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The effect of different periods of aestivation on recovery and nutritional composition of Apple snail (*Pomacea* sp.)

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Abstract The results showed a 100% recovery of all experimental groups. The survival rate of aestivation groups (2 weeks, 4 weeks, and 8 weeks) showed a significant difference, and the survival rate was 94.78±4.50, 90.82±3.11, and 88.78±2.86 percent, respectively. The average weight at the end of the experiment was 19.3±1.2, 15.1±3.7, 15.4±3. 6 and 13.8±2.9 g, respectively, which were not significantly different (P>0.05). The nutritional composition after aestivation showed a significant difference (P<0.05) in protein values among the groups. The protein value was 47.53±0.44, 51.17±0.53, 52.88±0.18, and 53.95±0.24 percent, respectively. The protein values showed an increasing trend over the aestivation period, while the fat was between 2.1-2.4 percent, and fiber was between 0.3-0.4 percent were not significantly different (P>0.05). The percentage of ash was significantly different (P<0.05), with values ranging from 17.0 to 19.4. It was shown that higher ash percentages were observed in the 4-week and 8-week groups. The percentages of NFE with values ranging from 24.1 to 33.1. Meanwhile, NFE percentages were higher in the control group and showed a decreasing trend over the study period. The percentage of calcium was significantly different (P>0.05), with values ranging from 5.6-6.7, while the percentage of phosphorus was about 0.6 percent, which was not statistically different (P>0.05). The survival rate trend of apple snails decreased with increasing aestivation period. Similarly, the body weight and NFE values decreased, but the body protein level increased.

Keywords: Apple snails, *Pomacea* sp., Aestivation, Nutritional composition

Introduction

The apple snails (*Pomacea* spp.) are large freshwater snails that feed on a variety of soft plants, such as algae, water spinach, water hyacinth, rice seedlings, and other aquatic plant debris (Burlakova *et al*., 2009). They are widely consumed, especially in the northeastern region of Thailand. They are prepared in various dishes such as Thai curry with freshwater snails and stir-fried freshwater snails with basil and chili. One of the most popular dishes featuring

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apple snails is the Thai Northeastern menu item "Som Tum" (papaya salad), which is commonly enjoyed with apple snails. In the past, apple snails were mostly harvested from natural water sources. However, natural water resources are becoming increasingly scarce due to environmental changes and water pollution caused by human activities, including the use of agricultural chemicals, habitat destruction, and the discharge of wastewater from households and industrial factories. Competition for food and shelter with other species has also led to a decrease in snail populations, which no longer meet the growing consumer demand. Many individuals have adopted simple farming methods to increase production, turning snail farming into a supplementary source of income (Mejía-Ramírez *et al*., 2020).

Currently, the trends in the manufacturing and aquaculture industries are expanding to accommodate the growing global population and diverse consumption patterns. The demand for protein remains a crucial factor for maintaining a healthy body and overall well-being, with increasing public interest in health. Generally, we can obtain protein from a variety of food sources such as meat, seafood, eggs, and legumes. One of the challenges associated with protein shortages arises from animal diseases or unsafe protein sources for consumers. Apple snails, therefore, present an alternative protein source, as they have a high protein content and low fat, with a dry weight protein content of 54.3±0.06% and fat content of 3.45±0.18%, respectively, when fed fresh mulberry leaves (Srijad *et al*., 2023). Other freshwater snail species also exhibit high protein content. Agbogidi and Okonta (2011) reported that a comparison of the nutritional value of snail meat with other meats showed that snail meat contains 20.70% protein, which is higher than that found in beef, pork, and lamb, while the fat content of snail meat is only 1.21%, lower than that of other meats. Additionally, snails are rich in minerals, easy to farm, grow rapidly, and yield high production (Qiu *et al*., 2011). Apple snails can also replace fishmeal in animal feed, which is often expensive, and are used as a supplement in the feed for various aquatic animals, including white shrimp, giant freshwater prawn, ducks, tilapia, eels, and crabs (Bombeo-Tuburan *et al*., 1995; Jintasataporn *et al*., 2004; Budiari *et al*., 2021; Chimsung and Tantikitti, 2014; Chetrakran *et al*., 2021; Rabia, 2015). Furthermore, apple snails have been used to supplement infant food in developing countries. Marsyha *et al*. (2018) reported that using golden apple snails (*P. canaliculata*) in infant food, which is rich in zinc, iron, omega-3, omega-6, and fatty acids, resulted in a reduced carbohydrate content while maintaining acceptable sensory qualities such as color, texture, and taste at concentrations of 0%, 5%, 10%, and 15%. The 5% supplement was deemed acceptable by 80-100% according to Indonesian Ministry of Health standards, offering a significant solution for alleviating protein shortages in impoverished countries.

