Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in climbing perch (*Anabas testudineus*) diet

[1]Supalug Kattakdad, [2]Narissara Suratip, [2]Bundit Yuangsoi, [1]Krittima Kasamawut, [1]Suriya Udduang

[1] Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin, Thailand; [2] Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. Corresponding author: S. Kattakdad, Supalug.ka@rmuti.ac.th

Abstract. Due to requiring of alternative protein ingredients in aquaculture, insect meal is a candidate for substitutes for fish meal. Thus, the objective of this study was to investigate the effect of substituting fish meal with black soldier fly pre-pupae meal (BSF) in diet of climbing perch on growth performance, feed utilization, biological indices, chemical composition in fillet, digestive enzyme activities and histopathological alteration in liver and intestine. The experiment was conducted as a completely randomized design with four treatments and three replicates. BSF was added to the diets to replace fish meal (FM); the replacement levels were 0% (BSF0), 25% (BSF25), 50% (BSF50), 75% (BSF75), and 100% (BSF100). Sex reversed Chomphon-1 climbing perch larvae (initial mean body weight = 3.12±0.05 g fish^{-1}; initial mean total length = 4.32±0.45 cm) were obtained from a reliable commercial farm. After a 60-day rearing period, the fish fed BSF0, BSF25 and BSF50 were found to have weight gain (WG) and specific growth rate (SGR) higher than the fish fed BSF75 and BSF100. Feed conversion ratio (FCR) was significantly lower ($p \leq 0.05$) in the fish fed BSF0. Protein efficiency ratio (PER) and condition factor (CF) showed increased trends and were significantly higher in the fish fed BSF0, BSF25 and BSF50 ($p \leq 0.05$). However, no significant differences were observed on survival rate and biological indices in terms of hepatosomatic index (HSI) and viscera index (VSI). Concerning the chemical composition in fillet, crude protein, moisture and ash were not affected among the dietary groups ($p > 0.05$). Whereas, crude fat increased with the increase in dietary BSF, the value was higher in fish fed with BSF75 and BSF100 than other dietary groups ($p \leq 0.05$). Experimental diets affected the digestive enzyme activities, which were lower protease and amylase in fish fed BSF diet while, higher lipase was observed in fish fed BSF diet than control ($p \leq 0.05$). About the histopathological alteration in the liver, no evident pathological changes were observed in the liver of each group. Intestinal histopathological and microvilli height were analyzed. The BSF75 and BSF100 groups were found to have more debris than other groups. Concerning microvilli height, there was an influence of experimental diets on the microvilli height which was higher in the BSF0, BSF25 and BSF50 groups than BSF75 and BSF100 groups ($p \leq 0.05$). Thus, this study indicates a successful replacement of 50% FM with black soldier fly pre-pupae meal (43.72% crude protein, 15.62% crude fat) in diets for climbing perch.

Key Words: black soldier fly, climbing perch, growth performance, insect meal, protein alternative.

Introduction. Climbing perch (*Anabas testudineus*) is an increasingly important fish for aquaculture in Thailand and Asian countries (Amornsakun et al 2005; Sarkar et al 2005; Zalina et al 2011; Piwpong et al 2016). This species is a suitable source of animal protein for humans due to it inhabiting both fresh and brackish water and its ability for breathing air, air-gulping behavior and tolerance for harsh environmental conditions, including impressive growth within a relatively short period of time (Sarkar et al 2005; Hasan et al 2010; Alam et al 2010). As climbing perch rearing increases, their demands for high quality feed has increased too. The growth of climbing perch is highly affected by the protein level. Previous studies have demonstrated that the optimal level of dietary protein for effective nursing of climbing perch should contain 45.00-50.92% (Alam et al 2010; Matthong et al 2015). The primary protein source in aquafeed is fishmeal (FM) which has highly digestible protein and amino acids, including high palatability (Li et al 2017). However, FM utilization is limited due to their products being unstable and the fact that the price of FM has increased by more than twofold in recent years (Xiao et al 2018). Therefore, it is necessary to replace FM by partial or total level of less expensive and high protein animal or plant ingredients. Several studies have reported that alternative plant-based proteins could serve as an aquafeed such as soybean, soy protein concentrate, corn gluten meal, vegetable meals, cottonseed meal, rapeseed meal and sunflower meal (Krogdahl et al 2003; Luo et al 2010; Nogales Merida et al 2010; Jiang et al 2013; Banuelos-Vargas et al 2014; Antonopoulou et al 2017; Torrecillas et al 2017). Nevertheless, plant-based protein ingredients often have deficiency in some amino acids, especially lysine or methionine, low palatability, contain antinutrients, and face competition with other food-feed industry sectors (Gatlin et al 2007; Glencross et al 2007; Krogdahl et al 2010; Magalhaes et al 2017).

