PARTIAL REPLACEMENT OF MARINE FISHMEAL BY BLACK SOLDIER FLY LARVAE MEAL IN DIET FOR SNAKEHEAD FISH (*Channa striata*)

Nguyen Phuc Cam Tu[1, *], Nguyen Ngoc Ha[2], Nguyen Nhu Tri[1]

ABSTRACT

This study aims to evaluate the replacement effect of marine fish meal (FM) protein with black soldier fly (BSF, *Hermetia illucens*) larvae meal protein on the growth, survival rate, and feed utilization of snakehead fish (*Channa striata*). BSF larvae were produced by feeding with the mixture of soybean milk by-product and ensiled elephant grass (*Pennisetum purpureum*) (ratio of 1: 1). Five iso-nitrogenous (44%) and iso-lipidic (8%) experimental diets were formulated to replace FM using BSF larvae meal at 0% (control), 25% (BSF_{25}), 50% (BSF_{50}), 75% (BSF_{75}) and 100% (BSF_{100}). There were three replicates for each treatment. Juvenile fish (19.75 g/fish) were randomly distributed into 15 hapas at 50 fish/hapa. At the end of 6 weeks of a feeding trial, the results showed that final weight, weight gain (WG), daily WG, and the specific growth rate of fish fed the control diet were statistically higher than those of fish fed the BSF_{50}, BSF_{75}, and BSF_{100} diets ($p < 0.05$), but not statistically different from those fed the BSF_{25} diet ($p > 0.05$). Compared to the control group, replacement of 25 - 75% of FM by BSF meal did not significantly affect feed conversion ratio (FCR), but the FCR of fish fed BSF_{100} treatment was statistically higher than that fed the control, BSF_{25}, and BSF_{50} diets ($p < 0.05$). There were no significant differences in survival rate among treatments ($p > 0.05$). This study suggests that BSF larvae meal protein could substitute 25% of FM protein without any negative effects on growth performance, survival rate, and feed efficiency of the snakehead fish.

Keywords: *Black soldier fly, fish meal, growth performance, snakehead fish.*

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1. INTRODUCTION

Larvae and prepupae of many insects are rich in protein, lipid, vitamins, minerals, etc. and are becoming a great source of nutrients for both humans and animals [1 - 2]. Compared to other protein sources such as fish meal (FM), soybean meal and animal and seafood by-products (blood meal, meat and bone meal, and other rendered products), insect meals are efficient and sustainable sources of protein for aquaculture [1 - 2]. In addition to being a source of food materials, insects also contain various bioactive substances with several pharmacological functions, such as antiviral and microbial activity as well as the immune response [3 - 4]. Insects such as the black soldier fly (BSF) (*Hermetica illucens*) have been studied more extensively, not only for aquaculture but also for livestock feed industry. In aquaculture, BSF meal was used as a replacement for FM for many species such as rainbow trout (*Oncorhynchus mykiss*) [5], whiteleg shrimp (*Litopenaeus vannamei*) [6], European seabass (*Dicentrarchus labrax*) [7], climbing perch (*Anabas testudineus*) [8],...

Snakehead fish (*Channa striata*) is an increasingly important fish for freshwater aquaculture in Vietnam. As snakehead fish farming production increases, there will be

[1] Faculty of Fisheries,
Nong Lam University - Ho Chi Minh city
[2] Research Institute for Biotechnology and Environment, Nong Lam University - Ho Chi Minh city
[*] Email: npctu@hcmuaf.edu.vn

