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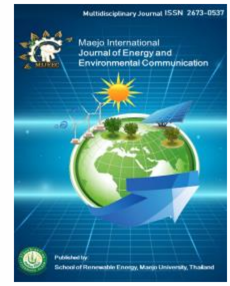

ARTICLE

Sustainable aquaculture leveraging black soldier fly larvae meal for economic and ecological gains in small-scale frog farming

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A B S T R A C T

The common lowland frog (*Hoplobatrachus rugulosus*) is an economically important species in Southeast Asia, with increasing demand for consumption. However, natural frog populations are rapidly declining, while farm production is insufficient to meet market demand. Frog feeds are also costly, prompting interest in using black soldier fly (BSF, *Hermetia illucens* L.) as a promising alternative protein source. BSF is nutritious and can be produced from household waste. This study aimed to establish the optimal inclusion level of BSF larvae (0, 30, 50, 70, and 100%) in the diet of common lowland frogs based on growth performance, feed conversion ratio (FCR), survival, and production cost. One hundred froglets (20.50±0.05 g) were stocked in triplicate plastic ponds and fed daily at 5% of their body weight for 120 days. Growth parameters, such as average weight gain, specific growth rate, average daily weight gain, and FCR, were significantly better for froglets-fed diets, including 100% and 70% BSFL, compared to lower inclusion levels. Also, higher survival rates were found with 70% and 100% BSFL inclusions, although not statistically significant compared to other treatments. Furthermore, diets containing 100% and 70% BSFL inclusions were more economical than those containing commercial feed pellets. The results showed that BSFL is a promising alternative protein source for replacing commercial feeds in frog diets since up to 100% inclusion had no negative impact on growth performance and survival.

1. Introduction

The common lowland frog (*Hoplobatrachus rugulosus*) is an economically important species in Southeast Asia, with increasing demand for consumption. However, natural frog populations are rapidly declining, while farm production is insufficient to meet market demand. Frog feeds are also costly, prompting interest in using black soldier fly (BSF, *Hermetia illucens* L.) as a promising alternative protein source. BSF is nutritious and can be produced from household waste. This study aimed to establish the optimal inclusion level of BSF larvae (0, 30, 50, 70, and 100%) in the diet of common lowland frogs based on growth performance, feed conversion ratio (FCR), survival, and production cost. One hundred froglets (20.50±0.05 g) were stocked in triplicate plastic ponds and fed daily at 5% of their body weight for 120 days. Growth parameters, such as average weight gain, specific growth rate, average daily weight gain, and FCR, were significantly better for froglets-fed diets, including 100% and 70% BSFL, compared to lower inclusion levels. Also, higher survival rates were found with

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70% and 100% BSFL inclusions, although not statistically significant compared to other treatments. Furthermore, diets containing 100% and 70% BSFL inclusions were more economical than those containing commercial feed pellets. The results showed that BSFL is a promising alternative protein source for replacing commercial feeds in frog diets since up to 100% inclusion had no negative impact on growth performance and survival.

2. Material and methods

2.1 Preparation of experimental units and animals

The 45-day-old tadpoles with similar average size and weight were acclimated in the experimental pond for about 1 week. The tadpoles were trained to eat small feed pellets with not less than 35% protein 3 times a day at 09:00 hrs, 12:00 hrs, and 15:00 hrs. Consequently, 15 experimental ponds (2 x 3 x 1.5 m) were prepared. Water was added to a depth of 5 cm, and morning glory and wooden beds were placed in ponds as hiding places and living areas for the froglets. Once the froglets were ready to accept feed pellets, they were weighed and randomly released into the experimental ponds at a density of 100 ind/m^2. The average weight of stocked froglets at the beginning of the experiment ranged from 20.50±0.05 to 21.10±0.50 g/ind.

Table 1. Nutritional values of prepared feed pellets and black soldier fly larvae used in the

Composition	Type of Feeds (% dry weight)	
	Feed pellets	BSFL
Crude protein	39.49±0.33	39.73±0.29
Crude fiber	2.84±0.17	9.50±0.24
Moisture	8.20±0.10	9.50±0.15
Fat	10.55±0.32	26.10±0.20
Ash	9.48±0.25	9.60±0.21

Table 2. The amount of essential amino acids of black soldier fly larvae used in the experiment.

