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Effects of the supplement of black soldier fly mix feeding on the growth performance, survival rate, and feeding behaviour of frogs, *Hoplobatrachus rugulosus* in captivity

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ABSTRACT
The objective of this research was to study the effects of processed food and a mixture of black soldier fly larvae on the growth rate, survival rate, and feeding behaviour of frogs in captive conditions, as well as to examine the water quality in the tanks where the frogs were fed with processed food, in addition to a mixture of worms and fruit flies. A comparison was made between feeding the frogs with 400 individuals of processed food, as well as 400 individuals of a mixture of worms and fruit flies over a period of 12 weeks. The results from the experiment showed that the average weight gain in frogs fed with processed food in stages 1, 2, and 3 was 67.10 ± 1.75, 46.75 ± 9.69, and 77.30 ± 0.11 g day^{-1}, respectively. The specific growth rate was recorded as 5.51% ± 0.71%, 1.06% ± 0.21%, and 1.05% ± 0.04% per day, respectively. The average survival rate was 94.61% ± 1.58%, 97.38% ± 0.27%, and 82.69% ± 7.06%, respectively. On the other hand, frogs fed with a mixture of worms and fruit flies showed an average weight gain of 55.98 ± 1.04, 37.88 ± 8.05, and 80.55 ± 3.08 g day^{-1} in stages 1, 2 and 3 respectively. The specific growth rate was 5.17% ± 0.69%, 1.02% ± 0.23%, and 1.22% ± 0.04% per day, respectively. The average survival rate was 93.10% ± 4.87%, 97.50% ± 1.28%, and 81.85% ± 02.89%, respectively. Statistical analysis showed significant differences ($p < 0.05$) in the growth rate between frogs fed with processed food as well as those fed with a mixture of worms and fruit flies. Regarding the water quality, frogs fed with processed food had temperatures ranging from 24.50 to 32.90 ℃, pH values between 6.89 and 8.90, dissolved solids ranging from 0.10 to 1.00 mg L^{-1}, electrical conductivity between 0.10 and 1.40 µS cm^{-1}, dissolved oxygen levels between 4.83 and 6.30 mg L^{-1}, and total ammonia levels ranging from 0.00 to 1.34 mg L^{-1}. For frogs fed with a mixture of worms and fruit flies, the water temperatures ranged from 24.10 to 32.60 ℃, pH values between 6.48 and 8.92, dissolved solids ranging from 0.10 to 1.00 mg L^{-1}, electrical conductivity between 0.10 and 1.20 µS cm^{-1}, dissolved oxygen levels between 4.33 and 6.44 mg L^{-1}, and total ammonia levels ranging from 0.00 to 0.96 mg L^{-1}. The water quality parameters did not show statistically significant differences ($p > 0.05$) between the group fed with processed food and the group fed with a mixture of worms and fruit flies.

Keywords: Black soldier fly, Growth rate, Survival rate, Frog Phayao.
Article type: Research Article.

INTRODUCTION
The frog is a beneficial animal for consumers, both directly and indirectly. It is a high-protein food source that helps control pests such as worms and plant-eating insects. Frog farming has gained significant interest among farmers due to its ease of rearing, low time investment, and high consumer demand. There is a growing international market for frog products, providing opportunities for Thai farmers to export their frog produce.

