

# Effects of protein levels in artificial pellet feed on growth and survival rate of black apple snail (*Pila polita*)

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**Abstract-** The present study was conducted to evaluate the effects of different protein levels in diet on the growth and survival rate of black apple snail (*Pila polita*) in grow-out period. There were 3 replicates in each treatment and snails were fed with protein levels at 15% (P15); 20 (P20); 25 (P25); 30 (P30); 35 (P35). Two month old juveniles with initial body weight, shell height and shell width of (2.52 g; 23.26 mm and 16.42 mm) were reared in the tarpaulin tanks (1 × 1 × 1 m; 40 cm water depth) at the density of 100 ind./tank. After 4 months of rearing period, the average body weight, shell height and shell width of the snail reached highest value in P20 (28.36 g; 54.59 mm; 39.74 mm, respectively) and that was significant difference ( $p < 0.05$ ) compared to the remaining protein contents (P15, P25, P30, P35). The survival rate of snails in P25 (75.3%) was higher than in P15 (74.7%), P20 (74.3%), P30 (73.3%) and P35 (71.7%). However, the survival rate was not significant difference among treatments ( $p > 0.05$ ). Snails in P20 obtained the highest productivity (2.79 kg/m<sup>2</sup>) and it was significant difference ( $p < 0.05$ ) from P15 (2.52 kg/m<sup>2</sup>), P25 (2.57 kg/m<sup>2</sup>), P30 (2.48 kg/m<sup>2</sup>) and P35 (2.31 kg/m<sup>2</sup>). The results of this study showed that the growth rate and productivity of black apple snail were highest when feeding diet contained 20% protein. The requirement of protein for the black apple snail in grow-out period was 22.12%.

**Index Terms-** Black apple snail, growth, protein contents, survival rate

## I. INTRODUCTION

Black apple snail (*Pila polita*) culture is a relatively new industry in Vietnam. It usually inhabits shallow ponds, lakes and plains field. This species has attracted a great interest of fish farmers because of its resistance to handling, rapid growth, delicious meat and high market price. Generally, meats of black apple snail, were highly nutritious, owing to its contents of proteins, rich vitamins and minerals (Bich *et al.*, 2003). In Vietnam, several studies were conducted to evaluate the efficiency of using some types of food for black apple snails in nursing (Binh and Thao, 2013; Thao *et al.*, 2013), grow-out period (Dat, 2010; Linh, 2011; Binh *et al.*, 2012; Binh and Thao, 2017b) and initially stimulates reproduction (Binh, 2011; Trieu, 2016).

Protein is the macronutrient whose requirement is prioritized in nutritional studies, because it represents the highest cost in commercial feeds, as well as playing an important role in

growth of shellfish. Inadequate protein in the diet results in a reduction or cessation of growth and a loss of weight due to withdrawal of protein from less vital tissue to maintain the functions of more vital tissue (Wilson, 2002). The minimum amount of dietary protein is needed to supply adequate amino acids and produce maximum growth. However, if too much protein is supplied in the diet, only part of it is used to make new protein, and the remainder will be converted to energy, which results in increased feed costs and increased ammonia nitrogen excretion. Protein requirement studies for shellfish have mainly focused on abalone (Britz and Hecht, 1997; Coote *et al.*, 2000; Gómez-Montes *et al.*, 2003; Green, 2009), babylon (Zhou *et al.*, 2007; Chaitanawisuti *et al.*, 2010; Chaitanawisuti *et al.*, 2011; Diana and Istiyanto, 2016; Chelladurai, 2017), golden apple snail (Mendoza *et al.*, 1999; Ramnarine, 2004) and Thai native apple snail (Thanathip and Dechnarong, 2017). Based on previous studies on abalone, reported protein requirements have ranged from 20 to 44% (Uki *et al.*, 1986; Mai *et al.*, 1995; Britz, 1996; Coote *et al.*, 2000). Similarly to babylon, reported protein requirements have ranged from 25 to 45% (Zhou *et al.*, 2007; Diana and Istiyanto, 2016; Chelladurai, 2017). The optimal dietary protein level for aquatic animals is influenced by the optimal dietary protein-to-energy balance, the essential amino acid compositions and digestibility of the protein, and the amount of non-protein energy sources in the test diet (Wilson, 2002). Some workers have investigated the protein digestibility of diets for *Pila ampullacea*. However, little information is concerning the dietary protein contents for black apple snail (*Pila polita*). From the above issues, it was found that the kind of processed food with appropriate protein content to improve the efficiency of black apple snail grow-out period, while also saving costs and reducing the impact on the water quality in culture environment, the research should be concerned.

