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

by Diana Rachmawati

Malays. Appl. Biol. (2019) 48(5): 1–10

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

DIANA RACHMAWATI[1*] and ASEP A. PRIHANTO[2,3,4]

[1]*Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, Semarang, Central Java 50275 Indonesia*
[2]*Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Brawijaya University, Jl. Veteran, Malang, East Java 65145, Indonesia*
[3]*Bio-Seafood Research Unit, Brawijaya University, Jl. Veteran, Malang, East Java, Indonesia*
[4]*Halal Thoyib Science Center, Brawijaya University, Jl. Veteran, Malang, East Java, Indonesia*
**E-mail: dianarachmawati1964@gmail.com*

Accepted 15 August 2019, Published online 31 December 2019

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 randomised design with four treatments and three replicates. Catfish fingerlings with an average weight of 2.23 g/fish were used as samples. The experimental diets were prepared to be isonitrogenous (31%) and isocaloric (252.06 kcal/g). Papain enzyme was added to the feed within various doses; A (0 g/kg feed), B (2 g/kg feed), C (4 g/kg feed), D (6 g/kg feed) and E (8 g/kg feed). The study parameters included apparent digestibility coefficient of protein (ADC_P), relative growth rate (RGR), the efficiency of feed utilisation (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR) and water quality. The results of the study show that the addition of papain enzyme significantly affected 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 utilisation of catfish (*Pangasius hypopthalmus*).

Key words: 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 much feed. One of the problems faced by fish farmers is low efficiency of feed utilisation and high feed cost (Hugues *et al.*, 2018). The efficiency of feed utilisation still needs further improvement. One of the efforts to increase efficiency is by adding enzyme in feed (Ebert, 2014).

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

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

* To whom correspondence should be addressed.

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

MATERIALS AND METHODS

Experimental design

The study was conducted from February to June 2017 at the Center for Hatchery and Freshwater Aquaculture, Muntilan, Central Java, Indonesia. The research employed a completely randomized design with four treatments and three replicates. The four treatments were by adding 0 g papain enzyme per kg feed (treatment A), 2 g papain enzyme per kg feed (treatment B), 4 g papain enzyme per kg feed (treatment C), 6 g papain enzyme per kg feed (treatment D), and 8 g papain enzyme per kg feed (treatment E) (Farrag *et al.*, 2013).

Fish preparation

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

Feed preparation

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

Table 1. The composition and the results of proximate analysis

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

a. The values were calculated based on Digestible Energy (Glencross *et al.*, 2011) for 1 g protein equals 3.5 kcal, 1 g fat equals 8.1 kcal, and 1 g carbohydrate equals 2.5 kcal.
b. According to Brooke and Daniel (2013), the optimal E/P ratio for growth ranges from 8 kcal/g to 12 kcal/g.
c. *Animal Nutrient Laboratory, Faculty of Husbandry and Agriculture, Diponegoro University (2017).

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

Performance analysis

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

The parameters were measured using the following equation:

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

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

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

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

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

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

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

Statistical analysis

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

RESULTS AND DISCUSSION

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

Apparent digestibility coefficient of protein (ADC_P)

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

Table 2. The values of investigated parameters

Experiment data	Diet treatments				
	A	B	C	D	E
ADC_P	55.67±0.02[d]	65.70±0.03[c]	80.83±0.05[a]	72.58±0.04[b]	70.25±0.05[b]
EFU (%)	50.12±0.24[c]	65.26±0.97[b]	75.09±0.75[a]	67.15±0.26[b]	66.25±0.57[b]
RGR (%/day)	3.48±0.10[d]	4.26±0.25[c]	8.01±0.27[a]	6.89±0.16[b]	5.33±0.14[b]
FCR	2.65±0,15[c]	2.26±0,14[b]	1.56±0,03[a]	2.20±0,22[b]	2.16±0,21[b]
PER	1,80±0,05[c]	2,00±0,26[b]	3.75±0,06[a]	2,34±0,27[b]	2,38±0,13[b]
SR (%)	92.33±5.77[a]	92.00±5.10[a]	92.33±5.77[a]	93.33±5.77[a]	93.00±5.78.0[a]

