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Addition of Ecoenzymes to Artificial Feed Formulations to Increase Survival Rate, Growth And Feed Efficiency of Climbing Perch Fish (*Anabas testudineus* Bloch)

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Abstract
Climbing perch fish (*Anabas testudineus* Bloch) is a high-value species in Indonesia, but its cultivation faces challenges like low survival rate and growth in early stages. Ecoenzymes, fermented organic solutions rich in amylase, protease, and lipase, offer potential to enhance feed efficiency. This study evaluated the effects of ecoenzyme-supplemented feed (0–15 ml/kg) on climbing perch fish growth, survival rate, and feed conversion ratio (FCR). A 30-day trial used a Complete Random Design (4 treatments, 3 replicates) with ecoenzyme doses of 0 (control), 5, 10, and 15 ml/kg feed. Parameters included survival rate, relative weight growth, and FCR. The 15 ml/kg dose significantly improved weight growth (229.3%) compared to control (193.7%) ($p < 0.05$), but survival (90–95%) and FCR (1.55–1.60) were unaffected. Ecoenzymes at 15 ml/kg enhance climbing perch fish growth, likely via nutrient digestibility. Future research should explore higher doses (>15 ml/kg), long-term effects, and ecoenzyme's bioactive components for aquaculture sustainability.

INTRODUCTION

Climbing perch fish (*Anabas testudineus* Bloch) is a change in the national name of betok fish based on the Decree of the Minister of Maritime Affairs and Fisheries Number 40/Kepmen.KP/2014 (GB 2014). Climbing perch fish is a highly economical fish and is very popular with the Banjar tribe, especially in South and Central Kalimantan, so the price can reach IDR 60,000.00 to 120,000.00 per kg, depending on the size (Ansyari and Slamat 2023). The habitat of climbing perch fish is in various types of shallow waters such as swamps, rice fields, small rivers, and ditches, and is able to survive in extreme environmental conditions, with low pH and oxygen levels, because it has a breathing apparatus in the form of a labyrinth that is able to take oxygen directly from the air (Nha Khanh et al. 2022; Li et al. 2024). Its spread is very wide, ranging from India, China, Southeast Asia, to Indonesia and parts of Indo-Australia, with a high concentration of populations in South Kalimantan and its surroundings (Dasuki et al. 2023). Climbing perch fish in Indonesia (especially in South Kalimantan) is an endemic freshwater fish in Indonesia, with high ecological, economic, and social value. However, its population in nature faces serious threats, as swamp waters as its habitat are increasingly degraded due to land conversion and environmental pollution, resulting in a significant population decline (Anabas and Di 2024)

The development of climbing perch fish cultivation has been carried out for a long time, but many obstacles are faced, including the low survival rate during the larval phase to the seed size (1 - 3 cm). Various studies on the reproductive aspects and management of Climbing Perch fish hatcheries have been carried out, including on reproductive aspects in their natural habitat (Mote et al, 2022). improvement of the quality of climbing perch fish broodstock through selection in Filial-2 offspring (Ansyari and Slamat 2017). and comparison of the growth of Filial-2 climbing perch fish seeds with natural seeds (Ansyari

and Slamat 2017). Gonadotropin hormone dosage for gonadal maturity (Priyatha and Chitra 2022), and vitamin C supplementation in climbing perch fish broodstock feed (Ansyari and Fatmawati 2022; M. et al. 2023). Another research is on the embryogenesis of climbing perch fish (Ansyari and Fatmawati 2022), and increased egg hatchability with a funnel system (Ansyari and Slamat 2022). However, there are still many problems, including the problem of high mortality of climbing perch fish seeds.

There are two causes of the high mortality of papuyu fish seeds, namely feed problems and media water quality management. Feed problems are a crucial problem in larval stadia to white-sized seeds (1-3 cm), because this stadia is a critical period and is greatly influenced by food and environmental compatibility. Food must be the right amount, on time, the right nutrition and the right mouth opening. Climbing perch fish breeders in South Kalimantan mostly use artificial feed in the form of flour to crumbles to feed larvae to seeds. This is because to feed them naturally, they are considered too troublesome. However, the provision of artificial feed in the size of crumbs still does not provide a solution for the survival and growth of climbing perch fish seeds, because it is still not optimal.

