The effectiveness of crude papain enzyme supplement for tilapia's (*Oreochromis niloticus*) growth at the floating nets of Cirata Reservoir

R Rostika[1]*, Sunarto[1], H N Sugiyanto[1] and L P Dewanti[1]

Fisheries Study Program–Fisheries and Marine Science Faculty, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, Hergamanah, Jatinangor, West Java, Indonesia
*Email: ritarostika_unpad@yahoo.com

Abstract. Papain is an enzyme capable of hydrolyzing protease into a more simple elements i.e. the peptide to amino acids. The enzyme in the feed can increase the absorption of protein and digestion rate in the digestive tract of fish. This research examined the effective level of enzyme papain to increase the Feed Utilization Efficiency (FUE), Protein Efficiency Ratio (PER) and Average Daily Gain (ADG). This research used Completely Randomized Design (CRD) with five treatments i.e. treatment A (control), treatment B (1.5 %), treatment C (2.25 %), treatment D (3 %) and treatment E (3.75 %) in triplicate. Tilapia (*Oreochromis niloticus*) with the average initial weight of 17 g, and initial total lenght of 8–10 cm was fed three times daily at feeding rate of 5 % of the total body weight. The results showed that supplementation of papain in the feed significantly increased the activity of protease, FUE, PER and ADG. The optimal dose of the enzyme papain at 3.75 % was able to increase 48.31 % of FUE, 2.13 % of PER and 2.07 % of ADG.

1. Introduction

Cirata Reservoir is one of sites to perform aquaculture activities in West Java. It is categorized as a multipurpose dam. Nowadays, people are highly enthusiastic to culture fish at Floating Net Cages (FNC) in Cirata Reservoir. However, those have already exceeded the reservoir carrying capacity that, at some point or when ignored, mass mortality of fish may occur due to its severe eutrophication. Eutrophication occurs when there are too many inputs coming from Citarum River upstream's from various activities, such as agriculture, livestock, industry, household and aquaculture itself.

One of the efforts to reduce eutrophication is by improving the feed quality for aquaculture through papain enzyme addition. Papain is a protease enzyme found in papaya sap. The enzyme is used to break down perfectly peptide bonds in protein that are decomposed into simpler peptide bonds as papain may catalyze the hydrolysis reaction [1].

The role of enzyme is greatly essential in protein digestive processes. In addition to endogenous enzymes, the exogenous ones are also required to speed up the protein hydrolysis processes. One exogenous enzyme which has the ability to rapidly hydrolyze proteins is papain enzyme. Papain is expected to improve protein feed utilization for tilapia growth. Efficient feed can improve body deposition as well as to reduce the total amount of dissolved solid in the waters. It means that the quality of water media in the Floating Net Cages is expected to be well maintained in accordance with the criteria for aquaculture.

The aims of this research was to examine the most appropriate dosage of papain enzyme to improve the value of Feed Efficiency Utilization (FEU), Protein Efficiency Ratio (PER) and Daily Growth Rate (DGR).

2. Materials and Methods

2.1. Research place and time

The research place was in Cirata Reservoir which was located in Cianjur Regency, West Java. Fish identification was performed at Animal Physiology Laboratory of Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor. The enzyme activity test was performed in the Laboratory of Researches and Services, Chemistry Department, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The research was conducted from August 2015 to January 2016.

2.2. Methods

This research was experimentally conducted with five treatments and three replications. Observations on tilapia weight were conducted every 15 days for 2 months. The cultivated tilapia size was about 10–12 cm with the initial weight of 16–18 g, fed with 5 % of its body weight, and then differently treated with papain enzyme addition in feed. The inclusion level used in this research is as follows:

A: Feed without enzyme (Control)
B: Feed hydrolyzed with papain with the dosage of 1.5 %
C: Feed hydrolyzed with papain with the dosage of 2.25 %
D: Feed hydrolyzed with papain with the dosage of 3 %
E: Feed hydrolyzed with papain with the dosage of 3.75 %

The variables studied were Survival Rate (SR), Feed Efficiency Utilization (FEU), Protein Efficiency Ratio (PER), Daily Growth Rate (DGR) and water quality.