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

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

The relation between 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 utilisation (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 hydrolyse protein; therefore, the protein was easily digested to increase feed utilisation efficiency. This phenomenon was also discovered by Hastuti (2001). Rachmawati and Samidjan (2018) also found that the addition of papain enzyme in the diet could improve feed usage efficiency. Treatment C (4 g/kg feed) gave the highest EFU (75.09%) followed by treatments D (67.15%), E (66.25%), B (65.26%) and A (50.12%). The high value of EFU in Catfish (*Pangasius hypopthalmus*) was expected due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyse protein; therefore, the protein was easily digested to increase feed utilisation efficiency. It was supported by the study that that dose resulted in the highest ADC_P. The high value of EFU suggested that less protein has been used for metabolism, but more protein has been used for fish growth (Tacon, 2002). Morover, NRC (2011) suggested that the high value of EFU indicated high quality of the feed; therefore, the feed can be utilised more efficiently. Manguti *et al.* (2014) also reported that the high value of EFU suggested that less protein has been used for metabolism, but more protein has been used for fish growth. The values of EFU in each treatment in the study was considered quite good since the values were higher than 50%. The good value of EFU was based on the Craig dan Helfrich (2002) opinion. They claimed that the value of EFU was good if the value was higher than 50% or even it reached 100%. Similar results were found by Patil and Singh (2014) in *Macrobrachium rosenbergii*, Muchlisin *et al*. (2016) in Keureling fish (Tor tambra), Rostika *et al*. (2018) in *Oreochromis niloticus*, Rachmawati *et al*. (2018) in *Cherax quadricarinatus*, Rachmawati and Samidjan (2018) in Sangkuriang Catfish (*Clarias* sp).

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

Relative growth rate (RGR)

The values of RGR in the Catfish (*Pangasius 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 hydrolyse protein; therefore, the protein was easily digested to increase fish growth. The finding was also supported by Singh *et al.* (2011). They suggested that papain enzyme is the protease enzyme that hydrolyses protein. The protease enzyme is the key enzyme to boost protein digestibility, accelerate absorption, and enhance growth. Wong *et al.* (1996) pointed out that papain can break down protein into amino acids; therefore, it increased protein digestibility, nutrients absorption, and fish growth. The highest value of RGR (8.10%/day) was in the treatment C (4 g/kg feed) followed by treatments D (6.89%/day), E (5.33%/day), B (4.26%/day) and A (3.48%/day). The highest value of RGR was obtained in the treatment C (4 g/kg feed). It was expected due to the right dose of papain enzyme addition in the diet (4 g/kg feed) to hydrolyse protein; therefore, the protein was easily digested to improve fish growth. The dose of papain enzyme addition in the diet (4 g/kg feed) brought about the highest value of RGR. It also generated the highest values of 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

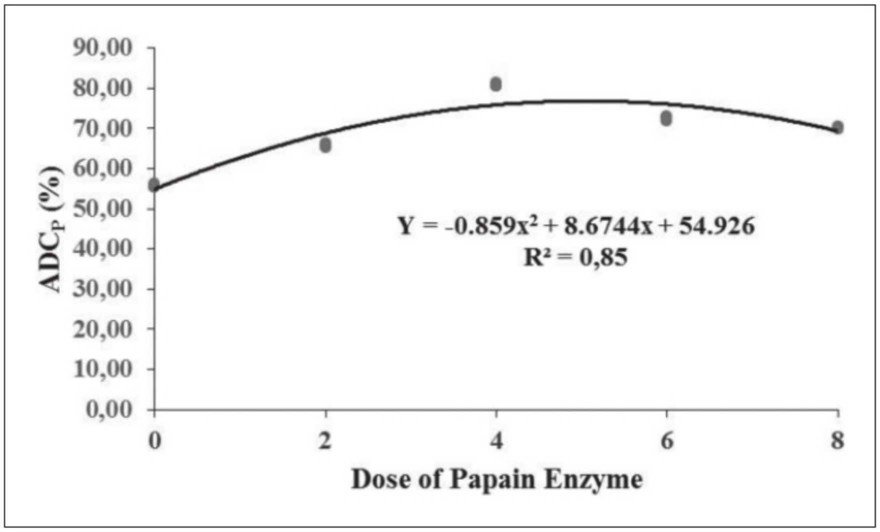

Fig. 1. The relationship between the dose of papain enzyme and ADC_P.

