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The Effect of Bromelain Enzyme Supplementation on the Growth Perform of Eels (*Anguilla Bicolor*)

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Abstract

Eel (*Anguilla bicolor*) is an important commodity and has a high economic value. This fish is rarely cultivated because, apart from the limited availability of seeds, the growth rate is relatively slow. This study aims to determine the value of growth rate, feed efficiency and fcr of Anguilla bicolor which is fed with bromelain enzyme supplementation. The method used was a completely randomized design (CRD) with four treatments and five replications. Data analysis used the ANOVA test with a confidence level of 95% and Duncan's advanced test (DMRT). Bromelain enzymes have a significant effect on FCR and feed efficiency. However, there was no significant effect on growth parameters in *Anguila bicolor*. However, the best treatment in this study was P4 with GR 0.38 ± 0.03 g/day, SGR 0.95 ± 0.03 %/day, FCR 1.97 ± 0.15 and feed efficiency of 50.9 ± 4.17%.
Keywords: *Anguilla bicolor*, bromelain enzyme and growth

INTRODUCTION

Anguilla bicolor is a fish with a high economic value and is one of Indonesia's most important exports. China receives 50.57 percent of all eel exports, followed by Japan with 33.01 percent, Malaysia with 11.69%, and Hong Kong with 4.73 percent. (Iskandar et al., 2021). Exports of eel from Indonesia in 2018 were 13,054 tons and increased to 61,691 tons in 2019 (Harianto, 2020). This fish contains up to 270 kcal/100g of energy and 45 times more Vitamin A than cow's milk, which contains 4700 IU/100g. Vitamin B1 is equivalent to 25 times the Vitamin B1 found in cow's milk, whereas Vitamin B2 is equivalent to 5 times the vitamin B2 found in cow's milk. In addition, the DHA (*docosahexanoic acid*) and EPA (*eicosapentaenoic acid*) concentrations are 1,337 mg/100 g and 742 mg/100 g, respectively. This content is higher than that of salmon, which contains 820 mg/100 g and 492 mg/100 g, respectively (Baedah, 2010). Consequently, demand for this species of fish continues to rise. However, growing an eel to a size suitable for human consumption is a lengthy process. This is due to the low appetite for seed at high stocking rates (Nursyahfira, 2017). Additionally, the cannibalism of eel seedlings impedes grow-out activities (Rahmawati et al. 2015). Eight to nine months are required for eels to reach a weight of 120 g with a feed utilization efficiency (FE) of 19.49% and a high feed conversion rate (FCR) of 13.25. (Arsyadana et al., 2017).

Efforts have been made to combat the slow growth rate by selecting seeds in advance, providing additional feed in the form of natural feed, and enhancing feed efficiency (Handoyo et al., 2012; Sandriyani et al., 2015; Ibrahim et al., 2021). Adding protease enzymes to feed

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is one way to improve feed efficiency (Zhou et al., 2010). The protease enzyme functions to hydrolyze protein in feed to make it more digestible and absorbable by the fish (Wiszniewski et al., 2018). Protein catabolism, blood clotting, cell growth and migration, tissue regulation, and inflammation are some of the physiological and pathological processes in which protease enzymes play a significant role (Motyan et al., 2013). Plants, animals, and microorganisms contain these enzymes. The enzymes papain and bromealin can be isolated from papaya and pineapple fruit, respectively (Jisha et al., 2013). However, the isolation of enzymes from fruit is highly seasonal dependent (Sugiarto et al., 2020). As a result, pure bromelain enzymes were selected as an alternative to fruit. Enzim bromealin dan enzim papain termasuk ke dalam protease golongan sufrihidil (Rachmania et al., 2017).

The bromealin enzyme is more suitable for carnivorous fish because it works more efficiently on animal protein than the papain enzyme does on vegetable protein (Taqwdasbriliani et al., 2013). The bromelain enzyme is capable of dissolving mucus and accelerating the kidneys' fat elimination. In addition to citric acid and malic acid, the bromelain enzyme also contains citric acid and malic acid, which can enhance the process of removing fat and manganese from certain enzyme components that are important for protein and carbohydrate metabolism (Winastia, 2011). This enzyme has been shown to increase growth and feed utilization efficiency in sterlet (*Acipenser ruthenus*) (Wiszniewski et al., 2018), *Puntius javanicus* (Subandiyono et al., 2018), *Osphronemus gourami* (Andriani et al., 2018), catfish (Clarias bathraca) (Yusni, et al., 2021), and Oreoch (Van Doan et al., 2021). Additionally, the addition of papain enzymes to eel feed can improve growth and feed efficiency (Sagita et al., 2017). The addition of the bromealin enzyme to eel feed has never been attempted in previous research.

