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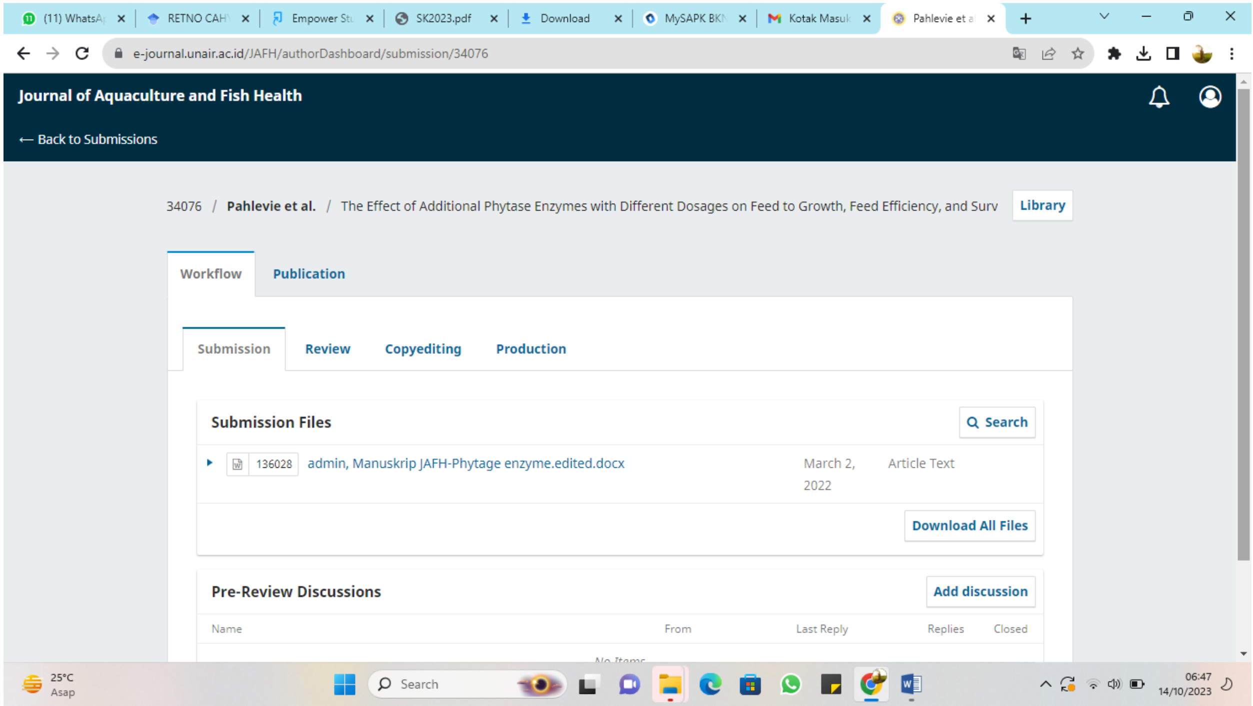

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The Effect of Additional Phytage Enzymes with Different Dosage on Manufactured Feed on the Growth, Efficiency of Feed Utilization and Survival Snakehead Fish (*Channa striata*)

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Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out but is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on growth, protein efficiency ratio, feed efficiency, feed consumption, and survival of snakehead. This research was carried out at the Fisheries Basic Laboratory in July – August 2021, this study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg 100 g^{-1} material). vegetable), P2 (addition of phytase 50 mg 100 g^{-1} vegetable material), P3 (addition of phytase 70 mg 100 g^{-1} vegetable material). The size of the fish used in this study was 3-4 cm with a stocking density of 2 L^{-1} fish, the maintenance of the study was 30 days. The results showed that the addition of phytase 70 mg 100 g^{-1} vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), feed consumption (37.68 g) protein efficiency ratio (2.03%), and survival (100%).

Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead fish *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia, including in Indonesia (War and Altaff, 2011). The availability of snakehead fish is still dominated by natural catches. Currently, snakehead fish can be cultivated with artificial feeding, but several factors cause limitations in cultivation such as low survival and relatively slow growth. This is due to the limited natural feed, high food competition that gives rise to cannibalism, and the limited space for fish to move (Muslim, 2007).

Based on Supandi *et al,* (2015), snakehead fish fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead fish is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead fish has been carried out but is still not optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean flour and bran. Soybean flour is one of the feed ingredients that contain high protein. However, soy flour also contains minerals combined with phytic acid, making it difficult for the body to absorb, as well as bran. According to Cao *et al,* (2007), the content of phytic acid in soybean flour reaches 3.88% or 59.9% of the total phosphorus. While the bran contains about 6.9% phytic acid. Sumiati *et al.* (2001). Phytic acid is the main storage form of phosphorus (P) contained in these vegetable raw materials (Amin *et al.,* 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and also proteins so that minerals and proteins cannot be digested by the fish's body as a whole and result in lower absorption. As a result, the utilization of soybean flour and bran is not optimal.

Phytic acid can be reduced by heating, but heating can damage the nutrients in the feed to be given. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Widowati *et al.*, 2004). Winata *et al,* (2018) stated that the phytase

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enzyme can break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.*, 2019). The addition of phytase enzymes to an artificial feed of 500 mg/kg of feed resulted in feed utilization efficiency of 84.55% in sangkuriang catfish (Kosim *et al*., 2016). The addition of the phytase enzyme 1000 mg/kg of feed was able to suppress the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). The addition of the phytase enzyme 1,200 mg/kg of feed was able to produce a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for larasati tilapia (Restianti *et al*., 2016). Based on the description above, the dose of the phytase enzyme in snakehead fish feed is not yet known. Therefore, it is necessary to research on the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Time and Place

This research was conducted at the Fisheries Basic Laboratory, Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University. Feed proximate analysis was carried out at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Dissolved phosphorus analysis of feed was carried out at the Palembang Industrial Center. This research was conducted in July – August 2021.

Research Materials

The materials used in this study were snakehead fish size 3-4 cm, fish meal, soybean meal, tapioca, bran, fish oil, corn oil, premix, phytase enzyme, potassium permanganate. The tools used in this research are aquarium, pH meter, thermometer, DO meter, spectrophotometer, UV-VIS spectrophotometer, digital scale, centrifuge, measuring cup, blender, sieve, basin, mixer, pellet printer, aerator, and oven.

Research Design

This study used a completely randomized design (CRD) with 4 treatments and 3 replications. The treatment in this study refers to the study of Amin *et al.* (2011) about the addition of phytase enzymes in catfish. The treatments included:

P0: feed not added with phytase enzymes
P1: feed added with phytase enzymes at a dose of 30 mg/100g vegetable ingredients
P2: feed added with phytase enzymes at a dose of 50 mg/100g vegetable ingredients
P3: feed added with phytase enzymes at a dose of 70 mg/100g vegetable ingredients

Feed Formulation

The feed formulations used are presented in Table 1.

Working Procedure

Preparation and Preparation of Test Feed

Prepare all the materials used and the feed raw materials are weighed according to the formulation. Next, the soybean flour and bran are mixed and stirred until homogeneous. The homogeneous mixture of ingredients is added as much as 350 mL $100g^{-1}$ of vegetable material. The material is stirred again until evenly distributed and the phytase enzyme is added according to the treatment dose. Then incubated for 2 h at 37°C (Matsumoto *et al*., 2001) the material mixture was used as the building blocks of feed.

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Making feed is done by mixing all the ingredients starting from the fewest ingredients to the most until all the ingredients are evenly and homogeneously mixed, after the ingredients are well mixed add warm water little by little until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, dried in the sun to dry. After the feed is dry, it is then put into a container and labeled with the treatment dose.

Table 1. The feed formulations used

Description	Treatment (g 1000g^{-1})			
Material (g)	P0	P1	P2	P3
Fish flour	375	375	375	375
Soy flour	402	402	402	402
Tapioca	44	43,84	43,73	43,62
Bran	144	144	144	144
Fish oil	2,5	2,5	2,5	2,5
Corn oil	2,5	2,5	2,5	2,5
Premix	30	30	30	30
Phytase enzyme	0	0,16	0,27	0,38
Jumlah (g)	1000	1000	1000	1000
Nutrient components (%)				
Protein (%)**	37,00	37,64	38,68	39,16
Acid (%)*	2,5	2,5	2,5	2,5
Fat (%)**	2,65	3,40	1,56	1,65
Carbohydrates (%)**	31,69	31,68	30,88	29,86
Ash (%)**	13,85	14,62	14,40	15,42
Water (%)**	7,91	6,84	9,67	9,16
Crude fiber (%)**	6,90	5,83	4,81	4,75

Note: *Phytic acid content: 3.88% soybean flour (Cao *et al*., 2007); bran 6.9% (Sumiati *et al*., 2001).
** Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2021)

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Matsumoto *et al*, (2001), namely vegetable material and the enzyme phytase which was mixed and incubated at 37°C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Preparation of Test Fish Rearing Media

The container used for the maintenance of snakehead fish is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g L^{-1} then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, every In the aquarium, an aeration hose is connected to the aerator and left overnight and then labeled according to treatment (Zainuri *et al*., 2017).

Maintenance

The snakehead fish seeds used were 3-4 cm in size, with a stocking density of 2 L-fish^{1} (Zainuri *et al*., 2017). Fish were acclimatized first for 3 days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method

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at satiation and given 3 times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed and their body length was measured first as initial data. Fish were kept for 30 days. During rearing the fish were fed with a frequency of three times a day at (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every 10 days during maintenance and sampling was carried out every 3 days.

Parameters

Parameters in this study include dissolved phosphorus content, growth length, absolute weight, feed efficiency, feed consumption, protein efficiency ratio, and survival.

Data Analysis

The data obtained were analyzed using analysis of variance, at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (BNT) test.

RESULTS AND DISCUSSIONS

Results

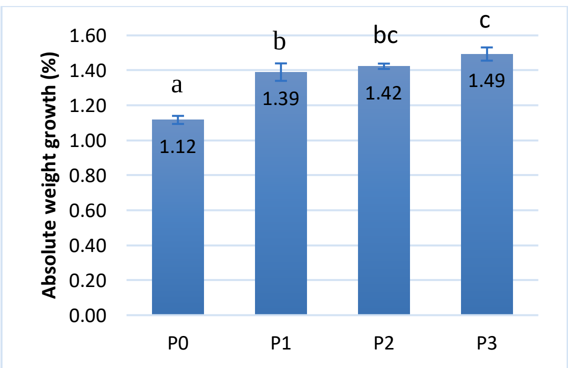
Dissolved phosphorus was measured to determine the action of enzymes that occur *in vitro*. The content of dissolved phosphorus in the feed-in each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

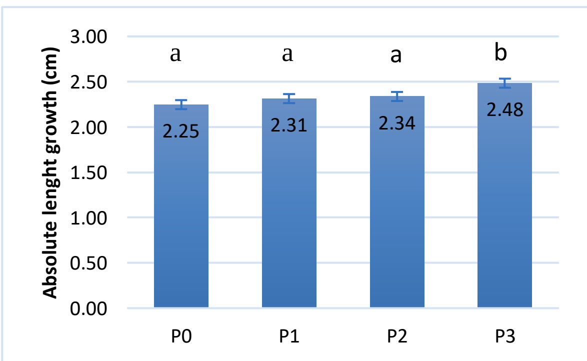
Treatment	Dissolved Phosphorus (mg L^{-1})
P0	3,87
P1	4,57
P2	5,12
P3	5,26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter) and the lowest was found in P0 (without the addition of phytase enzyme).

The absolute growth of length, weight, feed consumption, and feed efficiency of snakehead fish is presented in Figures 1, 2, and 3.



(a)



(b)

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Figure 1. Graph of snakehead absolute growth; (a) Absolute weight growth, (b) Absolute length growth

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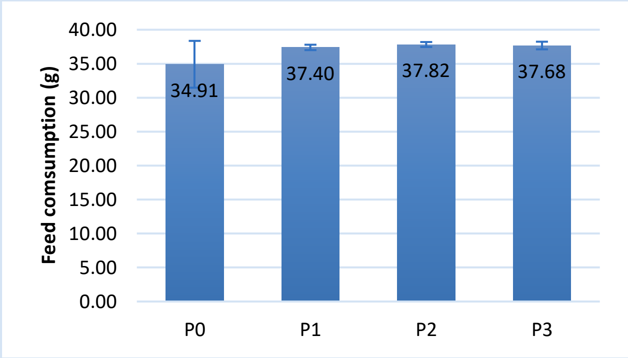

Figure 2. Graph of snakehead feed consumption

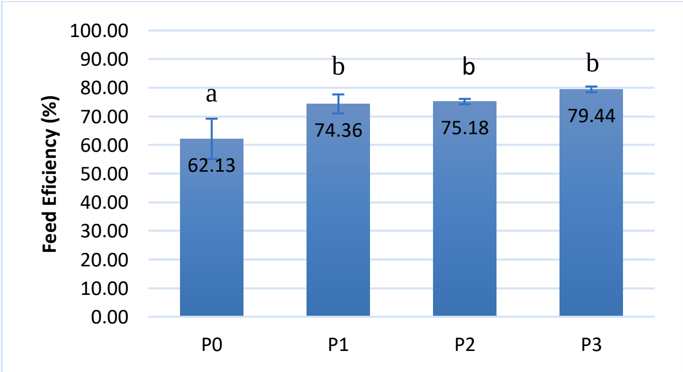

Figure 3. Graph of snakehead feed efficiency
Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

Based on the results of the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead fish, but had no significant effect on feed consumption. The results of the Least Significant Difference Test (BNT) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than other treatments, as well as the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

The protein efficiency ratio (REP) and survival of fish during the study of snakehead fish are presented in Figures 4, and 5.

