory of Halal Services, Institut Penyelidikan Produk Halal, Putra Malaysia University, Selangor, Malaysia. The determination of amino acid profile was done using High Performance Liquid Chromatography (HPLC) with Fluorescence Detector.

Apparent digestibility coefficient. Faeces collection was carried out throughout the feeding trial to determine the apparent digestibility coefficient of test diets. After feeding, the tanks were cleaned by siphoning out any uneaten feed and other detritus in the tanks. The fresh faeces were then carefully siphoned from the tanks 3 hours after feeding, rinsed with distilled water, dried on filter paper and immediately kept frozen until being used for analysis. Chromic oxide concentrations in the diets and faeces were determined using acid digestion method following Furukawa & Tsukahara (1966).

The apparent digestibility coefficient (ADC) for dry matter, and crude protein and crude lipid of the diets were calculated using the following equations (Cho & Kaushik 1990):

$$\text{ADC of dry matter} = 100 \times [1 - (\text{\% dietary chromic oxide} / \text{\% faeces chromic oxide})]$$

$$\text{ADC of nutrient} = 100 \times [1 - (\text{\% faeces nutrient} / \text{\% dietary nutrient}) \times (\text{\% dietary chromic oxide} / \text{\% faeces chromic oxide})]$$

The ADC of the test ingredients were calculated based on the digestibility of the RD and test diets using the equation by Bureau & Hua (2006):

$$ADC_I = ADC_{TD} + (ADC_{TD} - ADC_{RD}) \times (0.7 \times D_{RD} / 0.3 \times D_I)$$

Where: ADC_I = ADC of the test ingredients; ADC_{TD} = ADC of the test diet; ADC_{RD} = ADC of the reference diet; D_{RD} = % nutrient of the reference diet; D_I = % nutrient of the test ingredients.

Statistical analysis. Data on survival, growth performance, feed utilization, and apparent digestibility coefficient were subjected to one-way analysis of variance (ANOVA) and Duncan's post-hoc test with the significant difference of $p<0.05$ in Statistical Packages of Social Sciences Version 20. The average data collection was measured using mean ± standard error (SE) with triplicates in each treatment.

Results

Nutritional composition of BSFL meal. Table 2 shows the proximate analysis of each BSFL meal and fish meal (FM) used in the experimental diets and amino acid composition of the BSFL meals. The amount of protein in FM was the highest among all the ingredients. Among the three BSFL meals, the highest amount of protein (48.20%) was found in SPR followed by OVN1 (47.46%) and OVN2 (39.38%). The crude lipid content of all BSFL meals (25.69–38.36%) was higher than that of FM (10.34%). OVN2 had the highest amount of lipid followed by OVN1 and SPR. The ash content in BSFL ranged from 7.26 to 8.27% as compared to 12.37% in FM.

The amino acid composition of the three BSFL meals is presented in Table 2. In all the three BSFL meals, leucine, lysine, valine, and histidine were the most abundant essential amino acid (EAA) observed, whereas aspartic acid and glutamic acid were the most abundant non-essential amino acids. Highest amino acid values were observed in SPR as compared to OVN1 and OVN2.

Table 2

Proximate and amino acid composition ingredients used in the experimental diets

Proximate composition (%)				
Ingredients	*FM*	*SPR*	*OVN1*	*OVN2*
Moisture	7.91±0.06	7.10±0.05	3.21±0.03	5.32±0.03
Crude protein	71.31±0.17	48.20±0.05	47.46±0.14	39.38±0.16
Crude lipid	10.34±0.38	25.69±0.12	28.43±0.05	38.36±0.19
Ash	12.37±0.07	8.27±0.07	8.19±0.05	7.26±0.03
Amino acid composition (%)				
Amino acid type		SPR	OVN1	OVN2
Histidine		2.77	2.70	2.08
Threonine		1.94	1.85	1.42
Arginine		2.55	2.25	1.80
Methionine		1.07	0.93	0.61
Valine		3.09	2.92	2.29
Phenylalanine		2.11	1.98	1.35
Isoleucine		2.40	2.28	1.76
Leucine		3.62	3.51	2.66
Lysine		3.60	2.87	2.44
Aspartic acid		5.09	4.55	3.30
Cysteine		0.16	0.15	0.12
Glutamic acid		6.05	5.63	4.59
Serine		2.06	1.88	1.55
Glysine		0.25	0.22	0.12
Alanine		3.06	3.00	3.13
Proline		2.86	2.81	2.30
Hydroxyproline		0.03	0.03	0.03
Tyrosine		3.09	2.60	1.71

Growth performance. Fish fed with OVN1 performed better in terms of weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) than fish fed with other diets. There were no significant difference in all the measured parameters between fish fed with RD and fish fed with SPR and OVN2. Similarly, survival rate and condition factor (CF) were not impaired by the dietary treatments (Table 3).

