The effects of papain enzyme-enriched diet on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish (Clarias gariepinus) reared in tarpaulin pool

by Diana Rachmawati

The effects of papain enzyme-enriched diet on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish (*Clarias gariepinus*) reared in tarpaulin pool

Diana Rachmawati, Johannes Hutabarat, Istiyanto Samidjan, Seto Windarto

Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, 50275, Central Java, Indonesia. Corresponding author: D. Rachmawati, dianarachmawati1964@gmail.com

Abstract. Inefficiency of feed utilization in fish aquaculture is one of the main problems faced by fish farmers. The inefficiency of feed utilization lead to the high cost of the feed. The cost of feed is the largest portion of the total cost of production that accounted for around 40-60% of the total cost. Feed efficiency can be increased by enrichment of papain enzyme in the feed. The study aimed to examine the effects of papain enzyme-enriched feed on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish (*Clarias gariepinus*) reared in tarpaulin pools. The fish used in the research had an average weight of 1.48±0.17 g fingerling^{-1}. Experimental feed contained 30% protein which was added with papain enzyme. The papain enzyme enrichment into the feed were in four dosages namely treatments A (0% kg^{-1} feed), B (0.75% kg^{-1} feed), C (1.5% kg^{-1} feed), D (2.25% kg^{-1} feed), E (3% kg^{-1} feed). The variables observed were protease enzyme activities, efficiency of feed utilisation (EFU), relative growth rate (RGR), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (SR), apparent digestibility of protein (ADCp), and water quality. The outcomes showed that the papain enzyme-enriched feed was highly significant ($p < 0.01$) on the protease enzyme activities, EFU, RGR, PER, FCR, ADCp; otherwise it was insignificant on the SR. The dosage of 1.5% kg^{-1} feed (treatment C) was the best dosage of papain enzyme enrichment and it brought about the highest values of PEA, FEU and RGR which were 3.98 U g^{-1} protein, 70.35% and 4.68% day^{-1} respectively. The optimum dosages of papain enzyme supplementation FEU, RGR, PER and FCR ranged from 1.67 to 1.89% kg^{-1} feed. The water quality during study was in the good condition for rearing Sangkuriang catfish fingerlings.
Key Words: hydrolysis, survival rate, protein, protease.

Introduction. The fingerlings of Sangkuriang catfish (*Clarias gariepinus*) were attained from crossbreeding of second generation (F2) catfish with sixth generation (F6) which were readily available from the farmers (Ahmadi et al 2012). Sangkuriang catfish had high growth, high adaptability in the environment, delicious meat, and high amounts of nutrients. These features made farmers interested in the rearing of Sangkuriang catfish (Arief et al 2014).

The Sangkuriang catfish highly depends on the feeding, meanwhile the fish farmers still have inefficiency of feed utilization which causes high cost production. Mo et al (2016) reported that due to feed inefficiency, the variable cost of feed accounted to the biggest portion of the total production cost (40-60%). Oyakhilomen et al (2016) suggested that the reduction of production cost could be done through improving feed efficiency.

The diet inefficiency has occurred because of feeding type for the Sangkuriang catfish. It is a carnivorous animal, so it has difficulty in digesting plant-based diet, as reported by Olmos et al (2011). On the other hand, Olmos et al (2011) uncovered that papain enzyme-enriched diet can boost diet efficiency. Same suggestion was endorsed by Patil & Singh (2015). The enrichment of papain enzyme into the diet can improve protein utilization; it is due to the ability of papain enzyme to break down protein into simpler elements as amino acids. In turn those are easier to consume (Amri & Mamboya 2012; Paul et al 2013; Dawood et al 2014). Similar finding was obtained by Patil & Singh (2015) that the papain enzyme supplementation could promote the protein digestibility in the shrimp (*Macrobrachium rosenbergii*) and the dose of 0.1% gave the highest growth. They found that the dose of 0.1% gave the highest growth. Muchlisin et al (2016) found that the 27.5 mg papain enzyme per kg in the feed made keureling fish (*Tor tambra*) was the best dose for the fish growth with the average weight of 0.30 g and the average length of 3.5 cm. The dosage of 10 g kg^{-1} diet brought about the best growth and protein efficiency ratio in *Labeo rohita* (Khati et al 2015). Moreover, Rachmawati & Samidjan (2018) discovered that the 6 g kg^{-1} diet of papain enzyme enrichment could lead protein digestibility and growth of Sangkuriang catfish with the weight of 3.43 g $fingerling^{-1}$ to go up. Rostika et al (2018) also revealed that the papain enzyme enrichment in the feed with the dosage of 3.75% kg^{-1} diet was able to raise the diet utilization efficiency, protein efficiency ratio, and growth the weight of 17 g $fingerling^{-1}$ of tilapia. Patil & Singh (2015) explained that the need for papain enzyme depends on the type of fish and their weight. The information about the study of papain enzyme enrichment in the diet for Sangkuriang catfish was still scarce; therefore, the study to probe the effects of papain enzyme-enriched diet of Sangkuriang catfish became very relevant. Thus, the present study aimed to examine the effects of papain enzyme-enriched diet on protease enzyme activities, diet efficiency, and growth of fingerlings of Sangkuriang catfish (*Clarias gariepinus*) reared in tarpaulin pools.