Amino acid	Quantity (g/kg; dry)
Alanine	24.2
Arginine	20.0
Aspartate	35.9
Cystine	2.1
Glutamine	41.3
Glycine	22.2
Histidine	12.4
Iso-leucine	17.3
Leucine	28.0
Lysine	22.6
Methionine	7.6
Phenylalanine	16.3
Proline	21.4
Serine	15.0
Threonine	15.4
Tryptophan	5.8
Valine	24.8

2.2 Feeding management

The study explored the use of floating frog food pellets (35% protein) in combination with Fresh black soldier fly larvae (BSFL) produced from households' vegetable scraps and leftover food (Figure 1). The nutritional value of prepared feed pellets and BSFL meal is shown in Table 1, while the amount of essential amino acids of BSFL meal is provided in Table 2. The feeding rate was set at 10% body weight/day, with three feeding frequencies at 09:00 hrs, 12:00 hrs, and 15:00 hrs.

Figure 1. Fresh black soldier fly

2.3 Experimental **design**

The experimental units were laid in Complete Randomized Design (CRD). Five diets were formulated containing different replacements of commercial pellet feeds with BSFL; the substitution percentages were as follows: 0% (T_1; control), 30% (T_2), 50% (T_3), 70% (T_4), and 100% (T_5), respectively. The experiment was conducted for 120 days. The feeding rate was adjusted based on increased body weight after weighing every two weeks. The water was exchanged every week throughout the experiment.

2.4 Data collection

Twenty percent (20%) of the froglet population from each experimental pond was randomly collected and weighed every 2 weeks using an electronic scale. The feed consumed, the final number of harvested froglets, and fixed and variable costs in each treatment were also recorded. The collected data were processed and computed using the below formulae:

Average weight gain (g/ind) = Final weight - initial weight

Specific growth rate (SGR) (%/day) = (ln final weight - ln initial weight) × 100/ Days of culture

Average daily weight gain (ADG) (g/ind/day) = (Final weight - initial weight)/ Days of culture

Feed conversion ratio = Total feed consumed (g/Weight gain (g)

Survival rate (%) = No. of froglets survived × 100 / Initial no. of froglets

Production cost per froglet (Baht/ind) = Total cost / No. of frogs survived

Where, Total cost = Fixed cost + variable cost

Nutritional value and essential amino acid content analysis of black soldier fly larvae according to the method of Spranghers et al. (2017).

2.4 Statistical analysis

Mean values ± SD were expressed in the results. One-way analysis of variance (ANOVA) were used to analyze data. Treatment differences were evaluated using a Duncan test at P£0.05. Statistical analysis for all was performed using SPSS statistic Bass 17.0 for Window EDU S/N 5065845 (SPSS Inc, Chicago, USA).

3. Results and discussion

3.1 Average weight gain

Although the use of BSFL as a feed ingredient is gaining momentum in other aquaculture and livestock industries, few publications have directly addressed the use of BSFL for frog feed. The gap in this research reinforces the need for further, more thorough research to consider the potential benefits of its application in the amphibian farming industry. Embedding BSFL into frog diets provides a viable, sustainable alternative to traditional protein sources, such as fish meal, which is expensive and has such environmental implications as overfishing and depletion of local resources.

Furthermore, this study investigated the use of BSFL as a feed ingredient and determined the impacts on the performance of froglets fed with BSFL diets. Initially, it seemed like these treatments made no difference with weight gain, but it became clear that they differed as time went on. After 30 days of rearing, the average weight gain of froglets was significantly different ($p < 0.05$) among treatments (Table 3). This implies that the BSFL meal may change froglet growth over time due to its high protein and nutrient content.

The results of BSFL consumption were gradual and cannot be observed after 30 days because the froglets may adapt themselves to the composition of feed and the bioavailability of the nutrients in BSFL. Moreover, the BSFL contained bioactive compounds, including antimicrobial peptides and fatty acids, which may have enhanced nutrient absorption and overall growth. These results suggest that the future adoption of BSFL as a feed ingredient could significantly improve the growth performance of frogs and reduce dependence on traditional feed ingredients in frog farming.

