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Original Article

Performance of black apple snail (*Pila polita* Deshayes, 1830) juveniles reared using different lettuce and pellet feed ratios in small devices

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Abstract: The black apple snail (*Pila polita* Deshayes, 1830) is a crucial food source rich in protein and essential nutrients in many Asian countries. This omnivorous species exhibits a preference for plant-based feeds. Incorporating vegetables into its artificial diets may thus enhance the species' nutritional profile balance and reduce the farming environmental impact. This study aimed to assess the performance of *P. polita* juveniles raised under five different combinative ratios of lettuce (*Lactuca sativa* L.) and pellet feeds. The combinations were labeled Diet 1 (100% lettuce), Diet 2 (100% pellet feed containing 20% protein), Diet 3 (100% pellet feed containing 30% protein), Diet 4 (35% pellet feed containing 20% protein and 65% lettuce), and Diet 5 (35% pellet feed containing 30% protein and 65% lettuce). The study was conducted in 0.185 m³ styrofoam containers and followed a completely randomized design with three replicates. *Pila polita* juveniles (mean weight of 0.22 g and shell height of 6.40 mm) were raised at a density of 216 individuals per cubic meter (ind m^{-3}) using the tested diets over 60 days. While diets had no significant effect on survival rate, Diet 5 yielded the best rearing performance, as evidenced by significantly higher values for all growth parameters (weight and shell height), productivity, and feed conversion ratio compared to the other diets ($P \leq 0.05$). Additionally, Diet 5 exhibited the lowest coefficient of variation in weight, which was significantly lower than those of Diets 1 and 2 ($P \leq 0.05$). The findings showed that Diet 5 was the most effective under the conditions of this study.

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Introduction

Edible snails have long been an important component of human diets worldwide. They are appreciated for their food, not only for its deliciousness but also for its nutritional value. Snail meat is low in fat and calories, yet rich in essential amino acids and beneficial fatty acids (Çağıltay, 2011; Morei, 2012; Shathi et al., 2022). Additionally, it provides essential minerals, including phosphorus, calcium, sodium, and potassium (Chukwudebe, 2024). In Europe, particularly in Italy, Spain, and France, snail consumption is on the rise (Elmslie, 2005; Zagata and Sutherland, 2015). However, domestic production satisfies only 60-70% of national demand, and France imports approximately 95% of the 20,000-40,000 tonnes annually (Massari and Pastore, 2014; Tkachenko et al., 2020).

There are many benefits to raising edible snail species instead of traditional livestock. For instance, it can be done indoors or outdoors, requires little space, labor, or infrastructure, and has minimal negative environmental impacts (Hatziioannou et al., 2014; Zucaro et al., 2016; Rygało-Galewska et al., 2022). The production of 1 kg of fresh snail meat results in approximately 0.7 kg of CO_2 emissions, which is considerably less than the emissions associated with beef, poultry, or pork. Additionally, snail shells can sequester around 0.1 kg of CO_2 per kilogram produced (Massari and Pastore, 2014; Zucaro et al., 2016; de Vries and de Boer, 2010; Forte et al., 2016; Rygało-Galewska et al., 2022). Snail farming is thus regarded as a promising element of organic and sustainable

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agricultural systems (Hatziioannou et al., 2014; Zucaro et al., 2016; Rygało-Galewska et al., 2022).

The black apple snail (*P. polita*) is a large edible snail that belongs to the Ampullariidae family. It is native to tropical and subtropical Asia and is often found in wetlands, rice fields, ponds, and canals that are covered in plants (Thaewnon-ngiw et al., 2003; Bich et al., 2006; Binh and Thao, 2019b). Owing to its nutritional and medicinal value, it is widely farmed in Thailand, Vietnam, and other native countries (Thaewnon-ngiw et al., 2003; Bich et al., 2006; Binh and Thao, 2017; Ng et al., 2020). However, wild populations have declined due to overexploitation, agricultural waste pollution, and climate change, highlighting the growing importance of aquaculture for wild stock recovery and production expansion (Binh and Thao, 2019; Ng et al., 2020; Annate et al., 2023).