## II. MATERIALS AND METHODS

### Experimental design

The samples of *Pila polita* with initial weight ranging from 2.21 to 3.35 g, shell height from 22.56 to 24.88 mm and shell width from 15.56 to 17.44 mm were nursed at Cao Lanh district, Dong Thap province (10°20'40,6"N; 105°47'04,8"E). Fresh water was pumped from river to the settling tanks (10 m<sup>3</sup>) for 5 - 7 days and then supplied into the culture tanks.

This experiment included five feeding treatments with different protein levels with three replicates per each as follow: 15% (P15); 20 (P20); 25 (P25); 30 (P30); 35 (P35). *Pila polita*

juvenile were cultured in 1m<sup>2</sup> (1 × 1 × 1 m; 40 cm water depth) tarpaulin tanks at the density of 100 ind./m<sup>2</sup> (Binh *et al.*, 2012; Binh and Thao, 2017a). Two feeding trays (diameter of 20 cm) and two nylon bunch substrate (diameter of 50 cm) were laid at

the bottom of each tank. The experiment was conducted for 4 months the ingredients used and proximate composition values of the diets are presented in Table 1.

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

Dietary crude protein levels	P15	P20	P25	P30	P35	P40
<b>Ingredient (%)</b>						
Fish meal <sup>1</sup>	10.10	14.60	19.10	23.60	28.10	32.60
Soybean meal <sup>2</sup>	14.66	20.35	26.04	31.71	37.40	43.10
Wheat flour	68.24	58.05	47.86	37.69	27.50	17.30
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00
Vitamine premix <sup>3</sup>	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix <sup>4</sup>	1.00	1.00	1.00	1.00	1.00	1.00
CMC	3.00	3.00	3.00	3.00	3.00	3.00
<b>Proximate composition (%)</b>						
Crude protein	13.97	20.74	23.99	30.09	34.09	39.84
Crude lipid	2.19	2.20	1.95	2.11	2.04	2.64
Crude ash	3.82	5.15	5.52	7.31	8.54	10.41
Crude fibre	0.88	1.13	0.95	1.41	1.67	2.00
Moisture	12.88	13.73	14.52	12.10	14.16	11.02
NFE	66.26	57.05	53.07	46.98	39.50	34.09
Energy (KJ/g)	16.97	16.98	16.92	16.93	16.78	16.64
P/E ratio (mg protein/KJ)	8.84	11.78	14.78	17.72	20.85	24.03
Canxi	0.74	0.94	1.08	1.31	1.68	1.73

*Parameters: Protein, lipid, ash, fibre, moisture, NFE-Nitrogen free extract and canxi analyzed by Quality Assurance and Testing Center Can Tho;* <sup>1</sup> *Fish mea Kien Giang (Viet Nam);* <sup>2</sup> *Soybean meal (Argentina);* <sup>3,4</sup> *Vitamine premix and mineral premix: Vitamin A, 2.000.000 IU; Vitamin D, 400.000 IU; Vitamin E, 6g; Vitamin B1, 800mg; Vitamin B2, 800mg; Vitamin B12, 2mg; Calcium D. Panthotenate, 2g; Folic acid, 160mg; Vitamin C, 15g; Cholin Chloride, 100g; Ferous (Fe<sup>2+</sup>), 1g; Zinc (Zn<sup>2+</sup>), 3g; Manganese (Mn<sup>2+</sup>), 2g; Copper (Cu<sup>2+</sup>), 100mg; Iodine (I), 20mg; Cobalt (Co<sup>2+</sup>), 10mg.*

Snails were fed twice a day (at 7:00 AM and 6:00 PM) with the quantity of 2 - 4% of snail body weight in each tank. Water in the cuture tank is renewed 30 - 40% after a cycle of 7 - 10 days.

