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Original Research Articles

Using black soldier fly larvae as feed for Thai frog (*Rana rugosa* Temminck and Schlegel, 1838) – Preliminary study of the effect on production parameters

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This study aimed to evaluate the effect of the inclusion of either fresh or dried black soldier fly larvae (BSFL) in diets on the production parameters and chemical composition of Thai frogs (*Rana rugosa*) cultured in net cages. Frog fingerlings have an initial weight of 20.4 g and were distributed randomly into fifteen net cages (2 x 3 x 1.5 m) at a density of 240 frog.net cage-1. Including five dietary treatments named: T1 (100% commercial feed used as a control), T2 (100% dried BSFL), T3 (50% dried BSFL + 50% commercial feed), T4 (50% fresh BSFL + 50% commercial feed), and T5 (100% fresh BSFL) with three replicates for each treatment. The results showed that the survival rate of frogs ranged from 64.3% to 87.0%, improved feed conversion ratio (1.43 to 1.90%), increased live weight (194.7 – 244.6 g), daily weight gain (2.9 – 3.7 g.day-1), and frog's yield (6.30 – 7.96 kg.m-2). The contents of crude protein (18.21 – 23.19%), ether extract (0.28 – 0.53%), and total ash (0.99 – 1.05%) of frog meat in this study were affected by the inclusion of either fresh or dried BSFL in the diets of frogs. In recommendation, the diet comprised from 50% fresh BSFL + 50% commercial feed could be applied successfully in Thai frog cage production.

INTRODUCTION

Thai frogs (*Rana rugosa*) are easy to raise, suitable for Vietnam's weather conditions, use commercial pellets, and can catch motionless prey. This is a species of aquaculture with high economic value and potential for the domestic market in Vietnam. However, frog farmers face many difficulties such as poor seed quality, farming environment, diseases, and high price of food.[1] In aquaculture, the cost of food provided to animals often accounts for the highest proportion of the product's cost.[2] The current problem is that commercial feed prices are increasing, leading to increased production costs of aquatic products. So, the increasing costs of production in aquaculture due to the rising cost of feeding threaten the sustainability of the sector (Dawood, 2021). This is primarily caused by the overdependence of intensive aquaculture production on fishmeal and fish oils as major feed ingredients whose prices continue to rise due to declining production.[3] On the other hand, the food-feed-fuel competition for the limited resources under the current changing climatic conditions has drastically affected the availability of conventional feedstuffs such as fishmeal, soybean, and cereals, leading to a decline in availability and high volatility in feed ingredient prices.[2] Therefore, finding a rich-protein feed source that is easy to produce and low-cost to replace commercial feed to reduce costs and bring more profits to farmers is essential.

Currently, many insect species have been investigated in aquaculture. The black soldier fly (*Hermetia illucens*) is the most studied and promising insect to replace fishmeal in aquafeed. The black soldier fly has been identified as one of the most promising potential alternative protein sources for aquafeeds in the coming decades and the most promising insect species for mass-rearing for animal feed.[4] Recent studies have focused on evaluating potentially sustainable alternatives as black soldier fly with a short life cycle (40 – 45 days), and high reproductive capacity (500 – 1,200 eggs/female).[5] Black soldier fly larvae (BSFL) have high protein and fatty acid content, especially lauric acid, so they are an ideal raw material to replace part or all protein-rich food sources for commercial food production.[6,7] The application of black soldier fly larvae in aquatic feed has been studied on various fish species[8-12] and is considered a breakthrough in efforts to replace fishmeal in many aquatic species.

The growth performance and feed utilization effects of BSFL have been studied in aquaculture. However, optimum

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feed differs according to the species, size, and rearing system. Therefore, knowledge of the optimum feeding affects the usage of the nutrients in the feed, improves feed efficiency, and consequently results in better growth performance.[10] Hence, it is important to investigate and design a proper and applicable feeding regime to optimize cultured frog growth. In addition, replacing insects in aquatic feed showed conflicting results depending on species, growth stage, feed formulation, insect biomass processing method, and dietary administration period.

Frog flesh quality and safety are of primary importance to consumers, and thus, those parameters should be evaluated in frog-fed insect-derived products. These studies showed no adverse effects on growth and meat quality.[5] Most existing studies have investigated the effect of replacing fishmeal at different levels in the diets of aquatic animals to partially or fully replacing fishmeal. However, no studies have been published on the use of black soldier fly larvae as direct or indirect feed in frog farming. This study to our knowledge, represents the first designed to investigate the growth performance of frogs.