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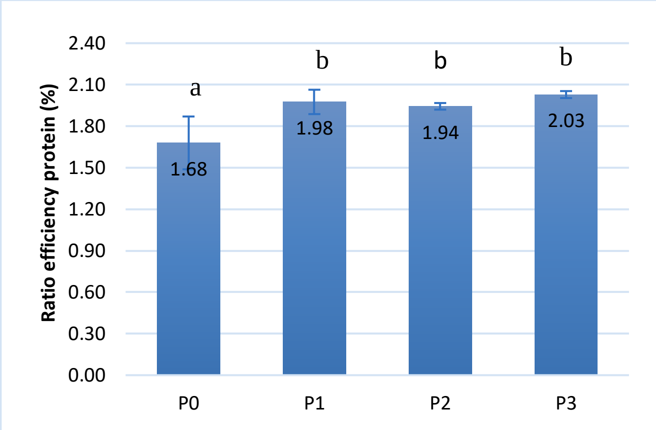

Figure 4. Graph of snakehead ratio efficiency protein

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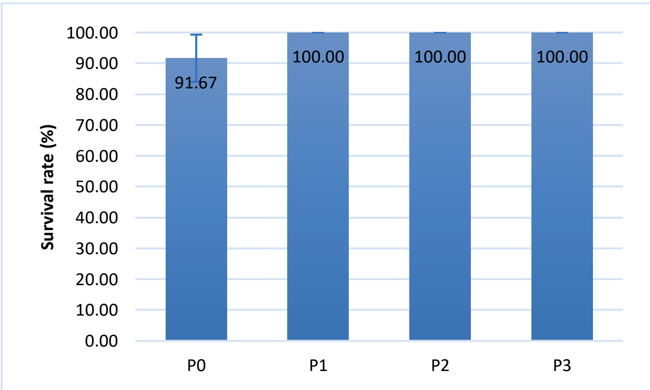

Figure 5. Graph of snakehead ratio efficiency protein

Based on the results of the analysis of variance showed that the addition of phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead fish feed. The results of the Least Significant Difference Test showed that the ratio of protein efficiency in P3 was significantly higher than P0 but not significantly different than P1 and P2. Based on the results of the analysis of variance showed that the addition of phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead fish.

Discussion

Phytase which is applied to feed can hydrolyze phytic acid in the feed. This can be seen from the dissolved phosphorus content of the feed, the higher the dissolved phosphorus content indicates the better the performance of phytase in hydrolyzing phytic acid. The results showed that the P3 treatment with a dose of 70 mg 100 g^{-1} vegetable ingredients caused higher dissolved phosphorus while the lowest phosphorus yield was found in P0 (treatment without phytase enzyme).

Based on the research results of Amin *et al*. (2011) feed with the addition of the phytase enzyme was able to increase the mineral P in the body of catfish. Mineral P is needed by the fish body for metabolic activities. Increased metabolic processes in the fish's body, will spur fish to consume more feed and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed and less is wasted as feces, it can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that the more efficient the feed is used by fish for growth (Amin *et al.,* 2020). Feed efficiency is influenced by many factors including feed quality. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the results of the study, the highest feed efficiency was found in the P3 treatment of 79.44%, however, the value of feed efficiency was high because according to Craig and Helfrich (2017) the value of good feed efficiency was more than 50%. This is following the opinion of Tacon (1987) which states that high feed efficiency indicates that the feed provided can be utilized efficiently so that the protein is reformed to meet fewer energy needs and more for growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, it was suspected that in the P0 treatment the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that the addition of phytase enzymes to feed, especially at P3 (70 mg/100g vegetable matter) caused the highest ratio of feed protein efficiency to snakehead fish. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. Rachmawati and Samidjan, 2014). According to Hoffman and Falvo (2004), the ratio of protein efficiency determines the effectiveness of protein by measuring weight gain in grams per gram of protein consumed. Feed protein based on the results of this study, the lowest protein was found in treatment P0 37.00 and the highest protein was found in treatment P3 which was 39.16. Snakehead fish fed feed without the addition of phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed, as a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish, and will even be wasted as feces. Amin *et al*. (2011) stated that fish cannot digest phytic acid so it will be released into the waters.

Based on the results of the analysis of variance showed that the addition of phytase with different doses in the feed had no significant effect on the survival of snakehead fish. The survival of fish is influenced by several factors, one of which is including water quality during maintenance. The water quality in this study was following the life needs of snakehead fish. The water temperature during the study ranged from 25.1 to 29.9^{o}C, according to Makmur (2003), the optimal temperature for the development of snakehead fish life ranged from 26.5 to 31.5^{o}C. The pH of the water ranged from 6.1 to 7.6 and Dissolved oxygen in snakehead fish rearing water during the study ranged from 4.01 to 4.90 mg L^{-1}. According to Muflikhah *et al*. (2008), a good pH for the maintenance of snakehead fish fry ranged from 4-9 and dissolved oxygen at least 3 mg L^{-1}. Ammonia content 0.02 – 0.37 mg L^{-1}. According to Jianguang (1997), the good ammonia content for snakehead fish is less than 1 mg L^{-1}.

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses gave a significant effect on growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead

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fish. However, it has no significant effect on feed consumption and the survival of snakehead fish. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg 100 g^{-1} vegetable ingredients (P3).

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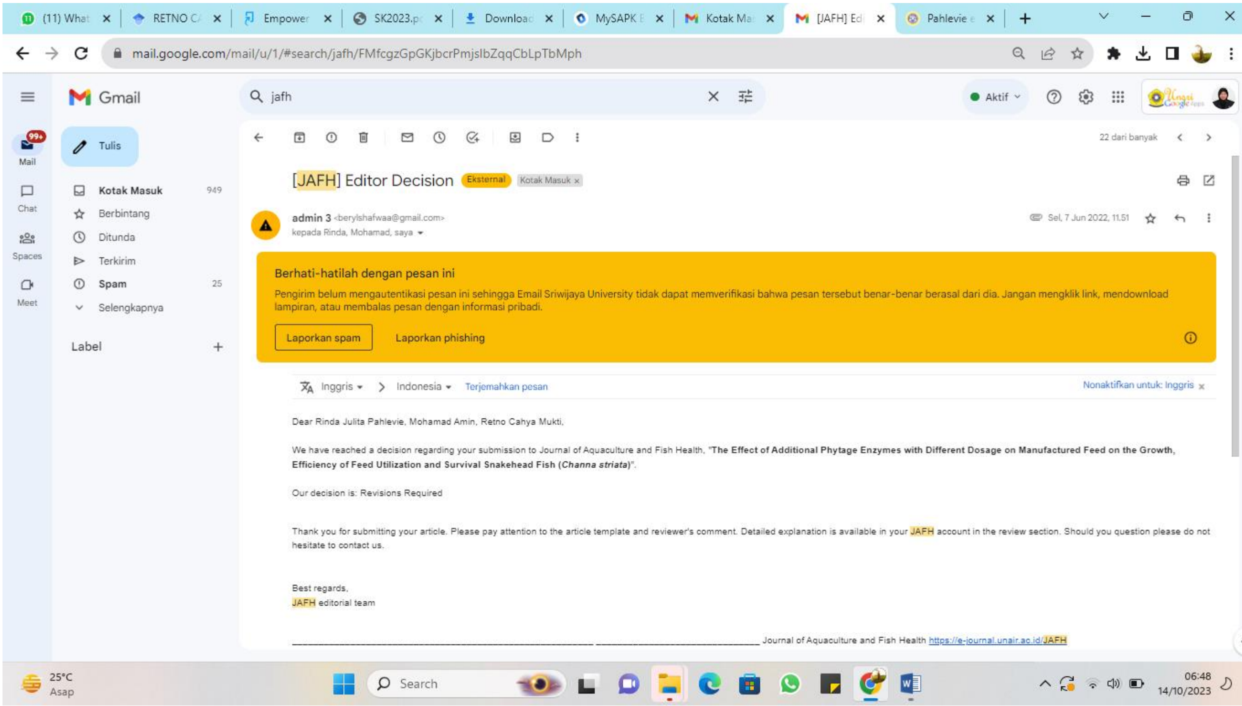

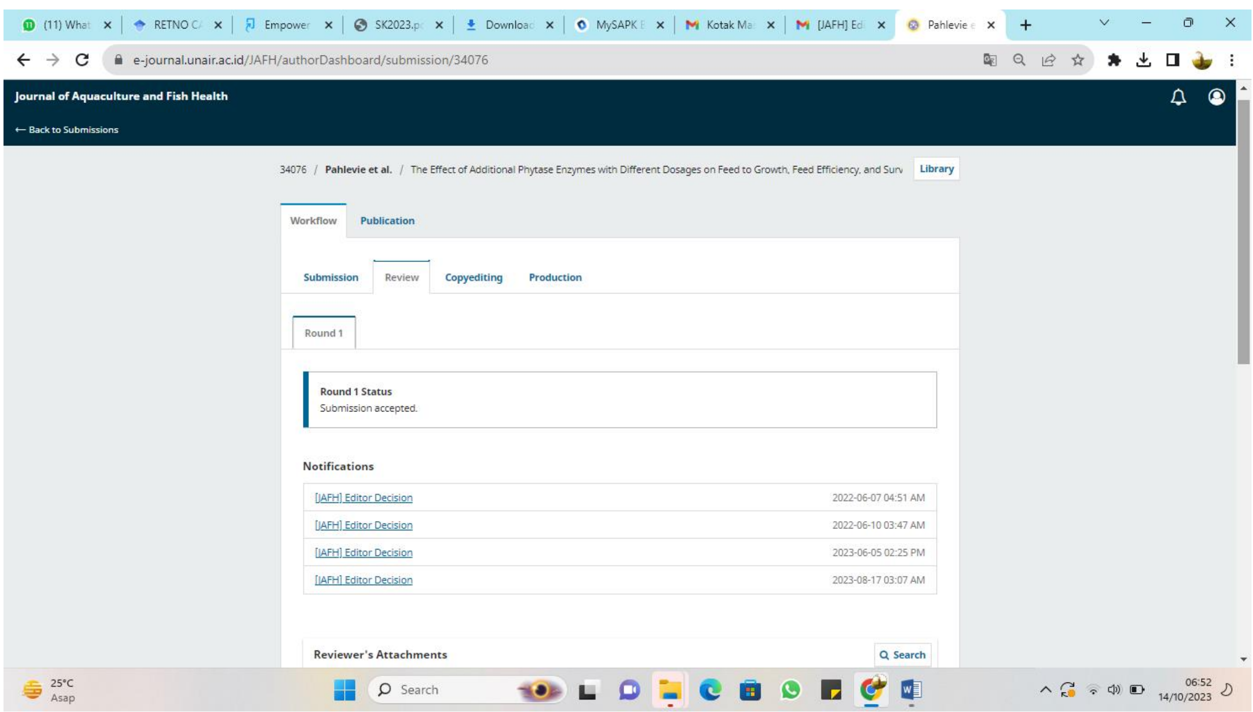

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The Effect of Additional Phytage Enzymes with Different Dosage on Manufactured Feed on the Growth, Efficiency of Feed Utilization and Survival Snakehead Fish (*Channa striata*)

Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out but is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on growth, protein efficiency ratio, feed efficiency, feed consumption, and survival of snakehead. This research was carried out at the Fisheries Basic Laboratory in July – August 2021, this study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg 100 g^{-1} material). vegetable), P2 (addition of phytase 50 mg 100 g^{-1} vegetable material), P3 (addition of phytase 70 mg 100 g^{-1} vegetable material). The size of the fish used in this study was 3-4 cm with a stocking density of 2 L^{-1} fish, the maintenance of the study was 30 days. The results showed that the addition of phytase 70 mg 100 g^{-1} vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), feed consumption (37.68 g) protein efficiency ratio (2.03%), and survival (100%).

Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead fish *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia, including in Indonesia (War and Altaff, 2011). The availability of snakehead fish is still dominated by natural catches. Currently, snakehead fish can be cultivated with artificial feeding, but several factors cause limitations in cultivation such as low survival and relatively slow growth. This is due to the limited natural feed, high food competition that gives rise to cannibalism, and the limited space for fish to move (Muslim, 2007).

Based on Supandi *et al,* (2015), snakehead fish fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead fish is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead fish has been carried out but is still not optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean flour and bran. Soybean flour is one of the feed ingredients that contain high protein. However, soy flour also contains minerals combined with phytic acid, making it difficult for the body to absorb, as well as bran. According to Cao *et al,* (2007), the content of phytic acid in soybean flour reaches 3.88% or 59.9% of the total phosphorus. While the bran contains about 6.9% phytic acid. Sumiati *et al*. (2001). Phytic acid is the main storage form of phosphorus (P) contained in these vegetable raw materials (Amin *et al.,* 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and also proteins so that minerals and proteins cannot be digested by the fish's body as a whole and result in lower absorption. As a result, the utilization of soybean flour and bran is not optimal.

Phytic acid can be reduced by heating, but heating can damage the nutrients in the feed to be given. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Widowati *et al*., 2004). Winata *et al,* (2018) stated that the phytase enzyme can break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al*., 2019).

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The addition of phytase enzymes to an artificial feed of 500 mg/kg of feed resulted in feed utilization efficiency of 84.55% in sangkuriang catfish (Kosim *et al*., 2016). The addition of the phytase enzyme 1000 mg/kg of feed was able to suppress the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). The addition of the phytase enzyme 1,200 mg/kg of feed was able to produce a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for larasati tilapia (Restianti *et al*., 2016). Based on the description above, the dose of the phytase enzyme in snakehead fish feed is not yet known. Therefore, it is necessary to research on the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Time and Place

This research was conducted at the Fisheries Basic Laboratory, Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University. Feed proximate analysis was carried out at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Dissolved phosphorus analysis of feed was carried out at the Palembang Industrial Center. This research was conducted in July – August 2021.

Research Materials

The materials used in this study were snakehead fish size 3-4 cm, fish meal, soybean meal, tapioca, bran, fish oil, corn oil, premix, phytase enzyme, potassium permanganate. The tools used in this research are aquarium, pH meter, thermometer, DO meter, spectrophotometer, UV-VIS spectrophotometer, digital scale, centrifuge, measuring cup, blender, sieve, basin, mixer, pellet printer, aerator, and oven.