Table 3

Growth performance of juvenile hybrid grouper after 12 weeks feeding trial

Parameters	*RD*	*SPR*	*OVN1*	*OVN2*
Final WG	103.18±3.75[a]	88.49±4.15[a]	124.60±8.26[b]	100.54±3.99[a]
WG	818.73±26.06[ab]	659.02±33.48[a]	978.36±71.32[b]	773.94±35.34[a]
SGR	2.64±0.03[ab]	2.41±0.05[a]	2.83±0.08[b]	2.58±0.05[a]
FI	97.59±0.26[ab]	84.59±4.39[a]	110.04±5.79[b]	88.76±5.11[a]
FCR	1.06±0.04	1.10±0.02	0.98±0.02	1.00±0.01
Survival	100.00±0.00	100.00±0.00	100.00±0.00	97.78±2.22
CF	1.76±0.02	1.75±0.01	1.86±0.01	2.42±0.38

Values are means ± S.E. Within a row, means with the same letters are not significantly different (P>0.05).

Apparent digestibility coefficient. The apparent digestibility coefficient (ADC) for dry matter (ADC_{DM}), crude protein (ADC_{CP}) and crude lipid (ADC_{CP}) of the test diets was significantly affected by the types of BSFL meal (Table 4). The highest ADC for dry matter and crude protein was found in RD (85.88% and 94.32%, respectively), followed by OVN1 (84.20% and 92.56%, respectively), SPR (81.77% and 91.04%, respectively), and OVN2 (78.28% and 90.93%, respectively). In contrast, ADC_{CL} was the highest in OVN2 (99.12%) followed by RD (98.94%), OVN1 (98.57%) and SPR (97.13%). The ADC_{DM} of the test diets was lower than the values of ADC_{CP} and ADC_{CL}. Meanwhile, the ADC values of the ingredients were slightly lower than those of the test diets, with a similar trend, except for OVN2 BSFL (Table 4).

Table 4

Apparent digestibility coefficient (ADC) for dry matter (ADC_{DM}), crude protein (ADC_{CP}) and crude lipid (ADC_{CL}) of the ingredients and test diets used in the experiment (%)

ADC	*RD*	*SPR*	*OVN1*	*OVN2*
		Test diets		
ADC_{DM}	85.88±0.50^{d}	81.77±0.31^{b}	84.20±0.37^{c}	78.28±0.17^{a}
ADC_{CP}	94.32±0.25^{c}	91.04±0.15^{a}	92.56±0.16^{b}	90.93±0.04^{a}
ADC_{CL}	98.94±0.06^{c}	97.13±0.11^{a}	98.57±0.06^{b}	99.15±0.10^{c}
		Ingredients		
ADC_{DM}	-	72.46±2.12^{b}	80.52±1.21^{c}	61.36±0.60^{a}
ADC_{CP}	-	83.24±1.08^{a}	88.29±0.74^{b}	81.07±1.28^{a}
ADC_{CL}	-	95.24±0.29^{a}	98.21±0.16^{b}	99.30±0.13^{c}

Values are means ± S.E. Within a row, means with similar superscript letters are not significantly different (P>0.05).

Discussion. The proximate composition of BSFL meals in the present study was either similar or slightly better than the proximate composition of BSFL used in previous studies (Kroeckel et al 2012; Magalhães et al 2017). The crude protein content of SPR1 and OVN1 obtained in this study was equivalent to the value reported by Kroeckel et al (2012), while the value for OVN2 is slightly lower (Table 2). The difference in protein content depends on the stage of development and also the processing method of the larvae used in the study (Aniebo & Owen 2010). In terms of amino acid, SPR had a slightly better profile compared to OVN1 and OVN2. In general, amino acid profile of BSFL was quite similar to fishmeal (Henry et al 2015) which had been considered as the protein with the best amino acid for fish (Oliva-Teles et al 2015). The amino acid composition of BSFL meals in the present study was similar to the amino composition of BSFL meal in the previous investigation based on Pacific white shrimp (*Penaeus vannamei*) as the target species (Cummins et al 2017). Study of Barroso et al (2014) found that amino acid profile in insects varies with the species. The amino acid profile of insects from order Diptera such as *H. illucens* and *Musca domestica* is better than soybean meal and can be a better replacement for fishmeal the fish feed formulation compare to soybean meal (Barroso et al 2014).