The purpose of this study was to evaluate the effect of direct use of black soldier fly larvae in fresh or dried form or plus with commercial feed on growth, survival rate, some carcass traits and meat chemical composition of the Thai frogs raised in net cages placed in freshwater ponds. The hypothesis of the study was that the inclusion of fresh or dry BSFL in the frog's diet improves production parameters.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND FEED

A total of 4,000 juveniles of frog were obtained from the Hatchery Centre, Hue province, Vietnam (16°23′1″N 107°35′46″E). Before the experiment, these frogs were held in five net cages (24 m^3/cage) under simulated – ambient summer photoperiod and temperature (28 – 29 ^oC) for acclimation for two weeks before the experiment (net cages were placed in the pond). All animal protocols were approved by the Animal Ethics Committee of Hue University, Vietnam (permit No. HUVN0026).

Commercial frog feed produced by CP Group-Vietnam was used to prepare the control feed containing 30% protein, 3.5% lipid, 14% ash, and 5% fibre. Fresh black soldier fly larvae (Fresh BSFL-**Figure 1A**) are fed biomass with tofu by-products and collected on day 7 after rearing.[13] Larvae were washed with water three times to remove all impurities on the body (Clean Fresh-BSFL was directly used for the frog). Subsequently, dried black soldier fly larvae (Dried BSFL-**Figure 1B**): Fresh-BSFL were boiled at 60°C for 30 minutes and then dried in the oven at 60°C (Dryer VN-100, Vietnam) for 48 hours before being stored at 4 °C until used. The chemical composition of BSFL is shown in **Table 1**.

Figure 1. (A) – Fresh black soldier fly larvae; (B) – Dried black soldier fly larvae

EXPERIMENTAL DESIGN

After two weeks of acclimation, 3,600 juveniles of frog (BW = 20.0 ± 1.27 g) were randomly selected and assigned to fifteen net cages with 2 x 3 x 1.5 m measurement (240 animals in each cage) under the same conditions as the stock cage. Subsequently, these cages were randomly assigned to five experimental groups corresponding to the different inclusion levels of fresh and dried BSFL named: T1 (100% commercial feed used as a control), T2 (100% dried BSFL), T3 (50% dried BSFL + 50% commercial feed), T4 (50% fresh BSFL + 50% commercial feed), and T5 (100% fresh BSFL) by completely randomized design method (CRD) with three replicates. The amount of feed in the diets was determined by the ratio of fresh BSFL/dry BSFL = 3/1. The frog was reared for 60 days at Huong Chu Research Farm in Hue City, Vietnam.

FEEDING AND MANAGEMENT

The frog was fed four times a day (7h, 11h, 15h, and 19h) until apparent satiation. The satiation level was determined based on apparent visual satiety. For the treatment T3 (50% dried BSFL + 50% commercial feed) and T4 (50% fresh BSFL + 50% commercial feed), frogs were fed commercial feed at 11 hours and 15 hours. The uneaten feed was removed for 30 minutes after feeding each meal daily.

Daily water quality was maintained through a gravity-driven water exchange between the stream and pond with approximately 30% daily water renewal. The temperature, pH, and DO in experimental cages were measured using a constant temperature meter, pH meter (Model: HI 98127; Manufacturer Hanna Instruments, USA), and DO meter (Model: YSI 550A; Manufacturer: Yellow Spring Instrument Company, Ohio, USA), respectively. Water samples from the pond were collected daily at 08:00 and 16:00 to determine $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$. Water quality parameters (mean ± SD) were recorded during the experimental period as follows: water temperature 28.5 ± 0.5 °C, pH 7.6 ± 0.4, DO 5.5 ± 0.5 $mg.L^{-1}$, ammonia and nitrite nitrogen < 0.1 and 0.05 $mg.L^{-1}$, respectively.

Table 1. Chemical composition of black soldier fly larvae and commercial feed.

