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Effects of commercial feed, mulberry leaves, and mixed feed on growth in apple snails (*Pomacea* sp.)

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Abstract This study determined the effects of different feeds on the growth performances of apple snails by using three treatments (commercial feed: CF, mulberry leaves: ML, and mixed feed: MF (commercial feed mixed with mulberry leaves). Apple snails at the age of four-five weeks were an average weight of 0.09 g/snail. These were reared in the CS which managed water circulation twice a day (one-hour morning-evening) and fed feed once an evening-day for 6 weeks.

The results showed that the average weight of apple snails (*Pomacea* sp.) in a closed system (CS) were significantly differed ($P<0.05$). The highest average weight was found in the MF with 4.3±0.4 g/snail. The CF and ML groups were displayed as 2.5±0.2, and 1.8±0.1 g/snail, respectively. The average length (mm/snail) of the MF group was significantly differed ($P<0.05$) in the ML and CF groups. These were 23.38±1.13, 24.07±1.14, and 28.12±1.59 of average length (mm/snail) in the ML, the CF, and the MF groups, respectively. The survival rate of apple snails in the CF (91.6±5.0%), ML (99.4±0.7%), and MF (94.5±6.1%) groups was significantly differed ($P<0.05$) among the ML and the CF groups. The lower FCR was found at 1.16 in the CF group which significantly differed when compared to the ML (1.34) and the MF (1.34) groups. Nutrient contents (protein, fat, fiber, ash, nitrogen-free extract (NFE), calcium, and phosphorus content) were significant differences ($P<0.05$). The highest protein content and lowest fat content of dried weight found in the ML group were 54.3±0.06% and 3.45±0.18 % respectively. These findings indicated that the easy way to culture apple snails using the commercial feed, and the alternative way to rear apple snails could be used appreciative feeds.

Keywords: Apple snails, *Pomacea* sp., Mulberry leaves, Commercial feed, Growth performance

Introduction

Apple snail (*Pomacea* sp.) is exotic species that originated in South America, they were brought into Thailand through Japan and the Philippines (Chankao, 2004). They are the largest freshwater mollusks having lengths of 35 millimeters to more than 165 millimeters (Hayes *et al*., 2012). These freshwater snails can be fast breeding, has rapid growth, and adapts well to

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harsh environmental conditions (Ngoc, 1997). In the past 20 years, apple snails are reported to be a serious pest of rice in Southeast and East Asia because they can damage young rice plants. In the 1980s, Japan was widely bred for human food (Kenji, 2003). Nowadays, this snail is acceptable and used to cook on many Thai menus such as Thai curry freshwater snail, and stir-fried freshwater snail with basil and chili. The most popular menu is Somtum or Papaya Salad with freshwater snails. Apple snail is a source of protein for animal feed such as fish, ducks, pigs, crocodiles, etc., and is also used to compost into bio-fertilizers (Ranamukhaarachchi and Wickramasinghe, 2006). Nowadays, this specie has a high price and use to cook traditional Esan menus. Then, the natural production of apple snails is decreased. Because these are affected by farmland ducks, poultries, insecticides, and agricultural chemicals in rice fields. Moreover, fermented golden apple snails were displayed as a highly potent alternative protein source to transgender tilapia as a replacement for fish meal (Chimsung and Tantikitti, 2014).

The world's higher population needs diverse consumption behaviors. Freshwater snails are therefore an alternative protein source. In addition, apple snails are also able to grow rapidly, become large and produce high yields (Qiu *et al*., 2011). They are also used in a variety of alternative protein sources, such as the use of fresh meat in cooking a variety of foods and used to replace the protein in high-priced animal feed. Broto *et al*. (2020) reported that apple snails are produced in the pond, which could be safe for human food. Moreover, these are having a high percentage of nutritional composition including protein content of up to 57.76, fat of 14.62, carbohydrate of 0.68, and ash of 15.3. In addition, the apple snail culture has an advantage over aquatic species, which can eat a wide variety of foods such as plant leaves, aquatic plants, vegetable scraps, and commercial feed. According to apple snail production in the closed-rearing system is free from disease or parasites and safe for humans. Besides being used as food for consumption or used in the field of cultivation.

