

Feed utilization efficiency and growth of Java barb (*Puntius javanicus*) fed on dietary pineapple extract

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Abstract. Pineapples extract is rich in bromelain content. Bromelain is the protease enzyme that acts in the digestive tracts to hydrolyze the proteins as peptides and amino acids. The proteins that are consumed become more easily absorbed into fish body. This study aimed to examine dietary pineapple extract on the total feed consumption (TFC), feed utilization efficiency (FUE), protein efficiency ratio (PER), relative growth rate (RGR), length-relative growth rate (RGR_L) and survival rate (SR) of Java barb (*Puntius javanicus*). The average body weight of *P. javanicus* used as trial fish was 2.47 ± 0.09 g fish⁻¹. The fish were held in 20 liter plastic containers with density of 1 fish L⁻¹ for 35 days. Experimental method with completely randomized design (CRD) consisting of 5 treatments and 4 replicates was applied in this study. The treatments were dietary pineapple extract of 0.00, 0.75, 1.50, 2.25 and 3.00%. The results showed that dietary pineapple extract influenced the values of FUE, PER, RGR and RGR_L of the trial fish ($P < 0.05$). However, the TFC and SR were not influenced by dietary pineapple extract. The pineapple extract in the trial feed of 1.50% lead for the highest value for FUE, PER, RGR and RGR_L, that were $37.63 \pm 5.99\%$ day⁻¹, $1.14 \pm 0.18\%$ day⁻¹, $1.94 \pm 0.39\%$ day⁻¹ and $0.84 \pm 0.13\%$ day⁻¹, respectively. Water quality parameters during the study were within a suitable range for the fish life.

Key words: *Puntius javanicus*, pineapples extract, bromelain, protein hydrolyzing, E/P ratio.

Introduction. Java barb (*Puntius javanicus*) is a herbivorous and one of the original cultivated freshwater fish in Indonesia. This fish is easily cultivated even in high stocking density and becomes one of the important economical fish species whose price is affordable by consumers (Hanief et al 2014; Moniruzzaman et al 2015). The meat protein content is 18.43% that is higher than carp and tilapia, both of 16% and 18.72% respectively (Dani et al 2005; Munthe et al 2016). The increase of *P. javanicus* production can be conducted by applying an intensive aquaculture system. The fish is similar to *Barbonymus gonionotus*. Moniruzzaman et al (2015) observed that the higher production is resulting from the stocking density up to 90 fish m⁻³. In the business of fish farming, better qualitative and quantitative supply of feed is important. Feed is the most important component in fish farming. Feed supply with complete nutrient compositions and appropriate of daily feeding schedule to fish is an important factor in aquaculture to increase the growth and survival rate of the fishes (Sulawesty et al 2014; Asma et al 2016).

The availability of sufficient, timely, sustainable feeding, fulfilling nutritional adequacy, fish-favored and easily digested is essential in intensive fish farming (Utami et al 2012). Therefore, feeding should be optimized to obtain better growth of *P. javanicus*. One of the problems in aquaculture is the feed cost. The efficiency of feeding could reduce the production costs. Moreover, an appropriate nutritional value needed by the fish is an alternative solution that needs to be pursued. Several ways to improve feed efficiency included optimizing feed digestion and absorption and increasing the efficiency of protein utilization by adding enzymes that helps on feed digestion (Isnawati et al 2015).

Pineapples extract contain a small amount of bromelain. Bromelain belongs to a group of sulfhydryl proteases which could hydrolyze proteins into simpler amino acids, contain different thiol endopeptidases and a complex mixture of proteases including several protease inhibitors. Moreover it is dissolved in water (Pavan et al 2012; Rathnavelu et al 2016). The enzyme can hydrolyze peptide's bonds of protein into amino acids. The similarity characteristic of bromelain to protease enzyme ensures its ability to hydrolyze proteins (Christy 2012; Rathnavelu et al 2016). Bromelain can be obtained from pineapple crop (*Ananas comosus*), either from its stems, rinds, leaves, fruits or rods in varying amounts (Pavan et al 2012; Masniar et al 2016). The content of bromelain found in pineapple's pulp varied between 0.080-0.125%, in the shell 0.050-0.075% and in the stem base 0.040-0.060% (Muniarti 2006). Bromelain might act as an exogenous enzyme and could also dissolve collagen by hydrolyzing the protein (Anugraha et al 2014). In fact, the use of bromelain was found to be an effective method to tenderize of meats, including squid muscle (Gokoglu et al 2017). The active substance of bromelain contains cysteine and histidine groups that are important in enzyme activation. Thus, the enzyme specifically intercepts the peptide bonds of the carbonyl groups as found in arginine or aromatic amino acids, i.e. phenylalanine and tyrosine (Hardiany 2013).