Material and Method

Fish preparation. An amount of 50 thousands of Sangkuriang catfish fingerlings were used in the study. The fingerlings had the average weight of 1.48±0.17 g. They were obtained from the fish farmers association in Tambaksari Village, Rowosasri District, Kendal Regency, Central Java, Indonesia. The fingerlings used in the study were only the healthy individuals. The signs of healthy fingerlings were such highly active, no disability, free of diseases, and normal movement. To test the normality of the fish movement was by putting all of the fish into the strong stream water, if the fish was able to swim to the opposite of the water stream direction, they were considered healthy fish. Then the fingerlings were acclimated for one week in which the fingerings were fed with the feed without papain enzyme enrichment. Before the start of the experiment, the fingerlings were let to fast for one day to guarantee that there will be no-effect of the previous feed consumed (Rachmawati et al 2017).

Feed preparation. The study used feed that contained 30% protein enriched with papain enzyme based on the dosages of treatments. The treatments were papain enzyme enrichment in the feed with the various dosages in the treatments A (0% kg^{-1} feed), B (0.75% kg^{-1} feed), C (1.5% kg^{-1} feed), D (2.25% kg^{-1} feed), E (3% kg^{-1} feed). Papain enzyme was supplied by The Center for Brackish Water Aquaculture, Jepara, Central Java Province, Indonesia. To mix feed with papain enzyme was by spraying the enzyme into the feed according to Merrifield et al (2011) method.

Research design. The research was conducted on April-June 2019 in the fish farmers association of Sangkuriang catfish in the Tambaksari Village, Rowosari District, Kendal Regency, Central Java Province, Indonesia. The feeding experiment that used Completely Randomized Design (CRD) was conducted for 49 days with 5 (five) treatments and 4 (four) replicates for each treatment. Three times of feeding were performed in the morning, the mid-noon, and the afternoon with fixed feeding rate of 5% of mass weight. The gain weight of the fish was measured every 7 (seven) days for rearing cycle.

Container preparation. The tarpaulin pools were used to do experiment for Sangkuriang catfish. There were 20-unit tarpaulin pools with 2 (two) meter diameter and 1 (one) meter water depth to raise the fingerlings. The density of the Sangkuriang catfish fingerlings was 2500 fingerlings per tarpaulin pool. Each pool was furnished with recirculation system.

Digesting enzyme analysis. The measurement of protease enzyme activities used extract of the digesting system of Sangkuriang catfish following the Sandeepa & Ammani (2015) method.

Water quality observation. Observed parameters to analyze water quality were pH, dissolved oxygen (DO), temperature, and ammonia. Water pH was measured using Jenway 3510, while DO was measured using JENWAY 970. Temperature and ammonia were measured using HANNA: HI 8633.

Observation of feed utilization and growth parameters. The parameters observed were efficiency of feed utilization (EFU), feed conversion ratio (FCR), relative growth rate (RGR), protein efficiency ratio (PER), apparent digestibility of protein (ADCp) and survival rate (SR) following Tacon et al (2002) method, protein digestibility following Fenucci (1981) method, and water quality analysis following APHA (2005) method. The equations of the parameters were as the following:

$$EFU = \frac{\text{final weight} - \text{initial weight}}{\text{the weight of feed consumed}} \times 100$$

$$FCR = \frac{\text{the weight of feed consumed}}{(\text{final weight} + \text{total weight of dead fish}) - \text{initial weight}} \times 100$$

$$PER = \frac{\text{final weight} - \text{initial weight}}{\text{the weight of feed consumed x protein content of feed}} \times 100$$

$$RGR = \frac{\text{final weight} - \text{initial weight}}{\text{onitial weight x experiment lenght}} \times 100$$

$$SR = \frac{\text{final count}}{\text{initial count}} \times 100$$

$$ADCp = \frac{\%\ Cr_2O_3 \text{ in the diet } \times \%\text{ protein in the feces}}{\%\ Cr_2O_3 \text{ in the feces } \times \%\text{ protein in the feed}} \times 100$$

Data analysis. To observe the treatment influences, analysis of variance (ANOVA) with 95% and 91% of confidence intervals was employed. The treatment influences were found very significant ($p < 0.01$) or significant ($p < 0.05$). If one found the treatment significant, double Duncan test was implemented to investigate the difference of the treatments. The following test was Polynomial Orthogonal to determine optimum treatment (Steel et al 1996). Water quality was descriptively analyzed by comparing water quality in the pond to water quality references for rearing Sangkuriang catfish.