Aestivation is the process by which snails reduce their internal bodily activities to survive. Apple snails adapt by closing their shell operculum and burrowing into the mud or dry grass to survive (Syobu *et al*., 2001). When environmental conditions become favorable, apple snails can recover and continue to live. This adaptation of gastropods, or apple snails, to survive in the wild is referred to as "temporary aestivation". In nature, aestivation occurs when there are changes in the environment, such as seasonal shifts, humidity, water, temperature, and vegetation, which can trigger aestivation in amphibians and other animals (Basavaraju and Krupanidhi, 2013). The ability of apple snails to adapt varies between species. For example, apple snails in India can survive between 25-35°C but cannot tolerate temperatures of 20°C and 40°C (Meenakshi, 1964). A study by Kalinda *et al*. (2018) on *Bulinus globosus* snails in a laboratory setting at 24-25°C showed that after 28-49 days of drought, snails began to die. The survival rate depended on the size and age of the snails, with larger snails having a higher survival rate due to their ability to burrow deeper into the soil. This is consistent with the findings of Yusa *et al*. (2006), who reported that the survival rate of *P. canaliculata* apple snails during drought conditions in a lab at 20-26°C showed that medium-sized snails (with shell lengths around 15 mm) had a survival rate of up to 60% for 22 months, while large snails (with shell lengths around 30 mm) had only a 17% survival rate. Aestivation can occur at any age, and when favorable conditions return, apple snails can recover and continue to live in the wild. The objective of this research was to test the mortality rate, recovery after aestivation, and nutritional value after different periods of aestivation, and to provide the managing production for sale, benefits for transportation, and reducing feed costs when apple snails reach full maturity.

Materials and methods

Lab animal preparation

A total of 1,000 apple snails were taken from Rungtawan's farm. At the age of 3 months, the average length of 28.12 millimeters showed an average weight of 4.3 grams per individual. Apple snails as lab animals are approved for use by the Ethics Committee on the Use of Animals KMITL, having reference number 014-2024. The apple snails were cultured in six round cement ponds (each with a volume of 138 liters) at the freshwater aquaculture laboratory, Faculty of Agricultural Technology, KMITL. The apple snails were fed with CP commercial feed at 15.5% of the protein contained with 4% by individual weight feeding once a day with mulberry leaves supplementation until the age of 5 months of the apple snails, resulting in a body size of 20-30 snails per kilogram. The water management of the system was 30% of the water changed every 4-5 days.

Experimental design

The experiment was divided into four trials with 3 replicates in a completely randomized design (CRD). The first treatment (T1) was a control treatment (as no aestivation), as the second treatment (T2) was aestivation for 2 weeks, the third treatment (T3) was aestivation for 4 weeks, and the fourth treatment (T4) was aestivation for 8 weeks. In this experiment, a total of 420 apple snails were acclimated for 5 days before being divided into 12 units with 35 apple snails per treatment. The apple snail was acclimated at room temperature (approximately 25 ^{O}C) for 5 days in a paper box (box size 10x20x10 centimeters). The paper box was placed with rice straw on the bottom base and covered on top of the apple snail with rice straw (Figure 1). Aestivation started for 2, 4, and 8 weeks at the end of acclimation in the laboratory room of the fisheries science department, agricultural faculty, KMITL. The control treatment was frozen after 5 days of acclimation for nutritional composition analysis.

Figure 1. Apple snails were aestivation in the paper box

The recovery evaluation: the 10 apple snails of each group were randomized to study the recovery after the end of the aestivation experiment for 2, 4, and 8 weeks. These were separated to the rear in the plastic pond, having 5

5-liter water volume to observe these snails' open operculum and movement for 24 hours.

The nutritional composition analysis: all residual frozen snails of each group were used to study the nutritional composition as follows: dry matter, moisture, protein, fat, fiber, ash, NFE, calcium, and phosphorus by modifying the AOAC (1995) method. Samples were taken from the refrigerator and left at room temperature until defrosted. Then, only take the whole body out from the shell to place on tissue paper to absorb excess water. The sample weight was recorded and then used to evaluate the nutritional composition.