A classical problem also has been commonly found in *P. javanicus* aquaculture is the slow growth of the fish that suspected of both less of feed consumed and low rate of feed efficiency. A low rate of feed efficiency it means that digestibility of the feed would not be optimal and the growth rate could be hampered. Protein might not be optimally digested due to the difficulties of the fish in hydrolyzing of some ingredients in the feed. Bromelain is an enzyme that plays an important role in the feed digestibility of fish. The presence of bromelain in diets can help and accelerate nutrients digestion process, and furthermore the nutrient availability for fish growth (Nisrinah et al 2013; Anugraha et al 2014; Choi et al 2016). Researches on dietary bromelain fed *Clarias gariepinus* (Nisrinah et al 2013) and *Cyprinus carpio* (Anugraha et al 2014), both indicated that diets containing 2.25% of pineapple extract as a bromelain source resulted on the best growth performances. When bromelain and papain supplements were added in the grass carp diet, the growth and immunity of the fishes were improved (Choi et al 2016). The increase of feed efficiency and protein efficiency ratio has also been found when common lowland frog *Rana rugulosa* (Wiegmann) was fed on diets containing bromelain from pineapple extract (Klahan & Sirithanawong 2015). Similar results were found by Li et al (2014) in broilers. Based on those researches, the presence of bromelain in feed may be a premise of a better hydrolyzing of nutrients from feed, especially the proteins. Researches on dietary bromelain in relation to the growth performances of java barb are still limited. Therefore, the experiment on dietary pineapple extract for *P. javanicus* is required to be conducted in order to test the improvement of feed utilization efficiency and growth of the fishes.

Material and Method

Fish preparation. Trial fishes with its total body length ranged from 5 to 7 cm, the average body weight of 2.47 ± 0.09 g and a total count of 300 fishes were used. After being acclimatized in the experimental container and growth medium for 7 days, the fishes were selected to gain the uniformity in size, health and complete body organs and used as the experimental fish. Then, the fishes were reared into prepared and treated growth medium with the density of 1 fish L⁻¹.

Container preparation. The experimental containers used in the study were plastic buckets of 20 liters capacity for each. A total of 20 containers (i.e. for 5 treatments and 4 replicates each) used were. Every container was filled with treated water as much as 70% of the total volume capacity, i.e. 15 liters. Furthermore, each container was equipped with aeration apparatus as an oxygen supply.

Feed preparation and bromelain extract. The experimental feed used was hydrolysis product of two ingredients, i.e. fish meal and soybean meal, with bromelain as treatment doses. Bromelain was obtained from the extraction of pineapple fruit. The ingredient composition and percentage of each feeding trial are presented in Table 1. All the ingredients were mixed and placed into a glass jar before pelletized and then placed into an oven with a temperature of 40 °C until dry (Nisrinah et al 2013). Pineapple-bromelain was extracted as the following steps. Purely ripe pineapple fruits were cleaned with distilled water, cut into smaller slices, and blended. The juice was extracted using a homogenizer and then filtered. Phosphate buffer solution (pH=7) was added to the filtered mass. Further 60% ammonium sulfate was added and stirred for 45 minutes with magnetic stirrer, and then the homogenate was incubated for a night in a refrigerator at 4 °C. The bromelain crude extract was centrifuged for 25 minutes at 3500 rpm, and the supernatant and precipitation were obtained.

