

EFFECT OF PAPAIN ENZYME SUPPLEMENTATION ON GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF CATFISH (*Pangasius hypophthalmus*)

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ABSTRACT

This study aims to evaluate the effect of papain enzyme diet on growth performance of Catfish (*Pangasius hypophthalmus*) through feed efficiency and growth parameters. The research employed a completely randomised design with four treatments and three replicates. Catfish fingerlings with an average weight of 2.23 g/fish were used as samples. The experimental diets were prepared to be isonitrogenous (31%) and isocaloric (252.06 kcal/g). Papain enzyme was added to the feed within various doses; A (0 g/kg feed), B (2 g/kg feed), C (4 g/kg feed), D (6 g/kg feed) and E (8 g/kg feed). The study parameters included apparent digestibility coefficient of protein (ADC_p), relative growth rate (RGR), the efficiency of feed utilisation (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR) and water quality. The results of the study show that the addition of papain enzyme significantly affected ADC_p, RGR, EFU, FCR, and PER, but not the SR of catfish. Based on regression analysis, the optimum dose of ADC_p and RGR were 4.05 g/kg, RGR was 4 g/kg feed, respectively. The EFU had an optimum dose of 3.93 g/kg feed, meantime the FCR and PER had an optimum dose of 4 g/kg feed each. In conclusion, the diet of papain in the amount of 4 g/kg feed increased the growth performance and nutrient utilisation of catfish (*Pangasius hypophthalmus*).

Key words: Diet, papain enzyme, catfish (*Pangasius hypophthalmus*), growth performance

INTRODUCTION

Catfish (*Pangasius hypophthalmus*) is one of freshwater fish which has good potential because it has a high market price and also easy to culture (Rathod *et al.*, 2018). An increase of catfish production will result in high demand for feed since catfish aquaculture requires much feed. One of the problems faced by fish farmers is low efficiency of feed utilisation and high feed cost (Hugues *et al.*, 2018). The efficiency of feed utilisation still needs further improvement. One of the efforts to increase efficiency is by adding enzyme in feed (Ebert, 2014).

The addition of enzyme has been proven to increase feed nutrient and reduce environmental

pollutions (Jiang *et al.*, 2013). Moreover, Ivar *et al.* (2013), reported that the addition of enzyme was able to improve feed nutrient, especially to boost protein utilisation. One of the enzymes is papain enzyme (Patil & Singh, 2014). Papain enzyme can break down amino acids. Thus, they are easy to digest (Amri & Mamboya, 2012). Ana *et al.* (2016) suggested that papain has numerous functions and can break down the main tissue (tissue, collagen, and myofibrillar protein).

Some studies on papain found that the addition of papain enzyme in feed can increase the growth of several species of fish. Singh *et al.* (2011) found that the addition of 2% enzyme per kg feed has the best result for *Cyprinus carpio* growth. Further, Farrag *et al.* (2013) reported that the addition of 6 g enzyme per kg feed promoted the best growth of *Oreochromis niloticus*. Patil and Singh (2014)

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suggested that the addition of 0.1% papain enzyme in feed resulted in the best growth of *Macrobrachium rosenbergii*. Khati *et al.* (2015) reported that 10 g addition of enzyme per kg feed gave the highest growth and protein efficiency of *Labeo rohita*. Muchlisin *et al.* (2016) also found that the addition of 27.5 g papain enzyme per kg feed can improve the best growth of Keureling fish (*Tor tambra*). Rostika *et al.* (2018) described that the papain enzyme 3%/kg feed boosted growth and protein efficiency ratio for *Oreochromis niloticus*. Rachmawati *et al.* (2018) proclaimed that the dose of papain enzyme of 0.3%/kg feed brought about the best protein digestibility, feed utilization efficiency, and growth for *Cherax quadricarinatus*. Moreover, Rachmawati and Samidjan (2018) expressed that the addition of papain enzyme 6 g/kg feed gave the best protein digestibility, feed utilisation efficiency, and growth for Sangkuriang Catfish (*Clarias* sp). However, there has been no research on the addition of papain enzyme in feed for catfish. Therefore, research on papain enzyme addition in feed for catfish needs to be conducted.