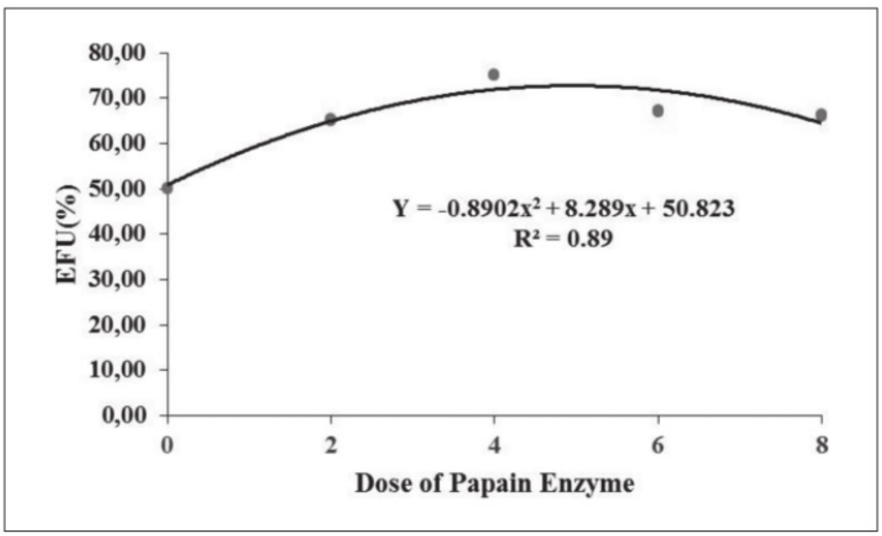

Fig. 2. The relationship between papain enzyme addition in the feed and EFU of catfish (*Pangasius hypopthalmus*).

digestibility and diet utilisation 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 hydrolyse lipid and carbohydrate in the diet (Ryosuke & Kazuhiko, 2004). The inappropriate dose of the papain enzyme in the diet, whether the dose was a deficiency or excessed, could hinder fish growth (Kazerani & Shahsavani (2011). Nutrient digestibility and absorption were obstructed because non-starch polysaccharide was nonsoluble. The release of galactose and xylose from not-starch polysaccharide was due to the addition of papain enzyme in the diet. Similar findings were discovered in the studies of *Chanos channos* (Singh *et al*., 2011), *Macro-brachium rosenbergii* (Patil & Singh, 2014), *Oreochromis niloticus* (Manguti *et al*., 2014), *Labeo rohita* (Khati *et al*., 2015), *Trachinotus blochii* (Mo *et al*., 2016), *Cherax quadricarinatus* (Rachmawati *et al*., 2018), *Clarias* sp. (Rachmawati & Samidjan, 2018).

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

Feed conversion ratio (FCR)

The values of FCR in which the feed was supplemented with papain enzyme with the doses of 2-8 g/kg feed were lower than that without enzyme addition. It was suggested that the addition of papain enzyme could boost protein digestibility and feed usage efficiency; in turn, it decreased feed conversion ratio. The lowest FCR was found in the treatment C (4 g/kg feed). It was suggested that at the dose of 4 g/kg feed protein digestibility and feed usage efficiency were maximised; therefore, it decreased FCR. The low value of FCR indicated that the fish was optimally digested and absorbed the nutrient (Steffens, 1989). Singh *et al*. (2011) studied in *Chanos channos* that the low value of FCR was due to the 2% addition of papain enzyme in the feed. Khati *et al*. (2015) reported that the FRC in *Labeo rohita* decreased after the feed had been supplemented with ten papain enzymes per kg feed. Muchlisin *et al.* (2016) stated that Keureling fish (*Tor tambra*) had low FCR after the fish had been given papain enzyme supplemented diet with the dose of 27.5 mg papain enzyme per kg feed. Rachmawati *et al.* (2018) studied in *Cherax quadricarinatus*, the lowest FCR (1.76) was obtained by adding 0.3% papain enzyme in the diet. 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 fulfil 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