MATERIAL AND METHOD

Site study

This research was conducted from May to June 2022 in the Wet Laboratory of the School of Health and Life Sciences at Airlangga University in Banyuwangi.

Materials and equipment

This study utilized 200 elver eel fish obtained from eel cultivators in the Kalipuro area, Banyuwangi Regency, with an average length of 28.76 ±0.14 cm and weight of 32.80 ±0.2 g, as well as chlorine in water reservoirs, commercial feed with a protein content of 37 %, and factory-made commercial bromelain enzyme. The investigation utilized aquariums with a volume of 40×30×30 cm and up to 20 pieces, hoses and aeration stones, water hoses, baking sheets, slides, analytical scales, feed containers, basins, rulers, raffia ropes. The used of water quality checker i.e: Disolved oxygen meter, nitrification test kits, thermometers, pH pen, and an ammonia test kit.

Design Studies

This research employed a completely randomized design (CRD) with five treatments and four replications. This study aims to determine the effect of adding bromelain enzymes to eel feed on

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its growth and efficiency. The dosage employed in this study is based on Rese's research (2020). Where (P0) there is no addition of bromealin enzyme, (P1) there is addition of 0.75 g/kg bromealin enzyme, (P2) there is addition of 1.5 g/kg bromealine enzyme, (P3) there is addition of 2.25 g/kg bromealin enzyme, and (P4) there is addition of 3 g/kg bromealin enzyme.

Preparation

Fish tank

The aquarium is washed with soap and water until it is thoroughly clean, then sterilized with chlorine for twenty-four hours. Three grams per liter of chlorine is used to kill microorganisms. In order to damage the enzymes in microorganisms, chlorine must be added to an aquarium for one to two days (Pratama and Mukti, 2015). The aquarium was then filled with water that had been stored for three days in a water reservoir, until it reached a height of 20 centimeters and a volume of 24 liters. Additionally, it is aerated for twenty-four hours before the aquarium is used as a fish breeding container (Nuryati and Rahman, 2015).

Fish specimen

First, the eels used in the fish are acclimatized to their new environment (Pratama and Mukti, 2015). The remaining eels were acclimatized by placing them in plastic packaging for 20 minutes, then opening and stocking thems (Effendi et al., 2006). Each aquarium contained ten fish seeds, which fasted for 24 hours. This is meant to increase feed consumption and eliminate the residual feed's effect on the fish's body (Nurhuda et al., 2018).

Fish diet

The commercial feed used in this study contained 37 percent protein. By combining the enzymes, a predetermined amount of bromealin is added to animal feed. In addition, for each treatment, the feed was mixed with water in a ratio of 1:1 to create a paste feed.

Pisciculture

Ad-satiation feeding is utilized in pisciculture, with feedings occurring twice daily at 10:00 a.m and 19:00 p.m. Additionaly, 20-50% of water is siphoned and added daily to maintain optimal water quality (Mulyana et al., 2012).

Data collection

Every ten days during the study, random measurements of weight and length growth were collected. By sampling eel seeds in each treatment and repetition, weight and length measurements were taken. Using an analytical balance, the eel fish's mass was determined by weighing eel seeds. Using a ruler, the length of an eel is determined by measuring its body from the base of its head to the tip of its tail.