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However, the natural frog population has drastically decreased due to rapid human population growth, resulting in the conversion of frog habitats into human settlements. Additionally, the demand for frog consumption has increased, leading to efforts to promote frog farming, which is favored due to its ease of rearing and fast growth rate, making it a popular choice for consumption. Currently, frog farming faces challenges in terms of expensive frog feed, which increases the overall cost of farming and reduces farmers' profits. To address this, research has been conducted to study the suitability of different types of frog feed. Three types of feed were compared: homemade feed composed of yellow bean dregs, groundfish, boiled and finely crushed chicken bones, rice bran, and boiled water spinach; fish feed (protein 28.88%); and frog feed (protein 33.23%). The results showed that fish feed and frog feed were more cost-effective compared to homemade feed, with costs per kilogram of frog weight at 35.69 baht and 32.40 baht, respectively, while homemade feed costs 42.74 baht. In addition, to feed challenges, frog farming also faces environmental issues related to waste produced by frogs and leftover food in the tanks. Inadequate water exchange leads to higher acidity levels in the frog tanks, resulting in increased mucus production, slower adaptation, and increased susceptibility to diseases. Given the importance and challenges above, the researchers were interested in studying and comparing the effects of processed food and a mixture of worms and fruit flies on the growth rate, survival rate, feeding behavior of frogs in captivity, and water quality. Parameters measured included dissolved oxygen (DO), pH, temperature, electric conductivity (EC), total dissolved solids (TDS), and ammonia (NH_3) levels. DO and NH_3 were measured every 7 days, while pH, temperature, and EC were measured every other day, with recorded observations. This research aimed to provide insights into the most suitable feeding practices and optimal water conditions for successful frog rearing by evaluating the water quality parameters and their suitability for frog farming. The environment is a factor that influences the growth of tadpoles. When comparing tadpoles raised in a semi-natural laboratory setting with those raised under natural conditions, it was found that the maximum growth rate in terms of weight and length before metamorphosis into frogs was similar. However, during the stage of morphological transformation from tadpole to frog, a loss in weight by 32% and a loss in length by 67% were observed. Additionally, tadpoles collected from natural water sources tended to be larger than those raised in the laboratory under the same timeframe. This could be attributed to factors such as density, nutrition, temperature, or other factors that affect growth. Temperature is a significant factor that influences the behavior and growth of amphibians. Most aquatic and terrestrial amphibians tend to avoid high temperatures. They seek out water bodies or seek shelter under materials that provide more excellent conditions. Nocturnal feeding behavior is often observed as a means of adapting to lower temperatures during the daytime. Amphibians in predominantly hot regions tend to be nocturnal feeders, while those in colder or semi-cold regions or at higher elevations above sea level tend to be diurnal feeders. Each species of amphibian has its preferred temperature range, and what is suitable for one species may not be suitable for another. Therefore, temperature is an important factor that governs the distribution of amphibians. Frogs, in general, are associated with hot regions, but they can be found in diverse habitats and may even inhabit outside of hot regions. Frogs in hot regions are not typically found in areas with temperatures below 15-20 ℃ and have a low tolerance for cold temperatures. Temperature also influences the evaporation of water from frog bodies, and various frog species employ mechanisms to facilitate water loss and lower internal body temperature. Many frog species, especially in the Ranidae family, spend significant time on land and increase their internal body temperature through sun basking. This promotes higher rates of water evaporation from their bodies, which can be problematic for maintaining water balance. Therefore, sun basking is primarily observed in frog species that inhabit water sources continuously. The pH value, which represents the acidity or alkalinity of water, is an essential characteristic of frog pond environments. In different seasons, the pH values of the water vary significantly, ranging from 5.5 to 9.0. This range indicates a wide variation in the acidity and alkalinity of the water. The suitable pH range for frog rearing should be between 6.5 and 8.5. The fluctuation in pH values is caused by the presence of organic substances in the water used for frog rearing, the waste produced by the frogs, and the decomposition of leftover food in the pond. Irregular water changes contribute to a decrease in the pH value of the water in the frog pond, resulting in excessive mucus production, inadequate adaptation, weakened immunity, and increased susceptibility to diseases in frogs. Oxygen is a gas that is vital for nearly all forms of life because it is required for various physiological processes that generate energy. Therefore, controlling and maintaining an adequate level of dissolved oxygen in water to support aquatic organisms is essential. Insufficient oxygen levels can be detrimental to aquatic life. It is necessary to ensure that the dissolved oxygen concentration remains within a safe range for the well-being of aquatic organisms. The dissolved oxygen (DO) concentration is typically measured in mg L^{-1}.

