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The effects of different stocking densities and feed types on frogs' growth and survival rates (*Rana tigerina* Dubois, 1981) reared in composite tanks

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Abstract

We performed two independent experiments, each with a completely random design in three repetitions and lasting 80 days, on the growth and survival of the artificially reared Thailand frog (*Rana tigerina* Dubois, 1981). Experiment 1 compared three stocking densities: 75 (D75), 100 (D100), and 125 (D125) individuals m^{-2}. Experiment 2 used a stocking density of 100 individuals m^{-2} to study the effects of different feed types: frog feed, snakehead fish feed, catfish feed, and tilapia feed. Daily weight gain (DWG) and specific growth rate (SGR) did not differ between D75, D100, and D125, but these parameters tended to be higher at D100. At the end of the experiment, the average final weight and survival rate in D75 (204.59 ± 35.4 g and 79.67 ± 4.04%) and D100 (215.05 ± 38.65 g and 79.00 ± 2.75%) were not different, but both were significantly higher than those in D125 (184.64 ± 37.75 g and 57.00 ± 6.56%). In addition, frogs fed snakehead fish feed showed the highest DWG, SGR, and yield and the lowest feed conversion ratio (FCR), followed by frogs given frog feed, tilapia feed, and catfish feed, which showed decreasing yields and increasing FCR. In conclusion, a stocking density of 100 individuals m^{-2} using snakehead fish feed is suitable for rearing the Thailand frog under our experimental conditions.

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Introduction

Frog meat is rich in protein, so it can be used as a substitute for other animal protein sources (Omoniyi et al., 2012). It is also a good source of Zn, K, Cu, Mg, Mn, folic acid, and thiamine and has a low saturated fat content (Tokur et al., 2008). Therefore, frog meat has contributed to meals worldwide, especially in continents such as America, Europe, Asia, etc. (Hince, 2011). This has promoted the cultivation of frogs throughout Southeast Asia (Somsiri, 1994). The Thailand frog (*Rana tigerina* Dubois, 1981) and the bullfrog (*Rana catesbeiana* Shaw, 1802) were the most commercially essential anurans due to biological characteristics such as size, body mass, and adaptability to humid tropical climates, which resulted in a culture in many countries (Pariyanonth & Daorerk, 1994).

Nowadays, the world's population growth has led to high rates of consumption of natural resources like water, energy, and food (Beddington, 2011), together with global climate change, resulting in many negative impacts, such as the increase in greenhouse gas emissions, water source pollution, as well as the exhaustion of resources (Froehlich et al., 2018). New cultivation approaches that minimize environmental problems while maximizing production to meet protein requirements are necessary (Munguia-Fragozo et al., 2015). In aquaculture, the growth and survival rate of cultured animals depends on the stocking density, food quality, the energy content of the diet, its physiological status, reproductive state, and environmental factors such as temperature, pH, etc. (Lovell, 1989), in which stocking density and diet are crucial to achieving economic goals. Previous studies addressed the adverse effects of stocking density on the growth and development of frogs, but most focused on individual stages from tadpole to complete metamorphosis (Dash & Hota, 1980; Martinez et al., 1996; Browne et al., 2003; Godome et al., 2018). Furthermore, feed costs account for roughly 60% of variable production costs in intensive aquaculture systems (Ayinla, 2007).

Consequently, feed and feeding stratagems are of paramount interest for feed utilization and profit maximization (Eriegha & Ekokotu, 2017). Formulated diets are essential to support frogs' continuous and specific nutritional requirements (Tacon, 1990). However, research on edible frog nutritional requirements is limited now, which poses many challenges in improving the culture of these frogs from the dietary perspective (Toledo et al., 2014). The nutritional composition of frog feed appropriate to each stage has been studied by several researchers (Sretarugsa et al., 1997; Mustapha and Bello, 2018; Godome et al., 2019), but studies that use commercially available feed remain scarce. This study evaluates the effects of different stocking densities, and types of feed on the growth and survival rates of the Thailand frog reared in composite tanks.