Results. The results of the parameters, the protease enzyme activities in the digesting system of Sangkuriang catfish and the water conditions were displayed in the Tables 1, 2 and 3. The result data of EFU, ADCp, FCR, PER, RGR and SR of Sangkuriang catfish during the study were displayed in the Table 1.

Table 1

The values of EFU, FCR, PER, RGR and SR of Sangkuriang catfish during the study

Experiment data	*Treatments*				
	A	*B*	*C*	*D*	*E*
EFU (%)	49.25 ± 0.28^e	55.67 ± 0.23^d	70.35 ± 0.29^a	64.43 ± 0.28^b	60.31 ± 0.23^c
ADCp (%)	51.25 ± 0.32^e	56.42 ± 0.35^d	69.83 ± 0.37^a	63.74 ± 0.31^b	60.35 ± 0.36^c
FCR	2.45 ± 0.16^c	2.32 ± 0.13^c	1.45 ± 0.19^a	1.98 ± 0.22^b	2.26 ± 0.24^c
PER	1.67 ± 0.13^d	2.08 ± 0.18^c	4.36 ± 0.14^a	3.62 ± 0.16^b	2.68 ± 0.15^c
RGR (% day^{-1})	2.21 ± 0.28^c	2.89 ± 0.26^c	4.68 ± 0.25^a	3.59 ± 0.27^b	3.12 ± 0.26^b
SR (%)	90.33 ± 2.36^a	88.33 ± 3.46^a	88.33 ± 3.48^a	90.33 ± 3.52^a	90.33 ± 3.43^a

Note: Mean values in different superscript alphabets showed significant influences ($p < 0.05$).

The results of Polynomial Orthogonal test to determine the papain enzyme-enriched diet relation to the parameters of EFU, ADCp, FCR, PER, and RGR were depicted in the Figures 1-5.

The equation $Y= -5.2927x^2 + 19.995x + 47.872$ (Figure 1) showed optimum dosage of papain enzyme at 1.89% kg^{-1} diet with the value of EFU at 66.75%. The value of R^2 was 0.8403. It indicated that 84.03% EFU was affected by the papain enzyme enrichment in the feed, while 15.97% was affected by other factors.

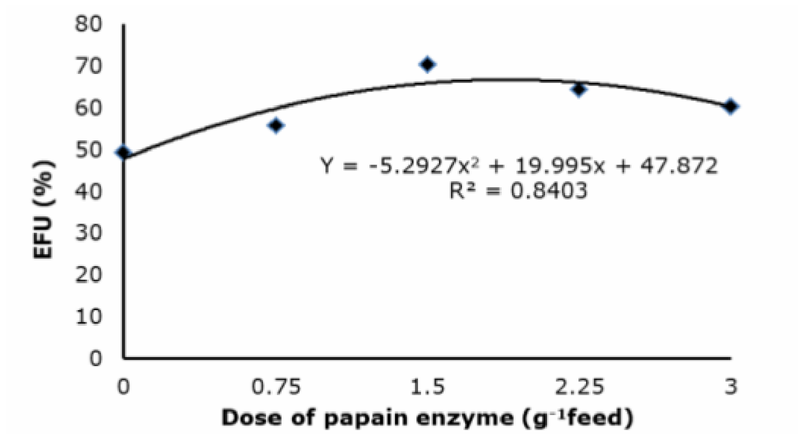

Figure 1. The papain enzyme-enriched feed relation to EFU of Sangkuriang catfish fingerlings.

The optimum dosage of papain enzyme enrichment could be seen on the equation $Y= -4.6502x^2 + 17.353x + 49.983$ (Figure 2). The optimum dosage was 1.87% kg^{-1} feed with maximum value of ADCp at 66.16%. The value $R^2 = 0.8061$ meant that 80.61% of ADCp was influenced by the papain enzyme supplementation, while 19.39% of ADCp was influenced by other factors.