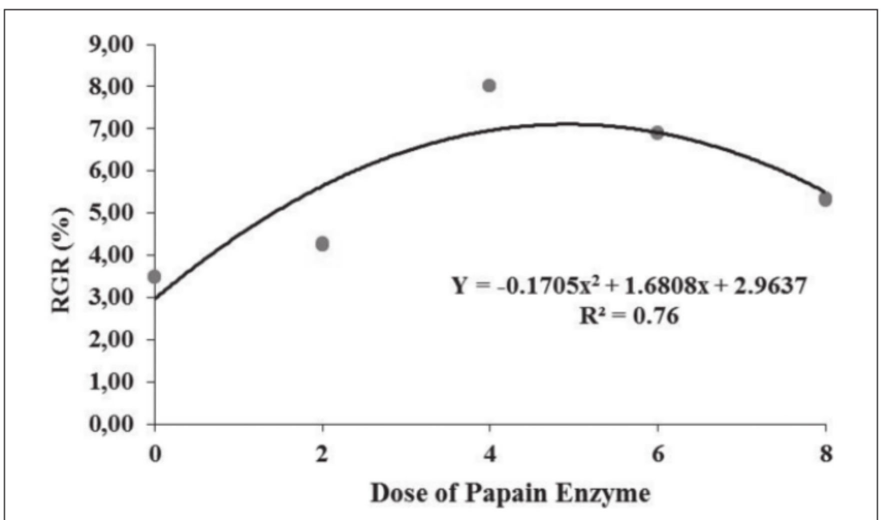

Fig. 3. The relationship between papain enzyme addition in the feed and RGR of catfish (*Pangasius hypopthalmus*).

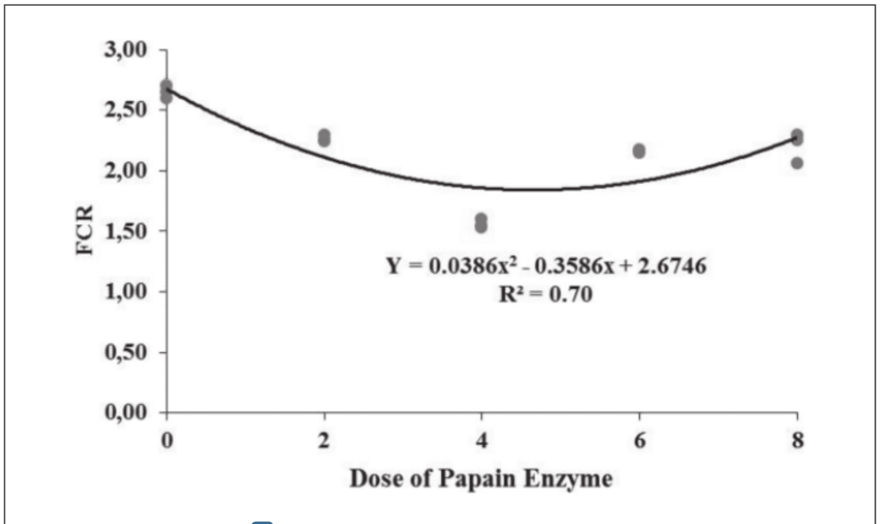

Fig. 4. The relation of papain enzyme addition in the feed and FCR of catfish (*Pangasius hypopthalmus*).

