Study on Gonadosomatic Index of Thai Native Apple Snail (*Pila ampullacea* Linneaus, 1758) in the Rice Fields of Srimuang-mai District, Ubon Ratchathani and Effect of Diet on the Growth of Juveniles

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ABSTRACT

With climate change and environmental destruction, Thai native apple snail (*Pila ampullacea* Linneaus, 1758) population decreased drastically in wetlands and rice fields. Information on the distribution of Thai apple snail and its feeding types is deficient. For this study, Thai native snails were collected annually from the rice fields and prepared for spawning in tanks. In July, wild females could spawn and egg clutches were moved and nursed with fresh lettuce (*Lactuca sativa*) for 1 month. Feeding studies consisted of four experiments with four treatments, namely, the control (fresh lettuce), 15 % (15P), 25 % (25P), and 40 % (40P) protein level in artificial pellet feed. Different food types were given to 1 month old juveniles for 120 days. The findings revealed that total weight, body weight, shell length, shell width and gonadosomatic index (GSI) of wild snails differed significantly over time ($P < 0.05$). Total weight, shell length, and shell width of spawning female ranged from 10.4-20.2 g, 30.26-40.79 mm and 26.25-38.92 mm, respectively. The percentage of hatching success (70.09-96.05 %) had a negative relationship with total weight, shell length, and shell width. In the feeding experiments, total weight, shell length, and shell width of juveniles fed on 15 % protein level in diet were significantly different ($P < 0.05$). No mortality was found in all the experiments. It was concluded that artificial pellet feed at 15% protein level can potentially induce growth of snails in captivity.

Keywords: Gonadosomatic Index, Apple snail

INTRODUCTION

The Thai native apple snail (*Pila ampullacea* Linneaus, 1758) is a native mollusc commonly found throughout the Southeast Asian region, especially Cambodia, Lao PDR, Malaysia, Thailand and Vietnam (Sri-aroon and Richter, 2012). The shell length and width ranged from 90-100 and 85-90 mm, respectively. The smooth surface of the shell is bright green or orange-brown. The snails are found in rice fields and ponds (Komalamisra *et al*., 2009). This snail belongs to Family Ampullariidae which is similar to the golden apple snail (*Pomacea canaliculata*), an exotic species also found in Thailand. In the natural waters, the growth of golden apple snail is faster than that ofThai native apple snail. These invaders may have an effect on the Thai native apple snail population (Sumpan and Chaichana, 2013).

P. ampullacea prefers several species of aquatic plants, e.g. floating mass (*Salvinia cucullata*), swamp morning glory (*Ipomoea aquatica*) and lettuce (*Lactuca sativa*) (Punha, 1985). During starvation period, *P. ampullacea* can change feeding preference from aquatic plants to decaying animals (Komalamisra *et al*., 2009). Several studies reported that lettuce provided a suitable food source for growth of freshwater mollusc, e.g. ramshorn snail (*Marisa cornuarietis*) (Selck *et al*., 2006), *Pomacea canaliculata* and *Pomacea insularum* (Boland *et al*., 2008), and *Pomacea maculata* (Horn *et al*., 2008).

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The growth rate of snails may be affected by energy content, nutrient composition, food digestibility and absorption of food, therefore appropriate diet and nutrient are key parameters (Foster *et al*., 1999; Selck *et al*., 2006). The aims of this study were to investigate breeding performance (GSI, total weight, body weight, shell length) of the wild broodstock in captivity, and test the growth performance of snails fed on vegetables compared with artificial feed containing different protein concentrations.