2.3. Research parameters

2.3.1. Survival Rate (SR). Survival Rate was calculated by using the following formula [2]:

$$SR = \frac{Nt}{N0} \times 100\ \%$$

Description:
SR = Survival Rate (%)
Nt = fish number day-t (fish)
N0 = fish number day-0 (fish)

2.3.2. Feed Efficiency Utilization (FEU). Feed Efficiency Utilization (FEU) was calculated by using the following formula [3]:

$$FEU = \frac{(Wt - W0)}{F} \times 100\ \%$$

Description:
FEU = Feed Efficiency utilization (%)
W0 = fish biomass day-0 (g)
Wt = fish biomass day-t (g)
F = fish feed amount consumed during the study (g)

2.3.3. Protein Efficiency Ratio (PER). Protein Efficiency Ratio was calculated by using the following formula [3]:

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

Description:
PER = Protein Efficiency Ratio (%)
W_0 = fish biomass day-0 (g)
W_t = fish biomass day-t (g)
Pi = the consumed feed protein weight (g)

2.3.4. Daily Growth Rate (DGR). Daily Growth Rate was calculated by using the following formula [4]:

$$DGR = \frac{(lnWt - lnW0)}{t} \times 100\ \%$$

Description:
DGR = Daily Growth Rate
lnW0 = fish biomass day-0 (g)
lnWt = fish biomass day-t (g)
t = Length of Cultivation (day)

2.4. Data analysis

Data were analyzed using variant investigation analysis (F-test) with confidence level of 95 %. If the treatment had a significant influence on variant analysis (ANOVA), then Duncan's multiple range test was conducted to determine the difference between treatments [5]. Water quality data were descriptively analyzed and used as the research supporting data.

3. Results and Discussion

3.1. Protease enzyme activities on papain fish digestion

The tested treatments are control treatment A and treatment B which was given the lowest papain enzyme inclusion in the feed by only 1.5 %. Protease enzyme activities in treatment B was higher than those in treatment A with the difference of 0.95 activity $U \cdot mg^{-1}$ Protein. It is assumed that in treatment B, the exogenous papain enzyme which was added to the fish feed might improve the activities of fish protein break down within fish intestine that amino acid absorption is much better and might result in the improving feed digestibility level that the body might utilize for its metabolism and growth.

3.2. Survival rate

Tilapia's survival rate was not significantly different in each treatment and still within the most favorable range due to the fish positive reactions to the enzyme- added feed characterized by the eaten enzyme-added feed. In addition to feeding factors, the environmental conditions could also influence tilapia's survival rate.

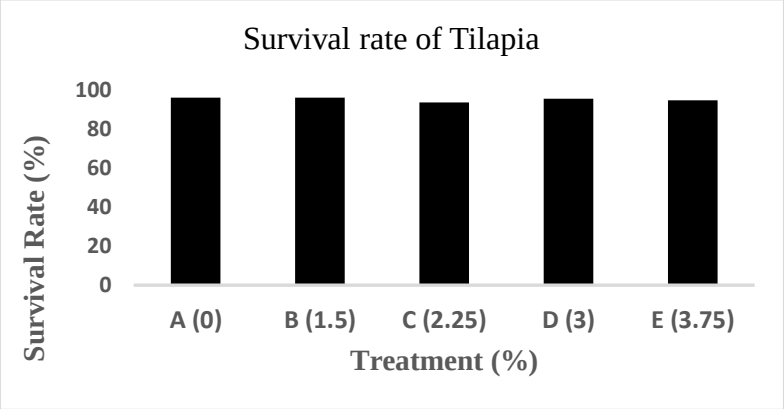

Figure 1. Survival rate of tilapia.

Feed was not only serves as energy sources, but also to maintain fish's survival, growth and reproduction. The protein content in merkartha feed given was ± 25 %, then supplementation of papain protease enzyme accelerate the process of complex protein break down into simple protein up to amino acid that protein might be easily digested as energy sources, growth and reproduction.

3.3. Feed Efficiency Utilization (FEU)

Feed Efficiency Utilization value showed the significant difference and tended to improve at higher dosages. When treatment A (control) was compared with the lowest papain enzyme inclusion (treatment B), the difference was about 5.77 %.

Papain was protease enzyme which might hydrolyze proteins into simpler elements, such as peptides up to amino acids. Papain addition as exogenous enzyme into feed might improve its protein hydrolysis which might result in the improving feed digestibility level. Higher digestibility level might improve its amino acid absorption ability to its body to grow [6]. In addition, feed quality is one factor which could influence protein efficiency value.

Table 1. Duncan test feed efficiency utilization fed with papin-supplemented feed at various dose.

Treatment of Papain Enzyme	Feed Efficiency Utilization (%)
A (0 %)	40.26 ± 0.07^{a}
B (1.5 %)	46.03 ± 0.05^{b}
C (2.25 %)	47.58 ± 0.03^{bc}
D (3 %)	48.01 ± 0.04^{cd}
E (3.75 %)	48.31 ± 0.09^{cd}

Mean value followed by different superscript indicates the significant different ($p < 0.05$).

Results of this study were also in line with those conducted by previous researchers [6] who reported that feed efficiency utilization is directly proportional to the exogenous enzyme dosage added to the feed. Their study used catfish fed with enzyme-supplemented feed in doses of A (0 %), B (0.75 %), C (1.5 %) and D (2,25 %) exhibiting Feed Efficiency Utilization value of 44.63 %; 52.71 %; 53.85 % and 62.83 %, respectively.