Table 1
The composition and percentage of ingredients in each trial feed for *P. javanicus*

Ingredient composition	Ingredient percentage in the trial feed (% dry matter)				
	0 (A)	0.75 (B)	1.50 (C)	2.25 (D)	3.00 (E)
Pineapple extract	0.00	0.75	1.50	2.25	3.00
Fish meal	25.25	25.25	25.25	25.25	25.25
Soybean meal	39.63	39.78	39.85	39.90	39.95
Wheat flour	7.00	7.00	7.00	7.00	6.65
Bran meal	12.17	12.17	13.15	14.15	15.15
Corn meal	6.95	6.05	4.25	2.45	1.00
Fish oil	1.00	1.00	1.00	1.00	1.00
Corn oil	2.00	2.00	2.00	2.00	2.00
Vit.-min. mix ¹	5.00	5.00	5.00	5.00	5.00
CMC ²	1.00	1.00	1.00	1.00	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00
<i>Proximate analysis results</i>					
Protein (%)	33.69	34.33	32.99	34.71	34.48
Fat (%)	8.68	8.39	8.62	8.23	8.45
NFE (%)	42.02	39.31	40.91	37.79	37.42
Total energy (kJ) ³	1231.73	1199.44	1207.75	1187.00	1187.26
E/P (kJ g ⁻¹ protein)	36.56	34.94	36.61	31.48	34.43

Note: ¹ Vit.-min. mix: vitamins and minerals mixture, ² CMC: carboxy methyl cellulose, ³ Based on the digestible energy (DE) value for tilapia with the assumption for protein = 14.7 kJ g⁻¹, fat = 34.02 kJ g⁻¹, non-nitrogenous free extract (NFE) = 10.5 kJ g⁻¹ (after Wilson 1982).

Fish management. Acclimatized fishes were held for 35 days in a 20 L plastic container with a density of 1 fish L⁻¹. During the rearing time, the fishes were fed on trial feed twice a day in the morning and afternoon by applying *ad satiation* method. Water quality was maintained and managed by siphoning out the water, so the dirt from the bottom of the container was not accumulated. At the time of siphoning, the lost water volume was replaced by a similar volume of clean water. The process of siphoning out and filling new-clean water into the container was done simultaneously. The aim of this manner was that fishes could adapt to the new water temperature and to decrease the level of stress.

Experimental design. The experimental design used a complete randomized design (CRD), which consisted of 5 treatments and each treatment was replicated 4 times. The 5 treatments were:

1. Treatment A: Feeding trial with a dose of dietary pineapple extract of 0.00%;
2. Treatment B: Feeding trial with a dose of dietary pineapple extract of 0.75%;
3. Treatment C: Feeding trial with a dose of dietary pineapple extract of 1.50%;
4. Treatment D: Feeding trial with a dose of dietary pineapple extract of 2.25%; and
5. Treatment E: Feeding trial with a dose of dietary pineapple extract of 3.00%.

Variables and measurement methods. The response variables measured were total feed consumed (TFC), feed utilization efficiency (FUE), protein efficiency ratio (PER), relative growth rate (RGR), length-relative growth rate (RLG_{-L}) and survival rate (SR). The formula for each measurement was:

a. Total feed consumed (TFC), by Pereira et al (2007):

$$TFC = F_0 - F_1$$

where:

TFC: total amount feed consumed (g)

F₀: initial amount of feed weight (g)

F₁: final amount of feed weight (g).

b. Feed utilization efficiency (FUE), by Tacon (1987):

$$FUE = \frac{Wt - W0}{F} \times 100$$

where:

FUE: feed utilization efficiency (%)

Wt: total fish weight at the end of the experiment (g)

W0: total fish weight at the beginning of the experiment (g)

F: total amount of feed consumed (g).

c. Protein efficiency ratio (PER), by Bake et al (2014):

$$PER = \frac{Wt - W0}{Pi} \times 100$$

where:

PER: protein efficiency ratio (%)

Wt: total fish weight at the end of the experiment (g)

W0: total fish weight at the beginning of the experiment (g)

Pi: total amount of dietary protein consumed (g).

d. Relative growth rate (RGR), by De Silva & Anderson (1995)

$$RGR = \frac{Wt - W0}{W0 \times t} \times 100$$

where:

