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# Effects of Different Dietary Mineral Contents on the Survival and Growth Rate of Black Apple Snails *(Pila polita)* in the Juvenile Stage

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## Abstract

The purpose of this study was to determine the effects of different dietary mineral contents on the growth, survival rate, and biomass increase of the black apple snail, *Pila polita*, during the nursing phase. Juveniles (0.26 g snail$^{-1}$) were fed diets with five different mineral supplementation levels: (1) Control (No.Mi); (2) Mineral supplement 3% (Mi3); (3) Mineral supplement 5% (Mi5); (4) Mineral supplement 7% (Mi7); and (5) Mineral supplement 9% (Mi9), with three replicates per treatment. Snails were reared in composite tanks with a density of 300 ind m$^{-2}$ for 56 days. After 56 days, the snail survival rate in Mi7 (98.4%) was higher ($P$ <0.05) than in No.Mi (95.8%). Snails in Mi7 also obtained the highest height, weight, and yield (23.16mm, 2.58g, and 742 g m$^{-2}$), which were significantly different from the other treatments ($P$ <0.05). The results also showed that the dietary mineral concentration of 7% generated an acceptable mineral ratio, which could increase snail rearing efficiency.

## Keywords

Black apple snail, growth, mineral supplementation, survival rate

## Introduction

The *Pila polita* snail is distributed across many Asian countries, specifically Indonesia, Cambodia, Laos, Vietnam, China, and Thailand (Cowie *et al.,* 2015). In Vietnam, the *Pila polita* snail lives in ponds, lakes, canals, ditches, and rice fields in the plains, midlands, and mountains, and is also a native snail species in the Mekong Delta (Đang Ngoc Thanh *et al.,* 2003). Yamashita *et al.* (2008) stated that the *Pila* and *Pomacea* snails have been used as food by people in most tropical and subtropical countries because it is a food source rich in protein and minerals. According to Le Van Binh & Ngo Thi Thu Thao (2020) and Truong Van Xa & Tran Kim Thoa (2024), the apple snail has high economic value due to its delicious meat that is rich in nutrients (fresh apple snail contains 11.9% protein; 0.7% fat;

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vitamins B1, B2, and PP; calcium, phosphorus, magnesium, zinc, copper, and essential amino acids), and the apple snail is considered one of the freshwater snail species with an important role in medicine (Pusadee *et al*., 2005).

Several mineral components, such as calcium, magnesium, zinc, sodium, potassium, copper, and phosphorus, are involved in shell formation of molluscan shellfish (Ireland, 1991). The hardness and thickness of snail shells greatly depend on the calcium content (Glass & Darby, 2009). In addition, calcium functions as a buffer solution, participating in metabolism and cell production (Ireland, 1993), and accounts for 30% of the total body mass of gastropods (Badmos *et al.*, 2016). Hunter & Lull (1977) suggested that calcium affects the fertility of mother snails. Thus, calcium is considered one of the limiting nutrients that affects the distribution of mollusks, as these species need calcium for survival, development, and reproduction (Glass & Darby, 2009; Karamoko *et al.*, 2014; Ngo Thi Thu Thao & Le Van Binh, 2017). The structure of snail shells contains minerals (calcium, phosphorus, magnesium, potassium, zinc, and copper), and snails also need to absorb and use these minerals to maintain acid-base balance. Consequently, minerals make an important contribution to the formation of shells that support the growth process (Ireland, 1991; Rygało-Galewska *et al.*, 2023). In addition, minerals also play a role in egg creation during the reproductive stage of female snails (Jatto *et al.*, 2010; Beeby & Richmond, 2011). Minerals have been shown to increase cells involved in fertility in aquatic animals (Gaál *et al.*, 2004; Chandra *et al.*, 2013). These components also influence the mineralization process in aquatic organisms, affecting muscle contractions, enzyme activation, cell differentiation, the immune response, neurological activity (Pu *et al.*, 2016), and biochemical processes that generate cellular energy (Huskinson *et al.*, 2007). Several studies have also mentioned that phosphorus plays an indispensable role in cellular functions, as it is a major component of nucleic acids, phospholipids, phosphoproteins, ATP, and several important enzymes. Studies on the mineral supplementation of aquatic animals, especially on fish and crustaceans, are quite abundant, but on mollusks they are still limited. A high stocking density (200,000 to 240,000 snail seeds $m^{-2}$) has been applied in the practice of rearing the black apple snail, *Pila polita* (Ngo Thi Thu Thao & Le Van Binh, 2018), and snail hatcheries always supplement calcium and some minerals into the snails' diet. However, excessive levels of minerals not only increase the cost of feed unnecessarily but also increase the input of minerals into the aquatic environment, which may affect the bioavailability of other nutrients. Therefore, this study was designed to determine the effects of mineral levels in artificial pellet feed on the rearing efficiency of black apple snails.