The Covid situation in the past 2 years, apple snails originated from the natural reservoir (Tambon Pho-Ngam, Prachantakham District, Prachinburi Province) which transported to Ban-saun Rungtawan (Nong-Chok district, Bangkok, Thailand). These are reared in a closed water system and fed with commercial feed, aquatic plants, and many plants culturing at Ban-saun Rungtawan. These snails as the safe material to support cooks in the house. Moreover, the benefit of gastropods in Thailand has been reported that the nautilus *Pila polita* is used as a traditional medicine to treat skin diseases (Thaewnon-ngiw *et al*., 2003). In addition, snail meat was low in fat and cholesterol, so it makes good medicine to help cure vascular diseases such as heart attack, cardiac arrest, and stroke (Babalola and Akinsoyinu, 2009). The mode of nutritional compositions in different animals is a need to know of human consumption, which could be

traced to animal origin/ farm and animal feeds. There is a gap in current knowledge of the nutritional composition of apple snails feeding with mulberry leaves.

Mulberry leaves contain a percentage of dry matter of high protein levels from 15.31 to 30.91. It has many mineral contents that can be used for many purposes. Mulberry leaves are relished by sheep and goats (Srivastava *et al*., 2006; Yu *et al*., 2018). Ouyang *et al*. (2019) reported that mulberry powder could be used to supplement ruminant feed enhancing digestion and absorption. Some 7.5% dried mulberry leaves fortified in masala biscuits showed an incredible increase in micro-nutrient content (Ramya and Chandrashekar, 2020).

The mulberry tree is a popular plant culturing inside many houses in all parts of Thailand. It grows extensively for leaves and fruit is delicious having high in vitamin C that can be occurred all year round. Other parts of the mulberry can also be used for a variety of purposes such as tea, cooks, and animal feed. Therefore, the study was to investigate the effect of mulberry leaves on growth performances, survival rate, and nutritional values in apple snails to compare with the commercial feed, and mixed feed.

Materials and methods

Lab animal preparation

Apple snails at the age of four-five weeks had an average length of 6.72±1.40 mm/snail and an average initial weight of 0.09±0.04 g/snail. Twenty experimental units of apple snails were tested at Ban-saun Rungtawan using the CS (Figure 1). Two hundred and fifty snails of each experimental unit were reared in a cement pond (33L), which opens water flowing twice a day (morning-evening at a rate of 3L/min). Water qualities in the water pond with the pump (Figure 1; A) such as water temperature, electrical conductivity (EC), pH, and dissolved oxygen (DO) were automatically recorded using the Aqualitic system.

Experimental design

The experiment was divided into three trials (Figure 2), the first treatment was given only commercial feed (CF) with 12 replicates, the second treatment was given only fresh mulberry leaves (ML) with 4 replicates and the last treatment is mixed between commercial feed and fresh mulberry leaves (MF) with 4 replicates. The experiment was conducted from June to October 2021. Growth performances were monitored for forty-two days.

At the beginning trial, the apple snails were fed twice a day (morning and evening) at 8% of the total weight in each experimental unit

and every week was adjusted the feed weight. The CF group used a commercial feed (CP 9932; 15.5% protein). In the ML group, the weight of ML was recorded and then fed to apple snails in each pond. The mulberry leaves were changed every three days, and these recorded the residue weight. The MF, the apple snails were fed twice a day (morning and evening) at 4% of total weight, and added the mulberry leaves to feed these snails. The mulberry leaves were changed every three days, and these recorded the residue weight at the same as the ML group. Sediments in the cement ponds were removed every week.