Nowadays, insect meal has become a focus for aquafeeds due to it having several advantages, including being reared on discarded organic by-products which requires less land and low water input (Panini et al 2017), high quality and quantity of protein (9.3-76%), lipids (7.9-40%), minerals, vitamins and energy (Barroso et al 2014; Sánchez-Muros et al 2015; Henry et al 2015; Nogales-Merida et al 2019), well-balanced essential amino acid profile (Alegbeleye et al 2012; Barroso et al 2014; Henry et al 2015), high feed conversion efficiency (Shelomi 2015), low discharge of greenhouse gases and ammonia (Oonincx et al 2010). Insect meal has a few animal welfare issues and low risk of transmission of infection in animals including low environmental contamination in rearing process. Thus, it has sustainable materials for the production of aquafeed. Among different candidate species to produce insect meal, black soldier fly (*Hermetia illucens*) pre-pupae meal (BSF) is of particular interest. BSF have been used as a feed ingredient for a variety of aquatic animals, such as European seabass (*Dicentrarchus labrax*) (Magalhães et al 2017), Atlantic salmon (*Salmo salar*) (Belghit et al 2019), hybrid grouper (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂) (Mohamad-Zulkifli et al 2019), Jian carp (*Cyprinus carpio* var. Jian) (Li et al 2016; Li et al 2017), yellow catfish (*Pelteobagrus fulvidraco*) (Xiao et al 2018), Pacific white shrimp (*Litopenaeus vannamei*) (Cummins et al 2017), rainbow trout (*Oncorhynchus mykiss*) (Stadtlander et al 2017; Dumas et al 2018), clownfish (*Amphiprion ocellaris*) (Vargas-Abúndez et al 2019) and turbot (*Psetta maxima*) (Kroeckel et al 2012). The dietary replacement level of FM by BSF ranging from 6 to 25% has been reported depending on the fish species (Henry et al 2015). Considering nutritional profiles, BSF is considered to have a nutritional value close to FM (Magalhaes et al 2017). It contains 39-43% protein (Spranghers et al 2017) complete with essential amino acids, especially, tyrosine, phenylalanine and histidine (Caligiani et al 2018). BSF has a high lipid content (33.1% on dry matter basis) and is rich in lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1n9), linoleic acid (C18:2n6) (Sealey et al 2011). The fatty acid composition depends on cultivation method (Sealey et al 2011; St-Hilaire et al 2007). In addition, BSF contains minerals such as iron, zinc, potassium, phosphorus, manganese and magnesium including a variety of vitamins (Belghit et al 2018). Moreover, BSF has a natural attractant component. It can improve feed palatability and stimulate feed consumption (Nogales-Merida et al 2019).

Therefore, this study aims to evaluate the effect of partial or complete dietary replacement with BSF pre-pupae meal on growth performance, feed utilization, digestive enzyme activity, fillet quality and histopathological changes of climbing perch.

Material and Method

Experimental diets. The experimental diets were formulated with five isonitrogenous (target at 40 % crude protein). The experimental design was a Completely Randomized Design (CRD) with three replicates. BSF pre-pupae meal was obtained from the Department of Entomology Faculty of Agriculture, Khon Kaen University, the chemical composition is presented in Table 1. BSF was added to the diets to replace FM; the replacement levels were 0% (BSF0), 25% (BSF25), 50% (BSF50), 75% (BSF75), and 100% (BSF100). All diets were designed using FM, BSF, soybean meal, defatted rice bran, cassava starch and corn meal. They were prepared and pelleted (2.5 mm pellet diameter) by extruder. After drying in a cool and well-ventilated place at room temperature for 12 h, the pellets were collected and stored at -20°C until use. The experimental diets formulation and chemical composition are presented in Table 2.

Table 1

Chemical composition of dry weight basis of black soldier fly pre-pupae meal

Chemical composition (%)	*Mean±SD*
Crude protein	43.72±3.42
Crude fat	15.62±4.02
Crude fiber	9.92±1.08
Moisture	11.62±1.84
Ash	5.89±1.10
Gross energy (Kcal Kg^{-1})	4784.40±256.05

Table 2

Formulation and proximate composition of the experimental diets

Ingredients (%)	*Experimental diets*				
	BSF0	*BSF25*	*BSF50*	*BSF75*	*BSF100*
Fish meal	35	26.25	17.5	8.75	0
BSF	0	8.75	17.5	26.25	35
Soybean meal	40	42	45	47	50
Defatted rice bran	11	9	8	6	4
Cassava starch	5	5	4	4	4
Corn meal	5	5	4	4	3
Soybean oil	1	1	1	1	1
Di-calcium phosphate	1	1	1	1	1
Vitamin and mineral premix[a]	1	1	1	1	1
Vitamin C	1	1	1	1	1
Proximate composition (% dry weight basis)					
Crude protein	40.30	40.24	40.26	40.03	40.18
Crude fat	5.75	6.20	6.73	7.18	7.63
Crude fiber	4.67	5.37	6.19	6.88	7.04
Ash	12.15	12.01	12.18	12.10	12.11
Moisture	10.02	9.50	9.40	10.03	9.40
Gross energy (kcal kg^{-1})	2650	2710	2890	3002	3088

[a] Vitamin premix provided per kg of diet: V_A 12,000,000 IU; V_D 2,000,000 IU; V_E 6000 IU; V_K 2000 mg; V_{B1} 800 mg; V_{B2} 2500 mg; V_{B6} 800 mg; V_{B12} 10 mg. Mineral provided per kg of diet: Mn 18 mg; Mg 200 mg; Co 0.1 mg; I 0.25 mg; Fe 140 mg; Cu 2.5 mg; Zn 65 mg; Se 0.2 mg