## Materials and Methods

### Experimental design

Black apple snail eggs were purchased from Dong Thap province and transferred to the Mollusc Experimental Laboratory, College of Fisheries and Aquaculture, Can Tho University to hatch into baby snails. After 20-25 days of rearing, healthy snails with an average weight of 0.26g and a height of 10.70mm were selected to arrange the experiment.

The experiment was set up in composite tanks with the dimensions of 80×60cm. The water column height was maintained at 30cm, an aeration system was installed, and one nylon bunch was located as substrates for the baby snails.

Snails were reared at a density of 300 individuals $m^{-2}$ for 56 days. The experiment was arranged with five treatments corresponding to five different mineral contents: Control (No.Mi), 3% mineral (Mi3), 5% mineral (Mi5), 7% mineral (Mi7), and 9% mineral (Mi9). Each treatment was replicated three times. Self-prepared diets were used in the experiment, and their nutritional compositions corresponding to each treatment are presented in **Table 1**.

### Food preparation

The food used in the experiment was prepared at the Experimental Laboratory, College of Fisheries and Aquaculture, Can Tho University and the feed formulas were designed

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

| Dietary mineral levels | No. Mi | Mi3 | Mi5 | Mi7 | Mi9 |
|---|---|---|---|---|---|
| Ingredient (%) | | | | | |
| Fish meal[1] | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Soybean meal[2] | 31.42 | 31.43 | 31.45 | 31.48 | 31.50 |
| Tapioca flour[3] | 40.97 | 37.96 | 35.94 | 33.91 | 31.89 |
| Soy oil | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin[4] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Mineral premix[5] | 0.00 | 3.00 | 5.00 | 7.00 | 9.00 |
| CMC | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Calcium[7] | 3.61 | 3.61 | 3.61 | 3.61 | 3.61 |
| Proximate composition (%) | | | | | |
| Crude protein | 28.1 | 27.9 | 28.3 | 28.0 | 28.4 |
| Crude lipids | 2.70 | 2.77 | 2.59 | 2.94 | 2.76 |
| NFE | 40.41 | 41.49 | 39.31 | 38.58 | 36.97 |
| Crude ash | 9.88 | 10.3 | 11.0 | 11.6 | 12.2 |
| Crude fiber | 2.79 | 2.55 | 3.04 | 3.01 | 3.18 |
| Moisture | 9.67 | 8.31 | 8.83 | 8.64 | 8.93 |
| Calcium | 5.03 | 5.05 | 5.12 | 5.26 | 5.32 |
| Phosphorus | 1.09 | 1.18 | 1.31 | 1.41 | 1.52 |
| Magnesium | 0.094 | 0.114 | 0.126 | 0.133 | 0.136 |
| Sodium | 0.233 | 0.337 | 0.378 | 0.427 | 0.581 |
| Energy | | | | | |
| Energy (Kcal $g^{-1}$)[7] | 3.98 | 3.85 | 3.77 | 3.68 | 3.60 |
| Protein/energy ratio (mg $Kcal^{-1}$)[7] | 62.88 | 64.90 | 66.34 | 67.86 | 69.44 |
| Mineral ratios | | | | | |
| Calcium:Phosphate | 4.61 | 4.27 | 3.90 | 3.73 | 3.50 |
| Calcium:Magnesium | 53.51 | 44.29 | 40.63 | 39.54 | 38.23 |
| Calcium:Sodium | 21.58 | 14.98 | 13.54 | 12.31 | 9.15 |