The nutritional values, Ca, and P of apple snail meat at the end of the trial were analyzed using a proximate analysis method as referenced by AOAC (1995). Apple snails of each group were pooled together and then 1 kg of each group was taken into the refrigerator for 24 hours. These samples removed the shell and only used apple snail meat. The weight of each group was recorded. The dry matter at 105 ℃ (Binder model: FD 56) was determined. The crude protein was determined by the Kjeldahl method (Gerhardt model: KT-L 20s and model: VAPODEST 400). The Soxtherm method (Gerhardt model: SOX 416) was used to analyze the crude fat of samples using an extractant (Petroleum Ether). Samples determined the ash content by using the gravity method. These samples were incinerated at a temperature of 600 ℃ to constant weight. The content determination of nitrogen-free extractives (NFE) was calculated using the following formula: NFE = dry matter (g/kg) − [MS(moisture) content (g/kg) + fiber content (g/kg) + fat content (g/kg) + crude protein(g/kg) + ash content (g/kg)]. The contents of Ca by precipitation and P were determined by using the atomic absorption spectrophotometer wavelength 400 nm (Shimadzu model: UV-1800) in acetylene-air flame with deuterium background correction. These parameters were analyzed at the Food Materials and Feed Quality Analysis, Industrial University Collaborative Research Center (IUCRC), Agricultural Technology, KMITL.

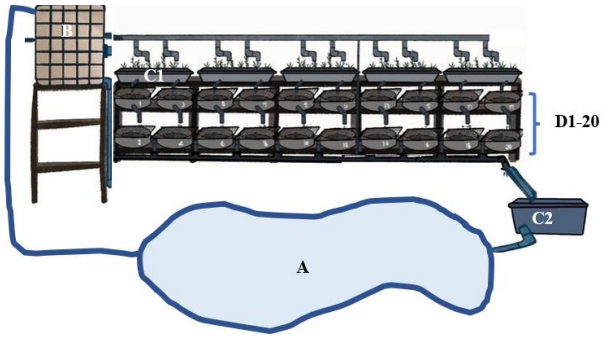

Figure 1. Schematic of closed circulation system; (A) water pond with water pump, (B) the water tank to distribute water, (C1) water treatment before flowing to the cement ponds, (D1-20) the cement ponds, and (C2) water treatment before flowing to the water pond

Figure 2. Apple snails fed different diets (A: CF, B: ML, and C: MF) were reared in the cement ponds, where CF= the commercial feed, ML=mulberry leaves, and MF=mixed feed

Data analysis

Mean values and standard deviation of weight and length were analyzed. Feed conversion and survival rates at the end of the trial were calculated using the following equations:

$$\text{Feed conversion rate (FCR)} = \frac{\text{Total feed (g)}}{\text{Weight gain (g)}}$$

$$\text{Survival rate (SR) \%} = \frac{\text{No. of animals survived (snail)}}{\text{No. of animals leased (snail)}} \times 100$$

One-way ANOVA with unequal sample sizes using the SYSTAT version 13 for Windows was used to analyze data, which accepted significantly different at $p<0.05$.

Results

The average weight, average length, FCR, and SR of apple snails fed different diets are displayed in Table 1. Significant differences ($P<0.05$) for these growth variables were found. The highest weight was found in the MF (4.3 ± 0.4) which was differently significant in the CF (2.5 ± 0.2) and ML (1.8 ± 0.2). There was a significant difference between the CF and the ML. The highest length was found in the MF (28.12 ± 1.59) which differed significantly in the CF (24.07 ± 1.14) and ML (23.38 ± 1.13). There was no significant difference between the CF and the ML. The highest SR was found in the ML (28.12 ± 1.59) which differed significantly in the CF. There was no significant difference between the ML and the MF. At the same of the SR between the MF and the CF was found no different significance. The results also indicated that the FCR of the CF was significantly different from the ML and the MF. However, no difference between the FCR of the

ML and the MF. The lower FCR was displayed in the CF group (1.16±0.03).

Table 1. Mean growth performances at age of 42 days of apple snails fed different diets

Growth variables	**CF**	**ML**	**MF**
Average weight (g/snail)	2.5 ± 0.2^{b}	1.8 ± 0.2^{a}	4.3 ± 0.4^{c}
Average Length (mm/snail)	24.07 ± 1.14^{a}	23.38 ± 1.13^{a}	28.12 ± 1.59^{c}
Survival rate (%)	91.6 ± 5.00^{b}	99.4 ± 0.77^{a}	94.5 ± 6.06^{ab}
Feed conversion ratio (FCR)	1.16 ± 0.03^{b}	1.34 ± 0.10^{a}	1.34 ± 0.04^{a}

*The difference letters have shown a significant difference($P<0.05$), where CF= the commercial feed, ML=mulberry leaves, and MF=mixed feed.