MATERIALS AND METHODS

Experimental animals

Wild samples of Thai native apple snail were collected from a 1 rai rice field in Srimuang-mai district, Ubon Ratchathani province, Thailand, from January to December 2015 (Fig. 1). The rectangular grid (1 m x 1 m) was placed randomly on the soil for 5 replications. The snails were stocked into plastic containers (1 m x 1 m x 1 m) and transferred to the laboratory. The snail taxonomy was identified by following Brandt (1974) and Kaewjam (1987). Approximately 300 snails were measured for total weight and shell length (from the tip of the shell to the longest diagonal point on the lip at the extreme aperture) and shell width with a caliper to the nearest 0.1 mm (Fig. 2A). The 115 females and 99 males were checked monthly for gonadosomatic index. The remaining snail samples were acclimatized and maintained in the plastic tank (1x1x1 m) at 20 snails/m^3 under ambient light and temperature conditions (approximately 28±1^{o}C). Fresh lettuce was fed until the experiment ended.

Breeding studies

The sexes of snails were identified based on the method of Garr *et al*. (2012). Only males have the penis sheath in the mantle cavity (Fig. 2B). The body weight and shell length of broodstock were recorded. In July, sixteen pairs of mature snails were marked with the color on the shell and placed into a 20 L tank at the ratio of 1:1. The bottom of the tank was covered by sterile soil and divided into 2 parts: dry and wet zones. After spawning, the breeding performances, egg clutch weight, egg clutch diameter, and number of total egg per clutch were measured. The egg clutches were then moved into small plastic containers (5x5x5 cm). The total number of juveniles and the percentage of hatching success were recorded.

Feeding trials

A batch of Thai native apple snail fingerlings of the wild broodstock was cultured in the aquarium and fed with fresh lettuce for 1 month. Two hundred and forty individuals (average total weight = 0.143± 0.027 g; shell length = 7.909±0.568 mm for overall specimens) were randomly placed into each aquarium (24x28x30 cm) at the rate of 20 snails/treatment and 10 snails/L. The snail juveniles were fed with the different diets: Diet1 = fresh lettuce (*L. sativa*) (C), Diet2 = pellet feed with 15% protein (15P), Diet3 = pellet feed with 25 % protein (25P) and Diet4 = pellet feed with 40 % protein (40P). The body weights were measured monthly. The moisture content in fresh lettuce (93.5 %) was higher than in the pellet feed (7.1-7.2 %). A 120 - day experiment was carried out in triplicate. The rate of feed fed to the experimental snails was 5 % of total weight twice daily and changed monthly by increasing total weight. The water supply was aerated and dechlorinated in a 3,000 L container. Two-thirds of the water in the aquarium was exchanged daily to remove debris and maintain water quality. The experiments were conducted at ambient temperature (28±1^{o}C and natural light).

Proximate analysis of diets was conducted according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1984). Growth performance parameters (feed conversion ratio, specific growth rate, shell length increase, survival rate and average daily weight gain) were recorded monthly for 4 months.

Statistical analysis

Total weight, shell length, shell width, gonadosomatic index of snails, egg clutch weight, egg clutch diameter, number of total egg per clutch, total number of juvenile and percentage of hatching success were presented as means ± standard deviation. The relationship of female and spawning and hatching steps was analyzed with Principle Component

Figure 1. The rice field area of *P. ampullacea* sampling in Srimuang-mai district, Ubon Ratchathani, Thailand.

