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Effect of Proteolytic Plant-Derived Enzyme on Gourami (*Osphronemus goramy* Lac.) Growth Rate

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ABSTRACT

This study aims at identifying the influence of proteolytic enzymes, papain and bromelin, addition on growth rate and feed utilisation efficiency in gourami fry. Four different concentrations of papain from papaya (0%, 0.75%, 1.5% and 2.25%) and bromelin from pineapple (0%, 1%, 1.5% and 2%) were used. The parameters investigated in this study were: protease enzyme activity, daily growth rate, survival rate, feed efficiency rate and water quality. Papain and bromelin activities were 2.16 and 12.41 units/mg protein, respectively. The addition of papain and bromelin did not affect the growth rate, survival and feed efficiency in a statistically significant way. It is likely that more than 60 days is required to observe the impact of enzyme addition on growth rate and feed efficiency. Thus, more experiments are needed to validate our results.

Keywords: Bromelin, feed efficiency, gourami fry, growth parameters, papain

INTRODUCTION

Gourami (*Osphronemus goramy)* is a freshwater species known for high economic value and nutritive contents. However, its ineffective digestive system during the larval stages has hampered the culturing of gourami. A completely functioning digestive system in gourami is achieved only after the fish reaches 2.4 cm at the age of forty days, after that the fry can receive artificial feed (Mokoginta et al., 1994). Before the digestive system is completely developed the production of endogen enzymes is low. Consequently, feed hydrolysation necessary for nutrients to be absorbed by

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the body, is limited. In order to solve this problem, Aslamyah et al. (2009) added a source of exogenous enzymes, such as amylase, protease, lipase and cellulose, to the artificial feed to improve the activity of the endogenous enzymes and consequently the growth and survival rate of gourami.

Proteases can be derived from plants, such as papaya and pineapple, natural sources of papain and bromelin respectively, that are capable of hydrolysing proteins into peptides or amino acids. Both enzymes are involved in the complete breaking down of a protein in peptides through their ability to catalyse hydrolytic reactions of a substrate (Muchtadi et al.,1992).

This forms the background of this study on the effects of papain and bromelin when added to artificial feed on growth, survival, and feed efficiency parameters of gourami fry.

MATERIALS AND METHODS

Tools and Materials

Twelve aquariums with the volume of (40x20x40) cm^3, DO meter digital Hanna HI-3810 with precision rate of 0,01 mg/L, pellet compactor machine, blower, pH meter Orion model 420, thermometer with precision rate of 0,1^oC, digital scale AND EK-120G with precision rate of 0,01 g, Amara water heater, gourami fry as test fish weighing between 5 and 7 grams obtained from the Center of Fresh Water Fish Fry in Sukabumi, raw papayas dried into simplicia (papain enzyme), powdered pineapple extract (bromelin enzyme) from Rumah Sehat Indonesia Jogjakarta, sprayer, and commercial feed with 36% protein content were used in this study.

Experimental design. The experimental design employed in this study is the Completely Randomized Design with four treatments and four repeats. Each treatment consisted of 10 gouramy fish fry. The experimental design was as follows:

Treatment A: formulated feed without papain

Treatment B: formulated feed with 0.75% papain

Treatment C: formulated feed with 1.5 % papain

Treatment D: formulated feed with 2.25% papain

Treatment E: commercial feed without bromelin enzyme

Treatment F: commercial feed with 1% bromelin

Treatment G commercial feed with 1.5% bromelin

Treatment H: commercial feed with 2% bromelin

Preparation of formulated Feed. The papain-containing feed and bromelin-containing feed were prepared using different methods. The formulated feed with papain enzyme contained 30% of proteins and was designed using the square method, while bromelin was added to commercial feed according to the treatment dose using the spray method. Bromelin

was dissolved in 100 ml of distilled water and then sprayed onto the feed and dried at room temperature. After getting dried, the pellets were smashed into crumbs.

Enzyme Activity Test. Papain enzyme was made using raw papayas dried into simplicia, while bromelin enzyme was produced from Rumah Sehat Indonesia, Yogyakarta. 0.1 g of powdered papain and bromelin were dissolved in 1 ml of distilled water 1 mL of casein was then added (10mg were dissolved in 1mL of phosphate buffer 0,1M pH 8). The sample was incubated for 30 minutes at 37^{O}C. After incubation, 3 mL of TCA 8% solution were then added and stirred until the solution became homogenous. The amount of 0.1 g of sample was then centrifuged at 10,000 rpm for 5-10 minutes and then measured using spectrophotometer at the wave length of 280 nm.

