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Eco-Enzyme Supplementation in the Fish Commercial Feed on Growth Performance of Nile Tilapia *(Oreochromis niloticus)*

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Abstract **Original Research Article**

Nile tilapia (Oreochromis niloticus) is one of the aquaculture commodities in Indonesia. One of the challenge of fish production is the high cost of the feed. Thus, the efficiency of fish feed should be improved by adding supplementation that could promotes fish growth and health. This study aimed to to investigate the effects of eco-enzyme supplementation into commercial feed on growth, feed utilization and survival rate in O. niloticus. The fish juvenile which used in this research was 4.95±0.95 g which obtained from the local fisheries in Yogyakarta. The research done using completely randomized design (CRD), four treatments, and three replications. The treatments were different eco-enzyme supplementation in commercial fish feeds: A: control/commercial feed, B: commercial feed supplemented with 20mL eco-enzyme, C: commercial feed supplemented with 40mL eco-enzyme, and D: commercial feed supplemented with 60mL eco-enzyme. Relative growth rate, food conversion ratio, feed efficiency, protein efficiency ration, and survival rate were used as parameters tested. The results showed that feed supplemented with 40ml eco-enzyme gave best effect of relative growth rate (RGR), feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency rate (PER) of fingerlings tilapia fish. While highest survival rate (SR) were obtained by the addition of 60 ml of eco-enzyme.

Keywords*:* Oreochromis niloticus, fish feed, eco-enzyme, fruit and vegetable waste, supplementation, growth.

INTRODUCTION

Aquaculture production is a large industry in Indonesia. In 2019, Indonesia produces 6 million tonnes of aquaculture products (excluding aquatic plants and non-food product) which count as top three producers (FAO 2021). One of source of the aquaculture commodities is nile tilapia (*Oreochromis niloticus*) farming. Tilapia farming is technically and economically more sustanaible because of its profit. However, sustainable aquaculture requires high quality of feeds which should be nutritious to promote fish growth and health. In fresh-water fish farming, commercial feeds significantly contributes to more than half of production cost (Rana *et al*., 2009). Thus, it is crucial to enhance the feed efficiency and growth performance in cultured fish. Low feed efficiency may be solved by feed supplementation using catalytic enzimes. This may solved the digestion problem of fish, since many of fish could not digest fibre while feeds contain high fibre content that decreased the nutrient utilitazion up to 10% (Halver 2002).

The supplementation of catalytic enzymes from rumen fluid of sheep into fish feed could improve growth performance in tilapia (Suprayudi *et al*., 2011). This enzyme has cellulases and hemicellulase activity that could hydrolyzed crude fibre to provide more nutrient availability (Agarwal 2003). In the other hand, there is another source of catalytic enzyme that generated from food waste fermentation. Eco-enzyme, also known as garbage enzyme, is produced by the fermentation of fresh fruit and vegetable wastes such as stalks and peels as substrate with sugar (Vama & Cherekar 2020). This enzyme was invented by Dr. Rhosukon from Thailand in 2006. This enzyme characterized as a dark brown liquid product with pungent vinegary smell (Hemalatha & Visanthini 2020). Previously, eco-enzyme was used as waste water and sludge treatment as well as biopesticide due its enzymatic and antimicrobial activity (Arifin 2009, Hemalatha & Visanthini 2020, Galintin *et al*., 2021). In addition, eco-enzyme also has thought as multipurpose solution for plant growth and comestic cleaning (Tang

& Tong 2011). However, there are few study that explore the potency of eco-enzyme for promoting animal growth and health. Thus, in this study we aimed to investigate the effects of eco-enzyme supplementation into commercial feed on growth, feed utilization and survival rate in *O. niloticus* fingerlings.

MATERIALS AND METHODS

Eco-enzyme production

To produce eco-enzyme, we collected fruit and vegetable waste (fvw) from local vegetable and fruit store, while we purchased the coconut brown sugar in the same store. The water used were normal tap water. The waste washed with running water to removes the soil and dirt. The fvw then shreded into small pieces to increase the surface are for chemical reaction. Furthermore, the sugar was mixed with water and was stirred until it completely dissolve. The ratio for components used for eco-enzyme was 1:3:10 stands as sugar, fvw, and water, as shown in Table 1. The mixtures were incubated in airtight plastic container to promote simple fermentation process. The fermentation process produced gases thus the container should have around 20% space left empty to prevent explosion (Arifin *et al*., 2009, Vama *et al*., 2020). After 90 days of fermentation, the remaining solid materials were separated from the liquid by filtration. Filtered eco-enzyme solution was placed in a closed container and were characterized by the measurement of pH as well as quantification of acetic acid and alcohol content.