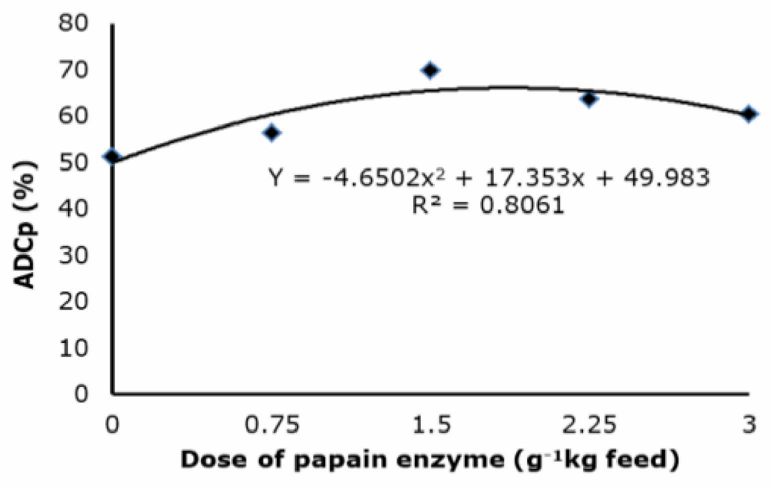

Figure 2. The papain enzyme-enriched feed relation to ADCp of Sangkuriang catfish fingerlings.

The equation $Y= 0.2819x^2 - 0.9417x + 2.5531$ (Figure 3) exhibited the optimum dosage of papain enzyme. The dosage was 1.67% kg^{-1} diet which resulted in the minimum value of FCR at 1.76. The value of $R^2 = 0.6379$ meant that 63.79% of FCR was affected by the papain enzyme supplementation in the feed, while 36.21% of FCR was affected by other factors.

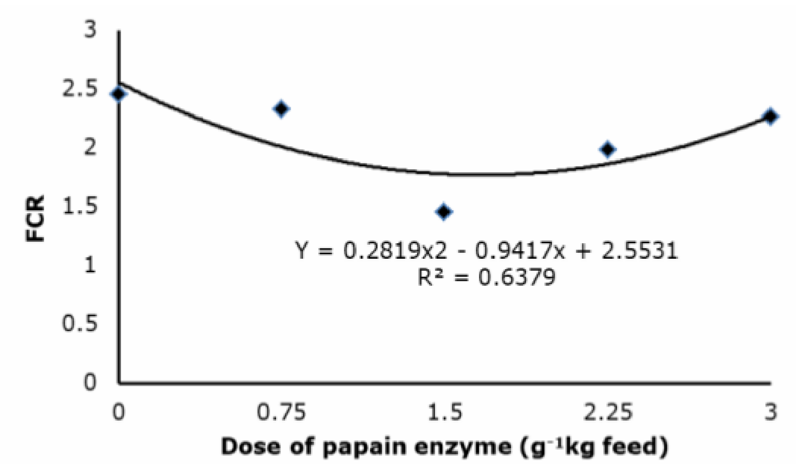

Figure 3. The papain enzyme-enriched feed relation to FCR of Sangkuriang catfish fingerlings.

The equation $Y= -0.7263x^2 + 2.6537x + 1.3529$ (Figure 4) pointed that the dosage of 1.83% kg^{-1} diet of papain enzyme was the optimum dosage which resulted in the PER of 3.77. The R^2 value of 0.7383 showed that 73.83% PER was influenced by papain enzyme enrichment in the diet, while 26.17% was influenced by other factors.

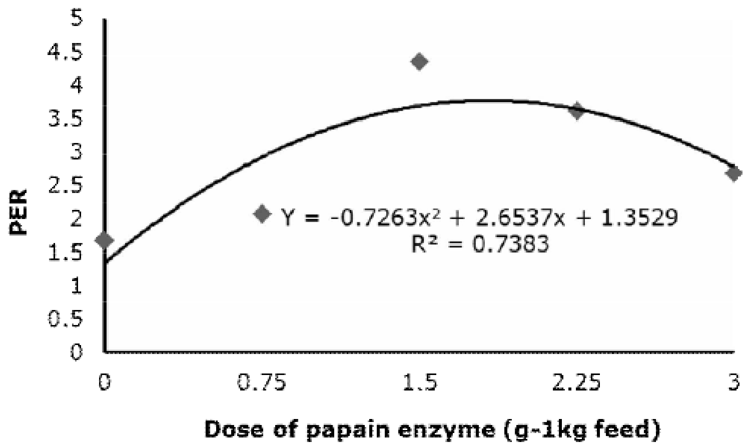

Figure 4. The papain enzyme-enriched feed relation to PER of Sangkuriang catfish fingerlings.