The values of lipid content in BSFL meals in this study were higher than the lipid values of fishmeal and BSFL meal obtained by Barroso et al (2014) with reported range value of 15.6-18.0%. However, the values are quite similar to the values observed by Newton et al (1977) and Sheppard et al (2002), with the value of ≈30%. Highest lipid content was observed in OVN2 followed by OVN1 and SPR. The developmental stages of the BSF used in the study can be one of the factors that affect the lipid composition (Barroso et al 2014) which highest lipid composition can be observed during their pre-pupae stage due to metamorphosis process (Panini et al 2017). Besides the developmental stages, feed consumed by the insects is another factor that causes variations in the fat composition of the insects (Barroso et al 2014). In this study, the supplier of SPR and OVN1 claimed that the BSFL were fed with organic industrial by-products while OVN2 were fed with kitchen and food waste. High lipid content of the OVN2 is obviously resulted from the food waste that usually contains high fat. Hence, food waste is not a healthy substrate for insects that will be used as aquaculture feed. The ash content of BSFL obtained in this study was less than the value observed by Barroso et al (2014) and Devic et al (2018) (9.3-11.6%). In BSF pupae, the ash content is much greater than the larvae ash value (Barroso et al 2014). It was also reported that the ash content of the BSF pupae contained higher amounts of minerals than the larvae.

The growth performance of fish observed in this study showed that BSFL meal can be incorporated at least up to 30% in the diets of juvenile hybrid grouper without giving any negative effects on the growth performance. Different fish species fed with BSFL meal showed similar outcome as the present study (Devic et al 2018; Karapanagiotidis et al 2014; Magalhães et al 2017). Furthermore, OVN1 is more preferable by the fish compare to OVN2 and SPR. Thus, it is suggested that the BSFL should be fed with organic by products rather than food waste and can be processed by oven-dried method to obtain a good quality of BSFL meal. It is reported that feeding substrate and processing method has impacts on the nutritional profile of the insects (Aniebo & Owen 2010; van Huis et al 2013). Further analysis on other nutritional content like minerals, fatty acid and chitin of the BSFL should be evaluated in order to investigate the full potential of the BSFL meal.

There were significant differences in ADC values in all treatments. The test diets were significantly lower in terms of ADC_{DM} than the RD. Among the test diets, OVN1 was more digestible than SPR and OVN2. Though there was a significant different in term of ADC_{CL}, the ADC values in all diets were tremendously high. The ADC_{CP} values of the BSFL-based test diets observed in this study (90-92%) are similar to those found for BSFL meal in *D. labrax* diets (91-93%) (Magalhães et al 2017) and higher than those found for *S. maximus* (81.1%) (Kroeckel et al 2012). Besides, the ADCs values for crude lipid, crude protein and dry matter in this study are also higher than the ADCs of poultry-by-product meal (Gunben et al 2014; Shapawi et al 2007) and soy protein concentrate (Mohd-Faudzi et al 2018) based-diets that were tested on grouper. ADC_{DM} (45.9%) and ADC_{CP} (76.1%) of mealworm (*Tenebrio molitor)* fed to Pacific white shrimp (*Penaeus vannamei*), were also not compatible with the ADC_{DM} and ADC_{CP} of the BSFL fed to hybrid grouper. While, North African catfish (*Clarias gariepinus*) fed with cricket meal also show high digestibility in terms of crude protein (81.21%), lipid (89.82%) and dry matter (73.97%) (Taufek et al 2016) but, they are still lower than ADCs of BSFL observed in this study. Generally, fish fed with insect-based meal diet had a greater tendency to digest dry matter, protein and lipid efficiently than those fed with ingredients from terrestrial animals and plants. Apparently, hybrid grouper can digest insect-based meal diets much better compared to other fish fed with BSFL diets and insect meal diets.

To the best of our knowledge, this is the first report on the ADC of BSFL meal in grouper species. The ADC values of BSFL meal in the present study were higher than the ADC values of BSFL meal tested in *P. maximus* (Kroeckel et al 2012). Furthermore, the ADC_{DM}, ADC_{CP}, and ADC_{CL} of OVN1, especially, were comparable to the ADC_{DM}, ADC_{CP}, and ADC_{CL} of white fishmeal and brown fishmeal fed to orange-spotted grouper (*Epinephelus coioides*) (Lin et al 2004). Besides, the ADC values of OVN1 were also better than the ADC values of other alternative ingredients such as soybean meal, peanut meal and yeast fed to the grouper, *E. coioides* (Lin et al 2004).