Table 1: Composition of ingredients for making eco-enzyme

No.	Component	Ratio	Weight/Volume
1	Coconut brown sugar	1	100g
2	Pineapple (*Ananas comosus*): peels	3	100g
	Chinese flowering cabbage (*Brassica rapa* var. parachinensis): leaves and stalks		100g
	Lettuce (*Lactuca sativa*): leaves and stalks		100g
3	Water	1	1000mL

Fish feed preparation

Commercial feeds were purchased from the local fish farm in Yogyakarta, Indonesia. We prepared four types of feed which treated with different amount of eco-enzyme. First is control (A) which have no eco-enzyme addition. Then B, C, and D with 20, 40, and 60mL of eco-enzyme addition per kg of fish feed. The mixture then fermented for 24 hours before used for feeding the fishes. The feeds supplemented eco-enzyme addition were analysed for proximate composition, including water, ash, protein, lipid, and carbohydrate in the laboratory of Food and Nutrition Center Studies, Gadjah Mada Univeristy, Yogyakarta, Indonesia.

Experimental procedure

Twelve (12) aquariums (25-litre) each filled with 17-litre water were aerated continuously using air compressor. We acclimated 60 individuals of nila fish (*O. niloticus*) from local fisheries in Yogyakarta, Indonesia to laboratory condition before distributed randomly to the 12 aquariums representing four types of dietary treatments (A: control/commercial feed, B: commercial feed supplemented with 20mL eco-enzyme, C: commercial feed supplemented with 40mL eco-enzyme, and D: commercial feed supplemented with 60mL eco-enzyme). Each treatment has three time replications. In each aquarium, we placed five fish. Fish were fed two times per day at 5% of their body weight. All fish were weighed every three day and the amount of feed were adjusted to the latest weight. The fish were kept for 21 days, and we measured their growth and feed utilization by calculating several parameters.

1. Relative growth rate (RGR) (Tacon 1995)
   RGR = (Wt-W0)/W0 x (t1-t0) x 100%
   Wt = final body weights of fish (g)
   W0 = initial body weights of fish (g)
   t1-t0 = duration of the experiment
2. Food conversion ratio (FCR) (Dada 2015)
   FCR = dry weight of feed (g) / weight gain of fish (g)
3. Protein efficiency ratio (PER) (Rachmawati et al.2019)
   PER = (Wt-W0)/P x 100%
   Wt-W0 = wet weight gain by fish
   P = protein (%) in feed x total diet weight (g) of feed consumed /100
4. Food Efficiency (Tacon 1995)
   FE= weight gain of fish (g)/amount of feed consumed during experiment (g)
5. Survival rate (SR) (Biswas et al. 2011)
   SR = (Nt-N0) x 100%.
   Nt = Number of fish harvested
   N0 = number of fish stocked

All data means of each parameter were analysed using ANOVA at 95% significance level to test significant differences between the various feed treatments.

RESULTS AND DISCUSSION

Eco-enzyme was produced using pineapple, Chinese cabbage and lettuce wastes by three months fermentation. The product has similar pH value, about 3.5, with previous study (Muliarta & Darmawan 2021, Galintin *et al*., 2021). The lower pH indicated higher organic acid such as acetic acid. In addition, the eco-enzyme contained several biocatalytic enzyme such as lipase, amylase, and protease (Galintin *et al*., 2021). One of the components of our eco-enzyme is the peel of pineapple which may possessed high amount of holocellulose (80%) which can produce cellulases and hemicellulases (Sarvanan *et al*., 2013). Previously, biological conversion of food and vegetable wastes into a value-added product as eco-enzyme have been major of interests. Most of eco-enzyme products were used as fertilizer (Arifin *et al*., 2009, Vama *et al*., 2020, Hemalantha & Visantini 2020, Hasanah 2020), aquaculture sludge treatment (Rasit *et al*., 2019, Galintin *et al*., 2021), disinfectant (Hasanah 2020) and even have potential antibacterial activity for endodontic treatment (Mavani *et al*., 2020). However, the potency of eco-enzyme for promoting animal growth still not explored yet. Previous study showed that supplementation of crude enzyme from rumen fluid into commercial fish feed have significant effect on feed efficiency in nile tilapia (Suprayudi *et al*., 2011). Thus, in this experiment, we used the eco-enzyme from fruit and vegetable wastes as to supplemented commercial feed for promoting fish growth and feed utilization.