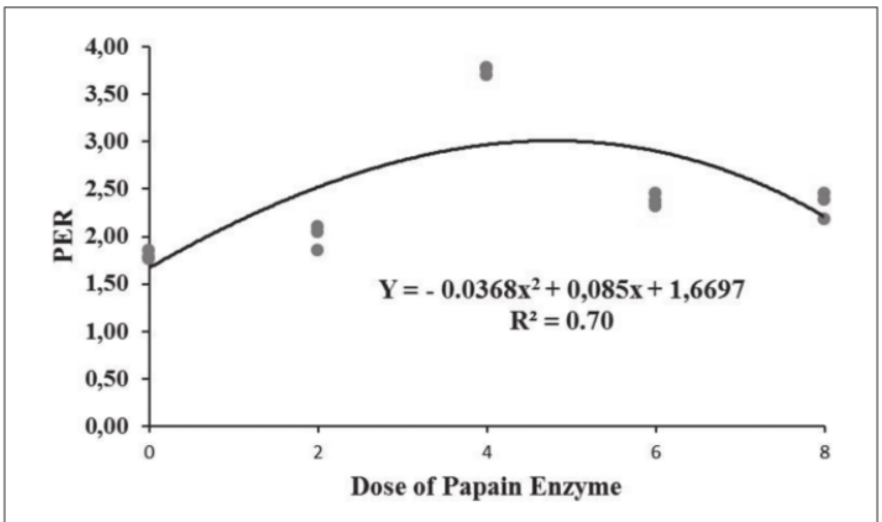

Fig. 5. The relationship between papain enzyme addition in the feed and PER of catfish (*Pangasius hypopthalmus*).

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

The relationship between papain enzyme and PER was presented in Figure 5. The relationship had a quadratic equation of $Y = -0.0386x^2 - 0.3586x + 2.6746$, $R^2 = 0.70$. The graph showed that the optimum dose of papain enzyme for PER was 4 g/kg feed with the maximal PER value of 3.75.

Survival rate (SR)

In this study, the survival rate of the fish was quite high (92.33%–93.33%), although the addition of papain enzyme did not affect SR of Catfish (*P. hypopthalmus*). The finding was supported by the findings of Patih and Singh (2014); Dabrowski and Glogowski (1977). They claimed that proteolytic enzyme does affect the fish survival rate. Yakuputiyage (2013) also discovered that survival rate was not influenced by the feed intake; otherwise, the survival rate was influenced by the initial condition of the fish and water quality. Similar results were obtained in *Channos channos* (Singh *et al*., 2011), *Macrobrachium rosenbergii* (Manush *et al*., 2013), *Labeo rohita* (Khati *et al*., 2015), *Cherax quadricarinatus* (Rachmawati *et al*., 2018), Sangkuriang Catfish (*Clarias* sp.) (Rachmawati & Samidjan, 2018).

CONCLUSION

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

ACKNOWLEDGMENTS

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

REFERENCES

Amri, E. & Florence, M. 2012. Papain, a plant enzyme of biological importance: a review. *American Journal of Biochemical Technology*, **8:** 99-104.

Ana, S.D., Antonio, C. & Ana, C.E. 2016. Bacterial collagenases - a review. *Journal Microbiology*. **42(1):** 106-126.

AOAC. 2016. *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington DC.

Bo-Li, Meiying, Q. & Fei-Lu. 2012. Composition, nutrition, and utilization of okara (soybean residue). *Journal Food International*, **28(3):** 231-252.

Brooke, C. & Daniel, P. 2013. Mariculture: a global analysis of production trends since 1950. *Marine Policy*, **39:** 94-100.

Craig, S. & Helfrich, L.A. 2002. *Understanding Fish Nutrition, Feeds and Feeding*. Cooperative Extension Service Publication, Virginia State University, USA.

Dabrowski, K. & Glogowski, K. 1977. A study of application of proteolytic enzymes to fish food. *Aquaculture*, **12:** 249-360.

Dasuki, A., Auta, J. & Oniye, S.J. 2013. Effect of stocking density on production of *Clarias gariepinus* (Tuegels) in floating bamboo cages at Kubanni Reservoir, Zaria, Nigeria. *Pure and Applied Sciences*, **6(1):** 112-117.

Ebert, A.W. 2014. Potential of underutilized traditional vegetables and legume crops to contribute to food and nutritional security, income and more sustainable production systems. *Sustainability*, **6(1):** 319-335.

