

Effects of Calcium Levels in Artificial Pellet Feed on the Growth and Survival Rate of Black Apple Snails (*Pila polita*)

Le Van Binh & Ngo Thi Thu Thao

College of Aquaculture and Fisheries, Can Tho University, Can Tho 94115, Vietnam

Abstract

The present study was conducted to evaluate the effects of different dietary calcium levels on the growth and survival rate of black apple snails (*Pila polita*) in the grow-out period. There were 3 replicates for each treatment and the snails were fed with five calcium levels (% dry matter) in diet as follows: 1% (Ca1), 3% (Ca3), 5% (Ca5), 7% (Ca7), and 9% (Ca9). Two-month-old juveniles with an average initial body weight of 2.13g, shell height of 21.71mm, and shell width of 16.35mm were reared in tarpaulin tanks (1 × 1 × 1m; 40cm water depth) at the density of 100 individuals per tank. After 4 months of the rearing period, the average body weight, shell height, and shell width of the snails reached the highest values in the Ca5 treatment (28.43g, 54.97mm, and 40.09mm, respectively) and these values were significantly different compared to the remaining calcium contents (Ca1, Ca3, Ca7, and Ca9). The survival rate of the snails in Ca3 (75.7%) was higher than in Ca1 and Ca7 (74.7%), Ca5 (73.7%), and Ca9 (71.7%). However, the survival rate was not significantly different among the treatments. Snails in Ca5 obtained the highest productivity (2.88 kg m⁻²) and this value was significantly different from Ca1 (2.55 kg m⁻²) and Ca9 (2.35 kg m⁻²). The results of this study showed that the growth rate and productivity of black apple snails were highest when they consumed a diet containing 5% calcium. The optimum requirement of calcium for the black apple snails in the grow-out period was 4.51%.

Keywords

Black apple snail, growth, dietary calcium, survival rate

Introduction

Mollusks rely on calcium for the growth of their shell and thus, are highly dependent on calcium availability for survival. Harrison *et al.* (1970) already reported the reduced growth rate, survival, and reproductive output of snails in low calcium environments. The most important site of calcium deposition in gastropods is the shell, where it constitutes 97% of the shell weight (Heller & Magaritz,

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Correspondence to
lvbinh654@gmail.com

1983; Marxen & Becker, 2000). Calcium is also deposited in the digestive gland, connective tissue cells, foot tissue, and around major blood vessels (Tompa & Watabe, 1976; Marxen & Becker, 2000). Although shell-building animals in particular need to obtain calcium, it is also required for other functions, including blood clotting, muscle function, and nerve transmission in other animals (Ireland, 1993). Chaitanawisuti *et al.* (2010) indicated that the calcium needs of species belonging to Gastropoda class are higher than others. Under certain conditions, calcium removed from the shell will lead to edgrowth in some gastropods (Harrison *et al.*, 1970; Tompa & Watabe, 1976). Besides, the growth and survival of species belonging to Gastropoda are affected when calcium levels decrease or come from a different origin (Oluokun *et al.*, 2005; Egonmwan, 2008; Kouassi, 2008; Badmos *et al.*, 2016; Binh *et al.*, 2017). Dietary calcium is an essential nutrient for the growth, reproduction, and construction of the shell in terrestrial gastropods (Marxen *et al.*, 2003; Egonmwan, 2008; Glass & Darby, 2009; Dalesman & Lukowiak, 2013). The growth rate is directly proportional to the concentration of calcium in the diet. Pawson & Chase (1984) showed that the growth rate of giant African snails (*Achatina fulica*) is reduced as calcium availability decreases. However, little information concerning the dietary calcium content for black apple snails (*Pila polita*) is available. From the above issues, an experiment was developed to test different kinds of processed food diets and determine the appropriate calcium content to improve efficiency of the black apple snail grow-out period, while also saving costs and reducing the impact on the water quality in the culture environment.