MATERIALS AND METHODS

Experimental design

The study was conducted from February to June 2017 at the Center for Hatchery and Freshwater Aquaculture, Muntilan, Central Java, Indonesia. The research employed a completely randomized design

with four treatments and three replicates. The four treatments were by adding 0 g papain enzyme per kg feed (treatment A), 2 g papain enzyme per kg feed (treatment B), 4 g papain enzyme per kg feed (treatment C), 6 g papain enzyme per kg feed (treatment D), and 8 g papain enzyme per kg feed (treatment E) (Farrag *et al.*, 2013).

Fish preparation

The fingerlings used in this study were catfish fingerlings with an average weight of 2.23 ± 0.30 g. The fish were acclimatized in disinfected-tank ($50 \times 30 \times 30$ cm) for a week and fed with manufactured feed. Prior to performing the experiment, the fish fasted for a day (Rachmawati *et al.*, 2017). The catfish were cultured for forty-two days (Dasuki *et al.*, 2013).

Feed preparation

The composition of the diets is depicted in Table 1. The diets were prepared to be isonitrogenous (31%) and isocaloric (252 kcal/g). The experimental diets were added 0.5% Cr₂O₃ and five different papain enzyme diets (NRC, 2011). Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish Water Aquaculture, Jepara, and Central Java, Indonesia. Before the experiment, feed nutrition was analysed by using proximate analysis (AOAC, 1990). The results of proximate analysis are presented in the following table.

Table 1. The composition and the results of proximate analysis

Ingredients diet composition	Treatment				
	A	B	C	D	E
Papain	0	2	4	6	8
Fish meal	34.76	34.55	34.32	34.20	34.08
Soybean meal	34.32	34.22	33.99	33.77	33.55
Corn meal	10.52	9.79	8.71	7.44	6.17
Rice bran	8.03	6.87	6.82	6.78	6.74
Dextrin	8.37	8.57	8.16	7.81	7.46
Fish Oil	1.5	1.5	1.5	1.5	1.5
Corn Oil	0.5	0.5	0.5	0.5	0.5
Min.Vit	1	1	1	1	1
CMC	1	1	1	1	1
TOTAL	100	100	100	100	100
Proximate analysis results					
Protein (%)	31.32	31.37	31.37	31.40	31.40
Fat (%)	7.03	7.04	7.04	7.04	7.04
BETN (%)	32.75	32.85	32.81	32.29	32.29
Energy (kcal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kcal/g Diet)	8.02	8.05	8.03	8.02	8.02

a. The values were calculated based on Digestible Energy (Glencross *et al.*, 2011) for 1 g protein equals 3.5 kcal, 1 g fat equals 8.1 kcal, and 1 g carbohydrate equals 2.5 kcal.

b. According to Brooke and Daniel (2013), the optimal E/P ratio for growth ranges from 8 kcal/g to 12 kcal/g.

c. *Animal Nutrient Laboratory, Faculty of Husbandry and Agriculture, Diponegoro University (2017).

Ingredient mixing to produce feed was done first by mixing fish meal and soybean meal. Then, papain enzyme was mixed into the mixed ingredients until the ingredients were mixed evenly. The mix was put for one hour to let the mix hydrolysed. At the same time, vitamin, mineral, and fish oil were mixed and diluted with water evenly. Then, the three was put into the dried mix (NRC, 2011). The mix of all ingredients was formed into pellet and dried. The feed pellet was then stored in a refrigerator before the feed pellet was given to the fish.

Performance analysis

The measured parameters were Apparent Digestibility Coefficient of Protein (ADC_p), Relative Growth Rate (RGR) and Survival Rate (SR), Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) (Maurício, 2011). The formula of the parameters is as follows:

The parameters were measured using the following equation:

$$ADC_p : 100 \left\{ \frac{\% Cr_2O_3 \text{ in the feed}}{\% Cr_2O_3 \text{ in the feces}} \times \frac{\% \text{ protein in the feces}}{\% \text{ protein in the feed}} \right\}$$

$$RGR : \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight} \times \text{Time experiment}} \times 100\%$$

$$SR : \frac{\text{Final count}}{\text{Initial count}} \times 100\%$$

$$EFU : \frac{(\text{Final weight} - \text{Initial weight})}{\text{The amount of feed consumed}} \times 100\%$$

$$FCR : \frac{\text{The amount of feed consumed}}{(\text{Final weight} + \text{Total weight fish death}) - \text{Initial weight}} \times 100\%$$

$$PER : \frac{\text{Final weight} - \text{Initial weight}}{\text{The amount of feed consumed} \times \text{Protein content of feed}} \times 100\%$$

Water quality, such as pH, dissolved oxygen (DO), temperature, and ammonia, were also examined.