Fish feeding and management. Sex reversed Chomphon-1 climbing perch larvae were obtained from a reliable commercial farm. In order to acclimatize to the rearing environment, the fish were cultured and fed a commercial diet three times daily in a floating cage for 2 weeks. Before the beginning of the feeding experiment, the experimental fish were starved for 12-16 h and weighed (initial mean body weight = 3.12±0.05 g $fish^{-1}$; initial mean total length = 4.32±0.45 cm). A total of 750 fish were randomly distributed into 15 floating cages, at a density of 50 fish per floating cage (1 x 1 x 1 m) and supplemental aeration were provided. The fish used in the experiment were equivalent in size and weight across treatments. During the 60-days feeding, the fish were hand-fed to apparent satiation 3 times daily (at 8:30, 12:30 and 16:30). The trial was conducted at the Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Thailand.

Sampling. Fish were starved for 12-16 h prior to sampling. Total feed intake was recorded weekly and 25 fish per cage were randomly weighed by total weight every two weeks with a 0.1 g sensitive electronic balance. The duration of this experiment was two months. At the end of experiment, all fish were measured for final body weight (FBW) and 10 fish per cage killed to collect data on hepatosomatic index (HSI), viscera index (VSI), chemical composition and digestive enzyme activities. Among the 10 sampled fish, 7 fish per cage were randomly selected for liver and intestinal sampling and then immediately frozen for digestive enzyme activities analysis. Muscle from another 3 fish were excised and then stored in -20°C for chemical composition analysis. Weight gain (WG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), viscera index (VSI), hepatosomatic index (HSI) were calculated via the following accepted formulae:

Weight gain (WG, g fish^{-1}) = final weight - initial weight
Specific growth rate (SGR, % day^{-1}) = ((Ln final weight -Ln initial weight) x 100)/60 days
Survival rate (SR, %) = (No. of fish harvested/ No. of fish stocked) x 100
Feed conversion ratio (FCR) = amount of feed given (g) / weight gain (g)
Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)
Condition factor (CF, g/cm^3) = body weight (g) / body length (cm)
Hepatosomatic index (HSI, %) = (liver weight (g) / body weight (g)) x 100
Viscera index (VSI, %) = (viscera weight (g) / body weight (g)) x 100

Chemical composition analysis and digestive enzyme activities

Chemical composition. Dry matter, crude protein, crude lipid, and ash analysis were undertaken for ingredients, experimental diets and fillet samples by using proximate analysis (AOAC 2005). Analysis was conducted in triplicate for each sample. Three fish from each replication were investigated for the chemical composition of fillet. The fillets were dissected from the dorsal body section. All fillets were cut above the lateral line into cubes approximately 1 × 1 × 1 cm.

Digestive enzyme activities. The fish liver and intestines of seven individuals from each cage were carefully weighed and homogenized in 0.01 M Tris buffer, pH 7.4, at a ratio of 1:3 (tissue:buffer) under an ice bath. The extract was subsequently centrifuged at 3000×g at 4°C for 10 min, the supernatant below lipid layer was collected and kept at -20°C until investigation for protein content by using Lowry method (Lowry et al 1951), and the supernatant used as the crude enzyme. The protease specific activity was determined by the modified casein method of Pan et al (2005). One unit of protease activity (U mg^{-1} protein) was defined as 1 mM of tyrosine liberated by hydrolyzing casein in 1 min. Amylase specific activity was determined by assessing the increase of reducing sugar (maltose) from the hydrolysis of ɑ-D (1,4) glycosidic bonds in polysaccharides, and stained with 3,5-dinitrosalicylic acid (DNS) by using the Bernfeld (1955) method. One unit of amylase activity (U mg^{-1} protein) was defined as 1 mM of glucose per min per mg protein. Lipase specific activity was measured according to Markweg-Hanke et al (1995) using a substrate as p-nitrophenylpalmitate (pNPP). One unit of lipase activity (U mg protein^{-1}) was defined as 1 mM of p-nitro phenol per min per mg protein.

Histological observation of liver and intestine. The fixed liver and middle intestine (3 individuals/cage) were wrapped in gauze and rinsed in running water for 24 h. For the intestinal morphology, approximately 0.5 cm length segments of midgut samples and liver were fixed with 10% formalin solution and dehydrated in ethanol, embedded in paraffin, cut by microtome at 4 µm sections, and stained with hematoxylin and eosin (H&E) according to standard histology procedures (Peng et al 2013). Tissue slides were digitally photographed with a light microscope (Nikon Eclipse Ci) equipped with a CCD camera and NIS-elements D software.

Statistical analysis. Mean values and standard deviations (S.D.) were calculated. The significance of difference among treatments was tested using One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at 0.05 of significance level. Statistical analysis was performed with IBM SPSS statistics version 20.