Research Design

This study used a completely randomized design (CRD) with 4 treatments and 3 replications. The treatment in this study refers to the study of Amin *et al.* (2011) about the addition of phytase enzymes in catfish. The treatments included:

P0: feed not added with phytase enzymes
P1: feed added with phytase enzymes at a dose of 30 mg/100g vegetable ingredients
P2: feed added with phytase enzymes at a dose of 50 mg/100g vegetable ingredients
P3: feed added with phytase enzymes at a dose of 70 mg/100g vegetable ingredients

Feed Formulation

The feed formulations used are presented in Table 1.

Working Procedure

Preparation and Preparation of Test Feed

Prepare all the materials used and the feed raw materials are weighed according to the formulation. Next, the soybean flour and bran are mixed and stirred until homogeneous. The homogeneous mixture of ingredients is added as much as 350 mL $100g^{-1}$ of vegetable material. The material is stirred again until evenly distributed and the phytase enzyme is added according to the treatment dose. Then incubated for 2 h at 37◦C (Matsumoto *et al*., 2001) the material mixture was used as the building blocks of feed.

Making feed is done by mixing all the ingredients starting from the fewest ingredients to the most until all the ingredients are evenly and homogeneously mixed, after the ingredients are well mixed add warm water little by little until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, dried in the sun to dry. After the feed is dry, it is then put into a container and labeled with the treatment dose.

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Table 1. The feed formulations used

Description	Treatment (g 1000g^{-1})			
Material (g)	P0	P1	P2	P3
Fish flour	375	375	375	375
Soy flour	402	402	402	402
Tapioca	44	43,84	43,73	43,62
Bran	144	144	144	144
Fish oil	2,5	2,5	2,5	2,5
Corn oil	2,5	2,5	2,5	2,5
Premix	30	30	30	30
Phytase enzyme	0	0,16	0,27	0,38
Jumlah (g)	1000	1000	1000	1000
Nutrient components (%)				
Protein (%)**	37,00	37,64	38,68	39,16
Acid (%)*	2,5	2,5	2,5	2,5
Fat (%)**	2,65	3,40	1,56	1,65
Carbohydrates (%)**	31,69	31,68	30,88	29,86
Ash (%)**	13,85	14,62	14,40	15,42
Water (%)**	7,91	6,84	9,67	9,16
Crude fiber (%)**	6,90	5,83	4,81	4,75

Note: *Phytic acid content: 3.88% soybean flour (Cao *et al*., 2007); bran 6.9% (Sumiati *et al*., 2001).
** Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2021)

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Matsumoto *et al,* (2001), namely vegetable material and the enzyme phytase which was mixed and incubated at 37°C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Preparation of Test Fish Rearing Media

The container used for the maintenance of snakehead fish is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g L^{-1} then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, every In the aquarium, an aeration hose is connected to the aerator and left overnight and then labeled according to treatment (Zainuri *et al*., 2017).

Maintenance

The snakehead fish seeds used were 3-4 cm in size, with a stocking density of 2 L-fish^1 (Zainuri *et al*., 2017). Fish were acclimatized first for 3 days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given 3 times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed and their body length was measured first as initial data. Fish were kept for 30 days. During rearing the fish were fed with a frequency of three times a day at (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every 10 days during maintenance and sampling was carried out every 3 days.

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Parameters

Parameters in this study include dissolved phosphorus content, growth length, absolute weight, feed efficiency, feed consumption, protein efficiency ratio, and survival.

Data Analysis

The data obtained were analyzed using analysis of variance, at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (BNT) test.

RESULTS AND DISCUSSIONS

Results

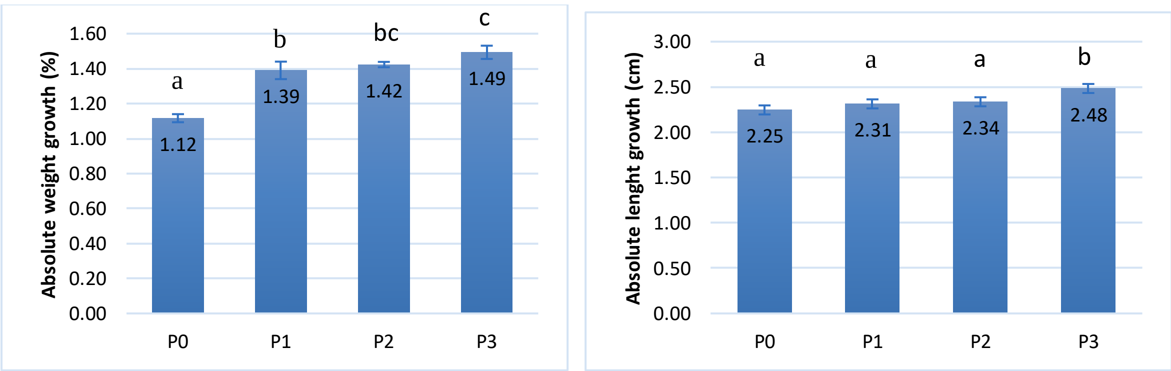
Dissolved phosphorus was measured to determine the action of enzymes that occur *in vitro*. The content of dissolved phosphorus in the feed-in each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

Treatment	Dissolved Phosphorus (mg L^{-1})
P0	3,87
P1	4,57
P2	5,12
P3	5,26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter) and the lowest was found in P0 (without the addition of phytase enzyme).

The absolute growth of length, weight, feed consumption, and feed efficiency of snakehead fish is presented in Figures 1, 2, and 3.



(a) (b)

Figure 1. Graph of snakehead absolute growth; (a) Absolute weight growth, (b) Absolute length growth

Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

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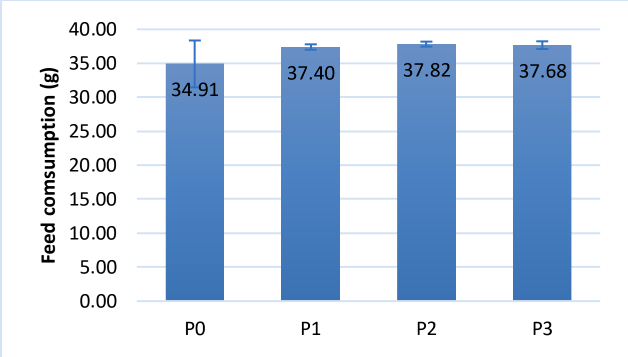

Figure 2. Graph of snakehead feed consumption

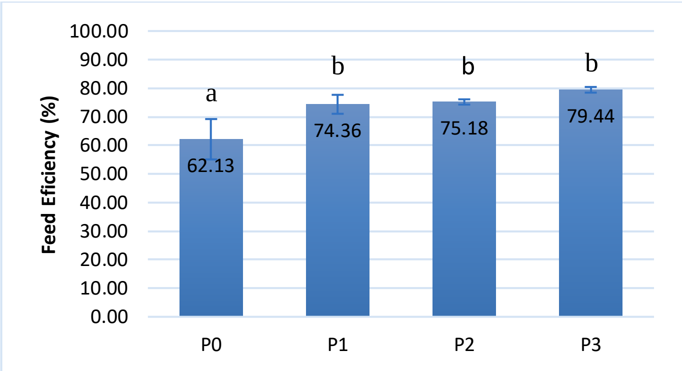

Figure 3. Graph of snakehead feed efficiency

Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

Based on the results of the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead fish, but had no significant effect on feed consumption. The results of the Least Significant Difference Test (BNT) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than other treatments, as well as the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

The protein efficiency ratio (REP) and survival of fish during the study of snakehead fish are presented in Figures 4, and 5.

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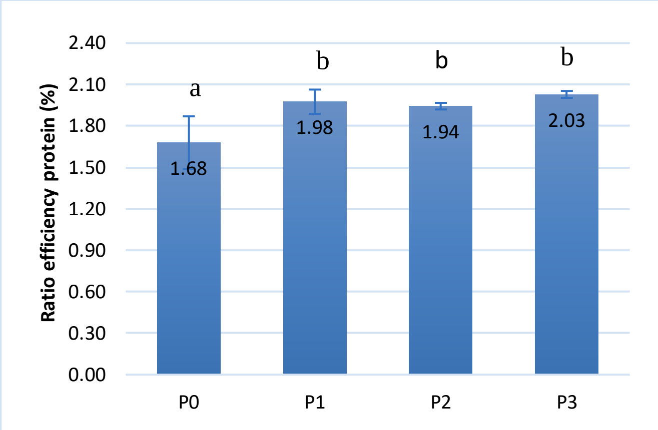

Figure 4. Graph of snakehead ratio efficiency protein
Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

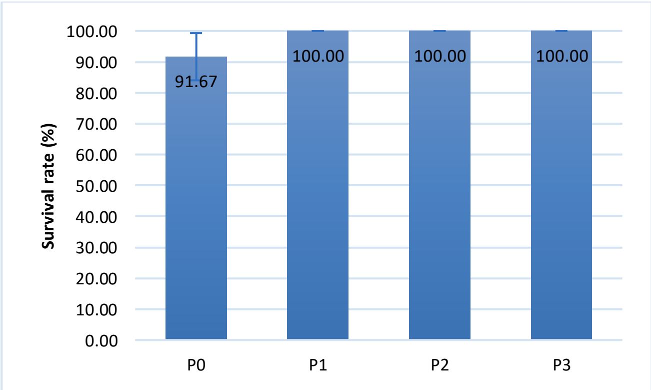

Figure 5. Graph of snakehead ratio efficiency protein

Based on the results of the analysis of variance showed that the addition of phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead fish feed. The results of the Least Significant Difference Test showed that the ratio of protein efficiency in P3 was significantly higher than P0 but not significantly different than P1 and P2. Based on the results of the analysis of variance showed that the addition of phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead fish.

Discussion

Phytase which is applied to feed can hydrolyze phytic acid in the feed. This can be seen from the dissolved phosphorus content of the feed, the higher the dissolved phosphorus content indicates the better the performance of phytase in hydrolyzing phytic acid. The results showed that the P3 treatment with a dose of 70 mg 100 g^{-1} vegetable ingredients caused higher dissolved phosphorus while the lowest phosphorus yield was found in P0 (treatment without phytase enzyme).

Based on the research results of Amin *et al*. (2011) feed with the addition of the phytase enzyme was able to increase the mineral P in the body of catfish. Mineral P is needed by the fish body for metabolic activities. Increased metabolic processes in the fish's body, will spur fish to consume more feed and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed and less is wasted as feces, it can be seen from the

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results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that the more efficient the feed is used by fish for growth (Amin *et al.,* 2020). Feed efficiency is influenced by many factors including feed quality. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the results of the study, the highest feed efficiency was found in the P3 treatment of 79.44%, however, the value of feed efficiency was high because according to Craig and Helfrich (2017) the value of good feed efficiency was more than 50%. This is following the opinion of Tacon (1987) which states that high feed efficiency indicates that the feed provided can be utilized efficiently so that the protein is reformed to meet fewer energy needs and more for growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, it was suspected that in the P0 treatment the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that the addition of phytase enzymes to feed, especially at P3 (70 mg/100g vegetable matter) caused the highest ratio of feed protein efficiency to snakehead fish. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. Rachmawati and Samidjan, 2014). According to Hoffman and Falvo (2004), the ratio of protein efficiency determines the effectiveness of protein by measuring weight gain in grams per gram of protein consumed. Feed protein based on the results of this study, the lowest protein was found in treatment P0 37.00 and the highest protein was found in treatment P3 which was 39.16. Snakehead fish fed feed without the addition of phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed, as a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish, and will even be wasted as feces. Amin *et al.* (2011) stated that fish cannot digest phytic acid so it will be released into the waters.

Based on the results of the analysis of variance showed that the addition of phytase with different doses in the feed had no significant effect on the survival of snakehead fish. The survival of fish is influenced by several factors, one of which is including water quality during maintenance. The water quality in this study was following the life needs of snakehead fish. The water temperature during the study ranged from 25.1 to 29.9°C, according to Makmur (2003), the optimal temperature for the development of snakehead fish life ranged from 26.5 to 31.5°C. The pH of the water ranged from 6.1 to 7.6 and Dissolved oxygen in snakehead fish rearing water during the study ranged from 4.01 to 4.90 mg L^{-1}. According to Muflikhah *et al.* (2008), a good pH for the maintenance of snakehead fish fry ranged from 4-9 and dissolved oxygen at least 3 mg L^{-1}. Ammonia content 0.02 – 0.37 mg L^{-1}. According to Jianguang (1997), the good ammonia content for snakehead fish is less than 1 mg L^{-1}.

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses gave a significant effect on growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead

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fish. However, it has no significant effect on feed consumption and the survival of snakehead fish. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg 100 g^{-1} vegetable ingredients (P3).

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Article Type:Full Paper

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Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead fish *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia, including in Indonesia (War and Altaff, 2011). The availability of snakehead fish is still dominated by natural catches. Currently, snakehead fish can be cultivated with artificial feeding, but several factors cause limitations in cultivation such as low survival and relatively slow growth. This is due to the limited natural feed, high food competition that gives rise to cannibalism, and the limited space for fish to move (Muslim, 2007).

Based on Supandi *et al,* (2015), snakehead fish fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead fish is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead fish has been carried out but is still not optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean flour and bran. Soybean flour is one of the feed ingredients that contain high protein. However, soy flour also contains minerals combined with phytic acid, making it difficult for the body to absorb, as well as bran. According to Cao *et al,* (2007), the content of phytic acid in soybean flour reaches 3.88% or 59.9% of the total phosphorus. While the bran contains about 6.9% phytic acid. Sumiati *et al*. (2001). Phytic acid is the main storage form of phosphorus (P) contained in these vegetable raw materials (Amin *et al.,* 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and also proteins so that minerals and proteins cannot be digested by the fish's body as a whole and result in lower absorption. As a result, the utilization of soybean flour and bran is not optimal.

