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JURNAL : Malaysian Applied Biology

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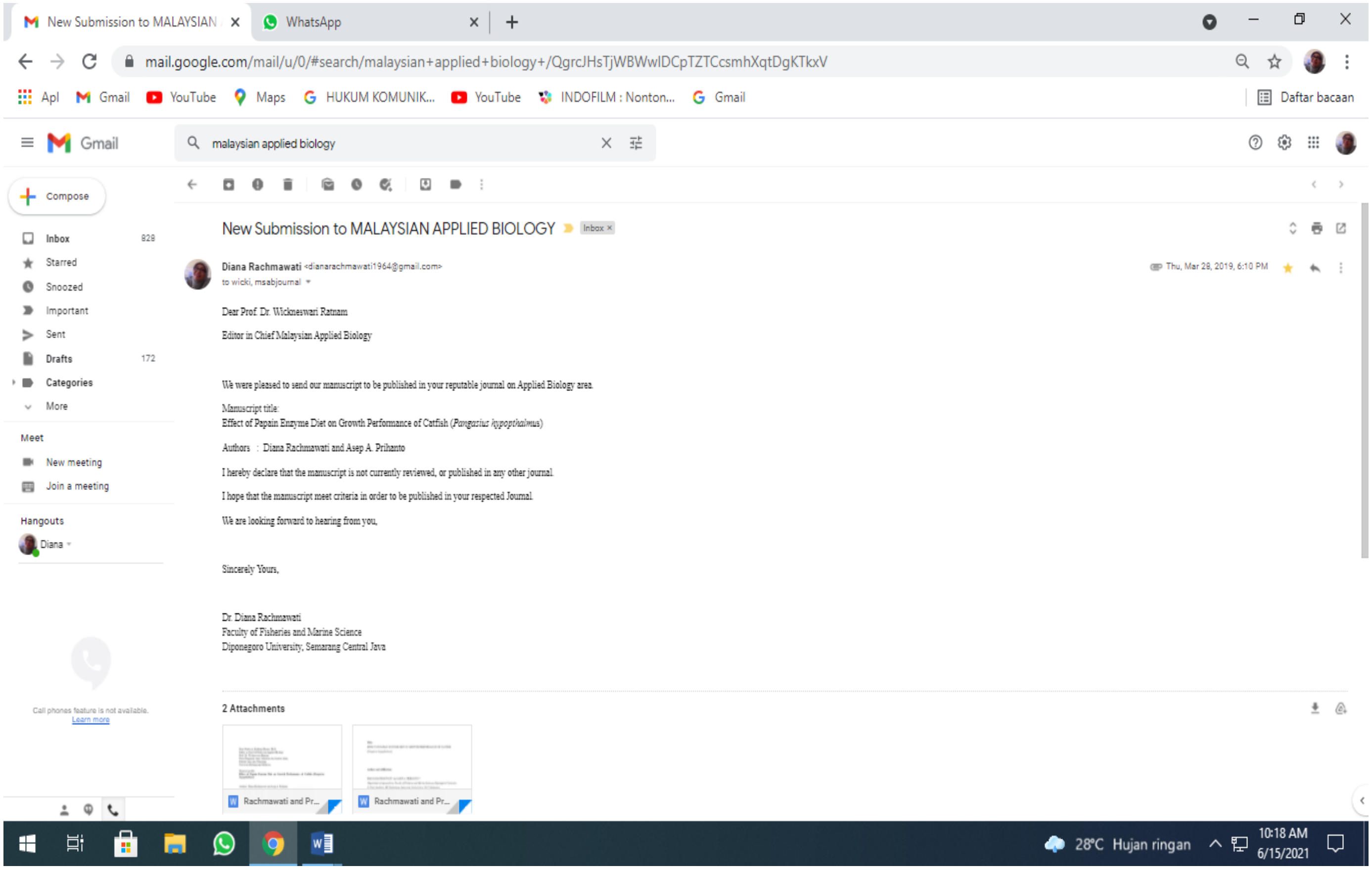
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Authors : Diana Rachmawati and Asep A. Prihanto

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We are looking forward to hearing from you,

Sincerely Yours,

Dr. Diana Rachmawati
Faculty of Fisheries and Marine Science
Diponegoro University, Semarang Central Java

2 Attachments

Rachmawati and Pr...

Rachmawati and Pr...

Dear Professor Kuldeep Dhama, Ph.D.

Editor in Chief of Malaysian Applied Biology

Prof. Dr. Wickneswari Ratnam

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Manuscript title:

Effect of Papain Enzyme Diet on Growth Performance of Catfish (*Pangasius hypopthalmus*)

Authors: Diana Rachmawati and Asep A. Prihanto.

Prof. Dr. Wickneswari Ratnam,

We were pleased to send our manuscript to be published in your reputable journal on Malaysian Applied Biology.

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The manuscript investigate effect of enzyme papain diet on growth performance of Cat Fish. In this study we showed that the diet of the papain enzyme positively affected the growth performance of the catfish.

I hope that the manuscript meet criteria in order to be published on your respected Journal.

Sincerely Yours,

Diana Rachmawati,

Department of Aquaculture,

Faculty of Fisheries and Marine Sciences,

Diponegoro University,

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Title:

EFFECT OF PAPAIN ENZYME DIET ON GROWTH PERFORMANCE OF CATFISH (*Pangasius hypopthalmus*)

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Running Title:

Papain on Catfish (*Pangasius hypopthalmus*) growth perrformance

Keywords:

Feed, Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

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ABSTRACT

This research aims to evaluate the effect of papain enzyme diet on growth performance of Catfish (*Pangasius hypopthalmus*) through feed efficiency and growth parameters. The research employed completely random design. Four treatments were conducted and each treatment was repeated 3 times. Catfish fingerlings with an average weight of 2.23 g/fish were used as samples. The experimental feed diets were designed based on isoprotein (31%) and isoenergy (252.06 kKal/g). The treatment was the addition of papain enzyme in feed with various doses, namely A (0 g/kg feed), B (2g/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), efficiency of feed utilization (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 ADCp, RGR, EFU, FCR, and PER, but not the SR of catfish. The optimum doses of ADCp, RGR, EFU, FCR, and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed respectively. In conclusion, a diet diet of papain enzyme increased the growth performance of catfish (*Pangasius hypopthalmus*).

Keywords: Feed, Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

INTRODUCTION

Catfish (*Pangasius hypopthalmus*) is one of fresh water fish which has a good potential because it has a high market price and also easy to culture. An increase of catfish production will result in a high demand of feed, since catfish aquaculture requires a lot of feed. One of the problems faced by fishermen is low efficiency of feed utilization and high feed cost compared to the total production cost (Hugues et al., 2018). The efficiency of feed utilization 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 additon of enzym was able to improve feed nutrient, especially to boost protein utilization. One of the enzymes is papain enzyme (Patil and Singh, 2014). Papain enzyme is able to break down amino acids. Thus, they are easy to digest (Amri and Mamboya, 2012). Ana et al. (2016) suggested that papain has numerous functions and can break down main tissue (tissue, collagen, and myofibrilar 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 supported the best growth of *Oreochromis niloticus.* Patil and Singh (2014) suggested that the addition of 0.1% papain enzyme in feed resulted in the best growth of *Macrobrachium rosenbergi*. 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*). 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 Fingerlings and Freshwater Aquaculture, Muntilan, Central Java, Indonesia. Research design and analysis employed ANOVA in Completely Random Design. The research was performed using four different experiments with three replications. Four different diet concentration of papain enzyme (extracted from Papaya Latex), A = 0 g/kg, B = 2 g/kg), C = 4 g/kg, D=6 g/kg, and E=8 g/kg diet, respectively, were added to the feed (Farrag et al., 2013). The fish species for this study is Catfish (*Pangasius hypopthalmus*).

Fish preparation

The fingerlings used in this study were catfish fingerlings with an average weight of 2.23±0.30 g/individual. The fish were adapted in disinfected-tank (50x30x30 cm^3) 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 feeds (Table 1) was designed with isoprotein (31%) and isoenergy (252 Kkal/g). The experimental pellet feeds were added 0.5% Cr2O3 and five different papain enzyme diets (NRC, 1993). Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish Water Aquaculture, Jepara, Central Java, Indonesia. Prior to the experiment, feed nutrition was analyzed by using proxymate analysis (AOAC, 1990). The result of proxymate analysis is presented in (Table 1).

Table 1. insert here..

Ingredient mixing to produce feed was done by firstly 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 hidrolyzed. At the same time, vitamin, mineral and fish oil were mixed and dillated with water evenly. Then, the mix was put into the dried mix (NRC, 1993). 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; Julie, 2014). 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). P value of $p<0.05$ and $p<0.01$ are considered as significant and highly significant, respectively. To determine the optimal dose of papain enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12.

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).

Table 2. insert here…

Apparent Digestibility Coefficient Of Protein (ADC_P)

The ADC_P increased after the addition of papain enzyme with the dose of 2-8g/kg feed. Among all treatments, the addition of 6 g/kg papain (treatment C) was the highest ADCp followed by the treatment D (72.58%), E (70.25%), B (65.70%) and A (55.67%). Papain enzyme successfully hydrolized protein. Hence, the food digestibility increased. This study is in line with (Fateme et al., 2012; Patih and Singh, 2014) who concluded that the protease enzyme increased protein digestibility. Moreover, Kumar et al. (2011) found that papain enzyme can unbind phytate acid and phophor so that the availability of phophor in the feed increases.

The dose of 2 g papain enzyme per kg feed resulted in the highest ADCp. This was followed by the high EFU (75.09%) and low FCR (1.75). It is suggested that the higher the protein digestibility, the higher the efficiency of feed utilization, but the lower the feed conversion ratio. Similar results were also reported by Singh et al. (2011), Muchlisin et al. (2016) and Mo et al. (2016).

The effect of papain enzyme and ADCp was explained, as depicted in (Figure 1.), it followed quadratic function, $Y = -0.859x^2 + 8.6744x + 54.926$, $R^2 = 0.85$. Based on the equation, the optimum dose was obtained at 4.05 g/kg feed.

Efficiency of Feed Utilization (EFU)

The highest EFU (75.09%) was obtained by C treatment (4 g/kg feed). In contrast, the lowest EFU of 50.12% was obtained by A treatment (0 g/kg feed). The high value of EFU suggested that less protein has been used for methabolism, but more protein has been used for fish growth. From this result, it is suggested that papain enzyme can improve feed utilization efficiency. This result is suported by Muchlisin et al. (2016), indicating that the addition of papain enzyme increased feed efficiency on Keureling fish (*Tor tambra*). Compared to our result, Catfish (*Pangasius hypopthalmus*) needs higher papain enzyme than Keureling fish (*Tor tambra*)**.** The Catfish needs 4 g/kg feed while Keureling fish needs only 27.5 mg/kg.

The relationship between papain enzyme and EFU is presented in (Figure 2.), it followed quadratic equation, $Y = -0.8902x^2 + 8.829x + 50.823$, $R^2 = 0.89$. From the equation, the optimum dose for EFU (75,09%) was at the level of 4 g/kg feed.

Relative Growth Rate (RGR)

The catfish fed with papain enzyme has higher RGR. The highest value of RGR (8.10%) was in C treatment (4 g/kg feed), while the lowest value of RGR (3.48%) was in A treatment (0 g/kg feed). It seemed that the absence of enzyme negatively affected the RGR. This result is in line with the earlier result. The addition of papain enzyme can increase the growth of several species, such as *Chanos channos* (Singh et al., 2011), *M. rosenbergii* (Patil and Singh, 2014), *Oreochromis niloticus* **(**Manguti et al., 2014), *Labeo rohita* (Khati et al., 2015), Goldlined seabream (*Rhabdosargus sarba*), brown spotted grouper (*Epinephelus bleekeri*) and pompano (*Trachinotus blochii*) (Mo et al., 2016).

Without papain enzyme, the hydrolisys of long chain peptide into short chain peptide will not occurr. As a result, protein digestibility and feed utilization efficiency decrease. Fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolizes polypeptide into 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 hydrolize lipid and carbohydrat in the feed (Ryosuke and Kazuhiko, 2004).

The relationship between papain enzyme and RGR was presented in the 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 the dose of 3.93 g/kg feed. The optimum value of RGR is 7.11%.

Feed Conversion Ratio (FCR)

The addition of papain enzyme with the dose of 2-8 g/kg feed can reduce FCR. It was found that the enzyme addition resulted in better FCR. The dose of 4 g/kg feed had the lowest FCR (1.56). This result is different from the research with different fish. Similar studies on *Chanos channos* and prawn (*Macrobrachium rosenbergii*) were conducted by Singh et al., (2011) and Patil and Singh (2014). They found that the doses of 2% and 0.1% of papain enzyme on the diet were the best doses for producing low FCR, high growth, high protein digestibility, and high ratio of protein efficiency. Khati et al. (2015) also reported that the addition of 10 g/kg feed of papain enzyme in the feed increased feed digestibility and reduced FCR on *Labeo rohita*.

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

Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) is an indicator which shows the performance of protein in the feed to provide amino acids for fish growth (Manush et al., 2013). PER of fish which was fed with the addition of papain enzyme increased on all tretments (2-8 g/kg feed) as much as 2.00-3.75. The value of PER was always higher than that (1.80) without papain enzyme. The highest PER was obtained in the treatment C (4 g/kg feed). It was expected due to the right dose that can provide amino acids. Similar results were also reported by Singh et al. (2011) and Khati et al. (2015). Singh et al. (2011) found that the addition of 2% papain enzyme in the feed on *Channos*

channos increased protein efficiency ratio by 2.24, while Khati et al. (2015) found that the addition of 10 g/kg feed increased protein efficiency ratio by 2.30.

The relationship between papain enzyme and PER is presented in Figure 5. The relationship has a quadratic equation of $Y = - 0.0386x^2 - 0.3586x + 2.6746$, $R^2 = 0.70$. The graph shows that the optimum dose of papain enzyme for PER was 3.77 g/kg feed with the PER value of 3.

Survival Rate (SR)

In this study, the addition of papain enzyme had no effect on SR of catfish (*Pangasius hypopthalmus*). As reported by Patih and Singh (2013), proteolitic enzyme does affect the fish survival rate. Moreover, Yakuputiyage (2013), reported that feed is not a factor influencing survival rate, but initial treatment and media quality affect survival rate. Similar results were obtained on *Channos channos* (Singh et al., 2011), *Macrobrachium rosenbergii* (Patil and Singh, 2014) and *Labeo rohita* (Khati et al., 2015).

CONCLUSION

Papain enzyme increased apparent digestibiity of protein, growth rate, efficiency of feed utilization, and protein efficiency ratio. On the other hand, it reduced feed conversion ratio of catfish. The optimum doses on ADC_P, RGR, EFU, FCR and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed, respectively. In conclusion, the papain enzyme has a positive effect on the growth performance of catfish (*Pangasius hypopthalmus*).

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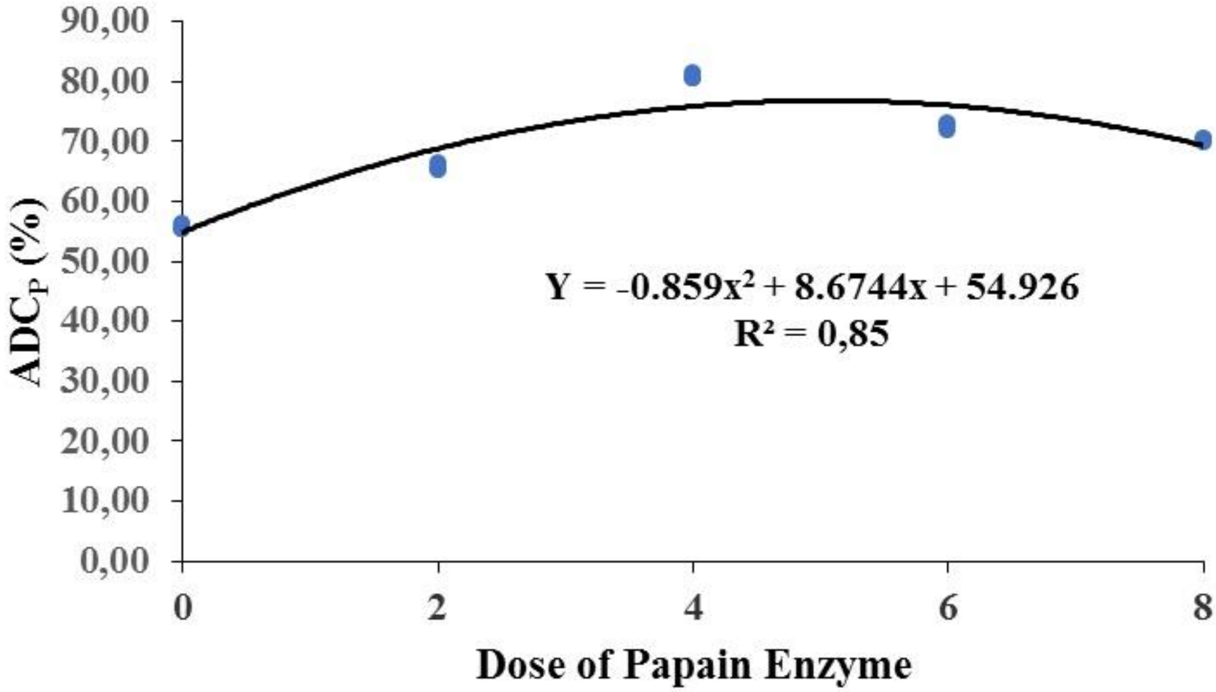


Fig. 1. The relationship between the dose of papain enzyme and ADC_P.

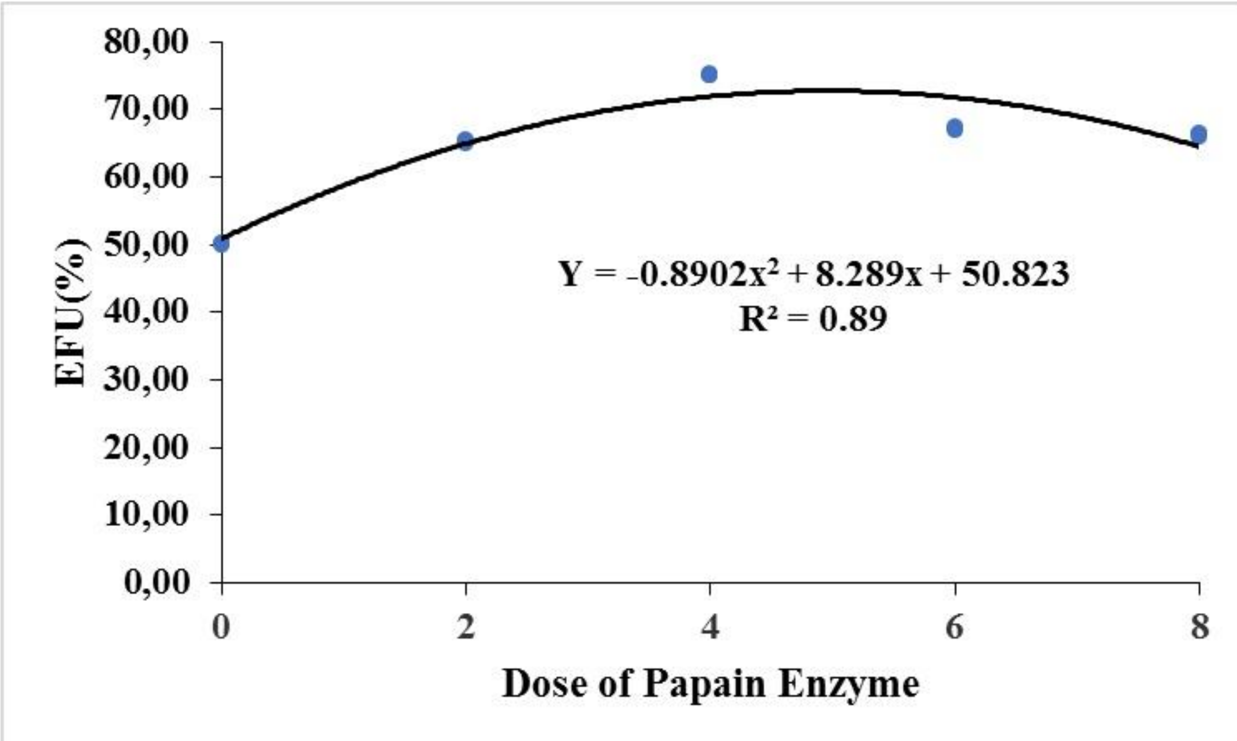


Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*)

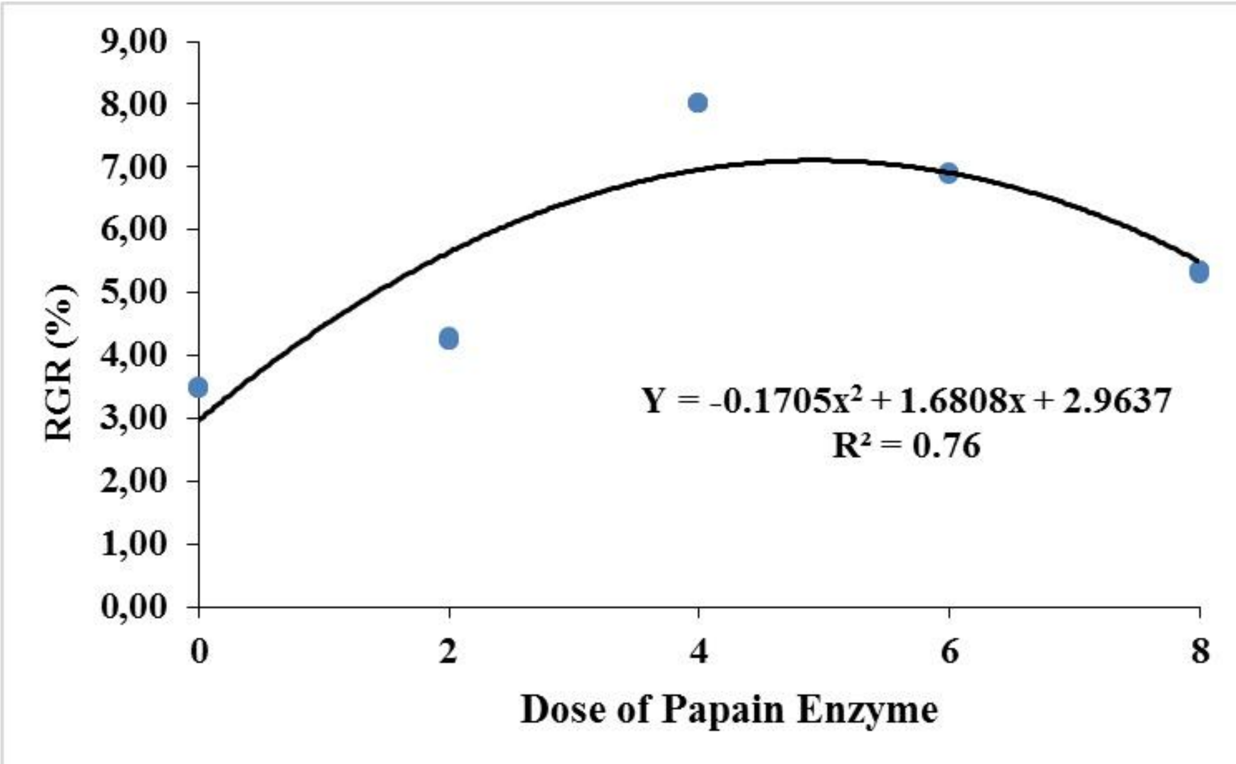


Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*)