Measurement parameters

This study measured the formula-calculated specific growth rate (*Sgr*) (Mulqan, et al., 2017). Formulas for daily growth rate (*Gr*) and feed convention ratio (Effendi, 1997). Feed utilization efficiency is calculated as (Iskandar and Elrifadah, 2015).

$$SGR = (ln\ (W_t) - In\ (W_0))/T \quad x\ 100\%$$

Which:

SGR: specific growth rate (%)
W_t: final average weight (g/ind)
W_0: initial average weight (g/ind)
T : time (day)

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$GR = (W_t-W_0)/t$
Which:
GR: daily growth (g/hari)
W_t: final average weight (g/ind)
W_0: initial average weight (g/ind)
t : time (day)

$FCR = F: ((Wt+D)-W0)$
Which:
FCR: feed convention ratio
F : total feed (g)
W_0: final average weight (g/ind)
W_t: initial average weight (g/ind)
D : weight of fish die (g)

$FE = ((Wt +D)-W0)/F \quad x\ 100\%$
Which:
FE: feed efficiency
W_t: final average weight (g/ind)
D : weight of fish die (g)
W_0: initial average weight (g/ind)
F : total feed (g)

Data analysis

The obtained data were analyzed using the Analysis of Variance (ANOVA) test with 95% confidence intervals and Duncan's multiple range extension test (DMRT) was used to determine differences in growth and feed efficiency. In addition, the analysis of water quality is described in order to examine the relationship between water quality and growth parameters and to draw conclusion.

RESULT AND DISCUSSION

Growth of Anguila bicolor

Each treatment had different growth and specific growth rates for Anguilla bicolor (Table 1). The highest growth and specific growth rates was found at (P4) was 0.38 ± 0.03 g/day and 0.95 ± 0.03 %/day. The control treatment (P0) had a lowest value of 0.18 ± 0.1 g/day and 0.49 ± 0.29 %/day. Bromelain enzyme in feed increases growth rate and specific growth rate linearly with dose. Collagen in the bromelain enzyme forms cartilage tissue to boost growth. This enzyme did not significantly affect eel growth ($P > 0.05$). Protein hydrolysis into amino acids increases with feed enzymes (Nurhidayah et al., 2013).

The bromelain enzyme has the capacity to degrade crude protein into amino acids that fish can utilize optimally for growth (Setiyani, et al., 2017). Moreover, it can convert collagen into gelatin and hydrolyze gelatin molecules to form cartilage in fish, thereby increasing their weight (Putri, 2012; Novita et al., 2017). The specific growth rate in this study ranged from 0.12% to 1.42 %, which is lower than the 2.4% observed in catfish treated with bromealin (Rese, 2020). We believe that one of the factors influencing the value difference is the species used. Due to variations in the protein content of the feed consumed, the activity of protease enzymes in fish digestion varies by species (Sagita et al., 2017).

Table 1. The parameter measurement (mean ± SD), on *Anguilla bicolor* which was fed with the addition of the enzyme bromelain.

Parameters	Unit	P0	P1	P2	P3	P4
Initial weight	($g.ind^{-1}$)	33.15±2.39	32.70±2.01	32.70±1.77	33.00±0.81	32.75±1.36
Final weight	($g.ind^{-1}$)	41.37±1.33	45.25±9.31	47.91±2.85	47.65±3.13	49.17±0.61
Initial Biomass	(g)	331.50±23.90	327.0±20.03	327.0±17.77	330.0±8.16	327.50±13.69

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Parameters	Unit	P0	P1	P2	P3	P4
Final Biomass	(g)	403.5±22.5	433±110.33	454±42.11	465±49.80	479.5±23.05
Growth rate	(g)	0.18 ± 0.10	0.29 ± 0.25	0.32 ± 0.14	0.34 ± 0.13	0.38 ± 0.03
Specific groth rate	(g.day^{-1})	0.49 ± 0.29	0.72 ± 0.53	0.81 ± 0.33	0.85 ± 0.30	0.95 ± 0.03
Fcr	-	4.63 ± 1.42^{a}	2.91 ± 1.22^{b}	2.47 ± 0.79^{b}	2.29 ± 0.38^{b}	1.97 ± 0.15^{b}
Feed efficiency	(%)	23.23 ± 7.16^{a}	40.47±22.11^{ab}	42.98±10.51^{b}	44.60 ± 7.50^{b}	50.90 ± 4.17^{b}
Feed consumption	(g)	355.35±9.22	342.07±9.75	359.22±9.53	340.22±10.60	324.22±9.08

Note. (P0): without the addition of enzymes, (P1): 0.75g/Kg, (P2): 1.5g/Kg, (P3): 2.25g/Kg, (P4): 3g/Kg. Different letters above the bar indicate that there is a significant difference (P <0.05).