### Food preparation

Experimental diets are formulated into pellets from ingredients including fish meal, soybean meal, wheat flour, soy oil, vitamine premix, mineral premix and CMC- Carboxymethyl cellulose. Scale raw materials according to the ratio of fish meal, wheat flour, vitamine premix, mineral premix and CMC for the first time and mix well (dry mix), after the cooking, soybean meal wasled to cool down (about 40 - 50 °C) and then mix in dry ingredients together with soy oil. After mixing, the pellets was dried in oven at 60°C in 24 hours and then stored in fridge at 4°C for feeding.

### Data collection and sample analysis

**Water quality:** Daily water temperature was recorded twice a day at 7:00 and 14:00 using a thermometer. The concentration of NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> (TAN), NO<sub>2</sub><sup>-</sup>, DO, pH and alkalinity was monitored weekly using test-kit (Sera, Germany).

**Growth performance and feed utilization:** At the beginning and 15 day intervals during the experiment, numbers of snails in each tank was counted for checking the survival rate. Shell height and shell width, body weight of 20 snails/tank was measured and weighed to determine the growth rate. The shell height and shell width were measured with an electronic digital caliper (0.01 mm), snail weight were determined with an electronic scale (0.01 g error).

Weight gain (WG), shell height gain (HG), shell width gain (WG), daily weight gain (DWG), daily height gain (DHG), daily height gain (DWG), specific growth rate in body weight (SGR<sub>w</sub>), specific growth rate in shell height (SGR<sub>H</sub>), specific growth rate in shell width (SGR<sub>w</sub>), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival were calculated using the following equations:

$$\text{Survival rate (\%)} = \frac{\text{Final number of snails}}{\text{Initial number of snails}} \times 100$$

$$\text{Biomass increase rate (BIR, \%)} = \frac{\text{Biomass increase}}{\text{Biomass initial}} \times 100$$

$$\text{Feed conversion rate (FCR)} = \frac{\text{Total feed intake (dry weight)}}{\text{Weight gain (wet weight)}}$$

$$\text{Productivity (P, kg/m}^2\text{)} = \frac{\text{Total final body weight} \times \text{S (survival)}}{\text{S}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain}}{\text{Protein intake}}$$

The data on specific growth rate in body weight (SGR<sub>w</sub>-%/day) were subjected to the quadratic regression analysis model (Zeitoun *et al.*, 1976; Sales *et al.*, 2003) and the optimal protein requirement of black apple snail was estimated.

### Statistical analysis

The data were analyzed for mean values, standard deviation by using Excel software and one way anova analysis followed by

Duncan post hoc test was applied to compare the significant difference of collected parameters among treatments at  $p < 0.05$  using SPSS program version 22.0.

### III. RESULTS

#### Water quality parameters

Mean values of environmental parameters were illustrated in Table 2. The average temperature ranged from 24.5°C to 30.5°C during the experiment, with daily fluctuation around

2.3°C (0.5 - 3.9°C). There was no significant difference of temperature among treatments.

pH values and DO were slightly varied during the treatments (7.69 - 7.83; 4.40 - 4.45 mgO<sub>2</sub>/L, respectively) and no significant difference ( $p > 0.05$ ) was found. Meanwhile, mean alkalinity values in P20 treatment (65.6 mg CaCO<sub>3</sub>/L) was lower and they have differences ( $p < 0.05$ ) compared to P15 and P35 (68.2 mg CaCO<sub>3</sub>/L).

Concentrations of TAN and NO<sub>2</sub><sup>-</sup> in P35 treatment (0.86 mg/L; 0.77 mg/L, respectively) were higher than other treatments ( $p < 0.05$ ).