Materials and Methods

Experimental design

The experimental *P. polita* with initial weights ranging from 1.52 to 2.72g, shell heights from 20.94 to 23.50mm, and shell widths from 14.47 to 17.34mm were nursed in the Cao Lanh district, Dong Thap province

(10°20'40,6"N; 105°47'04,8"E), Vietnam. Freshwater was pumped from the river to the settling tanks (10m³) for 7 days and then supplied to the culture tanks.

This experiment included five feeding treatments with different calcium levels and three replicates per treatment as follows: 1% (Ca1), 3% (Ca3), 5% (Ca5), 7% (Ca7), and 9% (Ca9). *P. polita* juveniles were cultured in 1m² (1 × 1 × 1m; 40cm water depth) tarpaulin tanks at the density of 100 ind. m⁻² (Binh *et al.*, 2012; Binh & Thao, 2018). Two feeding trays (diameters of 20cm) and two nylon bunch substrates (diameters of 50cm) were laid at the bottom of each tank. The experiment was conducted for 4 months. The ingredients used and proximate compositions of the experimental diets are presented in **Table 1**.

The snails were fed twice a day (at 7:00 and 18:00) with a quantity of 4% of the average snail body weight in each tank. The water volume in the cultured tanks was renewed 30% after a cycle of 10 days.

Food preparation

The experimental diets were formulated into pellets from ingredients including fish meal, soybean meal, wheat flour, soy oil, calcium powder (CaCO₃ 99% - calcium carbonate), vitamin premix, mineral premix, and CMC. The dry ingredients, fish meal, wheat flour, vitamin premix, mineral premix, and CMC, were weighed according to the ratios in **Table 1** and mixed well under dry conditions. The soybean meal was cooked, allowed to cool down to a temperature around 40-50°C, and then mixed with the dry ingredients together with soy oil. After mixing, the mixture was pressed into a pasta machine to make the pellets. After drying in the oven at 60°C for 24h, the pellets were stored in the fridge at 4°C for experimental feedings. The proximate compositions of each diet were analyzed and the results (**Table 1**) showed that the calcium levels were in accordance with the experimental design.

Data collection and sample analysis

Water quality

The water temperature in the cultured tanks was recorded twice a day at 7:00 and 14:00

Table 1. Formulation and proximate compositions of the experimental diets (% dry matter)

Treatments	Ca1	Ca3	Ca5	Ca7	Ca9
<i>Ingredient (%)</i>					
Fish meal ¹	12.50	12.50	12.50	12.50	12.50
Soybean meal ²	22.80	22.95	23.08	23.22	23.36
Wheat flour	58.70	56.42	54.28	52.14	49.99
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Vitamins, Minerals ³	3.00	3.00	3.00	3.00	3.00
CMC ⁴	2.00	2.00	2.00	2.00	2.00
Calcium ⁵	0.00	2.13	4.14	6.14	8.15
<i>Nutrition compositions (%)</i>					
Crude protein	18.50	19.50	19.30	18.50	18.90
Crude lipid	3.38	3.78	4.08	3.66	3.71
NFE	57.18	51.29	47.51	41.28	37.30
Crude ash	4.46	7.91	9.08	13.34	15.73
Crude fiber	1.61	1.62	1.33	1.70	1.45
Moisture	13.63	12.68	12.85	13.77	13.41
Calcium	0.88	2.87	5.48	7.39	9.15
Phosphorus	0.36	0.35	0.37	0.36	0.35

Note: Parameters: Protein, lipid, ash, fiber, moisture, NFE-Nitrogen free extract, and calcium were analyzed by the Quality Assurance and Testing Center Can Tho; ¹Fish meal Kien Giang (Viet Nam); ²Soybean meal (Argentina); ³Vitamin premix and mineral premix: Vitamin A, 2,000,000 IU; Vitamin D, 400,000 IU; Vitamin E, 6g; Vitamin B1, 800mg; Vitamin B2, 800mg; Vitamin B12, 2mg; Calcium D. Panthotenate, 2g; Folic acid, 160mg; Vitamin C, 15g; Choline chloride, 100g; Iron (Fe²⁺), 1g; Zinc (Zn²⁺), 3g; Manganese (Mn²⁺), 2g; Copper (Cu²⁺), 100mg; Iodine (I), 20mg; Cobalt (Co²⁺), 10mg; ⁴Carboxymethyl cellulose; and ⁵Calcium carbonate powder.

using a thermometer. pH levels, the concentrations of NH₄⁺/NH₃ (TAN), NO₂⁻, and DO, and alkalinity in the cultured tanks were checked weekly using a Sera test-kit (Made in Germany).