RGR: relative growth rate (% day⁻¹)

Wt: total fish weight at the end of the experiment (g)

W0: total fish weight at the beginning of the experiment (g)

t: rearing period of the experimental fish (day).

e. Length-relative growth rate (RLG_{-L}), by Effendie (1997):

$$RLG_{-L} = \frac{Lt - L0}{L0 \times t} \times 100$$

where:

RGR_{-L}: relative growth rate of fish body length (% day⁻¹)

Lt: total body length of fish at the end of the experiment (g)

L0: total body length of fish at the beginning of the experiment (g)

t: rearing period of the experimental fish (day).

f. Survival rate (SR):

$$SR = \frac{\sum Lt1}{\sum Lt0} \times 100$$

where:

SR: survival rate of the experimental fish (%)

$\sum Lt1$: total number of survived fish at the end of the experiment (fish)

$\sum Lt0$: total number of fish at the beginning of the experiment (fish).

g. Water quality parameters. Measurements of water quality parameters included temperature, dissolved oxygen (DO), total ammonia nitrogen (TAN) and pH level. The level of TAN was measured at the beginning, middle and the end of the research period. Water temperature was recorded daily, while DO and pH were recorded weekly. Ammonia content was analyzed by using a spectrophotometer, while pH measurement was measured by using pH-meter. Dissolved oxygen (DO) was measured by DO-meter.

Statistical analysis. The variance values of TFC, FUE, PER, RGR, RLG_L and SR were analyzed by using ANOVA model CRD with confidence interval of 95%. The difference of each treatment to the response was analysis by Duncan's test. The water quality parameters were analyzed descriptively.

Results. The biological parameter measurements of TFC, FUE, PER, RGR, RLG_L and SR are presented in Table 2. Table 2 shows that dietary pineapple extract affected most of all biological parameters of *P. javanicus* ($P < 0.05$), except for the TFC and SR ($P > 0.05$).

Table 2

The biological parameters of TFC, FUE, PER, RGR, RLG_L and SR in *P. javanicus* fed on various doses of dietary pineapples extract for 35 days

Biological parameters	Dose of dietary pineapple extract in each treatment (%) and the results				
	A (0.00)	B (0.75)	C (1.50)	D (2.25)	E (3.00)
TFC (g)	65.73±3.15 ^a	67.60±1.64 ^a	67.11±4.13 ^a	66.88±3.16 ^a	65.86±4.70 ^a
FUE (%)	18.23±4.12 ^a	28.68±4.19 ^b	37.63±5.99 ^c	35.18±4.54 ^{bc}	34.84±5.12 ^{bc}
PER (%)	0.52±0.12 ^a	0.84±0.12 ^b	1.14±0.18 ^c	1.01±0.13 ^{bc}	1.01±0.15 ^{bc}
RGR (% day ⁻¹)	0.89±0.21 ^a	1.55±0.27 ^b	1.94±0.39 ^b	1.82±0.36 ^b	1.76±0.33 ^b
RGR _L (% day ⁻¹)	0.44±0.09 ^a	0.66±0.08 ^b	0.84±0.13 ^b	0.73±0.08 ^b	0.68±0.21 ^b
SR (%)	85.00±12.62 ^a	83.33±12.77 ^a	81.67±10.00 ^a	80.00±12.17 ^a	80.00±5.44 ^a

Note: Values of variable with same superscripts in the same row shows non-significant differences ($P > 0.05$).

TFC: total feed consumed, FUE: feed utilization efficiency, PER: protein efficiency ratio, RGR: relative growth rate, RLG_L: length-relative growth rate, SR: survival rate.

The polynomial orthogonal and regression model of variables FUE, PER, RGR and RGR_L are presented in Figure 1. Figure 1A-D shows that biological response variables of *P. javanicus* to the experimental feed containing various levels of pineapples extract are quadratic pattern, with the determination coefficient values (R^2) ranges between 0.92 and 0.97.

Furthermore, the calculation for an optimum value of dietary pineapple extract concentration which affected the best response of each variable is presented in Table 3. Figure 1 and Table 3 showed that the optimum levels of dietary pineapples extract that responded to the FUE, PER, RGR and RGR_L ranged between 1.85 to 2.13%.