Feeding trial. The feeding trial lasted sixty days. Feed was given three times a day at5% of the fish biomass (Watanabe. 1988). It was given at 08.00, 12.00 and 16.00 local time. The remainder of the feed and fish excrement were siphoned every morning before feed was distributed. Observation of fish growth and water quality were conducted every ten days. The growth observation was conducted by weighing the biomass of fish fry, which was then noted as reference for adjustment of feed mixture stock and enzyme dosage. Measurement of water quality entailed temperature, diluted oxygen content and pH. In the event of the death of fish fry during the observation, the mass of the dead fish was weighed and the number of the dead fish fry counted and noted.

Observation Parameter

a. Daily Growth Rate

Growth rate was calculated using the mass formula according to Effendie (1997):

$$G=\frac{\ln Wt - \ln Wo}{t} \times 100\% \quad [1]$$

where:
G = Daily growth rate (%)
Wt= Biomass at end of study (gr)
Wo= Biomass at start of study (gr)
t= Duration of observation (day)

Survival Rate

The survival rate was calculated by counting the number of dead fish every day during the feeding trial. The percentage of survival rate was calculated using the survival rate formula according to Effendi (1997):

$$SR = \frac{Nt}{No} \times 100\% \quad [2]$$

where:
SR = Survival rate
Nt = Number of fish at the end of observation
No = Number of fish at the end of observation

Efficiency of Feed Use

The efficiency of feed use was calculated using this formula:

$$EPP = \frac{Wt-Wo}{F} \; x \; 100\% \qquad [3]$$

where:
EPP = Efficiency of feed use (%)
Wt = Completely mass of animal at end of study (g)
Wo = Completely mass of animal at start of study (g)
F = Amount of fish feed consumed during study (g)

Data Analysis

Data collected during the study was analysed using the variance analysis (F test). If the analysis exhibited significantly different results, then a Duncan test with confidence interval of 95% was conducted to compare the resulting values among treatments.

RESULT AND DISCUSSION

Daily Growth Rate

The addition of different percentages of exogenous enzymes in artificial feed resulted in variable results. The addition of papain and bromelin in fish feed contributed to the growth rate.

Figure 1 shows that the average mass of fish fed with feed enriched with enzymes was higher than the control variable. However, the variance analysis shows that the daily growth rate of gourami fry fed with papain and bromelin was not significantly different from the control (Table 1). The values of the daily growth rate can be classified as good, since the minimum acceptable growth rate is 1% (Retnosari, 2007). Our results are in agreement with Hepher (1988), reporting that the digestive ability is influenced by the existence of enzymes in the digestive system, the activity rate of the enzymes and the time needed for the feed to react with the enzymes. In this study, the activity of papain was only 2.16 unit/mg, while the bromelin activity reached 12,41 unit/mg protein. In a study by Hasan (2000), papain activity was 32.88 unit/mg protein, a value that caused more proteins to be hydrolysed into amino acids, and therefore absorbed by the body (Muchtadi, 1989). According to Suhermiyati and Setyawati (2008), bromelin is a proteolytic enzyme that functions by hydrolysing complex proteins into their constituting elements. Gantiawan's study results (2002) show that hydrolysis using bromelin can improve the quality of fish powder and digestibility according to the parameters of amino acids and digestive ability, where the amino acids from fish powder increased for every type of fish powder hydrolysate.