The contents in apple snail meat as protein, fat, ash, fiber, and nitrogen-free extract (NFE), Ca, and P are shown in Figure 3. These variables were significant differences ($P<0.05$). The highest percentage of protein content was found in the ML which differed significantly in the CF and the MF groups. However, it remarked to note that the fat percentage was found to be lower in the ML (3.4). The highest fat was displayed in the CF (7.6) and followed by the MF (6.2). The fiber was the highest percentage in the ML group (1.3) which differed significantly in the CF and the MF groups. There was no significant difference between the CF and the MF. The ash percentage in apple snail meat in the CF, ML, and MF was displayed at 16.7, 16.3, and 15.1, respectively. The CF and ML were shown no significant difference, while these groups displayed a significantly different from the MF. The NFE was shown no significant difference between the CF and MF however, these groups were significant difference from the ML group. The result of the Ca percentage in apple snail meat was displayed as the highest in the CF group (5.6), which differed from the ML (4.9) and MF groups (5.1). However, the phosphorus content was the highest in the ML group (0.71), which was a significant difference from the other groups. The P content in the CF and MF was no significant difference.

The mean water qualities during the experiment were found 27.8±0.8(℃) of water temperature, 180.1±57(µs/cm) of EC, pH 7.1±0.3, and DO 2.4±0.5(mg/L) of dissolved oxygen.

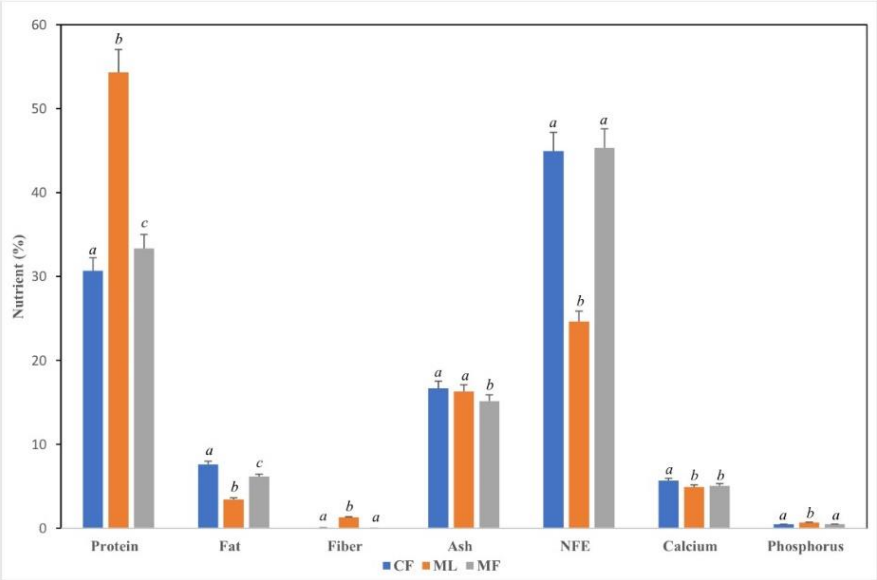

Figure 3. Comparison of proximate variables in apple snail meat fed different diets (% in dry weight), where CF=the commercial feed, ML=mulberry leaves, and MF=mixed feed