In order for aquatic organisms to thrive, it is recommended to maintain a DO level between 5-7 mg L^{-1}. This range provides sufficient oxygen for the metabolic needs of aquatic organisms and supports their normal respiration and other essential biological functions. Regular monitoring and management of dissolved oxygen levels in aquatic environments are crucial for the health and survival of aquatic life. The ammonia concentration in water is an important parameter affecting the aquatic environment's quality. According to the standards set by the Department of Fisheries, the total ammonia concentration in water should not exceed 0.02 mg L^{-1}. High levels of ammonia in a pond or aquatic environment can negatively affect aquatic organisms' health. When the ammonia concentration surpasses the recommended standard of 0.02 mg L^{-1}, it can cause stress, weakness and make aquatic organisms more susceptible to infections and diseases. Ammonia is particularly detrimental to aquatic organisms like frogs, as they are semi-aquatic and can respire through various means, such as their skin and lungs. The presence of high ammonia levels in water can negatively impact the oxygen uptake and overall well-being of aquatic organisms. Therefore, monitoring and maintaining ammonia levels within the acceptable range is crucial to ensure the health and survival of aquatic organisms in a pond or any aquatic habitat. Implementing proper water management practices and taking appropriate measures to control ammonia levels can help mitigate the risks associated with high ammonia concentrations. The *Hermetia illucens* L., commonly known as the black soldier fly, belongs to the family Stratiomyidae in the order Diptera. The adult flies have a black body with clear white markings on the tips of all legs. Their wings appear bluish or reddish-gold when reflecting light. The first abdominal segment of the backside has two distinct round white spots, while the lower abdomen has a band-like pattern (as shown in Fig. 3). The females have a large, elongated, and black ovipositor for egg deposition, while the males have a long, slender abdomen with deep furrows and brown or reddish-gold coloration. These flies reproduce through sexual reproduction, with the males actively seeking out the females and mating occurring on various surfaces. The males approach the females or engage in rapid mid-air copulation. The females lay eggs in groups in dry areas near cracks or crevices of food sources. Black soldier flies are commonly found in hot and subtropical climates. They are non-disease carriers and are not considered plant pests. According to the study by Tomberlin & Sheppard (2001), the life cycle of black soldier flies that feed on pineapple waste involves larvae that consume and decompose organic matter, making them helpful in reducing organic waste. Black soldier flies are also capable of controlling house flies (Muscadomestica) as they release an oviposition deterrent (allomone; Bradley & Sheppard 1984). Additionally, they have been found to reduce *Escherichia coli* bacteria when reared in dairy cow manure (Liu *et al.* 2008). Forensic entomology also uses these flies to estimate postmortem intervals (Pujol-Luz *et al.* 2008). In terms of nutritional value, an analysis of black soldier fly larvae revealed approximately 42% protein, 35% fat, 2,900 kilocalories of energy per kilogram, amino acids, and other nutrients (Sheppard *et al.* 2002). The protein content is comparable to pork, which is approximately 43.20%. This makes black soldier fly larvae suitable as supplemental feed for animals such as poultry, pigs, and tilapia (Newton *et al.* 2005). Research has shown that the larvae of the black soldier fly (*H. illucens*) can be beneficial in raising frogs. The black soldier fly larvae are known for their ability to consume and break down organic matter, making them useful in waste management and reducing organic waste. They have been used as feed for various animals, including frogs. One of the significant advantages of using black soldier fly larvae in frog rearing is their nutritional value. Studies have found that larvae are rich in protein (approximately 42%), fats (around 35%), amino acids, and other essential nutrients. The protein content in black soldier fly larvae is comparable to that in pork. This makes them a suitable and cost-effective supplemental food source for raising frogs. Additionally, the larvae of the black soldier fly have been found to inhibit the reproduction of house flies (Muscadomestica) by releasing a substance that acts as an oviposition deterrent. This can be beneficial in frog-rearing environments where house flies can be a nuisance. Furthermore, the black soldier fly larvae have been investigated for their potential use in forensic entomology, specifically in estimating postmortem intervals. This field utilizes insects and their life cycles to determine the time of death in forensic investigations. The research suggests that black soldier fly larvae can be a valuable resource in frog rearing. They offer a sustainable and nutrient-rich food source while aiding in waste management and potentially controlling pest insects. However, it is important to note that specific research on the use of black soldier fly larvae for frog rearing may vary, and further studies in this specific context may be necessary for more comprehensive insights.

MATERIALS AND METHODS

The number of frogs initially raised in the enclosure was 800 individuals. After leaving them for 2 weeks, the remaining number of frogs was 780. The frogs were then divided into 6 enclosures, with each enclosure containing

130 frogs. The experiment was conducted using a Completely Randomized Design (CRD) and the statistical analysis involved performing an independent T-test to compare two independent sample groups: the group fed with processed food and the group fed with a mixture of black soldier fly larvae. The experiment was divided into two sets, each consisting of three replicates. Set 1 (T_1R_1, T_1R_2 and T_1R_3) involved the processed food group, while Set 2 (T_2R_1, T_2R_2 and T_2R_3) the group fed with the mixture of black soldier fly larvae.

Preparation of the rearing tanks

The rearing tanks for the frogs were prepared by setting up wooden frames or posts according to the planned layout. Six rearing tanks were arranged in two rows, with each tank measuring 2.20 m in length and 1 m in width. The tanks were equipped with a shading net around the perimeter to prevent the entry of animals such as dogs and snakes. Additionally, the tanks were filled with water to a level of 2-5 cm.