Materials and Methods

Materials.

Experimental frogs were purchased from a private hatchery in Vietnam in Tra Vinh province. Before being used, they were tested for pathogens, morphology (no disability), and agility. The average initial weight of the frogs was 6–8 g. The water used for the experiment was river water with sediment removed. It was disinfected with potassium permanganate ($KMnO_4$) at 50 mg L^{-1}, continuously aerated for 3–5 days, and tested for pH and alkalinity using a test kit (sera, Germany) before being used in the experiment. The artificial pellet feed used was products from Vietnam Greenfeed Corporation Company, with the nutritional composition shown in **Table 1**. The composite tanks used in this study were 0.5 m^3 in volume, with a bottom area of 1 m^2 and a water depth of 0.15 m. The US National Research Council's guide for the Care and Use of Laboratory Animals was followed.

Table 1 The approximate composition of the experimental feed types

Composition	Frog feed	Snakehead fish feed	Catfish feed	Tilapia feed
Moisture (%)	11	11	11	11
Crude fiber (%)	6	5	7	6
Crude protein (%)	30	40	28	35
Crude fat (%)	5-7	7-10	5-6	5-6
Exchange energy (kcal kg^{-1})	2,060	2,910	2,860	1,700

Source: Vietnam Greenfeed Corporation Company

Experimental design.

This study was conducted in Tra Vinh province, Vietnam, with two independent experiments as follows to investigate the effects of stocking densities and feed on the growth and survival rates of Thailand frogs:

Experiment 1: The effect of different stocking densities on the growth and survival rates of Thailand frogs reared in composite tanks. In this experiment, three stocking densities of 75, 100, and 125 individuals m^{-2} were tested with three replicates based on a survey of households farming Thailand frogs in Tra Vinh province, Vietnam. The survey found a stocking density ranging from 70 to 150 individuals m^{-2}, and a stocking density of 150 m^{-2} resulted in poor growth for the frog. The frogs were randomly assigned to nine composite tanks and fed twice a day (at 07:00 and 16:00) with artificial feed pellets for frogs. The size of the feed pellets was changed from time to time depending on the size of the frog’s mouth; in the first 20 days, the frogs were provided feed at 4–6% of total body weight and then at 3–4% after that (as recommended by the manufacturer). The water in the tanks was changed twice a day, with 100% of the volume of treated water being replaced each time.

Experiment 2: Different types of feed on the growth and survival rates of Thailand frogs reared in composite tanks. Based on a survey on aquafeed used in Thailand's frog farming, four types were selected for this investigation: frog feed, snakehead fish feed, catfish feed, and tilapia feed, with each feed type being replicated three times. The experiment was performed using 12 tanks at a stocking density of 100 individuals m^{-2} based on Experiment 1. The care and management of this experiment were the same as in Experiment 1.

Biological evaluation.

In both experiments, samples for calculating growth performance were randomly obtained from 20 frogs per tank every 20 days. An electrical analytical balance calibrated to 0.01 g was used for the weight determination of the frog at sampling time. Data on survival rate (SR), feed conversion ratio (FCR), and yield was collected and calculated at the end of the experiment.

The daily weight gain (DWG), specific growth rate (SGR), SR, FCR, and yield were calculated using the following equations:

DWG (g day^{-1}) = (final body weight – initial body weight)/duration of experiment;

SGR (% day^{-1}) = 100 × (ln(final weight) – ln(initial weight))/duration of experiment;

SR (%) = 100×(final number of frogs/initial number of frogs);

Feed conversion ratio (FCR) = Consumed feed (g)/Weight gain (g); and

Yield (g m^{-2}) = biomass/rearing area.

Statistical analysis.

All variables were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan test to identify significant differences between mean values at $p < 0.05$. Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0 was used in this study.

Results

Water quality parameters.

The river water used in the experiments had a pH of 7.54 ± 0.30 and alkalinity of 80.60 ± 10.4 mg L−1, indicating slightly alkaline.

Effects of stocking densities on the growth and survival of Thailand frogs.