Based on the description above, it is very important to research on the addition of enzymes to the artificial feed. In this study, the addition of ecoenzymes, which is the result of fermentation of organic waste, which contains the activity of enzymes amylase, proteases and lipases added to artificial feed in the form of flour to be given to climbing perch fish seeds that are still white (1-3 cm) in size. Ecoenzymes are complex organic solutions that can be used as an alternative to environmental management (Santosa et al, 2023) and for artificial feed supplements for fish (Purba et al, 2022). The method of making ecoenzymes is similar to composting, but there is a difference when the ecoenzyme is added to water, molasses, which has a formulation of 10:3:1, requires a fermentation time of at least three months (Sarkar et al. 2006).

Based on this scientific information, research was conducted on the use of ecoenzymes, as a supplement to artificial feed formulated in the form of flour, and tested on climbing perch fish seeds to find out if there was an increase in survival and growth. The current study presents a novel application of ecoenzymes—derived from organic waste fermentation—in artificial feed formulations for climbing perch fish (*Anabas testudineus*), a high-value species in South Kalimantan facing survival and growth challenges. Unlike prior research on ecoenzymes in tilapia (*Oreochromis niloticus*) (Fernanda and Hariani 2021; Natsir et al. 2024) and catfish (*Clarias gariepinus*) (Bambang et al. 2024), this study uniquely (1) optimizes ecoenzyme doses (5–15 ml/kg feed) for papuyu fish, showing significant weight growth improvement (229.3% at 15 ml/kg) without impacting survival or FCR; (2) targets larval/seed stages (1–3 cm), a critical high-mortality phase, while earlier studies focused on juveniles/adults (e.g., Purba et al. 2022 tested 40–60 ml/kg in tilapia); (3) employs a self-formulated feed with uniform protein (30%) to isolate ecoenzyme effects, unlike hybrid feeds (Simanjuntak et al. 2022); and (4) emphasizes nutrient digestibility over water quality (Kusumawati et al. 2018), suggesting direct metabolic benefits for climbing perch fish.

RESEARCH METHOD

The research was conducted for 30 days from July to September 2024, located in the fish farming area of the "Rawa Sejahtera" Fish Cultivation Group, Malang River, Amuntai, North Hulu Sungai Regency, South Kalimantan. The equipment and materials used include: aquarium measuring 60 cm x 40 cm x 40 cm (filled with three-quarters of water), digital scales, rulers with millimeter blocks, stationery, label paper, mobile phones, aerators, small buckets, thermometers, pH meters, DO meters, ammonia test kits, scrapers, basins, scissors, sprayers, water hoses. The materials used are papuyu fish seeds in size 2 – 3 cm, ecoenzyme liquid, water, fish salt, and potassium permanganate. The stocking density of climbing perch

fish seeds in this study is 20 fish/aquarium or 20 fish/0.072 m3, feeding 5% of body weight (BB) and frequency of feeding 3 times a day.

The study was experimental using a Complete Random Design (RAL) with 4 treatments and 3 replications. The study treatment was the addition of ecoenzymes with different doses in the feed formulation, namely treatment O = artificial feed without ecoenzymes (control); treatment A = artificial feed with ecoenzyme 5 mL/kg; B = ecoenzyme 10 mL/kg and D = ecoenzyme 15 mL/kg. The ecoenzymes are mixed in a self-made feed with a formulation calculated according to the Pearson's Square method and the results are as presented in the following Table 1:

Table 1. Artificial feed formulation for climbing perch fish with the addition of ecoenzymes

No.	Material	Feed formulation (%)			
		O	A	B	C
1.	Trash fish meal	46.34	46.34	46.34	46.34
2.	Soybean flour	16.41	16.41	16.41	16.41
3.	Fine bran	23.63	23.63	23.63	23.63
4.	Coconut meal	11.82	11.82	11.82	11.82
6.	Vitamin mix	1.00	1.00	1.00	1.00
7.	Mineral mix	1.00	1.00	1.00	1.00
8	Total	100.00	100.00	100.00	100.00
9.	Addition of ecoenzymes	0 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg
9.	Estimated protein levels	30.00	30.00	30.00	30.00