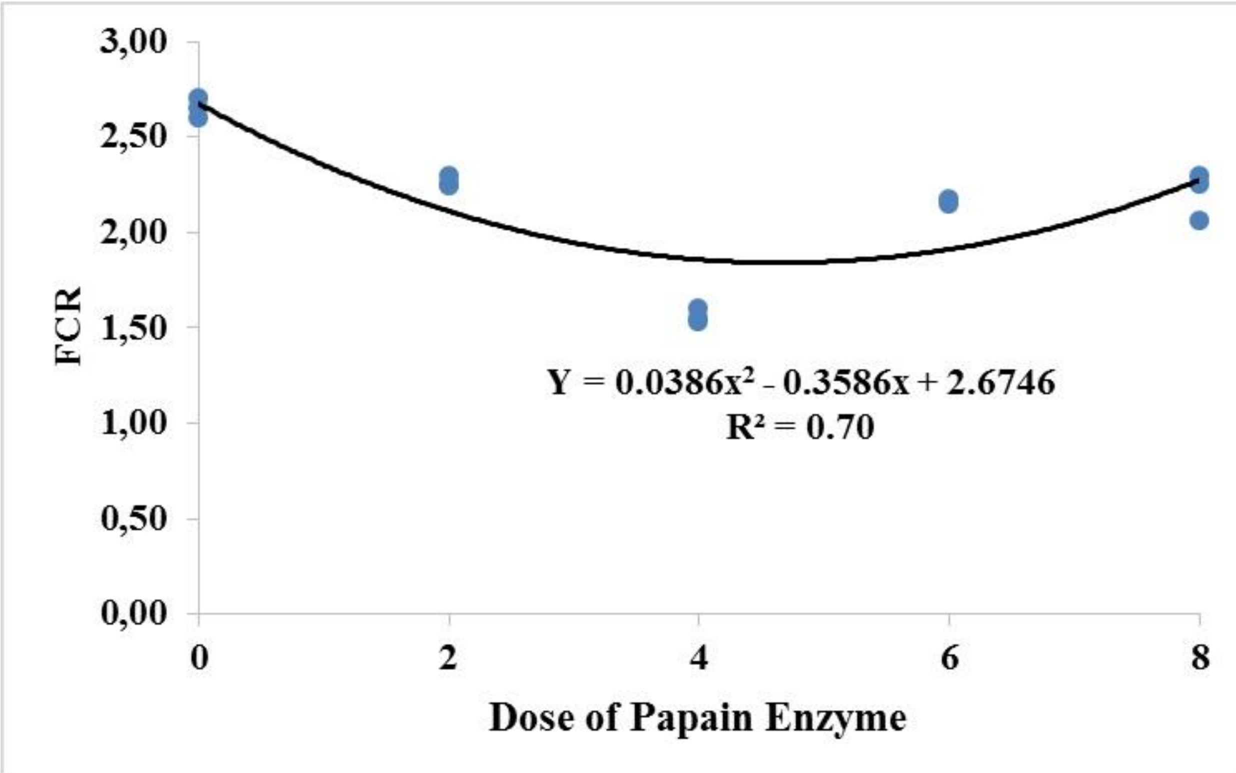


Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*)

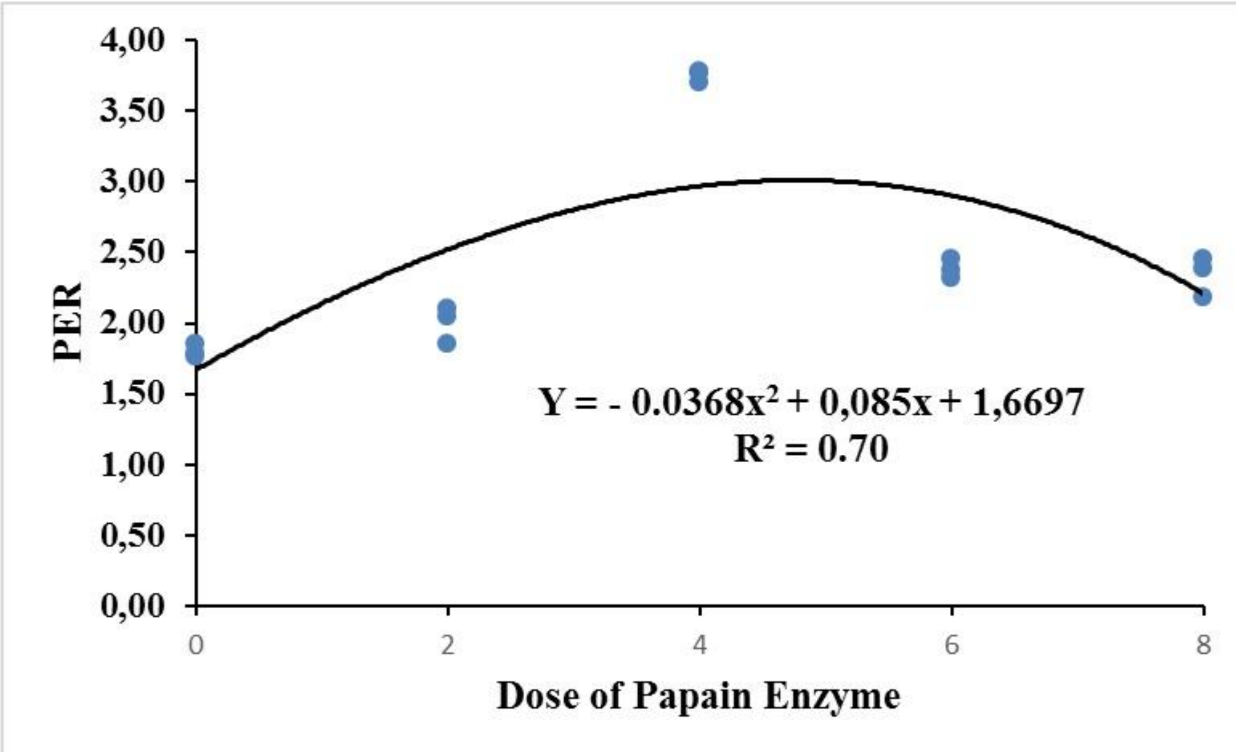


Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*)

Table 1. The composition and the results of proxymate 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 (kkal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kkal/g Diet)	8.02	8.05	8.03	8.02	8.02

Notes:

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.

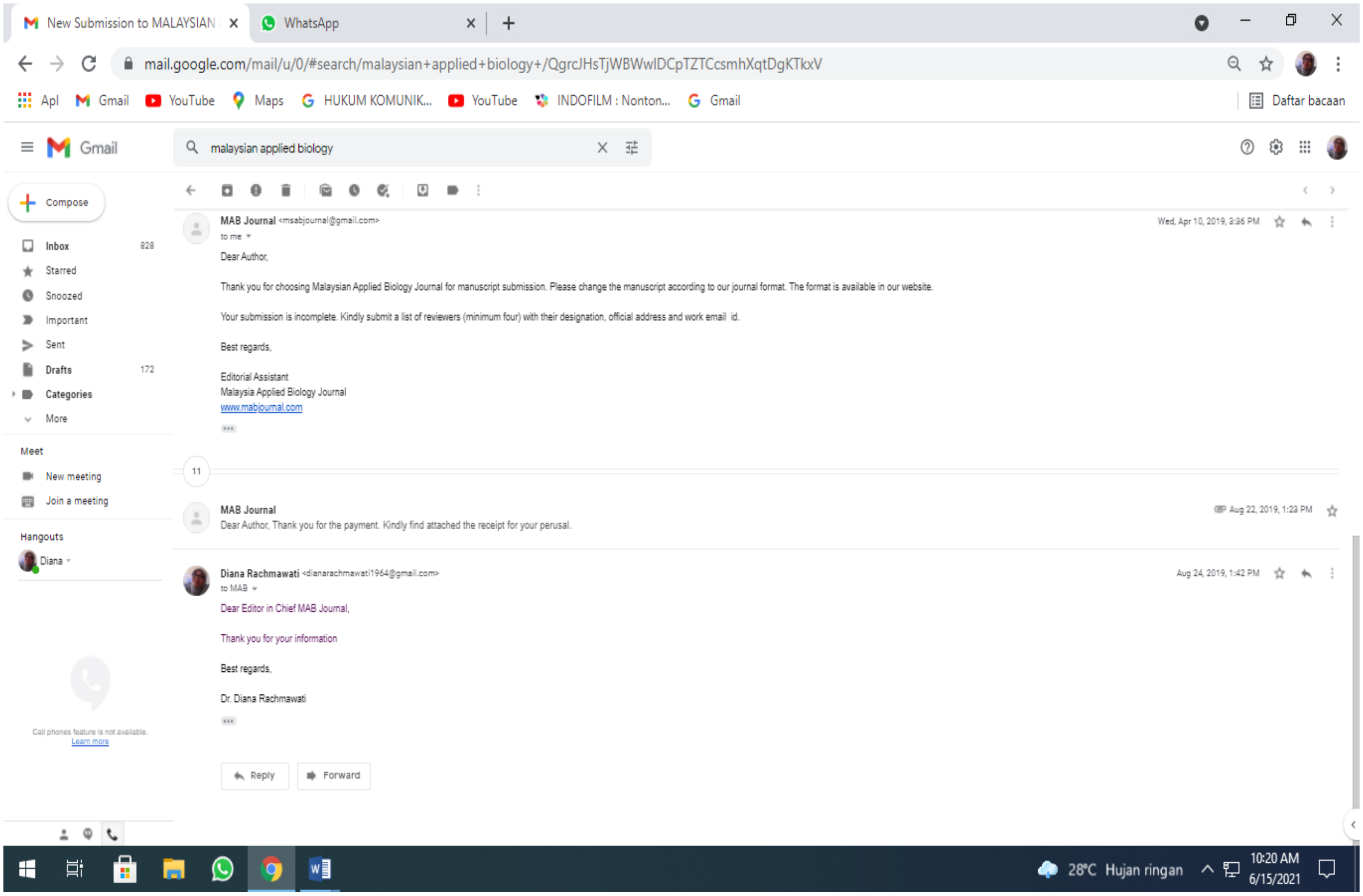
b. According Brooke and Daniel (2013), the optimal E/P ratio for growth ranges from 8 kcal/g to 12 kcal/g.

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

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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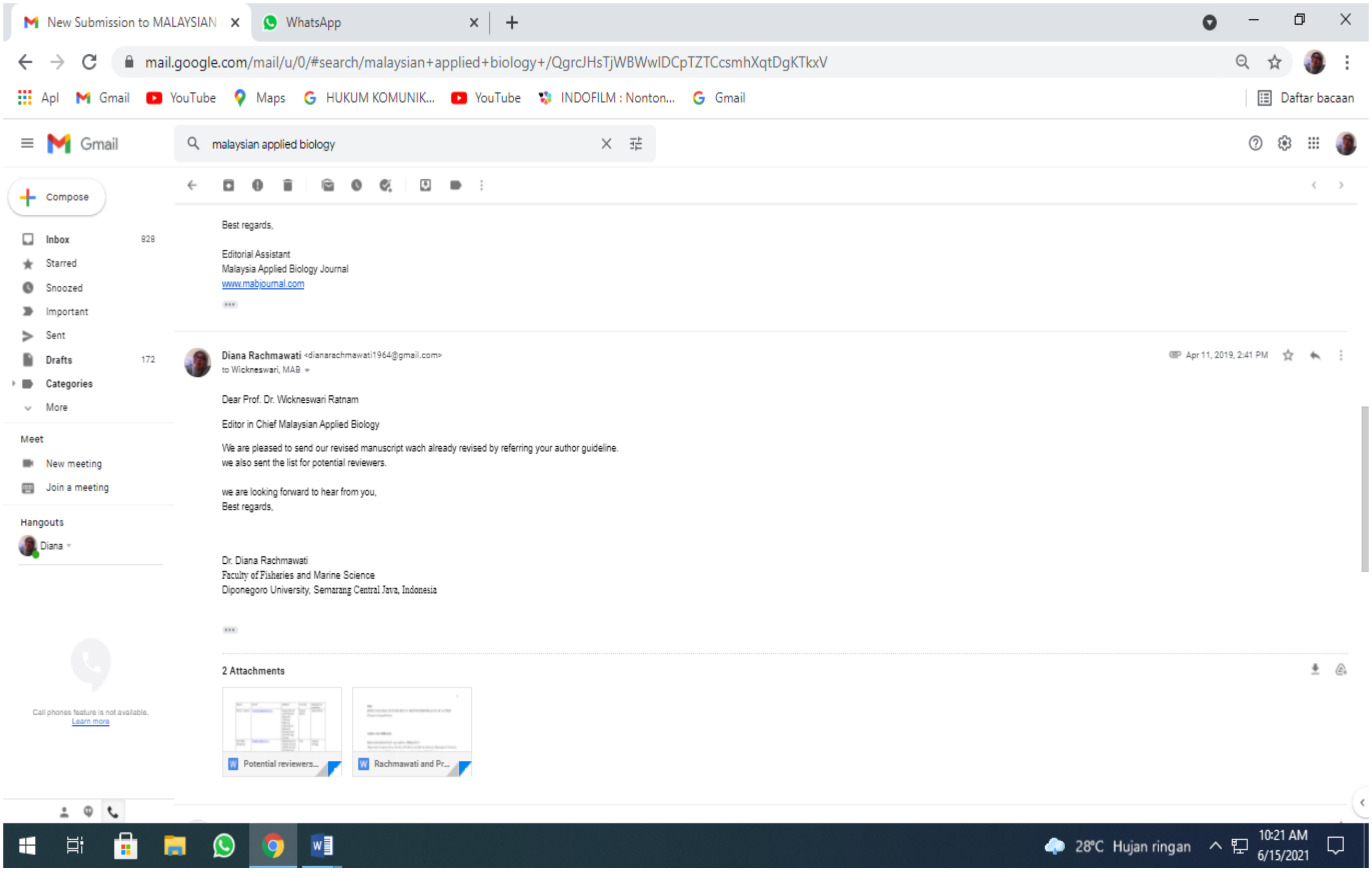

Best regards,

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Diana Rachmawati <dianarachmawati1964@gmail.com>
to Wickneswari, MAB

Apr 11, 2019, 2:41 PM

Dear Prof. Dr. Wickneswari Ratnam

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We are pleased to send our revised manuscript wach already revised by referring your author guideline.
we also sent the list for potential reviewers.

we are looking forward to hear from you,
Best regards,

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Faculty of Fisheries and Marine Science
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2 Attachments

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Rachmawati and Pr...

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Title:

EFFECT OF PAPAIN ENZYME DIET ON GROWTH PERFORMANCE OF CATFISH (*Pangasius hypopthalmus*)

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Papain on Catfish (*Pangasius hypopthalmus*) growth perrformance

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EFFECT OF PAPAIN ENZYME DIET ON GROWTH PERFORMANCE OF CATFISH (*Pangasius hypopthalmus*)

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ABSTRACT

This research aims to evaluate the effect of papain enzyme diet on growth performance of Catfish (*Pangasius hypopthalmus*) through feed efficiency and growth parameters. The research employed completely random design. Four treatments were conducted and each treatment was repeated 3 times. Catfish fingerlings with an average weight of 2.23 g/fish were used as samples. The experimental feed diets were designed based on isoprotein (31%) and isoenergy (252.06 kKal/g). The treatment was the addition of papain enzyme in feed with various doses, namely A (0 g/kg feed), B (2g/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), efficiency of feed utilization (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 ADCp, RGR, EFU, FCR, and PER, but not the SR of catfish. The optimum doses of ADCp, RGR, EFU, FCR, and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed respectively. In conclusion, a diet diet of papain enzyme increased the growth performance of catfish (*Pangasius hypopthalmus*).

Keywords: Feed, Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

INTRODUCTION

Catfish (*Pangasius hypopthalmus*) is one of fresh water fish which has a good potential because it has a high market price and also easy to culture. An increase of catfish production will result in a high demand of feed, since catfish aquaculture requires a lot of feed. One of the problems faced by fishermen is low efficiency of feed utilization and high feed cost compared to the total production cost (Hugues et al., 2018). The efficiency of feed utilization 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 additon of enzym was able to improve feed nutrient, especially to boost protein utilization. One of the enzymes is papain enzyme (Patil and Singh, 2014). Papain enzyme is able to break down amino acids. Thus, they are easy to digest (Amri and Mamboya, 2012). Ana et al. (2016) suggested that papain has numerous functions and can break down main tissue (tissue, collagen, and myofibrilar 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 supported the best growth of *Oreochromis niloticus.* Patil and Singh (2014), suggested that the addition of 0.1% papain enzyme in feed resulted in the best growth of *Macrobrachium rosenbergi*. 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*). 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 Fingerlings and Freshwater Aquaculture, Muntilan, Central Java, Indonesia. Research design and analysis employed ANOVA in Completely Random Design. The research was performed using four different experiments with three replications. Four different diet concentration of papain enzyme (extracted from Papaya Latex), A = 0 g/kg, B = 2 g/kg), C = 4 g/kg, D=6 g/kg, and E=8 g/kg diet, respectively, were added to the feed (Farrag et al., 2013). The fish species for this study is Catfish (*Pangasius hypopthalmus*).

Fish Preparation

The fingerlings used in this study were catfish fingerlings with an average weight of 2.23±0.30 g/individual. The fish were adapted in disinfected-tank (50x30x30 cm^3) 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 feeds (Table 1) was designed with isoprotein (31%) and isoenergy (252 Kkal/g). The experimental pellet feeds were added 0.5% Cr2O3 and five different papain enzyme diets (NRC, 1993). Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish Water Aquaculture, Jepara, Central Java, Indonesia. Prior to the experiment, feed nutrition was analyzed by using proxymate analysis (AOAC, 1990). The result of proxymate analysis is presented in (Table 1).

Table 1. insert here..

Ingredient mixing to produce feed was done by firstly 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 hidrolyzed. At the same time, vitamin, mineral and fish oil were mixed and dillated with water evenly. Then, the mix was put into the dried mix (NRC, 1993). 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; Julie, 2014). 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). P value of $p<0.05$ and $p<0.01$ are considered as significant and highly significant, respectively. To determine the optimal dose of papain enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12.

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).

Table 2. insert here…

Apparent Digestibility Coefficient Of Protein (ADC_P)

The ADC_P increased after the addition of papain enzyme with the dose of 2-8g/kg feed. Among all treatments, the addition of 6 g/kg papain (treatment C) was the highest ADCp followed by the treatment D (72.58%), E (70.25%), B (65.70%) and A (55.67%). Papain enzyme successfully hydrolized protein. Hence, the food digestibility increased. This study is in line with (Fateme *et al*, 2012; Patih and Singh, 2014) who concluded that the protease enzyme increased protein digestibility. Moreover, Kumar *et al*. (2011) found that papain enzyme can unbind phytate acid and phophor so that the availability of phophor in the feed increases.

The dose of 2 g papain enzyme per kg feed resulted in the highest ADCp. This was followed by the high EFU (75.09%) and low FCR (1.75). It is suggested that the higher the protein digestibility, the higher the efficiency of feed utilization, but the lower the feed conversion ratio. Similar results were also reported by Singh *et al*. (2011), Muchlisin *et al*. (2016) and Mo *et al*. (2016).

The effect of papain enzyme and ADCp was explained, as depicted in (Figure 1.), it followed quadratic function, $Y = - 0.859x^2 + 8.6744x + 54.926$, $R^2 = 0.85$. Based on the equation, the optimum dose was obtained at 4.05 g/kg feed.

Efficiency of Feed Utilization (EFU)

The highest EFU (75.09%) was obtained by C treatment (4 g/kg feed). In contrast, the lowest EFU of 50.12% was obtained by A treatment (0 g/kg feed). The high value of EFU suggested that less protein has been used for methabolism, but more protein has been used for fish growth. From this result, it is suggested that papain enzyme can improve feed utilization efficiency. This result is suported by Muchlisin *et al*. (2016), indicating that the addition of papain enzyme increased feed efficiency on Keureling fish (*Tor tambra*). Compared to our result, Catfish (*Pangasius hypopthalmus*) needs higher papain enzyme than Keureling fish (*Tor tambra*)**.** The Catfish needs 4 g/kg feed while Keureling fish needs only 27.5 mg/kg.

The relationship between papain enzyme and EFU is presented in (Figure 2.), it followed quadratic equation, $Y = -0.8902x^2 + 8.829x + 50.823$, $R^2 = 0.89$. From the equation, the optimum dose for EFU (75,09%) was at the level of 4 g/kg feed.

Relative Growth Rate (RGR)

The catfish fed with papain enzyme has higher RGR. The highest value of RGR (8.10%) was in C treatment (4 g/kg feed), while the lowest value of RGR (3.48%) was in A treatment (0 g/kg feed). It seemed that the absence of enzyme negatively affected the RGR. This result is in line with the earlier result. The addition of papain enzyme can increase the growth of several species, such as *Chanos channos* (Singh *et al*., 2011), *M. rosenbergii* (Patil and Singh, 2014), *Oreochromis niloticus* **(**Manguti *et al*., 2014), *Labeo rohita* (Khati *et al*., 2015), Goldlined seabream (*Rhabdosargus sarba*), brown spotted grouper (*Epinephelus bleekeri*) and pompano (*Trachinotus blochii*) (Mo *et al*., 2016).