Phytic acid can be reduced by heating, but heating can damage the nutrients in the feed to be given. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Widowati *et al*., 2004). Winata *et al,* (2018) stated that the phytase enzyme can break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.,* 2019). The addition of phytase enzymes to an artificial feed of 500 mg/kg of feed resulted in feed utilization efficiency of 84.55% in sangkuriang catfish (Kosim *et al*., 2016). The addition of the phytase enzyme 1000 mg/kg of feed was able to suppress the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). The addition of the phytase enzyme 1,200 mg/kg of feed was able to produce a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for larasati tilapia (Restianti *et al*., 2016). Based on the description above, the dose of the phytase enzyme in snakehead fish feed is not yet known. Therefore, it is necessary to research on the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Time and Place

This research was conducted at the Fisheries Basic Laboratory, Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University. Feed proximate analysis was carried out at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Dissolved phosphorus analysis of feed was carried out at the Palembang Industrial Center. This research was conducted in July – August 2021.

Research Materials

The materials used in this study were snakehead fish size 3-4 cm, fish meal, soybean meal, tapioca, bran, fish oil, corn oil, premix, phytase enzyme, potassium permanganate.The tools used in this research are aquarium, pH meter, thermometer, DO meter, spectrophotometer, UV-VIS spectrophotometer, digital scale, centrifuge, measuring cup, blender, sieve, basin, mixer, pellet printer, aerator, and oven.

Research Design

This study used a completely randomized design (CRD) with 4 treatments and 3 replications. The treatment in this study refers to the study of Amin *et al.* (2011) about the addition of phytase enzymes in catfish. The treatments included:

P0: feed not added with phytase enzymes
P1: feed added with phytase enzymes at a dose of 30 mg/100g vegetable ingredients
P2: feed added with phytase enzymes at a dose of 50 mg/100g vegetable ingredients
P3: feed added with phytase enzymes at a dose of 70 mg/100g vegetable ingredients

Feed Formulation

The feed formulations used are presented in Table 1.

Working Procedure

Preparation and Preparation of Test Feed

Prepare all the materials used and the feed raw materials are weighed according to the formulation. Next, the soybean flour and bran are mixed and stirred until homogeneous. The homogeneous mixture of ingredients is added as much as 350 mL $100g^{-1}$ of vegetable material. The material is stirred again until evenly distributed and the phytase enzyme is added according to the treatment dose. Then incubated for 2 h at 37◦C (Matsumoto *etal.*,2001) the material mixture was used as the building blocks of feed.

Making feed is done by mixing all the ingredients starting from the fewest ingredients to the most until all the ingredients are evenly and homogeneously mixed, after the

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ingredients are well mixed add warm water little by little until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, dried in the sun to dry. After the feed is dry, it is then put into a container and labeled with the treatment dose.

Table 1. The feed formulations used

Description	Treatment (g $1000g^{-1}$)			
Material (g)	P0	P1	P2	P3
Fish flour	375	375	375	375
Soy flour	402	402	402	402
Tapioca	44	43,84	43,73	43,62
Bran	144	144	144	144
Fish oil	2,5	2,5	2,5	2,5
Corn oil	2,5	2,5	2,5	2,5
Premix	30	30	30	30
Phytase enzyme	0	0,16	0,27	0,38
Jumlah (g)	1000	1000	1000	1000
Nutrient components (%)				
Protein (%)**	37,00	37,64	38,68	39,16
Acid (%)*	2,5	2,5	2,5	2,5
Fat (%)**	2,65	3,40	1,56	1,65
Carbohydrates (%)**	31,69	31,68	30,88	29,86
Ash (%)**	13,85	14,62	14,40	15,42
Water (%)**	7,91	6,84	9,67	9,16
Crude fiber (%)**	6,90	5,83	4,81	4,75

Note: *Phytic acid content: 3.88% soybean flour (Cao *et al*., 2007); bran 6.9% (Sumiati *et al*., 2001).
** Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2021)

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Matsumoto *et al,* (2001), namely vegetable material and the enzyme phytase which was mixed and incubated at $37^{\circ}C$ for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Preparation of Test Fish Rearing Media

The container used for the maintenance of snakehead fish is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g L^{-1} then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, everyIn the aquarium, an aeration hose is connected to the aerator and left overnight and then labeled according to treatment (Zainuri *et al*., 2017).

Maintenance

The snakehead fish seeds used were 3-4 cm in size, with a stocking density of 2 L-fish^{1} (Zainuri *et al*., 2017). Fish were acclimatized first for 3 days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given 3 times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed and their body length was measured first

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as initial data. Fish were kept for 30 days. During rearing the fish were fed with a frequency of three times a day at (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every 10 days during maintenance and sampling was carried out every 3 days.

Parameters

Parameters in this study include dissolved phosphorus content, growth length, absolute weight, feed efficiency, feed consumption, protein efficiency ratio, and survival.

Data Analysis

The data obtained were analyzed using analysis of variance, at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (BNT) test.

RESULTS AND DISCUSSIONS

Results

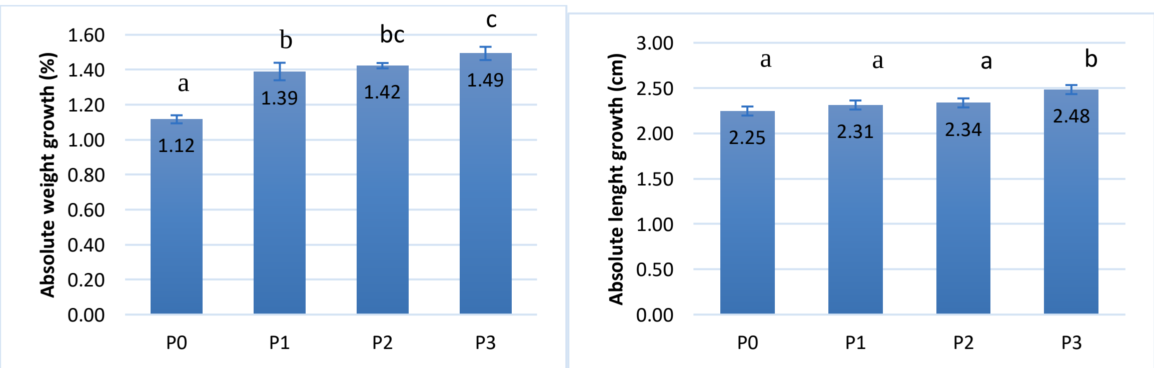
Dissolved phosphorus was measured to determine the action of enzymes that occur *in vitro*. The content of dissolved phosphorus in the feed-in each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

Treatment	Dissolved Phosphorus (mg L^{-1})
P0	3,87
P1	4,57
P2	5,12
P3	5,26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter) and the lowest was found in P0 (without the addition of phytase enzyme).

The absolute growth of length, weight, feed consumption, and feed efficiency of snakehead fish is presented in Figures 1, 2, and 3.



(a) (b)

Figure 1. Graph of snakehead absolute growth; (a) Absolute weight growth, (b) Absolute length growth

Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

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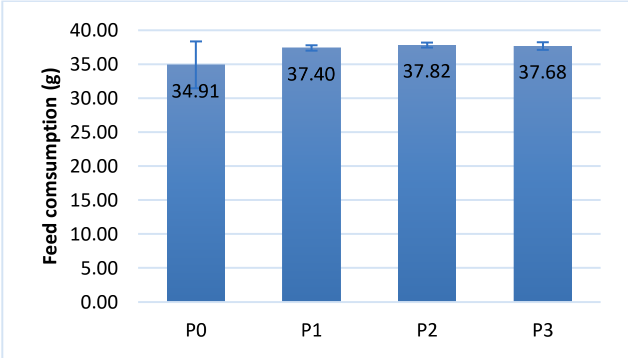

Figure 2. Graph of snakehead feed consumption

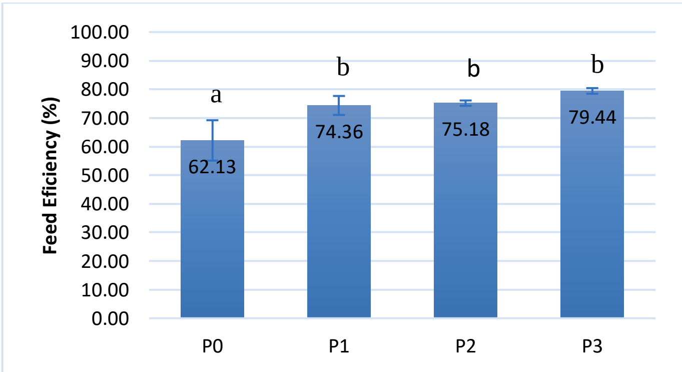

Figure 3. Graph of snakehead feed efficiency

Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

Based on the results of the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead fish, but had no significant effect on feed consumption. The results of the Least Significant Difference Test (BNT) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than other treatments, as well as the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

The protein efficiency ratio (REP) and survival offish during the study of snakehead fish are presented in Figures 4, and 5.

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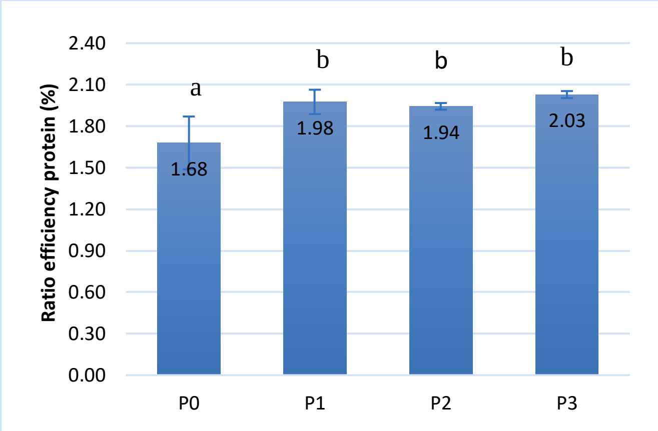

Figure 4. Graph of snakehead ratio efficiency protein
Information: The letters *superscript* different show a significantly different effect on the 95% BNT test.

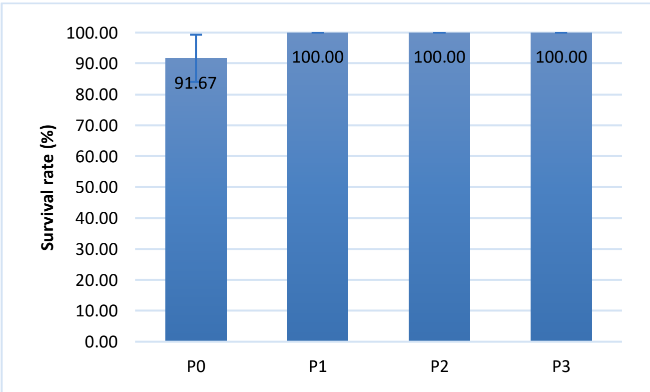

Figure 5. Graph of snakehead ratio efficiency protein

Based on the results of the analysis of variance showed that the addition of phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead fish feed. The results of the Least Significant Difference Test showed that the ratio of protein efficiency in P3 was significantly higher than P0 but not significantly different than P1 and P2.Based on the results of the analysis of variance showed that the addition of phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead fish.

Discussion

Phytase which is applied to feed can hydrolyze phytic acid in the feed. This can be seen from the dissolved phosphorus content of the feed, the higher the dissolved phosphorus content indicates the better the performance of phytase in hydrolyzing phytic acid. The results showed that the P3 treatment with a dose of 70 mg 100 g^{-1} vegetable ingredients caused higher dissolved phosphorus while the lowest phosphorus yield was found in P0 (treatment without phytase enzyme).

Based on the research results of Amin *et al*. (2011) feed with the addition of the phytase enzyme was able to increase the mineral P in the body of catfish. Mineral P is needed by the fish body for metabolic activities. Increased metabolic processes in the fish's body, will spur fish to consume more feed and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more

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dissolved phosphorus increases, the more phosphorus is absorbed and less is wasted as feces, it can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that the more efficient the feed is used by fish for growth (Amin *et al.,* 2020).Feed efficiency is influenced by many factors including feed quality. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the results of the study, the highest feed efficiency was found in the P3 treatment of 79.44%, however, the value of feed efficiency was high because according to Craig and Helfrich (2017) the value of good feed efficiency was more than 50%. This is following the opinion of Tacon (1987) which states that high feed efficiency indicates that the feed provided can be utilized efficiently so that the protein is reformed to meet fewer energy needs and more for growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, it was suspected that in the P0 treatment the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that the addition of phytase enzymes to feed, especially at P3 (70 mg/100g vegetable matter) caused the highest ratio of feed protein efficiency to snakehead fish. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. Rachmawati and Samidjan, 2014). According to Hoffman and Falvo (2004), the ratio of protein efficiency determines the effectiveness of protein by measuring weight gain in grams per gram of protein consumed. Feed protein based on the results of this study, the lowest protein was found in treatment P0 37.00 and the highest protein was found in treatment P3 which was 39.16. Snakehead fish fed feed without the addition of phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed, as a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish, and will even be wasted as feces. Amin *et al*. (2011) stated that fish cannot digest phytic acid so it will be released into the waters.

Based on the results of the analysis of variance showed that the addition of phytase with different doses in the feed had no significant effect on the survival of snakehead fish. The survival of fish is influenced by several factors, one of which isincluding water quality during maintenance. The water quality in this study was following the life needs of snakehead fish. The water temperature during the study ranged from 25.1 to 29.9^{o}C, according to Makmur (2003), the optimal temperature for the development of snakehead fish life ranged from 26.5 to 31.5^{o}C. The pH of the water ranged from 6.1 to 7.6 and Dissolved oxygen in snakehead fish rearing water during the study ranged from 4.01 to 4.90 mg L^{-1}. According to Muflikhah *et al*. (2008), a good pH for the maintenance of snakehead fish fry ranged from 4-9 and dissolved oxygen at least 3 mg L^{-1}. Ammonia content 0.02 – 0.37 mg L^{-1}. According to Jianguang (1997), the good ammonia content for snakehead fish is less than 1 mg L^{-1}.

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CONCLUSION

The addition of phytase enzyme in artificial feed with different doses gave a significant effect on growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead fish. However, it has no significant effect on feed consumption and the survival of snakehead fish. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg 100 g^{-1} vegetable ingredients (P3).