Average weight: The frogs' average weights were recorded for all stocking densities every 20 days. **Figure 1** shows that the average weight of frogs at a stocking density of 125 individuals m^{-2} (D125) was significantly lower than that at D75 and D100. Still, the latter two stocking densities did not differ at any recording time. On the final day of rearing, the highest average weight (215.05 ± 38.65 g) was found for D100, but it did not differ significantly ($p > 0.05$) from D75. The lowest weight (184.64 ± 37.75 g) was at D125, which was significantly different ($p < 0.05$) from the other two stocking densities.

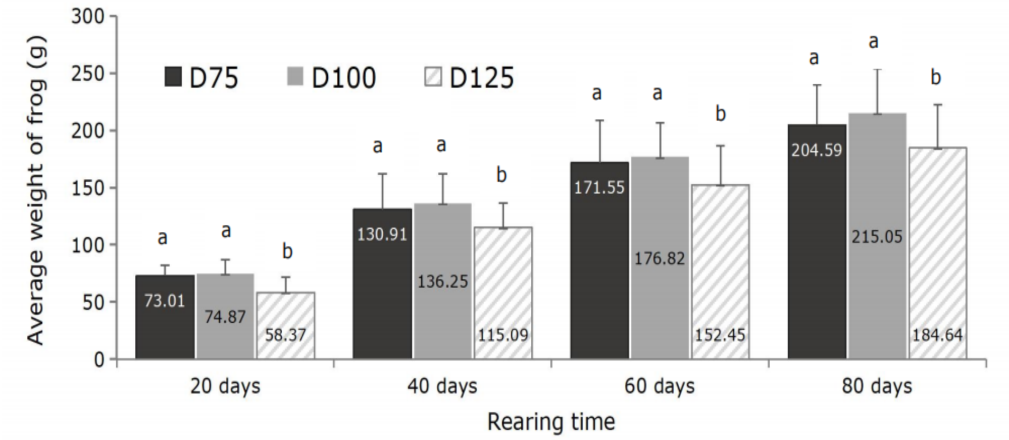

Figure 1 Average weight of Thailand frogs (*Rana tigerina* Dubois, 1981) at different stocking densities during the rearing period. D75, D100, and D125 are stocking densities of 75, 100, and 125 individuals m^{-2}, respectively [Different letters (a, b) above bars at the same sampling time indicate a significant difference ($p < 0.05$)]

Growth parameters: **Table 2** shows the parameters representing the frogs' growth rate, including daily weight gain (DWG) and specific growth rate (SGR), on the final day (80 days) of rearing. The average DWG reached the highest value at D100 (1.91 ± 2.21 g day^{-1}), but there was no statistically significant difference ($p > 0.05$) between the stocking densities. Similarly, the SGR was highest at D100 (1.17 ± 1.30% day^{-1}) and lowest at D125 (0.73 ± 1.28% day^{-1}), but it did not differ significantly between stocking densities ($p > 0.05$).

Survival rate: The results in **Table 2** show that the survival rate of the frogs was lower at higher stocking densities (D75, 79.67 ± 4.04%; D100, 79.00 ± 2.75%; D125, 57.00 ± 6.56%), but it was not significantly different between D75 and D100. In addition, it decreased strongly at D125 (it was 38.59% lower than at D100) and was significantly different from the survival rate at D75 and D100.

Table 2 Daily weight gain (DWG), specific growth rate (SGR), and survival rate (SR) of Thailand frog (*Rana tigerina* Dubois, 1981) at the different stocking densities on the final rearing day

Parameters	D75	D100	D125
DWG ($g.day^{-1}$)	1.65 ± 2.27^a	1.91 ± 2.21^a	1.61 ± 2.61^a
SGR ($\%.day^{-1}$)	0.97 ± 1.57^a	1.17 ± 1.30^a	0.73 ± 1.28^a
SR (%)	79.67 ± 4.04^a	79.00 ± 2.75^a	57.00 ± 6.56^b

D75, D100, and D125 are stocking densities of 75, 100, and 125 individuals m^{-2}, respectively
Values are presented as mean±SD. Values with different letters (a, b) in the same row show a significant difference ($p < 0.05$)

Effects of feed types on the growth and survival of Thailand frogs.

