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ENHANCING GROWTH PERFORMANCE AND FEED EFFICIENCY OF JUVENILE GOURAMI *Osphronemus gouramy* FED A PAPAIN ENZYME AND PROBIOTIC-SUPPLEMENTED DIET

Peningkatan Kinerja Pertumbuhan dan Efisiensi Pakan Juvenil Ikan Gurami *Osphronemus gouramy* yang Diberi Pakan dengan Suplementasi Enzim Papain dan Probiotik

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ABSTRACT

Gourami (*Osphronemus gouramy*) farming faces challenges, including slow growth rates and high feed costs. This study aimed to evaluate the effects of probiotics, papain enzymes, and their combination on the growth performance and feed utilization of gourami. The study was conducted using a completely randomized design with four treatments and three replicates each. The treatments consisted of a control group (feed without papain enzyme or probiotics), treatment A (feed supplemented with 8 mL/kg probiotics), treatment B (feed supplemented with 32.5 g/kg papain enzyme), and treatment C (feed supplemented with a combination of 0.25 g/kg papain enzyme and 15 mL/kg probiotics). Juvenile gourami with average weight of 5.82±0.09 g/fish were kept in nine fiberglass tank (3 m diameter and 1.2 m height) for 42 days with a stocking density of 15 fish/m^2. After 42 days of rearing, treatment C exhibited significantly higher specific growth rate, protein efficiency ratio, and protein retention than the other treatments ($P<0.05$), with values of 3.03±0.15%/day, 2.78±0.20%, and 44.71±3.06%, respectively. The lowest feed conversion ratio was also observed in treatment C, with a value of 0.94±0.06 ($P<0.05$). In conclusion, supplementation with 0.25 g/kg papain enzyme and 15 mL/kg probiotics in the feed enhanced growth performance and feed utilization in gourami.

Keywords: papain enzyme, gourami, growth, probiotics

ABSTRAK

Budidaya ikan gurami *Osphronemus gouramy* memiliki kendala yaitu pertumbuhan yang lambat serta tingginya biaya pakan. Penelitian ini bertujuan mengevaluasi pangaruh suplementasi probiotik, enzim papain, dan kombinasinya terhadap kinerja pertumbuhan dan efisiensi pakam ikan gurami. Penelitian ini dilakukan dengan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan. Perlakuan terdiri dari kontrol (pakan tanpa diberi enzim papain dan probiotik), A (pakan dengan penambahan 8 mL/kg probiotik), B (pakan dengan penambahan 32,5 g/kg enzim papain), dan C (kombinasi 0,25 g/kg enzim papain dan 15 mL/kg probiotik pada pakan). Benih ikan gurami berukuran 5,82±0,09 g/ekor dipelihara pada bak fiber (diameter 3 m dan tinggi 1,2 m) selama 42 hari dengan padat tebar 15 ekor/m^2. Setelah pemeliharaan 42 hari, perlakuan C menunjukkan laju pertumbuhan spesifik, rasio efisiensi protein dan retensi protein lebih baik dari perlakuan lainya (P<0,05), berturut-turut sebesar 3,03±0,15 %/hari, 2,78±0,20 dan 44,71±3,06%. Hasil *feed conversion ratio* dengan nilai paling rendah didapat pada perlakuan C sebesar 0,94±0,06 (P<0,05). Simpulan penelitian ini yaitu suplementasi 0,25 g/kg enzim papain dan 15 mL/kg probiotik melalui pakan terbukti dapat meningkatkan kinerja pertumbuhan dan efisiensi pakan ikan gurami.

Kata Kunci: enzim papain, gurami, pertumbuhan, probiotik

INTRODUCTION

The gourami (*Osphronemus gouramy*) is a freshwater fish with high economic value in Indonesia, with market demand steadily increasing in recent years. This increased demand has driven gourami production to increase by 88.54% from 2021 (41,184 tons) to 2022 (77,648 tons) (KKP, 2022). Gourami is also considered a leading commodity because it has a high selling price ranging from IDR 35,000.00–IDR 50,000.00/kg at the retail level (KKP, 2021). Challenges in gourami cultivation include slow fish growth (Arifin *et al.,* 2018), so reaching market size takes a relatively long time (8–10 months) and requires high feed costs (Afriyanti, 2020).