Without papain enzyme, the hydrolisys of long chain peptide into short chain peptide will not occurr. As a result, protein digestibility and feed utilization efficiency decrease. Fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolizes polypeptide into 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 hydrolize lipid and carbohydrat in the feed (Ryosuke and Kazuhiko, 2004).

The relationship between papain enzyme and RGR was presented in the 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 the dose of 3.93 g/kg feed. The optimum value of RGR is 7.11%.

Feed Conversion Ratio (FCR)

The addition of papain enzyme with the dose of 2-8 g/kg feed can reduce FCR. It was found that the enzyme addition resulted in better FCR. The dose of 4 g/kg feed had the lowest FCR (1.56). This result is different from the research with different fish. Similar studies on *Chanos channos* and prawn (*Macrobrachium rosenbergii*) were conducted by Singh *et al*. (2011) and Patil and Singh (2014). They found that the doses of 2% and 0.1% of papain enzyme on the diet were the best doses for producing low FCR, high growth, high protein digestibility, and high ratio of protein efficiency. Khati *et al*. (2015) also reported that the addition of 10 g/kg feed of papain enzyme in the feed increased feed digestibility and reduced FCR on *Labeo rohita*.

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

Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) is an indicator which shows the performance of protein in the feed to provide amino acids for fish growth (Manush et al., 2013). PER of fish which was fed with the addition of papain enzyme increased on all tretments (2-8 g/kg feed) as much as 2.00-3.75. The value of PER was always higher than that (1.80) without papain enzyme. The highest PER was obtained in the treatment C (4 g/kg feed). It was expected due to the right dose that can provide amino acids. Similar results were also reported by Singh *et al*. (2011) and Khati *et al*. (2015). Singh *et al.* (2011) found that the addition of 2% papain enzyme in the feed on *Channos channos* increased protein efficiency ratio by 2.24, while Khati *et al.* (2015) found that the addition of 10 g/kg feed increased protein efficiency ratio by 2.30.

The relationship between papain enzyme and PER is presented in Figure 5. The relationship has a quadratic equation of $Y = - 0.0386x^2 - 0.3586x + 2.6746$, $R^2 = 0.70$. The graph shows that the optimum dose of papain enzyme for PER was 3.77 g/kg feed with the PER value of 3.

Survival Rate (SR)

In this study, the addition of papain enzyme had no effect on SR of catfish (*Pangasius hypopthalmus*). As reported by Patih and Singh (2013), proteolitic enzyme does affect the fish survival rate. Moreover, Yakuputiyage (2013), reported that feed is not a factor influencing survival rate, but initial treatment and media quality affect survival rate. Similar results were obtained on *Channos channos* (Singh *et al*., 2011), *Macrobrachium rosenbergii* (Patil and Singh, 2014) and *Labeo rohita* (Khati *et al*., 2015).

CONCLUSION

Papain enzyme increased apparent digestibiity of protein, growth rate, efficiency of feed utilization, and protein efficiency ratio. On the other hand, it reduced feed conversion ratio of catfish. The optimum doses on ADC_P, RGR, EFU, FCR and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed, respectively. In conclusion, the papain enzyme has a positive effect on the growth performance of catfish (*Pangasius hypopthalmus*).

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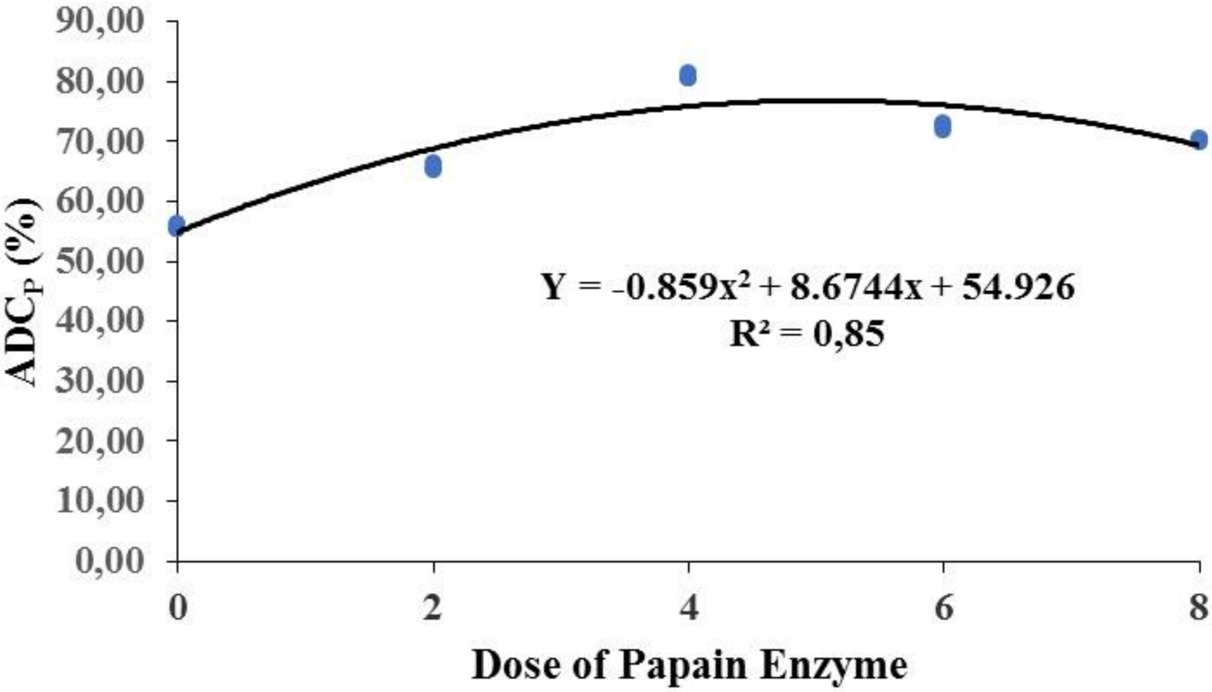


Fig. 1. The relationship between the dose of papain enzyme and ADC_P.

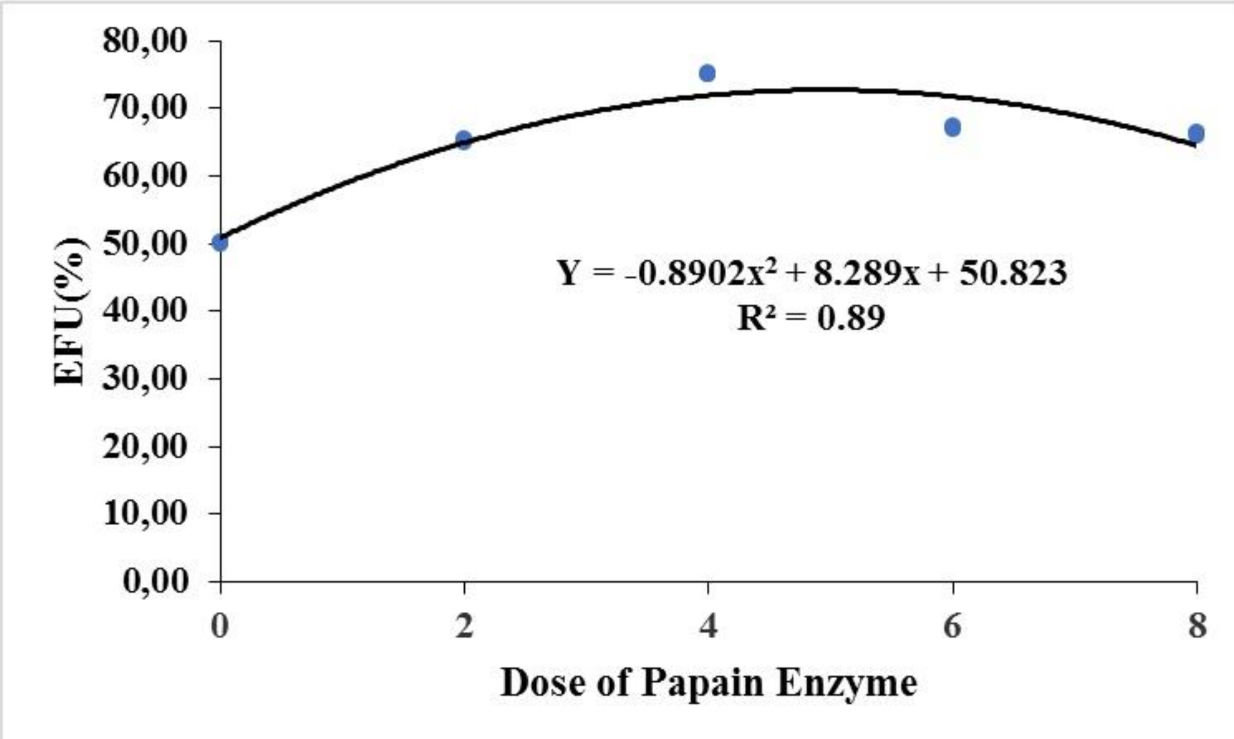


Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*)

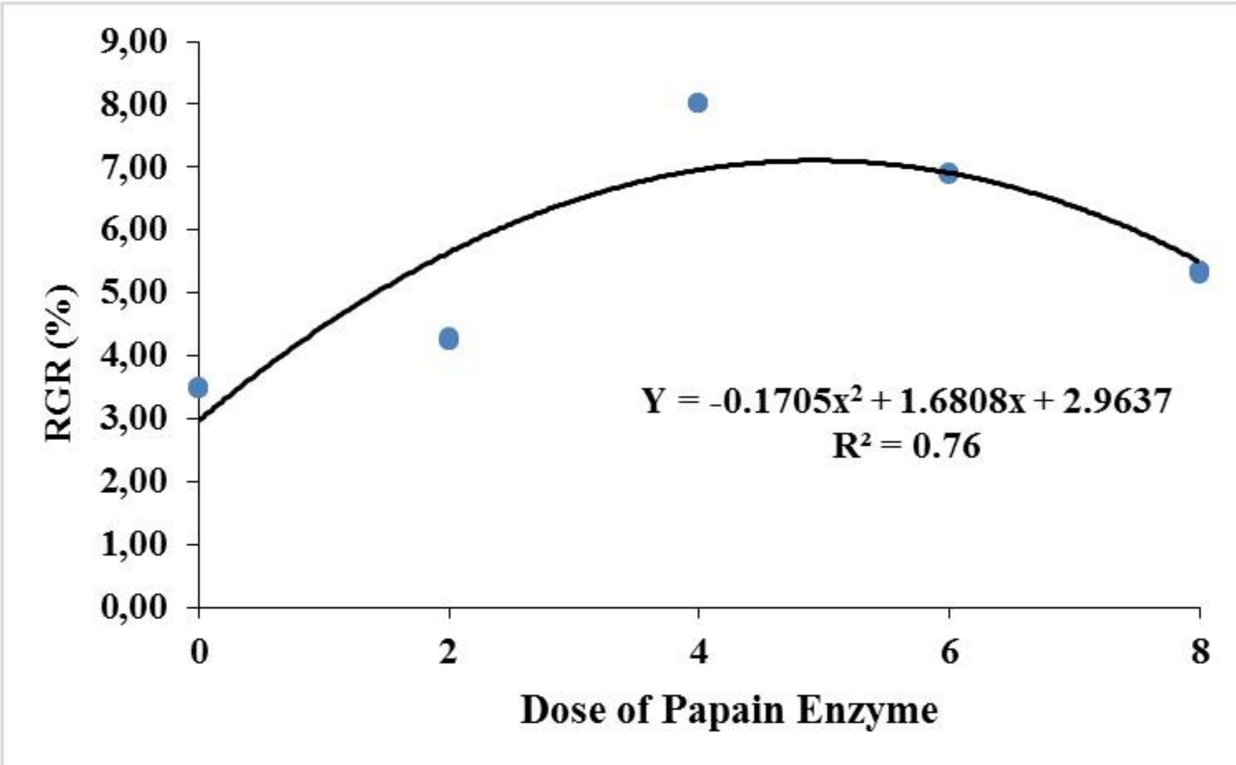


Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*)

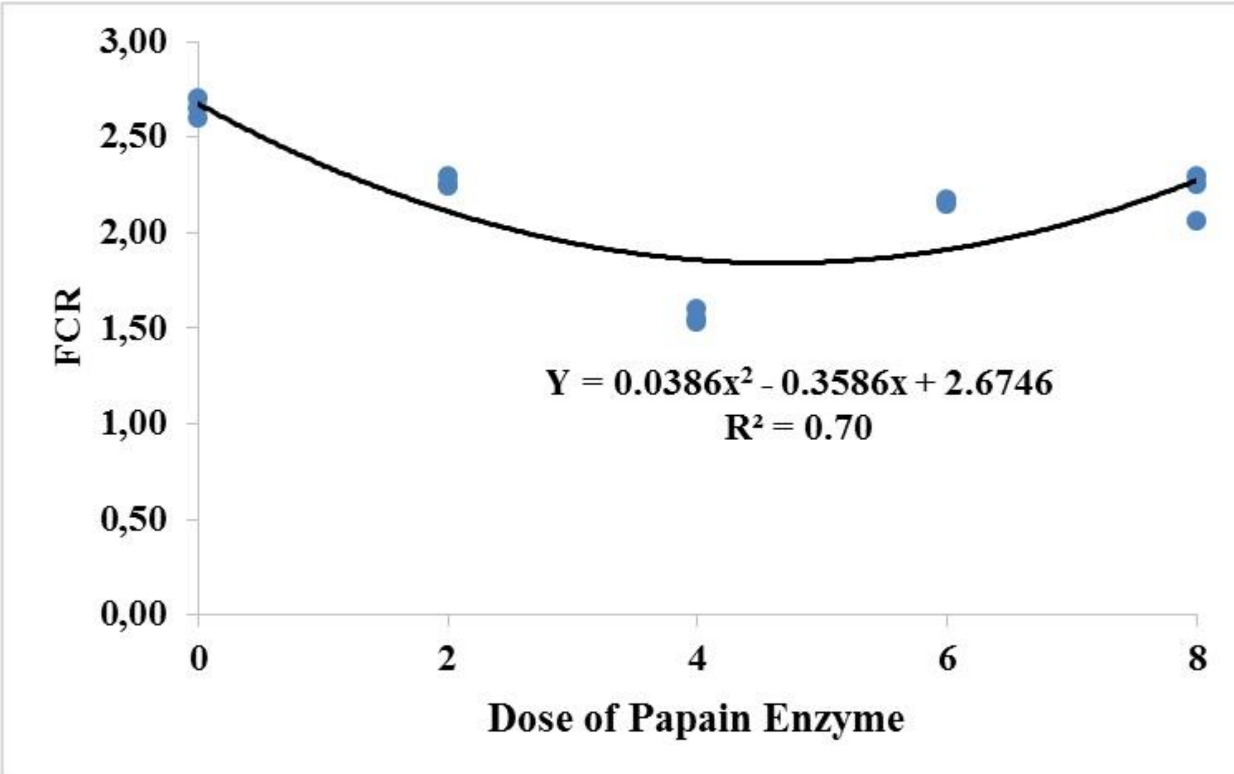


Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*)

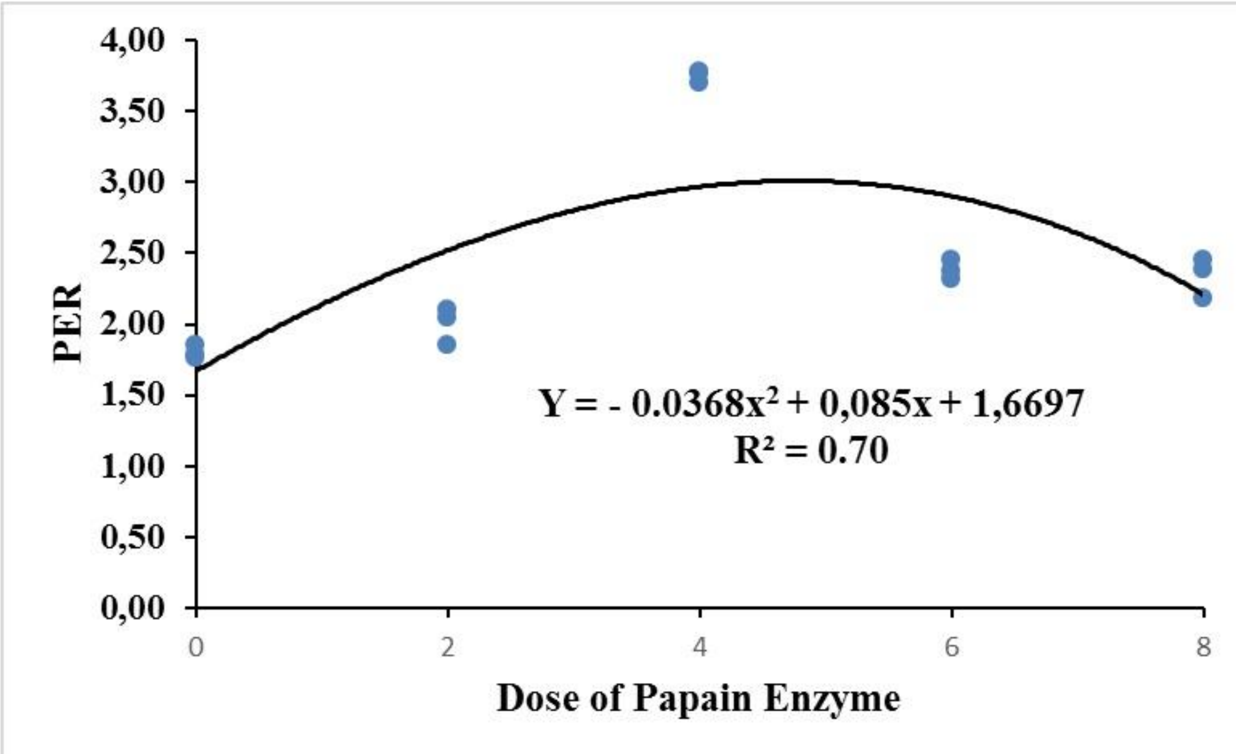


Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*)

Table 1. The composition and the results of proxymate 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 (kkal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kkal/g Diet)	8.02	8.05	8.03	8.02	8.02

Notes:

d. 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.

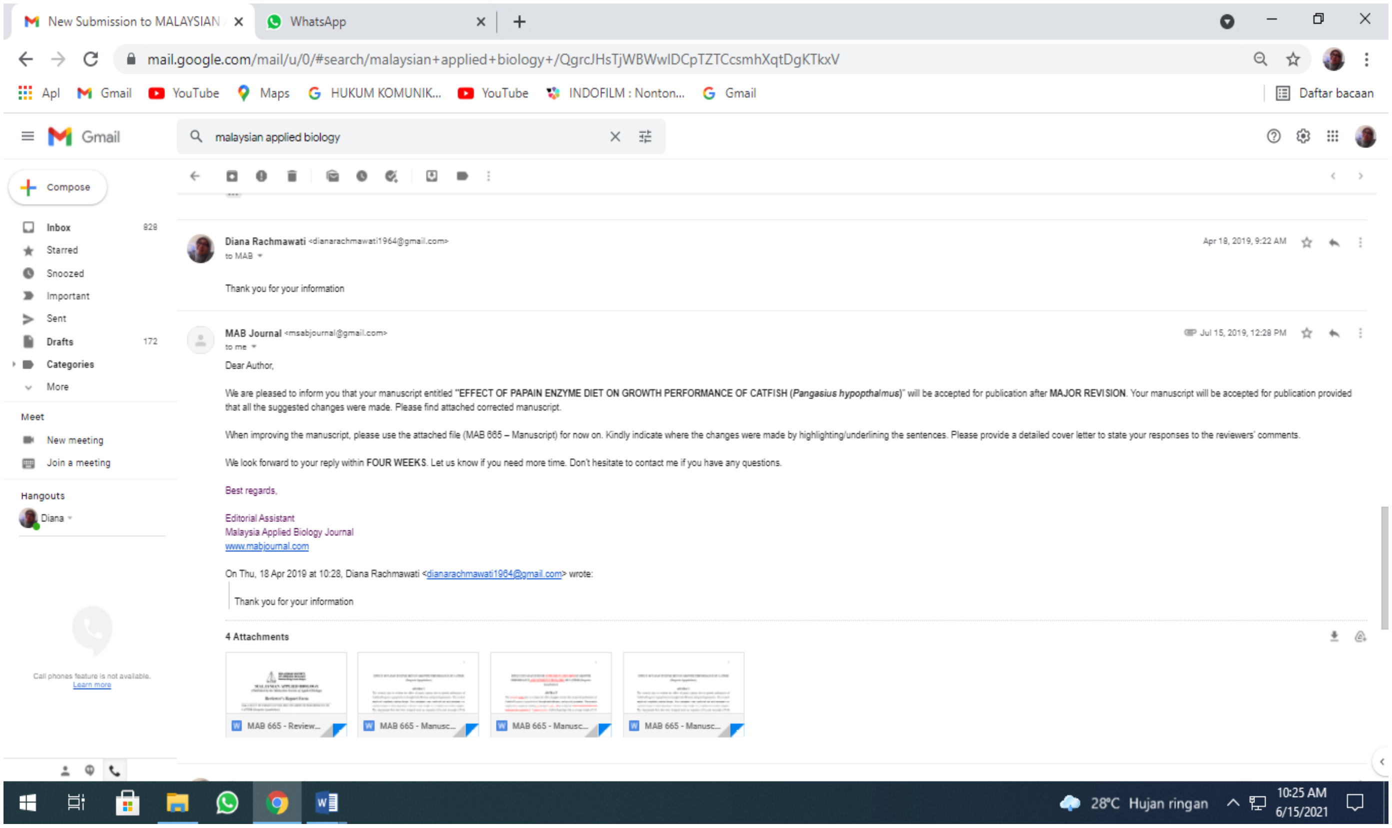
e. According Brooke and Daniel (2013), the optimal E/P ratio for growth ranges from 8 kcal/g to 12 kcal/g.

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

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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When improving the manuscript, please use the attached file (MAB 665 – Manuscript) for now on. Kindly indicate where the changes were made by highlighting/underlining the sentences. Please provide a detailed cover letter to state your responses to the reviewers' comments.