The obtained Feed efficiency utilization value ranges from 40.26–48.31 % that this could not be yet considered in a good category. Feed might be considered in good category when the feed efficiency value is more than 50 % or even close to 100 %. It was assumed that the water quality at the research

sites was less optimal, especially due to the dissolved oxygen which inhibited the fish growth [7]. The cultivation pools' DO ranges from 0.7–3.9 $mg \cdot L^{-1}$. This range was below the quality standard for cultivation according to BBPBAT Sukabumi (> 5 $mg \cdot L^{-1}$). However, it was still within the reasonable limit that tilapia could still live and develop, yet not for its optimal growth.

3.4. Protein Efficiency Ratio (PER)

This study showed that there were significant differences ($p \leq 0.05$) between the values of Protein Efficiency Ratio (PER) among treatments The highest average value of PER was obtained in treatment E by 2.13 %, followed by treatments D (2.10 %), C (2.06 %), B (2.03 %) and A (1.75 %) (table 2).

The value of Protein Efficiency Ratio obtained in this study was 2.13 %. Meanwhile, in research conducted by previous researchers [8], the value of Protein Efficiency Ratio was 2.37 % which examined young black tilapia fish (*Oreochromis niloticus* Bleekery). In the reasearch conducted by previous researchers [6] with the tested catfish, the highest Protein Efficiency Ratio value was 1.97 %. Similarly, the research conducted by previous researchers [9] with the tested carp fish shows that the highest Protein Efficiency Ratio value was 2.24 %.

Table 2. Test of Protein Efficiency Ratio (PER) fed with papin-supplemented feed at various dose.

Treatment of Papain Enzyme	PER (%)
A (0 %)	1.75 ± 0.02^{a}
B (1.5 %)	2.03 ± 0.01^{b}
C (2.25 %)	2.06 ± 0.00^{c}
D (3 %)	2.10 ± 0.01^{cd}
E (3.75 %)	2.13 ± 0.02^{cd}

Mean value followed by different superscript indicates the significant different ($p < 0.05$).

PER values was significantly difference among treatment ($P \leq 0.05$). This result might be caused by the different concentrations of protease in intestines of tilapia. This results supported by a research conducted by previous researchers [10], on the development of tilapia digestive enzymes concluding that all digestive enzymes, including protease enzymes are already exist in fish intestines. Due to the papain exogenous enzyme addition to the feed, it was expected that the addition might accelerate the intestinal digestive processes to increase the breakdown proteins to amino acids.

3.5. Daily Growth Rate (DGR)

Results indicated that the different papain enzyme supplemented in the feed generated various daily growth rate. Papain enzyme-containing feed given to tilapia had positive response to the fish growth indicated by increasing tilapia weight average in each sampling time (15 days). The average weight of tilapia in each treatment increased during the culture period.

This study provided the Daily Growth Rate averages that might be considered in good category, as it was above the minimum good growth rate value of 1 % [11]. Variant analysis indicated that the treatment influenced the growth rate of tilapia. The results of Duncan's multiple range test showed that daily growth rate of tilapia fed with papain supplemented feed was significantly different ($p \leq 0.05$) comparing to the control (table 3).

Tabel 3. Result of Daily Growth Rate (DGR) fed with papin-supplemented feed at various dose.

Treatment	DGR (%)
A (0 %)	2.11 ± 0.01^{a}
B (1.5 %)	2.44 ± 0.02^{b}
C (2.25 %)	2.54 ± 0.02^{bc}
D (3 %)	2.68 ± 0.01^{d}
E (3.75 %)	2.67 ± 0.02^{d}

The administration of papain enzyme 3 % in treatment D of this study showed the highest daily growth rate by 2.68 %. The results of research conducted by previous researchers [6], showed that the supplementation of papain up to 2.25 % resulted in daily growth of 2.89 % for catfish. In addition, the results of research conducted by previous researchers [8] showed that the supplementation of papain enzyme up to 2.25 % resulted in daily growth of 1.83 % for young black tilapia (*Oreochromis niloticus* Bleeker). Thus, it was then assumed that papain might hydrolyze proteins contained in feed that the feed given to the fish might be efficiently utilized and increase its daily growth rate.

4. Conclusion

Based on the research results, it could be concluded that the administration of feed-containing papain enzyme significantly improves protease enzyme activity in tilapia's digestive system. The optimal dose of 3.75 % papain enzyme improves Feed Utilization Efficiency value by 48.31 %, Protein Efficiency Ratio value by 2.13 % and Daily Growth Rate by 2.67 % of tilapia.

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