Results. The chemical composition of BSF pre-pupae meal is shown in Table 1. It consisted of 43.72% crude protein, 15.62% crude fat, 9.92% crude fiber, 11.62% moisture, 5.89% ash and 4784.40 kcal Kg^{-1} of gross energy. The chemical composition of experimental diets was found to have crude protein that ranged from 40.03 to 40.30%, crude fat ranging from 5.75 to 7.63%, crude fiber ranging from 4.67 to 7.04% ash ranging from 12.01 to 12.18%, moisture ranging from 9.40 to 10.03% and gross energy ranging from 2650 to 3088 kcal kg^{-1} (Table 2).

The effect of experimental diets on growth performance, feed utilization and biological indices are presented in Table 3. Growth performance showed significant difference ($p \leq 0.05$) in terms of WG and SGR in fish fed with experimental diets for 60 days. The fish fed with BSF0, BSF25 and BSF50 were showed higher WG and SGR than fish fed BSF75 and BSF100. In addition, there was significant difference in FCR, PER and CF. At the end of feeding trial, FCR was significantly lower ($p \leq 0.05$) in the fish fed BSF0. PER and CF showed increased trend and was significantly higher in fish fed BSF0, BSF25 and BSF50 ($p \leq 0.05$). However, no significant differences were observed on SR and biological indices in terms of HSI and VSI.

Table 3

Growth performance, feed utilization and biological indices of climbing perch after being fed with experimental diets for 60 days

Performance	*Experimental diets*					*P-value*
	BSF0	*BSF25*	*BSF50*	*BSF75*	*BSF100*	
Initial weight (g $fish^{-1}$)	3.16±0.07	3.15±0.05	3.09±0.03	3.09±0.03	3.12±0.03	0.175
WG (g $fish^{-1}$)	13.89±0.66^{b}	13.86±0.44^{b}	13.47±0.42^{b}	12.41±0.26^{a}	12.32±0.26^{a}	0.002
SGR (% day^{-1})	2.80±0.10^{b}	2.81±0.02^{b}	2.80±0.05^{b}	2.68±0.04^{a}	2.66±0.01^{a}	0.014
Survival rate (%)	94.07±.129	95.56±2.23	96.30±1.28	95.56±2.23	96.30±1.28	0.533
FCR	1.50a±0.05^{a}	1.59±0.05^{b}	1.61±0.02^{b}	1.67±0.09^{b}	1.66±0.01^{b}	0.008
PER	1.64±0.08^{b}	1.64±0.05^{b}	1.58±0.05^{b}	1.47±0.03^{a}	1.46±0.03^{a}	0.002
CF (g cm^{-3})	1.87±0.02^{b}	1.80±0.09^{ab}	1.80±0.04^{ab}	1.70±0.06^{a}	1.75±0.07^{a}	0.028
HSI	2.89±0.17	2.86±0.08	3.02±0.13	2.93±0.05	2.94±0.06	0.438
VSI	9.89±0.18	10.64±0.12	11.09±0.39	11.05±0.87	11.11±0.53	0.056

The values are presented as mean±SD. Different superscripts in the same row indicate significant differences at $p \leq 0.05$.

The effects of experimental diets on chemical composition in fillet are shown in Table 4. Crude protein, moisture and ash were not influenced among the dietary groups ($p > 0.05$). Whereas, crude fat increased with an increase in the dietary BSF with a high value for the fish fed with BSF75 and BSF100 than other dietary groups ($p \leq 0.05$).

Table 4

Chemical composition in fillet of climbing perch after being fed with experimental diets for 60 days (%, wet basis)

Chemical composition	*Experimental diets*					*P-value*
	BSF0	*BSF25*	*BSF50*	*BSF75*	*BSF100*	
Crude protein	24.93±0.83	25.03±0.44	24.82±0.15	24.50±0.56	24.85±0.13	0.735
Crude fat	3.27±0.06^{a}	3.30±0.10^{a}	3.30±0.09^{a}	3.59±0.03^{b}	3.64±0.05^{b}	< 0.001
Moisture	78.76±0.19	78.89±0.29	78.84±0.49	79.02±0.10	78.63±0.59	0.774
Ash	1.48±0.12	1.42±0.03	1.39±0.05	1.38±0.10	1.37±0.05	0.422

The values are presented as mean±SD. Different superscripts in the same row indicate significant differences at $p \leq 0.05$.

Protease, amylase and lipases in the liver and intestine were analyzed (Table 5). The experimental diets affected the digestive enzyme activities which were found to have lower protease and amylase in the fish fed BSF diet while lipase was found to be higher in fish fed BSF diet than control. The protease in liver showed the highest value in fish fed BSF0 and BSF25 ($p \leq 0.05$). Similar to protease in intestine, the activity was found to be higher in fish fed BSF0 diet ($p \leq 0.05$). According to the result of amylase, the activity was significantly lower in fish fed with BSF diets in both liver and intestine ($p \leq 0.05$).

However, the BSF diets had positive effect on lipase whereby lipase in liver and intestine increased with an increase in the level of BSF in the diet ($p \leq 0.05$). When the digestive enzyme activities of both liver and intestine were compared, it was found that the activity in intestine tended to be higher than the one in liver.