Based on the Y= $-0.6578x^2 + 2.3093x + 2.054$ (Figure 5) equation, the optimum dosage was 1.76% kg^{-1} diet of papain enzyme and brought about maximum RGR with the value of 4.08% day^{-1}. The R^2 value of 0.7556 meant that 75.56% PER was influenced by the papain enzyme-enriched diet, while the rest (24.44%) was affected by the other factors.

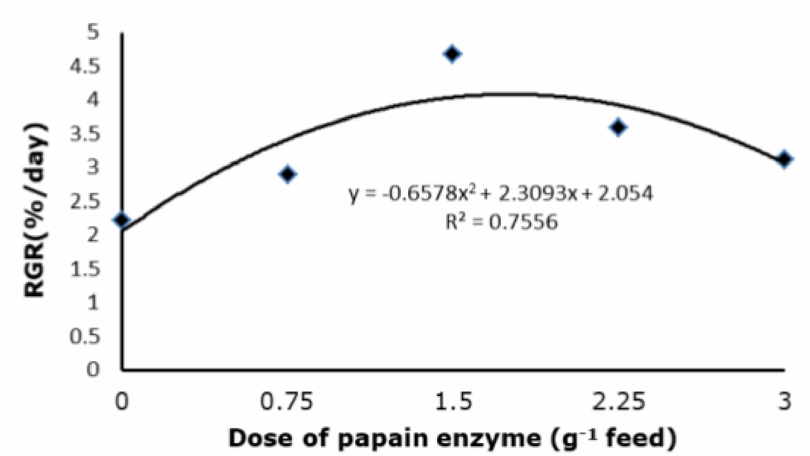

Figure 5. The papain enzyme-enriched feed relation to RGR of Sangkuriang catfish fingerlings.

Table 2 displays that the fingerlings of Sangkuriang catfish fed with papain enzyme-enriched diet (0.75-3% kg^{-1} diet) had higher activities of protease enzyme than those fed the diet without papain enzyme enrichment (0% kg^{-1} diet). The highest activities of protease enzyme of Sangkuriang catfish fingerlings were found in the treatment C (3.98 U g^{-1} protein), followed by B (3.04 U g^{-1} protein), D (2.95 U g^{-1} protein), E (2.05 U g^{-1} protein), and A (1.32 U g^{-1} protein).

Table 2

The activities of protease enzyme in the digesting system of fingerlings of Sangkuriang catfish fed with papain enzyme-enriched feed

Activity	*Treatments*				
(U g^{-1} protein)	*A*	*B*	*C*	*D*	*E*
Protease	1.32 ± 0.24^{d}	3.04 ± 0.28^{b}	3.98 ± 0.23^{a}	2.95 ± 0.27^{b}	2.05 ± 0.28^{c}

Note: Mean values with different superscripts refer to significant difference ($p < 0.05$).

The water conditions during the experiment were still in viable condition for cultivation of fingerlings of Sangkuriang catfish (Table 3).

Table 3

The parameters of water conditions for rearing the fingerlings of Sangkuriang catfish

No	*Variables*	*Values in each treatment*					*Feasibility*
		A	*B*	*C*	*D*	*E*	
1.	Temperature (°C)	25-29	25-30	26-29	26-30	25-29	25-32*
2.	pH	7.0-8.5	7.3-8.4	7.2-8.0	7.0-8.1	7.2-8.4	7-9*
3.	DO (mg L^{-1})	3.7-4.9	3.6-4.5	3.4-4.7	3.4-4.9	3.5-4.9	3-6*
4.	Ammonia (mg L^{-1})	0.01	0.01	0.01	0.01	0.01	< 1*

Note: * Boyd (2003).

