DOI: http://dx.doi.org/10.15578/iaj.18.2.2023.147-153 *Indonesian Aquaculture Journal, 18 (2), 2023, 147-153*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

THE SUITABILITY OF BLACK SOLDIER FLY LARVAE COMBINED WITH A COMMERCIAL DIET ON GROWTH AND FEED PERFORMANCES OF HYBRID CATFISH *(Clarias gariepinus x C. macrocephalus)*

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(Received: July 13, 2023; Final revision: October 18, 2023; Accepted: October 19, 2023)

ABSTRACT

The black soldier fly larvae (BSFL) have been numerous benefits for aquaculture. This study aimed to investigate the suitability of BSFL combined with a commercial diet (CMD) on growth and feed performances of hybrid catfish (*Clarias gariepinus* x *C. macrocephalus*). A completedly randomized design was used to consist of four treatments and three replications with 100:0 (control), 75:25, 50:50, and 25:75 of CMD and fresh BSFL blended feeding. Fish were reared in 2 m^2 inland canvas cages for 60 days. The results indicated that fish were fed 100% CMD had better average weight gain (AWG), daily weight gain (DWG), specific growth rate (SGR), food conversion ratio (FCR), feed efficiency (FE), and survival rate (SR) than the other treatments, but there was no statistically significant difference ($p > 0.05$) when compared to 75:25 and 50:50 CMD:BSFL feeding. At the end of the experiment, the SR of fish was 81-91% and showed no significant difference ($p > 0.05$) when compared between treatments. Therefore, hybrid catfish rearing can compensate CMD by fresh BSFL up to 50% of the feed amount per day. However, feeding with fresh BSFL can have side effects concerning steatosis.

KEYWORDS: BigOui; BSF; BSFL; growth; hybrid catfish

INTRODUCTION

In global production, Clarias catfish (*Clarias* spp.) is an economic aquatic animal and the tenth most significant aquaculture species after Grass carp, Silver carp, and Nile tilapia. In 2020, the productivity of Clarias catfish was 1,249 thousand tons (FAO, 2022). In ASEAN, Thailand had the second highest catfish production after Indonesia, and most of species have been hybrid catfish, commonly known in Thailand as mentioned in Pla Duk BigOui (Freshwater Aquaculture Research and Development Division, 2020). For domestic production in 2022, catfish production was 101.7 thousand tons which ranked second after Nile tilapia (Fisheries Statistics, 2022). These products were generally consumed by humans domestically, less than one percent was exported to ASEAN countries, with Singapore and Malaysia being the main markets. Catfish farming in Thailand is typically subsistence farming with most raised in ponds followed by cage farming. However, the main cost of cultured catfish is food which exceeds half of the total cost. The technologies or tools adopted by farmers to reduce costs are to provide organic complementary feed that contributes to fish growth rather than using artificial food alone to increase product value and reduce food costs. (Freshwater Aquaculture Research and Development Division, 2020).

Black Soldier Fly (BSF), *Hermetia illucens* (Linnaeus, 1758), is classified into the family Stratiomyidae, order Diptera. BSF larvae (BSFL) are highly nutritious, consisting of 35-56% protein, 5-37% fat, and great in ash, calcium, phosphorus, chitin, minerals, and vitamins which makes them suitable for aquafeed and especially for ornamental and commercial fish. In addition, they can also be fed to pigs, poultry, lizards, turtles, and shellfish species (Jeyaprakashsabari & Annand, 2021; Priyadarshana *et al*., 2021; Kamalii *et al*., 2022). Besides its nutritious contents, BSFL are also full of intestinal microorganisms and enzymatic activity to allow easy digestion of starch, pro-

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tein, and lipids. Likewise, the cultivation of BSFL is very easy to achieve a mass production. They can grow on animal dung, fruits waste, vegetable waste, household and restaurant waste and other organic waste. BSFL culture takes 44 days. BSFL are good biological conversion and produce organic fertilizer between growing periods (Sinansari & Fahmi, 2020; Priyadarshana *et al.*, 2021; Kamalii *et al.*, 2022).