Gourami growth is highly dependent on the feed provided. Feed with a high digestibility level can help fish obtain optimal nutrients from the feed. Protein, one of the main feed components, has a significant impact on feed price, growth, and the resulting metabolic waste (Suprayudi *et al.,* 2022). Therefore, research to optimize protein utilization in feed by increasing digestibility is crucial to enhance growth and feed utilization efficiency. A strategy to increase protein digestibility in feed is by administering protease enzymes, one of which is papain (Anggraini & Ariditadan, 2015). Papain is a proteolytic enzyme capable of breaking down protein into amino acids, thus optimizing protein utilization (Makmuri, 2022). The administration of papain enzyme to carp (Singh *et al.,* 2011), catfish (Amalia *et al.,* 2013), tilapia (Sari *et al.,* 2013), tiger grouper (Fadli *et al.,* 2013), giant freshwater prawn (Manush *et al.,* 2013), and Nilem fish (Sari *et al.,* 2020) has shown positive results on growth and feed efficiency.

Another effort to improve feed utilization is the administration of probiotics. Probiotics are live microorganisms in the host and provide benefits by modifying the microflora community, increasing nutritional value, improving feed utilization, enhancing health status, and improving aquaculture water quality (Verschuere *et al.,* 2000). The application of probiotics in aquaculture activities not only acts as a biocontrol agent or as a bioremediation, but can also increase the nutritional value of feed and the efficiency of nutrient absorption, thus enabling fish to achieve optimal growth (Fajri *et al.,* 2016; Putra *et al.,* 2015). The administration of probiotics is reported to change the composition of microbes in the intestine so that it can encourage the growth of beneficial bacteria well, and has the ability to produce enzymes that can help the digestive process, thereby increasing growth and feed efficiency (Suardani *et al.,* 2023; Raja & Arunachalam, 2011).

The addition of both papain and probiotics to feed aims to optimize the feed digestion process, thereby increasing growth and feed efficiency in gourami. Gourami growth has been successfully enhanced by adding papain at a dose of 32.5 g/kg (Sari & Andriani, 2018), probiotics at a dose of 8 mL/kg (Sutrisno *et al.,* 2022), and a combination of both with a papain enzyme dose of 0.25 g/kg feed and probiotics at 15 mL/kg (Mareta *et al.,* 2017). To date, there is no information available comparing the three methods, including the application of papain enzyme supplementation, probiotics, and their combination in semi-field and field-scale gourami nurseries. Therefore, this study aims to evaluate the effect of probiotic supplementation, papain enzyme, and their combination on gourami growth performance and feed efficiency.

RESSEARCH METHODS

Time and Place

The research was conducted for 42 days, starting from March 2024 to April 2024. The test fish were reared at the outdoor hatchery of the Sukabumi Campus of the IPB Vocational School. Proximate analysis of feed and meat was conducted at PT. Saraswanti Indo Genetech.

Tools and Materials

The tools used in making test feed are analytical scales, trays, 1 mL syringes, beakers (100 mL), spatulas, spray bottles, zip plastic. The tools used in fish maintenance activities are fiber tanks (3 m in diameter and 1 m in height), basins (20 L), rulers (0.1 cm), digital scales (1 g), analytical scales (0.0001 g), container boxes (100 L), sieves (30 cm x 25 cm), thermometers, pH meters, and DO meters. The materials used in this study are gourami fish seeds, papain enzymes, probiotics, binders, manure, floating pellets measuring 0.7–1.0 mm, and floating pellets measuring 1.3–1.7 mm (PF 1000).