increasing demand for high quality feed. The growth of snakehead fish is highly affected by the protein level. Previous study has determined that the dietary protein level for maximum specific growth rate of snakehead fish should contain 46.5 - 50.8% [9]. The main protein source in snakehead fish feed is FM, due to its high protein content with balanced essential amino acid profile [10]. However, the price of FM has been increased by more than twofold in recent years, leading to FM reduction trend in aquafeed [1]. Recent studies have shown that the larvae/prepupae meal of insects, including BSF, can be used as a substitute for FM in snakehead fish feed [11 - 14]. The advantage of BSF larvae is effectively convert a wide range of organic materials (domestic and livestock waste, agricultural by-products) into edible biomass. However, utilizing these rearing substrates for BSF production often resulted in accumulating a high heavy metal level of BSF meal [15 - 16]. Considering the heavy metal accumulation in larvae, and substrate availability and costs, several studies have focused on the use of plant leaves, such as sesbania (*Sesbania grandiflora*), as growing media for BSF larvae. Compared to utilizing kitchen and agricultural wastes, the sesbania-reared BSF larvae had the same protein content (43.5%), but lower content of fat (16.7%) and heavy metals [17]. Katya *et al.* (2017) [18] reported that the sesbania-reared BSF larvae meal could be used as a protein replacement for FM for Asian seabass (*Lates calcarifer*) juvenile. Napier or elephant grass (*Pennisetum purpureum*) is one of the highest yielding tropical forage grasses and has a high content of protein, lipid, minerals, and vitamins [19]. Elephant grass is often ensiled to improve its nutritional value (protein or energy). It has previously been utilized as a diet for tilapia and grass carp, and it has demonstrated good growth, survival, and feed utilization [20 - 21]. Therefore, this study was carried out to evaluate the effect of the partial or total replacement of dietary marine FM protein by protein meal of Napier grass reared BSF larvae on the growth, survival rate, and feed utilization of snakehead fish.

2. MATERIALS AND METHODS

2.1. Materials

Black soldier fly colony was established at the Research Institute for Biotechnology and Environment (RIBE), Nong Lam University - Ho Chi Minh city. Soymilk by-product (soy pulp) was obtained from the Vietnam Soya Products Company (Vinasoy), Tan Uyen district, Binh Duong province and transported to the BSF rearing experimental facility at the RIBE. Elephant grass was taken from a farmer in Trang Bang district, Tay Ninh province and delivered to the RIBE. Grass was ensiled as guided by Moran (2005) [22] with some modifications. Grass was chopped into 3 - 5 cm pieces by using a chaff cutter, wilted for 6 - 8 hours, and mixed with 3 - 5% molasses (on a fresh weight basis) as additive just prior to ensiling. The ensiling was conducted by packing the chopped grass materials in the ensiling plastic bags. The bag was squeezed, and the neck of the bag was twisted, tied with a rubber band, and stored in a roofed house. The silage was kept until pH dropped to 3.5 to 4.5, and silage was used to grow BSF larvae. Nutritional composition of elephant grass silage and soy pulp for rearing BSF larvae was presented in table 1.

Table 1. Nutritional composition (%, dry weight basis) of elephant grass silage and soy pulp for rearing BSF larvae

Parameters	Elephant grass silage	Soy pulp
Moisture	77.03	83.23
Protein	10.19	19.33
Fat	2.37	9.31
Fiber	35.45	23.13
Ash	7.65	6.10

BSFL were reared vertically in plastic containers (61 × 42 × 15 cm) in a greenhouse at 28°C with 90% air moisture. Based upon initial trials, about 1 g of BSF egg was put into each container and the larvae were grown on media containing grass silage mixed with soy pulp (1: 1). When about half of the larvae metamorphosed into prepupae, the larvae were harvested, gently cleaned, frozen at – 20ºC, dried at 60ºC to a constant weight and ground into a fine particle consistency (approx. 0.5 - 1 mm). Each container was added 1 kg of mixture daily for 15 days. After a 15 day growing period, the larvae were picked from the mixture residue, washed with distilled water, dried at 60ºC to constant weight, and then ground into homogenous powder for determination of the chemical composition. Contents (%, dry weight basis) of crude protein, fat, fiber, and ash of the BSF larvae were 50.17 ± 2.46, 20.39 ± 4.2, 11.8 ± 0.8, and 15.1 ± 0.9, respectively.