The parameters observed and measured in this study are:

1. Survival Rate, which is the percentage of fish that live at the end of the maintenance compared to the beginning of the rearing, with the equation:

$$SR = Nt / No \times 100\%$$

Information:

SR : Sintasan (*Survival Rate*) (%)
Nt : Number of larvae that lived at the end of the study (tail)
No : Number of larvae that lived at the beginning of the study (tail)

2. Relative Weight Growth, which is the percentage of fish population weight at the end of maintenance compared to the beginning of rearing, with the equation:

$$GR\ (\%) = \frac{Wt - Wo}{Wo} \times 100\%$$

Information:

GR : Relative weight growth (%)
Wt : Average weight at the end of the study (g)
Wo : Average weight of the initial study (g)

3. Feed Efficiency, which is calculated by the Feed Conversion Ratio parameter, which is the ratio of the increase in weight of the fish population during the maintenance period compared to the amount of feed weight given (Parker, 2012), with the equation:

$$FCR = \frac{F}{Wt-Wo}$$

Information:
FCR : Feed Conversion Ratio (*Feed Conversion Ratio)*
F : total amount of feed (g)
Wo : initial weight of fish population (g)
Wt : final weight of fish population (g)

The data obtained is presented in the form of tables and graphs and analyzed statistically. The data obtained was then searched on average using the help of *Microsoft Excel* after that it was tested with the normality test *of liliefors,* the homogeneity test using *the Bartlett procedure*, after the assumption was met, variance analysis (ANAVA) was carried out, then continued with a follow-up test, namely the Duncan Double Area Test.

RESULTS AND DISCUSSION

Survival Rate

The results of observations on survival parameters during the 30-day maintenance period of climbing perch fish in the aquarium with the provision of artificial feed that is self-formulated and added to various doses of ecoenzymes are presented in the following Table 2:

Table 2. The Average Survival Rate of Climbing Perch Fish in During 30 Days Rearing

Treatment	Number of fish (individual) on the day				Survival Rate (%)
	0	10	20	30	
O	20.0	18.7	18.3	18.0	90.0
A	20.0	19.7	19.3	18.7	93.5
B	20.0	20.0	19.3	18.7	93.5
C	20.0	19.7	19.3	19.0	95.0

The results of the analysis of the diversity of fish survival data above showed no significant differences between treatments (p > 5%). This means that all treatments have the same effect on the survival of climbing perch fish by giving artificial feed with ecoenzymes added at different doses and maintained for 30 days. The survival of climbing perch fish seeds shows an average yield that ranges from 90.0 - 95.0%. In tilapia research (*Oreochromis niloticus*), an ecoenzyme dose of 35 ml/kg, increased survival rate up to 86.67% higher than without ecoenzymes (Fernanda and Hariani 2021; Natsir et al, 2024;). Then to catfish (*Clarias gariepinus*), with the addition of ecoenzymes at a dose of 60 ml/kg is able to maintain survival above 90% (Bambang et al, 2024).

The results of papuyu fish survival in this study are relatively high and show that climbing perch fish seeds can survive with their environment. According to Natsir et al, (2024), ecoenzymes contain lipase, protease, and amylase enzymes that help ensure they are free of feed residues and organic waste, resulting in optimal oxygen quality and improved fish health. The results of the data analysis showed that there was no difference between ammonia and nitrite yields at different ecoenzyme concentrations and this was beneficial for the fish farming process (Kusumawati et al, 2018). In addition, ecoenzymes help suppress pathogenic bacteria, as they are natural disinfectants, thereby increasing fish survival rate, as they reduce the risk of death from disease (Suryani et al. 2022)

Relative Weight Growth

The results of measurements of relative weight growth during the 30-day maintenance period are presented in Table 3 below:

Table 3. Initial and Final of population weight, weight gain and relative weight growth (%) of climbing perch fish during 30-day rearing

Treatment	Initial Population Weight (gram)	Final Population Weight (gram)	Population Weight Gain (gram)	Relative Weight Growth (%)
O	28.4	83.4	55.0	193.7
A	28.5	87.1	58.6	205.6
B	28.7	91.4	62.7	218.5
C	28.7	94.5	65.8	229.3