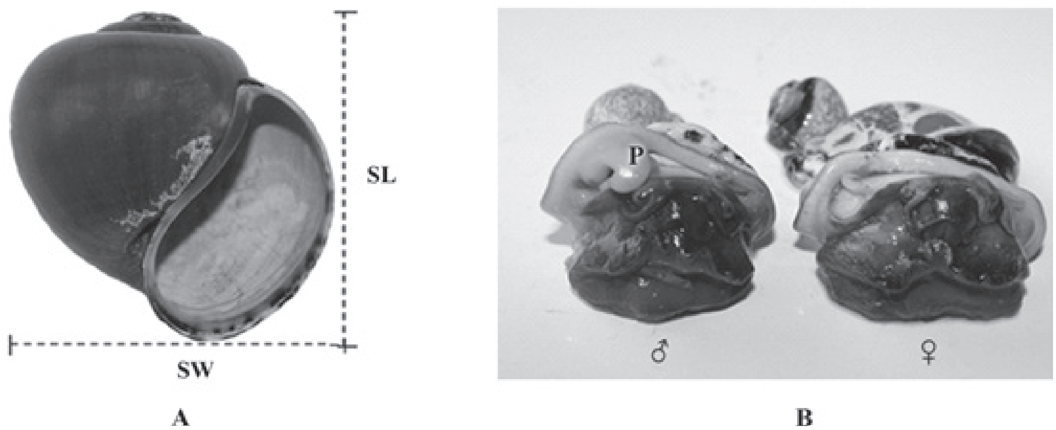

Figure 2. Shell (A) and sexual (B) characteristics of Thai native apple snail (*P. ampullacea*) (SL = shell length; SW = shell width; P = penis sheath)

Analysis (PCA). Differences in growth performance between different groups of samples were analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (R-package). Significance results were considered if $P < 0.05$.

RESULTS

Experimental animals

Collection of *P. ampullacea* samples from rice fields were conducted for 12 months, but the snails were found only for 8 months, from April to November. No snails were in the dry season (December to March). Total weight (TW), body weight (BW), shell length, shell width, and gonadosomatic index (GSI) of *P. ampullacea* (both male and female) were measured monthly (Table 1). The average TW and average BW of male snails were significantly different in August (15.35±6.92 g) and September (6.66±3.17 g), respectively ($P<0.05$), while the shell length and width of male snails in July (35.56±4.09 and 32.99± 3.77 mm) and August (36.77±5.42 and 33.97±4.79 mm) were significantly greater than in other months ($P<0.05$). The male GSI was significantly different in July (12.43±11.83 %) ($P<0.05$). The average TW of female snails was found to be significant in July (8.35±3.36 g), while the significant average BW was found in September (4.62±1.89 g) and November (5.92±1.39 g) ($P<0.05$). The average shell length and width of female snails were significant in November (35.67±1.89 and 34.15±2.43 mm), while the average GSI was found significant in May (8.09±1.65 %), June (8.08±2.79 %) and September (7.64±2.5 %) ($P<0.05$).

Breeding performance of wild *P. ampullacea*

Total weight, shell length, and shell width of spawning females ranged from 10.4 to 20.2 g (mean=14.68 g), 30.26 to 40.79 mm (mean=37.38 mm), and 26.25 to 38.92 mm (mean=33.91 mm), respectively. *P. ampullacea* spawned the egg clutch on the soil containing white hard shell. From 16 clutches, egg clutch weight, egg clutch diameter, and number of eggs per clutch ranged from 2.38 to 11.8 g (mean=5.61 g), 3.48 to 4.11 cm (mean=3.78 cm), and 107 to 301 eggs (mean=192.13 eggs), respectively. The period of hatching varied considerably, from 10 to 26 days (mean=21 days). The percentage of hatching success (70.09-96.05 %; mean=86.57 %) showed a negative relationship with total weight, shell length, and shell width. PCA revealed a negative relationship between size of female (TW, shell length, shell width) and breeding performance (percentage of hatching success and hatching period) during spawning and hatching stages (Fig. 3).

Growth performance of *P. ampullacea* fed with different diets

The proximate composition of 4 diets is presented in Table 2. Lettuce had the lowest protein content (1.3 %), while artificial feed had protein contents of 14.8, 24.7 and 39.5 % in 15P, 25P and 40P, respectively. The different energy in 100 g feed showed differently, 42.4, 284.5, 371.2 and 425.1 kcal in lettuce, 15P, 25P and 40P, respectively.

Total weight, shell length, and shell width of *P. ampullacea* juveniles fed different diets were measured monthly (Fig. 4). The diets had a marked effect on the growth of *P. ampullacea*. The average total weight, shell length, and shell width of *P. ampullacea* fed on 15 % protein level in diet were significantly different ($P < 0.05$). At 120 days, significant differences in SGR (2.68±0.05 %), SLI (0.12±0.04 mm/day) and ADG (0.03±0.00 g/day) in Table 3, were found in snails fed on 15 % protein level in diet ($P < 0.05$). FCR was lower for those fed on 15 % protein level (2.08±0.05) compared with the control diet (2.56±0.18) and 40% protein level (2.50±0.17) ($P<0.05$), but not significant at 25 % protein level (2.28±0.29) ($P>0.05$). Survival rates of snails fed with 15P (98.33±2.89 %) and control (98.33±2.89 %) were better than those fed with 40 % (96.67±2.89 %) and 25 % protein levels (91.67± 2.89 %) ($P < 0.05$).