Conclusions. It can be concluded that BSFL meal-based diets and BSFL meal ingredients were highly digestible by the hybrid grouper, and the performance of the BSFL meal was significantly influenced by the processing method. The ADC_{DM} and ADC_{CP} of the BSFL can be ranked as OVN2<SPR<OVN1. On the contrary, the ADC_{CL} of OVN2 yielded the best results among all the tested ingredients.

Acknowledgements. This study was supported by Ministry of Education Malaysia (Niche Research Grant Scheme, code NRGS 0004).

References

Alegbeleye W., Obasa S., Olude O., Otubu K., Jimoh W., 2012 Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for

African catfish *Clarias gariepinus* (Burchell 1822) fingerlings. Aquaculture Research 43:412-420.
Aniebo A. O., Owen O. J., 2010 Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica* Linnaeus) meal (HFLM). Pakistan Journal of Nutrition 9:485-487.
Barroso F., Haro C., Sánchez-Muros M., Venegas E., Martínez-Sánchez A., Pérez-Bañón C., 2014 The potential of various insect species for use as food for fish. Aquaculture 422-423:193-201.
Bondari K., Sheppard D., 1981 Soldier fly as feed in commercial fish production. Aquaculture 24:103-109.
Bondari K., Sheppard D. C., 1987 Soldier fly, *Hermetia ilucens* Diptera Stratiomyidae egg masses timing and site characteristics. Environmental Entomology 13(2):421-423.
Bureau D. P., Hua K., 2006 Letter to the editor of Aquaculture. Aquaculture 252:103-105.
Cho C. Y., Kaushik S. J., 1990 Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Review of Nutrition and Dietetics 61:132-172.
Cummins V. C., Rawles S. D., Thompson K. R., Velasquez A., Kobayashi Y., Hager J., Webster C. D., 2017 Evaluation of black soldier fly (*Hermetia ilucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 743:337-344.
Devic E., Leschen W., Murray F., Little D. C., 2018 Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing Black soldier fly (*Hermetia illucens*) larvae meal. Aquaculture Nutrition 24:416-423.
Furukawa A., Tsukahara H., 1966 On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish diet. Bulletin of the Japanese Society of Scientific Fisheries 32:502-506.
Gunben E. M., Senoo S., Yong A., Shapawi R., 2014 High potential of poultry by-product meal as a main protein source in the formulated feeds for a commonly cultured grouper in Malaysia (*Epinephelus fuscoguttatus*). Sains Malaysiana 43(3):399-405.
Henry M., Gasco L., Piccolo G., Fountoulaki E., 2015 Review on the use of insects in the diets of farmed fish: past and future. Animal Feed Science Technology 203:1-22.
Karapanagiotidis I., Daskalopoulou E., Vogiatzis I., Rumbos C., Mente E., Athanassiou C., 2014 Substitution of fishmeal by fly *Hermetia ilucens* prepupae meal in the diet of gilthead seabream (*Sparus aurata*). HydroMediT, November 13-15, Volos, Greece, pp. 110-114.
Kroeckel S., Harjes A.–G. E., Roth I., Katz H., Wuertz S., Susenbeth A., Schulz C., 2012 When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black soldier fly (*Hermetia ilucens*) as fish meal substitute. Aquaculture 364-365:345-352.
Lin H., Liu Y., Tian L., Wang J., Zheng W., Huang J., Chen P., 2004 Apparent digestibility coefficients of various feed ingredients for grouper *Epinephelus coioides*. Journal of the World Aquaculture Society 35(2):134-142.
Magalhães R., Sánchez-López A., Leal R., Martínez-Llorens S., Olivia-Teles A., Peres H., 2017 Black soldier fly (*Hermetia ilucens*) pre pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476:79-85.
Mohd-Faudzi N., Yong A., Shapawi R., Senoo S., Biswas A., Takii K., 2018 Soy protein concentrate as an alternative in replacement of fish meal in the feeds of hybrid grouper, brown-marbled grouper (*Epinephelus fuscoguttatus*) x giant grouper (*E. lanceolatus*) juvenile. Aquaculture Research 49:431-441.
Myers H. M., Jeffery K. T., Barry D. L., David K., 2008 Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Physiological Ecology 37(1):11-15.