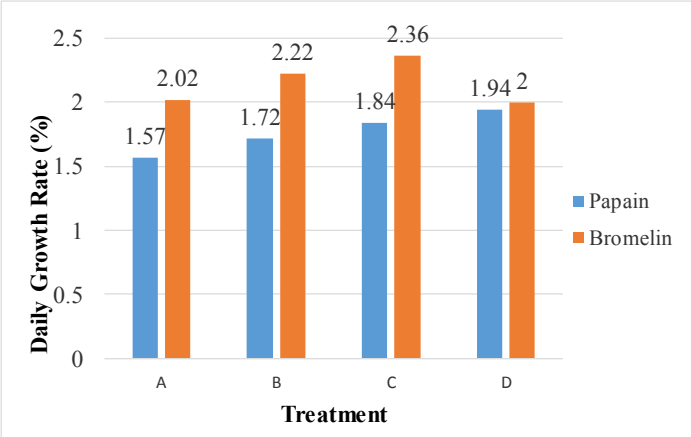

Figure 1. Daily growth of gourami fry according to different feeding treatments

Table 1
Average daily growth rate of gourami fry

Treatment	Average DGR (%)	
	Papain	Bromelin
A	$1.57^{a} \pm 0.09$	$2.02^{a} \pm 0.16$
B	$1.72^{a} \pm 0.23$	$2.22^{a} \pm 0.20$
C	$1.84^{a} \pm 0.11$	$2.36^{a} \pm 0.06$
D	$1.94^{a} \pm 0.15$	$2.00^{a} \pm 0.13$

Note. The values followed by superscript are not significantly different at the confidence interval of 95%.

Suhermiyati and Setiyawati (2008) suggested that bromelin is a proteolytic enzyme that works through hydrolysing complex protein into their individual building blocks. A study by Gantiawan (2002) showed that hydrolysis using bromelin enzyme can increase the quality of fish powder and feed digestibility.

The use of papaya simplicial papain enzyme did not significantly affect the daily growth rate. According to Fitri (1996), the growth of gourami fry weighing 10 grams can achieve the growth rate of 1.65% per day after the addition of enzymes. The results of the study by Pinandoyo et al. (2015) show that the use of 2,25% papain enzyme in black nile tilapia feed gave a daily growth rate of 1.83%. These results are similar to the study by Amalia et al. (2013) that concludes that the use of 2.25% concentration of papain enzyme in giant catfish feed resulted in a growth rate of 1.97%.

The results also show that the addition of exogen enzyme in the feed increases the digestive ability of gourami fry, although enzymes with higher protease activities are required to achieve maximum daily growth rate targets. Reed (1975) states that enzyme concentration is one of the factors which influences t the process of breaking down proteins. This indicates that the use of papain enzyme in the feed increases the daily growth rate of gourami fry.

Survival Rate

The survival rates at the end of the study varied. The percentage of survival rate in the papain treatments was between 73.3% and 80%, while in the bromelin treatments, the percentage was around 73.3%-82.5% (Figure 2).

The variance analysis results show that the difference in the survival rate of the gourami fry between the use of papain and bromelin in fish feed was not significant (Table 2).

The slight difference between treatments indicate that the addition of papain and bromelin in the feed did not have negative effects on the survival rate of gourami fry. Thus suggesting that the survival rate is determined, among others, by the necessity of nutrients in the feed. In a study by Mokoginta et al. (1994) it was established gourami larvae weighing 0.2 grams need protein at the percentage of 43.29% with C/P and 8 Kkal DE/g, in order to achieve daily growth rate, feed efficiency and high retention of protein. This protein percentage is not too far from the protein content of the commercial feed given in this study, which was about 36%.

Generally, the average survival rate of gourami found in this study was above 75%, which is an improvement of by Hasan (2000), where the artificial feed enriched with papain administration resulted in a survival rate of 45%. Subandiyono et al. (2013) in his study stated that the use of bromelin in artificial food for giant catfish did not significantly affect the survival rate

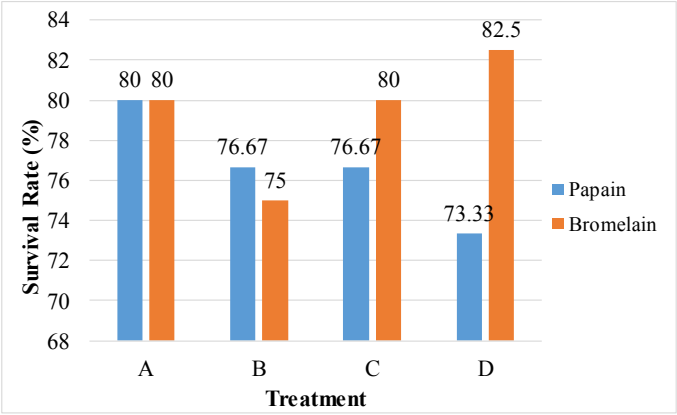

Figure 2. The survival rate of gourami fry according to different feeding treatments

Table 2
Average survival rate of gourami fry

Treatment	Average Survival Rate (%)	
	Papain	Bromelin
A	80.00[a]±7.40	80.00[a]±0.63
B	76.67[a]±10.48	75.00[a]±0.30
C	76.67[a]±3.83	80.00[a]±0.77
D	73.33[a]±10.70	82.5[a]±0.82

Note. The values followed by superscript are not significantly different at confidence interval of 95%.