Types	*DM*	*CP*	*EE*	*CF*	*Ash*
Dried BSFL	90.1	54.5	20.2	11.0	8.5
Fresh BSFL	29.7	16.2	6.0	3.3	2.5
Commercial feed	89.0	30.0	3.5	5.0	14

Note: Dried BSFL: Dried black soldier fly larvae; Fresh BSFL: Fresh black soldier fly larvae; DM: Dry matter; CP: Crude protein; EE: Ether extract; CF: Crude fibre; Ash: The mineral content

SAMPLING PROTOCOLS

Ten individuals in each net cage were randomly collected and anesthetized in 10 $mL.m^{-3}$ Aqui-S® before weighing. The growth parameters of frogs were estimated by determining body weight (BW) at the beginning and every fifteen days until 60 days. At the end of the experiment, nine frogs in each treatment were randomly collected and anesthetized with Aqui-S® at a concentration of 25 $mL.m^{-3}$ before slaughtering. Carcass traits were assayed at the Laboratory of Fisheries Faculty, University of Agriculture and Forestry, Hue University.

CALCULATION AND STATISTICAL ANALYSIS

All the parameters were calculated as follows: Daily weight gain (DWG, $g.day^{-1}$) was calculated by = $(W_i - W_0)/N$, in which W_0 and W_i were live weight at the initial day and day i, and N was the number day of stocking. Feed conversion ratio (FCR) was determined by a ratio between the amount of feed intake (FI - in dry matter) and weight gain (WG). Survival rate (SR, %) was determined by the ratio between the number of frogs at the final point of the experiment and the number of frogs at the initial point of the experiment. Yield ($kg.m^{-2}$) was calculated by the total live weight of frogs collected at the terminated experiment per square meter of water surface. The carcass traits of frogs were measured according to Ayres et al.[14]

All feed samples and frog meat were chemically analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and total ash according to the procedures of AOAC[15] at the Laboratory of Analysis of Feed and Livestock Products of the National Institute of Livestock Production, Ha Noi, Vietnam.

Data were presented in the form of the mean (M) and standard error of the mean (SEM) with three replicates (n=3). The data were statistically processed by analysis of variance (ANOVA) by the General Linear Model in Minitab v.16.2 (2010). The difference between the mean values was determined by the Tukey method at a confidence level of significance of $\alpha = 0.05$, and using Graph Pad Prism version 6.0 for Windows (Graph Pad Software, California, USA).

RESULTS

SURVIVAL RATE

In this study, the survival rate of frogs ranged from 64.3% to 87.0% (Figure 2). It was not significantly different among treatments T1, T2, T3, and T4 ($p>0.05$). However, there was a significant difference in the survival rate of frogs fed 100% fresh BSFL (T5) with the remaining treatments ($p<0.05$).

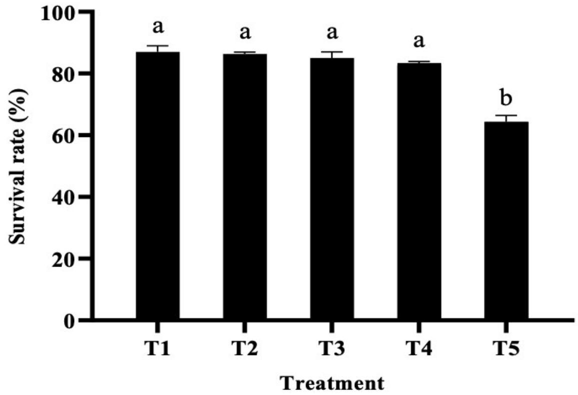

Figure 2. Survival rate of frogs in the different treatments

a, b: Mean in the columns without common letters are different at $p<0.05$.
T1 (100% commercial feed used as a control), T2 (100% dried BSFL), T3 (50% dried BSFL + 50% commercial feed), T4 (50% fresh BSFL + 50% commercial feed), and T5 (100% fresh BSFL)

GROWTH PERFORMANCE, FEED CONVERSION RATIO, AND YIELD

The results showed that feeding BSFL affected the growth of experimental frogs (Table 2). The frog's weight at the end of the experiment reached the highest in T5 (244.6 $g.frog^{-1}$), followed by T4 (236.9 $g.frog^{-1}$), there was no statistical difference between these two treatments ($p>0.05$). The frog weights in T2 and T3 were 204.5 and 209.2 $g.frog^{-1}$, respectively, and when comparing the weights between these two treatments, there was no statistical difference ($p>0.05$). Treatment T1 using only commercial feed achieved the lowest weight of 194.7 $g.frog^{-1}$ and there was no difference with T2.

At the end of the feeding trial, frogs' final weight and daily weight gain were highest in T5 (244.6 g, 3.7 $g.day^{-1}$, respectively) (Table 2). While FCR (1.43) was the lowest in T4, there was no difference with T5. The experimental diets affected these growth parameters ($p<0.05$). After 60 days of feeding, the yield of frogs ranged from 6.30 – 7.96 $kg.m^{-2}$, and the lowest in T5 (6.3 $kg.m^{-2}$).