Growth performance and feed utilization

At day 1 and at 15-day intervals during the experiment, the number of snails in each tank was counted to calculate the survival rate. To determine the growth rate, shell heights and shell widths were measured with an electronic digital caliper (0.01mm), and body weights were measured with an electronic scale (0.01g error) for 20 snails per tank.

Weight gain (WG), shell height gain (HG), shell width gain (SWG), daily weight gain (DWG), daily height gain (DHG), specific growth rate for body weight (SGR_{BW}), specific growth rate for shell height (SGR_H), specific

growth rate for shell width (SGR_{SW}), feed conversion ratio (FCR), calcium efficiency ratio (CaER), and survival were calculated using the following equations:

$$\text{Survival rate (\%)} = (\text{Final number of snails} / \text{Initial number of snails}) \times 100$$

$$\text{Biomass increase rate (BIR, \%)} = (\text{Biomass increase} / \text{Biomass initial}) \times 100$$

$$\text{Feed conversion rate (FCR)} = \text{Total feed intake (dry weight)} / \text{Weight gain (wet weight)}$$

$$\text{Productivity (P, kg m}^{-2}\text{)} = \text{Total snail biomass (kg) per m}^2 \text{ at the end of the experiment}$$

$$\text{Calcium efficiency ratio (CaER)} = \text{Weight gain} / \text{Calcium intake}$$

$$\text{Feed intake (FI-mg/ind./day)} = [\text{Total feed intake} / (\text{Initial number of snails} + \text{Final number of snails}) / 2] \times \text{Days of culture}$$

Statistical analysis

The data were analyzed for mean values and standard deviations using Excel software (2016), and one-way ANOVA analysis followed by the Duncan post hoc test was applied to determine significant differences of the collected parameters among the treatments at $P < 0.05$ using the SPSS program version 22.0.

The data on the specific growth rate (SGR_w, %/day) were subjected to the quadratic regression analysis model (Zeitoun *et al.*, 1976) and the optimal calcium requirement of black apple snails was estimated.

Results and Discussion

Water quality parameters

The mean values of the environmental parameters are shown in **Table 2**. During the experiment, the average temperature remained at 25.3°C in the morning and ranged from 27.5°C to 27.8°C in the afternoon, with daily fluctuations around 2.6°C (0.5-4.7°C; **Figure 1**). There were no significant differences in temperature among the treatments.

pH levels in all the treatments were in the range of 7.94-8.06. The highest pH was measured in Ca9 (8.06) and this value was not significantly different to those in Ca5 or Ca7. In addition, there were no significant differences among the pH values of 7.94, 7.96, and 8.01 for the treatments Ca1, Ca3, and Ca5, respectively.

DO levels varied slightly among the treatments (4.58-4.63 mgO₂ L⁻¹), and no significant differences were found.

The alkalinity value in Ca1 (67.6 mg CaCO₃ L⁻¹) was the lowest, and was significantly different compared to the values for Ca3, Ca7, and Ca9. The concentrations of TAN and NO₂⁻ varied from 0.65 to 0.74 mg L⁻¹ and from 0.59 to 0.73 mg L⁻¹, respectively. Ca5 showed the highest value of TAN (0.74 mg L⁻¹), but there was no significant difference with the other treatments. The value of NO₂⁻ in Ca5 (0.73 mg L⁻¹) was higher than in the other treatments.

Growth performance of black apple snails

The average body weights, shell heights, and shell widths of *P. polita* fed formulated diets with different concentrations of calcium for four months are illustrated in **Table 3**. The snails reached the highest values of these three traits in Ca5 (28.43g, 54.97mm, and 40.09mm, respectively) which were significantly different from the values in the other treatments.