Snakehead fish juvenile (2.5 g/fish) was obtained from a hatchery in An Giang province, shipped to the experimental farm, Faculty of Fisheries, Nong Lam University - Ho Chi Minh city, and then acclimated to the experimental conditions for three weeks before the feeding trial began. Fish were fed commercial pellets (Cargill Company) during the first week, and a control diet in the following two weeks to apparent satiation twice daily (7:00 and 15:00).

Feed ingredients were taken from the Lai Thieu Feed Mill Co., Ltd. The main protein sources were marine fish meal (60% protein, 7.12% lipid, Kien Giang province), soybean meal (Argentina), poultry by-product meal (65% protein, Brazil), and blood meal (Hungary); the lipid sources were marine fish oil (Peru) and soybean oil, while the carbohydrate sources were rice bran, and cassava meal.

2.2. Experimental diets

Five iso-nitrogenous (44%) and iso-lipidic (8%) experimental diets were formulated to replace FM using BSF larvae meal at 0% (control), 25% (BSF_{25}), 50% (BSF_{50}), 75% (BSF_{75}), and 100% (BSF_{100}). There were three replicates for each treatment. The formulation and the analyzed composition of the experimental diets are shown in table 2.

Table 2. Formulation and proximate composition of the five experimental diets

	Control	BSF_{25}	BSF_{50}	BSF_{75}	BSF_{100}
Ingredients (%)					
Marine fish meal 60% protein	12.00	9.00	6.00	3.00	0.00
Soybean meal	30.00	30.00	30.00	30.00	30.00
Cassava meal	14.50	14.22	14.43	14.26	13.98
Marine fish oil	1.00	1.00	1.00	1.00	0.80
Soybean oil	1.60	1.10	0.60	0.10	0.00
Rice bran	10.00	10.00	10.00	10.00	10.00
BSF larvae meal	0.00	3.50	7.00	10.50	14.00
Monocalcium phosphate	0.50	0.70	0.70	1.00	1.20
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Poultry by-product meal	24.30	24.30	24.00	23.80	23.60
Blood meal	2.00	2.00	2.00	2.00	2.00
Vitamin and mineral premix	0.35	0.35	0.35	0.35	0.35
Stay C 35%	0.03	0.03	0.03	0.03	0.03
Binder (Carboxymethylcellulose)	1.00	1.00	1.00	1.00	1.00
Methionine	0.20	0.21	0.23	0.24	0.25
Threonine	0.27	0.29	0.30	0.31	0.32
Lysine	0.02	0.09	0.17	0.24	0.31

Tryptophan	0.06	0.05	0.03	0.01	0.00
Choline chloride	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.03	0.03	0.03	0.03	0.03
Mold inhibitor	0.03	0.03	0.03	0.03	0.03
Proximate composition (%, dry weight basis)					
Crude protein	44.08	43.97	43.45	42.83	42.60
Crude fat	8.38	7.88	8.49	8.54	7.99
Ash	11.52	11.32	10.71	10.18	10.32
Crude fiber	3.02	3.04	3.66	3.72	5.01
Gross energy (Kcal/g, calculated values)	3.90	3.90	3.90	3.90	3.90

The ingredients were thoroughly mixed (about 30 min) using a Hobart mixer (Hobart Food Equipment Co. Ltd., Tianjin, China), then hot distilled water was added to produce a dough and pelleted by a 1.5 mm-die meat grinder. Pellets were dried at 60°C for 24 hours and kept in plastic bags in a refrigerator (4°C) until feeding.

2.3. Experimental design and management

Before the trial, fish were fasted for 24 hours for gut evacuation and weighed (initial mean body weight of 19.75 g/fish). A total of 750 snakehead juveniles were randomly allocated into 15 hapa at 50 fish/hapa (1 x 1 x 1.5 m) placed in an earthen pond. Fish were fed one of the trial diets *ad libitum* twice daily (7:00 and 15:00) using feeding trays (ϕ 40 cm) for six weeks. The sinking feed with known weight was placed in the feeding tray, then it was immersed into each hapa. One hour after feeding, left-over feed was collected and stored in a plastic container, then stored in a freezer. At the end of the experiment, feed in each container was dried in an oven to calculate total weight of left-over feed in 100% dry matter basis.