Average weight: **Figure 2** shows the mean weights of Thailand frogs fed different types of feed during the 80-day rearing period. The effect of different types of feed on weight tended to be relatively the same at recording times. Specifically, after every 20 rearing days, the frogs achieved the highest average weight when fed snakehead fish feed and the lowest when fed catfish feed and tilapia feed. In particular, after 80 days of rearing, the frogs' average weight of 221.04±34.97 g in snakehead fish feed was statistically higher than the weights in the other feed types.

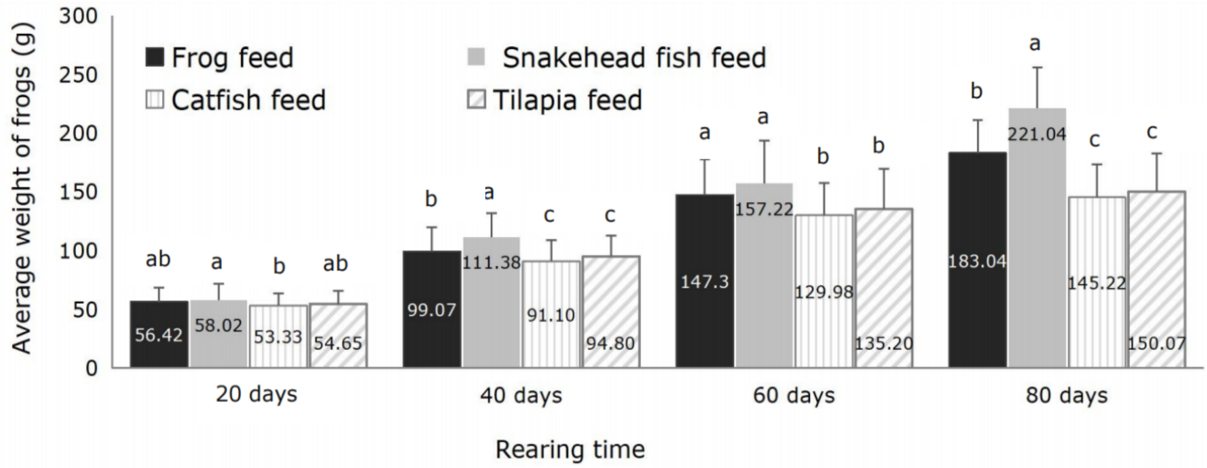

Figure 2 Average weight of Thailand frogs (*Rana tigerina* Dubois, 1981) given different types of feed at a stocking density of 100 individuals m^{-2} during the rearing period.
[Different letters (a, b, c) above bars at the same sampling time indicate a significant difference ($p < 0.05$)]

Growth parameters: **Table 3** shows the growth parameters of frogs after 80 days of rearing in Experiment 2. DWG was significantly higher in snakehead fish feed (3.19 ± 2.35 g day^{-1}) than in frog feed (1.78 ± 2.28 g day^{-1}), catfish feed (0.76 ± 1.93 g day^{-1}), and tilapia feed (0.75 ± 2.02 g day^{-1}) with no statistical difference between the last two feed types. SGR showed similar results, with it being significantly higher in snakehead fish feed (1.77 ± 1.29% day^{-1}) than in frog feed (1.09 ± 1.42% day^{-1}), catfish feed (56.00 ± 4.77% day^{-1}), and tilapia feed (0.56 ± 1.43% day^{-1}), with no significant difference between the last two feed types.

Feed conversion ratio: After 80 days of rearing, FCR reached higher values in frog feed, catfish feed, and tilapia feed (average of 1.19 ± 0.0, 1.29 ± 0.49, and 1.20 ± 0.03, respectively); however, there was no statistically significant difference between these three feed types. The lowest FCR value of 1.12 ± 0.01 was for snakehead fish feed, significantly different from catfish feed (**Table 3**).