Protein is an important macronutrient for growing shellfish and for aquaculture in general. Inadequate protein levels in diets can cause slow growth or even weight loss. Conversely, excessively high dietary protein levels can increase feed costs and lead to greater ammonia nitrogen excretion into the environment (Wilson, 2002). Furthermore, providing feeds with balanced macronutrient profiles (proteins, lipids, and carbohydrates) and trace elements is critical for enhancing growth and survival and for supporting environmental sustainability (Guillaume et al., 1999; Webster and Lim, 2002). Combining green feeds with formulated feeds in aquaculture is viewed as a sustainable production strategy that can enhance nutrient efficiency, decrease dependence on expensive feed inputs, and ultimately reduce both feed costs and environmental impacts (de Vries and de Boer, 2010; Ayadi et al., 2012; Farradia et al., 2022; Jamil et al., 2023; Shahin et al., 2023).

Lettuce (*Lactuca sativa* L.) is a leafy vegetable that is high in fiber, iron, folate, vitamins, and other bioactive compounds. It has been cultivated globally, with annual production exceeding 27.3 million tonnes across more than 1.27 million hectares (Johnson, 2014; Caliskan et al., 2014; Kim et al., 2016; FAOSTAT, 2018; Medina-Lozano et al., 2021; Yang et al., 2022). Owing to its nutritional and functional properties, lettuce is considered a valuable non-protein supplement in aquaculture, particularly in aquaponic systems, and has been successfully used as a green feed combined with pellet diets for various snail species (Selck et al., 2006; Thao et al., 2013; Babalola, 2016; Shahawy et al., 2018; Fatema and Hossain, 2018; Ciriminna et al., 2024; Hussain and Brown, 2024).

Pila polita is an omnivorous species that primarily prefers aquatic plants (Binh and Thao, 2022). The ideal protein content in its diets depended on factors such as the protein-to-energy ratio, amino acid composition, and non-protein energy sources (Wilson, 2002). This study assessed the rearing performance of *P. polita* juveniles fed different combinations of *L. sativa* and pellet feeds with varying protein levels using small styrofoam containers. The main objective was to develop space-efficient rearing methods applicable under constrained space conditions.

Materials and Methods

Experimental materials: The 25-day-old black apple snail juveniles, with an average weight of 0.22 g and a shell height of 6.40 mm, were sourced from artificially reproduced stocks at a local hatchery. The snails were transported to the experimental facility and acclimated to the trial conditions for 1 week prior to the experiment.

This study used fresh lettuce and commercial pellet feeds containing either 20% or 30% protein, produced by the CP manufacturer under the Starfeed brand in Vietnam. Five trial diets were developed using different dry matter-based ratios of lettuce and pellet feeds, including Diet 1 (100% lettuce), Diet 2 (100% pellet feed containing 20% protein), Diet 3 (100% pellet feed containing 30% protein), Diet 4 (35% pellet feed containing 20% protein and 65% lettuce), and Diet 5 (35% pellet feed containing 30% protein and 65% lettuce). Styrofoam containers with a volume of 0.185 m^3 (dimensions: 0.5×0.37×0.4 m) were used to rear juveniles. Freshwater from a drilled well was filtered to remove sediment and then maintained in containers at a depth of 20-30 cm, depending on the snails' developmental stage. Nylon fibers were added to the containers to provide refuge for the juveniles. This study was conducted in accordance with the guidelines of the U.S. National Research Council for the care and use of experimental animals (Directive 86/609/EEC).

Experimental procedure: This study was conducted from September to December 2024 at the Experimental Hatchery of the Faculty of Agriculture and Food Technology at Tien Giang University in southern Vietnam. It aimed to assess the performance of *P. polita* juveniles fed five different combinations of lettuce and pellet feeds. The combinations were labeled Diets 1-5, as described above. The completely randomized experiment, with three replicates, was conducted in 0.185 m^3 styrofoam containers and lasted 60 days. The experimental details are as follows: The snails were randomly distributed among 15 styrofoam containers at a stocking density of 216 ind m^{-3} (40 ind $container^{-1}$). They were fed experimental diets amounting to approximately 2-3% of their body weight each day, divided into two meals. Of the total daily ration, 30–40% was given at 07:00, while 60-70% was provided at 16:00. Waste and feces were removed from the containers daily, and 50% of the water volume in each container was replaced weekly.