The results of the analysis of the diversity of relative weight growth data showed a real difference between the treatments ($p < 5\%$) and after a follow-up test in the form of the Duncan Double Region test, it was found that treatment O was significantly different from treatment A, B and C, and treatment A was significantly different from B and C, and treatment B was also significantly different from C ($p < 5\%$). Based on the results of this diversity analysis, it is shown that the addition of ecoenzymes has an effect on increasing weight growth and is best in treatment C, which is a dose of 15 mL/kg of feed. This allows the dose of ecoenzymes to be added again to more than 15 mL/kg of feed to obtain optimal results. This is in accordance with the results of the research Cahyadi et al. (2020), the addition of ecoenzymes used as supplements to feed can increase growth in catfish. Then The best ecoenzyme optimization dose in aquaponic system tilapia cultivation using bucket media is 15 ml/L (Sikku et al, 2023).

The application of ecoenzymes to artificial feed can increase fish growth by the digestibility of nutrients, which helps in the breakdown of food components (carbohydrates, proteins, and fats), thereby increasing absorption (Natsir, 2024; Simanjuntak et al. 2022). The addition of ecoenzymes with a dose of up to 2 g/kg of feed can increase the length of the intestinal villi, but it has not been able to increase fish growth (Putra et al. 2020). In the treatment of artificial feed with enzymes added, it can increase the total weight and length by 6.25% and 13.4% compared to without ecoenzymes (Cahyadi et al. 2020). Furthermore, research Purba et al, (2022) obtaining the addition of 40 mL/kg of feed ecoenzymes had the best effect on relative growth and feed conversion ratio in tilapia fish, but survival was highest at the addition of 60 mL/kg of feed ecoenzymes. The following is a graph (Figure 1) illustrating the relative weight growth from day 0 to day 30 in maintenance with an artificial feed formulation with ecoenzymes added.

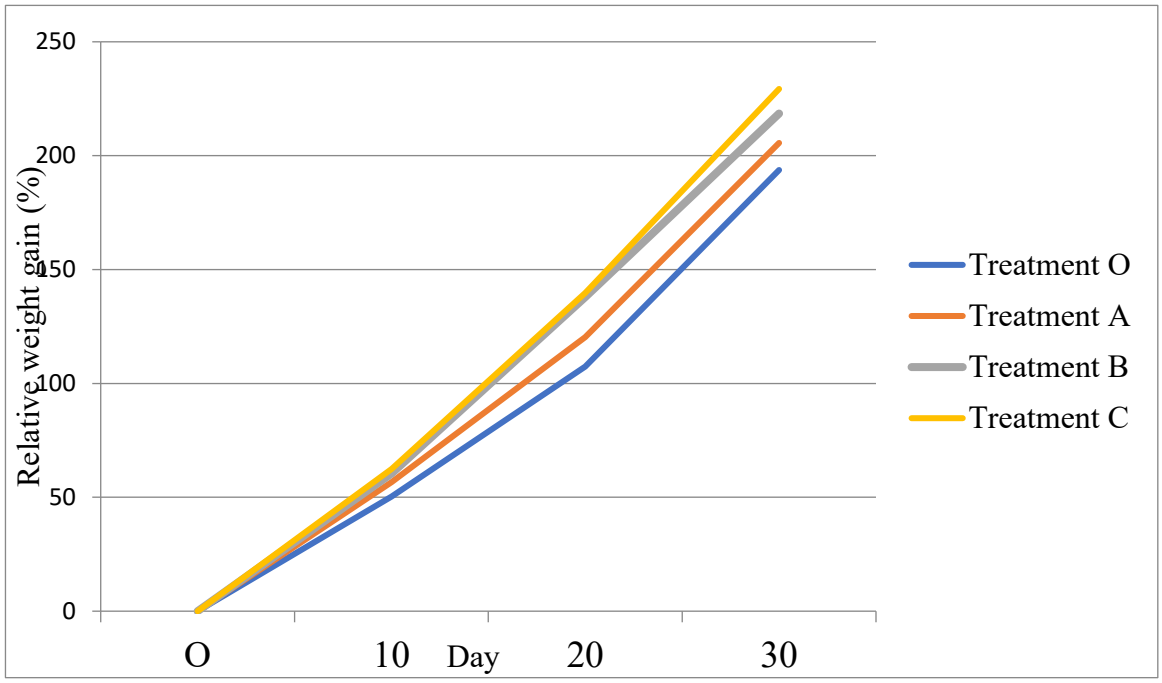

Figure 1. Relative weight growth of climbing perch fish during 30-day maintenance