Newton G. L., Booram C. V., Barker R. W., Hale O. M., 1977 Dried *Hermetia illucens* larvae meal as a supplement for swine. Journal of Animal Science 44(3):395-400.
Oliva-Teles A., Enes P., Peres H., 2015 Replacing fishmeal and fish oil in industrial aquafeed. Feed and feeding practices in aquaculture. In: Feed and feeding practices in aquaculture. Davis A. (ed), pp. 203-233, Woodhead Publishing Limited, Waltham, MA, USA.
Panini R. L., Freitas L. E. L., Guimarães A. M., Rios C., da-Silva M. F. O., Viera F. N., Fracalossi D. M., Samuels R. I., Predêncio E. S., Silva C. P., Amboni R. D. M. C., 2017 Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. Aquaculture 473:115-120.
Shakil Rana K. M., Salam M. A., Hashem S., Ariful Islam M., 2015 Development of Black soldier fly larvae production technique as an alternate fish feed. International Journal of Research in Fisheries and Aquaculture 5(1):41-47.
Shapawi R., Ng W. K., Mustafa S., 2007 Replacement of fish meal with poultry by-product meal in diets formulated for the humpback grouper, *Cromileptes altivelis*. Aquaculture 273:118-126.
Sheppard D. C., Tomberlin J. K., Joyce J. A., Kiser B. C., Sumner S. M., 2002 Rearing methods for the Black soldier fly (Diptera: Stratiomyidae). Journal of Medical Entomology 39(4):695-698.
Stamer A., Wesselss S., Neidigk R., Hoerstgen-Schwark G., 2014 Black soldier fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets. In: Proceedings of the 4th ISOFAR Scientific Conference: 'Building Organic Bridges'. Rahmann G., Aksoy U. (eds), Organic World Congress 2014, 13-15 Oct., Istanbul, Turkey.
St-Hilaire S., Cranfill K., McGuire M., Mosley E., Tomberlin J., Newton L., Sealey W., Sheppard C., Irving S., 2007 Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of The World Aquaculture Society 38:309-313.
Taufek N., Muin H., Ameenat A., Razak S. A., 2016 Apparent digestibility coefficients and amino acid availability of cricket meal, *Gryllus bimaculatus,* and fishmeal in African catfish, *Clarias gariepinus*, diet. Journal of The World Aquaculture Society 47(6):798-805.
van Huis A., Itterbeeck J., Klunder H., Mertens E., Halloran A., Muir G., Vantomme P., 2013 Edible insects: future prospects for food and feed security. In: FAO Forestry paper. No 171, 187 p.
*** Association of Official Analytical Chemists (AOAC), 1999 In Official methods of analysis of AOAC international, Cunniff P., (ed), volume 1, 5th revision, 16th ed. Washington: Association of Office Analytical Chemists International.

Received: 21 December 2018. Accepted: 11 April 2019. Published online: 20 April 2019.
Authors:
Nor Fatin Najihah Mohamad-Zulkifli, University of Malaysia Sabah, Borneo Marine Research Institute, Malaysia, Sabah, Kota Kinabalu, 88400, Jalan UMS, e-mail: naj.fatin93@gmail.com
Annita Seok Kian Yong, University of Malaysia Sabah, Borneo Marine Research Institute, Malaysia, Sabah, Kota Kinabalu, 88400, Jalan UMS, e-mail: annitay@ums.edu.my
Gunzo Kawamura, University of Malaysia Sabah, Borneo Marine Research Institute, Malaysia, Sabah, Kota Kinabalu, 88400, Jalan UMS, e-mail: kawamura@ums.edu.my
Leong-Seng Lim, University of Malaysia Sabah, Borneo Marine Research Institute, Malaysia, Sabah, Kota Kinabalu, 88400, Jalan UMS, e-mail: leongsen@ums.edu.my
Shigeharu Senoo, Kindai University, Aquaculture Technology and Production Centre, Japan, Wakayama, 649-2211, Nishimuro, 1-5, Shirahama, e-mail: sesige@mac.com
Emilie Devic, Entofood Sdn Bhd, Malaysia, 50470, Kuala Lumpur, e-mail: emilie.devic@entofood.com
Saleem Mustafa, University of Malaysia Sabah, Borneo Marine Research Institute, Malaysia, Sabah, Kota Kinabalu, 88400, Jalan UMS, e-mail: saleem@ums.edu.my
Rossita Shapawi, University of Malaysia Sabah, Borneo Marine Research Institute, Malaysia, Sabah, Kota Kinabalu, 88400, Jalan UMS, e-mail: rossita@ums.edu.my

How to cite this article:
Mohamad-Zulkifli N. F. N., Yong A. S. K., Kawamura G., Lim L.-S., Senoo S., Devic E., Mustafa S., Shapawi R., 2019 Apparent digestibility coefficient of black soldier fly (*Hermetia illucens*) larvae in formulated diets for hybrid grouper (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂). AACL Bioflux 12(2):513-522.