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Combination of papain enzyme and phytase enzyme in commercial feed and the protein and energy retention of tilapia *Oreochromis niloticus*

A Saifulloh[1], M B Santanumurti[2], M Lamid[2,3]* and W P Lokapirnasari[3]

[1] Undergraduate Program of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115
[2] Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115
[3] Faculty of Animal Husbandry Airlangga University, Campus C Mulyorejo – Surabaya

*Corresponding author: mirnylamid@fkh.unair.ac.id

Abstract. The nile tilapia (*Oreochromis niloticus*), is one of the important economic commodities in the fishery sector. In the process of the cultivation of the Nile Tilapia, there are many problems faced by cultivators; one of them is the expensive cost of the feed. An alternative to overcome this problem is by adding papain and fitase enzymes. This study aims to determine the effect and optimum dose of a combination of papain and fitase enzymes on the protein and energy retention of Nile Tilapia. This study used a Completely Randomized Design (RAL) with 4 treatments and 5 replications. The treatments used were P0 (control), P1 (papain 1% and fitase 0.05%), P2 (papain 3% and 0.1% fitase enzyme) and P3 (papain 5% and fitase 0.15%). The data was analyzed using ANOVA and continued with Duncan's multiple range test. The results of this study showed that the combination of papain and fitase enzymes in commercial feed affected the protein retention but did not affect the energy retention. A dose of 3% enzyme papain and 0.1% fitase enzyme (P2) was the optimum dose needed to increase the protein retention of Nile Tilapia fish.

1. Introduction

Nile tilapia (*Oreochromis niloticus)* is one of the more important economic commodities in the fishery sector. Every year, the production of Nile tilapia increases significantly. Based on the data collected by the Ministry of Marine Affairs and Fisheries in 2015, the production of Nile tilapia reached 63 million tons and this production is predicted to increase yearly. A solution is needed to improve the efficiency and productivity of feeding, so then the nutrients contained in the feed can be absorbed optimally and used by the fish for growth [1]. An effort that can be undertaken to optimize the utilization of the nutrient feed by Nile tilapia is by improving the quality of the feed itself by adding exogenous enzymes.

Nile tilapia require feed with particular nutrient content (protein, carbohydrate, and fat) that suits their needs related to supporting their bodily maintenance and growth. An effort that can be undertaken to maximize the absorption of the feeds protein is by adding the enzyme papain. The addition of the papain enzyme to feed can increase protein deposition into the proteolytic body, which means that the fish will be able to hydrolyze protein complex compounds into simple amino acids that

can be easily digested [2]. The addition of papain to commercial feed can improve protein retention, feed efficiency and the daily growth rate of carp [3].

Another obstacle in the absorption of nutrient feed is the presence of anti-nutritional substances in vegetable food substances called phytic acid. The phytase enzyme is one of the exogenous enzymes that is expected to decompose the anti-nutrients, especially phytic acid, to improve the efficiency of feed utilization and growth [4]. Phytase addition increases the concentration of minerals like magnesium, phosphorus, calcium, manganese and zinc in plasma, bone and the whole body [5].

Based on the description above, it is expected that the combination of these two enzymes can be complementary. The papain enzyme has the ability to break down proteins into simple elements, so then the absorption rate of the feed proteins will improve, while the fitase enzyme has the ability to hydrolyze the phytic acid contained in the feed ingredients.

The purpose of this study was to investigate the effect of the combination of the papain enzyme and the fitase enzyme to commercial feed related to the protein and energy retention of Nile tilapia *O. niloticus* and to determine the exact combination dose required for optimal results.

2. Materials and methods

2.1. Materials

The equipment required in this research included 20 aquariums measuring 40 cm x 30 cm x 30 cm, a tandon for the freshwater stock, a shelter, measuring cup, basin, aeration hose, aeration stone, strainer, ruler, seser, spray bottle, spoon, paper labels, thermometers, pH, DO meters and analytical scales.

The materials needed in this study were the seeds of Nile tilapia fish (*Oreochromisniloticus*) measuring from 7 - 10 cm with a body weight between 7 - 13 grams of 200 tails. The feed used was pellet-shaped commercial food under the "HI-PRO-VIT" brand. The feed treatment was added to by the papain enzyme from the "NEWZIME" brand from BBPBAP Jepara and the enzyme for fitase from the "SMIZYME" brand from a Chinese company.