Statistical analysis

The ADC_p, RGR, EFU, FCR, and PER data were evaluated using analysis of variance (ANOVA). The values were considered significant and highly significant at $p < 0.05$ and $p < 0.01$, respectively. The optimal dose of papain enzyme predicted using polynomial orthogonal test with SAS9 and Maple12 software.

RESULTS AND DISCUSSION

The results of Apparent Digestibility Coefficient of Protein (ADC_p), Relative Growth Rate (RGR), Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Survival Rate (SR) were presented in the (Table 2).

Apparent digestibility coefficient of protein (ADC_p)

The results of ANOVA showed that the supplementation of papain enzyme in the feed had a significant effect ($p < 0.05$) on ADC_p. The ADC_p increased after the addition of papain enzyme with the doses of 2-8 g/kg feed (treatments B, C, D, E). Among all treatments, the addition of 4 g/kg papain (treatment C) was the highest ADC_p (80.83%) followed by the treatments D (72.58%), E (70.25%), B (65.70%) and A (55.67%). It was expected that the highest value of ADC_p was due to the optimum dose that it could maximally hydrolyse protein; therefore, the digestibility of protein was higher in the treatment C than those in the treatments A,B,D,E. Mo *et al.* (2016) suggested that the addition of papain enzyme in the feed could increase the protein digestibility. The increase of the protein digestibility was because of the increase of protease enzyme in the fish digestive system after the fish had been fed with papain enzyme supplemented feed (Sing *et al.*, 2011; Dabrowski

Table 2. The values of investigated parameters

Experiment data	Diet treatments				
	A	B	C	D	E
ADC _p	55.67±0.02 ^d	65.70±0.03 ^c	80.83±0.05 ^a	72.58±0.04 ^b	70.25±0.05 ^b
EFU (%)	50.12±0.24 ^c	65.26±0.97 ^b	75.09±0.75 ^a	67.15±0.26 ^b	66.25±0.57 ^b
RGR (%/day)	3.48±0.10 ^d	4.26±0.25 ^c	8.01±0.27 ^a	6.89±0.16 ^b	5.33±0.14 ^b
FCR	2.65±0.15 ^c	2.26±0.14 ^b	1.56±0.03 ^a	2.20±0.22 ^b	2.16±0.21 ^b
PER	1.80±0.05 ^c	2.00±0.26 ^b	3.75±0.06 ^a	2.34±0.27 ^b	2.38±0.13 ^b
SR (%)	92.33±5.77 ^a	92.00±5.10 ^a	92.33±5.77 ^a	93.33±5.77 ^a	93.00±5.78.0 ^a

Note: Apparent Digestibility of Protein (ADC_p), Relative Growth Rate (RGR), Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Survival Rate (SR). The Values with the same superscripts in the column show that there was no difference.

& Glogowski, 1977). The high digestibility of the protein increases the digestion level on fish (Lanari *et al.*, 1998). According to Khati *et al.* (2015) the increase of the protein digestibility was because of the ability of the papain enzyme to hydrolyse protein. Moreover, Rachmawati *et al.* (2018) discovered that the addition of papain enzyme in the feed could hydrolyse protein that boosts protein digestibility. The findings showed that Patin catfish given feed with the addition of 4 g papain enzyme per kg feed resulted in the highest value of ADC_p. The dose of 4 g papain enzyme per kg feed also yielded the highest value of EFU (75.09%) and the lowest of FCR (1.75). The value of ADC_p was in line with the value of EFU, but the opposite with the FCR. Similar studies were also reported by Fateme *et al.* (2012), Patih and Singh, (2014), Kumar *et al.* (2011), Rachmawati *et al.* (2018), Rachmawati and Samidjan, (2018).

The relation between ADC_p and papain enzyme supplemented feed generated a quadratic function, $Y = -0.859x^2 + 8.6744x + 54.926$, $R^2 = 0.85$. Based on the equation, the optimum dose of ADC_p was obtained at 4.05 g/kg feed, while the optimum value of EFU is 75.09%.