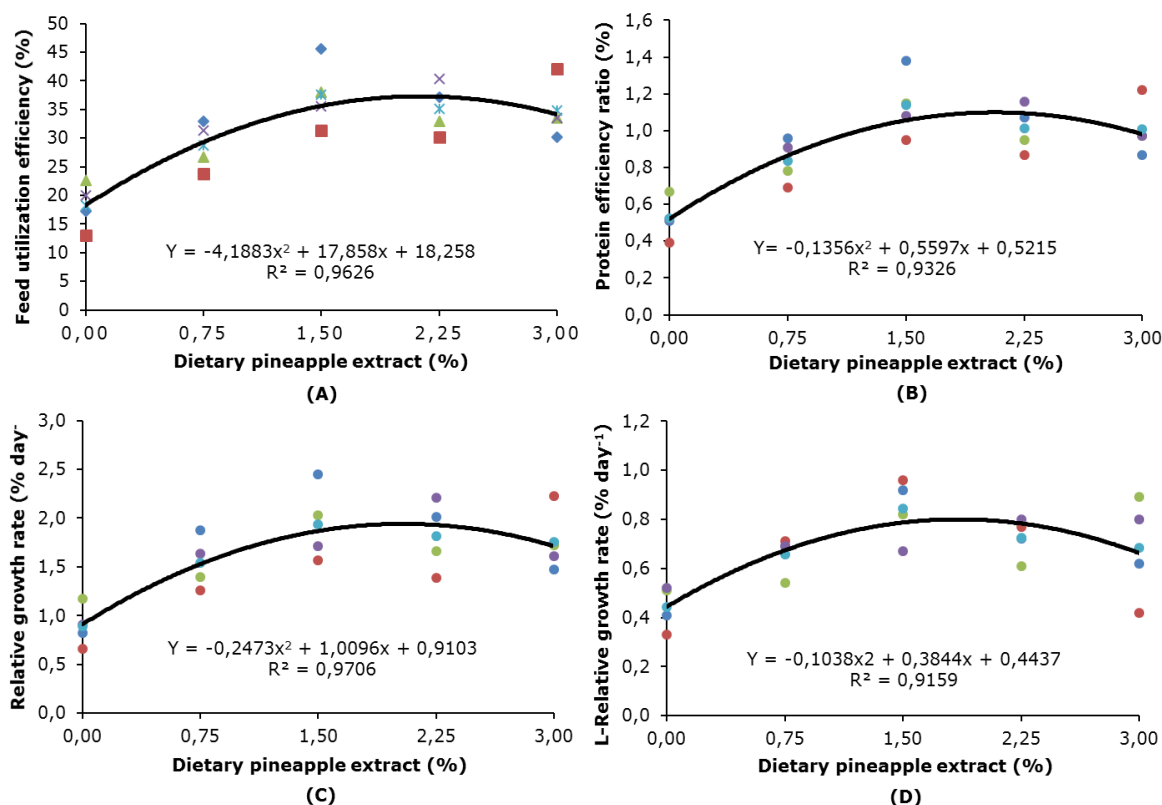


Figure 1. The polynomial orthogonal and regression model of response variables of *P. javanicus* to the experimental feed containing various levels of pineapple extract. Note: (A) Feed utilization efficiency, (B) Protein efficiency ratio, (C) Relative growth rate, (D) Length-Relative growth rate.

Table 3
Biological response variables and the regression equation of *P. javanicus* to the experimental feed containing various dietary pineapples extract

Response variable (Y)	Regression equation	Optimum level of dietary pineapples extract (%)	Maximum value of Y
FUE (%)	$Y = -4.1883x^2 + 17.858x + 18.258$	2.13	37.29
PER (%)	$Y = -0.1356x^2 + 0.5597x + 0.5215$	2.06	1.10
RGR (% day ⁻¹)	$Y = -0.2473x^2 + 1.0096x + 0.9103$	2.04	1.94
RGR _L (% day ⁻¹)	$Y = -0.1038x^2 + 0.3844x + 0.4437$	1.85	0.80

Note: FUE: feed utilization efficiency, PER: protein efficiency ratio, RGR: relative growth rate, RLG_L: length-relative growth rate.