Table 2: Mean values of environmental parameters during culture period

Parameters		Dietary crude protein levels				
		P15	P20	P25	P30	P35
Temperature (°C)	Morning	26.3±0.4 <sup>a</sup>	26.3±0.3 <sup>a</sup>	26.3±0.4 <sup>a</sup>	26.2±0.4 <sup>a</sup>	26.1±0.4 <sup>a</sup>
	Afternoon	28.6±0.6 <sup>a</sup>	28.5±0.6 <sup>a</sup>	28.7±0.6 <sup>a</sup>	28.5±0.6 <sup>a</sup>	28.6±0.4 <sup>a</sup>
DO (mg O <sub>2</sub> /L)		4.43±0.01 <sup>a</sup>	4.45±0.07 <sup>a</sup>	4.43±0.10 <sup>a</sup>	4.44±0.04 <sup>a</sup>	4.40±0.01 <sup>a</sup>
pH		7.83±0.06 <sup>a</sup>	7.69±0.04 <sup>a</sup>	7.73±0.05 <sup>a</sup>	7.74±0.03 <sup>a</sup>	7.79±0.01 <sup>a</sup>
NH <sub>4</sub> <sup>+</sup> /NH <sub>3</sub> -TAN (mg/L)		0.66±0.01 <sup>a</sup>	0.72±0.03 <sup>ab</sup>	0.79±0.04 <sup>c</sup>	0.77±0.04 <sup>bc</sup>	0.86±0.04 <sup>d</sup>
NO <sub>2</sub> <sup>-</sup> (mg/L)		0.60±0.03 <sup>a</sup>	0.70±0.03 <sup>b</sup>	0.72±0.01 <sup>b</sup>	0.76±0.02 <sup>c</sup>	0.77±0.01 <sup>c</sup>
Alkalinity (mg CaCO <sub>3</sub> /L)		68.2±1.0 <sup>b</sup>	65.6±1.5 <sup>a</sup>	66.6±1.1 <sup>ab</sup>	67.9±1.1 <sup>ab</sup>	68.2±1.7 <sup>b</sup>

The values in the same row with different letters indicating the significant difference ( $p < 0.05$ )

#### Growth of black apple snail

The average shell height, shell width and body weight of *Pila polita* fed with formulated diet for four months are shown in Table 3. The snail reached highest value in P20 (28.36 g; 54.59

mm; 39.74 mm, respectively) and that was significant difference ( $p < 0.05$ ) from other protein contents.