The efficiency of feed utilisation (EFU)

The Catfish (*Pangasius hypophthalmus*) fed with the addition of papain enzyme with various doses ranged from 2 to 8 g/kg feed had a higher value of EFU than that without addition of papain enzyme. It was due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyse protein; therefore, the protein was easily digested to increase feed utilisation efficiency. This phenomenon was also discovered by Hastuti (2001). Rachmawati and Samidjan (2018) also found that the addition of papain enzyme in the diet could improve feed usage efficiency. Treatment C (4 g/kg feed) gave the highest EFU (75.09%) followed by treatments D (67.15%), E (66.25%), B (65.26%) and A (50.12%). The high value of EFU in Catfish (*Pangasius hypophthalmus*) was expected due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyse protein; therefore, the protein was easily digested to increase feed utilisation efficiency. It was supported by the study that that dose resulted in the highest ADC_p. The high value of EFU suggested that less protein has been used for metabolism, but more protein has been used for fish growth (Tacon, 2002). Moreover, NRC (2011) suggested that the high value of EFU indicated high quality of the feed; therefore, the feed can be utilised more efficiently. Manguti *et al.* (2014) also reported that the high value of EFU suggested that less protein has been used for metabolism, but more protein has been used for fish growth. The values of EFU in each treatment in the

study was considered quite good since the values were higher than 50%. The good value of EFU was based on the Craig dan Helfrich (2002) opinion. They claimed that the value of EFU was good if the value was higher than 50% or even it reached 100%. Similar results were found by Patil and Singh (2014) in *Macrobrachium rosenbergii*, Muchlisin *et al.* (2016) in Keureling fish (Tor tambra), Rostika *et al.* (2018) in *Oreochromis niloticus*, Rachmawati *et al.* (2018) in *Cherax quadricarinatus*, Rachmawati and Samidjan (2018) in Sangkuriang Catfish (*Clarias* sp).

The relationship between papain enzyme and EFU is presented in the Figure 2. The relationship generated a quadratic equation, $Y = -0.8902x^2 + 8.829x + 50.823$, with the value of $R^2 = 0.89$. From the equation, the optimum dose of EFU was at the level of 4 g papain enzyme per kg feed. The maximum value of ADC_p was 81.25.

Relative growth rate (RGR)

The values of RGR in the Catfish (*Pangasius hypophthalmus*) fed with papain enzyme supplemented diet were higher than that without papain enzyme supplemented diet. It was due to the right dose of papain enzyme addition in the diet to hydrolyse protein; therefore, the protein was easily digested to increase fish growth. The finding was also supported by Singh *et al.* (2011). They suggested that papain enzyme is the protease enzyme that hydrolyses protein. The protease enzyme is the key enzyme to boost protein digestibility, accelerate absorption, and enhance growth. Wong *et al.* (1996) pointed out that papain can break down protein into amino acids; therefore, it increased protein digestibility, nutrients absorption, and fish growth. The highest value of RGR (8.10%/day) was in the treatment C (4 g/kg feed) followed by treatments D (6.89%/day), E (5.33%/day), B (4.26%/day) and A (3.48%/day). The highest value of RGR was obtained in the treatment C (4 g/kg feed). It was expected due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyse protein; therefore, the protein was easily digested to improve fish growth. The dose of papain enzyme addition in the diet (4 g/kg feed) brought about the highest value of RGR. It also generated the highest values of ADC_p, EFU, and PER. The lowest value of RGR was obtained in the treatment A (0 g/kg feed). It was suspected that protein hydrolysis was not in the best performance under zero addition of papain enzyme in the diet; therefore, protein digestibility and feed usage efficiency became low. The zero addition of papain enzyme in the diet leads to the low RGR and low ADC_p, EFU, and PER. Without papain enzyme, the hydrolysis of long-chain peptide into short-chain peptide will not occur. As a result, protein