Table 5

Digestive enzyme activities (U mg protein^{-1}) of climbing perch after being fed with experimental diets for 60 days

Digestive enzyme activities	*Experimental diets*					*P-value*
	BSF0	*BSF25*	*BSF50*	*BSF75*	*BSF100*	
Protease						
Liver	0.07 ± 0.00^{c}	0.07 ± 0.01^{c}	0.05 ± 0.01^{b}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	<0.001
Intestine	0.43 ± 0.01^{d}	0.31 ± 0.02^{c}	0.23 ± 0.00^{ab}	0.24 ± 0.01^{b}	0.21 ± 0.03^{a}	<0.001
Amylase						
Liver	0.48 ± 0.00^{c}	0.40 ± 0.01^{b}	0.30 ± 0.01^{a}	0.33 ± 0.01^{a}	0.32 ± 0.02^{a}	<0.001
Intestine	0.68 ± 0.02^{b}	0.67 ± 0.01^{b}	0.57 ± 0.02^{a}	0.57 ± 0.06^{a}	0.60 ± 0.02^{a}	0.002
Lipase						
Liver	788 ± 55.62^{a}	782 ± 93.28^{a}	953 ± 51.10^{b}	867 ± 25.29^{ab}	968 ± 53.85^{b}	0.008
Intestine	913 ± 27.48^{a}	928 ± 60.59^{a}	1078 ± 80.86^{b}	1053 ± 91.20^{b}	1102 ± 49.58^{b}	0.014

The values are presented as mean±SD. Different superscripts in the same row indicate significant differences at $p \leq 0.05$.

The histopathological alteration in the liver of the fish fed with experimental diets are showed in Figure 1. The hepatocytes of each group were polygonal in shape, spherical nucleus, surrounded by eosinophilic cytoplasm and clear cell boundaries. Sinusoid were found interspersed between the hepatocyte cells. No evident pathological changes were observed in the liver of each group. Thus, it was indicated that replacing fish meal with BSF had no negative effect on the liver structure. The effect of experimental diets on intestinal histopathological and microvilli height were analyzed (Figures 2 and 3). The intestinal microvilli of fish were mostly cylindrical in shape. The BSF75 and BSF100 groups were found to have more debris than other groups. Concerning microvilli height, there was influence of experimental diets on the microvilli height which was found to be higher in the BSF0 (466.29±13.97 um), BSF25 (501.92±93.19 um) and BSF50 (461.70±49.86 um) groups than BSF75 (374.45±26.64 um) and BSF100 (370.09±31.63 um) groups ($p\leq0.05$).

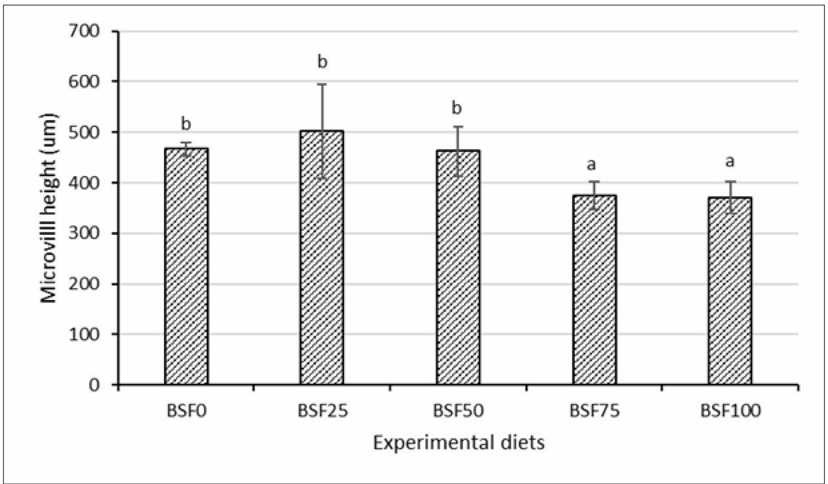

Figure 3. Microvilli height of the intestine of climbing perch after being fed with experimental diets for 60 days. The values are presented as mean±SD with different letters are significantly different ($p \leq 0.05$) from each other.