The results also indicated that the daily weight gain and specific growth rate were highest in the Ca5 treatment (0.22 g/day and 2.17 %/day, respectively), and lowest in Ca9 (0.18 g/day and 2.02 %/day, respectively).

Significant differences for these traits were detected among the treatments. Similarly, the highest daily height gain and specific growth

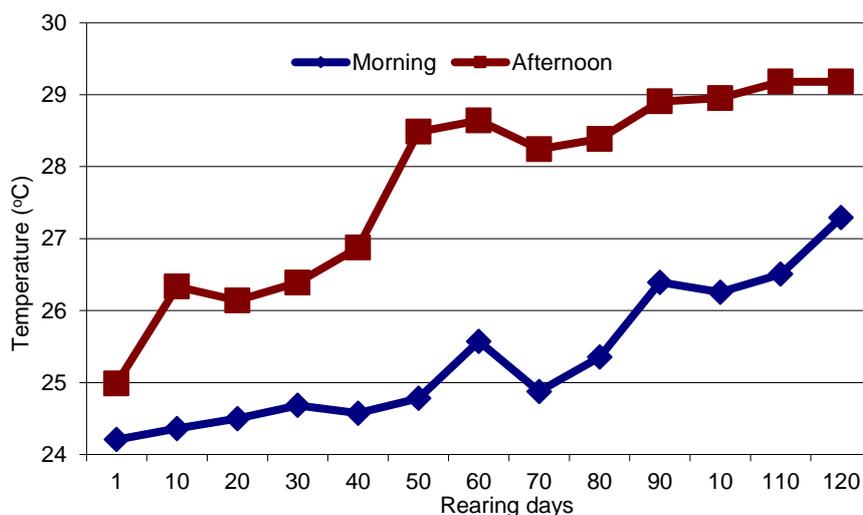


Figure 1. Fluctuations in the morning and afternoon temperatures during the experimental period

Table 2. Mean values of the environmental parameters during the experimental period

Diets		Ca1	Ca3	Ca5	Ca7	Ca9
Calcium levels (%DM)		1	3	5	7	9
Temperature (°C)	Morning	25.3 ^a ± 1.0	25.3 ^a ± 1.0	25.3 ^a ± 0.9	25.3 ^a ± 1.0	25.3 ^a ± 0.9
	Afternoon	27.6 ^a ± 1.6	27.6 ^a ± 1.4	27.8 ^a ± 1.4	27.5 ^a ± 1.6	27.5 ^a ± 1.5
Oxy (mg O ₂ L ⁻¹)		4.61 ^a ± 0.28	4.63 ^a ± 0.31	4.61 ^a ± 0.26	4.63 ^a ± 0.26	4.58 ^a ± 0.22
pH		7.94 ^a ± 0.32	7.96 ^{ab} ± 0.24	8.01 ^{abc} ± 0.28	8.03 ^{bc} ± 0.23	8.06 ^c ± 0.21
TAN (mg L ⁻¹)		0.65 ^a ± 0.10	0.71 ^a ± 0.11	0.74 ^a ± 0.11	0.72 ^a ± 0.01	0.66 ^a ± 0.07
NO ₂ ⁻ (mg L ⁻¹)		0.59 ^a ± 0.03	0.66 ^b ± 0.03	0.73 ^c ± 0.02	0.67 ^d ± 0.02	0.69 ^{bc} ± 0.01
Alkalinity (mgCaCO ₃ L ⁻¹)		67.6 ^a ± 1.1	71.5 ^{bc} ± 2.5	70.2 ^{ab} ± 1.0	74.2 ^{cd} ± 1.7	75.2 ^c ± 2.6

Note: The mean values in the same row with different letters indicate significant differences ($P < 0.05$).