Yield: The highest yield was 23.23 ± 0.72 g m^{-2} in the snakehead fish feed, which was significantly different from the yields in the other feed types: 21.10 ± 0.79 g m^{-2} in the tilapia feed, 20.37 ± 1.27 g m^{-2} in the frog feed, and 18.27±1.08 g m^{-2} in the catfish feed. Yield did not differ significantly between frog and tilapia feeds (**Table 3**).

Survival rate: The highest survival rates were recorded in frog and snakehead fish feed (63.67 ± 4.37% and 64.67 ± 2.08%, respectively), which were not significantly different. The lowest survival rates were in catfish feed (56.00 ± 4.7%) and tilapia feed (56.17 ± 4.25%), which were not significantly different, but both were significantly lower compared to frog feed and snakehead fish feed (**Table 3**).

Table 3 Daily weight gain (DWG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), and yield of the Thailand frog (*Rana tigerina* Dubois, 1981) with different feed types on the final rearing day.

Parameter	frog feed	snakehead fish feed	catfish feed	tilapia feed
DWG (g.day^{-1})	1.78±2.28^{b}	3.19±2.35^{a}	0.76±1.93^{c}	0.75±2.02^{c}
SGR (%.day^{-1})	1.09±1.42^{b}	1.77±1.29^{a}	0.57±1.41^{c}	0.56±1.43^{c}
SR (%)	63.67±4.37^{a}	64.67±2.08^{a}	56.00±4.77^{b}	56.17±4.25^{b}
FCR	1.19±0.0^{ab}	1.12±0.01^{b}	1.29±0.49^{a}	1.20±0.03^{ab}
Yield (g.m^{-2})	20.37±1.27^{b}	23.23±0.72^{a}	18.27±1.08^{c}	21.10±0.79^{b}

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference ($p < 0.05$)

Discussion

The effects of stocking density and feed-use efficiency on growth and survival are considered one of the evaluators of profitability in production. Increased stocking density may significantly improve production per unit area, but it can also result in stress reactions that decrease feed conversion efficiency due to higher energy consumption, thereby negatively affecting farmed animals' health, growth, and survival and reducing their biomass (Rowland et al., 2006; Islam et al., 2006; Gibtan et al., 2008). Our results showed a marked negative effect on the growth parameters and survival rate of Thailand frogs reared for 80 days at a high stocking density (125 individuals m^{-2}) (**Figure 1** and **Table 2**). Few previous studies have focused on the same stages of development of frogs as in this study. The stocking density of tadpoles is inversely proportional to their growth rate; Godome et al. (2018) recorded the highest final biomass at the lowest stocking density (5 tadpoles L^{-1}) and lower biomass as the stocking density increased. Other studies have also reported similar results (Rodriguez-Serna et al., 1996; Browne et al., 2003; Munguia-Fragozo et al., 2015). In this study, the results after 20, 40, and 60 days of rearing (**Figure 1**) also showed that the two stocking densities with the highest average frog weight were D75, and D100, and the stocking density with the lowest was D125. This indicated that under the same rearing conditions, different stocking densities have different growth rates: too high stocking density led to the slow growth of frogs. Under the conditions of this experiment, the most suitable stocking density for the growth performance of the Thailand frog is 100 individuals m^{-2}.

Rodriguez-Sema et al. (1996) study of bullfrog froglets (*Rana catesbeiana* Shaw, 1802) also showed that final body weight, individual weight gain, and specific growth rate were higher at the two lower stocking densities of 50 and 100 individuals m^{-2} and the lowest at 200 individuals m^{-2}. Although the survival rate of frogs decreased gradually as stocking density increased, there was no statistically significant difference between the three stocking densities, possibly because mortality from cannibalism also rose as stocking density increased due to the predatory activity of the giant frogs in the same tank. Our study also obtained similar results: the survival rate of Thailand frogs at a stocking density of 125 individuals m^{-2} was significantly lower than that at 75 and 100 individuals m^{-2} (**Table 2**).