Discussion. The papain enzyme-enriched diet had significant influence ($p < 0.05$) on the EFU of fingerlings of Sangkuriang catfish. The fingerlings fed with the feed at the dosage of papain enzyme of 0.75-3% kg^{-1} diet had higher EFU than those at the dosage of 0% kg^{-1} diet. The findings were confirmed by Mo et al (2016) and Rostika et al (2018) findings. They advised that the papain enzyme-enriched diet increases the breakdown and the digestion of plant-based diet; in turn it could boost the diet utilization efficiency. The highest value of EFU was attained in the treatment C (1.5% kg^{-1} diet) at the 70.35%. The dosage was thought that the papain enzyme was the right amount to hydrolyze amino acids that were easier to be digested, absorbed and able to raise the efficiency of feed utilization of the fingerlings of Sangkuriang catfish. According to Hastuti (2001) the hydrolysis of protein was due to papain enzyme, therefore the diet could be used efficiently. The high efficiency of feed utilization was indicated by better utilization of the diet to make the fish grow better (Tacon et al 2002). Moreover, Huet & Timmermans (1970) discovered that high efficiency utilization of feed was manifested by using the most part of the feed for the growth and a little of it for energy. Those phenomena were exhibited in the Table 1 that treatment C (1.5% kg^{-1} diet) resulted in the highest RGR compared to other treatments. Similar findings were pointed out by Muchlisin et al (2016) in the keureling fish (*Tor tambra*) and Rostika et al (2018) in the *Oreochromis niloticus* fish.

Papain enzyme-enriched diet at the dosages of 0.75-3% kg^{-1} diet made the values of ADCp increased when it was compared to the dosage of 0% kg^{-1} diet. Papain enzyme-enriched diet also caused protein digestion to go up when it was compared to the dosage of 0% kg^{-1} diet. It was indicated by the increases of protein content and protease enzyme (Singh et al 2011). Moreover, Khati et al (2015) and Mo et al (2016) denoted that protease enzyme was the enzyme that hydrolyzes protein to improve digestibility and absorption. The highest value of ADCp (69.83%) was attained when the fingerlings of Sangkuriang catfish was fed with the papain-enriched diet at the level of 1.5% kg^{-1} diet (treatment C). The highest value of ADCp (69.83%) was also because of the highest activities of protease enzyme as in the treatment C (3.98 U g^{-1} protein). The higher the activities of protease enzyme the higher the values of ADCp.

The papain enzyme-enriched diet at the dosages of 0.75-3% kg^{-1} diet (treatments B, C, D, E) reduced the values of FCR of the fingerlings of Sangkuriang catfish when it was compared to without supplementation of papain enzymes in the treatment A. As shown in the Table 1 the lowest FCR (1.45) of the fingerlings of Sangkuriang catfish was achieved in the treatment C with the dosage of papain enzyme of 1.5% kg^{-1} diet. The dosage was presumed the appropriate dosage to boost metabolism of the fingerlings of Sangkuriang catfish; therefore, the nutrient absorption could be maximized. According to Steffens (1989), the low FCR means that the nutrients were well digested and optimally absorbed by the fish. Similar study on *Chanos channos* was reported by Singh et al (2011) that the enrichment of 2% papain enzyme yielded the lowest FCR. The study in the post-larvae of *M. rosenbergii* fed with papain enzyme enrichment of 0.1% kg^{-1} diet could improve the growth and efficiency of feed utilization (Patil & Singh 2015). Khati et al (2015) also informed that *L. rohita* fed with the enrichment of 10 g papain enzyme per kg diet lowered the FCR. Muchlisin et al (2016) also reported that in the case of *T. tambra* the FCR was low after it was fed with the enrichment of 27.5 mg of papain enzyme per kg diet.

PER is a barometer to show the quality of protein that is able to supply essential amino acids to help fish growing (Munguti et al 2014). Hepher (1988) declared that the higher PER the better the quality of the diet; therefore, the diet is more efficient. Table 1 showed that the highest PER (4.36) was achieved at the papain enzyme enrichment of 1.5% kg^{-1} diet and the lowest PER (1.67) was reached in the treatment A with the dosage of 0% kg^{-1} diet. The dosage of 1.5% kg^{-1} diet (treatment C) was the right dosage to increase protease enzyme activities which has active role to breakdown protein into amino acids. According to Singh et al (2011) that the rate of protein absorption was higher when the diet was enriched with papain enzyme. Khati et al (2015) assured that papain enzyme was one of the protease enzymes which could increase protein digestibility. The similar findings were reported by Singh et al (2011) that the enrichment of 2% papain enzyme per kg diet could improve protein efficiency ratio for *C. channos* as much as 2.24. Khati et al (2015) suggested that *L. rohita* fed with papain enzyme-enriched diet at the dosage of 10 g kg^{-1} diet could improve PER by 2.30.