Table 3. Mean (± SE) growth rates of black apple snails fed on different calcium levels

Diets	Ca1	Ca3	Ca5	Ca7	Ca9
Calcium level (% DM)	1	3	5	7	9
Body weight					
Initial (g)	2.11 ^a ± 0.01	2.14 ^a ± 0.05	2.10 ^a ± 0.06	2.14 ^a ± 0.07	2.15 ^a ± 0.03
Final (g)	25.47 ^b ± 0.30	27.21 ^d ± 0.22	28.43 ^e ± 0.35	26.47 ^c ± 0.33	24.20 ^a ± 0.56
WG (g)	23.36 ^b ± 0.30	25.07 ^d ± 0.17	26.33 ^e ± 0.40	24.33 ^c ± 0.26	22.04 ^a ± 0.57
DWG (g/day)	0.19 ^b ± 0.00	0.21 ^c ± 0.00	0.22 ^d ± 0.00	0.20 ^{bc} ± 0.00	0.18 ^a ± 0.00
SGR _{BW} (%/day)	2.08 ^b ± 0.01	2.12 ^c ± 0.01	2.17 ^d ± 0.03	2.10 ^{bc} ± 0.02	2.02 ^a ± 0.02
Shell height					
Initial (mm)	21.61 ^a ± 0.13	21.75 ^a ± 0.08	21.74 ^a ± 0.09	21.72 ^a ± 0.19	21.75 ^a ± 0.10
Final (mm)	52.73 ^b ± 0.15	53.93 ^c ± 0.63	54.97 ^d ± 0.58	53.29 ^{bc} ± 0.37	51.69 ^a ± 0.27
HG (mm)	31.12 ^b ± 0.03	32.18 ^c ± 0.67	33.23 ^d ± 0.62	31.57 ^{bc} ± 0.25	29.94 ^a ± 0.35
DLG (mm/day)	0.26 ^b ± 0.00	0.27 ^c ± 0.01	0.28 ^d ± 0.01	0.26 ^b ± 0.00	0.25 ^a ± 0.00
SGR _L (%/day)	0.74 ^b ± 0.00	0.76 ^{bc} ± 0.01	0.77 ^c ± 0.01	0.75 ^b ± 0.00	0.72 ^a ± 0.01
Shell width					
Initial (mm)	16.27 ^a ± 0.05	16.34 ^a ± 0.06	16.38 ^a ± 0.23	16.37 ^a ± 0.13	16.40 ^a ± 0.09
Final (mm)	38.17 ^b ± 0.06	39.25 ^c ± 0.48	40.09 ^d ± 0.64	38.66 ^{bc} ± 0.11	37.32 ^a ± 0.23
SWG (mm)	21.89 ^b ± 0.05	22.91 ^{cd} ± 0.43	23.72 ^d ± 0.86	22.29 ^{bc} ± 0.07	20.93 ^a ± 0.23
DWG (mm/day)	0.18 ^b ± 0.00	0.19 ^{cd} ± 0.01	0.20 ^d ± 0.01 ^d	0.19 ^{bc} ± 0.00	0.17 ^a ± 0.00
SGR _{SW} (%/day)	0.71 ^b ± 0.02	0.73 ^{bc} ± 0.02	0.75 ^c ± 0.02	0.72 ^b ± 0.01	0.69 ^a ± 0.01

Note: The mean values in the same row with different letters indicate the significant differences ($P < 0.05$).

rate for shell height were observed in the Ca5 treatment and showed significant differences with the other treatments.

The specific growth rates for body weight (SGR_{BW}, %/day) of the snails were fitted into quadratic models (**Figure 2**). The best fit for the

estimation of the optimal calcium level could be described as:

$$Y = -0.0067x^2 + 0.0602x + 2.0173, R^2 = 0.9295$$

where Y is the SGR_{BW} and x is the percentage of dietary calcium. The trend of the growth curve showed that the SGR_{BW} reached

the maximum value (2.154 %/day) when the diet contained 4.51% calcium.

Survival rate, biomass increase rate, productivity, feed conversion rate, and calcium efficiency ratio of black apple snails

The survival rate of the snails in all the treatments decreased gradually during the 4 months of culture (Figure 3) and was not significantly different among treatments, with values in the range of 71.7 to 75.7%.