Water temperature, pH, and dissolved oxygen (DO) were measured twice daily at 7:00 and 14:00 using Digital thermometer WT - 1, Denver pH meter UP- 10, and Milwaukee MW 600 Dissolved Oxygen, respectively. Total ammonia nitrogen (TAN) and nitrite (NO_2^-) were determined twice weekly using the phenate method (4500-NH_3 F) and colorimetric method (4500-NO_2^- B), respectively; both analytical methods were performed according to the APHA standards [23]. Chemical composition of the experimental diets (moisture, crude protein, crude fat, crude fiber, and ash) was analyzed using the standard methods of AOAC (1995) [24]. All analyzed chemical parameters were conducted at the RIBE.

2.4. Growth performance and feed efficiency

Fish were counted at the beginning and the end of the trial to calculate the survival rate (SR, %). At the end of the feeding trial, all the fish were weighed for the estimation of weight gain (WG), daily WG (DWG), specific growth rate (SGR), and feed conversion ratio (FCR). Growth performance parameters were estimated using the following equations:

Weight gain (g/fish) = $W_f - W_i$

Daily weight gain (g/day) = $(W_f - W_i) / t$

Specific growth rate (%/day) = $100 \times (\ln W_f - \ln W_i) / t$

Where: *t*: duration of the trial (42 days);

W_i: initial mean body weight (g/fish);

W_f: final mean body weight (g/fish).

Feed conversion ratio = Feed intake (dry weight basis)/Wet weight gain

2.5. Statistical analyses

Statistical procedures were carried out as recommended by Gomez and Gomez (1984) [25] and Bhujel (2008) [26]. Arcsine square-root transformation was applied for survival rate data. After verification of normality and homogeneity of variance using Kolmogorov -Smirnov's one sample test and Levene test, respectively, data were analyzed by one-way ANOVA with Duncan multiple range test as pairwise comparisons. Differences among treatment means were considered at $p < 0.05$ level of significance. All statistical analyses were conducted using the IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp). All data were presented as the mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Water quality parameters

Table 3. Water quality parameters monitored (mean ± SD) in earthen pond during the feeding trial of snakehead fish

Parameters	Morning		Afternoon
Temperature	29.5 ± 0.4		30.4 ± 0.7
pH	7.9 ± 0.2		8.3 ± 0.2
DO (mg/L)	3.7 ± 0.3		4.7 ± 0.4
TAN (mg/L)		0.87 ± 0.96	
NO_2^- (mg/L)		0.52 ± 0.60	

The water quality parameters taken during the feeding trial were shown in Table 3. Mean values of temperature, pH, and DO were ranged from 29.5 - 30.4°C, 7.9 - 8.3, and 3.7 - 4.7 mg/L, respectively, while TAN and nitrite levels were 0.87 ± 0.96 mg/L and 0.52 ± 0.60 mg/L, respectively. Optimal values of temperature, pH, DO, TAN, and nitrite for snakehead fish were in ranges of 20 – 30°C, 6.5 - 8.5, 3.0 - 6.5 mg/L, 0.25 - 5.20 mg/L, and 0.01 - 0.56 mg/L [27 - 28], respectively. The results of this study showed that these obtained values were maintained within acceptable levels for *C. striata* in earthen pond.

3.2. Growth performance, survival rate and feed efficiency

3.2.1. Growth performance

The growth performance, survival rate, and feed efficiency of snakehead fish fed five trial diets for six weeks were shown in table 4. At the end of the trial, there were no significant differences in the average W_f, WG, and DWG between the control and BSF_{25} diets ($p > 0.05$). However, fish fed a diet with 100% BSF larvae meal had a significantly lower SGR ($p < 0.05$) than those fed with the control and BSF_{25} diets. No significant difference in growth performance of fish fed 50%, 75%, and 100% substitution level diets were observed ($p > 0.05$). These results indicated that BSF larvae meal could substitute 25% of FM without any negative effects on the growth performance of *C. striata*.