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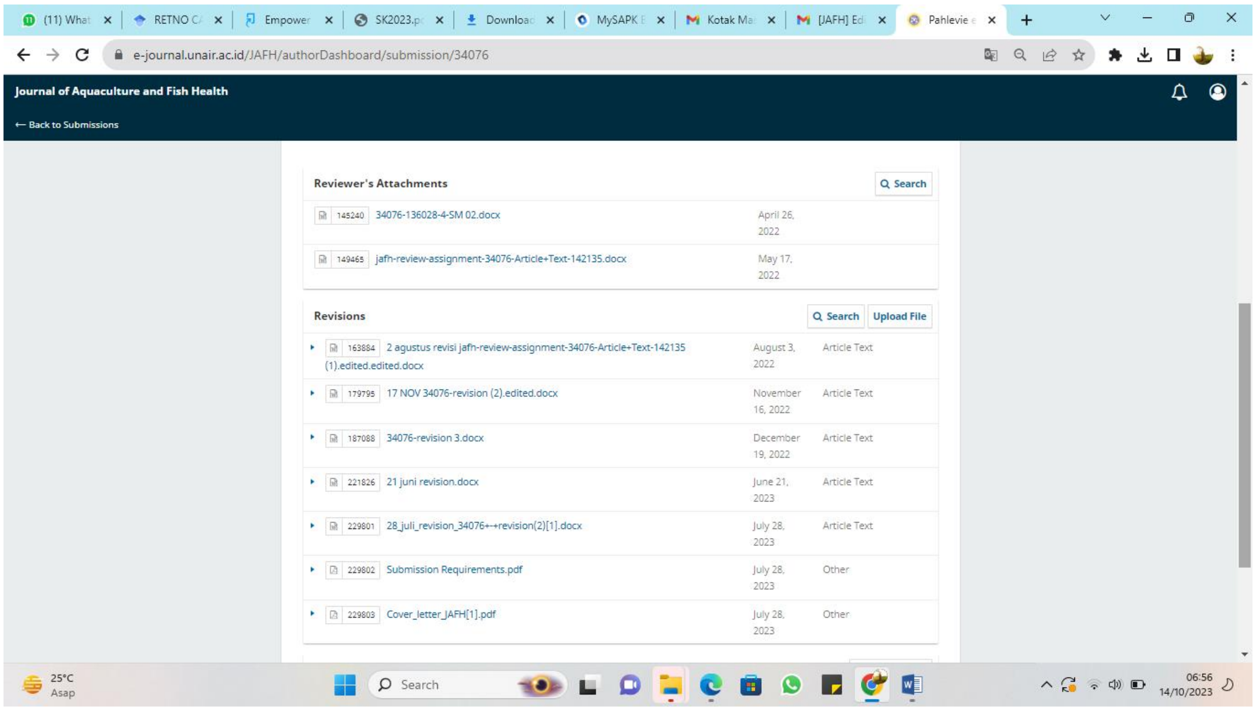

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Article Type: Full Paper

The Effect of Additional Phytase Enzymes with Different Dosage on Manufactured Feed on the Growth, Efficiency of Feed Utilization, and Survival of Snakehead Fish (*Channa striata*)

Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out but is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on growth, protein efficiency ratio, feed efficiency, feed consumption, and survival of snakehead. This research was carried out at the Fisheries Basic Laboratory in July – August 2021, this study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg/100 g vegetable material), P2 (addition of phytase 50 mg/100 g vegetable material), P3 (addition of phytase 70 mg/100 g vegetable material). The size of the fish used in this study was 3-4 cm with a stocking density of 2 fish/L fish, the maintenance of the study was 30 days. The results showed that the addition of phytase 70 mg/100g vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), feed consumption (37.68 g) protein efficiency ratio (2.03%), and survival (100%).

Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead fish *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia, including in Indonesia (War and Altaff, 2011). Natural catches still dominate the availability of snakehead fish. Snakehead fish can be cultivated with artificial feeding. However, several factors cause limitations in cultivation, such as low survival and relatively slow growth, because of the limited natural feed, high food competition that gives rise to cannibalism, and the limited space for fish to move (Muslim, 2007).

Based on Supandi *et al,* (2015), snakehead fish fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead fish is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead fish has been carried out but is still not optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean flour and bran. Soybean flour is one of the feed ingredients that contain high protein. However, soy flour also contains minerals combined with phytic acid, making it difficult for the body to absorb and bran. According to Cao *et al,* (2007), the phytic acid content in soybean flour reaches 3.88% or 59.9% of the total phosphorus. While the bran contains about 6.9% phytic acid. Sumiati *et al*. (2001). Phytic acid is the primary source of phosphorus (P) in these raw vegetable materials (Amin *et al.,* 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and proteins so that minerals and proteins cannot be digested by the fish's body as a whole, resulting in lower absorption. As a result, soybean flour and bran utilization are not optimal.

Phytic acid can be reduced by heating, but heating can damage the feed's nutrients. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Widowati *et al*., 2004). Winata *et al,* (2018) stated that the phytase enzyme could break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.,* 2019). The addition of phytase enzymes to an artificial feed of 500 mg/kg of feed resulted in a feed utilization efficiency of 84.55% in sangkuriang catfish (Kosim *et al*., 2016). Adding the phytase enzyme 1000 mg/kg of feed suppresses the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). Adding the phytase enzyme 1,200 mg/kg of feed produced a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for larasati tilapia (Restianti *et al*., 2016). Based on the description above, the dose of the phytase enzyme in snakehead fish feed is not yet known. Therefore, it is necessary to research the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Time and Place

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Testing methods phosphorus dissolved from the feed by Matsumoto *et al*, (2001), namely vegetable material and the enzyme phytase, which was mixed and incubated at 37 ^{O}C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Preparation of Test Fish Rearing Media

The container used to maintain snakehead fish is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g/L. Then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, and an aeration hose is connected to the aerator, left overnight, and then labeled according to treatment (Zainuri *et al*., 2017).

Maintenance

The snakehead fish seeds were 3-4 cm in size, with a stocking density of 2 fish/L (Zainuri *et al*., 2017). Fish were acclimatized first for three days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given three times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed, and their body length was measured first as initial data. Fish were kept for 30 days. During rearing, the fish were fed with a frequency of three times a day (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every ten days during maintenance, and sampling was carried out every three days.

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Parameters

Parameters in this study include dissolved phosphorus content, growth length, absolute weight, feed efficiency, feed consumption, protein efficiency ratio, and survival.

Data Analysis

The data obtained were analyzed using analysis of variance at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (BNT) test.

RESULTS AND DISCUSSIONS

Results

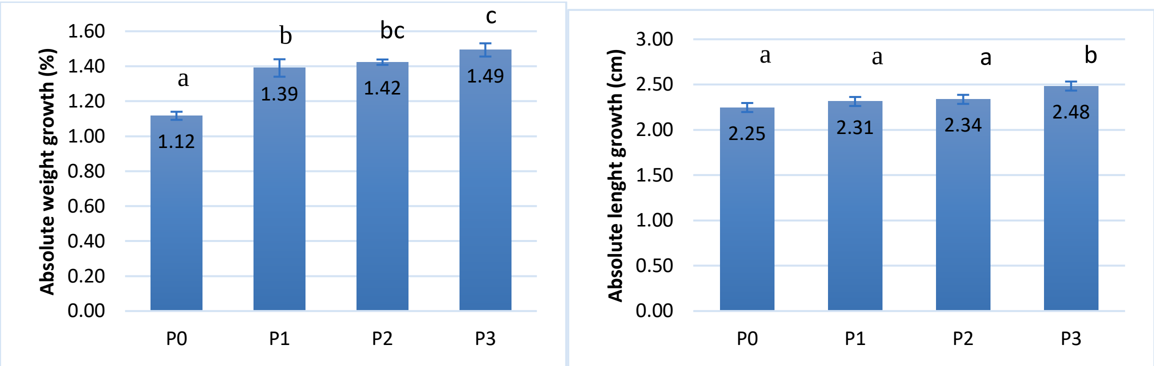
Dissolved phosphorus was measured to determine the action of enzymes *in vitro*. The content of dissolved phosphorus in the feed of each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

Treatment	Dissolved Phosphorus (mg/L)
P0	3,87
P1	4,57
P2	5,12
P3	5,26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter), and the lowest was found in P0 (without the addition of phytase enzyme).

The absolute growth of length, weight, feed consumption, and feed efficiency of snakehead fish is presented in Figures 1, 2, and 3.



(a) (b)

Figure 1. Graph of snakehead absolute growth; (a) Absolute weight growth, (b) Absolute length growth

Information: Different *superscript* letters show a significantly different effect on the 95% BNT test.

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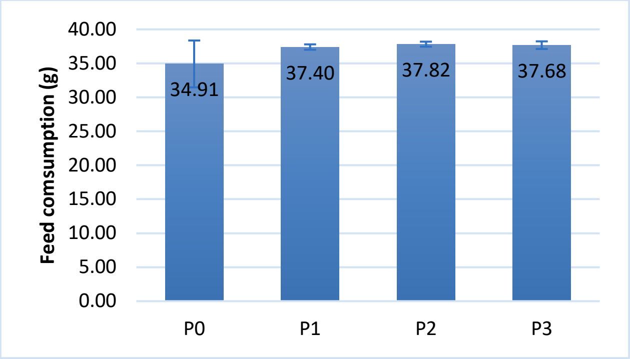

Figure 2. Graph of snakehead feed consumption

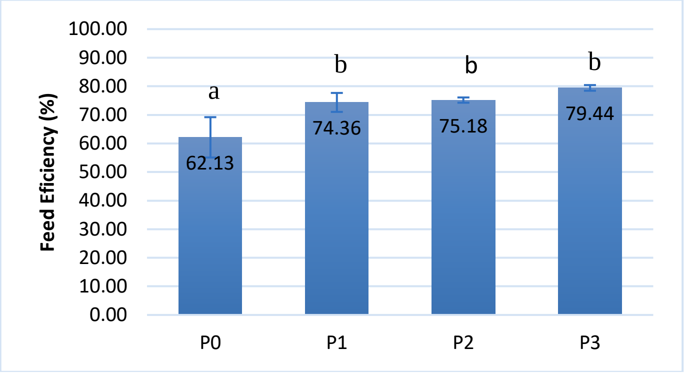

Figure 3. Graph of snakehead feed efficiency

Information: Different *superscript* letters show a significantly different effect on the 95% BNT test.

Based on the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead fish but had no significant effect on feed consumption. The Least Significant Difference Test (BNT) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than in other treatments, and the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

The protein efficiency ratio (PER) and survival of fish during the study of snakehead fish are presented in Figures 4 and 5.

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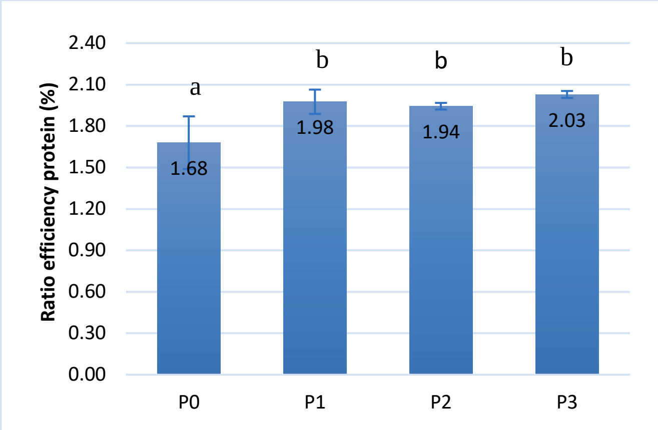

Figure 4. Graph of snakehead ratio efficiency protein
Information: Different *superscript* letters show a significantly different effect on the 95% BNT test.

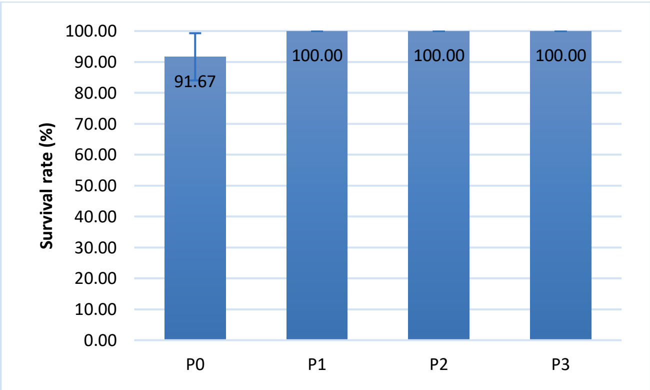

Figure 5. Graph of snakehead ratio efficiency protein

The variance analysis showed that adding phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead fish feed. The Least Significant Difference Test results showed that the protein efficiency ratio in P3 was significantly higher than P0 but not significantly different than P1 and P2. The variance analysis showed that adding phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead fish.

Discussion

Phytic acid in the feed can be hydrolyzed by phytase, which is added to it. The amount of dissolved phosphorus in the feed provides evidence of this. Phytase performs better at hydrolyzing phytic acid the higher the dissolved phosphorus content indicates. The findings demonstrated that P3 treatment, which had a dose of 70 mg/100 g of vegetable ingredients, led to higher levels of dissolved phosphorus, whereas P0 treatment had the lowest levels of phosphorus production (treatment without phytase enzyme).

Based on Amin *et al*. (2011), feeding with the addition of the phytase enzyme increased the mineral P in the catfish's body. The fish body needs mineral P for metabolic activities. Increased metabolic processes in the fish's body will spur fish to consume more feed, and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed, and less is wasted as feces. It can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that fish use, the more efficient the feed for growth (Amin *et al.,* 2020). Many factors, including feed quality, influence feed efficiency. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body, and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the study's results, the highest feed efficiency in the P3 treatment at 79.44%. However, feed efficiency was high because, according to Craig and Helfrich (2017), the value of good feed efficiency was more than 50%. Tacon (1987) states that high feed efficiency indicates that the feed provided can be utilized efficiently so that the protein is reformed to meet fewer energy needs and more for growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, and it was suspected that in the P0 treatment, the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes, so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that adding phytase enzymes to feed, especially at P3 (70 mg/100 g vegetable matter), caused the highest ratio of feed protein efficiency in snakehead fish. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed, which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. Rachmawati and Samidjan, 2014). According to Hoffman and Falvo (2004), the protein efficiency ratio determines protein's effectiveness by measuring weight gain in grams per gram of protein consumed. Feed protein based on the results of this study, the lowest protein was found in treatment P0 at 37.00%, and the highest protein was found in treatment P3, which was 39.16%. Snakehead fish fed feed without adding phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed. As a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish and will even be wasted as feces. Amin *et al*. (2011) stated that fish could not digest phytic acid so it will be released into the waters.