Experimental Design

This study used a completely randomized design (CRD) consisting of four treatments and three replications. The treatment designs included a control (feed without papain enzyme supplements and probiotics), treatment A (probiotic supplementation in commercial feed), treatment B (papain enzyme supplementation in commercial feed), and papain enzyme supplementation and probiotics in commercial feed (Table 1). These treatments refer to Sutrisno et al., (2022) who used probiotics at a dose of 8 mL/kg, Sari and Andriani (2018) who used papain enzyme at a dose of 32.5 g/kg, and Mareta *et al*. (2017) who used an additional dose of papain enzyme of 0.25 g/kg of feed and probiotics of 15 mL/kg.

Table 1. Treatment design for adding probiotics, papain enzyme, and a combination of both ingredients to gourami fingerlings

Treatment	Description
Control	Feeding without papain enzyme and probiotics
A	Feeding with the addition of probiotics at 8 mL/kg
B	Feeding with the addition of papain enzyme at 32.5 g/kg
C	Feeding with the addition of papain enzyme at 0.25 g/kg and probiotics at 15 mL/kg

Preparation of test feed and test fish

The test fish feed used in this study was commercial floating feed. The test feed was supplemented with 99% purity papain enzyme obtained from an online marketplace, while the probiotics used contained *Lactobacillus casei* and *Saccharomyces cereviceae* (EM4 Fisheries). These ingredients were mixed with a feed binder using a measuring cup and stirred until thoroughly mixed. The mixture was then added to the feed. After being thoroughly mixed, the feed was air-dried and placed in an airtight container. A 20 g sample of feed was taken from each treatment for proximate analysis.

The test fish used were gourami fingerlings from a Gourami Fish Hatchery near Sukabumi, weighing 5.82 ± 0.09 g per fish. The test fish were reared in round fiber tanks with a diameter of 2 m and a height of 1.2 m at a density of 15 fish/m² (SNI, 2000).

Maintenance of test fish

The test fish, which had been acclimatized for 7 days, were sampled for their weight. Thirty fish were randomly selected from each treatment. The test fish were then fed according to the experimental design and maintained for 42 days. Feeding was carried out three times a day, at 8:00 AM, 12:00 PM, and 4:00 PM WIB. Feed was provided at satiation, referring to the gourami feeding rate of 6%. The feed provided and any remaining feed were calculated and recorded as an evaluation of daily feeding. Water quality measurements were carried out daily by measuring temperature, pH, and dissolved oxygen.

Observation parameters

Observations of samples (feed and fish) were subjected to proximate analysis to determine protein, fat, carbohydrate, ash, and moisture content. The proximate analysis was conducted using the AOAC (2012) method. The results of the proximate analysis of the feed are presented in Table 2.

Table 2. Proximate analysis of feed with the addition of papain enzyme and probiotics to gourami fry.

Parameter	Treatment			
	K	A	B	C
Ash content (%)	8,75	9,08	10,88	9,81
Water content (%)	16,10	16,09	14,42	7,65
Protein (%)	35,36	36,66	35,93	38,00
Fat (%)	3,57	2,62	6,76	3,19
Carbohydrate (%)	36,22	35,55	32,00	41,34

The observed growth performance parameters included growth performance and feed digestibility, including the survival rate (SR), specific growth rate (SGR), protein efficiency ratio (REP), feed conversion ratio (FCR), and protein retention (RP). The units, formulas, and references for all observed growth performance parameters are shown in Table 3.

Table 3. Observation parameters for growth performance of gourami treated with papain enzyme and probiotics.

Parameter	Unit	Formulas	Reference
Survival rate (SR)	%	$\frac{Nt}{N0}$ x 100%	(Effendi, 2004)
Specific Growth Rate (SGR)	%/day	$\left[\sqrt[t]{\frac{Wt}{Wo}} - 1\right]$ x 100%	(Huisman, 1987)
Feed Conversion Ratio (FCR)	-	$\frac{F}{(Bt+Bm)-B0}$	(Zonneveld *et al.*, 1991)
Protein Efficiency Ratio (PER)	%	$\frac{Wt - W0}{F \times \%\text{feed protein}} \times 100$	(Tacon, 1987)
Protein Retention (PR)	%	$\left[\frac{Pt - P0}{Pc}\right] \times 100$	(Takaeuchi, 1988)

Description: Nt = final number of fish (tail), N0 = initial number of fish (tail), Wt = final average weight of fish (g), Wo = initial average weight of fish (g), t = maintenance period (days), F = amount of feed given (g), Bt = final fish biomass (g), B0 = initial fish biomass (g), Bm = dead fish biomass (g), Pt = final amount of fish protein (g), P0 = initial amount of fish protein (g), Pc = amount of protein consumed (g).