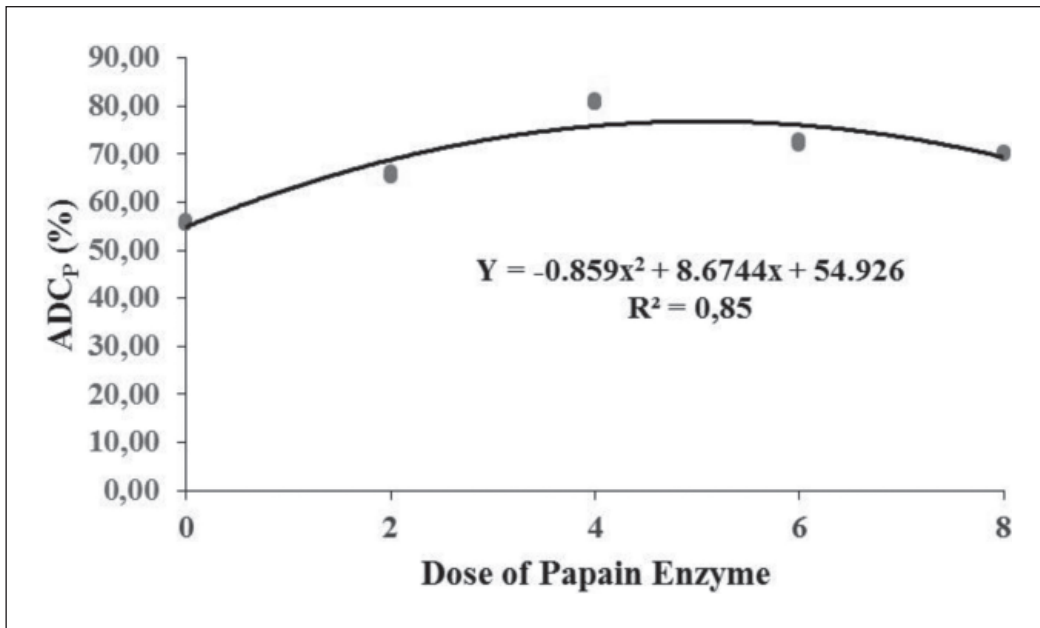


Fig. 1. The relationship between the dose of papain enzyme and ADC_p.

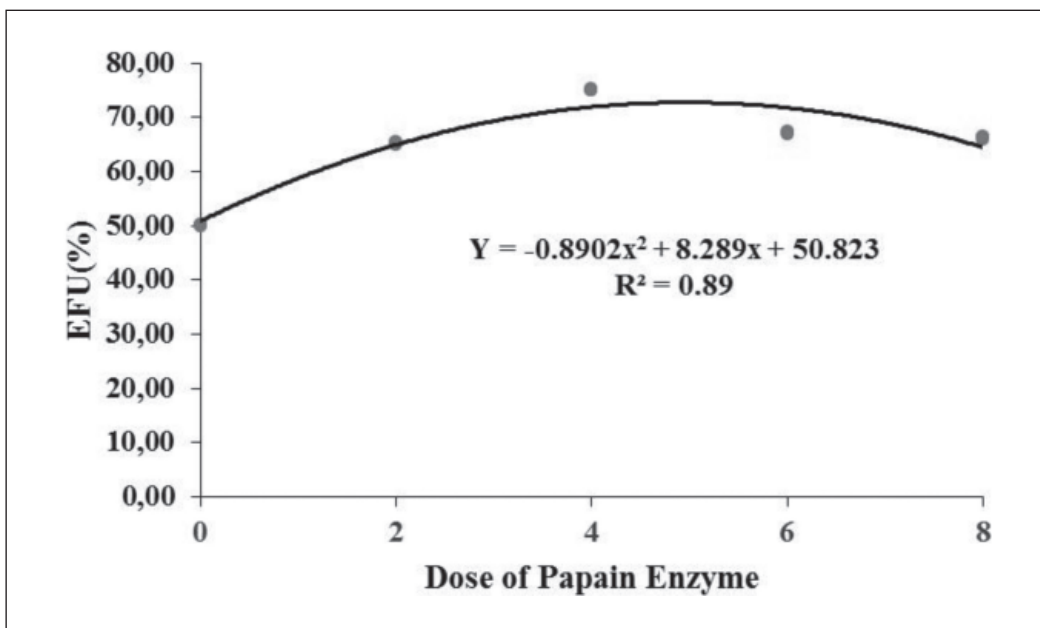


Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypophthalmus*).