The feed conversion ratio is considered helpful for evaluating feed efficiency in aquaculture. When fast growth with efficient food conversion is an objective of the culturist. Experiment 2 of the present study indicated that the frogs more effectively consumed snakehead fish feed, which may be explained by the differences in protein content between feed types. **Table 1** shows that the protein content of snakehead fish feed is 40%, while that of catfish feed is 28%. The protein content of the feed is significant for weight gain in frogs. According to studies on frog diets, the protein ratio suitable for tadpole and juvenile stage growth and development should be 40% (Carmona-Osalde et al., 1996; Olvera-Novoa et al., 2007; Godome et al., 2019). However, Browne (2009) discovered that proteins alone do not determine the growth performance of frogs under culture. Other nutrients, such as lipids, carbohydrates, and micro and macro-nutrients, also contribute to their growth. In particular, to make effective use of the protein ingested, there must be available energy supply components for protein metabolized into absorbents, which are primarily provided by fats and carbohydrates (Page & Andrews, 1973; Lovell, 1979). In this experiment, snakehead fish feed had the highest lipid content compared to the other three (**Tabe 1**). In addition, Zhang et al. (2016) demonstrated that the optimum carbohydrate/lipid ratio in the diet for bullfrog growth was approximately 2.07. This ratio was also **recorded** for the nutritional composition of the snakehead fish feed used in this study (**Table 1**). Perhaps these nutrition component ratios resulted in the frogs' needing less feed to gain weight, resulting in increased performance, which created an FCR that was lower than other feed types. It can be concluded that snakehead fish feed was the most suitable for the growth of Thailand frogs under our experimental conditions, as the frogs exhibited low feed intake but the highest growth indices and yield. In contrast, catfish feed was the least suitable. Although the frogs consumed the highest catfish feed, their growth performance and yield were the lowest (**Table 3**).

In this study, the survival rates of frogs fed different feed types were 56.00–64.67%. This rate **seemed** lower than that in previous studies on frogs, mostly tadpoles (Sretarugsa et al., 1997; Olvera-Novoa et al., 2007; Mustapha & Bello, 2018; Godome et al., 2019). However, it should be noted that the results have depended on several factors, such as species, stocking density, and environmental conditions (Hafedh, 1999). The highest survival rate was achieved in snakehead fish feed (64.67 ± 2.08%), which was significantly different from the rates in catfish feed (56.00 ± 4.7%) and tilapia feed (56.17 ± 4.25%) (**Table 3**). Although the apparent reason cannot be proved, differential FCR, WG, and SR confirmed the greater effectiveness of snakehead fish feed for commercial rearing of Thailand frogs compared to other feeds in this investigation.

The total alkalinity of the water environment indicated the concentration of carbonate and bicarbonate salts in the water, which maintained a stable pH level. The pH should be between 6.5 and 7.0, and the total alkalinity should be up to 40 mg L−1, appropriate for commercial frog farms (Mansano et al., 2019). Other researchers have found that pH levels between 6.0 and 8.0 are not harmful to animals, including frogs, and that the total alkalinity frequently observed in frog farms ranged from 10 to 80 mg L^{-1} (Ferreira, 2003; Mercante et al., 2014; Mansano et al., 2019). These parameters recorded in our study were not optimal but relatively stable and within a suitable range for the growth and development of frogs during the culturing period and had little impact on the experimental frogs.

Conclusion

The stocking densities of 75 and 100 individuals m^{-2} had a negligible impact on Thailand frogs' growth and survival rate. However, a stocking density of 100 individuals m−2 was highly effective under these experimental conditions for a high yield. In addition, snakehead fish feed was superior to the other feeds used in this study because of its higher protein and lipid content. A stocking density of 100 individuals m^{-2} and snakehead feed created the appropriate conditions for rearing frogs, enabling them to adapt and develop well. However, further studies that analyze the economic benefits of our rearing conditions are needed. Moreover, there may be opportunities to build aquaculture models like combined farming of frogs and snakehead fish to achieve high productivity and low investment costs.

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