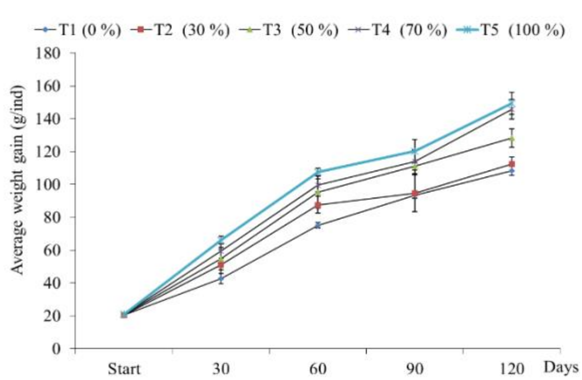

Figure 2. Average weight gain (g/ind) of frogs fed with 5 formulas of pellet feed mixed with black soldier fly larvae.

Future work will continue to explore the long-term effects of BSFL-based diets on growth and other KPIs relating to frog farming, such as feed conversion ratio (FCR), survival rate, and reproduction health. To date, minimal publications have been related to the direct use of BSFL as feed for frogs. The average weight gain of froglets among treatments began to differ significantly ($p<0.05$) after the 30th day of rearing (Table 3).

The froglets that received a diet containing 100% BSFL showed the highest final weight gain (66.06±2.40 g/ind), followed by the ones fed with 70% BSFL (59.39±4.46 g/ind). These were significantly higher compared with froglets received 50% (54.63±6.85 g/ind), 30% (51.15±8.92 g/ind), and 0% (42.67±3.14 g/ind) BSFL. The exact weight gain pattern was observed at the end of experiment (day 120), wherein froglets received 100% BSFL (149.34±6.60 g/ind) and 70% BSFL (145.67±6.03 g/ind) was significantly higher compared to those fed with 50% (128.29±5.72 g/ind), 30% (112.41±4.45 g/ind), and 0% (108.27±2.85 g/ind) BSFL (Table 3; Figure 2).

However, studies on various fish species have shown that using BSFL meal to replace traditional protein sources in fish feed yields positive results (Hoa & Dung, 2016; Vongvichith et al., 2020). The complete replacement of fishmeal with fermented BSFL meal did not adversely impact the growth, nutrient utilization, and immunity of pangasius catfish (*Pangasianodon hypophthalmus*) (Alfiko et al., 2022). Nile tilapia (*Oreochromis niloticus*) fed up with 100% BSFL meal grew perfectly without adverse effects on feed utilization, somatic indices, and hematological and skin mucus immunity (Tippayadara et al., 2021). The 100% replacement of fishmeal with BSFL meal was successful in common carp (*Cyprinus carpio*) at an inclusion level of 140 g/kg (Zhou et al., 2018) and Atlantic salmon (*Salmo salar*) at an inclusion level of 147.5 g/kg diet (Belghit et al., 2019).

3.2 Water quality

Results of this study revealed that growth parameters such as average weight gain, specific growth rate, average daily weight gain, and FCR were significantly better in froglets fed with 100% and 70% of BSFL inclusions than lower inclusions. This only proves the great potential of using BSFL diets, either in whole or partial proportions, in improving the growth efficiency of frogs. The better growth performance of froglets fed with BSFL diets might be attributed to their higher protein (39.73±0.29) and fat (26.10±0.20) percent dry weight compared to the feed pellet used in this study. The obtained percent protein dry weight in this study was within the 36 to 63% reported by Barragan-Fonseca et al. (2017).

This present finding was supported by the study of Nghia et al. (2023), wherein frogs cultured in net cages and fed with fresh total (100%) and partial (50%) inclusions of fresh and dried BSFL attained higher final weight at the end of the experiment compared to those that received 100% commercial diet. The best-recorded FCR (1.43) in the study of Nghia et al. (2023) was recorded in frogs fed with 50% fresh BSFL + 50% commercial feed, and this was better compared to the FCR (2.62) recorded in this study at 70% inclusion of BSFL.

Froglets received diet of 100% BSFL (0.016±0.001 %/day) had the highest SGR, followed by 70% BSFL (0.016±0.000 %/day), and these were statistically significant ($p<0.05$) when compared to diets of 50% (0.015±0.000 %/day), 30% (0.014±0.000 %/day), and 0 % (0.014±0.001 %/day) (Table 4). The BSFL diet also contained essential amino acids and vital nutrients for selecting a feed ingredient. These amino acids promote aquatic organisms' growth and reproduction (and disease resistance) (Andersen et al., 2016).