Experimental animals

One-month-old froglets obtained from the aquaculture facility of the Faculty of Agriculture and Natural Resources, Phayao University, were placed in the rearing tanks at a density of 200 individuals per tank. A total of four tanks were used to acclimatize the frogs to their new environment. After a two-week acclimatization period, the remaining frogs, totalling 780 individuals that had body sizes of about 2-2.5 inches or 10 g of frog masses, were divided into six rearing tanks with a density of 130 individuals per tank.

Frog rearing

During the frog-rearing process, regular care was provided to the frogs and the water quality in the rearing tanks. This included feeding the frogs with appropriate food according to their nutritional requirements and maintaining the water quality in the tanks. The water was refreshed daily and the tanks were cleaned to prevent excess food and frog waste buildup, which could lead to water contamination.

Feeding regimen

The frogs were fed twice a day, in the morning (08:30) and evening (16:30). The amount of food was approximately 10% of their body weight or 1.0 g per individual, which contained two weeks-old frogs. Afterward, providing food to several frogs increased according to their elevated body weight each week. (Table 1). In addition, the feeding process was observed for 30 min to ensure that the amount of food given was sufficient for the frogs' needs. If the food was depleted or there was excess remaining, adjustments or additional food were provided on the following day to meet their requirements.

Water quality management

Regular water changes in the rearing tanks are crucial to maintain water quality and promote optimal growth of the frogs. Therefore, the water will be replaced before each feeding session in the morning and evening, ensuring cleanliness by removing debris, frog waste, and leftover food. Water quality will be assessed based on clarity and odor to determine the need for water changes.

Table 1. A feeding schedule indicates the amount of food for rearing frogs within 12 weeks.

Age (weeks)	The amount of food (grams/individual)	Number of meals per day
2	1.0	2
4	2.5-3.0	2
6	3.5-4.0	2
8	5.0-5.5	2
10	5.5-6.0	2
12	5.5-6.0	2

Data recording and collection

1. Frog growth measurement

- Randomly selecting 45 frogs from each of the 6 rearing tanks, totaling 270 frogs.
- Weighing and measuring the length of each frog every week for a period of 85 days (12 weeks).
- Recording the weight and length of the frogs to calculate the total weight, average weight, and standard deviation.

2. Food Consumption

- Recording and calculating the amount of food consumed by the frogs on a weekly basis.

These data were provide valuable information on the growth and food consumption of the frogs throughout the experimental period.

Analysis for total protein content using the Kjeldahl method (AOAC 2000)

1. Sample preparation

- Collecting representative samples of frog tissues for protein analysis.
- Homogenizing the samples to ensure uniformity.

2. Digestion

- Weighing an appropriate amount of the homogenized sample and transferring it into a digestion flask.
- Adding a digestion mixture containing sulfuric acid and a catalyst (e.g., potassium sulphate) to the flask.
- Heating the flask in a digestion block or digester until complete digestion of the organic material occurs.

3. Distillation

- Connecting the digestion flask to a distillation apparatus.
- Adding an alkaline solution (e.g., sodium hydroxide) to the digestion flask to liberate the ammonia.
- Collecting the liberated ammonia in a receiving flask containing a known amount of acid (e.g., boric acid) solution.

4. **Titration**

- Titration of the collected ammonia solution with a standardized acid solution (e.g., hydrochloric acid).
- Determining the volume of acid solution required to neutralize the ammonia.

5. **Calculation**

- Calculating the nitrogen content in the sample based on the volume of acid solution used.
- Converting the nitrogen content to protein content using a conversion factor (e.g., 6.25 for animal tissues).

The Kjeldahl method is a widely accepted and accurate method for determining the total protein content in biological samples. By following the above steps, the protein content of the frog samples was analysed using this method.

Water quality analysis

To analyse the water quality, the following parameters were measured for each of the 6 tanks:

1. Temperature (Temp; °C)
2. pH (Positive potential of the Hydrogen ions; mg L^{-1})
3. Electric conductivity (EC; µs cm^{-1})
4. Total dissolved solids (TDS; mg L^{-1})

Measurement Equipment

For temperature, pH, EC, and TDS measurements, a Waterproof pH/EC/TDS & Temperature Meter (PH-3508) were used. Each parameter was measured three times for each tank.

Ammonia analysis
To determine the ammonia (NH_3) concentration in the water of each tank, a Handheld Colorimeter Ammonia MR (HANNA instrument) was utilized. The ammonia levels were checked for each tank.