Table 2. Growth performance, FCR, and yield

Parameters	*Treatment*					*SEM*	*p-value*
	T1	*T2*	*T3*	*T4*	*T5*		
Initial weight (g)	20.4	20.5	20.5	20.4	20.4	0.065	0.917
Final weight (g)	194.7^{c}	204.5^{cb}	209.2^{b}	236.9^{a}	244.6^{a}	2.301	<0.001
Daily weight gain ($g.day^{-1}$)	2.9^{b}	3.1^{ab}	3.1^{ab}	3.6^{a}	3.7^{a}	0.029	<0.001
FCR	1.90^{c}	1.76^{b}	1.66^{b}	1.43^{a}	1.51^{a}	0.048	<0.001
Yield ($kg.m^{-2}$)	6.80^{c}	7.15^{b}	7.13^{b}	7.96^{a}	6.30^{d}	0.147	<0.001

Note: ^{a,b,c}: Means (N= 30) in the same row without common letters are different at p<0.05. T1 (100% commercial feed used as a control), T2 (100% dried BSFL), T3 (50% dried BSFL + 50% commercial feed), T4 (50% fresh BSFL + 50% commercial feed), and T5 (100% fresh BSFL)

Table 3. Carcass traits and chemical composition of frog meat

Parameters	*Treatment*					*SEM*	*p-value*
	T1	*T2*	*T3*	*T4*	*T5*		
	Carcass traits						
Weight at slaughter (g)	190.3^{c}	194.0^{c}	207.2^{b}	237.1^{a}	240.7^{a}	3.245	0.001
Carcass dressing (%)	46.5^{c}	46.6^{c}	47.4^{b}	48.6^{a}	48.9^{a}	0.156	0.001
Thigh meat percentage (%)	23.4^{b}	24.2^{b}	24.3^{b}	26.4^{a}	25.6^{a}	0.168	0.001
Skin percentage (%)	8.1	8.3	8.2	8.4	8.5	0.149	0.473
	Chemical composition (%)						
Crude protein	18.21^{b}	19.72^{b}	20.43^{b}	23.01^{a}	23.19^{a}	0.539	0.001
Ether extract	0.28^{d}	0.39^{c}	0.38^{c}	0.46^{b}	0.53^{a}	0.023	0.001
Total ash	0.99	1.03	1.02	1.04	1.05	0.005	0.098

^{abc}Means (N= 30) in the same row without common letters are different at p<0.05.

CARCASS TRAITS AND CHEMICAL COMPOSITION OF FROG MEAT

There were significant differences between frog-feeding treatments. Live weight at slaughter ranged from 193.3 g to 240.7 g and differed among treatments (p<0.05). The live weight was higher in T4 and T5 (237.1 – 240.7 g) than in the others (190.3 – 207.2 g). The carcass and thigh meat ratio of frogs differed between treatments (p<0.05). In addition, frogs' carcass and thigh meat in treatments T4 and T5 were higher than in the other treatments. However, the value of skin ratio did not differ statistically (p>0.05) (**Table 3**).

The proximate composition of the meat of frogs at the end of the experiment, including the contents of crude protein (18.21 – 23.19%) and ether extract (0.28 – 0.53%), was affected by the inclusion of fresh and dried BSFL in the diets frogs (p<0.05) (**Table 3**). The crude protein and ether extract contents of frog meat were significantly higher in T4 and T5 than in other treatments (p<0.05). Total ash content in this study ranged from 0.99 – 1.05% and was not affected by including fresh and dried BSFL in the diets (p>0.05).