*Note: Parameters: Protein, lipids, ash, fiber, moisture, NFE- Nitrogen free extract; [1]Fish meal Kien Giang (Vietnam): Calcium: 6.54%; Phosphorus: 3.87%; Sodium: 19,717 mg $kg^{-1}$; Magnesium: 1,981 mg $kg^{-1}$; [2]Soybean meal (Argentina): Calcium: 0.181%; Phosphorus: 0.64%; Sodium: 2.820 mg $kg^{-1}$; Magnesium: 467 mg $kg^{-1}$; [3]Tapioca flour: Calcium: 0.066%; Phosphorus: <0.12%; Sodium: 49.1 mg $kg^{-1}$; Magnesium: <100 mg $kg^{-1}$; [4]Vitamins: Vitamin A (2,000,000 IU); Vitamin D (400,000IU); Vitamin E (6 g); Vitamin B1 (800 mg); Vitamin B2 (800mg); Vitamin B12 (2mg) and [5]mineral premix (PRO MIX, Golden Shrimp Biology Co., Ltd.): Calcium (min-max): 185-200 g $kg^{-1}$; Phosphorus (min-max): 50,000-50,500 mg $kg^{-1}$; Sodium (min-max): 1,850-2,100 mg $kg^{-1}$; Potassium (min-max): 950-1,100 mg $kg^{-1}$; Magnesium (min-max): 80,000-85,500 mg $kg^{-1}$; Manganese (min-max): 450-500 mg $kg^{-1}$; [6]Calcium: $CaCO_3$-Calcium Carbonate; 99.0% content $CaCO_3$ (China); [7]Source: Gómez-Montes et al. (2003).*

using Solver software in Excel. The experimental diets were formulated into pellets using several ingredients, including fish meal, soybean meal, tapioca flour, soy oil, vitamin premix, mineral premix, and CMC (carboxymethyl cellulose). The raw materials were scaled according to the ratio of fish meal, wheat flour, vitamin premix, mineral premix (PRO MIX, Golden Shrimp Biology Co., Ltd), and CMC and mixed well (dry mix). The soybean meal was cooked, allowed to cool down to a temperature of 40-50°C, and then mixed with the dry ingredients using soy oil. After mixing, the pellets were dried in an oven at 50°C for 24 hours and stored in the refrigerator at 4°C until feeding time.

Snails were fed twice a day (at 7:00 AM and 18:00 PM) with 7% of their total weight for the first 21 days, then 6% from days 22 to 35, and 5% from day 36 onward. The weights of the pellets were adjusted weekly after weighing the snails in each tank. The water in each culture tank was renewed by 30-40% after a cycle of 7-10 days.

### Data collection and sample analysis

*Water quality:* Water temperature was recorded twice a day at 7:00 AM and 14:00 PM using a thermometer. The concentrations of $NH_4^+/NH_3$ (TAN), $NO_2^-$, DO, pH, and alkalinity were monitored weekly using test kits (Sera, Germany).

*Growth performance and feed utilization:* After 56 days of rearing, the number of living snails in the tanks were counted to determine the survival rate, and the heights and weights of 20 snails/tank were measured. The shell height was measured using an electronic digital caliper (0.01mm resolution), and the snail weight was determined using an electronic scale (0.01g error).

Weight gain (WG), shell height gain (HG), daily weight gain (DWG), daily height gain (DHG), specific growth rate in body weight ($SGR_W$), specific growth rate in shell height ($SGR_H$), feeding rate (FR), and survival were calculated using the following equations:

Survival rate (%) = final number of snails / initial number of snails × 100.

Biomass increase rate (BIR,%) = (biomass increase / biomass initial) × 100.

Food conversion ratio (FCR) = Total feed intake (dry weight) / Weight gain (wet weight)

Feed intake (FI, mg/snail/day) = [Total food fed / (Number of arranged snails + Number of finished snails) / 2] × Culture time

After the experiment was completed, all of the snails in each tank were weighed and their shell heights were measured in order to quantify the rate of growth differentiation based on height and weight using the formula: CV (%) = S × 100/X; where CV stands for the coefficient of variation, S for the standard deviation, and X for the average snail weight and height at the end of the experiment.

Condition index (CI, mg $g^{-1}$) = DWM/DWS × 100; in which DWM is the weight of snail meat dried at 60°C after 24 hours and DWS is the snail shell weight (g).

The ratio of meat or meat/shell (%) of snails was calculated according to the formula: (Ps/Pt) × 100; in which Ps is the weight of snail meat (g) and Pt is the weight of the snail shell (g).