Table 1. Total weight, body weight, shell length, shell width and gonadosomatic index of Thai native apple snail (*P. ampullacea*) in the rice field. Results are given as mean±SD. Columns within each parameter with a different letter are significantly different (ANOVA, Tukey, $P<0.05$).

Month	Female						Male					
	n	TW (g)	BW (g)	SL (mm)	SW (mm)	GSI (%)	*n*	TW (g)	BW (g)	SL (mm)	SW (mm)	GSI (%)
April	14	6.63 ± 3.14^{c}	3.04 ± 1.22^{c}	30.16 ± 3.81^{b}	28.38 ± 3.51^{b}	6.23 ± 4.54^{b}	8	3.83 ± 1.18^{b}	2.25 ± 0.84^{b}	25.44 ± 2.59^{d}	23.78 ± 2.43^{d}	3.16 ± 1.01^{c}
May	18	9.12 ± 3.08^{bc}	3.89 ± 1.45^{bc}	33.83 ± 8.51^{ab}	30.99 ± 8.18^{ab}	7.33 ± 7.23^{b}	11	6.58 ± 2.59^{ab}	2.73 ± 1.14^{b}	28.29 ± 2.80^{cd}	26.01 ± 3.44^{cd}	8.09 ± 1.65^{a}
June	12	9.79 ± 4.44^{bc}	4.09 ± 2.13^{abc}	31.38 ± 5.94^{b}	28.62 ± 5.06^{b}	9.90 ± 9.04^{ab}	13	10.09 ± 4.05^{ab}	2.98 ± 0.68^{b}	30.70 ± 2.67^{bc}	28.16 ± 2.45^{bc}	8.08 ± 2.79^{a}
July	19	12.98 ± 4.39^{ab}	5.17 ± 1.95^{abc}	35.56 ± 4.09^{a}	32.99 ± 3.77^{a}	12.43 ± 11.83^{a}	21	8.35 ± 3.36^{a}	3.12 ± 1.27^{b}	30.67 ± 3.29^{bc}	28.46 ± 2.97^{bc}	6.74 ± 3.74^{ab}
August	21	15.35 ± 6.92^{a}	5.68 ± 2.93^{abc}	36.77 ± 5.42^{a}	33.97 ± 4.79^{a}	8.54 ± 7.49^{ab}	18	9.25 ± 3.42^{ab}	3.82 ± 1.98^{ab}	30.60 ± 3.71^{b}	28.72 ± 3.49^{b}	5.39 ± 2.20^{bc}
September	17	13.30 ± 5.57^{ab}	6.66 ± 3.17^{a}	34.75 ± 4.83^{ab}	32.33 ± 3.82^{ab}	8.07 ± 4.26^{ab}	19	9.84 ± 3.93^{ab}	4.62 ± 1.89^{a}	31.53 ± 4.12^{b}	29.57 ± 4.20^{b}	7.64 ± 2.50^{a}
October	5	14.38 ± 7.15^{ab}	6.36 ± 3.71^{abc}	34.89 ± 7.21^{ab}	32.76 ± 6.17^{ab}	6.31 ± 5.01^{b}	5	9.67 ± 3.43^{ab}	3.74 ± 0.96^{ab}	32.71 ± 3.86^{ab}	30.72 ± 4.14^{ab}	6.44 ± 3.66^{ab}
November	9	16.74 ± 8.04^{a}	6.55 ± 3.22^{ab}	36.59 ± 4.19^{a}	34.09 ± 4.50^{a}	4.43 ± 5.53^{b}	4	14.37 ± 2.17^{b}	5.92 ± 1.39^{a}	35.67 ± 1.89^{a}	34.15 ± 2.43^{a}	4.92 ± 0.58^{bc}

n = number of snail; TW = shell and body weight; BW = body weight; SL = shell length; SW = shell width; GSI = gonadosomatic index