Data collection and calculation: Every 10 days during the rearing period, 30 snails were randomly sampled from each styrofoam container to assess growth performance. Individual weights were measured using a digital scale with 0.01 g precision, while shell height was recorded to the nearest millimeter using a graduated ruler. At the end of the experiment, data were recorded on survival rate, productivity, feed conversion ratio, and coefficient of variation. The monitored parameters were determined using the following formulas (Stickney, 2000; Hieu et al., 2022):

Mean weight (MW, g) = total weight of 30 individuals / 30

Mean shell height (MH, mm) = total shell height of 30 individuals / 30

Daily weight gain (DWG, g day^{-1}) = (final weight - initial weight) / number of rearing days

Daily shell height gain (DHG, mm day^{-1}) = final shell height – initial shell height / number of rearing days

Specific growth rate in shell height (SGRh, % day^{-1}) = ((ln(final shell height) - ln(initial shell height)) / number of rearing days) × 100

Survival rate (SR, %) = (final snail number / initial snail number) × 100

Productivity (g m^{-3}) = biomass/ culture area

Feed conversion ratio (FCR) = total dry feed fed (g) / total wet weight gain (g)

Coefficient of variation (CV, %) = standard deviation of weight or shell height / mean of weight or shell height × 100

Water quality monitoring: During the experiment, temperature and pH were measured twice daily at 7:00 and 14:00 using a pH meter. Dissolved oxygen (DO), alkalinity, ammonia (NH_3), and nitrite (NO_2^-) were tested every three days at 7:00 using the Sera test kit (made in Germany).

Analyzing data: All data were analyzed using an analysis of variance (ANOVA) at a 95% confidence level. Tukey's post hoc test was applied to determine significant differences among treatment means. The assumption of homogeneity of variances was tested using Levene's test. Percentage data were arcsine-square-root-transformed prior to analysis. All statistical analyses were performed using SPSS software, version 20.0.

Results

Water quality parameters: Water quality parameters monitored during the experiment remained stable and did not differ significantly among diets. The mean values included a temperature range of 28.08 to 28.31°C, a pH range of 7.08 to 7.10, dissolved oxygen (DO) levels from 4.05 to 4.15 mg L^{-1}, alkalinity levels from 115.22 to 121.19 mg L^{-1}, ammonia (NH_3) at 0.02 mg L^{-1}, and nitrite at 0.01 mg L^{-1} (Table 1).

Rearing performance: During the first 10 days of rearing, no significant differences in MW and MH were observed among the tested diets ($P \geq 0.05$; Tables 2 and 3). However, from the 20th day onwards, Diet 5 exhibited the best growth performance, as evidenced by significantly higher values across all monitored

Table 1. Water quality parameters during experimental periods.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Temperature (°C)	28.15±0.06	28.31±0.12	28.26±0.22	28.17±0.28	28.08±0.10
pH	7.10±0.01	7.09±0.03	7.08±0.02	7.10±0.02	7.09± 0.02
DO (mg L^{-1})	4.09±0.04	4.12±0.08	4.15±0.12	4.05±0.05	4.08±0.07
Alkalinity (mg$CaCO_3$ L^{-1})	121.19±2.56	119.48±3.91	115.22±2.96	117.78±3.91	117.78±5.91
NH_3 (mg L^{-1})	0.02±0.04	0.02±0.04	0.02±0.04	0.02±0.04	0.02±0.04
NO_2^- (mg L^{-1})	0.01±0.01	0.01±0.01	0.01±0.00	0.01±0.01	0.01±0.01

Values are presented as mean±SD.