BSFL can be used as a substitute for fish meal, soybean meal, corn kernels, or expensive fish feed formulations and can be given directly as fresh fish feed (Sinansari & Fahmi, 2020; Kamalii *et al*., 2022). Dried meal from BSFL could replace up to 50% of fish meal in formulated diet without negative effect on fish (Jeyaprakashsabari & Annand, 2021; Priyadarshana *et al.*, 2021). In addition, Xiao *et al.* (2018); Zarantoniello *et al.* (2019); Madibana *et al.* (2020); Oteri *et al.* (2021) and Tippayadara *et al.* (2021) researched about fish meal replacement using BSFL meal in diets for yellow catfish, zebrafish, dusku kob (*Argyrosomus japonicus*), gilt-head bream, and tilapia respectively. The results found that BSFL meal did not adversely affect fish and there were no significant differences in the growth performances when compared to the control treatment. For some species, the growth performances were better than the control, and the immune improved. Aihyas *et al.* (2022) substituted corn grain and soybean meal with BSFL meal in a formulated diet for red hybrid tilapia, the result revealed that increasing the level of BSFL would increase the protein content in the fish. For whole feeding of BSFL, fish fed 50% BSFL accompanied with 50% commercial diet (CMD) presented similar growth performances and carcass yield when compared with fish fed only CMD (Ordoñez *et al.*, 2022).

Therefore, propose of this study aimed to estimate the suitable proportion of fresh BSFL in combined feeding with CMD on growth and feed performances of hybrid catfish.

MATERIALS AND METHODS

Preparation of experimental animals

A two month old group of hybrid catfish (*Clarias gariepinus* × *C. macrocephalus*), 10-13 g of average weight, brought from Rachen fish farm located in Wangchompoo Subdistrict, Mueang, Phetchabun Province. Fish were acclimated for 2 weeks in 2 m^2 inland canvas cages with one aeration point per cage and fed 50% fresh BSFL combined with 50% commercial diet (CMD). The feeding rate was between 6-10% of body wet weight and twice a day at 9.00 a.m. and 3.30 p.m. Cages were cleaned twice a week.

Black soldier fly larvae (BSFL) was cultivated at Chatree healthy organic farm, Huai Sakae Subdistrict, Mueang, Phetchabun Province using household organic waste as a growing media (Dortmans *et al*., 2017; Hanboonsong, 2017). Ten to twenty days old BSFL in the worm stage was collected to feed the fish (Figure 1).

Figure 1. BSFL was used in experiment.

Experimental design and executions

The experiment was conducted on May until July for 60 days at Chatree healthy organic farm. A completely randomized design (CRD) was used, consisted of four treatments and three replications mentioned by 100:0% (treatment 1; control), 75:25% (treatment 2), 50:50% (treatment 3), and 25:75% (treatment 4) of CMD (30-25% protein) and fresh BSFL of the total amount of food that was fed to the fish daily. After 2 weeks of acclimation, the fish were randomly weighed and 360 fish were divided into 12 cages (30 fish per cage). The next day, the fish were fed food following the experimental design. Feeding rate, feeding times per day and cleaning of the rearing system were performed as same as the acclimatization period. Water qualities such as pH, dissolved oxygen (DO), water temperature, and total ammonia nitrogen (TAN) were measured twice a week by pH meter model EZ-9902,

DO meter model WA-2017SD Lutron, thermometer, and Salifert ammonia test kit, respectively. Every 30 days, fish were randomly weighed to evaluate growth. During the experiment, fresh BSFL was collected and dried at 60°C, 48-72 hours. Dried BSFL was ground and nutritionally analyzed according to AOAC (1995). At the end of the experiment, fish were counted and randomly weighed including random sampling of liver and stomach tissue for histopathological study by hematoxylin and eosin stain. The growth parameters were calculated following the formulas of Tippayadara *et al.* (2021) described by:

$$Average\ weight\ gain; AWG\ (g) = final\ weight - initial\ weight$$

$$Daily\ weight\ gain; DWG\ \left(\frac{g}{day}\right) = \frac{final\ \ average\ \ weig\,ht - initial\ \ average\ \ weig\,ht}{days}$$