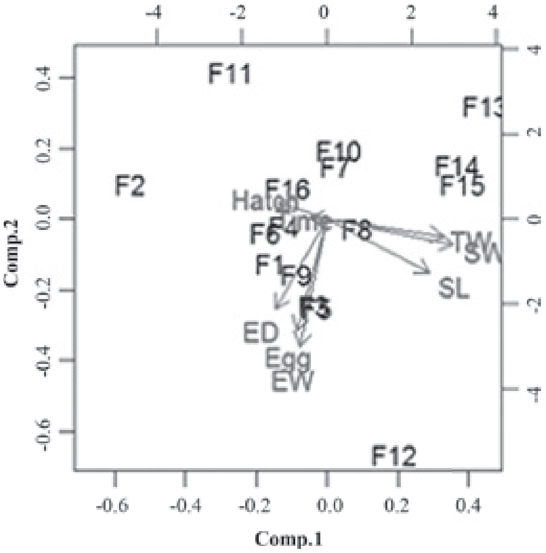

Figure 3. Percentage of hatching success and relevant parameters in Thai native apple snail (*P. ampullacea*) female. (TW = shell and body weight; SL = shell length; SW = shell width; Egg = number of egg per clutch; EW = egg weight per clutch; ED = diameter of clutch; Time = hatching period; Hatch = percentage of hatching success; F1-F16 = number of spawned female)

Table 2. Proximate composition of the experimental feeds for Thai native apple snail (*P. ampullacea*)

Proximate composition	Diet			
	C	15P	25P	40P
Protein (%)	1.3	14.8	24.7	39.5
Lipid (%)	0.2	3.6	3.6	3.8
Fiber (%)	0.5	8.2	8.1	8.1
Moisture (%)	93.5	7.1	7.2	7.2
Energy (kcal/100 g feed)	42.4	284.5	371.2	425.1

C = fresh lettuce (*Lactusa sativa*); 15P = pellet feed with 15% protein; 25P = pellet feed with 25% protein; 40P = pellet feed with 40% protein

Table 3. Growth performances of Thai native apple snail (*P. ampullacea*) fed on the different diet at fourth month. Results are given as a mean±SD. Columns within each parameter with a different letter are significantly different (ANOVA, Tukey, $p<0.05$).

Parameters	Diet			
	C	15P	25P	40P
FCR	2.56±0.18^{b}	2.08±0.05^{a}	2.28±0.29^{ab}	2.50±0.17^{b}
SGR (%)	2.03±0.08^{d}	2.68±0.05^{a}	2.44±0.02^{b}	2.29±0.08^{c}
SLI (mm/day)	0.08±0.01^{c}	0.12±0.004^{a}	0.104±0.003^{b}	0.09±0.01^{b}
SR (%)	98.33±2.89^{a}	98.33±2.89^{a}	91.67±2.89^{b}	96.67±2.89^{ab}
ADG (g/day)	0.01±0.00^{d}	0.03±0.00^{a}	0.02±0.00^{b}	0.02±0.00^{c}

FCR = feed conversion ratio; SGR = specific growth rate; SLI = shell length increase; survival rate; ADG = average daily weight gain