Water quality. Measurements of water quality parameters during 35 days-rearing periods of *P. javanicus* varied among a suitable range for the fish life (Table 4).

Table 4
The water quality parameters of each treatment during 35 days-rearing periods in *P. javanicus*

Feeding trial	The range values of water quality parameters			
	Temperature (°C)	pH	DO (mg L ⁻¹)	TAN (mg L ⁻¹)
A (0.00)	24 – 29	6.4 – 7.0	3.6 – 4.9	0.131 – 0.292
B (0.75)	24 – 29	6.1 – 7.0	3.6 – 4.9	0.136 – 0.221
C (1.50)	24 – 29	6.4 – 6.9	3.0 – 4.8	0.112 – 0.241
D (2.25)	24 – 29	6.4 – 7.0	3.9 – 5.0	0.119 – 0.293
E (3.00)	24 – 29	6.3 – 6.9	4.0 – 5.1	0.109 – 0.269

Discussion. Java barb (*P. javanicus*) consumed equal amounts of the experimental feed containing various levels of pineapples extract (Table 2). The presence of pineapples extract did not affect the total feed consumption (TFC). This indicates that the addition of pineapple extract into trial feeds (or dietary pineapple extract) in various levels did not affect the feed palatability within the trial fish. In terms of TFC, Fran & Junius (2013) stated that TFC was influenced more by the energy balance of proteins in the feed instead of feed palatability. Our research found that TFC of all trial feeds which contained equal dietary protein levels is similar ($P > 0.05$) (Table 2), with the average value of about 66.64 g. Dietary energy that exceeded the demands would decrease consumption rate, so that the intake of other nutrients would decrease too, including proteins (Fran & Junius 2013; Hanief et al 2014). Table 1 shows that the energy to protein ratio (E/P ratio) of the trial feeds is relatively equal, i.e. 31.48 to 36.61 kJ g⁻¹ protein, and therefore the TFC was not significantly different ($P > 0.05$) (Table 2).

The presence of pineapple extract in feeds consumed by the fish increased the feed utilization efficiency (FUE) rate ($P < 0.05$). The addition of pineapple extracts up to 1.50% seems to increase the FUE value. When the dietary pineapple extract increased up and till 3.00%, it was not followed significantly by the FUE response (Table 2). FUE response to various concentrations of dietary pineapple extracts is shown in a quadratic pattern (Figures 1, Table 3). The regression equation of FUE in Table 3 shows that the optimum level of dietary pineapple extract for *P. javanicus* was 2.13%, with the maximum FUE value of 37.29%. The pineapples extract contains bromelain, with the enzyme's activity value of 0.775 units (Maryam 2009). In this research, the presence of pineapple extract containing bromelain in the feed possibly affected the feed utilization and protein absorption, and eventually promoted the fish growth.

The value of protein efficiency ratio (PER) describes the feed protein utilization rate for fish growth. The experimental feeds contained either protein or energy to protein ratios (E/P ratios) at similar amount, i.e. 33.69 to 24.71% with E/P ratios of 31.48 to 36.61 kJ g⁻¹ proteins (Table 1). The PER values increased in the trial feeds containing pineapple extract (Table 2). The interaction between dietary protein level and E/P ratio influenced the efficiency of dietary protein utilization (Nisrinah et al 2013). Considering that, for the trial feeds with equal nutritional content and feed ingredients (Table 1), the increase in PER value was more likely due to the presence of pineapple extract in the feed.

When protein from feed is more easily digested, the protein could be better utilized by the fish body, and therefore, it leads to better growth as well (Isnawati et al 2015). Bromelain in pineapple extract could also accelerate the feeding process. Therefore, the feed consumed by *P. javanicus* could be more efficiently utilized, as well as the protein utilization. Taqwadasbriliani et al (2013) stated that PER was influenced by the ability of fish to digest the feed. The PER response of *P. javanicus* to the increase of the dietary pineapple extract dose shows a quadratic pattern (Figure 1, Table 3). Based on the regression equation, the optimum level of PER was gained when the concentration of dietary pineapple extract was 2.06%. At this point, the maximum value of PER was 1.10% (Table 3). The proteins are more easily absorbed when bromelain is supplied and the amount of protein deposit in the fish body increases. In the case of *P. javanicus* fishes which consumed feed containing pineapple extract of 2.06%, the PER value increased to 211.36% or 2.11 times compared to the fishes which consumed feed without pineapple extract.