$$Specific\ growth\ rate; SGR\ \left(\frac{\%}{day}\right) = \frac{\ln(final\ \ average\ \ weig\,ht) - \ln(initial\ \ average\ \ weig\,ht)}{days} \times 100$$

$$Food\ conversion\ ratio;\ FCR = \frac{total\ \ amount\ \ of\ \ food\ \ intake}{weig\,ht\ gain}$$

$$Feed\ efficiency;\ FE = \frac{weig\,ht\ gain}{total\ \ amount\ \ of\ \ food\ \ intake}$$

$$Survival\ rate;\ SR\ (\%) = \frac{final\ \ number\ \ of\ fish}{initial\ \ number\ \ of\ fish} \times 100$$

Statistical valuation

Three repeated data from each treatment were used to calculate the mean and standard deviation and the difference between treatments were considered at the 0.05 significance level by one way ANOVA and Duncan's multiple range test (DMRT). All processes were completed with IBM SPSS statistics version 21.

RESULTS AND DISCUSSION

Nutritional values of fresh BSFL

The main nutritional value of fresh BSFL in the experiment consisted of 36.06% protein and 21.76% fat, as shown in Table 1 which was similar to the studies of Barragan-Fonseca *et al.* (2017) and Priyadarshana *et al.* (2021) which revealed that BSFL comprised high protein levels at 37-63% and 40.4-56.2%, respectively and high fat levels at about 4.8-24.8%. In our experiment, the fresh BSFL appeared to have a little lower amount of protein than reviews and high fat content which could be due to the cultivated media of BSFL which mainly used household organic waste that was low in protein and high in carbohydrate and fat. However, our results correspond to the results of Chia *et al.* (2020) that studied the nutritional composition of BSFL fed on agro-industrial by-products. Their results showed that BSFL was protein and fat content between 29.9-45.7% and 9.5-49.0%, respectively.

Table 1. Nutritional values of fresh black soldier fly larvae used in the experiment

Analyzed parameters	Mean ± Standard deviation
Crude protein (%)	36.06 ± 0.19
Crude fat (%)	21.76 ± 1.15
Nitrogen free extract (%)	13.13 ± 1.40
Crude fiber (%)	4.54 ± 0.12
Ash (%)	9.51 ± 0.21
Gross energy (kcal/kg)	5,388.23 ± 60.30

Growth performances of hybrid catfish

After feeding CMD combination with fresh BSFL at the ratios of 100:0, 75:25, 50:50, and 25:75, the results indicated that fish in all treatments were grown following the experimental period revealed by AWG increasing (Figure 2a). Meanwhile, DWG, SGR, FCR and FE decreased (Figures 2b, 2c, 2d and 2e) due to increasing age or body mass of the fish so that the metabolic rate or specific oxygen consumption decreased which caused the growth rate to decrease. (Fidhiany & Winckler, 1998).

At 30 and 60 days, AWG, DWG, SGR, and FE of fish fed 100% CMD (control) showed higher performance than the other treatments and FCR was the lowest but there was no statistically significant difference ($p > 0.05$) when compared with the groups fed with 75:25 and 50:50 of CMD and fresh BSFL (Tables 2 and 3). At the end of the experiment, SR was about 81-91% and there was no statistically significant difference ($p > 0.05$) when compared between treatments (Table 3). Water qualities during the experiment in terms of pH, DO, water temperature, and TAN were between 7.13-7.44, 4.18-4.85 mg/l, 28.2-31.7 °C (10.50-11.30 a.m.), and 0.25-0.5 mg/l, respectively. In our experiment, fish fed CMD in combination with fresh BSFL had lower growth rate than fish fed CMD