digestibility and diet utilisation efficiency decreased. The fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolyzes polypeptide into a short-chain peptide. The availability of short-chain peptide is an important factor to increase protein digestibility, nutrient absorption, and growth (Bo Li *et al.*, 2012). Moreover, papain enzyme can also hydrolyse lipid and carbohydrate in the diet (Ryosuke & Kazuhiko, 2004). The inappropriate dose of the papain enzyme

in the diet, whether the dose was a deficiency or excessed, could hinder fish growth (Kazerani & Shahsavani (2011). Nutrient digestibility and absorption were obstructed because non-starch polysaccharide was nonsoluble. The release of galactose and xylose from not-starch polysaccharide was due to the addition of papain enzyme in the diet. Similar findings were discovered in the studies of *Chanos chanos* (Singh *et al.*, 2011), *Macrobrachium rosenbergii* (Patil & Singh, 2014),

Oreochromis niloticus (Manguti *et al.*, 2014), *Labeo rohita* (Khati *et al.*, 2015), *Trachinotus blochii* (Mo *et al.*, 2016), *Cherax quadricarinatus* (Rachmawati *et al.*, 2018), *Clarias* sp. (Rachmawati & Samidjan, 2018).

The relationship between papain enzyme and RGR was presented in Figure 3. The relationship has a quadratic equation, $Y = -0.1705x^2 + 1.6808x + 2.9637$, $R^2 = 0.76$. The optimum RGR was obtained from a dose of 3.93 g/kg feed. The maximum value of RGR is 7.11%/day.

Feed conversion ratio (FCR)

The values of FCR in which the feed was supplemented with papain enzyme with the doses of 2-8 g/kg feed were lower than that without enzyme addition. It was suggested that the addition of papain enzyme could boost protein digestibility and feed usage efficiency; in turn, it decreased feed conversion ratio. The lowest FCR was found in the treatment C (4 g/kg feed). It was suggested that at the dose of 4 g/kg feed protein digestibility and feed usage efficiency were maximised; therefore, it decreased FCR. The low value of FCR indicated that the fish was optimally digested and absorbed the nutrient (Steffens, 1989). Singh *et al.* (2011) studied in *Chanos channos* that the low value of FCR was due to the 2% addition of papain enzyme in the feed. Khati *et al.* (2015) reported that the FRC in *Labeo rohita* decreased after the feed had been supplemented with ten papain enzymes per kg feed. Muchlisin *et al.* (2016) stated that Keureling fish (*Tor tambra*) had low FCR after the fish had been given papain enzyme supplemented diet with the

dose of 27.5 mg papain enzyme per kg feed. Rachmawati *et al.* (2018) studied in *Cherax quadricarinatus*, the lowest FCR (1.76) was obtained by adding 0.3% papain enzyme in the diet. Moreover, Rachmawati and Samidjan, (2018) found that the low value of FCR in Sangkuriang Catfish (*Clarias* sp.) had occurred when the fish was fed with the feed supplemented with 6 g papain enzyme.

The relationship of papain enzyme and FCR had a quadratic equation (Figure 4). The equation was $Y = -0.0386x^2 - 0.3586x + 2.6746$ with the value of $R^2 = 0.70$. The optimum dose of papain enzyme on FCR is 4 g/kg feed. The maximal value of FCR is 1.56.

Protein efficiency ratio (PER)

Protein Efficiency Ratio is an indicator to measure the source of protein in the feed to fulfil the need for essential amino acids of fish (Manush *et al.*, 2013). Catfish (*P. hypophthalmus*) that was given papain enzyme supplemented diet with the doses of 2-8 g/kg feed had higher values of PER (2.00-3.75) compared to the PER (1.80) without the addition of papain enzyme. It was suggested that the addition of papain enzyme in the feed could increase protein hydrolysis into amino acids; therefore, it was easier to absorb and to build protein in the fish. Hephher (1988) indicated that the high value of PER represented high protein efficiency and high growth. The highest PER in the Catfish (*P. hypophthalmus*) was obtained at the dose 4 g papain enzyme per kg feed. The dose level was the right amount of papain enzyme addition into the feed to increase protease enzyme activities in the