Feed Convertion Ratio (FCR)

The addition of the bromealin enzyme to eel feed had a statistically significant effect (P <0.05) on the eel feed conversion ratio compared to the control treatment. However, it was not significantly different from bromelain enzyme treatment (Table 1). The treatment group (P0) had the lowest FCR value, at 4.63 ±1.43. While the highest enzyme (P4) treatment dose (1.97 ±0.15) yielded the best FCR, it was determined that the best FCR was achieved with the highest enzyme (P4) treatment dose. This is likely due to the fact that the bromealin enzyme aids in the optimal digestion of eel. *Anguilla bicolor* has a stomach large enough to accommodate and enzymatically digest food (Murtini, 2019).

The addition of bromelain improves the digestion of nutrients, particularly protein (Sagita et al., 2017). Pepsinogen is converted into the active enzyme pepsin in the stomach, which can convert proteins to peptides. Therefore, fish obtain a great deal of energy for metabolism and growth (Diana et al., 2017). In this study, the feed convention rate values varied between 1.97 and 4.63. This result is superior to those obtained by supplementing artificial feed with fish oil, which ranged between 5.79 and 7.81 (Perdana et al., 2016). Fish oil's high fat content diminishes the nutritional value of feed and interferes with the activity of enzymes in cell membranes, resulting in protein synthesis and low cell counts, which contribute to a high feed conversion rate (Takeuchi and Wattanabe, 1979). In addition, a high FCR is caused by the fact that the feed is not optimally absorbed by the fish's body and is instead eliminated in the feces (Arief et al., 2016).

Feed efficiencies

The average value of feed efficiency of *Anguilla bicolor* fed with the addition of the enzyme bromealin for fifty days had a significant (P<0.05) effect on the feed efficiency. According to the analysis of variance, there is a significant difference between P0 and P2 and between P3 and P4. There was however no significant difference between P1 and P0, P2, P3, and P4 (Table 1). P4 had the highest feed efficiency (50.9 ±4.17%), followed by P3 and P2 (44.6 ±7.5% and 42.98 ±10.57%, respectively). Growth rate is

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correlated positively with feed efficiency. Bromelain enzymes can degrade proteins into peptides and amino acids (Taqwdasbriliani et al., 2013).

The best feed efficiency in this study was comparable to Sagita et al., (2017) which was 59.12%, which applied the papain enzyme. Protein hydrolysis by endogenous enzymes and exogenous enzymes (bromealin and papain enzymes) can help generate more amino acids (Taqwdasbriliani et al., 2013). Through the Krebs cycle in mitochondrial cell organelles, amino acids are converted into energy. Thus, the energy requirements for maintenance and body function are met, resulting in an increase in fish growth (Harahap et al., 2019).

Quality of water

In this study, the water quality remained optimal for cultivating *Anguilla bicolor*. Temperatures range from 28 to 30 °C, pH ranges from 7 to 8, the dissolved oxygen in this study in range 4 and 6 ppm, and ammonia levels are between 0 and 1.5 ppm. Inadequate water quality disrupts the physiology of fish and affects their health (Demeke and Tassew, 2016). According to Sagita et al., (2017), the ideal water temperature for *Anguilla bicolor* is between 28 and 30 degrees Celsius. Temperatures between 10 and 11 ºC are lethal, 16 and 17 ºC reduce fish appetite, and temperatures below 21 ˚C hasten the spread of disease. In contrast, after forty days, the pH of the water ranges between 7 and 8. This value falls within the acceptable range for eel survival (Yudiarto et al., 2012). When the pH is too low, metals dissolve readily, resulting in water that is toxic. In contrast, a pH that is too high can lead to elevated ammonia levels (Tatangindatu et al., 2013).

In this study, dissolved oxygen was between 4 and 6 ppm, which is the optimal level for eel survival (Henditama et al., 2015). This study revealed that the concentration of ammonia was greater than the standard range of 0 - 1.5 ppm. This is likely attributable to a rise in eelfish metabolic results. The increase in ammonia is a result of metabolic waste products and fish growth (Verawati, 2017). While fish can tolerate excessive ammonia through excretion, excessive ammonia causes excretion disorders and death (Sinha et al., 2012).

CONCLUSION

The addition of bromelain enzymes to eel feed can boost growth rate, specific growth rate, fcr, and feed efficiency. The dose of bromelain enzyme was linear with fish growth, although there was no significant effect between treatments.

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