Dissolved oxygen analysis
The measurement of dissolved oxygen (DO) in the water was performed using the titration method. The exact technique was not specified in the given information. By conducting these analyses, the water quality parameters, including temperature, pH, EC, TDS, ammonia, and dissolved oxygen, were assessed for each tank.

Statistical analysis
The statistical analysis was involved the use of an independent t-test to compare two independent sample groups: processed food and insect larvae-based food. The purpose of the analysis was to determine if there was a significant difference between the two groups. A confidence level of 95% ($p < 0.05$) were used for assessing the statistical significance of the results.

RESULTS

1. Weight gain per day
The study was conducted in three phases, wherein frogs were farmed in tarpaulin ponds using various types of food, along with both an automatic water change system and a manual labour-based water change system. In phase 1, the automatic system showed an average daily weight gain of 67.10 ± 1.75 g, while the manual labour-based 55.98 ± 1.04 g. Moving on to phase 2, the automatic system exhibited an average daily weight gain of 46.75 ± 9.69 g, whereas the manual labour-based 37.88 ± 8.05 g. Finally, in phase 3, the automatic system resulted in an average daily weight gain of 77.30 ± 0.11 g, while the manual labour-based 80.55 ± 3.08 g.

2 .Specific growth rate
Through farming frogs in tarpaulin ponds with different types of food, including an automated water change system and a manual labour-based system for water change, the study was divided into three phases. In phase 1, the automatic system showed an average daily specific growth rate of 5.51 ± 0.71 g, while the manual labour-based 5.17 ± 0.69 g. In phase 2, the automatic system exhibited an average daily specific growth rate of 1.06 ± 0.21 g, whereas the manual labour-based 1.02 ± 0.23 g. In phase 3, the automatic system resulted in an average daily specific growth rate of 1.02 ± 0.23 g, while the manual labour-based system 1.22 ± 0.04 g.

3. Survival rate
The study comprised three phases, during which frogs were raised in tarpaulin ponds utilizing distinct water change systems, encompassing an automated system and a system reliant on manual labour. In phase 1, the automatic system showed an average survival rate of 94.61% ± 1.58%, while the manual labour-based system 94.10% ± 4.87%. In phase 2, the automatic system exhibited an average survival rate of 97.38% ± 0.27%, whereas the manual labour-based system 97.50% ± 1.28%. Finally, in phase 3, the automatic system resulted in an average survival rate of 82.69% ± 7.06% percent, while the manual labour-based system 81.85% ± 2.89% (Table 2).

4. Feed intake rate
Both the automatic and manual labour-based water change system were evaluated across three phases. In phase 1, the automatic system exhibited an average feed intake rate of 2.19% ± 0.03% per day, while the manual system 2.18% ± 0.02%. Moving to phase 2, the automatic system had an average feeding intake rate of 0.82% ± 0.10% per day, whereas the manual system 0.83% ± 0.07%. Finally, in phase 3, the automatic system had an average feed intake rate of 1.02% ± 0.03%, while the manual system 0.96% ± 0.04% (Table 2).

5. Feed weight of frogs
Frogs were reared in tarpaulin ponds using different types of food, along with an automatic and a manual labour-based water change system. The study was divided into three phases. In phase 1, the automatic system showed an average feed weight of 0.31 ± 0.00 g per body weight, while the diet mixed with striped fly larvae 0.32 ± 0.00 g. In phase 2, the automatic system exhibited an average feed weight of 0.06 ± 0.00 g per body weight, whereas the

diet mixed with striped fly larvae of 0.06 ± 0.00 g weight. In phase 3, the automatic system resulted in an average feed weight of 0.48 ± 0.04 g per body weight, while the diet mixed with striped fly larvae 0.46 ± 0.00. The observed difference was statistically significant ($p < 0.05$; Table 2).

6. Protein intake of frogs

The frogs were raised in tarpaulin ponds, utilizing various food options and employing both automatic water and manual labour-based water change systems. The study was divided into three phases. In phase 1, the automatic system showed an average protein intake of 384.13 ± 20.22 g per body weight, while the manual system by human labour 368.14 ± 9.77. Moving on to phase 2, the automatic water change system resulted in an average frog protein intake of 132.39 ± 4.40 g per body weight, whereas the normal water change system by human labour 102.89 ± 2.66 g. Finally, in phase 3, the automatic system exhibited an average frog protein intake of 1304.30 ± 74.57 g per frog body weight, while the manual system 1171.68 ± 28.62 g (Table 2).