Data Analysis

The data obtained were tabulated in Microsoft Excel and analyzed using Analysis of Variance (ANOVA) using the SPSS 26.0 statistical program. Data homogeneity and normality were evaluated using the Levene and Shapiro-Wilk tests, respectively. Significant differences were then further tested using Duncan's Multiple Range Test (DMRT) at a 95% confidence interval.

RESULT

The results of the analysis of growth performance and feed utilization of gourami fish maintained for 42 days with commercial feed treatment added with probiotics and papain enzymes. Based on the analysis of variance (One-way-ANOVA) with a 95% confidence interval in the parameters of LPS, FCR, REP, and RP there were significant differences (P <0.05). After further analysis using Duncan test fish in the control were significantly different from all treatments. Further tests of the specific growth rate parameters of treatment B resulted in no significant difference with treatments A and C, Meanwhile, FCR, REP, and RP obtained results from the control were significantly different from the treatment while each treatment between the other treatments was not significantly different. The lowest value for each parameter was obtained by the control and the highest was achieved by treatment C.

1. Specific Growth Rate

The results of the specific growth rate in the treatment without the addition of test materials (control) obtained results of 2.15 ± 0.11 % / day. The treatment of probiotic application with a dose of 8 mL / kg of feed resulted in a specific growth rate of 2.60 ± 0.12 % / day. The treatment of papain enzyme application with a dose of 32.5 g obtained results of 2.73 ± 0.15 % / day. The treatment of the combination of papain enzyme and probiotics at 0.25 g / kg and 15 mL / kg increased the specific growth rate by 3.03 ± 0.15 % / day. The results of the specific growth rate during maintenance are presented in Figure 1.

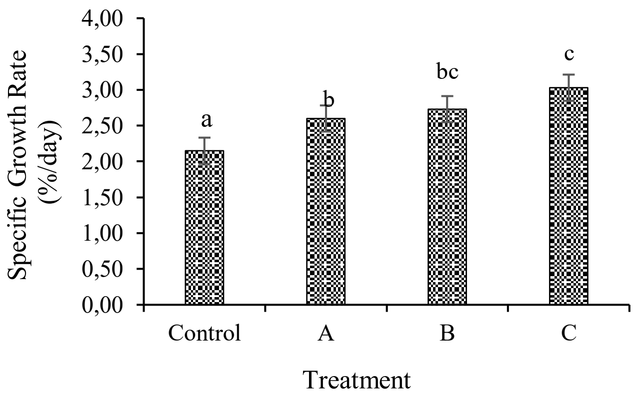

Figure 1. Specific growth rate of gourami fish seeds

2. Feed Conversion ratio

The administration of papain enzyme and probiotics to the feed conversion ratio resulted in a significant difference (P<0.05). The results of Duncan's further test showed that the control treatment was significantly different from the other treatments. However, treatments

A, B, and C showed no significant differences. The treatment with the lowest FCR in treatment C showed a value of 0.94±0.06, followed by treatment B with a value of 1.03±0.07. Treatment A with a result of 1.00±0.11, and the control treatment 1.59±0.13. The results of the feed conversion ratio during maintenance are presented in Figure 2.