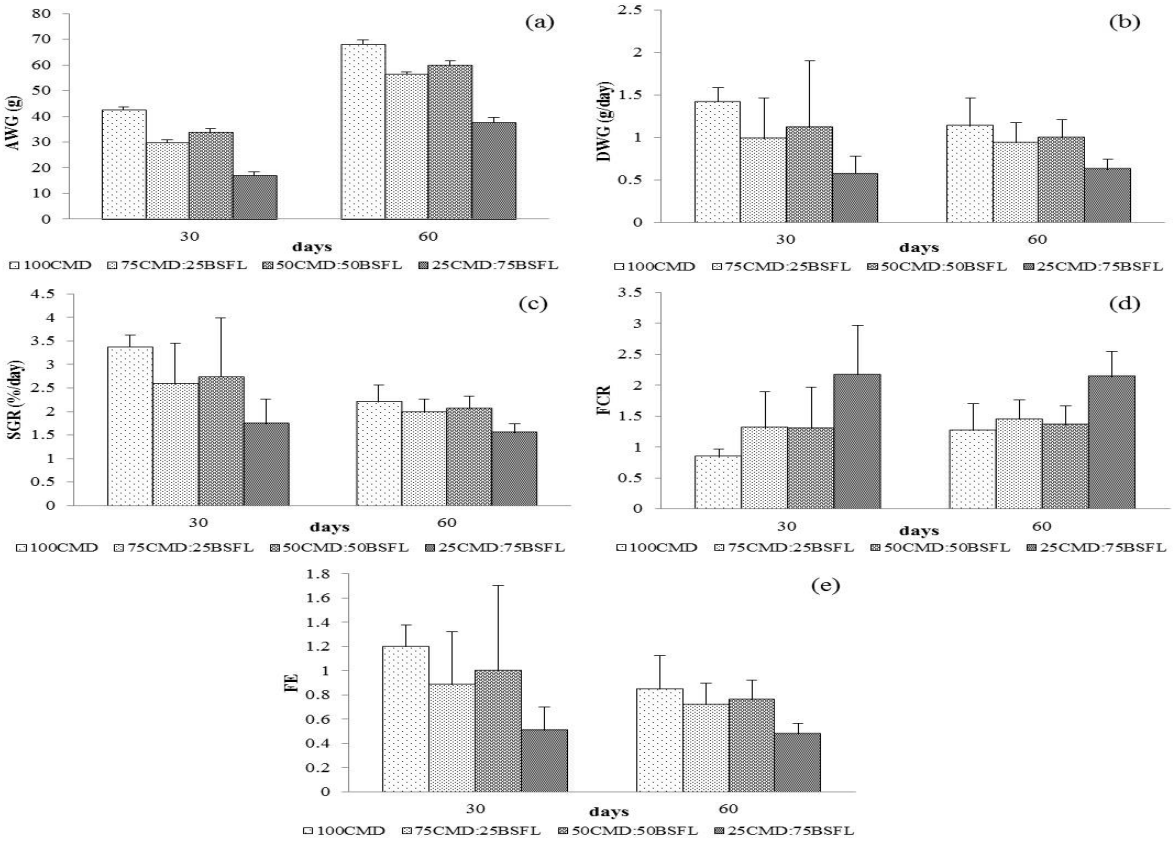

Figure 2. Growth performances (a, b and c), FCR (d) and FE (e) of hybrid catfish fed different proportions of commercial diet (CMD) combined with fresh black soldier fly larvae (BSFL).

Table 2. Growth indicators of fish fed different proportions of commercial diet (CMD) in combination with fresh black soldier fly larvae (BSFL) at 30 days of the experiment

Parameters	CMD:BSFL (%)			
	100:0	75:25	50:50	25:75
Average initial weight (g)	24.24 ± 1.59	24.24 ± 1.59	24.24 ± 1.59	24.24 ± 1.59
Average weight gain; AWG (g)	42.52 ± 1.13	29.57 ± 1.38	33.65 ± 1.52	16.99 ± 1.51
Daily weight gain; DWG (g/day)	1.41 ± 0.17	0.98 ± 0.47	1.12 ± 0.78	0.56 ± 0.21
specific growth rate; SGR (%/day)	3.37 ± 0.25	2.58 ± 0.86	2.73 ± 1.25	1.74 ± 0.52
Food conversion ratio; FCR	0.84 ± 0.11^{a}	1.30 ± 0.58^{ab}	1.30 ± 0.66^{ab}	2.16 ± 0.80^{b}
Feed efficiency; FE	1.19 ± 0.18	0.88 ± 0.43	1.00 ± 0.70	0.50 ± 0.19

Facts in the table represent the mean ± SD (n = 3) and the same superscripts in a row indicate no significant difference (p > 0.05).