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EFFECT OF PAPAIN ENZYME SUPPLIMENTATION ON GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF CATFISH (*Pangasius hypopthalmus*)

ABSTRACT

This study aims to evaluate the effect of papain enzyme diet on growth performance of Catfish (*Pangasius hypopthalmus*) through feed efficiency and growth parameters. The research employed a completely randomized design with four treatments and 3 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 kKal/g). Papain enzyme was added to the feed with in various doses; A (0 g/kg feed), B (2g/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), efficiency of feed utilization (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 ADCp, RGR, EFU, FCR, and PER, but not the SR of catfish. The optimum doses of ADCp, RGR, EFU, FCR, and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed respectively. In conclusion, a diet diet of papain enzyme increased the growth performance of catfish (*Pangasius hypopthalmus*).

Keywords: Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

INTRODUCTION

Catfish (*Pangasius hypopthalmus*) is one of fresh water fish which has a good potential because it has a high market price and also easy to culture (ref!!). An increase of catfish production will result in a high demand of feed, since catfish aquaculture requires a lot of feed. One of the problems faced by fishermen fish farmers is low efficiency of feed utilization and high feed cost compared to the total production cost (Hugues et al., 2018). The efficiency of feed utilization 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 additon of enzym was able to improve feed nutrient, especially to boost protein utilization. One of the enzymes is papain enzyme (Patil and Singh, 2014). Papain enzyme is able to break down amino acids. Thus, they are easy to digest (Amri and Mamboya, 2012). Ana et al. (2016) suggested that papain has numerous functions and can break down main tissue (tissue, collagen, and myofibrilar 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) More references !!! 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 supported the best growth of *Oreochromis niloticus.* Patil and Singh (2014), suggested that the addition of 0.1% papain enzyme in feed resulted in the best growth of *Macrobrachium rosenbergi*. 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*). 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 Fingerlings and Freshwater Aquaculture, Muntilan, Central Java, Indonesia. Research design and analysis employed ANOVA in Completely Random Design. The research was performed using four different experiments with three replications. Four different diet concentration of papain enzyme (extracted from Papaya Latex), A = 0 g/kg, B = 2 g/kg), C = 4 g/kg, D=6 g/kg, and E=8 g/kg diet, respectively, were added to the feed (Farrag et al., 2013). The fish species for this study is Catfish (*Pangasius hypopthalmus*).

Fish Preparation

The fingerlings used in this study were catfish fingerlings with an average weight of 2.23±0.30 g. The fish were acclimitized in disinfected-tank (50x30x30 cm^3) 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 are indicated in Table 1. The diets were prepared to be isonitogenous (31%) and isocaloric (252 Kkal/g). The experimental diets were added 0.5% Cr2O3 and five different papain enzyme diets (NRC, 1993). Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish Water Aquaculture, Jepara, Central Java, Indonesia. Prior to the experiment, feed nutrition was analyzed by using proxymate analysis (AOAC, 1990). The result of proxymate analysis is presented in (Table 1).

Table 1. insert here..

Ingredient mixing to produce feed was done by firstly 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 hydrolyzed. At the same time, vitamin, mineral and fish oil were mixed and dillated with water evenly. Then, the mix was put into the dried mix (NRC, 1993). 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; Julie, 2014). 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. To determine the optimal dose of papain enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12.

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 are presented in the (Table 2).

Table 2. insert here…

Apparent Digestibility Coefficient Of Protein (ADC_P)

The ADC_P increased after the addition of papain enzyme with the dose of 2-8g/kg feed. Among all treatments, the addition of 6 g/kg papain (treatment C) was the highest ADCp followed by the treatment D (72.58%), E (70.25%), B (65.70%) and A (55.67%). Papain enzyme successfully hydrolized protein. Hence, the food digestibility increased. This study is in line with (Fateme *et al*, 2012; Patih and Singh, 2014) who concluded that the protease enzyme increased protein digestibility. Moreover, Kumar *et al*. (2011) found that papain enzyme can unbind phytate acid and phophor so that the availability of phophor in the feed increases.

The dose of 2 g papain enzyme per kg feed resulted in the highest ADCp. This was followed by the high EFU (75.09%) and low FCR (1.75). It is suggested that the higher the protein digestibility, the higher the efficiency of feed utilization, but the lower the feed conversion ratio. Similar results were also reported by Singh *et al*. (2011), Muchlisin *et al*. (2016) and Mo *et al*. (2016).

The effect of papain enzyme and ADCp was explained, as depicted in (Figure 1.), it followed quadratic function, $Y = - 0.859x^2 + 8.6744x + 54.926$, $R^2 = 0.85$. Based on the equation, the optimum dose was obtained at 4.05 g/kg feed.

Efficiency of Feed Utilization (EFU)

The highest EFU (75.09%) was obtained by C treatment (4 g/kg feed). In contrast, the lowest EFU of 50.12% was obtained by A treatment (0 g/kg feed). The high value of EFU suggested that less protein has been used for methabolism, but more protein has been used for fish growth. From this result, it is suggested that papain enzyme can improve feed utilization efficiency. This result is suported by Muchlisin *et al*. (2016), indicating that the addition of papain enzyme increased feed efficiency on Keureling fish (*Tor tambra*). Compared to our result, Catfish (*Pangasius hypopthalmus*) needs higher papain enzyme than Keureling fish (*Tor tambra*)**.** The Catfish needs 4 g/kg feed while Keureling fish needs only 27.5 mg/kg.

The relationship between papain enzyme and EFU is presented in (Figure 2.), it followed quadratic equation, $Y = - 0.8902x^2 +8.829x + 50.823$, $R^2 = 0.89$. From the equation, the optimum dose for EFU (75,09%) was at the level of 4 g/kg feed.

Relative Growth Rate (RGR)

The catfish fed with papain enzyme has higher RGR. The highest value of RGR (8.10%) was in C treatment (4 g/kg feed), while the lowest value of RGR (3.48%) was in A treatment (0 g/kg feed). It seemed that the absence of enzyme negatively affected the RGR. This result is in line with the earlier result. The addition of papain enzyme can increase the growth of several species, such as *Chanos channos* (Singh *et al*., 2011), *M. rosenbergii* (Patil and Singh, 2014), *Oreochromis niloticus* **(**Manguti *et al*., 2014), *Labeo rohita* (Khati *et al*., 2015), Goldlined seabream (*Rhabdosargus sarba*), brown spotted grouper (*Epinephelus bleekeri*) and pompano (*Trachinotus blochii*) (Mo *et al*., 2016).

Without papain enzyme, the hydrolisys of long chain peptide into short chain peptide will not occurr. As a result, protein digestibility and feed utilization efficiency decrease. Fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolizes polypeptide into 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 hydrolize lipid and carbohydrat in the feed (Ryosuke and Kazuhiko, 2004).

The relationship between papain enzyme and RGR was presented in the 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 the dose of 3.93 g/kg feed. The optimum value of RGR is 7.11%.

Feed Conversion Ratio (FCR)

The addition of papain enzyme with the dose of 2-8 g/kg feed can reduce FCR. It was found that the enzyme addition resulted in better FCR. The dose of 4 g/kg feed had the lowest FCR (1.56). This result is different from the research with different fish. Similar studies on *Chanos channos* and prawn (*Macrobrachium rosenbergii*) were conducted by Singh *et al*. (2011) and Patil and Singh (2014). They found that the doses of 2% and 0.1% of papain enzyme on the diet were the best doses for producing low FCR, high growth, high protein digestibility, and high ratio of protein efficiency. Khati *et al*. (2015) also reported that the addition of 10 g/kg feed of papain enzyme in the feed increased feed digestibility and reduced FCR on *Labeo rohita*.

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

Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) is an indicator which shows the performance of protein in the feed to provide amino acids for fish growth (Manush et al., 2013). PER of fish which was fed with the addition of papain enzyme increased on all tretments (2-8 g/kg feed) as much as 2.00-3.75. The value of PER was always higher than that (1.80) without papain enzyme. The highest PER was obtained in the treatment C (4 g/kg feed). It was expected due to the right dose that can provide amino acids. Similar results were also reported by Singh *et al*. (2011) and Khati *et al*. (2015). Singh *et al.* (2011) found that the addition of 2% papain enzyme in the feed on *Channos channos* increased protein efficiency ratio by 2.24, while Khati *et al.* (2015) found that the addition of 10 g/kg feed increased protein efficiency ratio by 2.30.

The relationship between papain enzyme and PER is presented in Figure 5. The relationship has a quadratic equation of $Y = - 0.0386x^2 - 0.3586x + 2.6746$, $R^2 = 0.70$. The graph shows that the optimum dose of papain enzyme for PER was 3.77 g/kg feed with the PER value of 3.

Survival Rate (SR)

In this study, the addition of papain enzyme had no effect on SR of catfish (*Pangasius hypopthalmus*). As reported by Patih and Singh (2013), proteolitic enzyme does affect the fish survival rate. Moreover, Yakuputiyage (2013), reported that feed is not a factor influencing survival rate, but initial treatment and media quality affect survival rate. Similar results were obtained on *Channos channos* (Singh *et al*., 2011), *Macrobrachium rosenbergii* (Patil and Singh, 2014) and *Labeo rohita* (Khati *et al*., 2015).

CONCLUSION

Papain enzyme increased apparent digestibiity of protein, growth rate, efficiency of feed utilization, and protein efficiency ratio. On the other hand, it reduced feed conversion ratio of catfish. The optimum doses on ADC_P, RGR, EFU, FCR and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed, respectively. In conclusion, the papain enzyme has a positive effect on the growth performance of catfish (*Pangasius hypopthalmus*).

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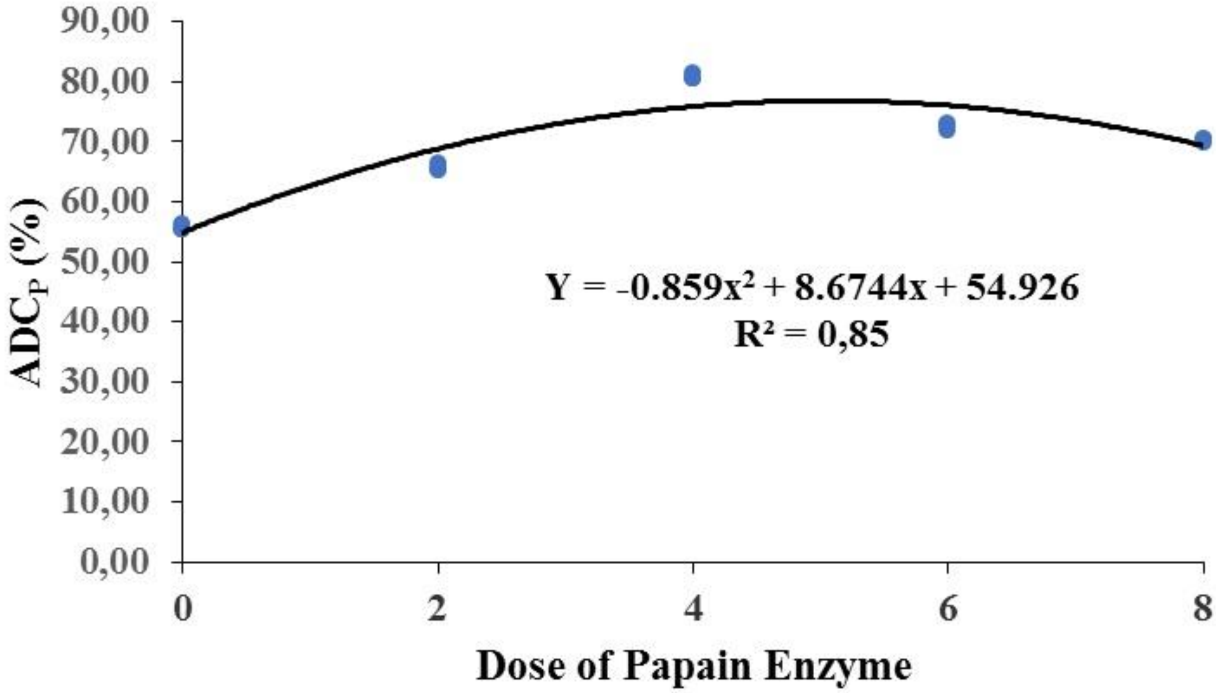

Fig. 1. The relationship between the dose of papain enzyme and ADC_P.

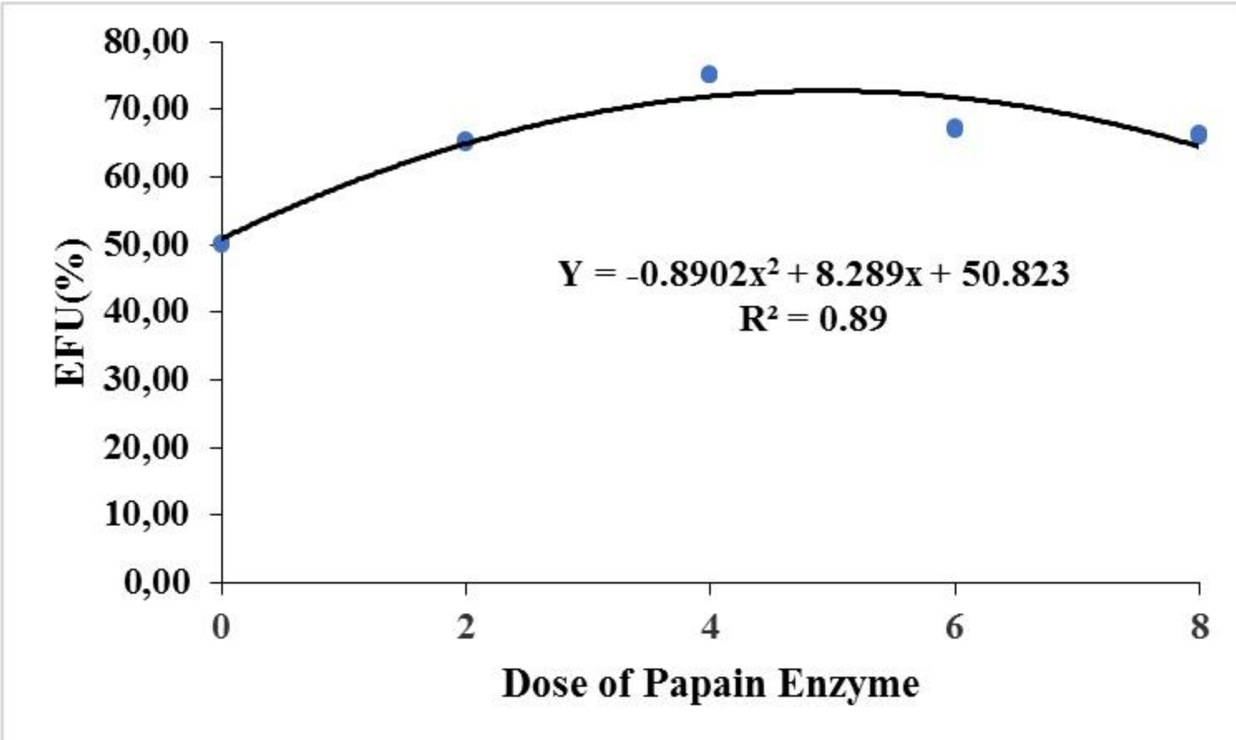

Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*)

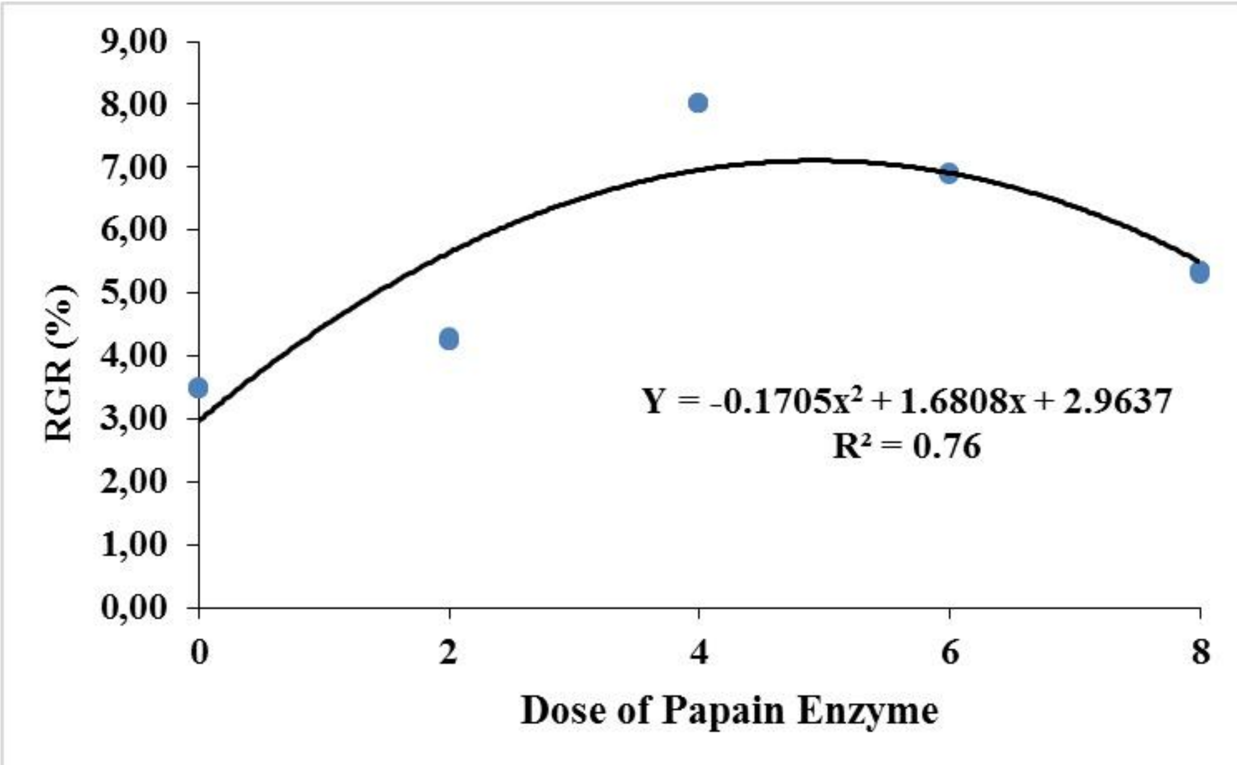

Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*)

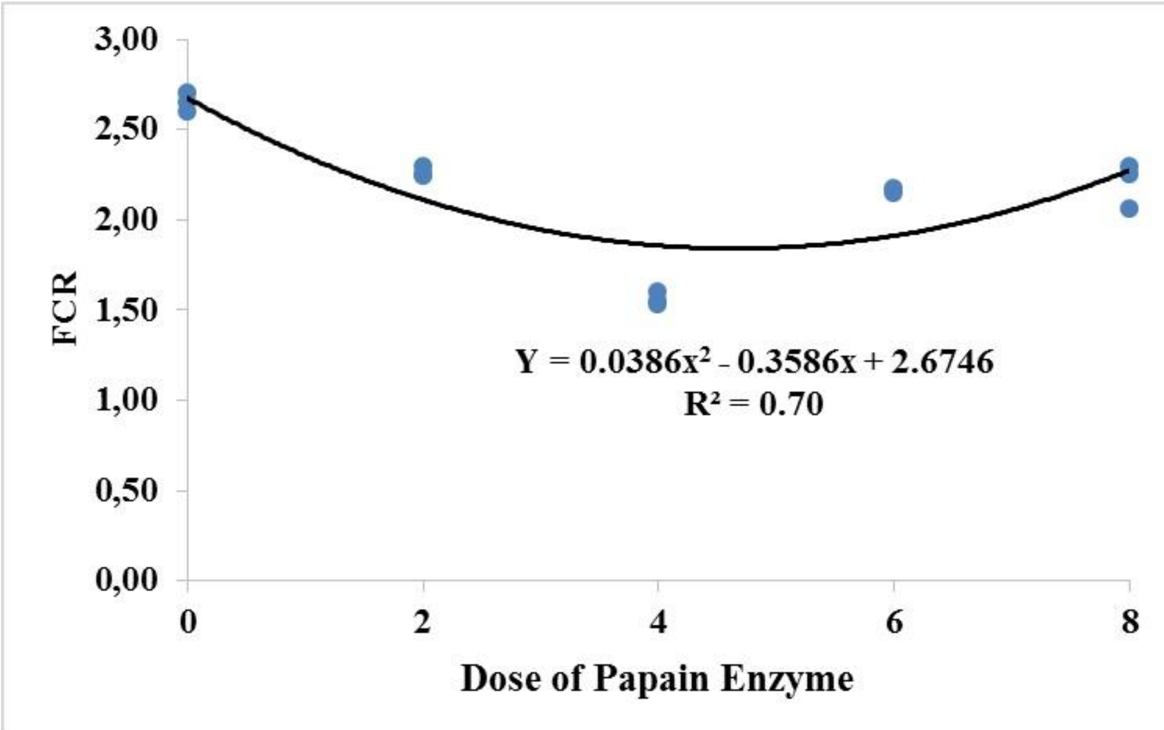

Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*)

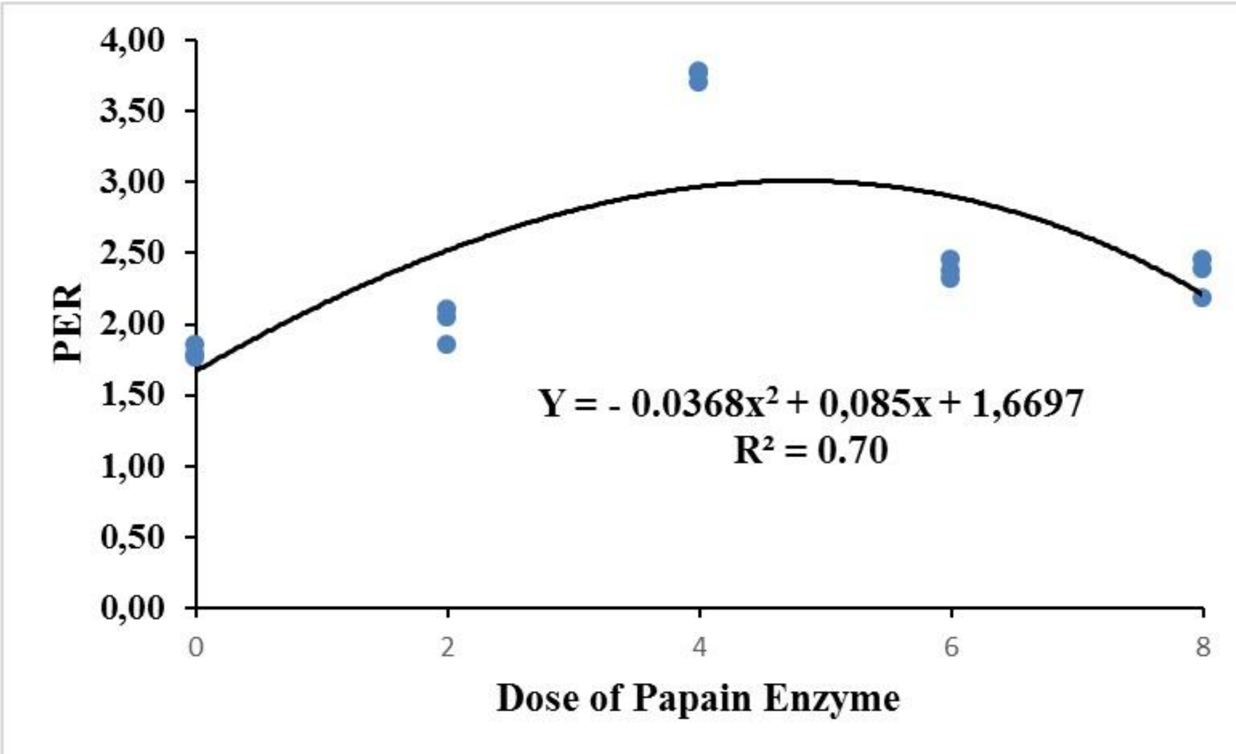

Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*)

Table 1. The composition and the results of proxymate 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 (kkal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kkal/g Diet)	8.02	8.05	8.03	8.02	8.02

Notes:

g. 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.