Data analysis

All parameters at the end of the trial were calculated using the following equations:

$$\text{Average weight (g)} = \frac{\text{Total weight}}{\text{Total no. of apple snails}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final no. of apple snails}}{\text{Initial no. of apple snails}} \text{x100}$$

$$\text{Hydration loss (\%)} = \frac{\text{The initial weight} - \text{The end of the trial weight}}{\text{The initial weight}} \text{x100}$$

$$\text{Recovering rate (\%)} = \frac{\text{Initial no. of apple snails} - \text{Death no. of apple snails}}{\text{Initial no. of apple snails}} \text{x100}$$

The average body weight, nutritional value, survival rate, recovery rate, and variance of the average were analyzed by using One-way analysis of variance (ANOVA). A statistical significance level of $p< 0.05$ was used in the program Systat Version 13.

Results

The result showed that the parameters of average initial weight, the end weight after aestivation, weight loss, hydration loss, and survival rate of *Pomacea* sp in different trials as shown in Table 1. The average initial weight was 19.29±1.17 grams per snail in all the treatments. The weight at the end of the trial has not differed significantly. The weights of T2, T3, and T4 were 15.07±3.68 15.39±3.56, and 13.75±2.93, respectively shown in Figure 2. The average weight of T3 was higher than T2. The average weight loss of the aestivation group was 4.22±0.81, 3.90±0.77, and 5.54±0.26 grams per snail, respectively, and the weight of the aestivation group was not significantly different. The hydration loss percentage was increased following aestivation time increased as 6.94±7.52, 13.05±7.17, and 19.90±4.81, respectively. The survival rate of the aestivation group was significantly differed, and the survival rate was 94.78±4.50, 90.82±3.11, and 88.78±2.86 percent, respectively (Table 1). The sample of the apple snail achieving 100% recovery within 24 hours of body part movement open the operculum (the protective lid that covers its shell) and stretch out its tentacles and foot as shown in Figure 2.

Table 1. Average of initial weight, end-weight, weight loss, hydration loss, and survival rate of *Pomacea* sp. on different trials

Treatments	**T1**	**T2**	**T3**	**T4**
Initial weight (g/snail)	19.29±1.17	19.29±1.17	19.29±1.17	19.29±1.17
End-weight (g/snail)	-	15.07±3.68	15.39±3.56	13.75±2.93
Weight loss (g/snail)	-	4.22±0.81	3.90±0.77	5.54±0.26
Hydration loss (%)	-	6.94±7.52	13.05±7.17	19.90±4.81
Survival rate (%)	-	94.78±4.50	90.82±3.11	88.78±2.86

*The different letters have shown a significant difference ($P<0.05$).

Figure 2. The apple snail was recovered in the plastic container for 24 hours, and the survival rate

The nutritional composition after aestivation is shown in Table 2. A significant difference ($P<0.05$) in protein values among the groups was found. The protein value was 47.53±0.44, 51.17±0.53, 52.88±0.18, and 53.95±0.24 percent, respectively. The protein values showed an increasing trend over the aestivation period, while the fat and fiber values did not significantly differ ($P>0.05$). The fat values were 2.08±0.07, 2.40±0.12, 2.16±0.10, and 2.26±0.22, respectively. The fiber values were 0.32±0.06, 0.39±0.08, 0.35±0.10, and

0.40±0.10, respectively. The values of ash and NFE was significantly differed (P<0.05), with higher ash percentages in the 4-week and 8-week groups. Meanwhile, NFE percentages were higher in the control group and showed a decreasing trend over the study period. Calcium values were significantly differed (P<0.05) between the aestivation groups, with higher values compared to the control group. The phosphorus value was not significantly different (P>0.05) among all experimental groups.

Table 2. Nutritional composition of the apple snails at the end of aestivation

Nutritional composition (% in dry weight)	T1	T2	T3	T4
Protein	47.53±0.44^{a}	51.17±0.53^{b}	52.88±0.18^{c}	53.95±0.24^{c}
Fat	2.08±0.07	2.40±0.12	2.16±0.10	2.26±0.22
Fiber	0.32±0.06	0.39±0.08	0.35±0.10	0.40±0.10
Ash	16.98±0.64^{a}	18.65±0.11^{a}	19.36±1.16^{ab}	19.28±0.69^{ab}
NFE	33.11±0.87^{a}	27.38±0.44^{b}	25.25±1.29^{bc}	24.10±1.07^{c}
Calcium	5.60±0.04^{a}	6.34±0.31^{b}	6.74±0.09^{b}	6.65±0.08^{b}
Phosphorus	0.55±0.01	0.64±0.09	0.63±0.09	0.68±0.01

*The different letters have shown a significant difference (P<0.05).