Table 4. Growth performance, survival rate, and feed efficiency of fish fed five trial diets after 6 weeks

	Control	BSF_{25}	BSF_{50}	BSF_{75}	BSF_{100}
W_i (g/fish)	19.7 ± 0.0^{a}	19.8 ± 0.0^{a}	19.7 ± 0.0^{a}	19.8 ± 0.0^{a}	19.7 ± 0.0^{a}
W_f (g/fish)	122 ± 11^{a}	109 ± 12^{ab}	98.4 ± 10.4^{bc}	98.2 ± 11.4^{bc}	80.4 ± 10.4^{c}
WG (g/fish)	102 ± 11^{a}	89.7 ± 11.6^{ab}	78.6 ± 10.3^{bc}	78.5 ± 11.4^{bc}	60.7 ± 10.4^{c}
DWG (g/day)	2.43 ± 0.26^{a}	2.13 ± 0.28^{ab}	1.87 ± 0.25^{bc}	1.87 ± 0.27^{bc}	1.45 ± 0.25^{c}
SGR (%/day)	4.33 ± 0.21^{a}	4.07 ± 0.25^{a}	3.82 ± 0.26^{ab}	3.81 ± 0.29^{ab}	3.33 ± 0.30^{b}
SR (%)	96.0 ± 2.0^{a}	96.0 ± 2.0^{a}	96.7 ± 2.3^{a}	96.0 ± 6.9^{a}	93.3 ± 3.1^{a}
FCR	1.48 ± 0.10^{a}	1.52 ± 0.12^{a}	1.78 ± 0.28^{a}	1.84 ± 0.34^{ab}	2.32 ± 0.36^{b}

Note: Data represents as mean ± SD (n = 3). Mean values in the same row with different superscript letters differ significantly (one-way ANOVA with Duncan test p < 0.05).

Similar to the findings of this study, most studies found that an optimal FM substitution level of 20 - 50% using BSF larvae meal would be possible without adverse effects on the growth performance of various fish species. Hoa and Dung (2016) [14] reported that snakehead fish (*C. micropeltes*) fed a diet including 40 and 50% BSF as substitution for the FM had a statistically lower growth rate compared to the other diets (containing 0, 10, 20 and 30% BSF). For barramundi juvenile (*L. calcarifer*), the growth rate including WG and SGR was significantly lower when BSF meal replacement levels were > 50% and an optimal substitution level of FM by BSF larvae meal in the diet was 28.4% [18]. In a feeding trial on rainbow trout (*O. mykiss*), fish fed a diet including 50% BSF as replacement for the FM obtained statistically lower W_f and WG as compared to the control diet [5]. Similarly, no significant change in the growth rate was observed in European sea bass (*D. labrax*) fed BSF meal at levels of up to 50% [7].

The decrease in growth rate responses to BSF larvae meal supplementation among carnivorous fish can be due to the substantial rise in chitin content besides the deficiency in essential amino acids such as cysteine, methionine and threonine [29]. Many studies stated that increasing inclusion level of BSF increased chitin content in the feed, and this may have affected its digestibility. The chitin levels in BSF biomass were in range of 8 - 24%, with shedding and cocoons being most rich in chitin [30]. Moreover, the growth decline in fish fed BSF could be due to reduced nutrient digestibility since protein digestibility for BSF was markedly lower than that of FM [29].