Table 3: Average growth rate of black apple snail fed on the different diets

Parameters	Dietary crude protein levels				
	P15	P20	P25	P30	P35
<b>Body weight</b>					
Initial (g)	2.54±0.10 <sup>a</sup>	2.53±0.06 <sup>a</sup>	2.51±0.04 <sup>a</sup>	2.54±0.04 <sup>a</sup>	2.56±0.02 <sup>a</sup>
Final (g)	26.09±0.50 <sup>bc</sup>	28.36±0.34 <sup>d</sup>	26.78±0.32 <sup>c</sup>	25.75±0.23 <sup>b</sup>	23.48±0.43 <sup>a</sup>
WG (g)	23.55±0.53 <sup>bc</sup>	25.84±0.39 <sup>d</sup>	24.26±0.27 <sup>c</sup>	23.21±0.27 <sup>b</sup>	20.92±0.45 <sup>a</sup>
DWG (g/day)	0.20±0.00 <sup>b</sup>	0.22±0.00 <sup>d</sup>	0.20±0.00 <sup>c</sup>	0.19±0.00 <sup>b</sup>	0.17±0.00 <sup>a</sup>
SGR <sub>w</sub> (%/day)	1.94±0.04 <sup>b</sup>	2.01±0.03 <sup>c</sup>	1.97±0.01 <sup>c</sup>	1.93±0.02 <sup>b</sup>	1.85±0.02 <sup>a</sup>
<b>Shell height</b>					
Initial (mm)	23.15±0.11 <sup>a</sup>	23.29±0.17 <sup>a</sup>	23.26±0.35 <sup>a</sup>	23.25±0.08 <sup>a</sup>	23.33±0.04 <sup>a</sup>
Final (mm)	52.84±0.83 <sup>b</sup>	54.59±0.78 <sup>c</sup>	53.46±0.48 <sup>b</sup>	52.65±0.30 <sup>b</sup>	50.63±0.20 <sup>a</sup>
HG (mm)	29.69±0.93 <sup>bc</sup>	31.30±0.63 <sup>c</sup>	30.20±0.80 <sup>bc</sup>	29.40±0.28 <sup>b</sup>	27.30±0.20 <sup>a</sup>
DLG (mm/day)	0.25±0.01 <sup>b</sup>	0.26±0.01 <sup>c</sup>	0.25±0.01 <sup>b</sup>	0.25±0.00 <sup>b</sup>	0.23±0.00 <sup>a</sup>
SGR <sub>L</sub> (%/day)	0.69±0.02 <sup>bc</sup>	0.71±0.01 <sup>c</sup>	0.69±0.02 <sup>bc</sup>	0.68±0.00 <sup>b</sup>	0.65±0.00 <sup>a</sup>
<b>Shell width</b>					
Initial (mm)	16.35±0.31 <sup>a</sup>	16.41±0.12 <sup>a</sup>	16.38±0.24 <sup>a</sup>	16.52±0.16 <sup>a</sup>	16.46±0.23 <sup>a</sup>
Final (mm)	38.27±0.21 <sup>bc</sup>	39.74±0.66 <sup>d</sup>	38.81±0.50 <sup>c</sup>	37.88±0.19 <sup>b</sup>	36.15±0.22 <sup>a</sup>
WG (mm)	21.92±0.47 <sup>bc</sup>	23.33±0.78 <sup>d</sup>	22.43±0.66 <sup>cd</sup>	21.36±0.35 <sup>b</sup>	19.69±0.37 <sup>a</sup>
DWG (mm/day)	0.18±0.00 <sup>bc</sup>	0.19±0.01 <sup>d</sup>	0.19±0.01 <sup>cd</sup>	0.18±0.00 <sup>b</sup>	0.16±0.00 <sup>a</sup>
SGR <sub>w</sub> (%/day)	0.71±0.02 <sup>bc</sup>	0.74±0.02 <sup>c</sup>	0.72±0.02 <sup>bc</sup>	0.69±0.01 <sup>b</sup>	0.66±0.01 <sup>a</sup>

The values in the same row with different letters indicating the significant difference ( $p < 0.05$ )

The results revealed that snails fed on treatment P20 have significantly higher performance in terms of daily height gain

and specific growth rate in shell height (0.26 mm/day and 0.71 %/day), while treatment P35 (0.23 mm/day and 0.65 %/day)

recorded the least (Table 3) and significant difference among these treatments ( $p < 0.05$ ). Similarly, there were significant ( $p < 0.05$ ) differences in daily width gain (WG) and specific growth rate in shell width (SGR<sub>W</sub>). The highest increase in WG and SGR<sub>W</sub> were recorded when snails were fed on P20 and the least was recorded in P35.

The specific growth rate of body weight (SGR<sub>W</sub>, %/day) of snails was fitted into quadratic models (Fig. 1). The best fit for the estimation of optimal protein level could be described as  $Y = -0.0009x^2 + 0.0395x + 1.5606$  ( $R^2 = 0.9408$ ). The trend of the growth showed that maximum SGR<sub>W</sub> (1.99 %/day) corresponding to the about of 22.12% protein in the diet.