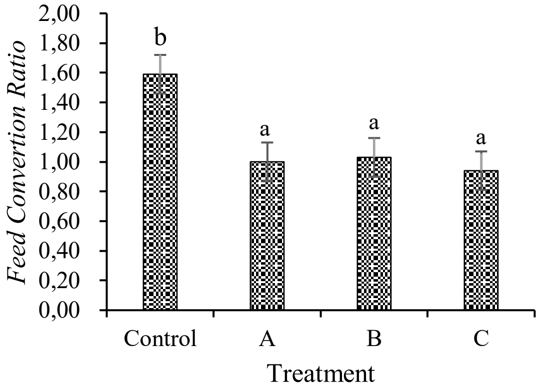

Figure 2. Feed Conversion Ratio of gourami fish seeds

3. Protein Efficiency Ratio

The administration of papain enzyme and probiotics on the protein efficiency ratio (Figure 3) resulted in a significant difference ($P<0.05$). The results of Duncan's further test showed that the control treatment was significantly different from the other treatments. However, treatments A, B, and C showed no significant difference. The treatment with the highest protein efficiency ratio value was treatment C with a value of 2.78±0.20, followed by treatment A with a result of 2.71±0.19, treatment B with 2.65±0.22, and the control treatment with 1.75±0.13.

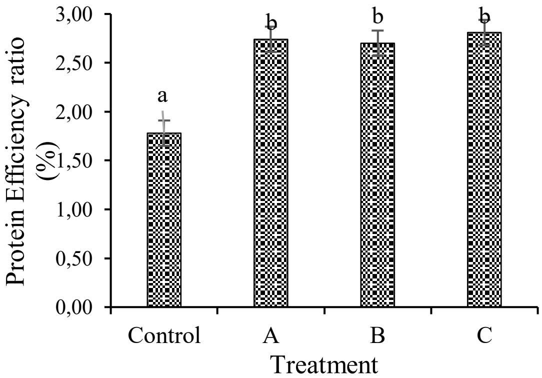

Figure 3. Protein efficiency ratio of gourami fish seeds

4. Protein Retention

The results of protein retention calculations in each treatment showed no significant differences, with the highest results obtained in treatment C (44.71±3.06%), followed by treatment B (41.85±3.31%), then treatment A (40.33±2.84%), and the control treatment (20.00±1.72%). The results of protein retention during maintenance are presented in Figure 4.

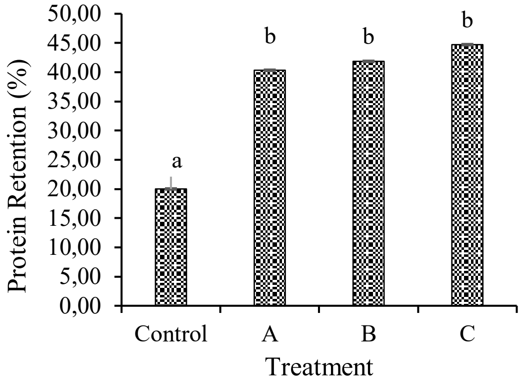

Figure 4. Protein retention of gourami fish seeds

5. Survival Rate

The effect of papain enzyme and probiotics on the survival rate can be seen in Figure 5. The survival rate of gourami fish after being given treatment showed various results with the highest results in treatment C of 82.07±0.00% in sequence, namely treatment B of 81.13±0.00%, then the control treatment of 74.52±0.00%, and treatment A with a value of 71.70±0.00%. This proves that the addition of papain enzyme and probiotics has an effect on the survival rate of gourami fish.