The biomass increasing rate and productivity reached their maximum values in the Ca5 treatment (640% and 2.88 kg m⁻², respectively) and these values were significantly different compared to the other treatments. Meanwhile, the Ca9 treatment had the lowest values (544% and 2.35 kg m⁻², respectively).

The FCR values ranged from 1.22 to 1.39. The Ca3 treatment had the lowest FCR value (1.22) which was not significantly different from the values of Ca1, Ca5, and Ca7 (Table 4).

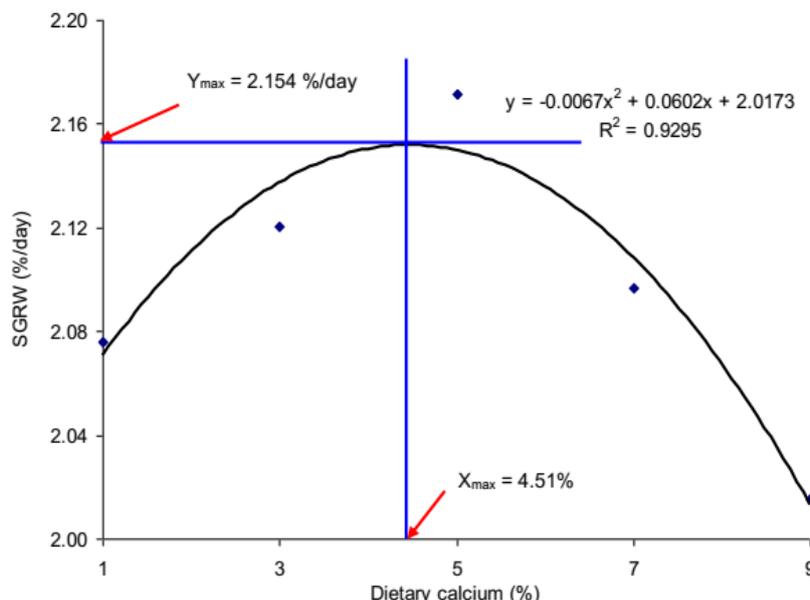


Figure 2. Relationship between the specific growth rate for body weight (SGR_{BW}) and dietary calcium levels (%DM) for black apple snails (*P. polita*)

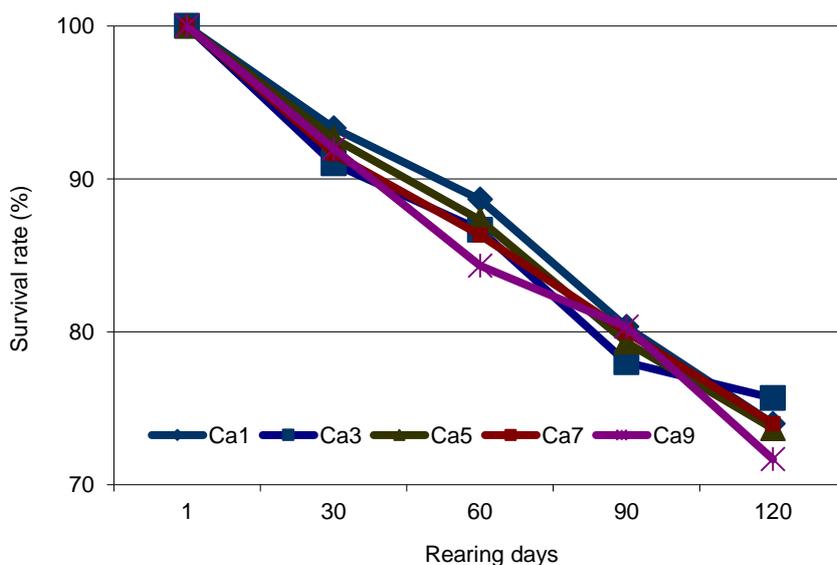


Figure 3. Survival rate of black apple snails during the experiment period

The highest CaER value was recorded in the Ca1 treatment (2343%) and was significantly different compared to Ca3 (868%), Ca5 (534%), Ca7 (354%), and Ca9 (237%). The FI values varied from 302 to 331 mg/ind./day and the Ca9 treatment presented the lowest FI value (302 mg/ind./day) (**Table 5**).