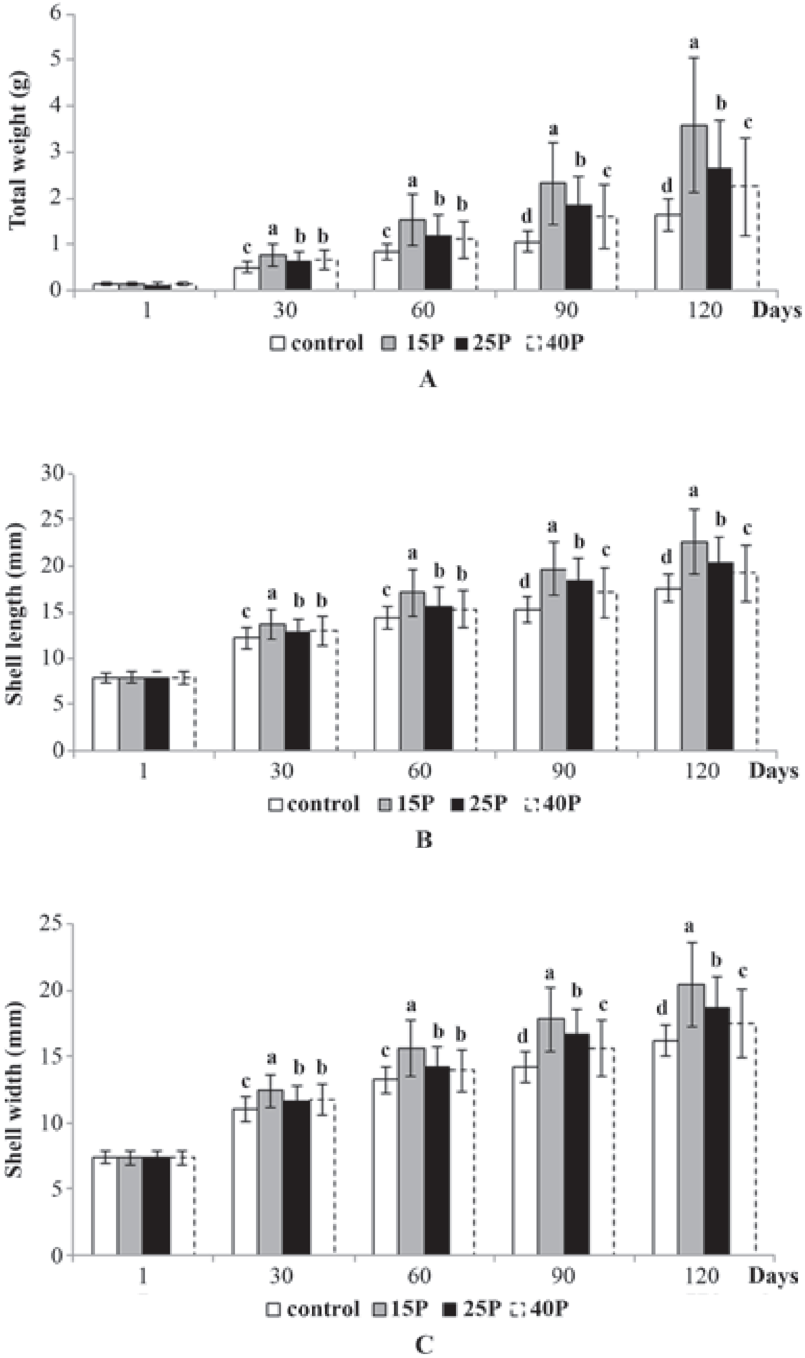

Figure 4. Growth performance (total weight (A), shell length (B) and shell width (C)) of Thai native apple snail (*P. ampullacea*) fed on different diets. Each value is mean±SD. Means with different letter superscripts are significantly different ($P<0.05$). (control = fresh lettuce (*L. sativa*); 15P = pellet feed with 15 % protein; 25P = pellet feed with 25% protein; 40P = pellet feed with 40% protein)

DISCUSSION

From April to November 2015, most of the *P. ampullacea* collected from the rice field were mature (shell length of female and male = 22.78-64.95 and 21.11-38.65 mm). Likewise, Kaneshima *et al*. (1986) reported that shell height of mature *Pomacea canaliculata* females and males was higher than 25 and 20 mm, respectively. The sexual maturation of *P. ampullacea* females occurred in July (in the middle of the rainy season), while significant GSI of males was found in May, June and September (early and late rainy season). Similarly, oviposition in *Pomacea canaliculata* females was found in late July to early August (Tanaka *et al*., 1999).