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

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

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

EFFECT OF PAPAIN ENZYME DIET ON GROWTH PERFORMANCE OF CATFISH (*Pangasius hypopthalmus*)

ABSTRACT

This research aims to evaluate the effect of papain enzyme diet on growth performance of Catfish (*Pangasius hypopthalmus*) through feed efficiency and growth parameters. The research employed completely random design. Four treatments were conducted and each treatment was repeated 3 times. Catfish fingerlings with an average weight of 2.23 g/fish were used as samples. The experimental feed diets were designed based on isoprotein (31%) and isoenergy (252.06 kKal/g). The treatment was the addition of papain enzyme in feed with various doses, namely A (0 g/kg feed), B (2g/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), efficiency of feed utilization (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 ADCp, RGR, EFU, FCR, and PER, but not the SR of catfish. The optimum doses of ADCp, RGR, EFU, FCR, and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed respectively. In conclusion, a diet diet of papain enzyme increased the growth performance of catfish (*Pangasius hypopthalmus*).

Keywords: Feed, Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

INTRODUCTION

Catfish (*Pangasius hypopthalmus*) is one of fresh water fish which has a good potential because it has a high market price and also easy to culture. An increase of catfish production will result in a high demand of feed, since catfish aquaculture requires a lot of feed. One of the problems faced by fishermen is low efficiency of feed utilization and high feed cost compared to the total production cost (Hugues et al., 2018). The efficiency of feed utilization 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 additon of enzym was able to improve feed nutrient, especially to boost protein utilization. One of the enzymes is papain enzyme (Patil and Singh, 2014). Papain enzyme is able to break down amino acids. Thus, they are easy to digest (Amri and Mamboya, 2012). Ana et al. (2016) suggested that papain has numerous functions and can break down main tissue (tissue, collagen, and myofibrilar 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 supported the best growth of *Oreochromis niloticus.* Patil and Singh (2014), suggested that the addition of 0.1% papain enzyme in feed resulted in the best growth of *Macrobrachium rosenbergi*. 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*). 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 Fingerlings and Freshwater Aquaculture, Muntilan, Central Java, Indonesia. Research design and analysis employed ANOVA in Completely Random Design. The research was performed using four different experiments with three replications. Four different diet concentration of papain enzyme (extracted from Papaya Latex), A = 0 g/kg, B = 2 g/kg), C = 4 g/kg, D=6 g/kg, and E=8 g/kg diet, respectively, were added to the feed (Farrag et al., 2013). The fish species for this study is Catfish (*Pangasius hypopthalmus*).

Fish Preparation

The fingerlings used in this study were catfish fingerlings with an average weight of 2.23±0.30 g/individual. The fish were adapted in disinfected-tank (50x30x30 cm^3) 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 feeds (Table 1) was designed with isoprotein (31%) and isoenergy (252 Kkal/g). The experimental pellet feeds were added 0.5% Cr2O3 and five different papain enzyme diets (NRC, 1993). Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish Water Aquaculture, Jepara, Central Java, Indonesia. Prior to the experiment, feed nutrition was analyzed by using proxymate analysis (AOAC, 1990). The result of proxymate analysis is presented in (Table 1).

Table 1. insert here..

Ingredient mixing to produce feed was done by firstly 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 hidrolyzed. At the same time, vitamin, mineral and fish oil were mixed and dillated with water evenly. Then, the mix was put into the dried mix (NRC, 1993). 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; Julie, 2014). 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). P value of $p<0.05$ and $p<0.01$ are considered as significant and highly significant, respectively. To determine the optimal dose of papain enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12.

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).

Table 2. insert here…

Apparent Digestibility Coefficient Of Protein (ADC_P)

The ADC_P increased after the addition of papain enzyme with the dose of 2-8g/kg feed. Among all treatments, the addition of 6 g/kg papain (treatment C) was the highest ADCp followed by the treatment D (72.58%), E (70.25%), B (65.70%) and A (55.67%). Papain enzyme successfully hydrolized protein. Hence, the food digestibility increased. This study is in line with (Fateme *et al*, 2012; Patih and Singh, 2014) who concluded that the protease enzyme increased protein digestibility. Moreover, Kumar *et al*. (2011) found that papain enzyme can unbind phytate acid and phophor so that the availability of phophor in the feed increases.

The dose of 2 g papain enzyme per kg feed resulted in the highest ADCp. This was followed by the high EFU (75.09%) and low FCR (1.75). It is suggested that the higher the protein digestibility, the higher the efficiency of feed utilization, but the lower the feed conversion ratio. Similar results were also reported by Singh *et al*. (2011), Muchlisin *et al*. (2016) and Mo *et al*. (2016).

The effect of papain enzyme and ADCp was explained, as depicted in (Figure 1.), it followed quadratic function, $Y = -0.859x^2 + 8.6744x + 54.926$, $R^2 = 0.85$. Based on the equation, the optimum dose was obtained at 4.05 g/kg feed.

Efficiency of Feed Utilization (EFU)

The highest EFU (75.09%) was obtained by C treatment (4 g/kg feed). In contrast, the lowest EFU of 50.12% was obtained by A treatment (0 g/kg feed). The high value of EFU suggested that less protein has been used for methabolism, but more protein has been used for fish growth. From this result, it is suggested that papain enzyme can improve feed utilization efficiency. This result is suported by Muchlisin *et al*. (2016), indicating that the addition of papain enzyme increased feed efficiency on Keureling fish (*Tor tambra*). Compared to our result, Catfish (*Pangasius hypopthalmus*) needs higher papain enzyme than Keureling fish (*Tor tambra*). The Catfish needs 4 g/kg feed while Keureling fish needs only 27.5 mg/kg.

The relationship between papain enzyme and EFU is presented in (Figure 2.), it followed quadratic equation, $Y = - 0.8902x^2 +8.829x + 50.823$, $R^2 = 0.89$. From the equation, the optimum dose for EFU (75,09%) was at the level of 4 g/kg feed.

Relative Growth Rate (RGR)

The catfish fed with papain enzyme has higher RGR. The highest value of RGR (8.10%) was in C treatment (4 g/kg feed), while the lowest value of RGR (3.48%) was in A treatment (0 g/kg feed). It seemed that the absence of enzyme negatively affected the RGR. This result is in line with the earlier result. The addition of papain enzyme can increase the growth of several species, such as *Chanos channos* (Singh *et al*., 2011), *M. rosenbergii* (Patil and Singh, 2014), *Oreochromis niloticus* **(**Manguti *et al*., 2014), *Labeo rohita* (Khati *et al*., 2015), Goldlined seabream (*Rhabdosargus sarba*), brown spotted grouper (*Epinephelus bleekeri*) and pompano (*Trachinotus blochii*) (Mo *et al*., 2016).

Without papain enzyme, the hydrolisys of long chain peptide into short chain peptide will not occurr. As a result, protein digestibility and feed utilization efficiency decrease. Fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolizes polypeptide into 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 hydrolize lipid and carbohydrat in the feed (Ryosuke and Kazuhiko, 2004).

The relationship between papain enzyme and RGR was presented in the 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 the dose of 3.93 g/kg feed. The optimum value of RGR is 7.11%.

Feed Conversion Ratio (FCR)

The addition of papain enzyme with the dose of 2-8 g/kg feed can reduce FCR. It was found that the enzyme addition resulted in better FCR. The dose of 4 g/kg feed had the lowest FCR (1.56). This result is different from the research with different fish. Similar studies on *Chanos channos* and prawn (*Macrobrachium rosenbergii*) were conducted by Singh *et al*. (2011) and Patil and Singh (2014). They found that the doses of 2% and 0.1% of papain enzyme on the diet were the best doses for producing low FCR, high growth, high protein digestibility, and high ratio of protein efficiency. Khati *et al*. (2015) also reported that the addition of 10 g/kg feed of papain enzyme in the feed increased feed digestibility and reduced FCR on *Labeo rohita*.

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

Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) is an indicator which shows the performance of protein in the feed to provide amino acids for fish growth (Manush et al., 2013). PER of fish which was fed with the addition of papain enzyme increased on all tretments (2-8 g/kg feed) as much as 2.00-3.75. The value of PER was always higher than that (1.80) without papain enzyme. The highest PER was obtained in the treatment C (4 g/kg feed). It was expected due to the right dose that can provide amino acids. Similar results were also reported by Singh *et al*. (2011) and Khati *et al*. (2015). Singh *et al.* (2011) found that the addition of 2% papain enzyme in the feed on *Channos channos* increased protein efficiency ratio by 2.24, while Khati *et al.* (2015) found that the addition of 10 g/kg feed increased protein efficiency ratio by 2.30.

The relationship between papain enzyme and PER is presented in Figure 5. The relationship has a quadratic equation of $Y = -0.0386x^2 - 0.3586x + 2.6746$, $R^2 = 0.70$. The graph shows that the optimum dose of papain enzyme for PER was 3.77 g/kg feed with the PER value of 3.

Survival Rate (SR)

In this study, the addition of papain enzyme had no effect on SR of catfish (*Pangasius hypopthalmus*). As reported by Patih and Singh (2013), proteolitic enzyme does affect the fish survival rate. Moreover, Yakuputiyage (2013), reported that feed is not a factor influencing survival rate, but initial treatment and media quality affect survival rate. Similar results were obtained on *Channos channos* (Singh *et al*., 2011), *Macrobrachium rosenbergii* (Patil and Singh, 2014) and *Labeo rohita* (Khati *et al*., 2015).

CONCLUSION

Papain enzyme increased apparent digestibiity of protein, growth rate, efficiency of feed utilization, and protein efficiency ratio. On the other hand, it reduced feed conversion ratio of catfish. The optimum doses on ADC_P, RGR, EFU, FCR and PER were 4.05, 4.0, 3.93, 4.0, 3.77 g/kg feed, respectively. In conclusion, the papain enzyme has a positive effect on the growth performance of catfish **(***Pangasius hypopthalmus*).

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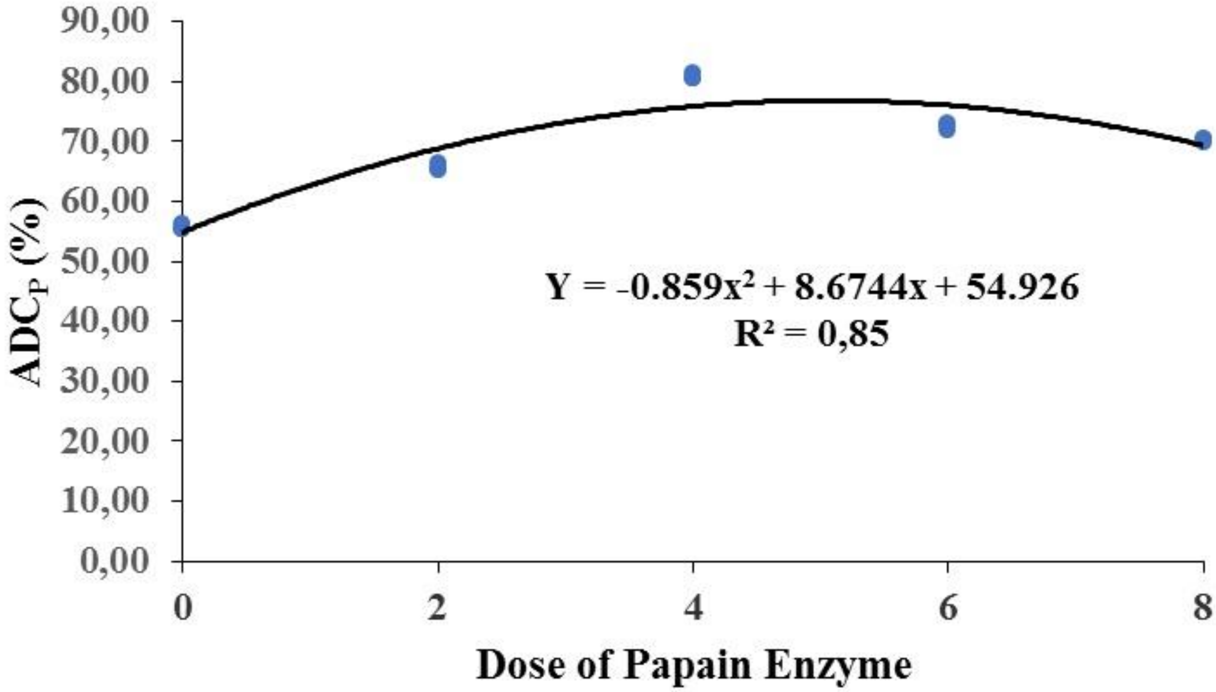

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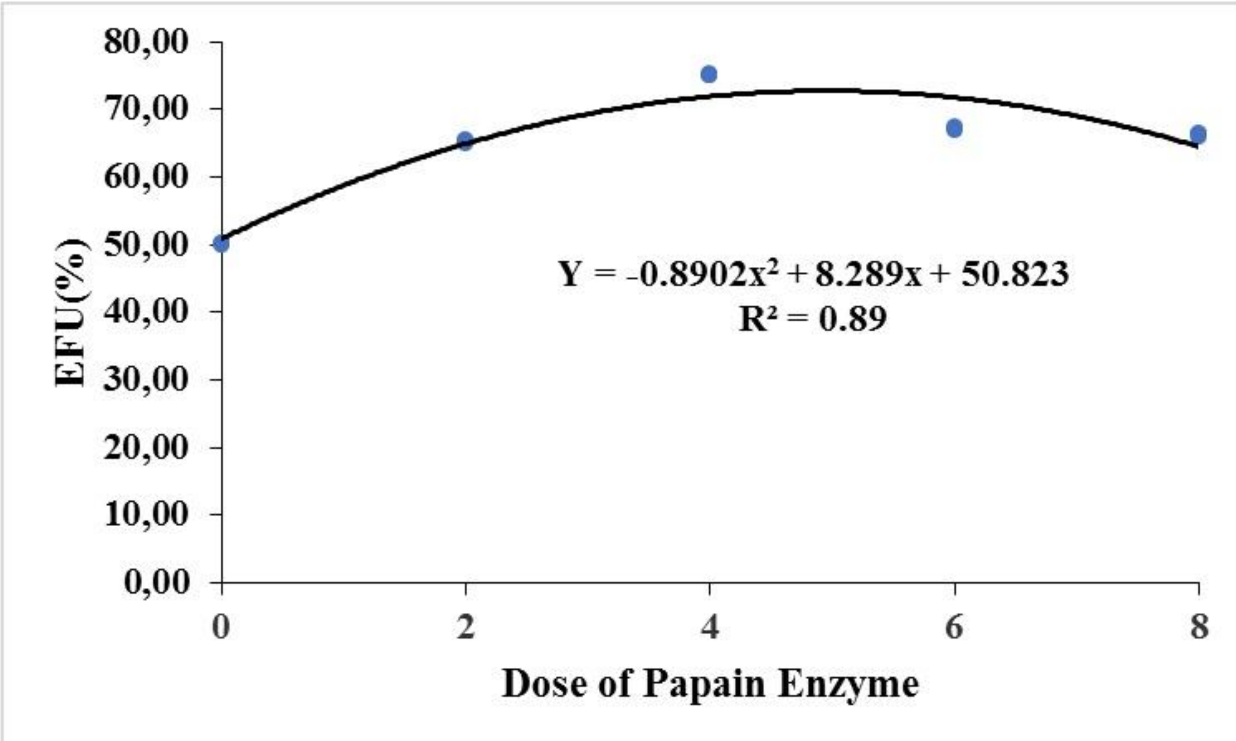

Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*)

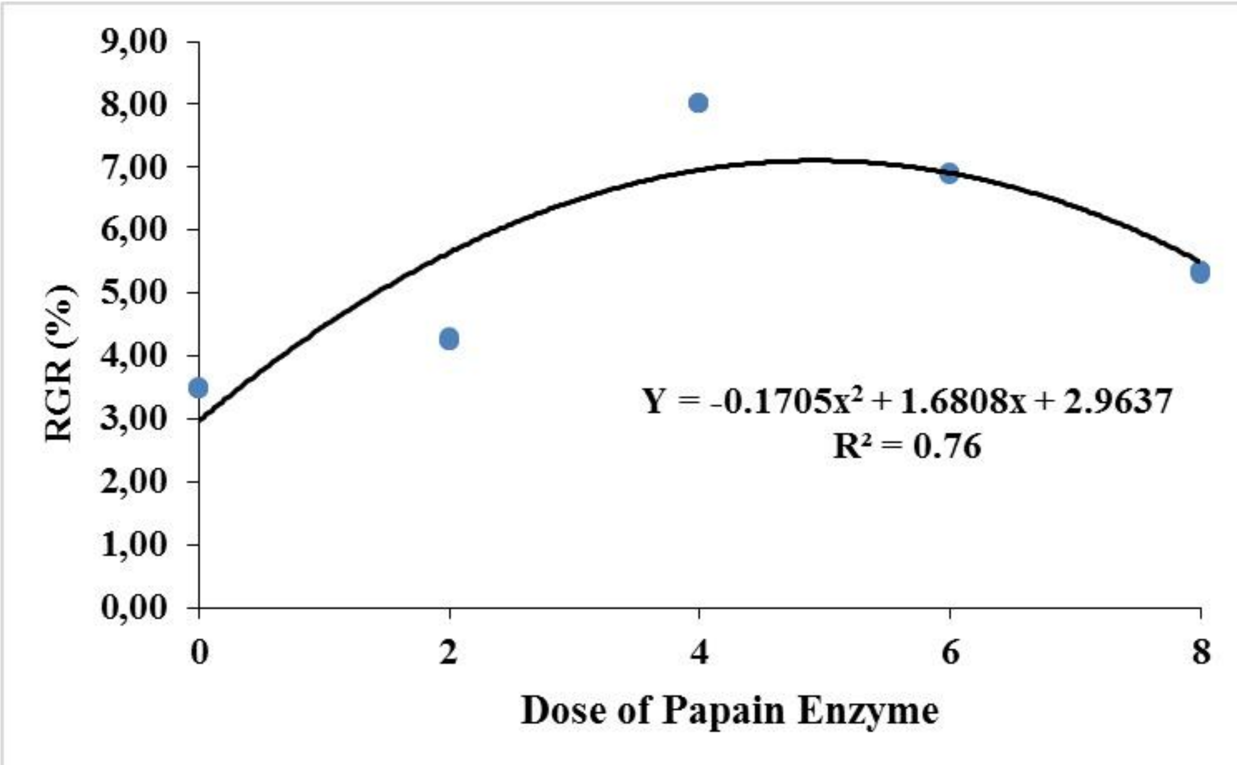

Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*)

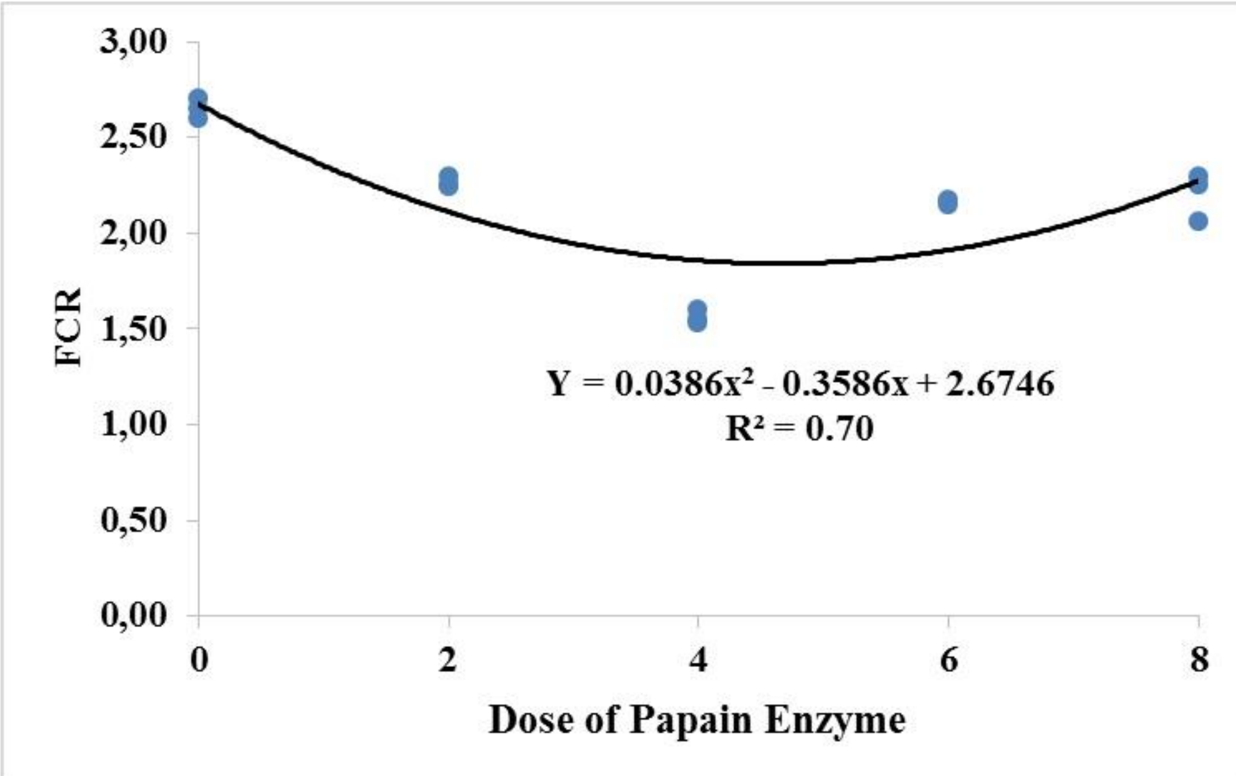

Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*)