DISCUSSION

The use of BSFL in aquafeed formulations has recently gained attention. Detailed knowledge about the inclusion levels of BSFL for the Thai frog, a promising candidate for intensive aquaculture in Vietnam, still needs to be improved due to insufficient scientific data and including fresh or dried BSFL in the frog diet during 60 days of culture positively affected the growth performance and chemical composition. Treatment T5-fed 100% fresh BSFL frogs achieved faster growth than other treatments. According to Binh and Thao,[16] frogs have a food protein content of 27 – 35%, and the initial seed weight was 22.1 $g.frog^{-1}$ and after 60 days of raising frog weight ranged from 246.2 to 253.7 $g.frog^{-1}$. In this study, treatments using BSFL in fresh or dried form yielded higher frog weights and shorter rearing times than other studies.[17,18] To date, no publications have related to the direct use of BSFL as food for frogs. However, studies have been conducted on some fish species[19-22] wherein BSFL meal used to replace protein sources in fish food has brought positive results. Lan et al.[13] reported that BSFL fed by tofu by-products contained very high LA (omega-6) ranging from 27.57 - 29.7% and ALA (omega-3) ranged from 1.89 – 2.04% as total fat. BSFL meal contains 2.7% omega-3 fatty acids and 22.3% omega-6 fatty acids as total fat.[5] Meantime, fishmeal just contained 1.1 - 1.3% linoleic acid (LA) and alpha-linolenic acid (ALA) 0.3-0.9% as total fat.[23] Therefore, BSFL will be a potential protein source to replace fishmeal protein to reduce the cost of aquafeed due to sustainability issues of fishmeal.[24] Besides, the broader adoption of BSFL utilization in aquafeed will likely depend, to a more considerable extent, on aquaculture farmers and consumer acceptance.[25]

Figure 3. The Frog has a bloated stomach

The survival rate results of this study are similar to previous publications.[17,18] The survival rate of frogs ranged from 75 to 92%. However, frogs reared in treatment T5 achieved a low survival rate (64.3%). The survival rate of frogs depends on frog resistance, quality of seed, care, management, and rearing environment. In frog culture, the period from 5 g.frog^{-1} to 20 g.frog^{-1} often has a high loss rate due to poor seed quality, so in this study, seed with an average weight of 20.4 g.frog^{-1} and this stage was adapted to the experimental foods. Frogs are a species that prefers to eat fresh food. In the treatment (T5) using 100% fresh BSFL directly after 45 days of rearing, the frogs had a bloated stomach (**Figure 3**). Maybe the frog needed to digest the fresh BSFL fully. In the treatment, where frogs were fed 100% fresh BSFL, the survival rate was low, possibly because the frogs could not absorb the entire chitin layer of the larvae.[5] However, the results vary depending on the different forms of BSFL and parts used in aquafeed. For example, dietary BSFL meal as a replacement for fishmeal showed deleterious effects on the transcription of antioxidant enzymes and stress-related genes in the leukocytes of the head kidney.[26]

On the other hand, the ether extract content in fresh BSFL was relatively high (**Table 1**), while commercial food for frogs has an ether extract content of 3.5 – 4%. This may be why frogs' abdominal distention resulted in lower survival than other treatments. Some previous studies on BSFL as food for aquatic animals recommended adding BSFL meal after defatting. This problem was noted by Renna et al.[27] in dietary defatted rainbow trout without any adverse effects on growth or survival or fish meat quality. In addition, there have been reports that using BSFL as fish feed leads to adverse changes in fatty acid composition in meat, such as a reduction of unsaturated fatty acids.[28,29] Therefore, it is necessary to evaluate further the effect of lipid content in BSFL on the survival rate of frogs. The high fat content of BSFL makes it challenging to balance diets according to energy requirements. Therefore, one of the effective solutions is defatting, but it requires additional technology and labor costs.[5,27] According to Schiavone et al.,[30] BSFL can be defatted down to 18% and 4.6% (DM) with the protein content of 55.3% and 65.5% (DM), respectively, and it will be more effective when using fresh BSFL.

In the present study, T4 and T5, the frogs caught prey more efficiently than the treatments T1, T2, and T3 because there was an alternation in feeding the frogs between the commercial feeds and fresh BSFL, so it stimulated feeding, making the frogs better at catching prey. Also, it relates to the habit of frogs that prefer catching fresh insects. Some previous publications[17,18,31] recorded FCR when raising frogs fluctuated from 0.8 to 1.6. However, the results of these studies mainly used commercial frog feed (protein 35%, lipid 3%). No studies have been conducted on feeding frogs with dried or fresh BSFL or combining these commercial feeds with BSFL.

The main products of frog meat are the carcass and skin, which are a source of food. The remaining ingredients that cannot be used can be processed as food for other farmed animals[32] (**Figure 4**). Slaughtering frogs with a weight greater than 201g has the best productivity and meat quality. Frogs reach 151 – 250g, carcass weight ratio is 48.97 – 49.96%, thigh meat is 25.94 – 26.75%, and skin is 9.42 – 9.98%. The results of our study showed that the carcass rate and thigh meat rate were not significantly different from previous publications.[14]

Frog meat is a product with high nutritional content and good meat quality. According to Cagiltay et al.,[33] every 100g of farmed frog meat can provide more than adults' daily requirement of amino acids. In this study, frogs fed BSFL had similar meat quality to previous studies.[14,33] Frog meat's protein, fat, and mineral content depends on the season, age, and feeding regime. However, the chemical composition of wild frog meat is not different from farmed frog meat.[33] In this study, the ether extract content of treatments T4 (0.46%) and T5 (0.53%) were higher than the other treatments, possibly because the frogs used fresh BSFL containing high ether extract content that affected the meat quality of frogs.