Discussion

The high growth of apple snails is the goal of this study. Our results showed that apple snails were displayed in the MF, which found an average weight of more than 4.0 g/snail. Generally, commercial feed is easy to rear apple snails and it has a positive effect to support the growth performances. This showed a lower FCR of apple snails rearing with a commercial feed. The average length was found at the same average weight, which occurred the highest in the MF. As Qiu *et al*. (2011) reported that *P. canaliculala* consumed fresh leaves of *Murdannia nudiflora* displaying the highest survival rate. These may be continued to eat leaves. Our results indicated that the mulberry leaves can be used to mix with commercial feed supporting the average weight. It is a good idea to attention farmers to culture the mulberry tree as an additive feed of apple snails as an alternative protein source or a safe food in many houses and reducing the cost. Research on apple culture has been reported by Garr *et al*. (2011), who used a commercial catfish diet mixing with *Ulva* macroalgae rearing the Florida apple snail. This research used the recirculating aquaculture system. In the same of this study, we use the closed system for snail rearing, which can control the water flow on-off. However, Aroonsrimorakot *et al*. (2017) reported that *Cyclophorus fulguratus* fed different diets as lettuce mixed with chicken food, lettuce with catfish food, and lettuce with grained gravel. It found that different diets were not differently significant in the growth

performances. In general, *Calothrix* sp. (Cyanobacteria) was superior to pellet carp food, as the former demonstrated that it can be efficiently consumed as food for *P. patula cuternacensis* in laboratory conditions (Ruiz-Ram ŕez *et al*., 2005). Ramnarine (2004) reported that the level of 30% protein in feed gave the best growth in juvenile *P. ureceus* however, the percentage of 20% protein was greater the feed conversion efficiency (1.77) than the 30% protein diet (1.90). Our study used a commercial feed (tilapia feed having 15.5% protein) to rear apple snails, which found a lower FCR (1.1).

Nutritional contents such as protein, carbohydrates, vitamins, microelements, and fiber are the main point to indicate growth performances in aquatic animals. Many plants are used as food in the diet of apple snails, such as mulberry leaves, papaya leaves, gourd leaves, etc. Their nutritional value varies depending on the food being fed. Apple snails are nutritious with high protein and many minerals, such as Zinc iron also contains omega-3 and omega-6, which are good for the immune system and brain development in children. Marsyha *et al*. (2018) reported that found protein content of golden apple snail flour (100g) was 12.73g, which is lower than apple snail at 54.31% when fed with mulberry (ML). Moreover, Each species of apple snail has a different protein content as in two species of freshwater snails (*Lanites varicus* and *Nucella lapillus*) for the nutritional assessment using their muscle tissue. The mean crude protein range was 70.00 ± 0.03. (Eneji *et al*., 2008). Agbogidi and Okonta, (2011) reported the protein content of apple snails is slightly higher than pork, lamb, and beef, but less in fat in snail meat 1.21% while we have done a slightly higher 3.45% in ML. In the fiber study by Jatto *et al*., (2010), the fiber has highest in *Achatina achatina* (4.06%), which feeding with *Centrosema molle* has a difference in the fiber of ML (1.32%). Whereas, the highest NFE was Broiler starter mash (control) at 47.8%, which is similar to the NFE values of the MF (45.32%) (Kalio and Etela, 2011). Ash in Marine snails (*Hexaplex trunculus*) collected from the fish market was 15.14 ± 0.77%, similar to MF feeding at 15.15±0.30% (Zarai *et al*., 2011). In this study, we found that the calcium content of the apple snails of all groups was higher than the phosphorus content, which indicated very low. Essien *et al*. (2016) reported that the calcium content in the giant land snail (*Archachatina marginata*) collected from nature was lower calcium content than in the culture system (0.049 %), and the difference in CF (5.68%). Phosphorus content was the highest with fed Centrosema mole 0.277% (Kalio and Etela, 2011), whereas that difference in CF was 0.71%.

In the experiment, it can be found that apple snails with the highest protein content were fed exclusively on mulberry leaves. The body weight and length are less than the two treatments of experiments. It would depend on the people who choose and want this protein from mollusks, then mulberry is recommended. If the needed quantity for trading can be

sustained as a ready meal and supplemented with mulberry leaves or supplemented with other plants.

Apple snails are easy to rear at subsistent and commercial levels with high returns on low input when we use mulberry leaves. These serve as valuable sources of alternative protein for human consumption.

Apple snails fed a commercial feed mixed mulberry leaves had a higher weight than the other groups. A lower feed conversion ratio was found in apple snails fed a commercial feed. The mulberry leaves are related to the highest protein and lower fat in apple snails as high-quality food sources for human consumption.

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