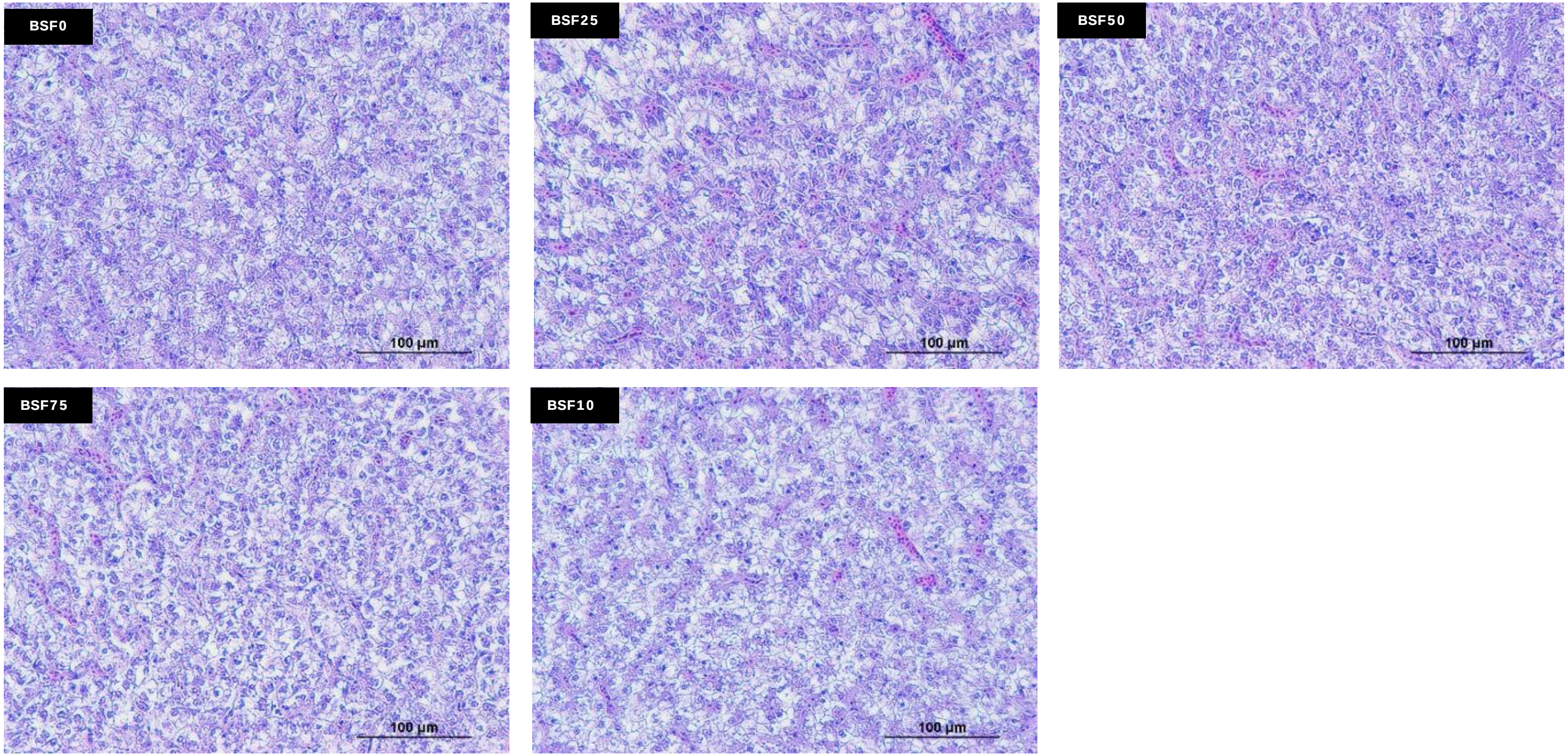

Figure 1. Histopathological alteration in liver of climbing perch after being fed with experimental diets for 60 days as displayed under microscope (200X).