Table 2. Initial mean weight (IMW), mean weight (MW), weight gain (WG), daily weight gain (DWG), and specific growth rate in weight (SGRw) of *Pila polita* juveniles during the 60-day rearing period under different diets.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
IMW (g ind^{-1})	$0.22±0.01^a$	$0.22±0.01^a$	$0.22±0.01^a$	$0.22±0.01^a$	$0.22±0.01^a$
MW10 (g ind^{-1})	$0.55±0.05^a$	$0.54±0.06^a$	$0.55±0.06^a$	$0.55±0.06^a$	$0.55±0.06^a$
MW20 (g ind^{-1})	$0.73±0.12^a$	$0.80±0.12^b$	$1.01±0.15^d$	$0.86±0.17^c$	$1.12±0.25^e$
MW30 (g ind^{-1})	$0.97±0.16^a$	$1.26±0.22^b$	$2.09±0.56^d$	$1.86±0.45^c$	$2.48±0.60^e$
MW40 (g ind^{-1})	$1.96±0.26^a$	$2.22±0.38^b$	$2.43±0.47^c$	$2.37±0.38^c$	$3.05±0.53^d$
MW50 (g ind^{-1})	$2.36±0.39^a$	$2.59±0.43^b$	$2.93±0.53^c$	$2.86±0.49^c$	$3.79±0.53^d$
MW60 (g ind^{-1})	$2.92±0.45^a$	$3.07±0.48^b$	$3.34±0.45^c$	$3.19±0.47^b$	$4.09±0.50^d$
WG (g ind^{-1})	$2.70±0.10^a$	$2.85±0.08^{ab}$	$3.12±0.04^c$	$2.97±0.11^{bc}$	$3.87±0.10^d$
DWG (g day^{-1})	$0.04±0.01^a$	$0.05±0.00^a$	$0.05±0.00^a$	$0.05±0.00^a$	$0.06±0.01^b$
SGRw (% day^{-1})	$4.35±0.07^a$	$4.43±0.04^{ab}$	$4.54±0.04^c$	$4.46±0.07^{bc}$	$4.90±0.06^d$

Values are presented as mean±SD. Values with different letters (a, b, c, d, e) in the same row show a significant difference (*P*≤0.05).

Table 3. Initial mean shell height (IMH), mean shell height (MH), shell height gain (HG), daily shell height gain (DHG), and specific growth rate in shell height (SGRh) of *Pila polita* juveniles during the 60-day rearing period under different diets.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
IMH (mm ind^{-1})	6.40±0.48	6.40±0.48	6.40±0.48	6.40±0.48	6.40±0.48
MH10 (mm ind^{-1})	$12.24±0.57^a$	$12.26±0.59^a$	$12.30±0.51^a$	$12.30±0.46^a$	$12.27±0.47^a$
MH20 (mm ind^{-1})	$13.96±1.06^a$	$14.43±1.12^b$	$15.66±0.71^d$	$14.81±1.46^c$	$15.89±1.02^d$
MH30 (mm ind^{-1})	$15.48±0.77^a$	$16.56±0.66^b$	$19.60±2.00^d$	$18.71±1.74^c$	$21.37±2.26^e$
MH40 (mm ind^{-1})	$19.58±1.26^a$	$20.59±1.84^b$	$21.39±1.78^c$	$21.19±1.80^c$	$23.11±1.29^d$
MH50 (mm ind^{-1})	$20.83±1.86^a$	$21.92±1.89^b$	$22.90±1.41^c$	$22.71±1.24^c$	$24.17±0.69^d$
MH60 (mm ind^{-1})	$22.84±1.05^a$	$23.18±0.99^b$	$23.71±0.67^c$	$23.56±0.77^c$	$24.50±0.71^d$
HG (mm ind^{-1})	$16.44±0.17^a$	$16.78±0.17^a$	$17.31±0.10^b$	$17.16±0.17^b$	$18.1±0.28^c$
DHG (mm day^{-1})	$0.28±0.00^a$	$0.28±0.00^a$	$0.29±0.00^b$	$0.29±0.00^b$	$0.30±0.00^c$
SGRh (% day^{-1})	$2.14±0.02^a$	$2.14±0.02^a$	$2.15±0.02^a$	$2.16±0.05^a$	$2.24±0.06^b$