Pineapple extract added into the experimental feed significantly affected RGR of *P. javanicus* ($P < 0.05$). The fishes that consumed feeds containing pineapple extract had a better growth rate (Table 2). This was probably due to the roles of pineapple extract that helped hydrolyzing the feed nutrients, especially the proteins, so it promotes a better feed utilization. Furthermore, this phenomenon resulted on the higher growth rate. The response variable of RGR to the feeds containing various concentrations of dietary pineapple extract shows a quadratic pattern (Figure 1, Table 3). According to Amalia et al (2013) and Choi et al (2016), the more amount of enzyme added into the feed promotes

the more amount of proteins to be hydrolyzed into amino acids, and thereby increases the protein absorption and growth of the feeding fishes. On the other hand, if the amount of enzyme passes the optimum point, it could respond to a negative effect and might inhibit the fish growth (Amalia et al 2013). The regression equation of RGR shown in Table 3 indicates that the optimum concentration of pineapple extract in feed for the best growth response was 2.04%. Based on the statistical analysis, dietary pineapple extract significantly affects the growth of *P. javanicus* ($P < 0.05$) (Table 2), with the maximum growth rate of $1.94\% \text{ day}^{-1}$ when the dietary pineapple concentration is 2.04% (Figure 1, Table 3). Within the present experiment, if the fishes consume trial feed containing dietary pineapple extract of 2.04%, the RGR increases to 218.06% or 2.18 times compare to the fishes that consume trail feed with no pineapple extract.

Similar responses to RGR were found in RGR_{-L} variable. Taqwadasbriliani et al (2013) found that bromelain could activate collagen conversion into gelatin. In this case, bromelain hydrolyzes the gelatin molecule. Gelatin is one of the protein types from skin collagen, the bone's connective tissue (ligament) of fish. Gelatin serves as a bone constituent, so it could be beneficial for bone's growth. The response variable of RGR_{-L} of *P. javanicus* that consume trial feeds containing pineapple extract as a source of bromelain is presented in Figure 1, Table 2 and Table 3. Those data informed that the optimum concentration of dietary pineapple extract of 1.85% might lead to the maximum value of RGR_{-L} that was $0.80\% \text{ day}^{-1}$. At the optimum concentration of dietary pineapple extract, the RGR_{-L} increased up to 181.72%.

The survival rate (SR) of *P. javanicus* was not influenced by the concentration of dietary pineapple extract (Table 2). The SR of trial fish is certainly influenced by water qualities, especially the pH level (Fitria 2012; Anugraha et al 2014; Nyanti et al 2017). In this experiment, SR values of the trial fish during 35 days-rearing periods ranged between 80.00 to 85.00%, with the average value of 82.00%. Those values are relatively higher than that of a research where bigger fishes were used (Hanief et al 2014; Moniruzzaman et al 2015). The similarity of SR values among the treatments indicates that the water quality parameters along the experimental period matches to *P. javanicus* life requirements (Table 4) and are within the desirable limits of the fishes (Bhatnagar & Devi 2013).

Conclusions. Based on the research, it is suggested that the addition of pineapple extract into *P. javanicus* feed could increase the feed utilization efficiency (FUE), protein efficiency ratio (PER), weight and length growth (RGR and RGR_{-L}) until 181.72 to 218.06%. However, total feed consumed (TFC) and survival rate (SR) values were not significantly different among the treatments. The optimum concentration of dietary pineapple extract of about 1.85 to 2.13% could gain the maximum values of FUE, PER, RGR and RGR_{-L} for 37.29%, 1.10%, $1.94\% \text{ day}^{-1}$ and $0.80\% \text{ day}^{-1}$, respectively. The average value for TFC and SR was 66.64 g and 82.00%, respectively.

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