Farrag, F.H., Khalil, F.F., Mehrim, A.I. & Refaey, M.M.A. 2013. Pawpaw (*Carica papaya*) seeds powder in nile tilapia (*Oreochromis niloticus*) diet 1-growth performance, survival, feed utilization, carcass composition of fry and fingerlings. *Journal of Animal and Poultry Science*, **4:** 363-379.

Fateme, K., Seyed, A. & Hosseini. 2012. Citric acid improves growth performance and phosphorus digestibility in Beluga (Huso huso) fed diets where soybean meal partly replaced fish meal. *Animal Feed Science and Technology*, **171(1):** 68-73.

Glencross, B., Hien, T.T.T., Phuong, N.T. & Cam-Tu, T.L. 2011. A factorial approach to defining the energy and protein requirements of Tra Catfish, Pangasianodon hypothalamus. *Aquaculture Nutrition*, **17(2):** 116-172.

Hastuti, D. 2001. Effect of bromelain extract as bating agents on mechanical strength and wrinkle temperature on tanning full Nabati local rabbit skin. BSc Thesis, Fakultas Peternakan Universitas Jenderal Soedirman, Purwokerto, 144 pp. [in Indonesian].

Hepher, B. 1988. *Nutrition of Pond Fishes*. Cambridge University Press, New York. 98 pp.

Huet, M. 1970. *Textbook of Fish Culture*. Fishing News (Book Ltd.), London, 436 p.

Hugues, de V., Komen, H., Quillet, E., Chatain, B., Allal, F., Benzie, J.A.H. & Vandeputte, M. 2018. Improving feed efficiency in fish using selective breeding: a review. *Aquaculture*, **10(4):** 833-851.

Ivar, R., Manuel, Y., Bernd, U., Laura, R., Oystein, S. & Clara, B. 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. *Aquaculture*, **5(1):** 107-114.

Jianga, Q., Wenyuan, G., Yanpeng, S., Xia, Li., Haiyang, W., Luqi, H. & Peigen, X. 2013. Physicochemical properties and *in vitro* digestion of starches from different dioscorea plants. *Aquaculture*, **32(2):** 432-439.

Kazerani, H.R. & Shahsavani. 2011. The effect of supplementation of feed with exogenous enzymes on the growth of common carp (*Cyprinus carpio*). *Iranian Journal of Veterinary Research*, **12(2):** 127-137.

Khati, A., Danish, M., Mehta, K.S. & Pandey, N.N. 2015. Estimation of growth parameters in fingerlings of *Labeo rohita* (Hamilton, 1822) fed with exogenous nutrizyme in Tarai region of Uttarakhand, India. *African Journal of Agricultural Research*, **10:** 3000-3007.

Kumar, V., Sinha, A.K., Makkar, H.P.S., Boeck, G. De. & Becker, K. 2011. Phytate and phytase in fish nutrition. *Journal of Animal Physiology and Animal Nutrition*, **96(2):** 335-365.

Lanari, D., Agaro, E. & Turri, C. 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **161:** 345-356.

Manguti, J.M., Ogello, E.O., Liti, D., Waidbacher, H., Straif, M. & Werner, Z. 2014. Effects of pure and crude papain on the utilization and digestibility of diets containing hydrolysed feather meal by Nile tilapia (*Oreochromis niloticus* L.). *International Journal of Advanced Research*, **2:** 809-822.

Manush, S.M., Srivastava, P.P., Kohli, M.P.S., Jain, K.K., Ayyappan, S. & Metar, S.Y. Combined effect of papain and vitamin-C levels on growth performance of Freshwater Giant Prawn, *Macrobrachium rosenbergii*. *Turkish Journal of Fisheries and Aquatic Sciences*, **13:** 479-486.

Maurício, E., Eduardo, L.C.B., Ronaldo, O.C. & Wilson, W. 2011. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817). *Aquaculture*, **43(3):** 63-75.

Mo, W.Y., Lau, R.S.S., Kwok, A.C.K. & Wong, M.H. 2016. Use of soybean meal and papain to partially replace animal protein for culturing three marine fish species: Fish growth and water quality. *Environmental Pollution*, **219:** 815-820.