3.3 Feed conversion ratio and survival rate

Differences in amino acids and nutritional value in BSFL are based on the feed material used to feed BSFL (Nguyen et al., 2015). The results of this study show that BSFL fed with vegetable scraps and household food scraps have relatively high amino acid content (Table 2), which is similar to the reports of St-Hilaire et al. (2007) and Shumo et al. (2019), who found that BSFL fed with cow dung has high amino acid content and that amino acids such as alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and tyrosine. The BSFL amino acid content primarily depends on how the larvae is processed.

The amino acid content of dried BSFL meal has increased through defatting, i.e., mechanical or chemical removal of fat (Andersen et al., 2016; Renna et al., 2017). In addition, the amino acid profile of BSFL was similar to soybean meal, a protein source frequently used in animal feeds (Newton et al., 1977). On an "as-is" basis, BSFL provides 3.39 g/kg combined cysteine and methionine, enough to supply most animals (National Research Council, 1995). BSFL meal contains 2.7% omega-3 fatty acids and 22.3% omega-6 fatty acids as total fat. In contrast, fishmeal contains only 1.1–1.3% linoleic acid (LA) and 0.3–0.9% alpha-linolenic acid (ALA) as total fat, according to Ngoan et al. (2021). The vitamin composition of BSFL is comparable to other feeder insects commonly used. However, water-soluble vitamins (B vitamins) are sufficient to meet animal nutritional requirements (National Research Council, 1995).

The best FCR was recorded in froglets fed with 70% BSFL (2.62±0.15), followed by 100% BSFL (2.70±0.18). These were statistically significant ($p<0.05$) compared in froglets that received 50% (2.86±0.19), 30% (3.18±0.14), and 0% (3.45±0.18) BSFL diets (Table 4). Although not statistically significant, the highest survival rate was noted in froglets fed with 70% BSFL (80.94±0.98%), followed by 100 (80.72±0.84%), 50 (80.28±0.51%), 0 (80.17±0.60%), and 30% (80.06±0.75%) BSFL diets ($p>0.05$) (Table 4). In addition to growth performance, studies have also investigated the digestibility of BSFL diets across various animal species, including fish.

Digestibility varies depending on the chemical and physical composition of the diet, food processing methods, and the animals' digestive physiology and feed intake (Khan et al., 2003). Overall, research shows that the dry matter digestibility of BSFL-based feeds ranges from 59% to 84% (Newton et al., 1977; Renna et al., 2017). In addition to growth performance, studies have also investigated the digestibility of BSFL diets across various animal species, including fish. Digestibility varies depending on the chemical and physical composition of the diet, food processing methods, and the animals' digestive physiology and feed intake (Khan et al., 2003). Overall, research shows that the dry matter digestibility of BSFL-based feeds ranges from 59% to 84% (Newton et al., 1977; Renna et al., 2017).

3.4 Production cost

The first two lowest production costs were obtained in froglets fed with 100% (3.56±0.13 Baht/ind) and 70% (3.89±0.22 Baht/ind) BSFL diets and these were statistically significant compared with 50% (5.63±0.05 Baht/ind), 30% (6.92±0.17 Baht/ind), and 0% (9.18±0.06 Baht/ind) BSFL diets (Table 4). This study also highlighted that 100% and 70% inclusion of BSFL in the diet of froglets was more cost-effective than 100% feed pellets. In recent years, the use of insect meal as a high-quality ingredient in the diets of chickens, pigs, and fish has grown rapidly, although research on its application in frogs remains limited (Onsongo et al., 2018; Van Huis, 2013). Insects are rich in high-quality protein and can be mass-produced with a minimal environmental footprint due to their low greenhouse gas emissions (Van Huis, 2013).

Several challenges exist in the large-scale production of BSF. Waste conversion efficiency can depend on the type of household waste used, and the nutritional profile of the larvae can be affected by the substrate on which they were reared. In addition, BSF larvae can be raised on household waste, as long as the management is such that no contaminants or pathogens may affect the quality of the larvae and the safety of the fish feed made from them.

Table 3. Average weight gain (g/ind) of froglets fed with formulated diets containing proportions of pellet feeds and black soldier fly larvae.