7. Protein efficiency

In phase 1, the average protein efficiency in the automatic water change system was 26.56 ± 1.09, while in the manual labour-based 27.12 ± 1.86. In phase 2, the average protein efficiency of the automatic system was 7.43 ± 1.39, while the manual system 6.48 ± 1.55. In phase 3, the automatic system exhibited an average protein efficiency of 4.77 ± 0.24, and the normal system for manual water change 5.69 ± 0.23 (Table 2).

8. Feed efficiency

In phase 1, the average feed efficiency of the automatic water change system was 2.14 ± 0.12, while the manual labour-based 2.07 ± 0.08. Moving on to phase 2, the automatic system exhibited an average feed efficiency of 1.90 ± 0.41, while the normal manual system 1.72 ± 0.33. Lastly, in the third phase, the automatic system showed an average feed efficiency of 0.71 ± 0.04, whereas the manual system 0.84 ± 0.04 (Table 2).

9. Feed conversion ratio

In phase 1, the automatic water change system exhibited an average feed conversion ratio of 0.46 ± 0.03, while the normal water change system by manual labour 0.48 ± 0.02. In phase 2, the automatic system showed an average feed conversion ratio of 0.59 ± 0.15, whereas the normal manual system 0.63 ± 0.14. Lastly, in phase 3, the automatic system demonstrated an average feed conversion ratio of 1.41 ± 0.08, while the normal manual system 1.18 ± 0.05 (Table 2).

10. Water quality

Water quality parameters were collected from frog farming systems utilizing an automatic water change system and a manual labour-based normal water change. The parameters included water temperature (Temp), water pH value (pH), total dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO), and ammonia content in the water. The temperature of the water ranged from 24.1 to 32.9 ℃, while the pH of the water ranged from 6.48 to 8.92 mg L^{-1}. The total dissolved solids in the water were within the range of 0.1 to 1.0 mg L^{-1}, and the electrical conductivity ranged from 0.1 to 1.3 µS cm^{-1}. The dissolved oxygen content fell between 4.74 and 6.30 mg L^{-1}, and the total ammonia values ranged from 0.04 to 0.62 mg L^{-1}. The water quality remained within the appropriate criteria, ensuring normal growth of the frogs.

DISCUSSION

This research study investigated the effects of processed food and the supplement of black soldier fly mix feeding on the growth performance and feeding behaviour of frog rearing in capacity for 12 weeks. At present, numerous studies related to the sustainable utilization of insects to replace protein from soybean production for animal feed)Tahamtani *et al.* 2021(. An Insect diet has been admitted that it is high alternative protein source for aquafeed, and the quantity levels of insect food also should be suitable for animal raising (Hua *et al.* 2019(. Especially, the black soldier flies)BSF, *Hermetia illucens*(have received a lot of attention associated with food substitution of processed food for farming frogs, since they are rich in 43.17% protein, a good source of lipids)approximately 31.08%(, and carbohydrate (Spranghers *et al.* 2017; Muin & Taufeket 2022).

Table 2. Growth Performance and Feed Efficiency of Frogs with Automatic Water Change System.

	Growth Stage						Average	
	Phase 1		Phase 2		Phase 3			
	automatic	manual	automatic	manual	automatic	manual	automatic	manual
Weight gain per day (g day^{-1})	67.10±1.75	55.98 ± 1.04	46.75 ± 9.69	37.88 ± 8.05	77.30 ± 0.11	80.55 ± 3.08	63.72 ± 8.98	58.14 ± 12.36
Specific growth rate (g day^{-1})	5.51±0.71	5.17 ± 0.69	1.06 ± 0.21	1.02 ± 0.23	1.05 ± 0.04	1.22 ± 0.04	2.54 ± 1.48	2.47 ± 1.35
Survival rate (%)	94.61±1.58	94.10 ± 4.87	97.38 ± 0.27	97.50 ± 1.28	82.69 ± 7.06	81.85 ± 2.89	91.56 ± 4.51	91.15 ± 4.75
Feed intake rate (g day^{-1})	2.19±0.03	2.18 ± 0.02	0.82 ± 0.10	0.83 ± 0.07	1.02 ± 0.03	0.96 ± 0.04	1.34 ± 0.43	1.32 ± 0.43
Feed weight of frogs (g/frog 100 g)	0.31±0.00	0.32 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.48 ± 0.04	0.46 ± 0.00	0.28 ± 0.12	0.28 ± 0.12
Protein intake of frogs (g/frog 100 g)	384.13±20.22	368.14 ± 9.77	132.39 ± 4.40	102.89 ± 2.66	1304.30 ± 74.57	1171.68 ± 28.62	606.9 ± 356.17	547.57 ± 321.31
Protein efficiency	26.56±1.09	27.12 ± 1.86	7.43 ± 1.39	6.48 ± 1.55	4.77 ± 0.24	5.69 ± 0.23	12.92 ± 6.86	13.1 ± 7.01
Feed efficiency	2.14±0.12	2.07 ± 0.08	1.90 ± 0.41	1.72 ± 0.33	0.71 ± 0.04	0.84 ± 0.04	1.58 ± 0.44	1.54 ± 0.37
Feed conversion ratio	0.46±0.03	0.48 ± 0.02	0.59 ± 0.15	0.63 ± 0.14	1.41 ± 0.08	1.18 ± 0.05	0.82 ± 0.3	0.76 ± 0.21