Table 3. Growth indicators of fish fed different proportions of commercial diet (CMD) in combination with fresh black soldier fly larvae (BSFL) at 60 days of the experiment

Parameters	CMD:BSFL (%)			
	100:0	75:25	50:50	25:75
Average initial weight (g)	24.24 ± 1.59	24.24 ± 1.59	24.24 ± 1.59	24.24 ± 1.59
Average weight gain; AWG (g)	68.01 ± 1.71^{a}	56.27 ± 1.09^{ab}	59.91 ± 1.71^{ab}	37.53 ± 1.94^{b}
Daily weight gain; DWG (g/day)	1.13 ± 0.32^{a}	0.93 ± 0.23^{ab}	0.99 ± 0.21^{ab}	0.62 ± 0.11^{b}
specific growth rate; SGR (%/day)	2.20 ± 0.36^{a}	1.98 ± 0.28^{ab}	2.06 ± 0.25^{ab}	1.55 ± 0.18^{b}
Food conversion ratio; FCR	1.26 ± 0.43^{a}	1.44 ± 0.32^{ab}	1.35 ± 0.31^{a}	2.14 ± 0.40^{b}
Feed efficiency; FE	0.85 ± 0.27	0.71 ± 0.18	0.76 ± 0.16	0.47 ± 0.08
Survival rate; SR (%)	91.11 ± 3.38	85.55 ± 3.84	86.66 ± 3.66	81.11 ± 3.84

Facts in the table represent the mean ± SD (n = 3) and the same superscripts in a row indicate no significant difference (p > 0.05).

alone and a few fish presented faintly yellow skin in the ventral area. The probable reasons were that BSFL had a fat content up to 21.76% and the fat may had poor quality from rancid fats in growing media, which resulted in decreasing of consumption, digestion and the slightly yellow coloring (Behera, 2015; Priyadarshana *et al.*, 2021).

The fish fed CMD combined with fresh BSFL appeared to have lower growth rates than the control treatment, with the DWG, SGR, and FCR of fish at 30 and 60 days of the experiment were between 0.99-1.41 g/day, 2.06-3.37 %/day, and 0.84-1.35, respectively. These results are consistent with the studies of Jintasataporn *et al.* (2003) and Munkit (2020) which used silkworm pupae as a substitution of fish meal at 0, 25, 50, 75, and 100% in hybrid catfish formulated diet and used BSFL meal at 0, 30, and 50% mixed with other ingredients of local-made feed compared to CMD for common climbing perch, respectively. Their results indicated that fish consuming fishmeal replacement with domestic silkworm pupae showed lower growth rates than fish eating nonreplaced food (0% silkworm), with the FCR was between 1.23-1.27. Fish eating local food mixed with 0, 30 and 50% BSFL meal also exhibited lower growth rates than fish eating CMD alone, with the SGR and FCR were between 3.47-4.16 %/day and 1.17-1.44, respectively. Moreover, according to Madibana *et al.* (2020) who studied the effects of BSFL meal substitution of fish meal in dusky kob diet and Aisyah *et al.* (2022) who studied supplanted corn grain and soybean meal with BSFL meal in red hybrid tilapia diet, showed that fish had the SGR between 1.69-1.90 and 0.76-2.70 %/day, respectively and the FCR of tilapia was 1.44-1.81 which were similar to the present study. However, the study of Ordoñez *et al.* (2022) which researched whole BSFL feeding combined with commercial for Tambaqui, showed 1.40-2.04 g/day of DWG which appears to be a few greater than our studied result due to the higher nutritional values of their fresh BSFL which contained up to 41.57% protein and 23.85% lipid.