### Statistical analysis

The effect of the different mineral levels on the rearing parameters was analyzed by one-way ANOVA followed by the Duncan post hoc test using SPSS software version 22.0 ($\alpha$ = 5%). Percentage (%) figures were arcsine transformed before statistical processing. To determine the correlations among survival rate, feed conversion ratio, and feed intake of black apple snails raised with different mineral contents the equation $y = ax^2 + bx + c$ was used (Coote *et al.*, 2000).

## Results and Discussion

### Water quality parameters

The mean values of the environmental parameters are listed in **Table 2**. The average temperature ranged from 26.4 to 27.8°C in the morning and from 28.5 to 31.0°C in the afternoon. There were no significant differences in the temperature among the treatments ($P$ >0.05). The pH and alkalinity values in all the treatments did not have large fluctuations (7.18-7.29 and 62.0-64.0 mg $CaCO_3$ $L^{-1}$, respectively) and were not significantly different among the treatments ($P$ >0.05).

### Growth performance of black apple snails

The snails in all the treatments had an average height of 10.7mm and weight of 0.26g at the beginning of the experiment (**Table 3**). After 56 days of rearing, in Mi7, the average height and weight were 23.16mm and 2.58g, respectively, which were considerably higher than the values of snails in the other treatments ($P$ <0.05). Snails in the Mi7 treatment also had the highest absolute weight gain (41.4 mg $day^{-1}$), which was substantially higher ($P$ <0.05) than the other treatments. Comparing the Mi7 treatment to Mi3 (3.92%) and No. Mi (3.60%), the snails' relative

**Table 2.** Mean values (± SD) of the environmental parameters in the different treatments

| | Dietary crude mineral levels | | | | |
|---|---|---|---|---|---|
| | No. Mi | Mi3 | Mi5 | Mi7 | Mi9 |
| Morning temperature (°C) | 26.9 ± 0.2 | 26.9 ± 0.2 | 27.0 ± 0.2 | 27.0 ± 0.2 | 26.9 ± 0.3 |
| Afternoon temperature (°C) | 29.9 ± 0.4 | 29.9 ± 0.3 | 29.9 ± 0.3 | 30.0 ± 0.4 | 30.0 ± 0.4 |
| Oxygen (mg $O_2$ $L^{-1}$) | 4.54 ± 0.26 | 4.60 ± 0.29 | 4.54 ± 0.25 | 4.54 ± 0.27 | 4.55 ± 0.22 |
| pH | 7.23 ± 0.40 | 7.25 ± 0.38 | 7.29 ± 0.32 | 7.18 ± 0.34 | 7.26 ± 0.41 |
| $NH_4$/$NH_3$-TAN (mg $L^{-1}$) | 0.59 ± 0.27 | 0.60 ± 0.29 | 0.62 ± 0.30 | 0.64 ± 0.31 | 0.62 ± 0.30 |
| $NO_2^-$ (mg $L^{-1}$) | 0.64 ± 0.31 | 0.59 ± 0.28 | 0.59 ± 0.28 | 0.62 ± 0.30 | 0.59 ± 0.28 |
| Alkalinity (mg$CaCO_3$ $L^{-1}$) | 62.0 ± 0.6 | 63.7 ± 2.1 | 63.4 ± 2.0 | 64.0 ± 1.6 | 63.3 ± 1.0 |

**Table 3.** Growth rates of shell height and weight of black apple snails (mean ± SD) with different dietary mineral contents