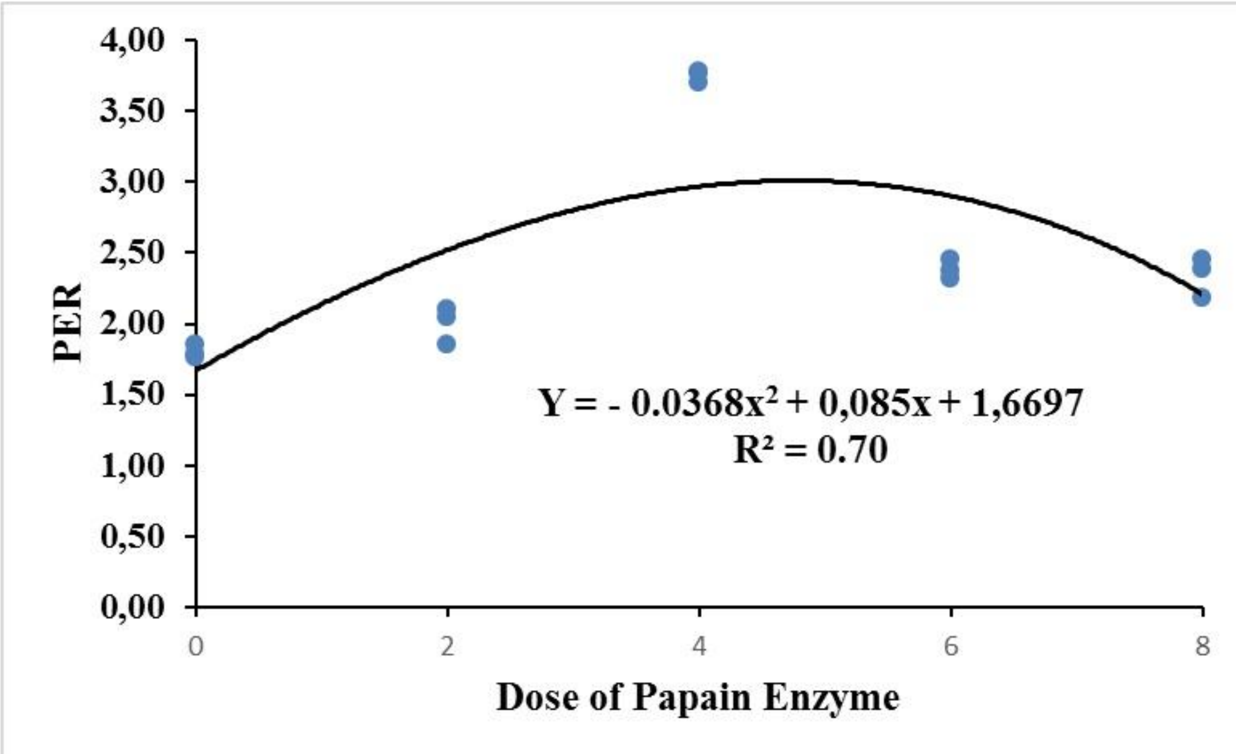

Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*)

Table 1. The composition and the results of proxymate 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 (kkal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kkal/g Diet)	8.02	8.05	8.03	8.02	8.02

Notes:

j. 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.

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

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

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

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Reviewer's Report Form

Title: EFFECT OF PAPAIN ENZYME DIET ON GROWTH PERFORMANCE OF CATFISH (*Pangasius hypopthalmus*)

Manuscript tracking number: MAB 665

Reviewer's comments:

Reviewer 1

1. The manuscript have got serious grammatical errors and spelling mistakes
2. The abstract to be revised
3. The title need to be revised
4. The authors need to work on the results and discussion and relate the work to previous published works
5. The manuscript to revised in totality

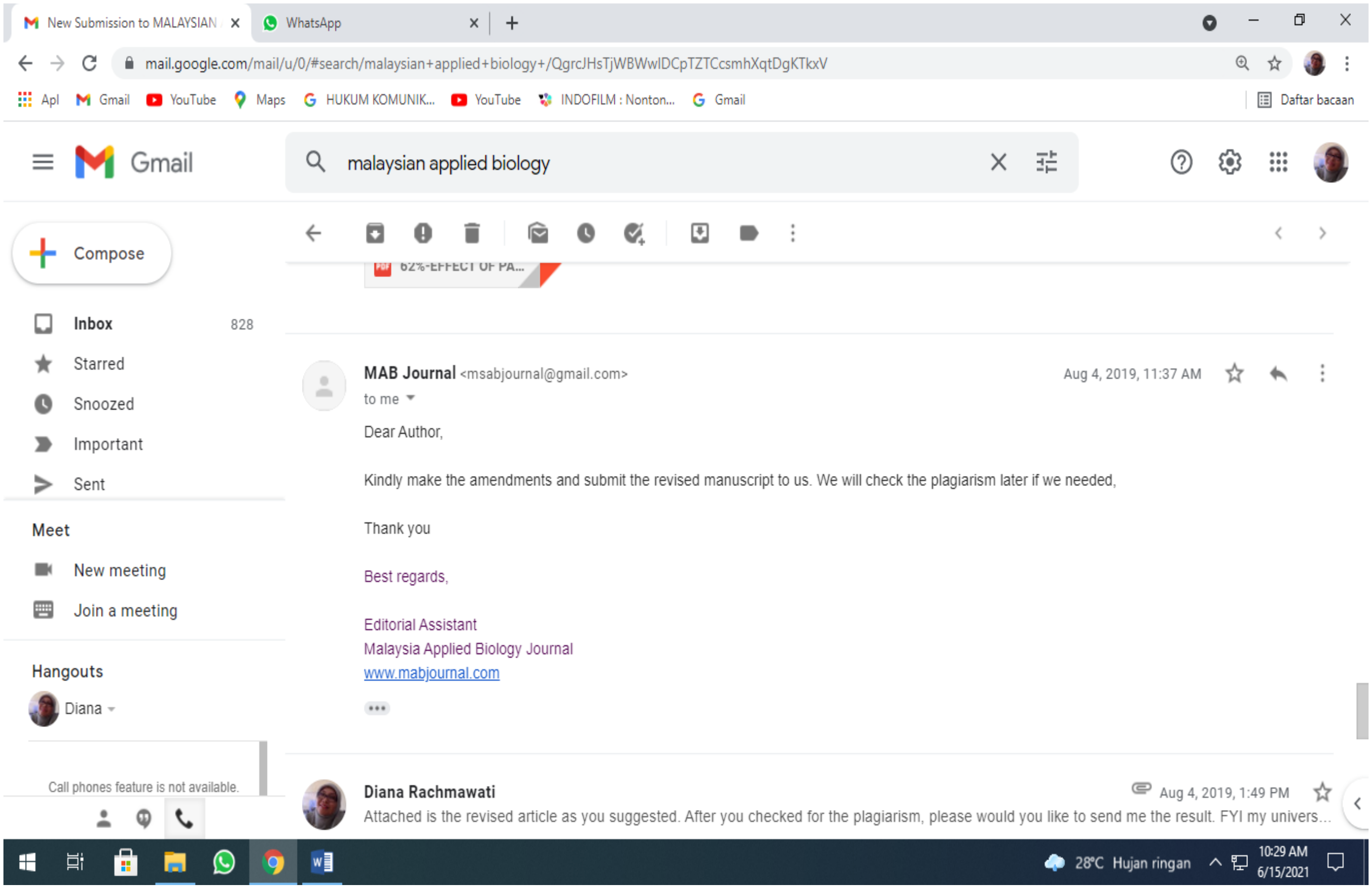
Reviewer 2

1. This manuscript confirmed that papain supplemented feed had significant effect on growth performance of Catfish (***Pangasius hypopthalmus***) without affecting survival. However, the effect was not linear. Some discussion could be offered for decreased growth performance at higher levels of papain.

2. Not clear where papain enzyme was extracted from. According to section on **Experimental design,** line 4: Four different diet concentration of papain enzyme (extracted from Papaya Latex). According to section on **Feed Preparation,** line 3**:** Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish.

3. Dasuki *et al*., 2013 not in list of Reference

4. There are some typo errors that require correction-please refer attachment, errors highlighted in red.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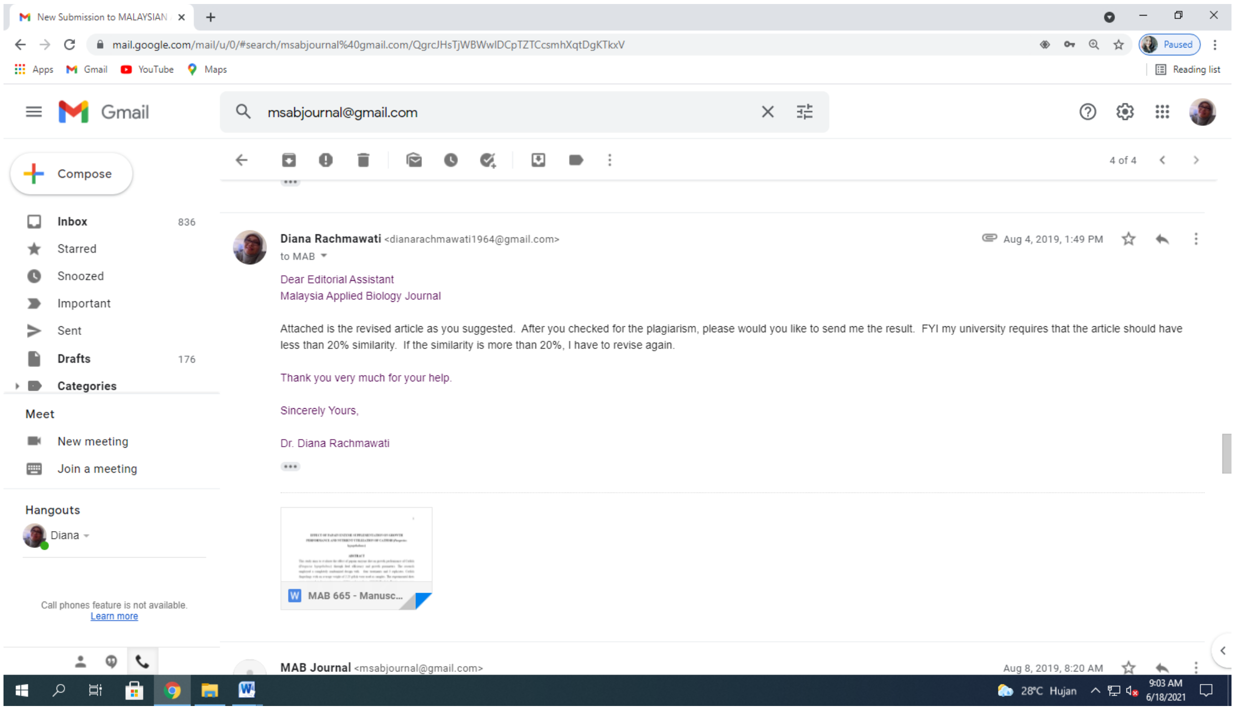
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EFFECT OF PAPAIN ENZYME SUPPLEMENTATION ON GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF CATFISH (*Pangasius hypopthalmus*)

ABSTRACT

This study aims to evaluate the effect of papain enzyme diet on growth performance of Catfish (*Pangasius hypopthalmus*) through feed efficiency and growth parameters. The research employed a completely randomized design with four treatments and 3 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 with in various doses; A (0 g/kg feed), B (2g/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), efficiency of feed utilization (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 ADCp, RGR, EFU, FCR, and PER, but not the SR 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 diet of papain enzyme increased the growth performance and nutrient utilization of catfish (*Pangasius hypopthalmus*).

Keywords: Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

INTRODUCTION

Catfish (*Pangasius hypopthalmus*) is one of fresh water fish which has a 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 a high demand of feed, since catfish aquaculture requires a lot of feed. One of the problems faced by fish farmers is low efficiency of feed utilization and high feed cost

(Hugues *et al*., 2018). The efficiency of feed utilization 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 additon of enzym was able to improve feed nutrient, especially to boost protein utilization. One of the enzymes is papain enzyme (Patil and Singh, 2014). Papain enzyme is able to break down amino acids. Thus, they are easy to digest (Amri and Mamboya, 2012). Ana *et al*. (2016) suggested that papain has numerous functions and can break down main tissue (tissue, collagen, and myofibrilar 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) 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 utilization 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 3 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 (treatmemnt 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 (50x30x30 cm^3) 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 the Table 1. The diets were prepared to be isonitrogenous (31%) and isocaloric (252 Kkal/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, Central Java, Indonesia. Prior to the experiment, feed nutrition was analyzed by using proxymate analysis (AOAC, 1990). The results of proxymate analysis are presented in the followint table.

Table 1. The Composition and The Results of Proxymate 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

Notes:

m. 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.

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

o. *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 hydrolyzed. At the same time, vitamin, mineral and fish oil were mixed and dillated with water evenly. Then, the mix 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. To determine the optimal dose of papain enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12 softwares.

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 are presented in the (Table 2).

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	$2.65\pm0,15^{c}$	$2.26\pm0,14^{b}$	$1.56\pm0,03^{a}$	$2.20\pm0,22^{b}$	$2.16\pm0,21^{b}$
PER	$1,80\pm0,05^{c}$	$2,00\pm0,26^{b}$	$3.75\pm0,06^{a}$	$2,34\pm0,27^{b}$	$2,38\pm0,13^{b}$
SR (%)	92.33 ± 5.77^{a}	92.00 ± 5.10^{a}	92.33 ± 5.77^{a}	93.33 ± 5.77^{a}	$93.00\pm5.78.0^{a}$

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

Apparent Digestibility Coefficient Of Protein (ADC_{P})

The results of ANOVA showed that the supplementation of papain enzyme in the feed had significant effect ($P<0.05$) on ADCp. The ADC_{P} increased after the addition of papain enzyme with the doses of 2-8g/kg feed (treatments B,C,D,E). Among all treatments, the addition of 4 g/kg papain (treatment C) was the highest ADCp (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 ADCp was due to the optimum dose that it could maximally hydrolyze protein; therefore, the digestibility of protein was higher in the treatment C than those in the treatmens 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 supplimented feed (Sing *et al*., 2011; Dabrowski and Glogowski, 1977). According to Khati *et al*. (2015) the increase of the protein digestibility was because of the ability of the papain enzyme to hydrolyze protein. Moreover, Rachmawati *et al*, (2018) discovered that the addition of papain enzyme in the feed could hydrolyze protein that boost 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 ADCp. 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 ADCp 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 ADCp 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 ADCp was obtained at 4.05 g/kg feed, while the optimum value of EFU is 75.09 %.

Efficiency of Feed Utilization (EFU)

The Catfish (*Pangasius hypopthalmus*) fed with the addition of papain enzyme with various doses ranged from 2 to 8 g/kg feed had the 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 hydrolyze protein; therefore, the protein was easily digested to increase feed utilization efficiency. This phenomenon was alaso discovered by Hastuti (2001). Rachmawati and Samidjan (2018) also found that the addition of papain enzyme in the diet could improve feed usage efficiency. The highest EFU (75.09%) was obtained by treatment C (4 g/kg feed) followed by treatments D (67.15%), E (66.25%), B (65.26%) and A (50.12%). The high value of

EFU in Catfish (*Pangasius hypopthalmus*) was expected due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyze protein; therefore, the protein was easily digested to increase feed utilization efficiency. It was supported by the study that that dose resulted in the highest ADCp. The high value of EFU suggested that less protein has been used for methabolism, but more protein has been used for fish growth (Tacon, 2002). Manguti *et al*. (2014) also reported that the high value of EFU suggested that less protein has been used for methabolism, 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 dan 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 perkg feed. The maxsimal value of ADCp was 81.25.

Relative Growth Rate (RGR)

The values of RGR in the Catfish (*Pangasius hypopthalmus*) 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 hydrolyze 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 hydrolyzes protein. The protease enzyme is the key enzyme to boost protein digestibility, accelerate absorption and enhance 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 hydrolyze 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 ADCp, EFU and PER. The lowest value of RGR was obtained in the treatment A (0 g/kg feed). It was suspected that protein hydrilysis 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 lead to the low RGR and low ADCp, EFU and PER. Without papain enzyme, the hydrolisys of long chain peptide into short chain peptide will not occurr. As a result, protein digestibility and diet utilization efficiency decreased. Fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolizes polypeptide into 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 hydrolyze lipid and carbohydrate in the diet (Ryosuke and Kazuhiko, 2004). The wrong dose of the addition of papain enzyme in the diet, whether the dose was shortage or excessed, could hinder fish growth. It was proven by Kazerani and Shahsavani (2011). Nutrient digestibility and absorption were obstructed because non-starch polysccharide was non soluble. The release of galactose and xilose from non-strach polysaccharide was due to the addition of papain enzyme in the diet. Similar findings were discovered in the studies of *Chanos channos* (Singh *et al*., 2011), *Macrobrachium rosenbergii* (Patil and 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 and Samidjan, 2018).

The relationship between papain enzyme and RGR was presented in the 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 the dose of 3.93 g/kg feed. The maximal 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 maximized; 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 10 papain enzyme per kg feed. Muchlisin et a.l (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. Morover, Rachmawati and Samidjan, (2018) found that the low value of FCR in Sangkuriang Catfish (Clarias sp) had occured 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 fulfill the need for essencial animo acids of fish (Singh *et al*., 2011). Catfish (*Pangasius hypopthalmus*) 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. The highest PER in the Catfish (*Pangasius hypopthalmus*) was obtained at the dose 4 g papin enzyme per kg feed. The dose level was the right amount of papain enzmye addition into the feed to increase protease enzyme activities in the digestive system; therefore, protein disgestibility and efficiency of feed utilization increased to build protein in the fish. The value of PER moved along the values of ADCp 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 waspresented 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 had no effect on SR of Catfish **(***Pangasius hypopthalmus*). The finding were supported by the findings of Patih and Singh (2013); Dabrowski and Glogowski (1977). They claimed that proteolitic 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* (Patil and Singh, 2014), *Labeo rohita* (Khati *et al*., 2015), *Cherax quadricarinatus* (Rachmawati *et al*., 2018), Sangkuriang Catfish (Clarias sp) (Rachmawati and Samidjan, 2018).

CONCLUSION

Papain enzyme increased apparent digestibiity of protein, growth rate, efficiency of feed utilization, 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 **(***Pangasius hypopthalmus*).

ACKNOWLEDGEMENTS

Appreciation expressed to those who already helped in this study, especially for the Head of the Center for Hatchery and Freshwater Aquaculture, Muntilan, Central Java, Indonesia who has provided laboratory to do the study.

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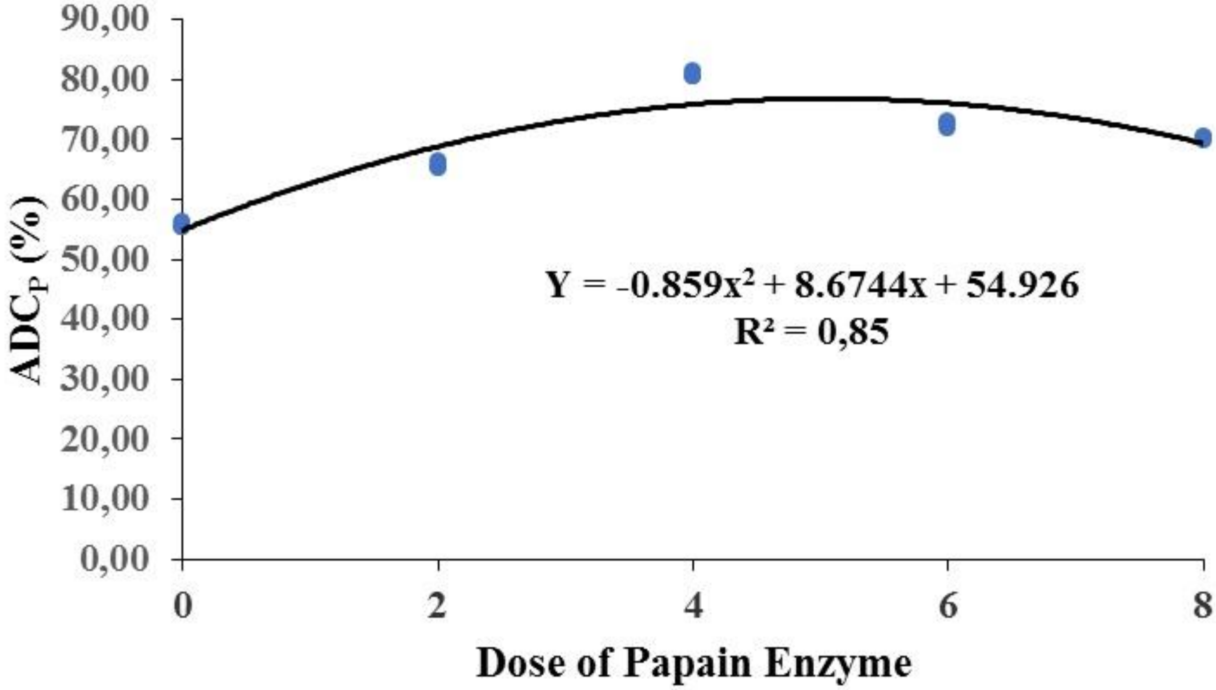


Fig. 1. The relationship between the dose of papain enzyme and ADC_P.