3.2.2. Survival rate

Survival rates ranged from 93.3% to 96.7% without significant differences among diets ($p > 0.05$) (Table 4). As stated above, all monitored water quality parameters were maintained at suitable levels for adequate growth and survival of snakehead fish. Furthermore, this result duplicates recent published studies, in which diets containing high inclusion levels of BSF, did not affect the SR of many fish species. Study of Abdel-Tawwab *et al.* (2020) [7] revealed that up to 50% of FM replacement would be possible without negative effects on the SR of European sea bass. Similarly, no significant difference was found on the SR when BSF larvae meal incorporated up to 100% in the climbing perch (*A. testudineus*) diet [8]. In contrast, Katya *et al.* (2017) [18] reported that there was significant difference in the SR among the treatments of barramundi fed different trial diets, but no clear trend in the effects of dietary treatments on fish SR could be taken.

3.2.3. Feed efficiency

Compared to control group, replacement of 25 - 75% of FM by BSF meal resulted in no significant effect on FCR ($p > 0.05$), but the FCR of fish fed BSF_{100} treatment was statistically higher than those fed the control, BSF_{25}, and BSF_{50} diets ($p < 0.05$) (Table 4). This result is in agreement with earlier studies on the inclusion of BSF larvae in feed of other farmed fish species. Hoa and Dung (2016) [14] recorded that FCR of the treatments with inclusion levels of BSF meal less than 30% in snakehead fish feed were not significantly different compared to the control. Similarly, no significant difference was found on the FCR when replacing 50% or 100% of BSF larvae meal in diets of the European sea bass [7], climbing perch [8], and Atlantic salmon (*Salmo salar*) [31]. In contrast, compared to the other diets (inclusion of 0 and 25% BSF meal), St-Hilaire *et al.* (2007) [5] discovered that rainbow trout fed a diet containing 50% BSF meal as substitution for the FM had a statistically higher FCR. Moreover, Nile tilapia *Oreochromis niloticus* fed housefly maggot meal exhibited a higher FCR compared to those fed a diet including 52% of FM [32]. In case of juvenile turbot *Psetta maxima*, the FCR was significantly higher when BSF meal replacement levels were > 33% [33].

4. CONCLUSION

This study is the first research work to offer a new technique for rearing BSF larvae, using silage of Napier grass (*P. purpureum*) as the substrate. Results revealed that substitution of FM by BSF meal at levels less than 50% had negative effects on growth performance, survival rate, and feed efficiency of snakehead fish as compared to control group. The acceptable level of FM protein replacement for the performances of snakehead fish was 25%. Our observations from the present study suggested that snakehead fish might have limited efficacy to consume chitin from BSF larvae. Further investigation on this matter is recommended to examine the effect of dietary chitin on predatory fish species.

REFERENCE

1. Makkar H. P. S., Tran G., Heuzé V. & Ankers P. (2014). "State-of-the-art on use of insects as animal feed", *Animal Feed Science and Technology,* 197, pp. 1-33, DOI: http://dx.doi.org/10.1016/j.anifeedsci. 2014.07.008.

2. Huis A. V., Itterbeeck J. V., Klunder H., Mertens E., Halloran A., Muir G. & Vantomme P. (2013). Edible insects: future prospects for food and feed security *FAO Forestry Paper* (Vol. 171, pp. 187): Food and Agriculture Organization of the United Nations.

3. Ido A., Iwai T., Ito K., Ohta T., Mizushige T., Kishida T., Miura C. & Miura T. (2015). "Dietary effects of housefly (*Musca domestica*) (Diptera: Muscidae) pupae on the growth performance and the resistance against bacterial pathogen in red sea bream (*Pagrus major*) (Perciformes: Sparidae)", *Applied Entomology and Zoology,* 50 (2), pp. 213-221, DOI: https://doi.org/10.1007/s13355-015-0325-z.

4. Maulu S., Langi S., Hasimuna O. J., Missinhoun D., Munganga B.P., Hampuwo B.M., Gabriel N.N., Elsabagh M., Van Doan H., Abdul Kari Z. & Dawood M. A. O. (2022). "Recent advances in the utilization of insects as an ingredient in aquafeeds: A review", *Animal Nutrition,* 11, pp. 334-349, DOI: https://doi.org/10.1016%2Fj.aninu.2022.07.013.