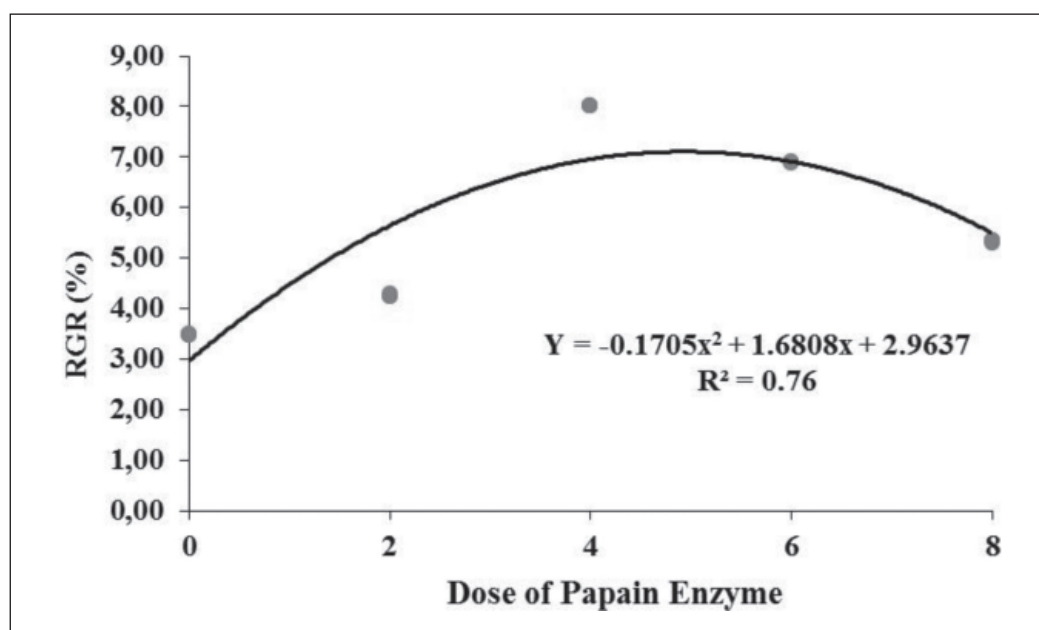


Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypophthalmus*).

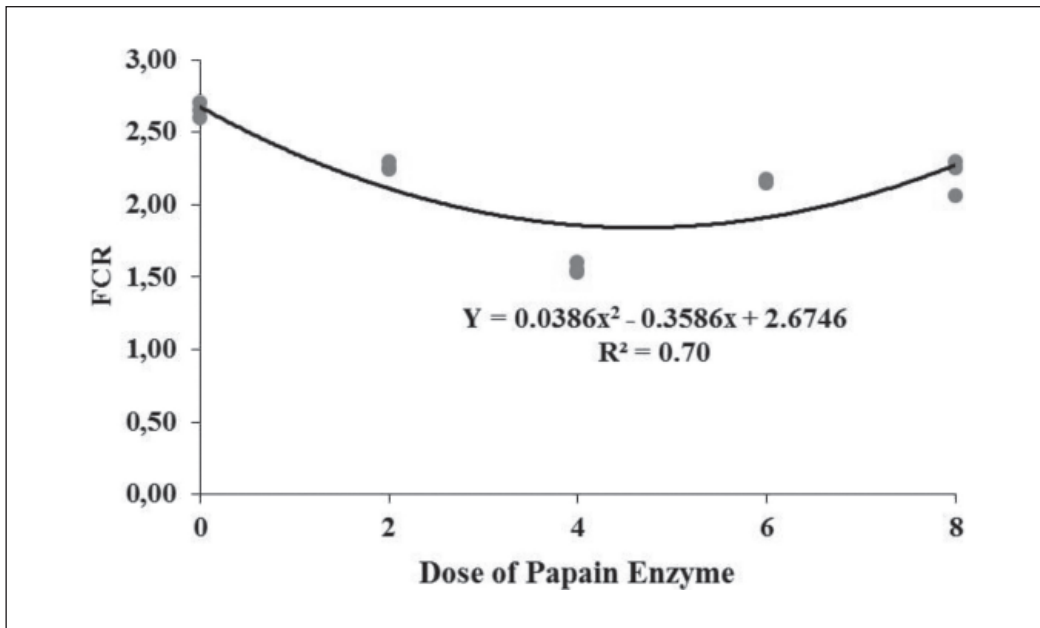


Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypophthalmus*).