| | Dietary crude mineral levels | | | | |
|---|---|---|---|---|---|
| | No.Mi | Mi3 | Mi5 | Mi7 | Mi9 |
| *Snail weight* | | | | | |
| Day 1 (g) | $0.26^a$ ± 0.00 | $0.26^a$ ± $0.00^a$ | $0.26^a$ ± 0.00 | 0.26a ± 0.00 | $0.26^a$ ± 0.00 |
| Day 56 (g) | $1.99^a$ ± 0.05 | $2.30^{ab}$ ± 0.05 | $2.41^b$ ± 0.01 | $2.58^d$ ± 0.01 | $2.36^c$ ± 0.02 |
| Weight gain (g) | $1.72^a$ ± 0.05 | $2.05^b$ ± 0.05 | $2.15^c$ ± 0.01 | $2.32^d$ ± 0.01 | $2.10^{bc}$ ± 0.01 |
| DWG (mg $day^{-1}$) | $30.8^a$ ± 0.8 | $36.6^b$ ± 0.9 | $38.3^c$ ± 0.3 | $41.4^d$ ± 0.1 | $37.5^{bc}$ ± 0.3 |
| $SGR_W$ (% $day^{-1}$) | $3.60^a$ ± 0.05 | $3.92^b$ ± 0.05 | $3.95^b$ ± 0.01 | $4.07^c$ ± 0.01 | $3.96^b$ ± 0.01 |
| *Shell height* | | | | | |
| Day 1 (mm) | $10.80^a$ ± 0.10 | $10.74^a$ ± 0.10 | $10.64^a$ ± 0.05 | $10.64^a$ ± 0.11 | $10.66^a$ ± 0.04 |
| Day 56 (mm) | $20.60^a$ ± 0.17 | $21.77^b$ ± 0.23 | $22.69^c$ ± 0.1 | $23.16^d$ ± 0.13 | $22.51^c$ ± 0.11 |
| Shell height gain (mm) | $9.81^a$ ± 0.08 | $11.03^b$ ± 0.14 | $12.05^d$ ± 0.06 | $12.52^e$ ± 0.02 | $11.85^c$ ± 0.06 |
| DHG (µm $day^{-1}$) | 175a ± 1 | $197^b$ ± 3 | $215^d$ ± 1 | $224^e$ ± 2 | $212^c$ ± 1 |
| $SGR_H$ (% $day^{-1}$) | $1.15^a$ ± 0.02 | $1.26^b$ ± 0.01 | $1.35^d$ ± 0.01 | $1.39^e$ ± 0.01 | $1.33^c$ ± 0.02 |

*Note: Values in the same row with different letters indicate significant differences (P <0.05).*

weight growth rate was also the greatest (4.07% $day^{-1}$) and distinct ($P$ <0.05). Also in the Mi7 treatment, the snails' relative and absolute height growth rates (1.39% $day^{-1}$ and 224 µm $day^{-1}$) were also higher than the other treatments ($P$ <0.05).

## Rearing efficiencies of black apple snails with different dietary mineral contents

After 56 days of rearing, the Mi7 treatment had the highest snail survival rate (98.4%), followed by Mi9 (97.3%), Mi5 (97.1%), and Mi3 (96.4%). There were also significant differences ($P$ <*0.05*) between the Mi7 treatment and No.Mi (95.8%) and Mi3 (96.4%), and there was a strong correlation between the survival rate and the amount of minerals added to the feed ($y = -0.0283x^2 + 0.4882^x + 95.607$, $R^2 = 0.748$).

Snails in the Mi7 treatment were the most productive (742 g $m^{-2}$) and had the largest biomass increase rate (597%), which was statistically different from the other treatments ($P$ <0.05). Although the snail condition index ranged from 282 to 288 mg $g^{-1}$ and the shell weight ratio ranged from 24.4 to 25.3%, there were no significant differences among the treatments ($P$ >0.05).

**Table 4** reveals that after 56 days of rearing, the Mi3 treatment had the lowest rate of growth differentiation in the snails' height and weight (4.75% and 12.3%), followed by Mi7 (5.28% and 12.8%), and this treatment differed from the others ($P$ <0.05).

**Feed intake of black apple snails with different dietary mineral contents**

The snails' feed coefficient increased progressively in Mi9 (0.67) but declined gradually in the Mi3 (0.66) and Mi7 (0.62) treatments. There were significant differences ($P$ <0.05) between the Mi7 treatment and Mi9 and No. Mi (**Table 5**).During the 56-day rearing period, snails in the No.Mi treatment consumed the least amount of feed (10.8 mg/snail/day), followed by Mi3 (11.4 mg/snail/day) and Mi5 (11.6 mg/snail/day). These differences were significant ($P$ <0.05) when compared to Mi7 (12.1 mg/snail/day).