Period (Days)	**Treatment**				
	T_1 (0%)	**T_2 (30%)**	**T_3 (50%)**	**T_4 (70%)**	**T_5 (100%**
Start	20.50±1.32^a	20.50±0.05^a	20.83±0.29^a	20.83±0.29^a	21.10±0.50^a
30	42.67±3.14^c	51.15±8.92^{bc}	54.63±6.85^b	59.39±4.46^{ab}	66.06±2.40^a
60	75.11±1.63^d	87.59±5.07^c	95.15±9.67^{bc}	99.48±3.94^{ab}	107.48±2.36^a
90	93.32±1.67^b	94.59±11.18^b	111.00±2.30^a	113.89±7.24^a	120.20±7.00^a
120	108.27±2.85^c	112.41±4.45^c	128.29±5.72^b	145.67±6.03^a	149.34±6.60^a

Note: Mean ± SD followed by different English letters horizontally indicates statistical difference at $p<0.05$.

Table 4. Growth efficiency, Survival rate and Production cost of froglets fed formulated diets containing proportions of pellet feeds and black soldier fly larvae.

Growth Efficiency	Treatment				
	T_1 (0%)	T_2 (30%)	T_3 (50%)	T_4 (70%)	T_5 (100%
Average weight gain (g/ind)	87.77±3.09^{c}	91.91±4.77^{c}	107.45±5.79^{b}	124.84±6.31^{a}	128.34±6.94^{a}
Specific growth rate (%/day)	0.014±0.001^{c}	0.014±0.000^{bc}	0.015±0.000^{b}	0.016±0.000^{a}	0.016±0.001^{a}
Average daily weight gain (g/ind/day)	0.73±0.03^{c}	0.77±0.04^{c}	0.90±0.05^{b}	1.04±0.05^{a}	1.07±0.06^{a}
Feed conversion ratio	3.45±0.18^{c}	3.18±0.14b^{c}	2.86±0.19^{ab}	2.62±0.15^{a}	2.70±0.18^{a}
Survival rate (%)	80.17±0.60^{a}	80.06±0.75^{a}	80.28±0.5^{a}	80.94±0.98^{a}	80.72±0.84^{a}
Production cost (Baht/ind)	9.18±0.06^{e}	6.92±0.17^{d}	5.63±0.05^{c}	3.89±0.22^{ab}	3.56±0.13^{a}

Note: Mean ± SD followed by different English letters horizontally indicates statistical difference at $p<0.05$.

Adding insect meals to animal diets is more cost-effective in livestock and aquaculture than conventional protein sources (Onsongo et al., 2018). In Kenya, the price of BSFL, for instance, is 0.8–1.2 USD/kg compared to fish meal (1.2–1.5 USD/kg) and soybean meal (0.9–1.6 USD/kg). BSFLs are relatively affordable because they can grow on low-value organic waste and because their systems are simple to produce (Papa et al., 2022; Van Huis, 2013).

4. Conclusion

The BSFL seems a promising alternative protein source to substitute commercial feeds in frog diets, up 100%, without affecting growth or survival. This is expected to decrease BSFL production prices as BSFL production increases, making this protein source a highly viable and sustainable alternative to feed future frogs. In the present study, we sought to determine the suitability of black soldier fly larvae (BSFL) meal as a sustainable alternative protein source to replace their commercial feed pellets in the diet of rice field frogs (Hoplobatrachus rugulosus). Five diets compared 0, 30, 50, 70, and 100% replacement of BSFL by feed pellet. Diet regimens containing 70 % and 100 % BSFL had a significantly higher weight gain, specific growth rate, and average daily weight gain than those containing lower BSFL inclusions. In the 100% BSFL group, this feed conversion ratio was also very good, and it was slightly better in the 70% BSFL group, followed by the 100% BSFL group. Treatments did not significantly differ in survival rates. The lowest production costs were seen on diets with 100% and 70% BSFL. The BSFL meal can replace commercial feed pellets up to 100% in frogs' diets without harm to growth performance and survival rates. At the same time, it reduces production costs, making it a promising and sustainable protein source for future frog farming.

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Conflict of Interest Declaration

The authors declare that no competing or personal interests jeopordize the results reported within this study.

CRediT authorship contribution statement

Teppitag Boonta: Writing – original draft, Methodology, Investigation, Conceptualization, Funding acquisition, Project administration. Niwooti Whangchai: Review & editing, Formal analysis. Sudaporn Tongsiri: Review & editing, Methodology, Investigation, Conceptualization. Redel L. Gutierrez: Review & editing, Investigation, Conceptualization. Alvin T. Reyes: Writing – review & editing, Conceptualization. Tipsukhon Pimpimol: Methodology, Formal analysis. Chanagun Chitmanat: Writing – review & editing, Investigation, Conceptualization.

Declaration of Competing Interest

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