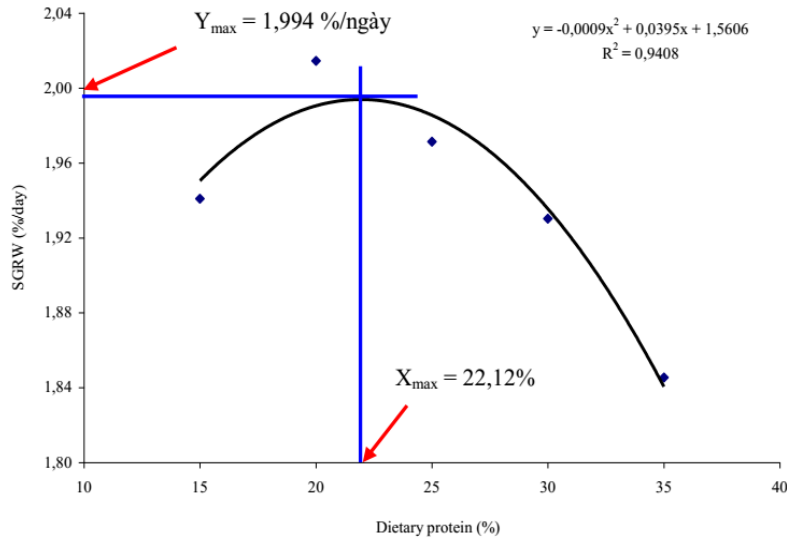


Fig. 1: Relationship between specific growth rate of body weight (SGR<sub>W</sub>) and dietary protein levels for black apple snail (*Pila polita*)

**Survival rate, biomass increase rate, productivity, feed conversion rate and protein efficiency ratio of black apple snail**

different among treatments ( $p > 0.05$ ), within the range of 71.7 - 75.3 %.

Survival rate of snail in all treatments decreased gradually during 4 months of culture (Fig. 2) and was not significantly

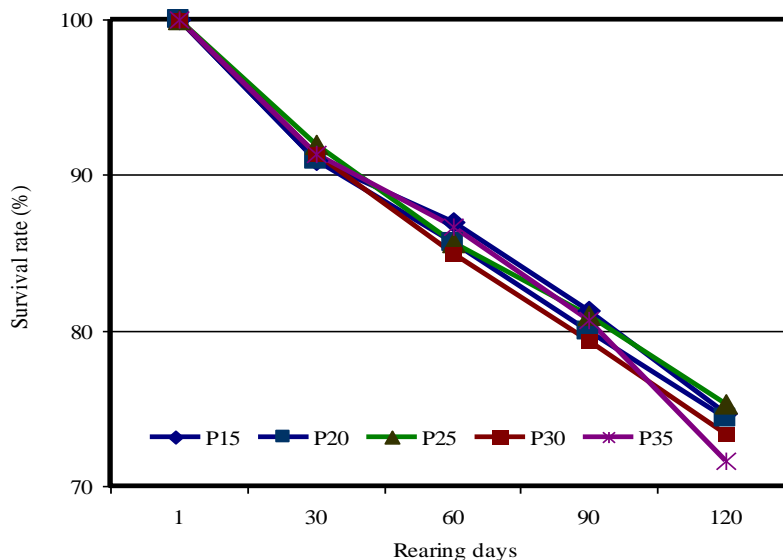


Fig. 2: Survival rate of black apple snail during experiment period

Biomass increase rate and productivity reached highest value in P20 (511%; 2.79 kg/m<sup>2</sup>, respectively) with significant difference ( $p < 0.05$ ) compared to other protein levels, especially P35 (436% and 2.31 kg/m<sup>2</sup>).

FCR values reached 1.28 - 1.42 whereas treatment P20 presented the lowest FCR values compared to the treatment P35

(Table 4). However, the FCR was not significant difference among treatments ( $p > 0.05$ ). The highest PER was observed in treatment P15 (151%) with significant difference ( $p < 0.05$ ) compared to treatment P20 (127%), P25 (93%), P30 (74%) and P35 (59%).