Discussion

The pH and alkalinity levels increased as the calcium levels increased due to the higher calcium content of the treatments. The calcium source was from CaCO₃. Ions of Ca²⁺ were absorbed by the snails for the growth of their shells and then ions of CO₃²⁻ were released into the water which led to the increase in alkalinity as the calcium level increased. According to Binh *et al.* (2017) and Thao & Binh (2017), the mean values of alkalinity ranged from 60.1-71.9 mg L⁻¹ when rearing snails with different calcium contents. In addition, Glass & Darby (2009) and Dalesman & Lukowiak (2013) suggested that fast-growing species of snails will have a greater demand for calcium to support shell formation, resulting in a lower alkalinity of the nursing environment.

The goal of this study was to determine the optimum level of calcium in the diet that would maximize the growth of black apple snails. Our results showed that the weight gain of black

apple snails increased when the calcium levels increased from 1 to 5%, and then decreased as the calcium levels continued to increase from 5 to 9%. These results are similar to those in other Gastropoda species (Coote *et al.*, 1996; Briers, 2003; Oluokun *et al.* 2005; Egonmwan, 2008; Chaitanawisuti *et al.*, 2010; Badmos *et al.*, 2016; Binh *et al.*, 2017; Thao & Binh, 2017). The best growth was observed in black apple snails fed a diet with 5% calcium content.

Brodersen & Madsen (2003) reported that snails raised at lower calcium concentrations were significantly smaller than snails raised at higher concentrations. Egonmwan (2008) found that after 30 weeks of culture, the mean weight and shell length of *Limicolaria flammea* fed food containing calcium carbonate were 1150mg and 20.75mm, respectively, which were significantly higher than the others fed with no calcium (950mg and 17.5mm, respectively). Oluokun *et al.* (2005) indicated that *Archachatina marginata* fed a diet with 8% calcium had the highest mean weight (234g), while those fed with 4% calcium had the lowest mean weight (198g). Similarly, Adou *et al.* (2011) showed that with the diet containing 12.02% calcium, *Achatina achatina* increased in body weight up to 96.78g and had a low rate of cumulative mortality (11%) compared to the 14% calcium diet (73.03g and 12%,

Table 4. Survival rate (SR), biomass increasing rate (BIR), and productivity (P) of black apple snails fed on different calcium levels

Diets	Ca1	Ca3	Ca5	Ca7	Ca9
Calcium level (% DM)	1	3	5	7	9
SR (%)	74.0 ^a ± 2.6	75.7 ^a ± 2.5	73.7 ^a ± 2.9	74.0 ^a ± 4.4	71.7 ^a ± 6.0
BIR (%)	594 ^{ab} ± 22	600 ^{ab} ± 10	640 ^b ± 17	593 ^{ab} ± 30	544 ^a ± 52
P (kg m ⁻²)	2.55 ^{ab} ± 0.12	2.82 ^{bc} ± 0.12	2.88 ^c ± 0.12	2.69 ^{bc} ± 0.17	2.35 ^a ± 0.19

Note: The values in the same row with different letters indicate the significant differences ($P < 0.05$).

Table 5. Feed conversion ratio (FCR), calcium efficiency ratio (CaER), and feed intake (FI) of black apple snails fed on different calcium levels

Diets	Ca1	Ca3	Ca5	Ca7	Ca9
Calcium level (% DM)	1	3	5	7	9
FCR	1.34 ^{ab} ± 0.10	1.22 ^a ± 0.05	1.24 ^a ± 0.06	1.27 ^{ab} ± 0.05	1.39 ^b ± 0.03
CaER (%)	2343 ^e ± 116	868 ^d ± 37	534 ^c ± 24	354 ^b ± 24	237 ^a ± 21
FI (mg/ind./day)	315 ^b ± 4	322 ^{bc} ± 5	331 ^c ± 6	317 ^b ± 2	302 ^a ± 11