Muchlisin, Z.A., Fardin, A., Tanzil, M., Nur, F., Zulkarnain, F. & Mu-hammadar, A.A. 2016. The effectiveness of experimental diet with varying levels of papa-in on the growth performance, survival rate and feed utilization of *Keureling* Fish (*Tor tambra*). *Biosaintifika*, **8:** 172-177.

National Research Council. 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington, DC.

Patil, D.W. & Singh, H. 2014. Effect of papain supplemented diet on growth and survival of post -larvae of *Macrobrachium rosenbergii*. *International Journal of Fisheries and Aquatic Studies*, **1(6):** 87-90.

Rachmawati, D., Samidjan, I., Maizirwan & Mel. 2017. Effect of phytase on growth performance, diet utilization efficiency and nutrient digestibility in fingerlings of (*Chanos chanos,* Forsskal 1775). *Journal Science*, **146:** 237-245.

Rachmawati, D, Asep, A.P., Roy Hendroko, S. & Olga, A. Effect of Papain Enzyme in Feed on Digestibility of Feed, Growth Performance, and Survival Rate in Post Larvaes of Freshwater Lobster [Cherax quadricarinatus (Von Martens, 1868)]. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences*, **55 (3):** 31-39.

Rachmawati, D. & Samidjan, I. 2018. The Effects of Papain Enzyme Supplement in Feed on Protein Digestibility, Growth and Survival Rate in Sangkuriang Catfish (Clarias sp.). *Omni-Akuatika*, **14 (2):** 91-99.

Rathod, N.B., Pagarkar, A.U., Pujari, K.H., Shingare P.E., Satam, S.B., Phadke, G.G. & Gaikwad, B.V. 2018. Status of valuable components from pangasius: a review. *International Journal of Current Microbiology and Applied Science*, **7(04):** 1-15.

Rostika, R., Sunarto, H.N. Sugiyanto & Dewanti, L.P. 2018. The effectiveness of crude papain enzyme supplement for tilapia's (*Oreochromis niloticus*) growth at the floating nets of Cirata Reservoir. The 2nd International Symposium on Marine and Fisheries Research. *IOP Conf. Series: Earth and Environmental Science*, **139:** 1-6.

Ryosuke, M. & Kazuhiko, I. 2011. Integrated functional nanocolloids covered with artificial cell membranes for biomedical applications. *Nanotoday*, **6(1):** 67-73.

Singh, P., Sajid, M., Munir, H.S., Vikas, P., Danish, M., Ramesh. & Singh, C. 2011. Exogenous supplementation of papain as growth promoter in diet of fingerlings of *Cyprinus carpio*. *International Aquatic Research*, **3:** 1-9.

Steffens, W. 1989. *Principles of Fish Nutrition*. Ellis Horwood Limited, John Willey and Sons, New York.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P. & Decamp, O.E. 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition*, **8:** 121-137.

Wong, M.H., Tang, L.Y. & Kwok, F.S.L. 1996. The use of enzyme digested soyaben residue for feeding common carp. *Journal of Biology, Hong Kong Bapist University*, **9:** 418-424.

Yakuputiyage, A. 2013. On-farm feeding and feed management strategies in tropical aquaculture. In: *On-Farm Feeding and Feed Management in Aquaculture*. M.R. Hasan and M.B. New (Eds.). FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO, 361-37.

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

ORIGINALITY REPORT

13%	9%	10%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Sánchez-Muros, M.J., F.G. Barroso, and C. de Haro. "Brief Summary of Insect Usage as an Industrial Animal Feed/Feed Ingredient", Insects as Sustainable Food Ingredients, 2016. Publication	1%
2	academicjournals.org Internet Source	1%
3	krishikosh.egranth.ac.in Internet Source	1%
4	garuda.ristekdikti.go.id Internet Source	1%
5	www.jol343.com Internet Source	1%
6	link.springer.com Internet Source	1%
7	Submitted to Universitas Brawijaya Student Paper	<1%