Values are presented as mean±SD. Values with different letters (a, b, c, d, and e) in the same row show a significant difference (*P*≤0.05).

growth parameters (MW, WG, DWG, SGRw, MH, HG, DHG, and SGRh) compared to the other diets (*P*≤0.05, Tables 2, 3). In contrast, Diets 1 and 2 yielded the poorest performance, as evidenced by lower values for most weight and shell height parameters, except for DWG and SGRh, compared with the other diets (*P*≤0.05, Tables 2, 3). Additionally, Diet 3 outperformed Diet 4, as indicated by values in MW20, MW30, MW60, WG, SGRw, MH20, and MH30 (*P*≤0.05, Tables 2, 3).

SRs did not differ significantly among the tested diets (*P*≥0.05; Table 4), whereas productivity varied across diets. Diet 5 achieved the highest productivity, which was significantly higher than that of the other diets (*P*≤0.05, Table 4). In contrast, no significant differences in productivity were observed among the remaining diets (*P*≥*0*.05; Table 4). FCRs in Diet 5 and Diet 3 were statistically similar (*P*≥0.05, Table 4), and both were significantly lower than those of the other diets (*P*≤0.05, Table 4). Among the others, FCRs increased significantly in the following order: Diet 2 < Diet 4 < Diet 1 (*P*≤0.05, Table 4). Diet 5 produced the lowest CVh values, which were significantly different from those of Diet 1 and Diet 2 (*P*≤0.05, Fig.

Table 4. Survival rate (SR), productivity, and feed conversion ratio (FCR) of *Pila polita* juveniles after 60 days of rearing under different diets.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
SR (%)	77.5±1.4[a]	75.8±2.2[a]	75.0±2.5[a]	73.3±1.6[a]	75.0±1.4[a]
Productivity (g m^{-3})	627.3±13.19[a]	647.4±35.5[a]	696.2±17.3[a]	651.0±42.2[a]	852.0±47.9[b]
FCR	0.87±0.02[d]	0.33±0.02[b]	0.28±0.01[a]	0.38±0.02[c]	0.27±0.02[a]

Values are presented as mean±SD. Values with different letters (a, b, c, d, and e) in the same row show a significant difference (P≤0.05).

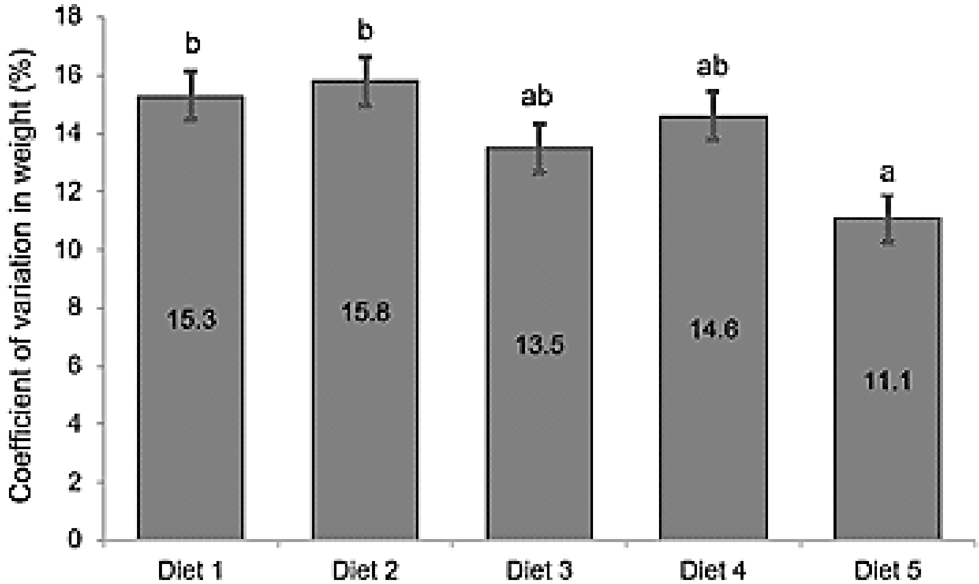

Figure 4. Coefficient of variation in weight (CVw) of *Pila polita* juveniles after 60 days of rearing under different diets. Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference (P≤0.05).