5. St-Hilaire S., Sheppard C., Tomberlin J. K., Irving S., Newton L., McGuire M. A., Mosley E. E., Hardy R. W. & Sealey W. (2007). "Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*", *Journal of the World Aquaculture Society,* 38 (1), pp. 59-67, DOI: https://doi.org/10.1111/j.1749-7345. 2006.00073.x.

6. Amaya E. A., Davis D. A. & Rouse D. B. (2007). "Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions". *Aquaculture,* 262 (2), pp. 393- 401, DOI: https://doi.org/10.1016/j.aquaculture. 2006.11.015.

7. Abdel-Tawwab M., Khalil R. H., Metwally A. A., Shakweer M. S., Khallaf M. A. & Abdel-Latif H.M.R. (2020). "Effects of black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, organs-somatic indices, body composition, and hemato-biochemical variables of European sea bass, *Dicentrarchus labrax*", *Aquaculture,* 522, pp. 735136, DOI: https:// doi. org/10. 1016/ j. aquaculture. 2020. 735136.

8. Kattakdad S., Suratip N., Yuangsoi B., Kasamawut K. & Udduang S. (2022). "Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in climbing perch (*Anabas testudineus*) diet", *AACL Bioflux,* 15 (1), pp. 68-82.

9. Hien T. T. T., Lan N. T. N., Yen D. T. & Tuan N. A. (2005). "Protein requirements of giant snakehead (*Channa micropeltes*, Cuvier, 1831) fingerlings", *Can Tho University Journal of Science,* 3, pp. 58-65.

10. El-Sayed A. -F. M. (2020). Chapter 7 - Nutrition and feeding. In A. -F. M. El-Sayed (Ed.), *Tilapia Culture (Second Edition)* (pp. 135-172): Academic Press.

11. Prachom N., Yuangsoi B., Pumnuan J., Ashour M., Davies S. J. & El-Haroun E. (2023). "Effects of substituting the two-spotted cricket (*Gryllus bimaculatus*) meal for fish meal on growth performances and digestibility of striped snakehead (*Channa striata*) juveniles", *Life,* 13 (2), pp. 594, DOI: https://doi.org/10.3390/life13020594.

12. Prachom N. & Suharman I. (2022). "The use of cricket (*Gryllus bimaculatus*) meal as protein source for snakehead fish (*Channa striata*)", *IOP Conference Series: Earth and*

Environmental Science, 1118, pp. 012020, DOI: https:// doi.org/10.1088/1755-1315/1118/1/012020.

13. Wallady A. A., Rahardja B. S. & Kenconojati H. (2021). "Dietary combination of maggot and commercial feed enhance the growth rate and feed conversion ratio of snakehead fish (*Channa striata*)", *IOP Conference Series: Earth and Environmental Science,* 1036, pp. 012085, DOI: https://doi.org/10.1088/1755-1315/1036/1/012085.

14. Hoa N. P. & Dung N. V. (2016). "Utilizing the black soldier fly larvae/pre-pupae (*Hermetia Illucens*) in feeds for snakehead fish (*Channa micropeltes*)", *Vietnam Journal of Agricultural Sciences,* 14 (4), pp. 590-597.

15. Attiogbe F. K., Ayim N. Y. K. & Martey J. (2019). "Effectiveness of black soldier fly larvae in composting mercury contaminated organic waste", *Scientific African,* 6, pp. e00205, DOI: https:// doi.org/10.1016/j.sciaf.2019.e00205.

16. Diener S., Zurbrügg C. & Tockner K. (2015). "Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle", *Journal of Insects as Food and Feed,* 1 (4), pp. 261-270, DOI: https://doi.org/10.3920/ JIFF2015. 0030.