In this study, we used BSFL as food for frogs in their original form, different from previous publications that often-used larvae powder to replace fish meal, and soybean meal to reduce costs in commercial frog farming in Vietnam. The issue of using fresh and dried BSFL on growth indicators and frog productivity has had positive results, but in this study, we did not evaluate the indicators of immune response as well as disease resistance of frogs. The response of immune function in aquatic animals to dietary supplementation has become an important criterion for evaluating the suitability of feed ingredients in aquaculture. Insect utilization in aquafeed has been evaluated on several immune-related parameters including blood biochemical composition, histopathology of related organs, gut health, related gene expression, and disease resistance in numerous aquaculture species.[34] The use of dietary BSF entails environmental benefits associated with the use of forage fish and, from a life cycle assessment viewpoint, on climate change, acidification, human toxicity, marine ecotoxicity, and abiotic depletion.[35] Therefore, research directions related to BSFL as food for aquatic animals have been and are being conducted by the Ministry of Agriculture and Rural Development in Vietnam, and many scientists are interested in clarifying professional issues.

In summary, the findings of this study suggest that using BSFL can offer high efficiency in terms of growth and meat

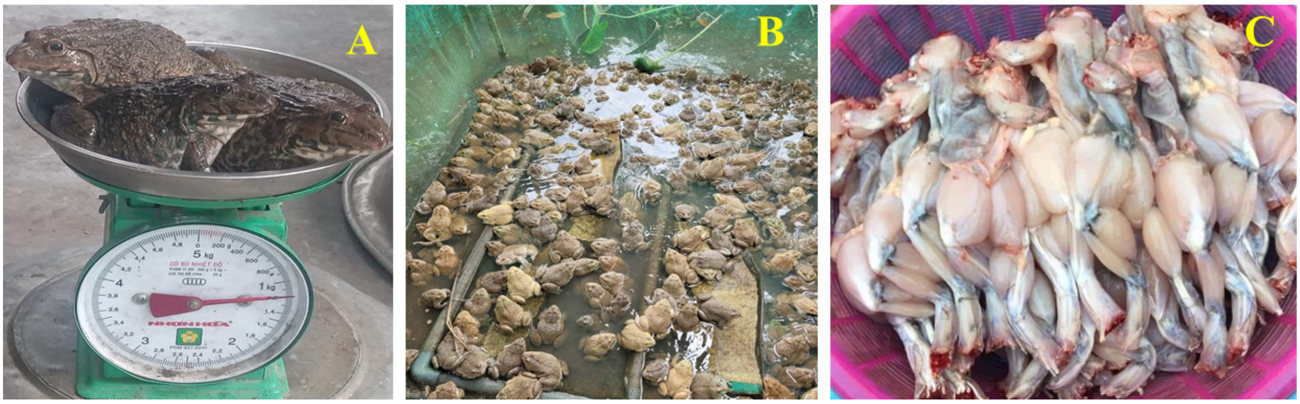

Figure 4. Frog after 60 days of experiment

Frog's mass (A); Mesh cage for raising experimental frogs (B); Frog meat after 60 days of experiment (C)

quality to meet food requirements, which will make an important contribution to the frog culture by supplementing cheap protein sources. In recommendation, the diet comprised of 50% fresh BSFL + 50% commercial feed could be applied successfully for Thai frog farm-scale production in Vietnam.

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AUTHORS' CONTRIBUTION

Conceptualization: Vo D. Nghia (Equal), Nguyen D.Q. Tram (Equal). Data curation: Vo D. Nghia (Equal), Pham T.P. Lan (Equal). Formal Analysis: Vo D. Nghia (Equal), Pham T.P. Lan (Equal). Writing – original draft: Vo D. Nghia (Lead). Investigation: Pham T.P. Lan (Lead). Methodology: Nguyen D.Q. Tram (Equal). Supervision: Nguyen D.Q. Tram (Lead). Writing – review & editing: Nguyen D.Q. Tram (Lead).

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