Table 4: Survival rate (SR), biomass increase (BIR), productivity (P) on the different diets

Parameters	Dietary crude protein level				
	P15	P20	P25	P30	P35
SR (%)	74.7±2.1 <sup>a</sup>	74.3±1.5 <sup>a</sup>	75.3±3.2 <sup>a</sup>	73.3±3.2 <sup>a</sup>	71.7±2.5 <sup>a</sup>
BIR (%)	471±24 <sup>abc</sup>	511±21 <sup>c</sup>	493±19 <sup>bc</sup>	463±35 <sup>ab</sup>	436±12 <sup>a</sup>
P (kg/m <sup>2</sup> )	2.52±0.06 <sup>b</sup>	2.79±0.04 <sup>c</sup>	2.57±0.10 <sup>b</sup>	2.48±0.05 <sup>ab</sup>	2.31±0.17 <sup>a</sup>
FCR	1.34±0.07 <sup>a</sup>	1.28±0.02 <sup>a</sup>	1.34±0.03 <sup>a</sup>	1.34±0.03 <sup>a</sup>	1.42±0.16 <sup>a</sup>
PER (%)	151±4 <sup>e</sup>	127±2 <sup>d</sup>	93±4 <sup>c</sup>	74±2 <sup>b</sup>	59±5 <sup>a</sup>

The values in the same row with different letters indicating the significant difference ( $p < 0.05$ )

#### IV. DISCUSSION

Water quality conditions (NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub><sup>-</sup>-TAN, NO<sub>2</sub><sup>-</sup>, DO, pH and alkalinity) remained within a suitable range for black apple snails throughout the experiment. However, in this experiment, the levels of TAN and NO<sub>2</sub><sup>-</sup> increased as protein levels increased, which could be explained by the higher protein content of the treatments. The amount of organic matter produced from food and excreta from snails will be transferred to ammonium ions leading to high levels of TAN and NO<sub>2</sub><sup>-</sup>. This result is quite consistent with studies by Thao *et al.* (2013); Binh and Thao (2013) that the levels of TAN and NO<sub>2</sub><sup>-</sup> concentrations on the treatments fed with pellets (18,0% protein) is always higher than the treatments fed just rice bran (6,4% protein), wheat flour (4,7% protein) or vegetable (3,4% protein).

Determination of optimum dietary protein level which produced the maximum growth of black apple snail was our goal in this study. Studies showed that pelleted foods containing fishmeal, soymeal, vegetable and wheat flour was preferentially consumed and improved the growth rate of black apple snail (Dat, 2010; Linh, 2011; Binh *et al.*, 2012; Binh and Thao, 2017a, b; Thanathip and Dechnarong, 2017). Our results found that weight gain of black apple snail increased with increasing dietary protein level from 15 to 20% and slightly decreased thereafter with further increases in dietary protein. These results are similar to those in other gastropoda species (Uki *et al.*, 1986; Britz, 1996; Coote *et al.*, 2000; Ramnarine, 2004; Lee and Lim, 2005; Diana and Istiyanto, 2016; Nhan and Harry, 2016; Thanathip and Dechnarong, 2017). The best growth and mean protein gain were observed in black apple snail fed 20% protein content.

Our results showed that the growth of juvenile black apple snail increased with increasing dietary protein level from 15 to 20%, and slightly decreased thereafter with further increases in dietary protein. These results are similar to those in other mollusk species (*Pila ampullacea*, *Achatina achatina*, *Pomacea bridgesii*, *Babylonia areolata*). Chaitanawisuti *et al.* (2011) reported that *Babylonia areolata* fed 35 to 36% protein increased weight (1.41 g/day) than 20% protein (0.51 g/day) and 28% protein (1.11 g/day). Similarly, Chelladurai (2017) confirmed that the highest growth at 40% level of protein diet (53.5 g; 69.1 mm) and the lowest was observed at 30% (51.9 g; 59.6 mm). For abalone, Britz and Hecht (1997) found that when *Haliotis midae* fed the diet content 24% protein (10% fat content) increased the body weight (9.2 g) and shell height (39.4 mm) lower than 34% (9.9 g; 40.1 mm, respectively) and 44% (11.9 g; 42.3 mm). Study on freshwater gastropods, Lee and Lim (2005) reported that