Note: The mean values in the same row with different letters indicate the significant differences ($P < 0.05$).

respectively) and the 16% calcium diet (76.75g and 13%, respectively). Aman *et al.* (2011) revealed that using oyster shell powder, which contains calcium, as the substrate could affect the growth of the snail *Archachatina marginata*. When the substrates were amended with 30% oyster shell powder with a calcium content of 7.7%, the bodyweight growth was 0.86 g/day and the shell growth was 0.39 mm/day. This value decreased when the amount of oyster shell powder was reduced. In *Babylonia areolate*, Chaitanawisuti *et al.* (2010) demonstrated that a diet supplemented with 7% calcium resulted in spotted Babylon snails that had a significantly higher specific growth rate for length (0.37 %/day) than those fed diets supplemented with 4% calcium (0.32%/day). Numerous studies have previously demonstrated the importance of calcium as a factor determining the development and wellbeing of snails.

Our findings showed that snails in the experiment had a good growth trend compared to previous studies. The results of this study and previous studies have shown that freshwater gastropods (*Pila polita*, *Pomacea paludosa*, *Achatina achatina*, and *Limicolaria flammea*) require a diet higher in calcium content than marine species (*Babylonia areolata* and *Huliotis laeuigata*). A diet with levels of calcium in a suitable range will ensure optimum growth. However, if the content of calcium exceeded the body's needs, then the growth rate of the snail would decrease (Coote *et al.*, 1996; Oluokun *et al.*, 2005; Kritsanapuntu *et al.*, 2006; Chaitanawisuti *et al.*, 2010; Badmos *et al.*, 2016; Binh *et al.*, 2017).

The survival rate of black apple snails after four months of culture ranged from 71.7 to 75.7% and there were no significant differences among the treatments. Ireland (1990) found that during four months culturing *Achatina fulica*, mortality was low and occurred in snails on the lower calcium diets. Chaitanawisuti *et al.* (2010) reported that the survival rate of *Babylonia areolate* with different levels of dietary calcium (1%, 4%, and 7%) did not affect survival (91.0-94.9%).

The biomass increasing rate (BIR) was highest in the 5% calcium diet (640%). This

result was comparable to that of Binh & Thao (2018) who found the BIR was 436-511%. The productivity of black apple snails after four months of culture was in the range of 2.35-2.88 kg m⁻², similar with the study results of Binh & Thao (2017) and (2018) who measured productivity to be 2.31-3.15 kg m⁻² at the snail density of 100 ind m⁻² when feeding with pellets (18% protein; 2.4% calcium).

The calcium efficiency rate (CaER) of black apple snails decreased as the calcium level in the diet increased. Our study found that the FCR of black apple snails increased when the calcium levels increased from 5% to 9%. Chaitanawisuti *et al.* (2010) demonstrated that *Babylonia areolate* fed with a 4% calcium diet had an FCR value (2.47) lower than those fed with a 7% calcium diet (2.76). Oulokun *et al.* (2005) studied *Archachatina marginata* and found that the FCR was highest (6.49) when the snails were fed a diet with a 4% calcium content and tended to decrease when the calcium content increased to 6% (5.36) and 8% (5.25). The feed intake (FI) of black apple snails increased as the calcium level increased from 1 to 5% and then decreased when calcium level increased to 9%. The explanation for this is related to the amount of calcium absorbed by the snails. With a diet of 5% calcium content, black apple snails had the highest growth rate and then the rate started to decrease as the calcium level increased.

Conclusions

The concentration of calcium in the diet did not affect the survival rate of black apple snails during the culture period.

After 4 months of culture, the growth of black apple snails (*Pila polita*) had maximum values in terms of body weight, shell height, and shell width when the snails were fed a diet containing 5% calcium.

The addition of calcium levels to 5% to the total diet of black apple snails (*Pila polita*) would be beneficial in terms of weight gain and productivity. The estimation for the optimum requirement of calcium for black apple snail growth was 4.51% dry matter of total diet.

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