The variance analysis showed that adding phytase with different doses in the feed had no significant effect on the survival of snakehead fish. The survival of fish is influenced by several factors, one of which is water quality during maintenance. This study's water quality followed the life needs of snakehead fish. The water temperature during the study ranged from 25.1 to 29.9 ^{o}C. Makmur (2003) states that the optimal temperature for developing snakehead fish life ranges from 26.5 to 31.5 ^{o}C. The pH of the water ranged from 6.1 to 7.6, and Dissolved oxygen in snakehead fish-rearing water during the study ranged from 4.01 to 4.90 mg/L. According to Muflikhah *et al*. (2008), a good pH for the maintenance of snakehead fish fry ranged from 4-9 and dissolved oxygen at least 3 mg/L. Ammonia content 0.02 – 0.37 mg/L. According to Jianguang (1997), the good ammonia content for snakehead fish is less than 1 mg/L.

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses significantly affected growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead fish. However, it has no significant effect on feed consumption and the survival of snakehead

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fish. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg/100 g of vegetable ingredients (P3).

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The Effect of Additional Phytase Enzymes with Different Dosages on the Growth, Efficiency of Feed Utilization, and Survival of Snakehead (*Channa striata*)

Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out. However, it is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on growth, protein efficiency ratio, feed efficiency, and survival of snakehead. This study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg/100 g vegetable material), P2 (addition of phytase 50 mg/100 g vegetable material), P3 (addition of phytase 70 mg/100 g vegetable material). The size of the fish used in this study was 3-4 cm, with a stocking density of 2 fish/L fish. The rearing of the study was 30 days. The results showed that the addition of phytase 70 mg/100g vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), protein efficiency ratio (2.03%), and survival (100%).

Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia (Song *et al.*, 2013). Natural catches still dominate the availability of snakehead. Snakehead can be cultivated with artificial feeding. The main obstacle in the cultivation of snakehead is maintaining a low life and relatively slow growth (Triyanto *et al.,* 2020).

Based on Supandi *et al*. (2015), snakehead-fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead has been carried out but still needs to be optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean flour and bran. Soybean flour is one of the feed ingredients that contain high protein. However, soy flour also contains minerals combined with phytic acid, making it difficult for the body to absorb and bran. According to Rasyid (2017), the phytic acid content in soybean flour reaches 0.605mg/g. While the bran contains about 0.370 mg/g phytic acid. Phytic acid is the primary source of phosphorus (P) in these raw vegetable materials (Amin *et al.,* 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and proteins so that minerals and proteins cannot be digested by the fish's body, resulting in lower absorption. As a result, soybean flour and bran utilization could be more optimal.

Phytic acid can be reduced by heating, but heating can damage the feed's nutrients. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Sari and Ginting, 2012). Winata *et al.* (2018) stated that the phytase enzyme could break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.,* 2019). The addition of phytase enzymes to an artificial feed of 500 mg/kg resulted in a feed utilization efficiency of 84.55% in catfish (Kosim *et al*., 2016). Adding the phytase enzyme 1000 mg/kg of feed suppresses the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). Adding the phytase enzyme 1,200 mg/kg of feed produced a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for tilapia (Restianti *et al.*, 2016). Based on the description above, the dose of the phytase enzyme in snakehead feed has yet to be discovered. Therefore, it is necessary to research the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Research Materials

The materials used in this study were snakehead size 3-4 cm, a fish meal with a protein content of 62.65%, soybean meal with a protein content of 37%, tapioca with a protein content of 0.5%, bran with a protein content of 13%, fish oil, corn oil, premix, phytase enzyme Bio-Phytase 5000®, and potassium permanganate. The tools used in this research are aquarium measuring 25 x 25 x 25 cm^3, pH meter (ATC digital tester®) with an accuracy of 0.1 pH units, thermometer (Oxygen meter®) with an accuracy of 0.1°C, DO meter (Oxygen meter®) with an accuracy of 0.01 mg/L, spectrophotometer (Thermo scientific Genesys 150®) (Oxygen meter®) with an accuracy of 0.001mg/L, UV-VIS spectrophotometer (Unico®) with a wavelength of 200-750 nm, a digital scale (Mini Digital Platform Scale I-2000®) with an accuracy of 0.01 g, centrifuge (Backman coulter®) with speed control ±50 rpm, measuring cup with an accuracy 1 mL, blender (Miyako BL-152 GF®) with a capacity 1,5 L, sieve with size 40-60 mesh, basin with volume 10 L, pellet machine (Mincer Manual VIPOO-A12®) with a capacity of 5 kg, aerator (Amara®), and oven (Memret®) with maximum temperature ±70°C.

Research Design

This study used a completely randomized design (CRD) with four treatments and three replications. The treatment in this study refers to the study of Amin *et al.* (2011) about the addition of phytase enzymes in catfish. The treatments included:

P0: feed not added with phytase enzymes
P1: feed added with phytase enzymes at a dose of 30 mg/100g of vegetable ingredients
P2: feed added with phytase enzymes at a dose of 50 mg/100g of vegetable ingredients
P3: feed added with phytase enzymes at a dose of 70 mg/100g of vegetable ingredients

Feed Formulation

The feed formulations used are presented in Table 1.

Table 1. The feed formulations used

Description	Treatment (g/1000 g)			
Material (g)	P0	P1	P2	P3
Fish flour	375	375	375	375
Soy flour	402	402	402	402
Tapioca	44	43.84	43.73	43.62
Bran	144	144	144	144
Fish oil	2.5	2.5	2.5	2.5
Corn oil	2.5	2.5	2.5	2.5
Premix	30	30	30	30
Phytase enzyme	0	0.16	0.27	0.38
Jumlah (g)	1000	1000	1000	1000
Nutrient components (%)				
Protein (%)**	37.00	37.64	38.68	39.16
Acid (%)*	2.5	2.5	2.5	2.5
Fat (%)**	2,65	3.40	1.56	1.65
Carbohydrates (%)**	31.69	31.68	30.88	29.86
Ash (%)**	13.85	14.62	14.40	15.42
Water (%)**	7.91	6.84	9.67	9.16
Crude fiber (%)**	6.90	5.83	4.81	4.75

Note: *Phytic acid content: 3.88% soybean flour (Cao *et al*., 2007); bran 6.9% (Sumiati *et al*., 2001).
** Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2021)

Working Procedure

Preparation and Preparation of Test Feed

Prepare all the materials used, and the feed raw materials are weighed according to the formulation. Next, the soybean flour and bran are mixed and stirred until homogeneous. The material is stirred again until evenly distributed, and the phytase enzyme is added according to the treatment dose. Then incubated for two hours at 37 ^oC (Matsumoto *et al.*, 2001 in Amin *et al.*, 2011), the material mixture was used as the building blocks of feed.

Making feed is done by mixing all the ingredients from the fewest to the most until all the ingredients are evenly and homogeneously mixed. After the ingredients are well mixed, add warm water until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, and dried in the sun to dry. After the feed is dry, it is put into a container and labeled with the treatment dose.

Preparation of Test Fish Rearing Media

The container used to maintain snakehead is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g/L. Then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, and an aeration hose is connected to the aerator, left overnight, and then labeled according to treatment (Zainuri *et al.*, 2017).

Fish Rearing

The snakehead was 3-4 cm in size, with a stocking density of 2 fish/L (Zainuri *et al.*, 2017). Fish were acclimatized first for three days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given three times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed, and their body length was measured first as initial data. Fish were kept for 30 days. During rearing, the fish were fed with a frequency of three times a day (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every ten days during rearing, and sampling was carried out every three days.

Parameters

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Matsumoto *et al.* (2001) *in* Amin *et al.* (2011), namely vegetable material and the enzyme phytase, which was mixed and incubated at 37 °C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Absolute Length Growth

The absolute length growth of snakehead is calculated using the formula:

$$L = Lt - L0$$

Information:
L: Growth in absolute length (cm)
Lt: Fish length at the end of rearing (cm)
L0: Fish length at the beginning of rearing (cm)

Absolute Weight Growth

The absolute weight growth of snakehead is calculated using the:

$$W = Wt - W0$$

Information:
W: Absolute weight growth (g)
Wt: Fish weight at the end of rearing (g)
W0: Fish weight at the beginning of rearing (g)

Feed Efficiency
Feed efficiency is calculated using the formula:

$$FE = \frac{(Wt+D)-Wo}{f} x100\%$$

Information:
FE: Feed efficiency (%)
Wt: Fish biomass at the end of rearing (g)
W0: Fish biomass at the beginning of rearing (g)
D: Dead fish biomass during rearing (g)
F: Feed consumed (g)

Protein Efficiency Ratio
Calculation of protein efficiency ratio (PER) using the formula:

$$\text{Protein efficiency ratio} = \frac{Wt-Wo}{Pi}$$

Information:
Wt: Fish biomass at the end of rearing (g)
W0: Fish biomass at the start of rearing (g)
Pi: Weight of feed protein consumed (g)

Survival
The survival of snakehead is calculated using the formula:

$$\text{Survival (\%)} = \frac{Nt}{No} x\ 100\%$$

Information:
Nt: Number of live fish at the end of rearing (fish)
N0: Number of fish at the beginning of rearing (fish)

Water quality

The water quality of the snakehead rearing media measured included temperature, dissolved oxygen, pH, and ammonia. Temperature (^{o}C) was measured every day, dissolved oxygen (mg/L) and pH were measured once every ten days. Ammonia was measured at the beginning and end of rearing.

Data Analysis

The data obtained were analyzed using analysis of variance at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Dissolved Phosphorus Content

Dissolved phosphorus was measured to determine the action of enzymes *in vitro*. The content of dissolved phosphorus in the feed of each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

Treatment	Dissolved Phosphorus (mg/L)
P0	3.87
P1	4.57
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P3	5.26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter), and the lowest was found in P0 (without the addition of phytase enzyme). Phytic acid in the feed can be hydrolyzed by phytase, which is added to it. The amount of dissolved phosphorus in the feed provides evidence of this. Phytase performs better at hydrolyzing phytic acid the higher the dissolved phosphorus content indicates. The findings demonstrated that the P3 treatment, which had a dose of 70 mg/100 g of vegetable ingredients, led to higher levels of dissolved phosphorus, whereas the P0 treatment had the lowest levels of phosphorus production (treatment without phytase enzyme).

Based on Amin *et al*. (2011), feeding with the addition of the phytase enzyme increased the mineral P in the catfish's body. The fish body needs mineral P for metabolic activities. Increased metabolic processes in the fish's body will spur fish to consume more feed, and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed, and less is wasted as feces. It can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing are presented in Table 3.

Table 3. Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing

Parameters	Treatments			
	P0	P1	P2	P3
L (cm)	2.25 ± 0.02 ^{a}	2.31 ± 0.04 ^{a}	2.34 ± 0.04 ^{a}	2.48 ± 0.08 ^{b}
W (g)	1.12 ± 0.02 ^{a}	1.39 ± 0.05 ^{b}	1.42 ± 0.02 ^{b}	1.49 ± 0.04 ^{c}
FE (%)	62.13 ± 7.06 ^{a}	74.36 ± 3.32 ^{b}	75.18 ± 0.93 ^{b}	79.44 ± 0.99 ^{b}
PER	1.68 ± 0.19 ^{a}	1.98 ± 0.09 ^{b}	1.94 ± 0.02 ^{b}	2.03 ± 0.03 ^{b}
SR (%)	91.67 ± 7.64	100 ± 0.00	100 ± 0.00	100 ± 0.00

Note: The numbers in the same row, followed by different superscript letters, show significantly different effects on the 95% LSD test.

Based on the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead. The Least Significant Difference Test (LSD) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than in other treatments, and the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that fish use, the more efficient the feed for growth (Amin *et al.*, 2020). Many factors, including feed quality, influence feed efficiency. According to

Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body, and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the study's results, the highest feed efficiency in the P3 treatment at 79.44%. However, feed efficiency was high because, according to Craig and Helfrich (2017), the value of good feed efficiency was more than 50%. Mukti *et al*. (2020) state the higher the feed efficiency value, the more efficient the feed is for its growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, and it was suspected that in the P0 treatment, the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes, so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

The variance analysis showed that adding phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead feed. The Least Significant Difference Test results showed that the protein efficiency ratio in P3 was significantly higher than P0 but not significantly different than P1 and P2. The variance analysis showed that adding phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that adding phytase enzymes to feed, especially at P3 (70 mg/100 g vegetable matter), caused the highest ratio of feed protein efficiency in snakehead. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed, which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. Rachmawati and Samidjan, 2014). According to Rachmawati *et al*. (2017), the protein efficiency ratio determines protein's effectiveness by measuring weight gain in grams per gram of protein consumed. Based on this study's results, the lowest protein was found in treatment P0 at 37.00%, and the highest protein was found in treatment P3, which was 39.16%. Snakehead-fed feed without adding phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed. As a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish and will even be wasted as feces. Amin *et al*. (2011) stated that fish could not digest phytic acid, so it will be released into the waters.

The variance analysis showed that adding phytase with different doses in the feed had no significant effect on the survival of the snakehead. The survival of fish is influenced by several factors, one of which is water quality during rearing. This study's water quality followed the life needs of snakehead.

Water Quality

Data on water quality during rearing is presented in Table 4.