Histopathology study of the liver and stomach

Stomach condition, fundic (Figures 3c and 3e) and pyloric (Figures 3d and 3f) of fish fed 50% fresh BSFL

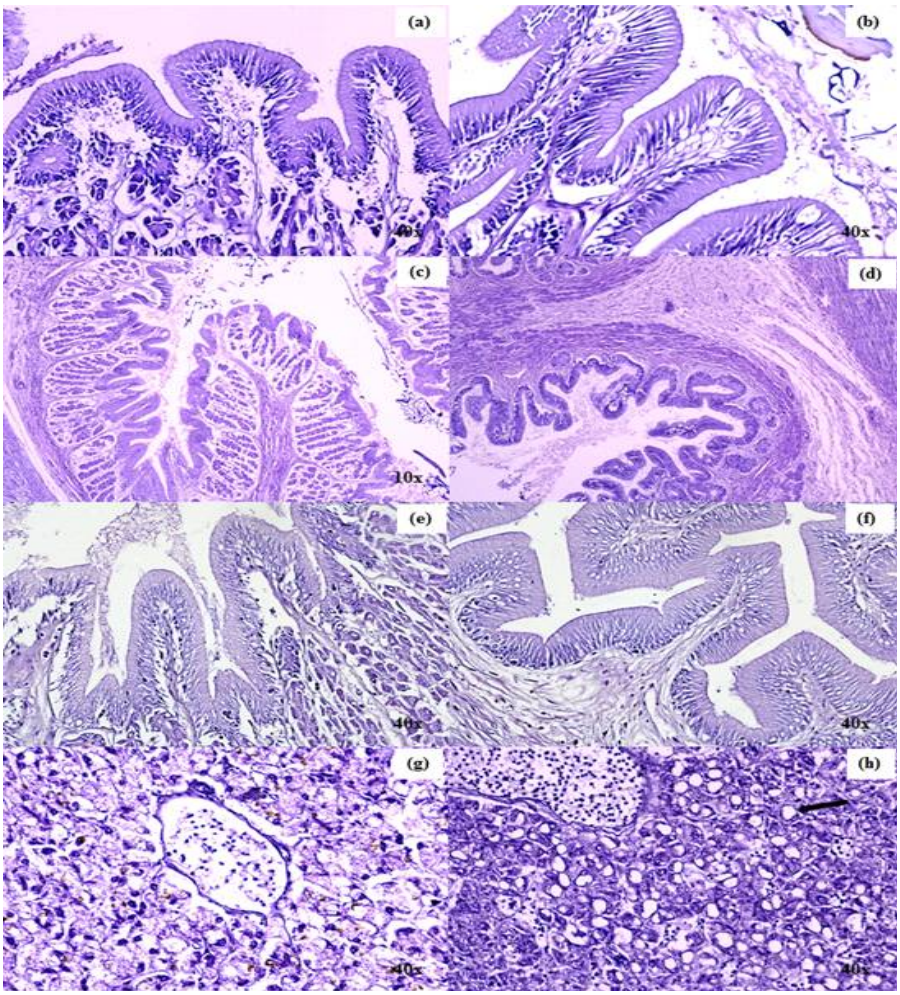

Figure 3. Histopathological studies of fundic (a, c and e), pyloric (b, d and f) and liver (g and h) of fish fed 100% commercial diet (CMD) and 50:50 of CMD:BSFL (black soldier fly larvae).

were normal as same as fish fed 100% CMD (Figures 3a (fundic) and 3b (pyloric)), but the liver appeared to have lipid accumulation in hepatocytes and greater presence of ballooning formation (Figure 3h) than fish fed 100% CMD (Figure 3g) which indicated steatosis condition. These results were similar to that of Zarantoniello *et al.* (2019) that estimated about zebrafish fed diets including 25% and 50% BSF full-fat prepupae meal compared with control group (0% BSF). Their results showed that the gut histological study did not show inflammation but the liver presented swollen hepatocytes and abundant intracytoplasmic lipid accumulation.

CONCLUSION

In conclusion, hybrid catfish can be fed fresh BSFL up to 50% combined with CMD 50% which do not has negative effect on growth and stomach function of fish. However, feeding with fresh BSFL can have side effect concerning steatosis.

ACKNOWLEDGEMENTS

This research could not have been accomplished without the help of all the participants. The researcher would like to thank all partners for their assistance to the successful completion of this work and special thanks is given to Phetchabun Rajabhat University for the funding provided.

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