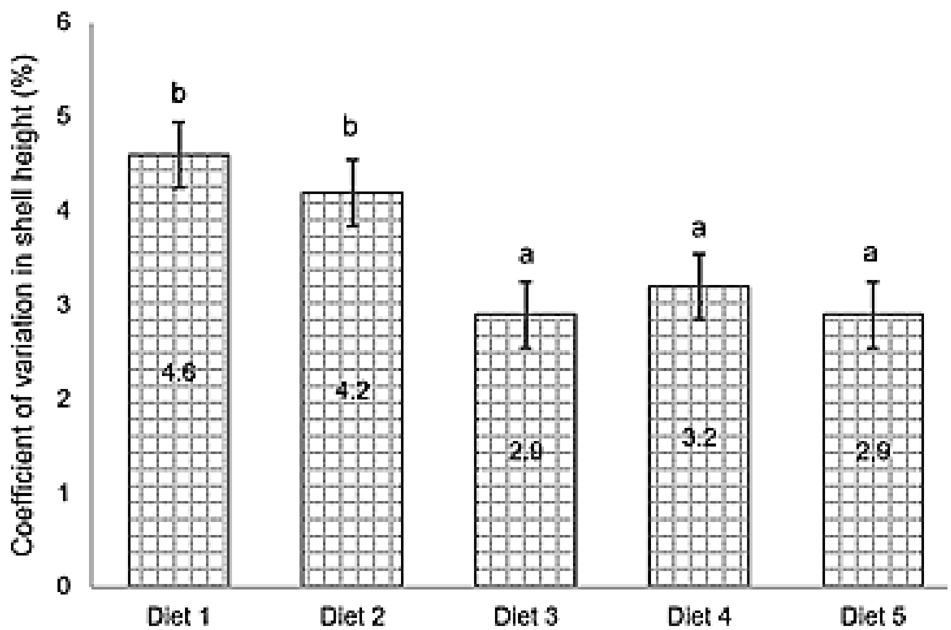

Figure 2. Coefficient of variation in shell height (CVh) of *Pila polita* juveniles after 60 days of rearing under different diets. Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference (P≤0.05).

1). However, there was no significant difference in CVh among Diet 3, Diet 4, and Diet 5 (P≥0.05, Fig. 2), and all three had significantly lower values compared to Diet 1 and Diet 2 (P≤0.05, Fig. 2).

Discussions

During the first 10 days, no significant differences in weight or shell height were observed among the different diets. This uniformity in the early stages aligns with previous findings regarding apple snails and other mollusks, where low metabolic demands, the development of digestive enzyme systems, and feeding behaviors are still maturing, buffering the effects of dietary variation (Guillaume et al., 1999; Dillon, 2000; Selck et al., 2006).

From day 20 onward, however, Diet 5 produced the best overall rearing performance, as assessed based on parameters including weight and shell height growth, productivity, FCR, CVw, and CVh. The findings suggest that the lettuce-pellet ratio in Diet 5 likely offered a nutritionally balanced profile that meets the dietary needs of *P. polita* juveniles. Pellet feeds are concentrated sources of protein, essential amino acids, and minerals that are indispensable for somatic growth and shell accretion in ampullariid snails (Wilson, 2002; Binh and Thao, 2018). At the same time, lettuce supplies dietary fiber, vitamins, and a range of bioactive compounds, including polyphenols, carotenoids, chlorophyll, and vitamins A and E, that are known to improve digestive efficiency, antioxidant defense, and overall physiological condition (Kim et al., 2016; Medina-Lozano et al., 2021; Yang et al., 2022; Shi et al., 2022). Lettuce also contains appreciable levels of calcium and iron (Katz and Weaver, 2003), which may indirectly facilitate shell mineralization, explaining the enhanced shell growth observed under balanced diet combinations in the present study. Similar advantages of mixed plant-based and formulated diets have been documented in *P. conica*, *P. globosa*, and other gastropods, where the appropriate incorporation of green feeds improved growth performance while reducing feed costs (Dillon, 2000; Thao et al., 2013; Binh and Thao, 2019a; Shathi et al., 2022).