The nutritional contents of the control (commercial feed) and eco-enzyme supplemented fish feed were tested by proximate analysis for its water, ash, fat, protein and carbohydrates percentage. The results were obtained for commercial feed (control) and feed supplemented with eco-enzyme (Table 2). There are little differences in nutritional content of supplemented fish feed compared to the control. However, usually the enzyme especially cellulase would help to hydrolyzed cellulose and hemicellulose in the fish food diet. Thus further analysis in crude fibre content should be performed for better understanding the effect of eco-enzyme in fish feed improvement.

Table 2: Proximate composition (% dry matter) of experimental fish diet

	Water	Ash	Fat	Protein	Carbohydrate
A: Commercial fish feed (0ml/kg)	11.94	8.205	3.79	26.86	49.21
B commercial feed supplemented with 20mL eco-enzyme/kg	12.555	8.15	3.79	31.28	49.225
C: commercial feed supplemented with 40mL eco-enzyme/kg	12.485	8.29	3.515	26.635	49.075
D: commercial feed supplemented with 60mL eco-enzyme/kg	13.075	8.21	3.84	26.795	48.075

Experimental diet of tilapia fish shown that feed supplemented with 40ml eco-enzyme gave highest value of relative growth rate (RGR), feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency rate (PER) of fingerlings tilapia fish. The highest RGR value (6,71%/day in C treatment exhibited that the energy from feed is more than enough therefore could be used for promoting growth. The relative growth might also increase by the help of some microbes from eco-enzyme supplementation (Effendi 1997). Furthermore, the smallest value in FCR, though it is not significantly different with the control. This indicated the highest improvement for feed use efficiency. This support the result of FE where 40 ml eco-enzyme supplementation have gained the efficiency of feed utilization by fish. The high food efficiency indicated fish need less amount of feed to gain their body weight because most of the energy from feed could promote fish growth (Marzuqi *et al.*, 2012). The higher FE value also represent the higher quality of feed that could promote fish growth (Setiawati *et al*., 2003). Most of fish feed were made from plant material thus it might contain high fibre. The cellulases in eco-enzyme would help to hydrolyzed cellulose from commercial feed. Accordingly, fish could obtain more energy from more simple carbohydrate. Hereinafter, the improvement of PER shown by the highest value on treatment C reflected that 40ml eco-enzyme could enhance nutrient utilization that important for protein absorption in fish (Rachmawati *et al*., 2019).

The survival in *O. niloticus* were improved by the supplementation of eco-enzyme into their diet. However, the highest survival rate (SR) were obtained by the addition of 60 ml of eco-enzyme. Similar trends obtained by the research of feed supplementation using rumen crude enzyme, the highest concentration gave highest support for fish survival rate compared to the commercial feed (Suprayudi *et al*., 2011). Future research should focus on the identificat of rearing technologies for different species of fish reared using feed additive as a feed supplement.

Table 3: Mean growth performance and feed utilization of *O. niloticus* fingerlings fed experimental fish diets

Parameter	A (0)	B (20ml/kg)	C (40ml/kg)	D (60ml/kg)
Initial average weight (g)	4,90 (0,42)	4,60 (0,28)	4,50 (0,14)	5,60 (0,00)
Final average weight (g)	9,50 (2,12)	9,25 (0,35)	10,50 (2,12)	9,65 (0,49)
Final average weight gain (g)	4,60 (1,70)	4,65 (0,64)	6,00 (2,26)	4,05 (0,49)
RGR (%/day)	4,51 (1,33)	5,09 (1,00)	6,71 (2,73)	3,62 (0,44)
FCR	1,48 (0,67)	1,53 (0,24)	1,14 (0,37)	2,65 (0,45)

Parameter	A (0)	B (20ml/kg)	C (40ml/kg)	D (60ml/kg)
PER	1,96 (0,08)	2,12 (0,33)	3,48 (1,14)	1,43 (0,24)
FE	0,75 (0,34)	0,66 (0,10)	0,93 (0,30)	0,38 (0,06)
SR (%)	20	40	33,33	60

Note: In all parameters, the means of supplemented feed were not significantly different at $p<0.05$ with the control. Values in parentheses are standard errors of means.

CONCLUSION

The eco-enzyme from fruit and vegetable wastes would added the value of food waste by its conversion into feed supplement. The supplementation of 40ml eco-enzyme per kg feed functions as growth promoter in *O. niloticus* fingerlings.

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