Feed Use Efficiency

The effect of different concentrations of papain and bromelin on feed efficiency is reported in Figure 3. The use of papain resulted in a feed rate between 27.70% and 34.88%, while the use of bromelin resulted in a feed rate between 36.19% and 40.14%.

Statistical analysis showed that the addition of papain and bromelin did not impart any significant difference in feed efficiency rate (Table 3).

Feed efficiency is used to evaluate feed quality. The higher the efficiency, the better the feed (Kordi, 2010). Feed efficiency is affected by factors such as fish size, fish physiological functions, feed quality and rate of consumption (Brown, & Bratzek, 1980). According to Affandi et al. (1992), gourami with a length between 13.5 and 15.0 cm has a ratio of intestine length to body length ranging from 1.31 to 2.31. The values indicate that the digestive system of the gourami is developing, although it structurally it remains immature. Besides, papain and bromelin used are protease of low activity, making the study sub-optimal. Indeed, the addition of high dosage to obtain better efficiency values is influenced by the activity of protease enzymes causing hydrolysation of complex proteins into amino acids and peptide chains. The study by Handayani et al. (2000) shows the relationship between growth and activity of digestive enzymes. The increase in the activity of protease enzymes, a-amylase and lipase positively correlates with the increase in growth rate and efficiency of feed used in gourami fry. The availability of digestive enzymes influences enzyme activity in digesting the given feed and hence both growth rates and efficiency of feed use.

According to Andriani (2009), the feed efficiency value is directly proportional to the growth rate, indicating that growth occurs with the change in feed efficiency. The bigger the value of efficiency, the better the utilization of the feed in achieving the required mass. This is in line with Amalia et al. (2013) study, indicating that the addition of papain in the artificial feed for giant catfish results in higher growth rate and efficiency. Similarly, the use of bromelin (or bromelain) in a study by Putri

(2012) on giant catfish indicates that the use of 2.25% concentration of bromelin results in a highest value of efficiency compared to other treatments and the control treatment.

In this study, the recorded feed efficiency values were between 36.19% and 40.14%. According to Craig and Helfrich (2002), feed is considered good if its efficiency is more than 50% or even closer to 100%. Result indicates the addition of redundant enzyme concentration in feed substrates causes lower activity rates of protease enzymes, resulting in inefficient hydrolysis and piling of bound proteins or inter-enzyme chains which in turn lower the fish digestive ability (Arqiya, 2002).

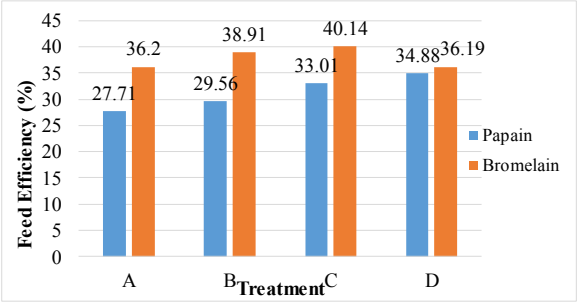

Figure 3. Feed efficiency of gourami fry according to different feeding treatments

Table 3
Average feed use efficiency for gourami fry

Treatment	Average Feed Use Efficiency (%)	
	Papain	Bromelin
A	$27.71^{a}\pm3.31$	$36.2^{a}\pm0.55$
B	$29.56^{a}\pm7.04$	$38.91^{a}\pm0.93$
C	$33.01^{a}\pm3.30$	$40.14^{a}\pm0.44$
D	$34.88^{a}\pm4.24$	$36.19^{a}\pm0.83$

Note. The values followed by superscript are not significantly different at confidence interval of 95%.

CONCLUSION

The addition of papain and bromelin enzymes in fish feed increases the growth rate, survival rate and feed efficiency of gourami fry although not in a statistically significant manner. It is likely that a period longer than 60 days is required to observe the impact of enzyme addition toward growth rate and feed efficiency. Thus, more experiments are need to validate the results of this study.

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