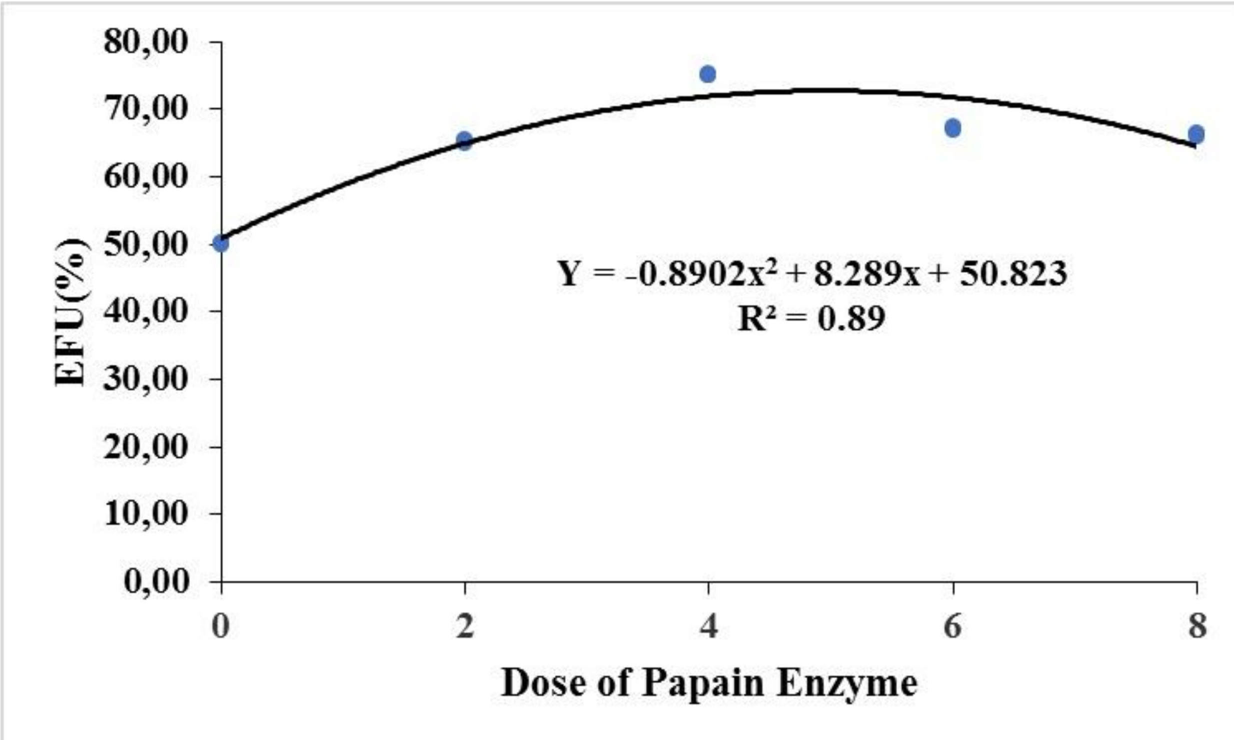


Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*)

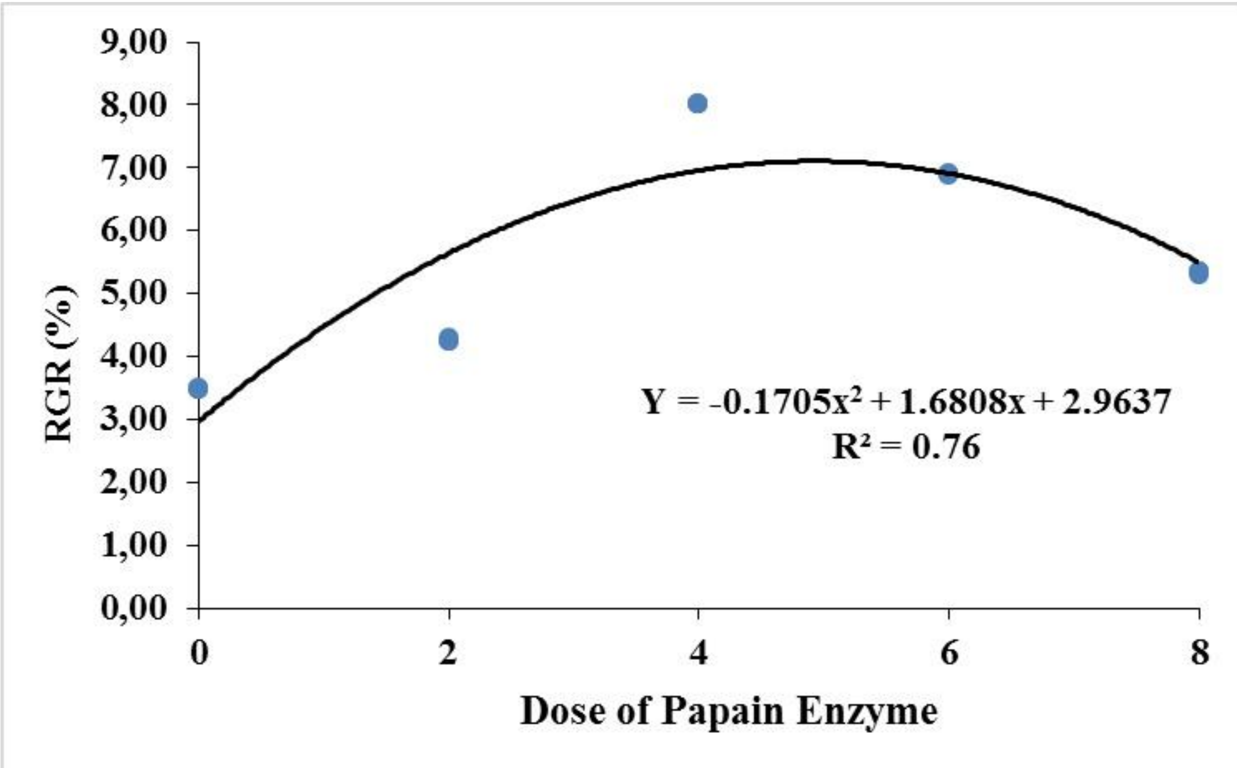


Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*)

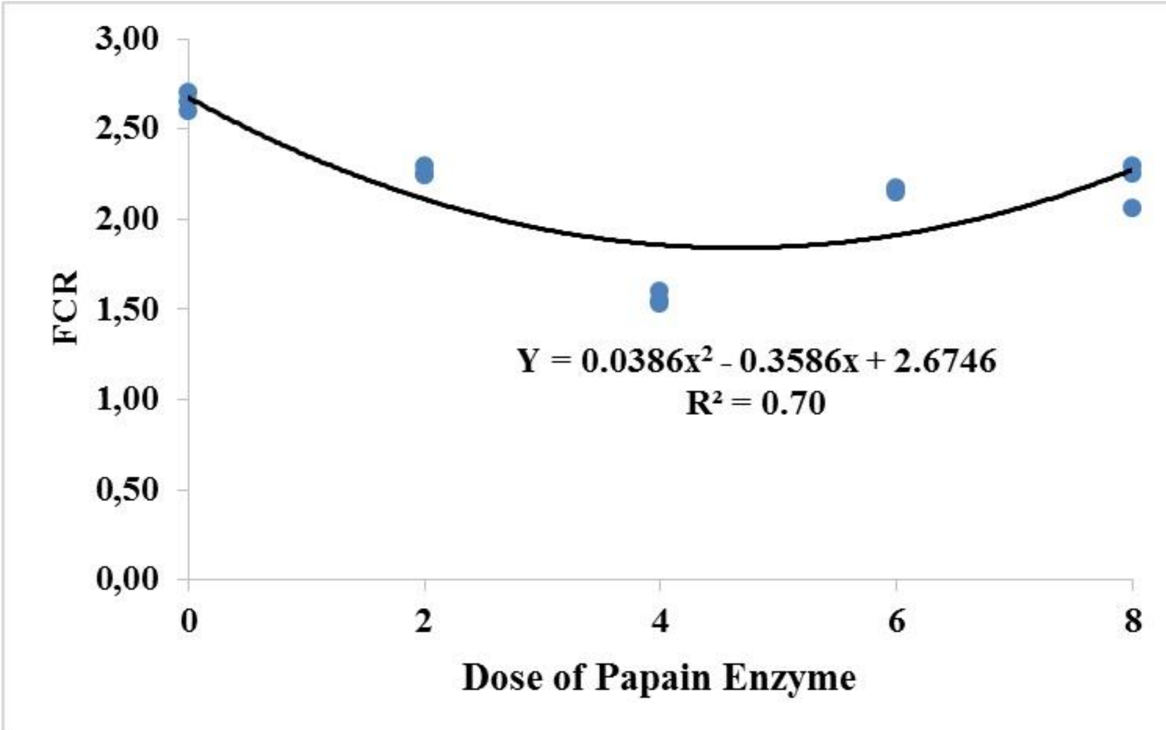


Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*)

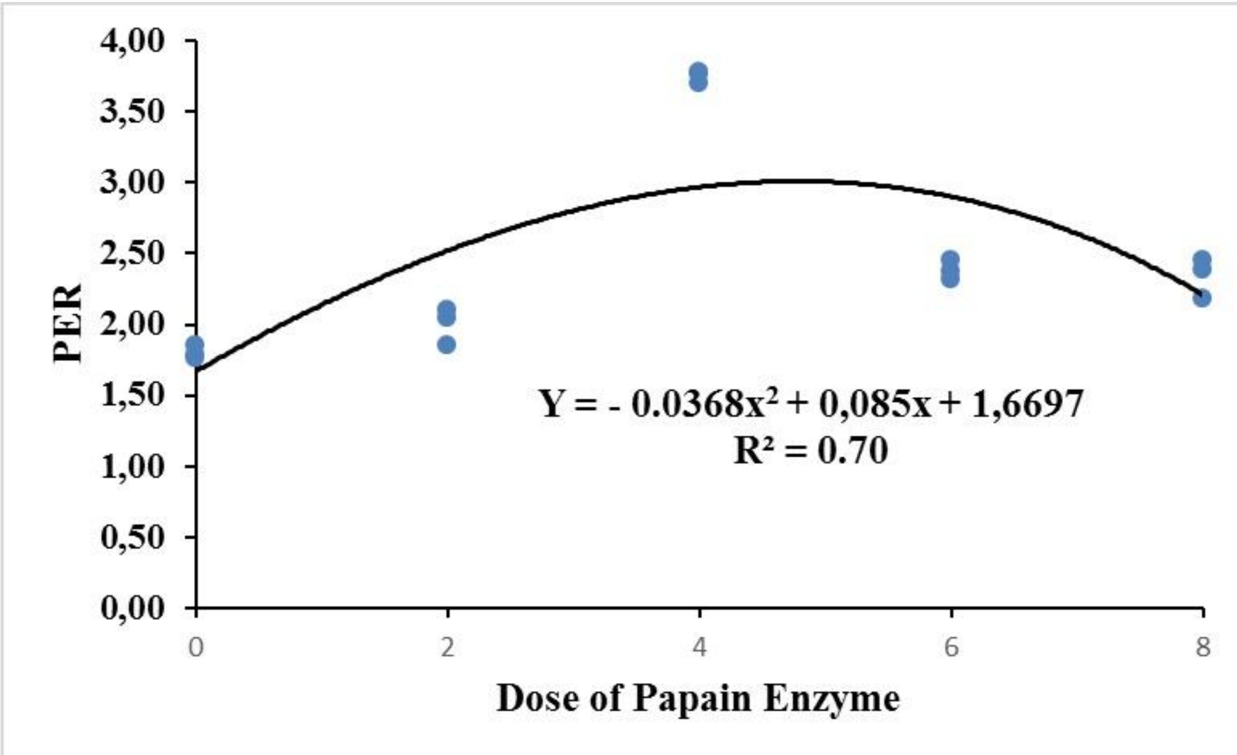


Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*)

Table 1. The composition and the results of proxymate 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 (kkal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kkal/g Diet)	8.02	8.05	8.03	8.02	8.02

Notes:

p. 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.

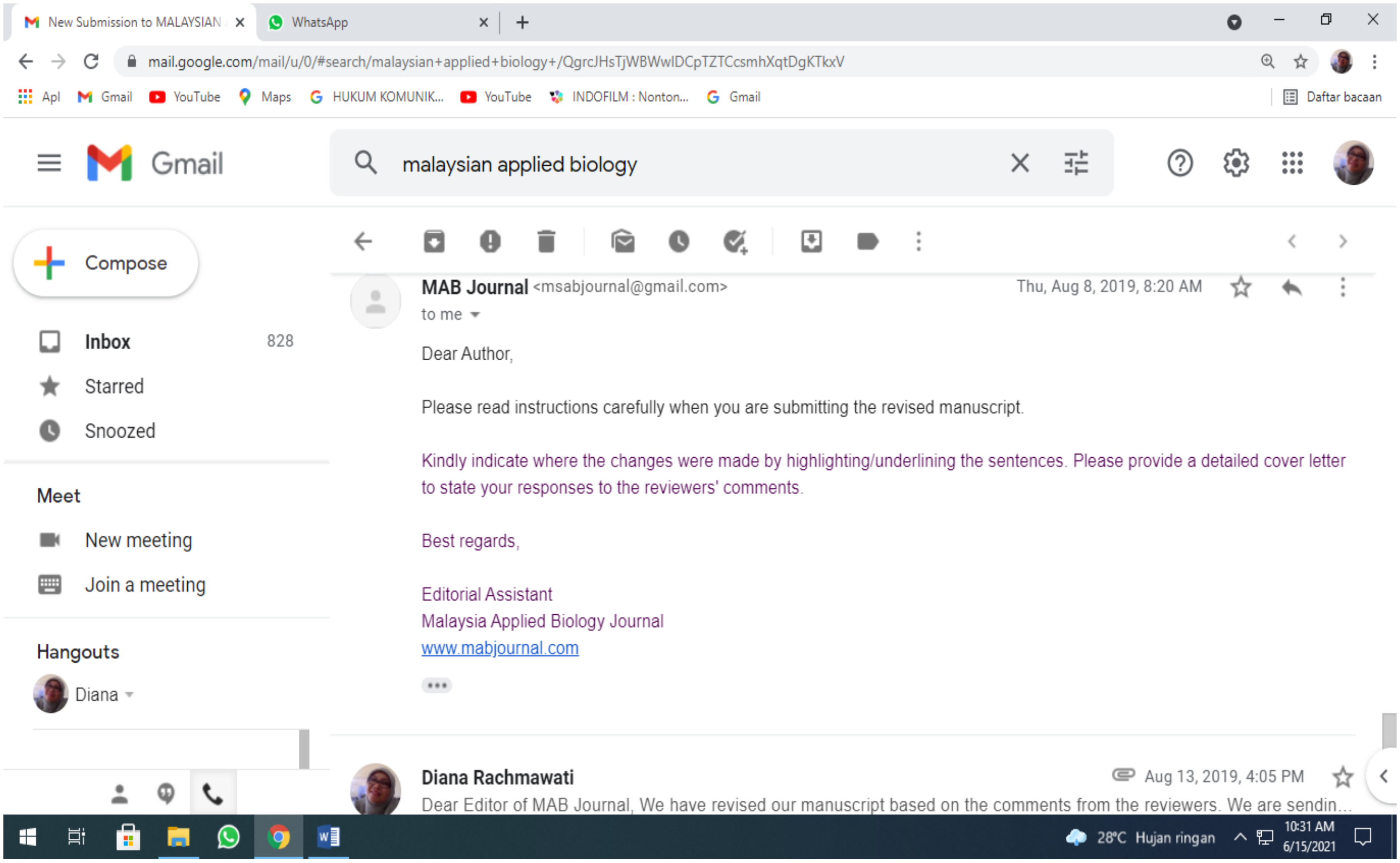
q. According Brooke and Daniel (2013), the optimal E/P ratio for growth ranges from 8 kcal/g to 12 kcal/g.

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

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



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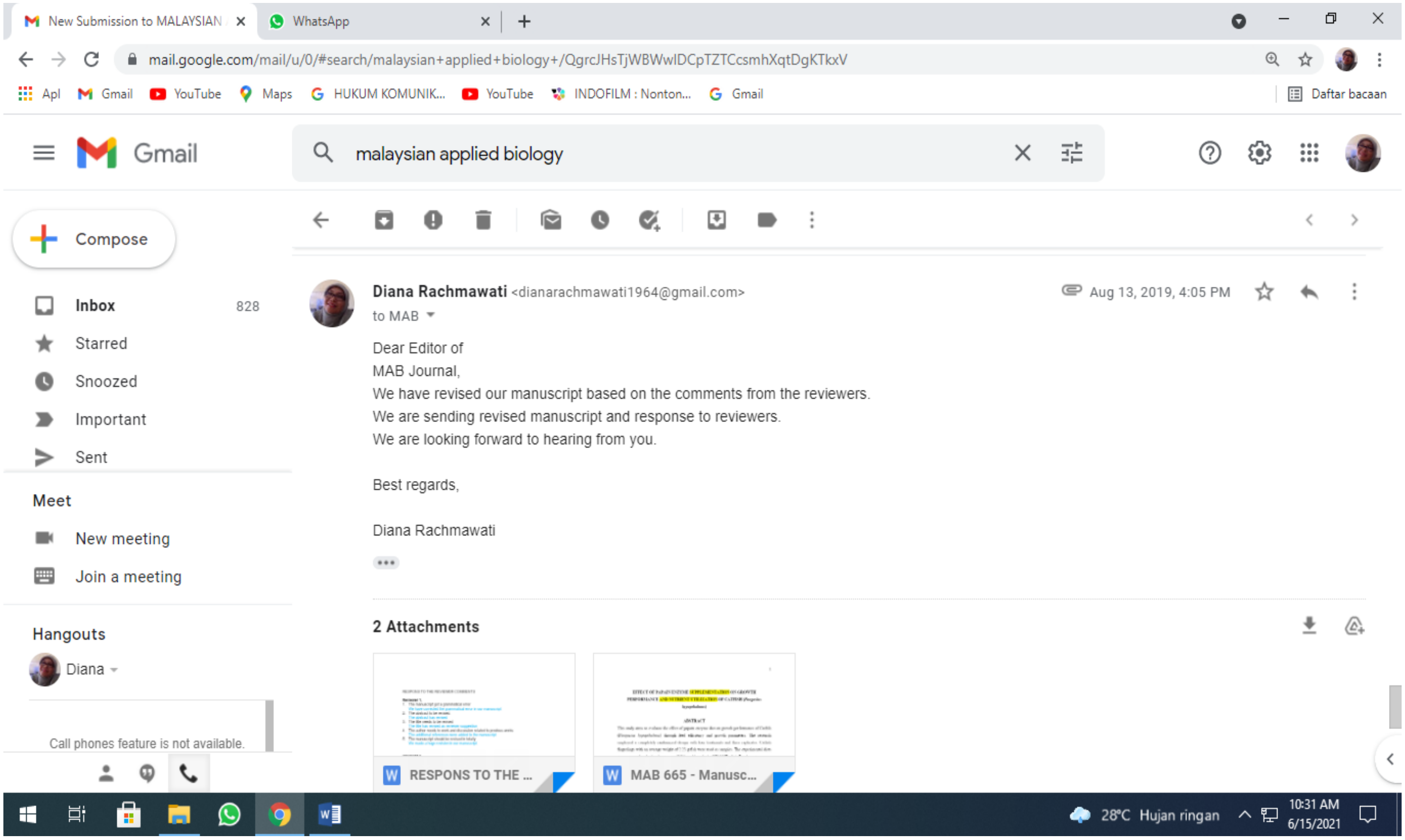
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RESPONS TO THE REVIEWER COMMENTS

Reviewer 1.

1. The manuscript got a grammatical error
   We have corrected the grammatical error in our manuscript
2. The abstract to be revised.
   The abstract has revised
3. The title needs to be revised
   The title has revised as reviewer suggestion
4. The author needs to work and discussion related to previous works
   The additional references were added to the manuscript
5. The manuscript should be revised in totally.
   We made a huge revision in our manuscript.

Reviewer 2.

1. This manuscript confirmed that papain supplemented feed had a significant effect on growth performance of Catfish **(*Pangasius hypopthalmus*)** without affecting survival. However, the effect was not linear. Some discussion could be offered for decreased growth performance at higher levels of papain.
   References were added to the discussion section

2. Not clear where papain enzyme was extracted from. According to section on **Experimental design,** line 4: Four different diet concentration of papain enzyme (extracted from Papaya Latex). According to section on **Feed Preparation,** line 3**:** Crude papain enzyme was extracted from Papaya fruit (*Carica papaya*) by Center for Brackish.

   The papain enzyme used in this study is from Carica papaya, which is prepared by the Center for brackish. The enzyme preparation has revised.

3. Dasuki *et al*., 2013 not in list of Reference

   Dasuki et al., 2013 was added to the reference list

4. There are some typo errors that require correction-please refer attachment, errors highlighted in red.

   The typo error has revised, and all manuscript was carefully re-checked.

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

ABSTRACT

This study aims to evaluate the effect of papain enzyme diet on growth performance of Catfish **(***Pangasius hypopthalmus*) through feed efficiency and growth parameters. The research employed a completely randomized 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 (2g/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 utilization (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 ADCp, 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 utilization of catfish **(***Pangasius hypopthalmus*).

Keywords: Diet, Papain enzyme, Catfish (*Pangasius hypothalamus*), Growth performance

INTRODUCTION

Catfish (*Pangasius hypopthalmus*) 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 a lot of feed. One of the problems faced by fish farmers is low efficiency of feed utilization and high feed cost

(Hugues *et al*., 2018). The efficiency of feed utilization 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 enzym was able to improve feed nutrient, especially to boost protein utilization. One of the enzymes is papain enzyme (Patil and Singh, 2014). Papain enzyme can break down amino acids. Thus, they are easy to digest (Amri and 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) 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 utilization 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 (50x30x30 cm^3) 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 Kkal/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. Prior to the experiment, feed nutrition was analyzed 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

Notes:

s. 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.

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

u. *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 hydrolyzed. 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 are presented in the (Table 2).