mean weight gain of snails *Semisulcospira gottschei* fed the 22% protein diets with 3.3 kcal/g diet was not significantly different from that of snails fed the 32 to 52% protein diets. Mendoza *et al.* (1999) revealed that crude protein affected the growth of the golden apple snail (*Pomacea bridgesii*), when using diets with 20 and 30% protein content, the growth rate was 5.39 %/day and 5.51 %/day. This value decreased when protein content decreasing to 10% (4.02 %/day) or increasing up to 40% (4.82 %/day). In *Pila ampullacea*, Thanathip and Dechnarong (2017) found that the diets had a marked effect on the growth, the average shell height increase and body weight of this snail species fed with 15% protein artificial diet revealed significantly growth (0.12 mm/day and 0.03 g/day) compared to 25% protein artificial diet (0.10 mm/day and 0.02 g/day) and 40% protein artificial diet (0.09 mm/day and 0.02 g/day). The results of this study and previous studies have shown that freshwater gastropods (*Pila ampullacea*, *Achatina achatina* and *Pomacea bridgesii*) require lower protein content than marine species (*Babylonia areolata*, *Haliotis laeugata*). The levels of protein in suitable range will ensure optimum growth. However if the content of this substance was exceeded the body needs, then growth of the snail would be able to decrease (Mendoza *et al.*, 1999; Ani and Ugwuowo, 2011; Chaitanawisuti *et al.*, 2011; Diana and Istiyanto, 2016; Nhan and Harry, 2016; Chelladurai, 2017; Thanathip and Dechnarong, 2017).

Survival rate of black apple snail after 4 cultured months in the range of 71.7 to 75.3% and was not significant difference among treatments ( $p > 0.05$ ). The survival rate of snails in this study was lower than that of the study of Thanathip and Dechnarong (2017) after 4 culture months, survival rates of *Pila ampullacea* fed 15% protein (98.3%) were better than those fed with 40% protein (96.7%) and 25% protein (91.7%). Chelladurai (2017) reported that the highest survival rate of *Babylonia spirata*, feed on diet containing 40% protein (91.0%), whereas the lowest was observed in 30% protein (89.0%).

The biomass increase rate was highest in the 20% protein content and was comparable to that of Thao (2015) with the average biomass increase rate from 494 to 527% (i.e. from 4.94 to 5.27 times to the original biomass). At a protein content of 20%, the growth rate in weight was highest, snail also presented high survival rate so that the BIR was the top ranking compare to other protein levels. Black apple snail productivity obtained after 4 months of culture ranged from 2.31 to 2.79 kg/m<sup>2</sup>, a similar study by Binh and Thao (2017a, b), with a density of 150 ind./m<sup>2</sup>, the highest productivity obtained (3.37 - 3.57 kg/m<sup>2</sup>) when feeding with pellets (18% protein).

The efficiency of protein rate (PER) of black apple snails decreases as protein levels increase in diet. Study on *Pomacea bridgesii*, Mendoza *et al.* (1999) reported that PER was highest when fed with 10% protein content (11.02%) and tended to decrease when protein content increased to 20% (8.28%) or 30% (4.43%). Similarly, Ugwuowo (2009) obtained PER in *Archachatina marginata* when feeding 16% protein (3.78%) and those values were higher when increasing protein levels in diet from 18% to 22%. Similarly, this study found that PER and FCR of black apple snails decreases as protein levels increase in the diet. Thanathip and Dechnarong (2017) reported that *Pila ampullacea* fed 15 to 25% protein showed FCR (2.08 to 2.28) lower than 40% protein (2.50). Mendoza *et al.* (1999) studied on *Pomacea bridgesii* and FCR was highest when fed with 10% protein content (0.90) and tended to decrease when protein content increased to 20% protein (0.60) and 30% (0.63). In the current study, protein efficiency ratio significantly decreased with increasing dietary protein level. This is probably because more dietary protein is used as energy when high protein diets are fed. In the isoenergetic diets, an increase in the dietary level of non-protein digestible energy increased nitrogen retention by decreasing nitrogen loss (Cho and Kaushik, 1985).

Considering the growth response obtained in the present study, the dietary protein level of about 20% is adequate for rearing *Pila polita* which had a good growth performance, survival and productivity. The estimation for optimum requirement of protein for the black apple snail growth was 22.12%.

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