8	R Rostika, Sunarto, H N Sugiyanto, L P Dewanti. " The effectiveness of crude papain enzyme supplement for tilapia’s ( ) growth at the floating nets of Cirata Reservoir ", IOP Conference Series: Earth and Environmental Science, 2018 Publication	<1 %
9	Submitted to Syiah Kuala University Student Paper	<1 %
10	agricultureandfoodsecurity.biomedcentral.com Internet Source	<1 %
11	trjfas.org Internet Source	<1 %
12	Submitted to University of Stirling Student Paper	<1 %
13	Goliomytis, M., H. Orfanou, E. Petrou, M. A. Charismiadou, P. E. Simitzis, and S. G. Deligeorgis. "Effect of hesperidin dietary supplementation on hen performance, egg quality and yolk oxidative stability", British Poultry Science, 2014. Publication	<1 %
14	ejournal.undip.ac.id Internet Source	<1 %
15	pinnacle.allenpress.com Internet Source	<1 %

16	Submitted to Universiti Sains Malaysia Student Paper	<1%
17	I Samidjan, D Rachmawati. " Polyculture Engineering technology of red tilapia ( ) and white shrimp ( ) based for protease enzyme ", IOP Conference Series: Earth and Environmental Science, 2018 Publication	<1%
18	S S Agustina. " The effect of commercial feed enrichment with leaf extract on the growth and survival rate of tilapia ( ) ", IOP Conference Series: Earth and Environmental Science, 2019 Publication	<1%
19	Azizah, Imroatul, Tria Wahyuningrum, Kusnarman Keman, Sanarto Santoso, and Dwi Hidayati. "The effects of Vigna unguiculata on aortic endothelial cells, endothelial nitric oxide synthase expression, lipid profile, and atherosclerosis in ovariectomized rats", Journal of Experimental and Integrative Medicine, 2014. Publication	<1%
20	hdl.handle.net Internet Source	<1%
21	www.ajol.info Internet Source	<1%
22	dergipark.gov.tr	

Internet Source

<1%

23 Filipe Coutinho. "Dietary protein requirements and intermediary metabolism response to protein/carbohydrate ratio of zebra seabream (Diplodus cervinus, Lowe 1838) juveniles", Repositório Aberto da Universidade do Porto, 2013.
Publication

<1%

24 Otchoumou Kraidy Athanase, Blé Mélécony Célestin, Saki Suomion Justin, Alla Yao Laurent. "Growth performance of Heterobranchus longifilis, (Valenciennes, 1840) Fingerlings Fed with Nymphaea lotus (Linné, 1753)", Journal of Agricultural Studies, 2019
Publication

<1%

25 Submitted to CVC Nigeria Consortium
Student Paper

<1%

26 onlinelibrary.wiley.com
Internet Source

<1%

27 www.bioflux.com.ro
Internet Source

<1%

28 mnet.mendelu.cz
Internet Source

<1%

29 www.thaiscience.info
Internet Source

<1%

30	www.e-fas.org Internet Source	<1%
31	M E Azim1. "Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-based aquaculture", Aquaculture Research, 9/2001 Publication	<1%
32	Rita Rostika, Camelia Panatarani, I Made Joni, Achmad Rizal, Lantun P. Dewanti, M. Rudyansyah Ismail. "Engineering of Papaya Simplicia Nanoparticles for Grouper Fish (Epinephelus fuscoguttatus lanceolatus) feed", Journal of Powder Technology and Advanced Functional Materials, 2019 Publication	<1%
33	www.intelaquares.com Internet Source	<1%
34	Submitted to Universiti Putra Malaysia Student Paper	<1%
35	biblio.ugent.be Internet Source	<1%
36	aquatecindonesia.com Internet Source	<1%
37	repository.hkbu.edu.hk Internet Source	

<1%

38 www.mdpi.com
Internet Source
<1%

39 A, Khati, Danish M, S Mehta K, N P N, and ey. "Estimation of growth parameters in fingerlings of Labeo rohita (Hamilton, 1822) fed with exogenous nutrizyme in Tarai region of Uttarakhand, India", African Journal of Agricultural Research, 2015.
Publication
<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

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

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10