Diet 4, which combined pellets containing 20% protein with lettuce, resulted in poorer growth performance than Diet 3. This outcome may be due to the insufficient protein content in diet 4, which failed to meet the nutritional requirements of the experimental snails. This deficiency likely impeded somatic growth and shell accretion, despite the potential benefits of dietary nutrient diversity from the inclusion of lettuce. Previous research indicated that maximum growth performance in *P. polita* was achieved with dietary protein concentrations of approximately 25% during the fry stage (Diem et al., 2018) and around 20% during the grow-out phase (Binh and Thao, 2018). The negative effects of failing to meet protein requirements were evident in Diet 1 (100% lettuce) and Diet 2 (100% pellets containing 20% protein), both of which exhibited the poorest growth performance and the highest. In Diet 1, the absence of pellet feeds likely limited protein and mineral intake, whereas in Diet 2, the exclusive use of pellets without green feed may have reduced feeding stimulation and dietary micronutrient diversity, ultimately impairing growth efficiency. Similar poor performance under nutritionally imbalanced diets has been reported in juvenile *Marisa cornuarietis* and land snails (Selck et al., 2006; Babalola, 2016), as well as in fish and invertebrates, such as tilapia and sea urchins (Jamil et al., 2023; Ciriminna et al., 2024).

Although Diet 3 (100% pellets with 30% protein) showed an FCR value comparable to that of Diet 5, most performance parameters, such as weight, DHG, and productivity, were lower than those achieved with Diet 5. The studies indicated that diets exceeding physiological requirements can diminish appetite by strongly stimulating satiety-related hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY. This hormonal regulation prolongs feelings of fullness, reduces feed intake, and may ultimately limit growth rate (Veldhorst et al., 2008; Morell and Fiszman, 2017). The lowest CVw observed in Diet 5 suggests improved size uniformity, which is advantageous for farm management and marketability. Balanced diets reduce competitive disparities among individuals and promote more synchronous growth, as several studies have found (Tkachenko et al., 2020; Supriyono et al., 2024).

Survival rates did not differ significantly among diets, indicating that all feeding regimes met the juveniles' basic maintenance requirements. This agrees with previous studies showing that survival is generally less sensitive to dietary composition than growth and feed efficiency, provided that minimum nutritional needs are met (Dat, 2010; Thao and Binh, 2022). However, Diet 5 yielded the highest productivity per unit area, underscoring its practical value for commercial snail farming.

Diet 5 yielded the highest SGRw (4.90% day^{-1}), which was similar to the value reported by Binh and Thao (2017), who achieved an SGRw of 4.82% day^{-1} when rearing *juvenile P. polita* in hapas using various ratios of lettuce and 18% protein pellet feed. Furthermore, the present study recorded higher values for both SGRh (2.14-2.24% day^{-1}) and SR (73.3-77.5%) compared to Binh and Thao (2017)'s findings, which ranged from 1.41-1.48% day^{-1} for SGRh and 64.5-71.7% for SR. The findings indicated that a well-balanced combination of lettuce and pellet feed enhances the growth performance, feed efficiency, productivity, and size uniformity of *P. polita* juveniles and showed the potential to practice cultivating black apple snails in small containers.

During the rearing periods, the monitored water quality parameters remained stable and within the appropriate ranges for *P. polita* (Thao et al., 2013; Binh and Thao, 2014, 2019b; Diem et al., 2018). They therefore did not significantly influence the main findings.

Conclusions

Diet 5 (35% pellets containing 30% protein and 65% lettuce) yielded the best rearing performance for *P. polita* juveniles under the study conditions, as reflected in the highest weight and shell height growth, productivity, FCR, CVw, and CVh. The results indicate a new trend toward cultivating black apple snails in small containers. Further research is suggested to investigate various combinations of these feed ingredients for the later life stages of this species in comparable rearing facilities.

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