Table 4. Data on water quality during rearing

Parameters	Treatments			
	P0	P1	P2	P3
Temperature (°C)	25.1 – 29.9	25.1 – 29.7	25.2 – 2.,9	25.2 – 29.7
pH	6.3 – 7.4	6.2 –7.6	6.1 – 7.5	6.1 – 7.5
Dissolved oxygen (mg/L)	4.19 – 4.81	4.15 – 4.74	4.08 – 4.90	4.01 – 4.82
Ammonia (mg/L)	0.02 – 0.37	0.02 – 0.25	0.02 – 0.25	0.02 – 0.18

The water quality temperature during the study ranged from 25.1 to 29.9 °C, pH ranged from 6.1 to 7.6, dissolved oxygen from 4.01 to 4.90 mg/L, and ammonia content 0.02 – 0.37 mg/L. Rukmini (2013) states that the optimal temperature for developing snakehead life ranges from 25 to 30 °C. According to Muslim and Syaifudin (2012), dissolved oxygen for snakehead from 4.2-5.6 mg/L. According to Extrada *et al.* (2013), the pH of the water to snakehead ranged from 6.0 to 7.1, and ammonia content was 0.04 – 1,1 mg/L.

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses significantly affected growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead. However, it has no significant effect on the survival of snakehead. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg/100 g of vegetable ingredients (P3).

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The Effect of Additional Phytase Enzymes with Different Dosages on Feed to Growth, Feed Efficiency, and Survival of Snakehead (*Channa striata*)

Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out. However, it is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on growth, protein efficiency ratio, feed efficiency, and survival of snakehead. This study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg/100 g vegetable material), P2 (addition of phytase 50 mg/100 g vegetable material), P3 (addition of phytase 70 mg/100 g vegetable material). The size of the fish used in this study was 3-4 cm, with a stocking density of 2 fish/L fish. The rearing of the study was 30 days. The results showed that the addition of phytase 70 mg/100g vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), protein efficiency ratio (2.03%), and survival (100%).

Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia (Song *et al.*, 2013). Natural catches still dominate the availability of snakehead. Snakehead can be cultivated with artificial feeding. The main obstacle in the cultivation of snakehead is maintaining a low life and relatively slow growth (Triyanto *et al.,* 2020).

Based on Supandi *et al*. (2015), snakehead-fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead has been carried out but still needs to be optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean meal and bran. Soybean meal is one of the feed ingredients that contain high protein. However, soy meal also contains minerals combined with phytic acid, making it difficult for the body to absorb and bran. According to Rasyid (2017), the phytic acid content in soybean meal reaches 0.605mg/g. While the bran contains about 0.370 mg/g phytic acid. Phytic acid is the primary source of phosphorus (P) in these raw vegetable materials (Amin *et al.*, 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and proteins so that minerals and proteins cannot be digested by the fish's body, resulting in lower absorption. As a result, soybean meal and bran utilization could be more optimal.

Phytic acid can be reduced by heating, but heating can damage the feed's nutrients. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Sari and Ginting, 2012). Winata *et al.* (2018) stated that the phytase enzyme could break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.,* 2019). The addition of phytase enzymes to an artificial feed of 500 mg/kg resulted in a feed utilization efficiency of 84.55% in catfish (Kosim *et al*., 2016). Adding the phytase enzyme 1000 mg/kg of feed suppresses the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). Adding the phytase enzyme 1,200 mg/kg of feed produced a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for tilapia (Restianti *et al*., 2016). Based on the description above, the dose of the phytase enzyme in snakehead feed has yet to be discovered. Therefore, it is necessary to research the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Time and Place

This research was conducted at the Fisheries Basic Laboratory, Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University. Feed proximate analysis was carried out at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Dissolved phosphorus analysis of feed was carried out at the Palembang Industrial Center. This research was conducted in July – August 2021.

Research Materials

The materials used in this study were snakehead size 3-4 cm, a fish meal with a protein content of 62.65%, soybean meal with a protein content of 37%, tapioca with a protein content of 0.5%, bran with a protein content of 13%, fish oil, corn oil, premix, phytase enzyme Bio-Phytase 5000®, and potassium permanganate. The tools used in this research are aquarium measuring 25 x 25 x 25 cm^3, pH meter (ATC digital tester®) with an accuracy of 0.1 pH units, thermometer (Oxygen meter®) with an accuracy of 0.1^{o}C, DO meter (Oxygen meter®) with an accuracy of 0.01 mg/L, spectrophotometer (Thermo scientific Genesys 150®) (Oxygen meter®) with an accuracy of 0.001mg/L, UV-VIS spectrophotometer (Unico®) with a wavelength of 200-750 nm, a digital scale (Mini Digital Platform Scale I-2000®) with an accuracy of 0.01 g, centrifuge (Backman coulter®) with speed control ±50 rpm, measuring cup with an accuracy 1 mL, blender (Miyako BL-152 GF®) with a capacity 1,5 L, sieve with size 40-60 mesh, basin with volume 10 L, pellet machine (Mincer Manual VIPOO-A12®) with a capacity of 5 kg, aerator (Amara®), and oven (Memret®) with maximum temperature ±70^{o}C.

Research Design

This study used a completely randomized design (CRD) with four treatments and three replications. The feed is made with isoprotein by 38%. The treatment in this study refers to the study of Amin *et al.* (2011). The treatments included:
P0: feed not added with phytase enzymes
P1: feed added with phytase enzymes at a dose of 30 mg/100g of vegetable ingredients
P2: feed added with phytase enzymes at a dose of 50 mg/100g of vegetable ingredients
P3: feed added with phytase enzymes at a dose of 70 mg/100g of vegetable ingredients

Feed Formulation

The feed formulations used are presented in Table 1.

Table 1. The feed formulations used

Description	Treatment (g/1000 g)			
Material (g)	P0	P1	P2	P3
Fish meal	375	375	375	375
Soybean meal	402	402	402	402
Tapioca	44	43.84	43.73	43.62
Rice Bran	144	144	144	144
Fish oil	2.5	2.5	2.5	2.5
Corn oil	2.5	2.5	2.5	2.5
Premix	30	30	30	30
Phytase enzyme	0	0.16	0.27	0.38
Total (g)	1000	1000	1000	1000

Nutrient components (%)				
Protein (%)	37.00	37.64	38.68	39.16
Phytic Acid (%)*	2.5	2.5	2.5	2.5
lipid (%)	2,65	3.40	1.56	1.65
Nitrogen free-extract (NFE) (%)	31.69	31.68	30.88	29.86
Ash (%)	13.85	14.62	14.40	15.42
Water (%)	7.91	6.84	9.67	9.16
Crude fiber (%)	6.90	5.83	4.81	4.75

Note: *Phytic acid content: 3.88% soybean meal (Cao *et al*., 2007); bran 6.9% (Sumiati *et al*., 2001).

Working Procedure

Preparation and Preparation of Experimental Feed

Prepare all the materials used, and the feed raw materials are weighed according to the formulation. Next, the soybean meal and rice bran are mixed and stirred until homogeneous. The material is stirred again until evenly distributed, and the phytase enzyme is added according to the treatment dose. Then incubated for two hours at 37^{o}C (Matsumoto *et al*., 2001), the material mixture was used as the building blocks of feed.

Making feed is done by mixing all the ingredients from the fewest to the most until all the ingredients are evenly and homogeneously mixed. After the ingredients are well mixed, add warm water until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, and dried in the sun to dry. After the feed is dry, it is put into a container and labeled with the treatment dose.

Preparation of Test Fish Rearing Media

The container used to maintain snakehead is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g/L. Then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, and an aeration hose is connected to the aerator, left overnight, and then labeled according to treatment (Zainuri *et al*., 2017).

Fish Rearing

The snakehead was 3-4 cm in size, with a stocking density of 2 fish/L (Zainuri *et al*., 2017). Fish were acclimatized first for three days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given three times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed, and their body length was measured first as initial data. Fish were kept for 30 days. During rearing, the fish were fed with a frequency of three times a day (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every ten days during rearing, and sampling was carried out every three days.

Parameters

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Matsumoto *et al.* (2001) *in* Amin *et al.* (2011), namely vegetable material and the enzyme phytase, which was mixed and incubated at 37 °C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Absolute Length Growth

The absolute length growth of snakehead is calculated using the formula according to Lugert *et al.* (2014):

$$L = Lt - L0$$

Information:
L: Growth in absolute length (cm)
Lt: Fish length at the end of rearing (cm)
L0: Fish length at the beginning of rearing (cm)

Absolute Weight Growth

The absolute weight growth of snakehead is calculated using the formula according to Lugert *et al.* (2014):

$$W = Wt - W0$$

Information:
W: Absolute weight growth (g)
Wt: Fish weight at the end of rearing (g)
W0: Fish weight at the beginning of rearing (g)

Feed Efficiency

Feed efficiency is calculated using the formula according to NRC (2011):

$$FE = \frac{(Wt+D)-Wo}{f} x100\%$$

Information:
FE: Feed efficiency (%)
Wt: Fish biomass at the end of rearing (g)
W0: Fish biomass at the beginning of rearing (g)
D: Dead fish biomass during rearing (g)
F: Feed consumed (g)

Protein Efficiency Ratio

Calculation of protein efficiency ratio (PER) using the formula according to Hardy and Barrows (2002):

$$\text{Protein efficiency ratio} = \frac{Wt-Wo}{Pi}$$

Information:
Wt: Fish biomass at the end of rearing (g)
W0: Fish biomass at the start of rearing (g)
Pi: Weight of feed protein consumed (g)

Survival

The survival of snakehead is calculated using the formula according to Wang *et al.* (2018):

$$\text{Survival (\%)} = \frac{Nt}{No} x\ 100\%$$

Information:
Nt: Number of live fish at the end of rearing (fish)
N0: Number of fish at the beginning of rearing (fish)

Water quality

The water quality of the snakehead rearing media measured included temperature, dissolved oxygen, pH, and ammonia. Temperature (^oC) was measured every day, dissolved oxygen (mg/L) and pH were measured once every ten days. Ammonia was measured at the beginning and end of rearing.

Data Analysis

The data obtained were analyzed using analysis of variance at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Dissolved Phosphorus Content

Dissolved phosphorus was measured to determine the action of enzymes *in vitro*. The content of dissolved phosphorus in the feed of each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

Treatment	Dissolved Phosphorus (mg/L)
P0	3.87
P1	4.57
P2	5.12
P3	5.26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter), and the lowest was found in P0 (without the addition of phytase enzyme). Phytic acid in the feed can be hydrolyzed by phytase, which is added to it. The amount of dissolved phosphorus in the feed provides evidence of this. Phytase performs better at hydrolyzing phytic acid the higher the dissolved phosphorus content indicates. The findings demonstrated that the P3 treatment, which had a dose of 70 mg/100 g of vegetable ingredients, led to higher levels of dissolved phosphorus, whereas the P0 treatment had the lowest levels of phosphorus production (treatment without phytase enzyme).

Based on Amin *et al*. (2011), feeding with the addition of the phytase enzyme increased the mineral P in the catfish's body. The fish body needs mineral P for metabolic activities. Increased metabolic processes in the fish's body will spur fish to consume more feed, and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed, and less is wasted as feces. It can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing are presented in Table 3.

Table 3. Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing

Parameters	Treatments			
	P0	P1	P2	P3
L (cm)	2.25 ± 0.02 ^{a}	2.31 ± 0.04 ^{a}	2.34 ± 0.04 ^{a}	2.48 ± 0.08 ^{b}
W (g)	1.12 ± 0.02 ^{a}	1.39 ± 0.05 ^{b}	1.42 ± 0.02 ^{b}	1.49 ± 0.04 ^{c}
FE (%)	62.13 ± 7.06 ^{a}	74.36 ± 3.32 ^{b}	75.18 ± 0.93 ^{b}	79.44 ± 0.99 ^{b}
PER	1.68 ± 0.19 ^{a}	1.98 ± 0.09 ^{b}	1.94 ± 0.02 ^{b}	2.03 ± 0.03 ^{b}

SR (%)	91.67 ± 7.64	100 ± 0.00	100 ± 0.00	100 ± 0.00

Note: The numbers in the same row, followed by different superscript letters, show significantly different effects on the 95% LSD test.

Based on the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead. The Least Significant Difference Test (LSD) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than in other treatments, and the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that fish use, the more efficient the feed for growth (Amin *et al.,* 2020). Many factors, including feed quality, influence the feed efficiency. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body, and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the study's results, the highest feed efficiency in the P3 treatment at 79.44%. However, feed efficiency was high because, according to Craig and Helfrich (2017), the value of good feed efficiency was more than 50%. Mukti *et al*. (2020) state the higher the feed efficiency value, the more efficient the feed is for its growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, and it was suspected that in the P0 treatment, the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes, so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

The variance analysis showed that adding phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead feed. The Least Significant Difference Test results showed that the protein efficiency ratio in P3 was significantly higher than P0 but not significantly different than P1 and P2. The variance analysis showed that adding phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that adding phytase enzymes to feed, especially at P3 (70 mg/100 g vegetable matter), caused the highest ratio of feed protein efficiency in snakehead. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed, which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. (Rachmawati and Samidjan, 2014). According to Bhilave *et al.,* (2012), the protein efficiency ratio determines protein's effectiveness by measuring weight gain in grams per gram of protein consumed. Based on this study's results, the lowest protein was found in treatment P0 at 37.00%, and the highest protein was found in treatment P3, which was 39.16%. Snakehead-fed feed without adding phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed. As a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish and will even be wasted as feces. Amin *et al*. (2011) stated that fish could not digest phytic acid, so it will be released into the waters.

The variance analysis showed that adding phytase with different doses in the feed had no significant effect on the survival of the snakehead. The survival of fish is influenced by several factors, one of which is water quality during rearing. This study's water quality followed the life needs of snakehead.

Water Quality

Data on water quality during rearing is presented in Table 4. This study's water quality followed the life needs of snakehead fish.

Table 4. Data on water quality during rearing

Parameters	Treatments				Optimum Value
	P0	P1	P2	P3	
Temperature (°C)	25.1 – 29.9	25.1 – 29.7	25.2 – 2.,9	25.2 – 29.7	25-30 (Rukmini, 2013)
pH	6.3 – 7.4	6.2 –7.6	6.1 – 7.5	6.1 – 7.5	4.0 – 9.0 (Muflikhah *et al*. 2008)
Dissolved oxygen (mg/L)	4.19 – 4.81	4.15 – 4.74	4.08 – 4.90	4.01 – 4.82	> 3 (Muflikhah *et al*. 2008)
Ammonia (mg/L)	0.02 – 0.37	0.02 – 0.25	0.02 – 0.25	0.02 – 0.18	< 1 (Jianguang,1997)

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses significantly affected growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead. However, it has no significant effect on the survival of snakehead. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg/100 g of vegetable ingredients (P3).