Based on the results of the study, the highest relative weight growth was 229.3% in treatment C (ecoenzyme dose of 10 mL/kg feed). The high growth is suspected to be due to the contribution of ecoenzymes that are able to improve the digestive process (Liang et al. 2022). The addition of enzyme composition to artificial feed formulations can improve the performance of catfish larvae and intestinal structural enzymes, increasing weight by 6.25% and length by 13% compared to feed without enzymes (Cahyadi et al. 2020). Ecoenzymes in the form of prebiotics, probiotics and synbiotics in some fish species are able to improve and increase survival and growth, such as in rainbow trout (Diken et al, 2021), catfish (Simanjuntak et al. 2022) and catfish (Putra et al. 2020) and tilapia (Fernanda and Hariani 2021).

Feed Efficiency

The feed efficiency in this study is illustrated through the *Feed Conversion Ratio (FCR*), which is the ratio of the weight of feed given during maintenance compared to fish meat production. The results of the calculation of feed conversion ratio in this study are presented in Table 4. The following:

Table 4. Feed Conversion Ratio of Climbing Perch Fish Feed during 30 days rearing

Treatment	Amount of feed during rearing (gram)	Population Weight Gain (gram)	Feed Conversion Ratio
O	86.90	55.00	1.58
A	93.76	58.60	1.60
B	97.81	62.70	1.55
C	102.65	65.80	1.56

The results of the analysis of the diversity of Feed Conversion Ratio (FCR) data above shows an intangible (insignificant) difference between treatments (p > 5%). All treatments had the same effect on the conversion ratio of climbing perch fish feed with artificial feed with ecoenzymes added at different doses and maintained for 30 days. It is suspected that in this study, the addition of ecoenzymes to artificial feed formulations only increases appetite, so that with more ecoenzymes the dosage causes the growth of climbing perch fish to increase, but feed conversion or feed efficiency is the same as artificial feed that does not add ecoenzymes. In essence, enzyme supplementation in fish feed can improve digestion and nutrient absorption and growth of farmed fish (Liang et al. 2022).

The results of the feed conversion ratio in Table 3 above show a range of 1.55 – 1.60. This is higher than the research Bugar et al, (2018) who raise climbing perch fish with a biofloc system and get a lower (more efficient) feed conversion ratio, which is 1.4 – 1.5. Furthermore, research Syarifudin et al. (2023) who raised climbing perch fish seeds with a small-scale aquaponic system with the Nutrient Film (NFT) Technique obtained a feed conversion ratio of 1.31. However, research Khatune-Jannat et al. (2012) Those who researched the rearing of climbing perch fish in open waters got a high, less efficient feed conversion ratio, which was 1.95, while Uddin et al. (2016) with a high stocking density of 100 heads/m^3 get a conversion score of 2.65 – 2.93 and (Ansyari and Slamat 2020) Those who keep in open swamp waters get a feed conversion ratio of 2.62 – 3.03. From some of these data, it can be concluded that the maintenance of papuyu fish in closed waters with low stocking density will get a feed conversion ratio of 1.3 – 2.0, but on the other hand, in open waters and high stocking density, the feed conversion ratio is > 2.0.

CONCLUSION

The study found that adding ecoenzymes to climbing perch fish feed for 30 days significantly improved weight growth (optimal dose: 15 ml/kg) but did not affect survival or feed conversion ratio (FCR). Future research should investigate higher doses (>15 ml/kg) to optimize growth performance, extend rearing periods (60–90 days) to assess long-term effects, and explore mechanisms like nutrient digestibility, gut microbiota, and metabolic responses. Additionally, analyzing ecoenzyme composition and conducting economic viability assessments would clarify its practical benefits for aquaculture. This deeper investigation could enhance understanding and application of ecoenzymes in fish farming.

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