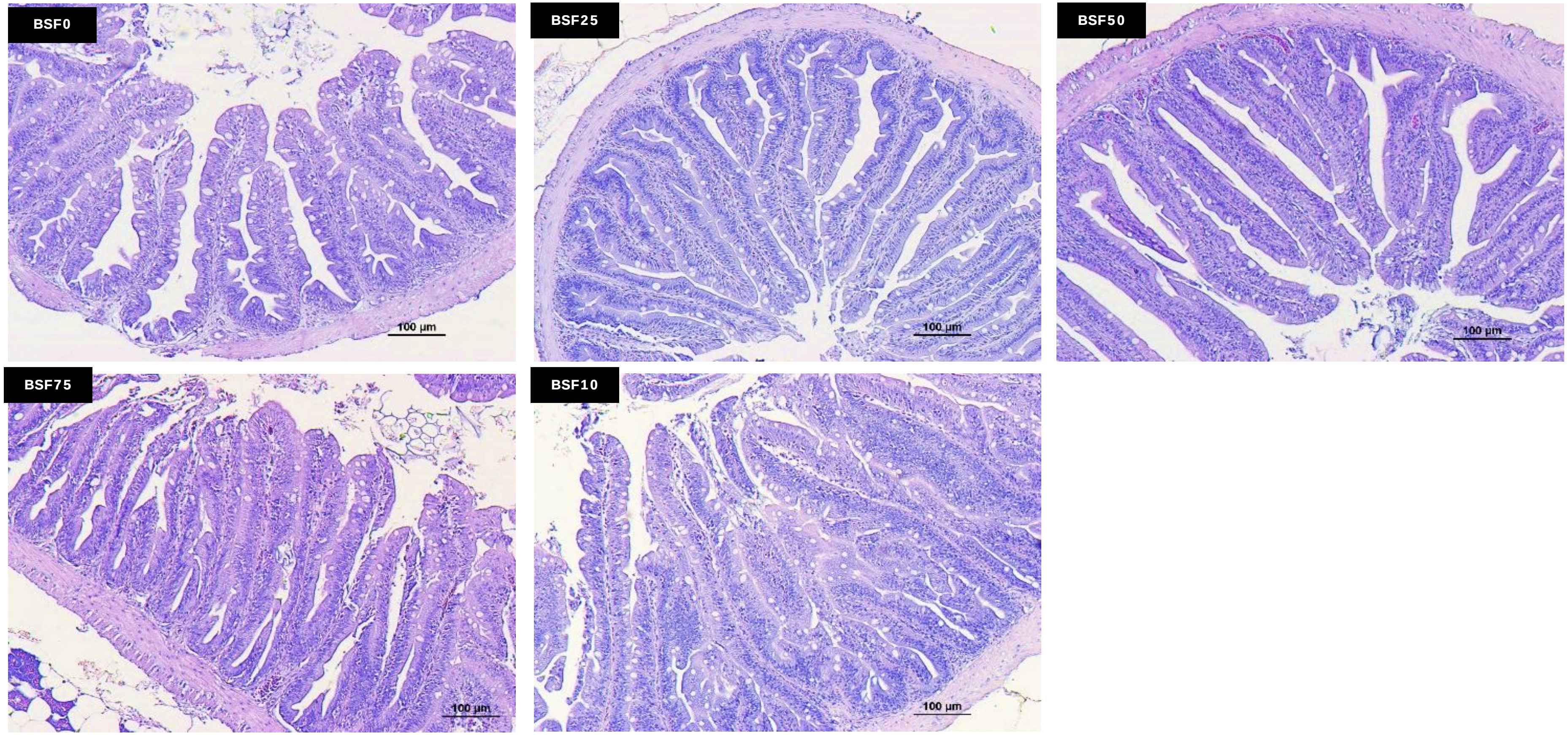

Figure 2. Intestinal microvilli of climbing perch after being fed with experimental diets for 60 days as displayed under microscope (100X).

Discussion. The analysis of chemical composition of BSF pre-pupae meal reveals a high crude protein (43.72 %) and high crude fat (15.62%) in accordance with the report of Wang & Shelomi (2017). The report showed that protein content is between 31.7 to 47.6% and fat is between 11.8 to 41.7% depending on the diet used to feed BSF and including the stage of BSF. St-Hilaire et al (2007) reported that BSF pre-pupae meal was higher in fat and lower in protein compared to the anchovy fish meal, consistent with the study of Magalhaes et al (2017), the authors suggested that BSF is considered to have a nutritional value close to FM. It was pointed out that BSF is a protein-rich raw material including a rich source of energy from fat. It was demonstrated by Caligiani et al (2018) that BSF has complete essential amino acids, especially, tyrosine, phenylalanine and histidine. The reports of Sealey et al (2011) and St-Hilaire et al (2007) suggested that BSF is rich in lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1n9), linoleic acid (C18:2n6). In addition, BSF contains minerals such as iron, zinc, potassium, phosphorus, manganese and magnesium including a variety of vitamins (Belghit et al 2018). Moreover, BSF has a natural attractant component, it can improve feed palatability and stimulate feed consumption (Nogales-Merida et al 2019).

In this study, a partial substitution of FM protein with BSF (BSF25, BSF50) in the diet did not negatively affect WG, SGR, FCR, PER and CF of climbing perch. When FM protein was substituted with BSF over 50% (BSF75, BSF100), negative effects on growth performance were observed. However, a partial or complete substitution of FM protein with BSF showed no significant differences on SR and biological indices in terms of HSI and VSI. The results indicate that BSF can replace FM with at least up to 50% in climbing perch diets. However, previous studies on the replacement of BSF in diets have suggested replacing 100% FM in Atlantic salmon diet (Belghit et al 2019), replacing 45% FM in European seabass juvenile diet (Magalhaes et al 2017), replacing 50% FM in rainbow trout diet (Stamer et al 2014), dietary replacement of 25% FM with BSF in yellow catfish (Zhang et al 2014), replacing up to 30% FM with BSF in gilthead seabream juvenile diet (Karapanagiotidis et al 2014) and replacing up to 50% of dietary FM protein from the current study though a complete substitution of FM protein with BSF was unsuccessful. This may have resulted from chitin content of BSF pre-pupae meal. Chitin is a component of the exoskeleton of crustaceans and insects (Finke 2007; Gasco et al 2018), BSF contains chitin of approximately 8.7% dry matter (Diener et al 2009). Several research studies have reported that chitin and its derivatives play an important role on decreasing nutrients digestibility and fatty acids synthesis and also reducing enzyme accessibility to substrates, including increasing hydrolysis of lipoproteins and triglyceride in the liver (Rust 2002; Belghit et al 2019; Zhang et al 2008; Li et al 2016; Magalhaes et al 2017). In addition, chitin content also had negative effect on nutrients absorption in intestine (Li et al 2017; Alegbeleye et al 2012). According to the results of intestinal histopathological alteration and microvilli height in this research, there was reduced microvilli height when over 50% FM was replaced with BSF in climbing perch diets. This intestine physiological change has a significant effect on absorption efficiency. (Caballero et al 2003). High level of BSF in diet inducing intestinal morphology changes were investigated by Jian carp (Li et al 2017) and rainbow trout (Dumas et al 2018) and intestinal folds' length was significantly reduced with an increase in BSF in the diet of clownfish (*A. ocellaris*) (Vargas-Abúndez et al 2019). However, optimal level of chitin and chitin derivatives in diet can stimulate innate immune cells and afford disease resistance in kelp grouper (*Epinephelus bruneus*) (Harikrishnan et al 2012), inhibit the growth of pathogens *Escherichia coli* and *Salmonella* in the intestine of broilers (Khempaka et al 2011). Bruni et al (2018) explained that the use of BSF larval meal in diet of rainbow trout could increase incidence of probiotics bacteria (*Carnobacterium* genus), stimulate non-specific immune response and improve *in vivo* disease resistance.