Moreover & Makkar *et al.* 2014and Shumo *et al.* (2019have reported that the BSF has a lot of amino acids such as alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and tyrosine, which are nutritional value for physiological structure development and growth performance in frogs. The parameters of this trial, related to growth performance)weight gain per day, specific growth rate, survival rate, feed intake rate, and feed conversion ratio(and nutritional value)protein efficiency (out of comparison trial of processed food and the BSF meal. The results showed that the growth performance)weight gain per day, specific growth rate, and protein efficiency(of phase 3 with BSF meal was significantly higher than the processed food. On the other hand, the feed conversion ratio was significantly lower in feeding with the BSF meal than the processed food. The BSF meal was the issue to further solve the recipe for the growth performance of frogs. A similar finding was reported by Muin & Taufek)2 (022who found that weight gain per day, specific growth rate, and protein efficiency of the fish supplemented with the BSF diet were significantly higher than those in the fish meal group. Also, Nghia *et al.* (2023) reported that the supplement of fishmeal by BSF meal in diets for frogs (*Rana rugosa* Temminck & Schlegel, 1838) developed the survival rate (75.6-86.8%), feed conversion ratio, increased live weight (194-240.7 g), daily weight gain (3.11-3.66 g) and frog's yield (5.9-8.41 kg m^{-2}), including chicken frogs (*Leptodactylus fallax*), good digestibility, which improved healthy frogs owing to the larvae of the BSF as an alternative, palatable, high-calcium (Ca) food, which contributed the necessary for additional gut-loading or dust supplements in frogs (Dierenfeld & King, 2008). Alternatively, the feed conversion ratio was significantly lower in those feds with BSF than in the fish meal group. In terms of the growth performance of phase 2 for both processed food and the BSF, the meal was significantly lower than the other stages, owing to overfeeding affecting palatability, digestibility, and absorption. A similar study by Day & González)2000(and Espe *et al.*)2007(reported the decreased feed intake and palatability in animals due to overfeeding and elevated dietary BSF, including the alternative protein source contains antinutritional factors)ANFs ;Francis *et al.* 2001; Tusche *et al.* 2011; Jaber & Najim 2023; Babashev *et al.* 2023 .(Thereby, the negative effect on aquatic animal growth, caused by increasing levels of insect diets, could refer to a nutrition imbalance. On the other hand, the dietary replacement of BFS at a modest level had no detrimental effect on growth performance)Hua 2021). Furthermore, the BSF contains the polysaccharide chitin)approximately 87.0 g kg^{-1}, DM ;Diener *et al.* 2009; Shiau & Yu 1999(, a main component of insect cuticle. The reduced specific growth rate in frogs was due to the BSFL containing a lot of chitins (Chapman, 1998; Tharanathan & Kittur 2003(. For instance, Fontes *et al.*)2019(reported decrease in the apparent digestibility coefficient)ADC %(because of the presence of high chitin in the BSF diet. Also, the lower dietary methionine results in an unusual rate of protein synthesis)Hien *et al.* 2018(, and the lower dry matter and crude protein digestibility is caused by high dietary chitin (Spranghers *et al.* 2017). Thus, chitin consumption of frogs at high levels harmed growth performance .Moreover, a high protein intake rate of frogs results in high growth performance, high energy efficiency, and a high survival rate)Wilson *et al.* 1986), However, the BSF is the replacement innovation of aquafeed manufacturing for sustainable aquaculture and nutritional value enhancement. The BSF has received increasing attention associated with raising it. It is the best source for converting organic waste into valuable biomass and high-quality feeds within a short time. In addition, it is not a disease vector, as it is not consumed by the adult fly)Veldkamp *et al.* 2012; Van Huis *et al.* 2015(. Currently, greenhouse gas is continuously released by the aquaculture industry, which is an important issue of climate change topic in the world. The aquaculture industry has been realizing to solution of increasing greenhouse gas for many years. The BSF is a good outstanding nutrients and a good alternative source of aquafeed production, since its production emits little greenhouse gas and ammonia emissions)Oonincx *et al.* 2010(, including lower environmental impact)Gasco *et al.* 2019(. Also, the BSF meal has been used as feed for various animals such as swine, cattle, poultry)Van Huis 2013; Oonincx *et al.* (2015, aquatic invertebrates, shrimps)Cummins *et al.* 2017(, including frogs.