Snail production in captivity may involve with many factors, e.g.species and environment. The average number of eggs per clutch of *P. ampullacea* (192.13 eggs) was similar to those of *Pomacea canaliculata* (213.48 eggs) and *Pomacea insularum* (200 eggs) (Tanaka *et al*., 1999; Barnes *et al*., 2008). The embryonic development may depend on temperature. The period of hatching times of *P. ampullacea* varied considerably during 10-26 days at 28±1^{o}C, while Aufderheide *et al*. (2006) reported hatching times of apple snail (*Marisa cornuarietis*) was 8 days at 28^{o}C and 17 days at 22^{o}C. At uncontrolled temperature, Demian and Yousif (1973) reported that the hatching times of *M. cornuarietis* took 8 days at the temperature of 25-30^{o}C and 20 days at 15-20^{o}C.

In *P. ampullacea*, total weight, shell length, and shell width have a negative influence on percentage of hatching success and hatching time. Similarly, *Pomacea canaliculata* female receiving less nutrients from their food can mature at a smaller size (low total weight, shell length and shell width) and male can mate with them (Estoy *et al*., 2002). Additionally, Tamburi and Martin (2009) reported that small *Pomacea* snails showed higher foraging and competitive abilities than larger snails. It is in disagreement with a study on *Helisoma* where it showed that body size had a positive relationship with the total number of eggs produced (Norton and Bronson, 2006).

The growth of *P. ampullacea* fed on artificial diet containing raw materials from plants and animals was better than those fed with lettuce. It showed that *P. ampullacea* can accept and digest the protein from animal source. It may be feeding behavior of *P. ampullacea* was similar to apple snail (*Pomacea bridgesi*) that can accept the artificial feed (Mendoza *et al*., 1999). At 120 days, the average shell length increase and total weight of *P. ampullacea* fed with 15% protein artificial diet (0.86±0.28 g/month and 3.68±1.45 mm/month) revealed significantly when compared with fresh lettuce (0.38±0.15 g/month and 2.44±1.42 mm/month), 25% protein artificial diet (0.63±0.14 g/month and 3.12±1.23 mm/month) and 40% protein artificial diet (0.53±0.09 g/month and 2.85±1.49 mm/month). Similarly, Dupont-Vivet *et al*. (2000) cultured the edible snail (*Helix aspersa*) at indoor condition and fed on an artificial feed containing 15% crude protein, 2% crude fat, 3 % cellulose and 37% ashes which can promote the growth of juveniles into adults.

The optimal protein level and dietary energy may affect the growth of snails. Overall results of *P. ampullacea* presented that the highest growth performances were detected in 15 % protein artificial diet. Similarly, *Pomacea bridgesii* fed on artificial diet (20 % protein level) can promote in better growth than snails fed with dehydrated lettuce and artificial diet containing 40 % protein (Mendoza *et al*., 1999). Additionally, the dietary energy in 15 % protein level (284.5 kcal/100 g feed) of *P. ampullacea* can improve the growth, while Mendoza (1999) reported that growth of apple snail *(Pomacea bridgesi*) fed on dietary energy (250 kcal/100 g feed) was higher than snails gained high dietary energy (350 kcal/100 g feed). Either lack or access of protein in diet can reduce the growth in the different mechanism. A lack of protein (control experiment, 1.3 % protein level) would contain less amino acids available for protein synthesis, while the excess of protein (25 % and 40 % protein level in diet) can stimulate a higher energetic expenditure for ammonia excretion and inadequate energy for growth (Catacutan and Coloso, 1995).

In a previous study, the feed mixture (rice bran, *Leucaena* sp. leaf meal, casein, calcium carbonate, mixed vitamin and agar) and lettuce was fed on *Bithynia siamensis goniomphalos* in laboratory (Lamkom and Phosri, 2015). It was difficult to prepare and maintain feed quality. The results of this study indicated that 15 % protein level of artificial feed can promote the growth of *P. ampullacea* juveniles. It is possible to develop parasite-free culture of *P. ampullacea* in captivity. In future studies, other factors such as temperature, stocking density, and digestive system of *P. ampullacea* should be investigated.

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