17. Kuppusamy G., Kong C. K., Segaran G. C., Tarmalingam E., Herriman M., Ismail M. F., Mehmood Khan T., Low L. E. & Goh B. -H. (2020). "Hummingbird-leaves-reared black soldier fly prepupae: assessment of nutritional and heavy metal compositions", *Biology,* 9 (9), pp. 274, DOI: https://doi.org/10.3390/biology9090274.

18. Katya K., Borsra M. Z. S., Ganesan D., Kuppusamy G., Herriman M., Salter A. & Ali S. A. (2017). "Efficacy of insect larval meal to replace fish meal in juvenile barramundi, *Lates calcarifer* reared in freshwater", *International Aquatic Research,* DOI: https://doi.org/10.1007/s40071-017-0178-x.

19. Maleko D., Mwilawa A., Msalya G., Pasape L. & Mtei K. (2019). "Forage growth, yield and nutritional characteristics of four varieties of napier grass (*Pennisetum purpureum* Schumach) in the west Usambara highlands, Tanzania", *Scientific African,* 6, pp. e00214, DOI: https://doi.org/ 10.1016/j.sciaf. 2019.e00214.

20. Dinesh Chandra S., Sampa Rani K. & Md. Nahid H. (2015). "Production of organic grass carp (*Ctenopharyngodon idella*) and GIFT tilapia (*Oreochromis niloticus*) using napier grass, *Pennisetum purpureum*", *Journal of Fisheries,* 3(1), DOI: https://doi.org/10.17017/j.fish.97.

21. Chikafumbwa F. J. K. (1996). "The use of napier grass (*Pennisetum purpureum*) and maize (*Zea mays*) bran as low-cost tilapia aquaculture inputs", *Aquaculture,* 146 (1), pp. 101-107, DOI: https://doi.org/10.1016/S0044-8486(96) 01341-5.

22. Moran J. (2005). Making quality silage. In J. Moran (Ed.), *Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmers in the Humid Tropics* (pp. 83-97): CSIRO Publishing.

23. APHA (2017). *Standard Methods for the Examination of Water and Wastewater* (23rd ed.). Washington DC, US: Water Environment Federation, American Public Health Association, American Water Works Association.

24. AOAC (1995). *Official Methods of Analysis. Association of Official Analytical Chemists* (16th ed.): Gaithersburg, Maryland, USA.

25. Gomez K. A. & Gomez A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). United States: John Wiley & Sons.

26. Bhujel R. C. (2008). *Statistics for aquaculture*: Wiley-Blackwell.

27. Ngo T. L. (2003). *Culture technique of eel, frog, softshell turtles and snakehead fish* (9th ed.): Agricultural Publishing House, Ho Chi Minh City, Vietnam.

28. Courtenay W. R. & Williams J. D. (2004). *Snakeheads (Pisces, Channidae) - a biological synopsis and risk assessment*: U.S. Department of the Interior and U.S. Geological Survey.

29. 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 illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*)", *Aquaculture,* 473, pp. 337-344, DOI: https://doi.org/10.1016/j.aquaculture.2017.02. 022.

30. Soetemans L., Uyttebroek M. & Bastiaens L. (2020). "Characteristics of chitin extracted from black soldier fly in different life stages", *International Journal of Biological Macromolecules,* 165, pp. 3206-3214, DOI: https://doi.org/10.1016/ j.ijbiomac. 2020.11.041.

31. Belghit I., Liland N. S., Gjesdal P., Biancarosa I., Menchetti E., Li Y., Waagbø R., Krogdahl Å. & Lock E.-J. (2019). "Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*)", *Aquaculture,* 503, pp. 609-619, DOI: https:// doi.org/10.1016/j.aquaculture.2018.12.032.

32. Slawski H., Schulz C. & Ogunji J. O. (2008). Evaluation of housefly maggot meal as an alternative protein source in the diet of *Oreochromis niloticus*. *World Aquaculture, 39,* 16-18.

33. 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 illucens*) as fish meal substitute - Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*)", *Aquaculture,* 364 (0), pp. 345-352, DOI: http://dx.doi. org/10.1016/j.aquaculture. 2012.08.041.