Discussion

In the aestivation experiment, apple snails approximately lost 20% weight at the end of the trial (8 weeks), and the survival rate tended to decrease with longer aestivation. The survival rate was 89%, consistent with those who reported that the survival rate of *Eobania vermiculata* was 88%. The recovery of the apple snails from hibernation in this study showed a 100% recovery ability (Şereflişan and Duysak, 2021), similar to the study of Glasheen *et al.* (2017), who reported that during aestivation of two species of apple snails, *P. canaliculata* and *P. maculata,* at a temperature of 15.2-28.17 ^{0}C, with a total of 156 individuals, 36% of the apple snails survived by burying under the sand for 47 days. They were put in water to observe the survival rate, and 93% survived performing normal activities. The recovery did not occur immediately; it gradually opened the shell cover, extended its tentacles, and started moving. After the initial placement of the apple snail, after 5 minutes, their shells were still closed. After 15 minutes, 44% of the apple snails began to open their shell flaps; 56% were still closed. After 30 minutes, only 23% of the apple snails were closed. After 90 minutes, 98% of the apple snails had opened their shell flaps, and after 3 hours, all living apple snails were moving. This is consistent with the study by Mueck *et al*. (2018), who reported that all the experimental clams moved and ate within 24 hours after aestivation at 20–21 ^{0}C. Şereflişan and Duysak (2021) reported that the aestivation of four land snail species, *Helix pomacella*, *E. vermiculata*, *H. melanostoma*, and *H. asemnis*, revealed that these four snails do not have a shell cover. Instead, they create an epiphragm, a mucus-like substance composed of calcium carbonate, which is like a shell cover to reduce water loss during aestivation when the temperature drops below 20 ^{O}C.

During the winter season of 1986-1987 in the Baltic Islands in Sweden, the lowest temperature was recorded at -16 ^{O}C. Baur and Baur (1991) reported that land snails *C. clienta* and *Balea perversa* were aestivated both individually and in a group. The *B. perverse* was found to be a more aestivated group than *C. clienta*. The adults of both species were aestivated in more groups than juveniles, which positively affected the survival rate. The average survival rate of *C. clienta* was 33.9 and *B. perversa* was 73.3 percent. During midwinter, the average survival rate of *C. clienta* was 83.2 and *B. perversa* was 91.1 percent. The adults of both species had higher survival rates than juveniles. Moreover, Kalinda *et al*. (2018) reported that study of *Bulinus globosus* simulated in the laboratory at 24-25 ^{O}C and dehydrated after 28-49 days, the apple snail started to die, and the survival rate depended on the size and age of the apple snail, with larger oysters having higher survival rates by being able to burrow deeper. This is consistent with the study by Yusa *et al*. (2006), who reported that the survival of *P. canaliculata* apple snails to drought in the laboratory at 20 to 26 ^{O}C showed that medium-sized apple snails (shell length approximately 15 millimeters) had a survival rate as high as 60 percent for 22 months.