The highest value of RGR of the fingerlings of Sangkuriang catfish was obtained at the dosage of 1.5% kg^{-1} diet (treatment C), its value was 4.68% day^{-1}. It was suggested that the dosage of 1.5 g kg^{-1} diet papain enzyme was the effective dosage to increase protease activities in the digesting system; in turn it can optimally hydrolyze protein and increase protein digestibility and diet utilization. The lowest value of RGR (2.21% $diet^{-1}$) of the fingerlings of Sangkuriang catfish was achieved at the dosage of 0% kg^{-1} diet (treatment A). It was thought that due to the absence of papain enzyme in the diet there would be no protease enzyme activities, in turn there would be no increase of protein digestibility and efficiency feed utilization. Papain enzyme is the protease enzyme which can hydrolyzes polypeptides into short-chained peptides. The short-chained peptides were the main influencer to raise protein digestibility, nutrient absorption and growth (Wong et al 1996). The papain enzyme-enriched diet that can increase growth was reported by Singh et al (2011) on *C. channos,* Khati et al (2015) on *L. rohita*, Mo et al (2016) on *Epinephelus bleekeri,* and Muchlisin et al (2016) on *T. tambra*.

The papain enzyme-enriched diet insignificantly ($p > 0.05$) influenced on survival rate (SR) of the fingerlings of Sangkuriang catfish. This finding was in accordance to the finding of Dabrowski & Glogowski (1977). They found that papain enzyme is the proteolytic enzyme which if added into the diet, it insignificantly influences on the survival rate. Moreover, Yakuputiyase (2013) advised that factor that affects the survival rate is the condition of the media culture. Similar results were reported by Patil & Singh (2015) for *M. rosenbergii*, Farraq et al (2013), Munguti et al (2014) and Rostika et al (2018) for *O. niloticus*.

Conclusions. The conclusion of the study was that papain enzyme-enriched diet could increase activities of protease enzyme, feed efficiency and growth of the fingerlings of Sangkuriang catfish. The enrichment of 1.5% kg^{-1} papain enzyme diet was the best dosage on the activities of protease enzyme, feed efficiency and growth of the fingerlings of Sangkuriang catfish.

Acknowledgements. The authors want to thank to the Ministry of Research, Technology and Higher Education which have given a research grant based on the letter of assignment of applied research, Research Directorate and Community Service, Directorate General Strengthening Research, the Ministry of Research, Technology and Higher Education the Ministry of Research, Technology and Higher Education, the Budget Year of 2019 No.: 257-84/UN7.P4.3/PP/2019.

References

Ahmadi H., Iskandar, Kurniawati N., 2012 Pemberian probiotik dalam pakan terhadap pertumbuhan lele sangkuriang pada pendederan II. Jurnal Perikanan dan Kelautan 4(3):99-107. [in Indonesian]

Amri E, Mamboya F., 2012 Papain, a plant enzyme of biological importance: a review. American Journal of Biochemistry and Biotechnology 8(2):99-104.

APHA, 2005 Standard methods for examination of water and wastewater. 21st edition, Washington DC, USA, 185 pp.

Arief M, Fitriani N., Sri Subekti, 2014 Pengaruh pemberian probiotik berbeda pada pakan komersial terhadap pertumbuhan dan efisiensi pemanfaatan pakan ikan lele sangkuriang (*Clarias* sp.). Jurnal Ilmiah Perikanan dan Kelautan 6(1):49-53. [in Indonesian]

Boyd C. E., 2003 Guidelines for aquaculture effluent management at the farm-level. Aquaculture, 226(1-4):101-112.

Dabrowski K., Glogowski J., 1977 A study of the application of proteolytic enzymes to fish food. Aquaculture, 12(4):349-360.

Dawood M. A. O., El-dakar A., Mohsen M., Abdelraouf E., Koshio S., Ishikawa M., Yokoyama S., 2014 Effects of using exogenous digestive enzymes or natural enhancer mixture on growth, feed utilization, and body composition of rabbitfish, *Siganus rivulatus*. Journal of Agricultural Science and Technology B 4:180-187.

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 Animal and Poultry Production 4(6):363-379.

Fenucci J.L, 1981 Studies on the nutrition of marine shrimp of the Penaeus. Ph.D. Thesis, Faculty of Department of Biology, University of Houston, Houston, Texas, USA, 185 pp.

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, 388 pp.

Huet M., Timmermans J. A., 1970 Textbook of fish culture: breeding and cultivation of fish. Fishing News (Books) Limited, 436 pp.

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(30):3000-3007.

Merrifield D. L., Bradley G., Harper G. M., Baker R. T. M., Munn C. B., Davies S. J., 2011 Assessment of the effects of vegetative and lyophilised *Pediococcus acidilactici* on growth, feed utilisation, intestinal colonisation and health parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture and Nutrition 17(1):73-79.

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.

Munguti J. M., Ogello E. O., Liti D., Waidbacher H., Straif M., Zollitsch W., 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(6):809-822.