## Discussion

The snails may have taken calcium directly from the water because the alkalinity was comparatively low in the treatments without mineral addition, presumably as a result of the diets' lack of calcium (Glass & Darby, 2009; Badmos *et al.*, 2016; Le Van Binh & Ngo Thi Thu Thao, 2019). According to previous studies, snails in low calcium levels grow more slowly, have thinner shells (Glass & Darby, 2009), and have lower fertility rates (Hunter & Lull, 1977). According to Ngo Thi Thu Thao & Le Van Binh (2017), raising snails with pellets supplemented with 1-3% calcium resulted in an alkalinity of 61.4-63.2 mgCaCO3 L-1 and the alkalinity increased to 67.4 mg CaCO3 L-1 when adding 7% calcium.

Adding 7% mineral content to the snails' diet resulted in high relative weight and height growth rates. According to Lee *et al.* (1999), feeding *Haliotis discus hannai* with 2% mineral supplementation resulted in greater weight and height gains than feeding them with 4% or no mineral supplementation. Similar findings were made by Tan *et al.* (2001), who studied the same abalone species and found that when 1% phosphorus was introduced to the diet, the growth rate reached 86.9 µm day$^{-1}$, and when 0.5% or 2% phosphorus was added, the growth rate dropped to 82.1 µm day$^{-1}$. According to Chaitanawisuti *et al.* (2010), *Babylonia areolata* fed a diet containing 1% phosphorus had larger shell heights and weights (29.7mm and 4.90g) than those fed a diet containing 3% phosphorus (28.2mm and 4.29g) or 5% phosphorus (27.7mm

**Table 4.** Survival rate, biomass increase rate (BIR), productivity, condition index (CI), shell ratio, weight and shell height differentiation (CV) of snails with different dietary mineral contents (mean ± SD)

| | | Dietary crude mineral levels | | | | |
|---|---|---|---|---|---|---|
| | | No. Mi | Mi3 | Mi5 | Mi7 | Mi9 |
| Survival rate (%) | | 95.8$^{a}$ ± 0.4 | 96.4$^{ab}$ ± 0.4 | 97.1$^{bc}$ ± 0.4 | 98.4$^{d}$ ± 0.4 | 97.3$^{c}$ ± 0.7 |
| BIR (%) | | 440$^{a}$ ± 24 | 501$^{b}$ ± 21 | 491$^{b}$ ± 17 | 597$^{c}$ ± 20 | 509$^{b}$ ± 16 |
| Productivity (g m$^{-2}$) | | 597$^{a}$ ± 10 | 685$^{b}$ ± 9 | 671$^{b}$ ± 7 | 742$^{c}$ ± 10 | 681$^{b}$ ± 17 |
| CI (mg g$^{-1}$) | | 284$^{a}$ ± 31 | 287$^{a}$ ± 37 | 282$^{a}$ ± 36 | 282$^{a}$ ± 33 | 288$^{a}$ ± 28 |
| Shell ratio (%) | | 25.0$^{a}$ ± 1.3 | 24.4$^{a}$ ± 2.5 | 24.9$^{a}$ ± 3.5 | 24.8$^{a}$ ± 2.8 | 25.3$^{a}$ ± 1.7 |
| CV (%) | Shell height | 5.46$^{ab}$ ± 0.11 | 4.75$^{a}$ ± 0.98 | 5.89$^{b}$ ± 0.34 | 5.28$^{ab}$ ± 0.12 | 5.62$^{ab}$ ± 0.45 |
| | Snail weight | 14.9$^{b}$ ± 1.8 | 12.3$^{a}$ ± 1.1 | 14.7$^{b}$ ± 1.1 | 12.8$^{a}$ ± 1.1 | 14.9$^{b}$ ± 1.0 |

*Note: Values in the same row with different letters indicate significant differences (P <0.05).*

**Table 5.** Feed conversion ratio (FCR) and feed intake (FI, mg/snail/day) of snails with different dietary mineral contents (mean ± SD)

| | Dietary crude mineral levels | | | | |
|---|---|---|---|---|---|
| | No. Mi | Mi3 | Mi5 | Mi7 | Mi9 |
| FCR | 0.72$^{c}$ ± 0.02 | 0.66$^{b}$ ± 0.01 | 0.65$^{ab}$ ± 0.01 | 0.62$^{a}$ ± 0.03 | 0.67$^{b}$ ± 0.01 |
| FI | 10.8$^{a}$ ± 0.4 | 11.4$^{b}$ ± 0.3 | 11.6$^{b}$ ± 0.2 | 12.1$^{c}$ ± 0.1 | 11.5$^{b}$ ± 0.2 |

*Note: Values in the same row with different letters indicate significant differences (P <0.05).*

and 4.91g). Previous studies have stated that phosphorus serves as a buffer to keep body fluids at the ideal pH. Body fluids with low phosphorus levels have an unbalanced pH. The development of gastropod mollusks will subsequently be impacted by this (Coote *et al.*, 1996; Tan *et al.,* 2001).