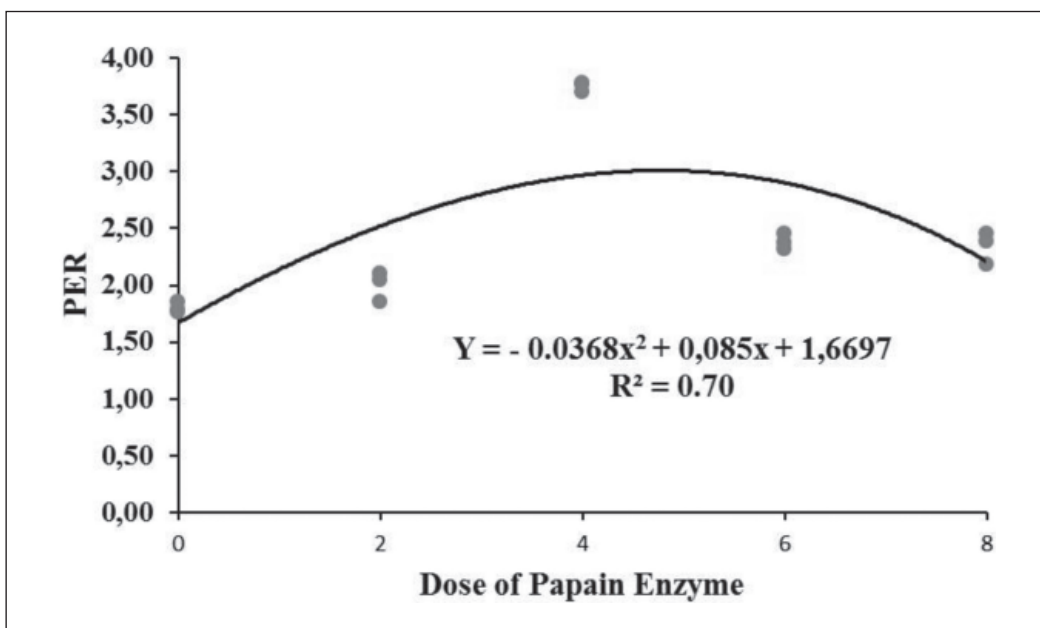


Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypophthalmus*).

digestive system; therefore, protein digestibility and efficiency of feed utilisation increased to build protein in the fish. The value of PER moved along the values of ADC_P and EPP. Similar results were found by Singh *et al.* (2011) in *Channos channos*, Khati *et al.* (2015) in a *Labeo rohita*, Rachmawati *et al.* (2018) in *Cherax quadricarinatus*, Rachmawati and Samidjan (2019) in *Clarias sp.*

The relationship between papain enzyme and PER was presented in Figure 5. The relationship had a quadratic equation of $Y = -0.0386x^2 - 0.3586x$

$+ 2.6746$, $R^2 = 0.70$. The graph showed that the optimum dose of papain enzyme for PER was 4 g/kg feed with the maximal PER value of 3.75.

Survival rate (SR)

In this study, the survival rate of the fish was quite high (92.33%–93.33%), although the addition of papain enzyme did not affect SR of Catfish (*P. hypophthalmus*). The finding was supported by the findings of Patih and Singh (2014); Dabrowski and Glogowski (1977). They claimed that proteolytic

enzyme does affect the fish survival rate. Yakuputiyage (2013) also discovered that survival rate was not influenced by the feed intake; otherwise, the survival rate was influenced by the initial condition of the fish and water quality. Similar results were obtained in *Channos channos* (Singh *et al.*, 2011), *Macrobrachium rosenbergii* (Manush *et al.*, 2013), *Labeo rohita* (Khatai *et al.*, 2015), *Cherax quadricarinatus* (Rachmawati *et al.*, 2018), Sangkuriang Catfish (*Clarias* sp.) (Rachmawati & Samidjan, 2018).

CONCLUSION

Papain enzyme increased apparent digestibility of protein, growth rate, efficiency of feed utilisation, and protein efficiency ratio. On the other hand, it reduced feed conversion ratio of catfish. The optimum dose of ADC_p parameter was 4.05 g/kg feed, while the optimum dose of RGR was 4 g/kg feed. The EFU had an optimum dose of 3.93 g/kg feed, meantime the FCR and PER had an optimum dose of 4 g/kg feed each. In conclusion, the papain enzyme has a positive effect on the growth performance of catfish (*P. hypophthalmus*).

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