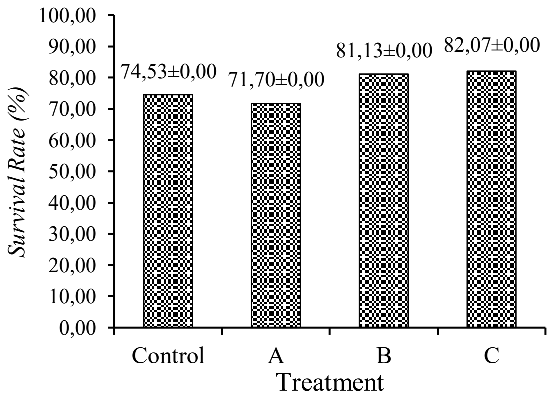

Figure 5. Survival rate of gourami fish seeds

DISCUSSION

The results of this study indicate that the increase in specific growth rate is consistent with previous research findings. Mareta *et al.* (2017) reported that administering papain enzyme and probiotics can increase specific growth rate, with the best result being 1.17±0.27%/day. Furthermore, Makmuri *et al.* (2022) found that administering papain enzyme at a dose of 0.25 g/kg to catfish resulted in a specific growth rate of 4.74±0.17% per day. The use of probiotics also provides the added benefit of increasing feed efficiency because they contain bacteria capable of producing lipase, amylase, and protease enzymes, which play a role in helping fish digest feed optimally (Mareta *et al.,* 2017).

Quality feed has a positive impact on gourami growth and production (Suwarsito & Susylowati, 2024), with protein being the most important nutrient component in fish feed. Feed quality can be measured by its ingredients, including protein content, which supports fish growth. Muchlisin *et al.* (2016) revealed that the addition of the enzyme papain can improve the digestion and nutrient absorption process from feed, thus optimizing feed utilization efficiency. This increased efficiency also improves protein deposition in the fish's body, which plays a direct role in supporting fish growth. Fish growth performance in this study served as an indicator of feed quality, which was assessed based on feed utilization and nutrient availability.

The highest feed conversion ratio in this study was found in treatment C (0.94±0.06). According to research by Sulatika *et al.* (2019), the feed conversion ratio for gourami ranged from 2.44 to 2.31. Feed quality impacts the feed conversion ratio because it impacts palatability, or the fish's reaction to the feed. Feed consumption will increase if the protein-energy content is higher than required, thus increasing the absorption of protein and other nutrients. Optimal protein utilization by the fish's body includes building blocks, the production of new cells during growth, as an energy source for hormone and enzyme formation, and oxygen transport. Increased protein absorption occurs when feed is consumed and utilized efficiently, thus boosting growth performance (Isnawati *et al.,* 2015).

A similar study conducted by Sulasi *et al.* (2018) on carp yielded the best protein efficiency value of 2.35±0.05 with the addition of 0.25 g/kg papain enzyme and 10 mL/kg probiotics. The results of the protein efficiency ratio of the treatment used by Mareta *et al.,* (2017) obtained the best results of 1.51 ± 0.12. These results indicate that this study provides better protein utilization in feed, this is indicated because of the differences in the containers used and the different levels of protein provided. According to research by Sulasi *et al.,* (2018) the difference in the type of fish used then the ability to digest feed has an impact on the value of the protein efficiency ratio.

Quality feed will be digested, absorbed, and utilized by the fish body to increase the retention value of the fish meat. Retention content in the fish body is influenced by the protein content of the feed and the level of digestibility of the feed. Digested, absorbed, and utilized protein is used for biological growth, metabolic energy production, and repair of damaged cells (Danu & Heltonika, 2015). The results of the calculations for each treatment showed no significant differences, but the highest results were obtained in treatment C (45.51±3.07%). Protein retention can be increased by providing a feed with high protein, balanced amino acids, and sufficient feed energy (Pohlenz *et al.,* 2012).

The results of this study align with Mareta *et al.* (2017) that found that a combination of papain enzyme at a dose of 0.25 g/kg and probiotics at 15 mL/kg resulted in a survival rate of 83.33±5.77%. The varying survival rates of gourami fish are thought to be due to optimal utilization of the feed provided by the gourami fish, allowing for effective and efficient use of energy for growth activity and survival rates. The survival rate of gourami fish is also reported to be in the range of around 76.67%-80.00% (Anggara *et al.,* 2018).

CONCLUSION

Supplementation of 0.25 g/kg papain enzyme and 15 mL/kg probiotics can improve growth performance and feed efficiency in gourami. The combined application of papain enzyme and probiotics to gourami feed showed higher protein content, protein efficiency ratio, and protein retention than other treatments and reduced the feed conversion ratio.

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