According to a study by Ademolu *et al.* (2004), the snail *Archachatina marginata* grew to a height of 21.3mm and a weight of 14.0g when minerals were added to their diet at a rate of 0.3 g $kg^{-1}$. Without the addition of minerals, the snail's size was only 17.5mm and 12.0g, respectively. The *Achatina achatina* snail lives on land and absorbs minerals through food and drinking water, whereas aquatic snails absorb minerals from food and also directly from the aquatic environment where they live (Nyameasem & Borketey-La, 2014).

Our findings showed that snail growth declined when the diet included dietary ingredients with a high mineral concentration (9%). The reason for this is that too much calcium restricts the absorption and digestion of calcium (Ireland, 1991; Tan *et al.,* 2001). The snails also decreased their food intake because of imbalanced dietary item consumption or excessive calcium buildup in the body (Ireland, 1991; Ireland & Marigomez, 1992). Certain important minerals in metabolism, including magnesium, zinc, and copper, can be blocked by too much calcium (Ireland & Marigomez, 1992). However, there was a definite trend in this study that the ratios of Ca:P, Ca:Mg, and Ca:Na in the diets fell as the mineral content increased, and the appropriate ratio could enhance the growth performance of snails.

The research results on black apple snails showed that the growth rate of snails in the juvenile stage decreased when the feed was supplemented with a high mineral content. This can be explained by the fact that when too many minerals are supplemented in feed or water, it limits the digestion and absorption of calcium and phosphorus due to the need to excrete excess calcium from the body or due to the imbalanced consumption between the components in the feed. In addition, high calcium prevents some divalent ions (magnesium, zinc, iron, copper, and manganese) from participating in metabolic processes (Ireland, 1991; Ireland & Marigomez, 1992). Phosphorus acts as a buffer to maintain optimal pH in body fluids, and a phosphorus deficiency causes a pH imbalance in body fluids, which affects the growth of gastropod mollusks (Tan *et al.,* 2001). Research by Tan *et al.* (2001) showed that a Ca:P ratio ranging from 0.1:1 to 9.0:l did not affect the growth, development, and reproduction of the abalone *Haliotis discus hannai*. However, Coote *et al.* (1996) recommended a Ca:P ratio from 0.72:1 to 2.68:l and Oluokun *et al*. (2005) recommended a ratio from 5.09:1 to 5.99:l to be suitable for the growth and development of the abalone *Haliotis laevigata* and the snail *Archachatina marginata*, respectively. Through research, black apple snails in the adolescent stage supplemented with minerals in their feed at a content of 7% (equivalent to the ratios of 3.7:1 for Ca:P, 39.5:1 for Ca:Mg, and 12.3:1 for Ca:Na) resulted in better growth and differentiation than other mineral contents.

Black apple snails fed with varying dietary mineral concentrations had significantly variable survival rates ($P$ <0.05) ranging from 97.1% to 98.4% for those with higher dietary mineral contents. Chaitanawisuti *et al.* (2010) demonstrated that the survival rate (91.0-94.9%) of *Babylonia areolata* increased with varying dietary calcium concentrations (1%, 4%, and 7%) but did not differ. According to Tan *et al.* (2001), the survival rates of the abalone *Haliotis discus* using dietary calcium concentrations of 0-2% varied from 96-100%. Wacker & Baur (2004) suggested that a calcium deficiency impairs metabolism and reduces survival rates of aquatic organisms. However, Ngo Thi Thu Thao & Le Van Binh (2017) showed that when black apple snails were fed a diet containing 1% to 5% calcium, their survival rate was 77.3 to 78.6%, but when they were fed a diet containing 7% calcium, their survival rate dropped to 75.3%. According to Kritsanapuntu *et al.* (2006), an increase in the calcium concentration (0.0-5.0 g $CaCO_3$ $m^{-3}$) led to a lower survival rate of *Babylonia areolata* snails (92.9-57.1%). Tan *et al.* (2001) also observed that the abalone *Haliotis discus hannai* maintained a survival rate of

89.0% when fed a diet supplemented with 2% minerals; however, when the content increased to 6%, the rate dropped to 84.0% (Lee *et al.,* 1999).