In the previous study, Srijad *et al*. (2023) reported the results of different feeds as commercial feed (CF), mulberry leaves (ML), and mixed feed (MF), commercial feed mixed with mulberry leaves, on the growth performances of apple snails. Apple snails at the age of four weeks were reared in the circulation system, which managed water circulation and fed feed once a day for 6 weeks. The nutritional contents of the MF group: the values of protein, fat, ash, fiber, starch and sugar, calcium, and phosphorus in apple snail meat were 33.35±0.18, 6.16±0.03, 0.03±0.02, 15.15±0.30, 45.32±0.06, 5.08±0.08, and 0.49±0.01%, respectively. These studies were applied to feed the apple snail until the age of 5 months with commercial feed with fresh mulberry leaves, which showed the highest average weight and length found in the MF was 4.3±0.4 g/snail and 28.12±1.59 millimeters from previous. The nutritional contents in the control group of this study displayed the values of protein, fat, ash, fiber, starch, sugar, calcium, and phosphorus in snail meat were 47.53±0.44, 2.08±0.07, 0.32±0.06, 16.98±0.64, 33.11±0.87, 5.60±0.04, and 0.55±0.01%, respectively. The results after the end of aestivation showed that all nutritional composition content increased in T2-T4. However, other studies that reported the result of protein levels fed to the growth performance and FCR also reported the protein content. Ramnarine (2004) reported that the crude protein level of 30% showed the highest weight gain but was not significantly different from the weight gain of the 20% protein diet. A diet containing 20% crude protein is recommended for the culture of *P. urceus*. Moreover, Şereflişan and Duysak (2021) reported that in the aestivation of four land snail species, the highest calcium content was found in *H. asemnis* with a value of 29.96±0.12 mg/g, followed by the highest iron content in *H. pomacella* with a value of 19.50±0.33 mg/g, and the highest phosphorus content in *E. vermiculata* with a value of 4.02±0.20 mg/g. In this aestivation experiment of apple snails, the highest calcium content was in the 4-week aestivation group (6.74±0.09%). In the experiment, the value was lower than our experiment because, in the study of 4 land snails, which are snails that do not have a shell cover during aestivation, they must create an epiphragm as a thin sheet like a shell cover to cover the body in the shell, so the calcium value is higher. The phosphorus value is the same. In the experiment, the highest value was found during the 8-week aestivation period, with a value of 0.68±0.01%. During aestivation from late November to March, the weight loss was 18-22%. However, these studies do not investigate the nutritional content of the whole apple snail (meat, shell, operculum, or shell cover).

Bhunia *et al*. (2016) reported that the study of immunity during aestivation and food starvation in *Pila globose* snails collected from nature after being adjusted at 25-30 ^{O}C was divided into 4 experimental groups: Group 1 control (normal activity), Group 2 aestivation (no water and food), Group 3 stimulated (water injection), and Group 4 fasted (no food for 15 days). The amount of protein in the blood in the aestivation group was the highest compared to the other experimental groups, with a value of approximately 16 mg protein per milliliter. The fact that the aestivation group had a higher blood protein value than the other groups, related to the maintenance of protein reserves both inside and outside the cells, is consistent with the apple snail experiment, which tends to increase when aestivating for a longer period. There is a reduction in various activities, drawing energy from other parts to use for survival first. In aestivation for 8 weeks, the value was equal to 53.95 ± 0.24%, which tends to increase with the longer aestivation period. This is because the percentage of water loss increases when the aestivation period is longer, resulting in a higher protein concentration. Kotsakiozi *et al*. (2012), reported that two land snails, *H. aspersa* distributed on Mount Hymettus and *H. figulina* distributed on the island of Lesvos in Greece, compared the physiological responses, found that the protein level of both land snails started to increase at the beginning of aestivation and peaked in May, then rapidly decreased during June to August. The lipids were not significantly different but tended to increase with longer aestivation.

Consistent with the study of Akande *et al.* (2010), the responses of the body's metabolic processes in the blood of two land snails, *Bulinus globosus* and *B. rohlfsi*, during aestivation and starvation simulated aestivation in the laboratory by keeping them in a glass tank for 30 days, showed a tendency to increase in both snails in lipids value. The hemolymph of *B. globosus* was 3.03 mg/dL during aestivation compared to 2.20 mg/dL in the normal period. And the fasting period was 2.70 mg/dL. The hemolymph of *B. rohlfsi* was equal to 2.30 mg/dL. During aestivation, compared to the normal period 1.60 mg/dL and fasting period 1.80 mg/dL. In terms of NFE or carbohydrates, there was a statistically significant difference, but it tended to decrease when aestivation was longer, because when the snails reduce their activities, the body still needs energy for various activities in the body. The first order of energy use is to draw out carbohydrates. After that, it will draw out fat and finally draw out energy from protein to use for survival.

In this experiment, the result after aestivation has shown that the weight and survival have decreased, hydration loss has increased, and all the nutritional composition content increases with longer aestivation. Aestivation of apple snails can keep apple snails alive longer, able to reduce the cost of raising bloodstock, and help with long-distance transportation. Moreover, longer aestivation may increase the protein content, which can be added to food or alternative protein.

In addition, comparative studies on growth and nutritional value before and after aestivation should be conducted, and nutritional values should be studied after recovery. Future studies should investigate the whole snail before and after aestivation or investigate the shell part before and after aestivation.

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