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	$2.65\pm0,15^c$	$2.26\pm0,14^b$	$1.56\pm0,03^a$	$2.20\pm0,22^b$	$2.16\pm0,21^b$
PER	$1,80\pm0,05^c$	$2,00\pm0,26^b$	$3.75\pm0,06^a$	$2,34\pm0,27^b$	$2,38\pm0,13^b$
SR (%)	92.33 ± 5.77^a	92.00 ± 5.10^a	92.33 ± 5.77^a	93.33 ± 5.77^a	$93.00\pm5.78.0^a$

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

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 ADCp. The ADC_P increased after the addition of papain enzyme with the doses of 2-8g/kg feed (treatments B,C,D,E). Among all treatments, the addition of 4 g/kg papain (treatment C) was the highest ADCp (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

ADCp was due to the optimum dose that it could maximally hydrolyze 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 and 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 hydrolyze protein. Moreover, Rachmawati *et al.*, (2018) discovered that the addition of papain enzyme in the feed could hydrolyze 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 ADCp. 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 ADCp 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 ADCp 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 ADCp was obtained at 4.05 g/kg feed, while the optimum value of EFU is 75.09 %.

The efficiency of Feed Utilization (EFU)

The Catfish (*Pangasius hypopthalmus*) 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 hydrolyze protein; therefore, the protein was easily digested to increase feed utilization 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 hypopthalmus*) was expected due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyze protein; therefore, the protein was easily digested to increase feed utilization efficiency. It was supported by the study that that dose resulted in the highest ADCp. 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). Morover, NRC (2011) suggested that the high value of EFU indicated high quality of the feed; therefore, the feed can be utilized 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 dan 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 ADCp was 81.25.

Relative Growth Rate (RGR)

The values of RGR in the Catfish **(***Pangasius hypopthalmus*) 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 hydrolyze 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 hydrolyzes 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 hydrolyze 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 ADCp, 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 ADCp, EFU, and PER. Without papain enzyme, the hydrolysis of long-chain peptide into short-chain peptide will not occur. As a result, protein digestibility and diet utilization efficiency decreased. The fish growth rate will consequently be not worthy. Papain enzyme is a protease enzyme that hydrolizes 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 hydrolyze lipid and carbohydrate in the diet (Ryosuke and 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 and 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 channos* (Singh *et al*., 2011), *Macrobrachium rosenbergii* (Patil and 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 and 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 maximized; 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 10 papain enzyme per kg feed. Muchlisin et a.l (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. Morover, 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 fulfill the need for essential amino acids of fish (Manush *et al*., 2013). Catfish **(***P.hypopthalmus*) 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. Hepher (1988) indicated that the high value of PER represented high protein efficiency and high growth. The highest PER in the Catfish **(***P.hypopthalmus*) 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 digestive system; therefore, protein digestibility and efficiency of feed utilization increased to build protein in the fish. The value of PER moved along the values of ADCp 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 **(***Pangasius hypopthalmus*). The finding was supported by the findings of Patih and Singh (2014); Dabrowski and Glogowski (1977). They claimed that proteolitic 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* (Khati *et al*., 2015), *Cherax quadricarinatus* (Rachmawati *et al*., 2018), Sangkuriang Catfish (Clarias sp) (Rachmawati and Samidjan, 2018).

CONCLUSION

Papain enzyme increased apparent digestibility of protein, growth rate, efficiency of feed utilization, 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 **(***Pangasius hypopthalmus*).

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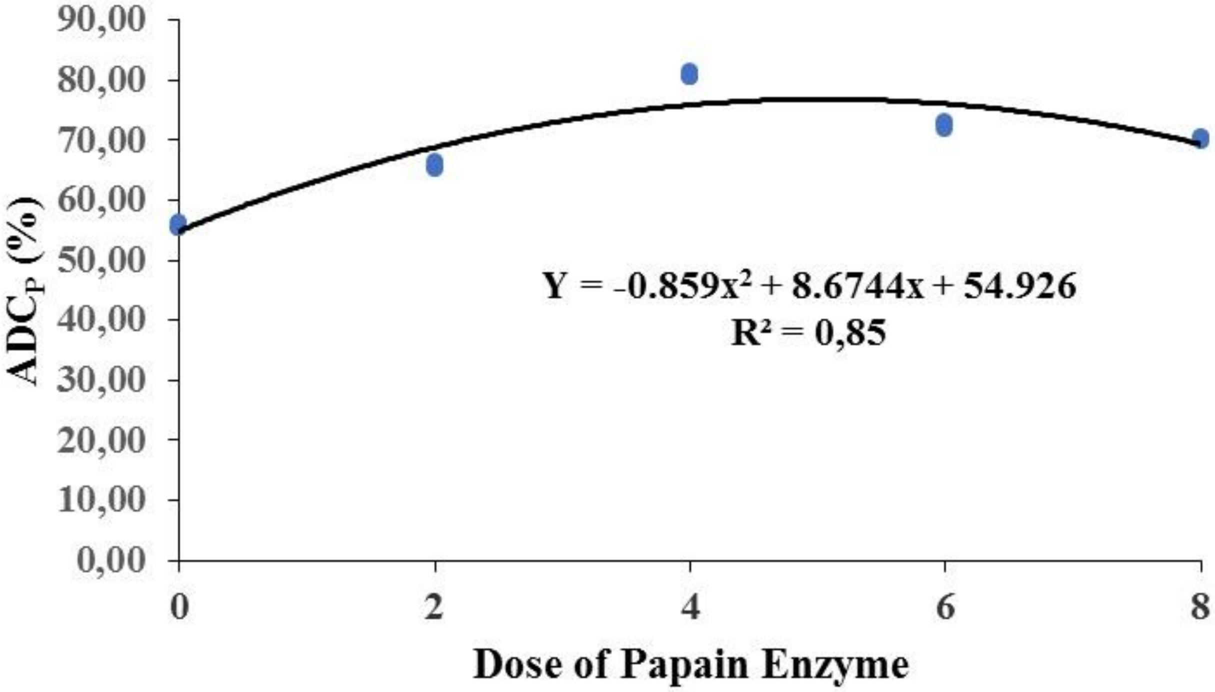


Fig. 1. The relationship between the dose of papain enzyme and ADC_P.

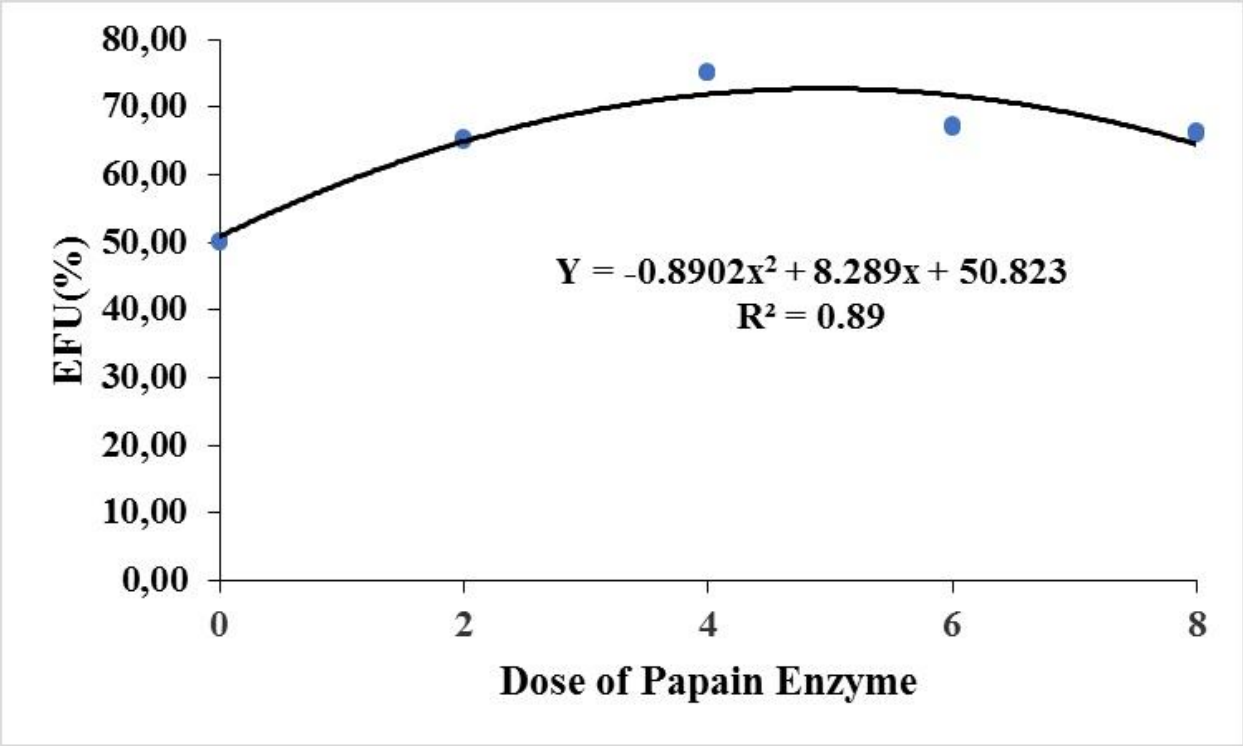


Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*)

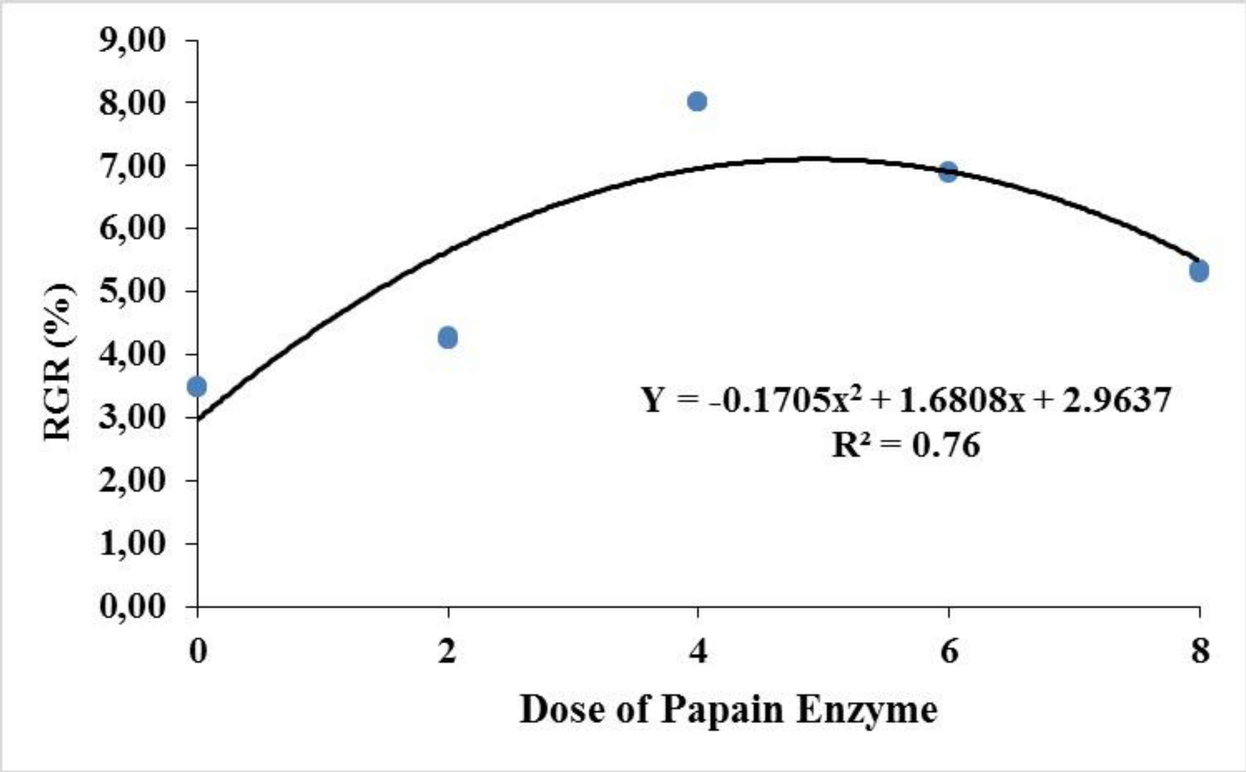


Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*)

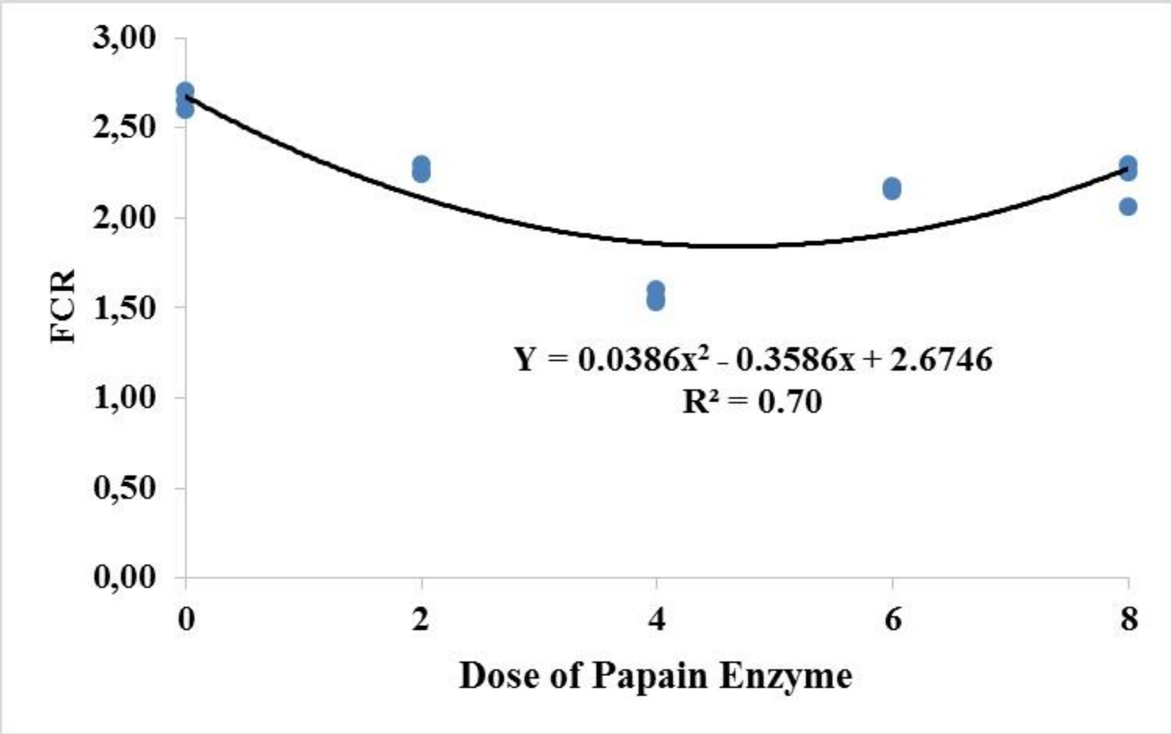


Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*)

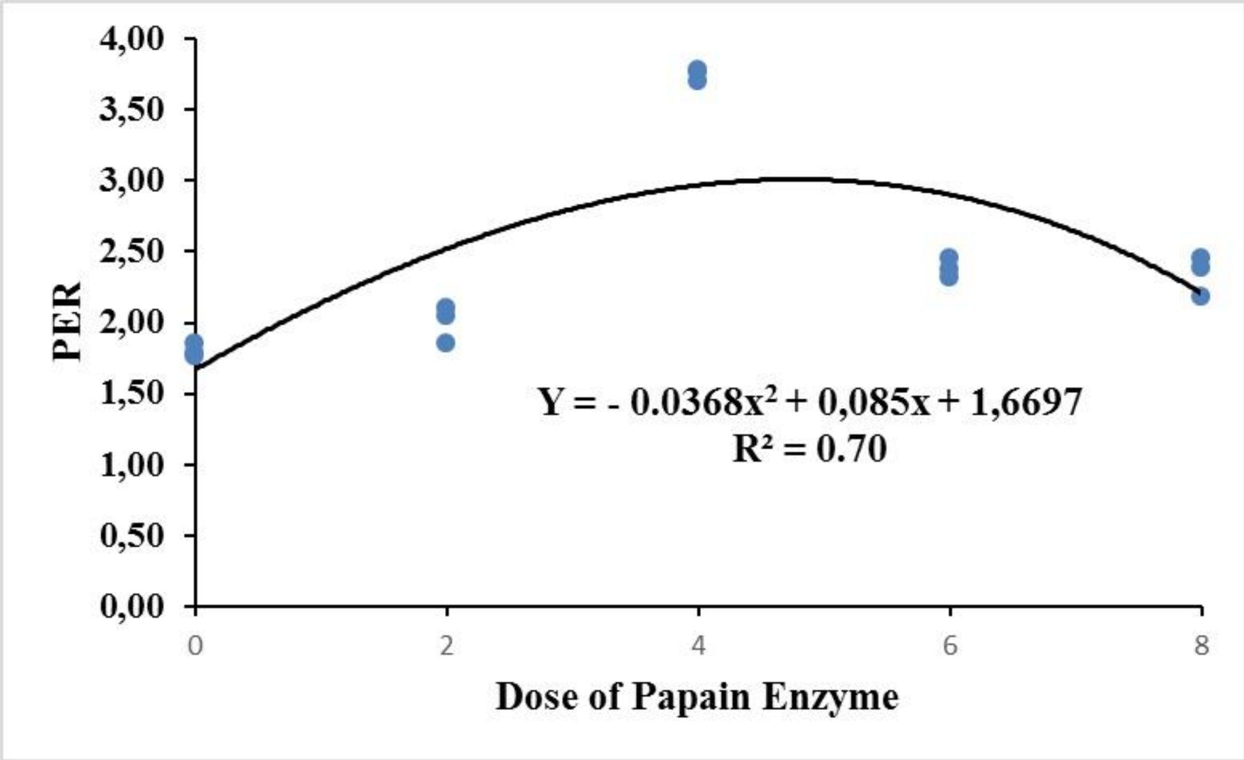


Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*)

Table 1. The composition and the results of proxymate 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 (kkal/g)	252.06	252.02	252.27	250.04	250.04
Ratio E/D (kkal/g Diet)	8.02	8.05	8.03	8.02	8.02

Notes:

v. 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.

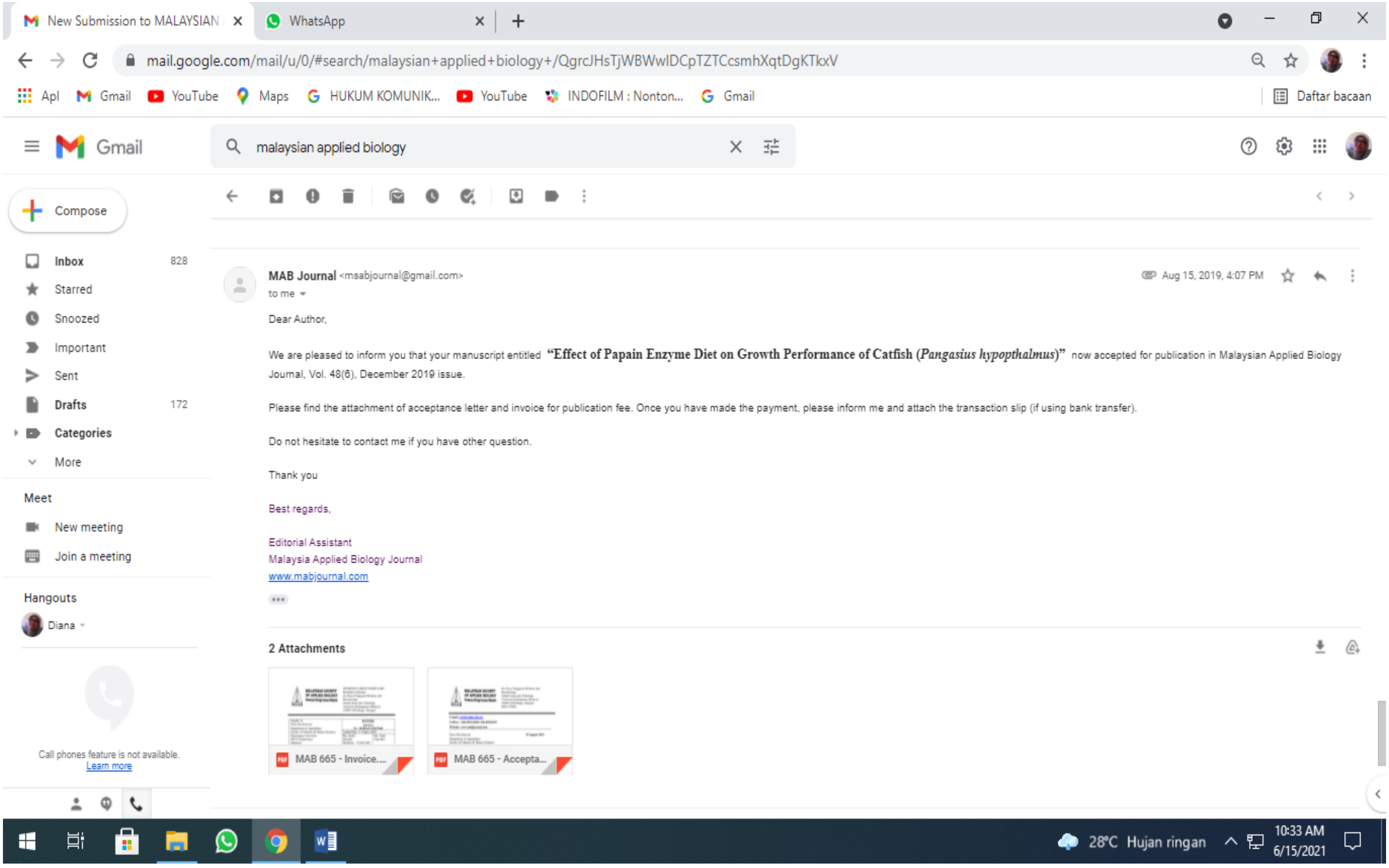
w. According Brooke and Daniel (2013), the optimal E/P ratio for growth ranges from 8 kcal/g to 12 kcal/g.

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

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



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15 August 2019

Dear Sir/Madam/Ms,

We would like to inform that your manuscript entitled **"Effect of Papain Enzyme Diet on Growth Performance of Catfish (*Pangasius hypopthalmus*)"** written by *Diana Rachmawati and Asep A. Prihanto.* has been accepted for publication in the Malaysian Applied Biology Journal (MABJ), Vol. 48 (6), December 2019 Issue.

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