In terms of water quality, it is significant for maintaining water quality and encouraging the growth performance of the frogs. Water quality parameters were investigated from frog farming systems with an automatic and manual labour-based normal water change systems. The parameters included water temperature (Temp), water pH value (pH), total dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO), and ammonia content in the water which were measured every 7 days. The results showed that the temperatures of the water ranged from 24.1to 32.9℃. A similar study by Fabiano *et al.* (2008) reported that the water temperature of aquaculture in tropical regions was between 17.1 and 29 ℃, which is an important level in the evaporation of water from tropical frog bodies. Body temperature is elevated to maintain water balance in the frog bodies. In contrast, when the temperature is higher- or lower- than the standard, the animals of tropical regions lose the maintaining water balance. The pH of the water ranged from 6.48 to 8.92, which is the suitable criterion for frog rearing. The pH range for aquaculture was reported by CONAMA) National Environment Council(number 357 that the pH of the water should be ranged from 6.0 to 9.0)CONAMA, 2005(, and Boyd)1982(who reported that the optimum pH for frog rearing should be ranged from 6.5 to 8.5. Certainly, draining waste water will contribute to the pH value of the water in the frog pond which is good apparently. Also, the pH fluctuation resulting in elevated tension and supressed immunity in frogs. Therefore, the pH of the water is a significant condition for the growth performance of frogs. The TDS in the water were within the range of 0.1 to 1.0 mg L^{-1}, and the EC ranged from 0.1 to 1.3 µS cm^{-1}, which were suitable TDS and EC ranges according to recommended information of CONAMA,)2005.(The DO ranged from 4.74 to 6.30 mg L^{-1}, which is the moderate criterion for frog rearing. The DO range for aquaculture reported by CONAMA (2005) should be above 6.0 mg L^{-1}. The total ammonia values ranged from 0.04 to 0.62 mg L^{-1}, which was higher than the appropriate standard of 0.02 mg L^{-1}. Owing to an overfeeding factor of processed food and BSF diet, which generate high organic matter and total ammonia values, resulting in increased TDS, EC, and total ammonia values. In addition, the DO was lower than the appropriate standard. The water quality parameters influence the negative and positive growth performance of frogs . The feeding management process is essential to the water quality, including weight gain per day, specific growth rate, survival rate, feed intake rate, and feed conversion ratio of frogs. Moreover, the water quality imbalance results in increased stress, excessive mucus production, inadequate adaptation, weak immunity, and increased susceptibility to diseases in frogs. Thus, the optimal feeding quantity and water quality parameters for successful frog rearing are important research to improve frog farming for aquaculture sustainability in the future. The cost-effectiveness of the BSF mixes feeding to frogs on farms and water quality, enhances the growth performance such as good health, increased energy, and enlarged weight or body size of frogs. These are excellent results for farmers to rear frogs with alternative food that has low costs but nutritional abundance when compared to processed food. Better quality of frogs (weight and health) would increase the earnings for farmers in aquaculture, and reduce overall costs such as processed food, tank renovation, purchasing tools, transportation, and electric bills, including labour costs. Moreover, farmers can rear the BSF themselves, since it is easy to rear. Larvae of the BSF can grow greatly on areas of organic material, such as fruits and vegetables, including waste from other animals. Also, adults can hatch 300 to 600 eggs, which grow into the larval stage 20 to 25 days later. Therefore, the BSF is an alternative insect species that could improve appropriate innovation, which is associated with adding food quality for aquaculture industries, and could enhance aquaculture sustainability. Also, the BSF can be beneficial in raising frogs, including other aquatic animals. Finally, the authors expect that this study will provide scientific insights and further information comprehensively that are important to improve raising frogs and aquaculture.

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