2.2. Methods

2.2.1. Preparation of the Nile tilapia's maintenance

The aquariums to be used were cleaned first; they were washed using soap until clean and then washed again with chlorine before being dried for 2-3 days. After the aquariums were clean, the aquariums were filled with water as high as 15 cm from the bottom of the aquarium and then a hose and aeration stone were put inside each aquarium. Prior to the treatment, the Nile tilapia seeds were acclimatized to the temperature and new water condition, and familiarized with the commercial feed used.

The commercial feed used was commercial dried pellet-shaped food. The papain enzyme and fitase enzyme used came in the form of powder. The enzymes were weighed by the % of the weight of the feed according to each treatment. Then, 1% tapioca from the weight of the feed which served as the adhesive was added. The enzymes and tapioca were put into a spray bottle and then mixed with aquades by as much as 10% of the weight of the feed until it was evenly distributed. The commercial feed was placed in a container and then sprayed by the enzyme that had been mixed with distilled water until it was evenly distributed. The feed that had been mixed with the enzyme was then dried for 1 hour. After it was dry, the feed was given.

2.2.2. Parameter of research

The main parameters of this study were growth rate, survival rate, feed conversion ratio, and feed efficiency. The supporting parameters observed were dissolved oxygen, pH, temperature, and ammonia.

2.2.3. Data analysis

The data obtained from the results of this study was analyzed using the ANOVA statistical test in order to determine whether there were differences among the treatments in accordance with the design used, which was a Completely Randomized Design. Duncan's multiple-range test was then performed

with a 5% significance level when it was known that the treatments showed significant differences, in order to know what the best treatment was [6].

3. Results

3.1. Protein and energy retention

The average of the protein retention and energy retention of the Nile Tilapia fish can be seen in Table 1.

Table 1. The average protein and energy retention of the Nile tilapia fish.

Treatment		P0	P1	P2	P3
Protein Retention	Average (%) ± SD	$7,46^{a}$ ±3,3837	$12,69^{b}$ ±2,3777	$17,36^{c}$ ±2,8185	$15,92^{bc}$ ±3,3831
	Transformation (√y+0,5) ±SD	2,758±0,6615	3,619±0,3494	4,221±0,3470	4,035±0,4115
Energy Retention	Average (%) ± SD	9,26±1,7458	10,72±1,7040	11,87±2,0859	12,61±2,9770
	Transformation (√y+0,5) ±SD	3,1148±0,2830	3,3420±0,2563	3,5085±0,2949	3,6031±0,4055

Noted: Different columns of *superscript show significant differences* ($p < 0,05$).

3.2. Water quality

The data of the water quality range value can be seen in Table 2.

Table 2. Water quality range as a maintenance media for 30 days.

Parameters	Range
Temperature (^{0}C)	26-28,9^{0}C
pH	7
Dissolved Oxygen (mg/l)	3,73-5,67 mg/l
Ammonia (mg/l)	0,01

4. Discussion

The highest protein retention rate was found in treatment P2, which showed that the feed that was had a combination of 3% papain enzyme and 0.1% fitase enzyme added to it was the optimal dose among all of the treatments. The combination of 3% papain enzyme and 0.1% fitase enzyme provided more optimal enzyme work than the other treatments. This was because papain has an active SH-group edge that forms a disulfide bond with a cysteine side that has broken or hydrolyzed the amide in amino acid residues such as cysteine, arginine, lysine, glutamine and histidine [7].

The combination of 3% papain enzyme and 0.1% fitase enzyme complemented one another and had a significant effect on feed protein absorption. The fitase enzymes in the feed increased the nutrient uptake, regulated the nutrient excretion (such as phosphor, nitrogen, and minerals) and hydrolyzed the phytic acid (phosphate elemental reserves) in fish feed into inositol and phosphoric acid [8]. With the decomposition of the phytic acid anti-nutrients, the metabolic processes involved, such as breaking down proteins and complex minerals in the body, worked well.

The combination of papain enzyme and fitase enzyme in the commercial feed had no significant effect on the energy retention of the Nile Tilapia fish. The energy produced from the feed was used for metabolic processes and later activity if there was excess energy from that used for growth [9].

Amino acids should be maximized properly by the Nile Tilapia to produce inhibited energy by the presence of phytic acid supplementation in feed containing nabati materials. A previous study [10] showed that phytic acid supplementation greatly impacts the decrease in amino acid digestion by up to 8 %. The phytic acid was able to decrease the energy absorption by binding energy-producing molecules such as carbohydrates, fats and proteins, and reducing the phytic acid carbohydrates by forming bonds with α-amylase, sucrose and maltose [11]. By breaking down the phytic acid bonds, the absorption of the feed nutrients could be maximized, which then affected the energy produced by the Nile tilapia.

5. References

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