Muchlisin Z. A., Fardin A., Tanzil M., Nur F., Zulkarnain J., Muhammadar A. A., Yulvizar C., 2016 The effectiveness of experimental diet with varying levels of papain on the growth performance, survival rate and feed utilization of Keureling fish (*Tor tambra*). Biosaintifika 8(2):172-177.

Olmos J., Ochoa L., Paniagua-Michel J., Contreras R., 2011 Functional feed assessment on *Litopenaeus vannamei* using 100% fish meal replacement by soybean meal, high levels of complex carbohydrates and *Bacillus* probiotic strains. Journal Marine Drugs 9(6):1119-1132.

Oyakhilomen O., Murtala M. O., Abraham F., Kwagyang S. M., 2016 Technical efficiency of catfish farming in Alimosho local government area of Lagos State, Nigeria: a gender perspective. Agricultura Tropica et Subtropica 49(1-4):45-49.

Paul B., Nasreen M., Sarker A., Islam M. R., 2013 Isolation, purification and modification of papain enzyme to ascertain industrially valuable nature. International Journal of Bio-Technology and Research 3(5):11-22.

Patil D. W., Singh H., 2015 Effect of papain supplemented diet on growth and survival of post-larvae of *Macrobrachium rosenbergii*. International Journal of Fisheries and Aquatic Studies 1(6):86-90.

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.

Rachmawati D., Istiyanto S., Maizirwan M., 2017 Effect of phytase on growth performance, diet utilization efficiency and nutrient digestibility in fingerlings of *Chanos chanos* (Forsskal 1775). Philippine Journal of Science 146(3):237-245.

Rostika R., Sunarto, Sugiyanto H. N., Dewanti L. P., 2018 The effectiveness of crude papain enzyme supplement for tilapia's (*Oreochromis niloticus*) growth at the floating nets of Cirata Reservoir. IOP Conference Series: Earth and Environmental Science 139(1):012006.

Sandeepa M. G., Ammani K., 2015 Effect of probiotic bacterium on growth and biochemical parameters of shrimp *Litopenaeus vannamei*. International Journal of Recent Scientific Research 6(2):2871-2875.

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

Steel R. G. D., Torrie J. H., Dickey D. A., 1996 Principles and procedures of statistics: a biometrical approach. 3rd edition, McGraw Hill, New York, pp. 352-358.

Steffens W., 1989 Principles of fish nutrition. Ellis Horwood limited, John Willey and Sons, New York, 384 pp.

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(2):121-137.

Wong M. H., Tang L. Y., Kwok F. S. L., 1996 The use of enzyme-digested soybean residue for feeding common carp. Biomedical and Environmental Science 9(4):418-442.

Yakupitiyage A., 2013 On-farm feeding and feed management strategies in tropical aquaculture. In: On-farm dieting and diet management in aquaculture. Hasan M. R., New M. B. (eds), Fisheries and Aquaculture Technical Paper No. 583, Rome, FAO, pp. 361-376.

Received: 19 July 2019. Accepted: 29 September 2019. Published online: 19 December 2019.
Authors:
Diana Rachmawati, Department of Aquaculture, Faculty of Fishery and Marine Science, Diponegoro University, Semarang 50275, Central Java, Indonesia, e-mail: dianarachmawati1964@gmail.com
Johannes Hutabarat, Department of Aquaculture, Faculty of Fishery and Marine Science, Diponegoro University, Semarang 50275, Central Java, Indonesia, e-mail: johannesfpik@gmail.com
Istiyanto Samidjan, Department of Aquaculture, Faculty of Fishery and Marine Science, Diponegoro University, Semarang 50275, Central Java, Indonesia, e-mail: istiyanto_samidjan@yahoo.com
Seto Windarto, Department of Aquaculture, Faculty of Fishery and Marine Science, Diponegoro University, Semarang 50275, Central Java, Indonesia, e-mail: seto.sidhartawan@gmail.com

How to cite this article:
Rachmawati D., Hutabarat J., Samidjan I., Windarto S., 2019 The effects of papain enzyme-enriched diet on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish (*Clarias gariepinus*) reared in tarpaulin pool. AACL Bioflux 12(6):2177-2187.

The effects of papain enzyme-enriched diet on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish (Clarias gariepinus) reared in tarpaulin pool

ORIGINALITY REPORT

9%	5%	5%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%

★ Submitted to Curtin University of Technology

Student Paper

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

The effects of papain enzyme-enriched diet on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish (Clarias gariepinus) reared in tarpaulin pool

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

PAGE 11