However, in the present study, no evident pathological changes and no intestinal inflammation were observed in the intestine histological analyses. This may be related to fatty acid composition in BSF. Spranghers et al (2017) reported that BSF pre-pupae was high in the medium-chain fatty acid lauric acid (C12:0). Lauric acid (C12) has been reported to improve gut health because of its intestinal anti-inflammatory, antibacterial and antiviral activity (Feng et al 2018; Vargas-Abúndez et al 2019). According to Sealey et al (2011), the increased BSF in the diet increased lauric acid content. Concerning histopathological alteration in liver, the liver plays an important role in lipid metabolism by controlling endogenous carbohydrate storage and glucose mobilization in fish (Meton et al 2004; Sangiao-Alvarellos et al 2005; Pérez-Jiménez et al 2007; Viegas et al 2012). In the present study, replacing fish meal with BSF had no negative effect on the liver structure. It was similar to the study on clownfish (Vargas-Abúndez et al 2019). However, an irregular shape of hepatocytes was observed in juvenile Jian carp fed with defatted BSF larvae meal replacing 100% FM (Li et al 2017). Even though histology is a standard technique to examine the internal architecture of various tissues and cells, it is difficult to provide sufficient information about the biochemical composition of tissue samples (Vargas-Abúndez et al 2019).

The effect of experimental diets on chemical composition in fillet (shown as % wet weight) showed crude protein, moisture and ash were not influenced among the dietary groups whereas, crude fat increased with an increase in the dietary BSF. The present study contrasted with the study of Belghit et al (2019) in that replacing FM with BSF larvae meal had no significant effects on whole fish dry matter, crude protein, crude lipid, ash or amino acid composition in Atlantic salmon. However, the authors suggested that rancid odor increased in the baked fillet obtained from salmon fed with increased BSF inclusion in the diet but it was similar to the values obtained for Atlantic salmon fed with other alternative diets. According to Sealey et al (2011), the increased BSF in the diet in turn increased lauric acid content and this may have allowed for increased lipid oxidation of the lauric acid as an energy substrate.

Concerning digestive enzyme activity, BSF dietary inhibited protease and amylase activity while it enhanced lipase activity. According to the research of Magalhaes et al (2017), the results revealed that no differences between intestinal portions were observed for protease and amylase activities. The dietary BSF only affected lipase activity in European seabass, it was lower in fish fed with 6.5% of BSF diets than the control. Conversely, the study of Li et al (2017) showed that defatted BSF larvae meal in diets for juvenile Jian carp did not influence the digestive enzyme activities in terms of trypsin, alpha-amylases, and lipases. No effect of BSF larvae meal was also observed for the activity of trypsin and leucine aminopeptidase in Atlantic salmon (Belghit et al 2019). However, the dietary compositions are the main causes that affect digestive enzyme activity and digestion transport time along the gastrointestinal tract (Castro et al 2016).

In addition, the use of insect meal is a process to reduce antibiotics in aquafeed because of the great diversity of anti-microbial peptides found in insects (Tonk & Vilcinskas 2017). Otvos (2000) identified and classified several antimicrobial activities in insect meal such as α-helical peptides, cysteinerich peptides, prolinerich peptides and glycine-rich proteins. Due to the global problem related to the increasing resistance of bacteria to antibiotics and environmental impact (Gasco et al 2018), including representing a valid alternative to FM in aquafeed, BSF pre-pupae meal can be a good candidate ingredient for aquafeeds for a more sustainable aquaculture.

Conclusions. In the present study, we evaluated the effect of partial and complete dietary replacement of fishmeal with black soldier fly (*Hermetia illucens*) pre-pupae meal on growth performance, feed utilization, digestive enzyme activity, fillet quality and histopathological changes of climbing perch (*Anabas testudineus*). This study indicates a successful replacement of 50% FM with BSF pre-pupae meal (43.72% crude protein, 15.62% crude fat) in diets for climbing perch without significantly affecting growth performance and histopathological alteration in liver and intestine. Negative effects on growth performance, high accumulation of crude fat in fillet and decreased protease and amylase activity were detected in substitution of FM protein with BSF over 50%. However, better understanding about the effect of chitin and lauric acid on physiological responses is still needed.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Supalug Kattakdad, Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin 32000, Thailand, e-mail: supalug.ka@rmuti.ac.th
Narissara Suratip, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand, e-mail: narissara.sur@gmail.com
Bundit Yuangsoi, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand, e-mail: bundyu@kku.ac.th
Krittima Kasamawut, Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin 32000, Thailand, e-mail: krittima2562@gmail.com
Suriya Udduang, Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin 32000, Thailand, e-mail: suriya.ud@rmuti.ac.th

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