For the sweet snail *Babylonia areolata*, Chaitanawisuti *et al.* (2010) found that food supplemented with 1% calcium increased biomass (662%) more than a diet containing 4% (388%) and 7% (560%). On the other hand, Ngo Thi Thu Thao & Le Van Binh (2017) found that the biomass increase rate in *Pila polita* was only 1,060% when 1% calcium was added, and it rose to 1,226% when 5% calcium was added. According to a related study by Ngo Thi Thu Thao & Le Van Binh (2017), adding 1% to 5% calcium to pelleted meals increased snail yield from 127 g $m^{-2}$ to 134 g $m^{-2}$. However, at higher supplemental calcium levels (7%), the output dropped to 119 g $m^{-2}$. Our findings revealed that increasing the mineral contents in the diet resulted in a decrease in the ratios of calcium and phosphorus, as well as calcium and sodium. This led to an appropriate scale among minerals, which could be beneficial to snail growth, survival, and biomass increase.

In the present study, snails in the 5 to 7% mineral supplementation groups had considerably lower FCR ($P$ <0.05) compared to those at the higher or not supplemented groups. Rygało-Galewska *et al.* (2023) found that the snail *Cornu aspersum* fed a diet with varying mineral contents (calcium from 8.9 to 20 g $kg^{-1}$ and magnesium from 0.8 to 4.7 g $kg^{-1}$) had FCR values ranging from 0.86 to 1.28. Chaitanawisuti *et al.* (2010) discovered that juvenile *Babylonia areolata* fed a diet with 4% calcium and 3% phosphorus had a higher FCR (2.63) than diets with lower or higher Ca:P ratios. The amount of feed consumed by snails in this study was greatly impacted by the mineral contents of the diets, which ranged from 0% to 7%. According to Rygało-Galewska *et al.* (2023), *Cornu aspersum* fed a diet with 2% calcium and 0.08% magnesium consumed 8.34 g/snail/day, which increased to 10.64 g/head/day when given 2% calcium and 0.47% magnesiumA sufficient mineral concentration, as well as an acceptable mineral ratio, may be able to boost food absorption because they are useful for metabolism.

The findings of this study indicated that when the mineral content of the diet increased to 9%, snails stored more calcium in their shells, resulting in a thicker shell and possibly a drop in meat proportion. Chaitanawisuti *et al.* (2010) discovered that *Babylonia areolata* fed a diet containing 4% calcium and 3% phosphorus had a shell weight ratio of 26.5%, however this value was larger when snails were fed a diet with a lower or higher calcium content. The findings of this study were consistent with previous studies on increasing shell bulk in mollusks in mineral-rich conditions (Beeby & Richmond, 2007; Glass & Darby, 2009; Dalesman & Lukowiak, 2013).

Different dietary mineral contents influenced snail growth differentiation, with adequate mineral content and mineral ratios causing snails to grow faster and be more similar in size. Previous studies have stated that high mineral contents (in the environment or in food), particularly calcium, inhibits some divalent mineral elements such as magnesium, zinc, copper, and iron from participating in metabolism and cell formation (Pu *et al.,* 2016). At the same time, more energy is required to eliminate calcium and phosphorus from the body. In contrast, a low mineral content in the diet may not offer enough minerals for the development process, limiting snail growth throughout the nursery phase (Garcia *et al.,* 2006; Lee *et al.,* 1999). Ngo Thi Thu Thao & Le Van Binh (2017) found that the growth differentiation ratio of weight and height of apple snails was higher when fed a diet containing 2.4% calcium (45.3% and 17.5%) or 9.4% calcium (45.4% and 16.5%) than in a diet containing 5.4% calcium (32.3% and 13.8%).

## Conclusions

The snails' weight and shell height (2.58g and 23.16mm) and survival rate (98.4%) were highest in the diet with 7% minerals after 56 days of rearing. Also this dietary mineral content resulted in the greatest increase in biomass and productivity of black apple snails during the rearing stage. The findings of this study can be applied in practical snail production to improve the growth performance, survival rate, and biomass of apple snails in hatcheries.

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