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The Effect of Additional Phytase Enzymes with Different Dosages on Feed to Growth, Feed Efficiency, and Survival of Snakehead Fish (*Channa striata*)

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Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out. However, it is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on growth, protein efficiency ratio, feed efficiency, and survival of snakehead. This study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg/100 g vegetable material), P2 (addition of phytase 50 mg/100 g vegetable material), P3 (addition of phytase 70 mg/100 g vegetable material). The size of the fish used in this study was 3-4 cm, with a stocking density of 2 fish/L fish. The rearing of the study was 30 days. The results showed that the addition of phytase 70 mg/100g vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), protein efficiency ratio (2.03%), and survival (100%).

Keywords: Feed, Phytase enzymes, Phytic Acid, Snakehead

INTRODUCTION

Snakehead *(Channa striata)* is one type of freshwater fish which is also one of the most popular consumption fish in Southeast Asia (Song *et al.*, 2013). Natural catches still dominate the availability of snakehead. Snakehead can be cultivated with artificial feeding. The main obstacle in the cultivation of snakehead is maintaining a low life and relatively slow growth (Triyanto *et al.,* 2020).

Based on Supandi *et al*. (2015), snakehead-fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead has been carried out but still needs to be optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean meal and bran. Soybean meal is one of the feed ingredients that contain high protein. However, soy meal also contains minerals combined with phytic acid, making it difficult for the body to absorb and bran. According to Rasyid (2017), the phytic acid content in soybean meal reaches 0.605mg/g. While the bran contains about 0.370 mg/g phytic acid. Phytic acid is the primary source of phosphorus (P) in these raw vegetable materials (Amin *et al.*, 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg and Fe) and proteins so that minerals and proteins cannot be digested by the fish's body, resulting in lower absorption. As a result, soybean meal and bran utilization could be more optimal.

Phytic acid can be reduced by heating, but heating can damage the feed's nutrients. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Sari and Ginting, 2012). Winata *et al.* (2018) stated that the phytase enzyme could break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.,* 2019). The addition of phytase enzymes to an artificial feed of 500 mg/kg resulted in a feed utilization efficiency of 84.55% in catfish (Kosim *et al*., 2016). Adding the phytase enzyme 1000 mg/kg of feed suppresses the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015).

Adding the phytase enzyme 1,200 mg/kg of feed produced a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for tilapia (Restianti *et al.*, 2016). Based on the description above, the dose of the phytase enzyme in snakehead feed has yet to be discovered. Therefore, it is necessary to research the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY

Ethical Approval

Not applicable.

Time and Place

This research was conducted at the Fisheries Basic Laboratory, Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University. Feed proximate analysis was carried out at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Dissolved phosphorus analysis of feed was carried out at the Palembang Industrial Center. This research was conducted in July – August 2021.

Research Materials

The materials used in this study were snakehead size 3-4 cm, a fish meal with a protein content of 62.65%, soybean meal with a protein content of 37%, tapioca with a protein content of 0.5%, bran with a protein content of 13%, fish oil, corn oil, premix, phytase enzyme Bio-Phytase 5000®, and potassium permanganate. The tools used in this research are aquarium measuring 25 x 25 x 25 cm^3, pH meter (ATC digital tester®) with an accuracy of 0.1 pH units, thermometer (Oxygen meter®) with an accuracy of 0.1^{o}C, DO meter (Oxygen meter®) with an accuracy of 0.01 mg/L, spectrophotometer (Thermo scientific Genesys 150®) (Oxygen meter®) with an accuracy of 0.001mg/L, UV-VIS spectrophotometer (Unico®) with a wavelength of 200-750 nm, a digital scale (Mini Digital Platform Scale I-2000®) with an accuracy of 0.01 g, centrifuge (Backman coulter®) with speed control ±50 rpm, measuring cup with an accuracy 1 mL, blender (Miyako BL-152 GF®) with a capacity 1,5 L, sieve with size 40-60 mesh, basin with volume 10 L, pellet machine (Mincer Manual VIPOO-A12®) with a capacity of 5 kg, aerator (Amara®), and oven (Memret®) with maximum temperature ±70^{o}C.

Research Design

This study used a completely randomized design (CRD) with four treatments and three replications. The feed is made with isoprotein by 38%. The treatment in this study refers to the study of Amin *et al.* (2011). The treatments included:

P0: feed not added with phytase enzymes
P1: feed added with phytase enzymes at a dose of 30 mg/100g of vegetable ingredients
P2: feed added with phytase enzymes at a dose of 50 mg/100g of vegetable ingredients
P3: feed added with phytase enzymes at a dose of 70 mg/100g of vegetable ingredients

Feed Formulation

The feed formulations used are presented in Table 1.

Table 1. The feed formulations used

Description	Treatment (g/1000 g)			
Material (g)	P0	P1	P2	P3
Fish meal	375	375	375	375
Soybean meal	402	402	402	402
Tapioca	44	43.84	43.73	43.62
Rice Bran	144	144	144	144
Fish oil	2.5	2.5	2.5	2.5

Corn oil	2.5	2.5	2.5	2.5
Premix	30	30	30	30
Phytase enzyme	0	0.16	0.27	0.38
Total (g)	1000	1000	1000	1000
Nutrient components (%)				
Protein (%)	37.00	37.64	38.68	39.16
Phytic Acid (%)*	2.5	2.5	2.5	2.5
lipid (%)	2,65	3.40	1.56	1.65
Nitrogen free-extract (NFE) (%)	31.69	31.68	30.88	29.86
Ash (%)	13.85	14.62	14.40	15.42
Water (%)	7.91	6.84	9.67	9.16
Crude fiber (%)	6.90	5.83	4.81	4.75

Note: *Phytic acid content: 3.88% soybean meal (Cao *et al*., 2007); bran 6.9% (Sumiati *et al*., 2001).

Working Procedure

Preparation and Preparation of Experimental Feed

Prepare all the materials used, and the feed raw materials are weighed according to the formulation. Next, the soybean meal and rice bran are mixed and stirred until homogeneous. The material is stirred again until evenly distributed, and the phytase enzyme is added according to the treatment dose. Then incubated for two hours at 37^{o}C (Matsumoto *et al*., 2001), the material mixture was used as the building blocks of feed.

Making feed is done by mixing all the ingredients from the fewest to the most until all the ingredients are evenly and homogeneously mixed. After the ingredients are well mixed, add warm water until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, and dried in the sun to dry. After the feed is dry, it is put into a container and labeled with the treatment dose.

Preparation of Test Fish Rearing Media

The container used to maintain snakehead is an aquarium measuring 25 x 25 x 25 cm^3. Before the aquarium was used, it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g/L. Then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water, and an aeration hose is connected to the aerator, left overnight, and then labeled according to treatment (Zainuri *et al*., 2017).

Fish Rearing

The snakehead was 3-4 cm in size, with a stocking density of 2 fish/L (Zainuri *et al*., 2017). Fish were acclimatized first for three days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given three times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat, 2013). The adapted fish were weighed, and their body length was measured first as initial data. Fish were kept for 30 days. During rearing, the fish were fed with a frequency of three times a day (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every ten days during rearing, and sampling was carried out every three days.

Parameters

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Matsumoto *et al.* (2001) *in* Amin *et al.* (2011), namely vegetable material and the enzyme phytase, which was mixed and incubated at 37 °C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Absolute Length Growth

The absolute length growth of snakehead is calculated using the formula according to Lugert *et al.* (2014):

$$L = Lt - L0$$

Information:
L: Growth in absolute length (cm)
Lt: Fish length at the end of rearing (cm)
L0: Fish length at the beginning of rearing (cm)

Absolute Weight Growth

The absolute weight growth of snakehead is calculated using the formula according to Lugert *et al.* (2014):

$$W = Wt - W0$$

Information:
W: Absolute weight growth (g)
Wt: Fish weight at the end of rearing (g)
W0: Fish weight at the beginning of rearing (g)

Feed Efficiency

Feed efficiency is calculated using the formula according to NRC (2011):

$$FE = \frac{(Wt+D)-Wo}{f} x 100\%$$

Information:
FE: Feed efficiency (%)
Wt: Fish biomass at the end of rearing (g)
W0: Fish biomass at the beginning of rearing (g)
D: Dead fish biomass during rearing (g)
F: Feed consumed (g)

Protein Efficiency Ratio

Calculation of protein efficiency ratio (PER) using the formula according to Hardy and Barrows (2002):

$$\text{Protein efficiency ratio} = \frac{Wt-Wo}{Pi}$$

Information:
Wt: Fish biomass at the end of rearing (g)
W0: Fish biomass at the start of rearing (g)
Pi: Weight of feed protein consumed (g)

Survival

The survival of snakehead is calculated using the formula according to Wang *et al.* (2018):

$$\text{Survival (\%)} = \frac{Nt}{No} x\ 100\%$$

Information:
Nt: Number of live fish at the end of rearing (fish)
N0: Number of fish at the beginning of rearing (fish)

Water quality

The water quality of the snakehead rearing media measured included temperature, dissolved oxygen, pH, and ammonia. Temperature (°C) was measured every day, dissolved oxygen (mg/L) and pH were measured once every ten days. Ammonia was measured at the beginning and end of rearing.

Data Analysis

The data obtained were analyzed using analysis of variance at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Dissolved Phosphorus Content

Dissolved phosphorus was measured to determine the action of enzymes *in vitro*. The content of dissolved phosphorus in the feed of each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment

Treatment	Dissolved Phosphorus (mg/L)
P0	3.87
P1	4.57
P2	5.12
P3	5.26

Based on Table 2. It is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter), and the lowest was found in P0 (without the addition of phytase enzyme). Phytic acid in the feed can be hydrolyzed by phytase, which is added to it. The amount of dissolved phosphorus in the feed provides evidence of this. Phytase performs better at hydrolyzing phytic acid the higher the dissolved phosphorus content indicates. The findings demonstrated that the P3 treatment, which had a dose of 70 mg/100 g of vegetable ingredients, led to higher levels of dissolved phosphorus, whereas the P0 treatment had the lowest levels of phosphorus production (treatment without phytase enzyme).

Based on Amin *et al*. (2011), feeding with the addition of the phytase enzyme increased the mineral P in the catfish's body. The fish body needs mineral P for metabolic activities. Increased metabolic processes in the fish's body will spur fish to consume more feed, and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed, and less is wasted as feces. It can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing are presented in Table 3.

Table 3. Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing

Parameters	Treatments			
	P0	P1	P2	P3
L (cm)	2.25 ± 0.02^{a}	2.31 ± 0.04^{a}	2.34 ± 0.04^{a}	2.48 ± 0.08^{b}

W (g)	1.12 ± 0.02 [a]	1.39 ± 0.05 [b]	1.42 ± 0.02 [b]	1.49 ± 0.04 [c]
FE (%)	62.13 ± 7.06 [a]	74.36 ± 3.32 [b]	75.18 ± 0.93 [b]	79.44 ± 0.99 [b]
PER	1.68 ± 0.19 [a]	1.98 ± 0.09 [b]	1.94 ± 0.02 [b]	2.03 ± 0.03 [b]
SR (%)	91.67 ± 7.64	100 ± 0.00	100 ± 0.00	100 ± 0.00

Note: The numbers in the same row, followed by different superscript letters, show significantly different effects on the 95% LSD test.

Based on the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute growth of length, weight, and feed efficiency of snakehead. The Least Significant Difference Test (LSD) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than in other treatments, and the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that fish use, the more efficient the feed for growth (Amin *et al.*, 2020). Many factors, including feed quality, influence the feed efficiency. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body, and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the study's results, the highest feed efficiency in the P3 treatment at 79.44%. However, feed efficiency was high because, according to Craig and Helfrich (2017), the value of good feed efficiency was more than 50%. Mukti *et al*. (2020) state the higher the feed efficiency value, the more efficient the feed is for its growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, and it was suspected that in the P0 treatment, the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes, so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

The variance analysis showed that adding phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead feed. The Least Significant Difference Test results showed that the protein efficiency ratio in P3 was significantly higher than P0 but not significantly different than P1 and P2. The variance analysis showed that adding phytase enzymes with different doses in the feed did not significantly affect the survival of snakehead.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that adding phytase enzymes to feed, especially at P3 (70 mg/100 g vegetable matter), caused the highest ratio of feed protein efficiency in snakehead. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed, which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. (Rachmawati and Samidjan, 2014). According to Bhilave *et al.* (2012), the protein efficiency ratio determines protein's effectiveness by measuring weight gain in grams per gram of protein consumed. Based on this study's results, the lowest protein was found in treatment P0 at 37.00%, and the highest protein was found in treatment P3, which was 39.16%. Snakehead-fed feed without adding phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed. As a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish and will even be wasted as feces. Amin *et al*. (2011) stated that fish could not digest phytic acid, so it will be released into the waters.

The variance analysis showed that adding phytase with different doses in the feed had no significant effect on the survival of the snakehead. The survival of fish is influenced by several factors, one of which is water quality during rearing. This study's water quality followed the life needs of snakehead.

Water Quality

Data on water quality during rearing is presented in Table 4. This study's water quality followed the life needs of snakehead fish.

Table 4. Data on water quality during rearing

Parameters	Treatments				Optimum Value
	P0	P1	P2	P3	
Temperature (°C)	25.1 – 29.9	25.1 – 29.7	25.2 – 2.,9	25.2 – 29.7	25-30 (Rukmini, 2013)
pH	6.3 – 7.4	6.2 –7.6	6.1 – 7.5	6.1 – 7.5	4.0 – 9.0 (Muflikhah *et al.* 2008)
Dissolved oxygen (mg/L)	4.19 – 4.81	4.15 – 4.74	4.08 – 4.90	4.01 – 4.82	> 3 (Muflikhah *et al.* 2008)
Ammonia (mg/L)	0.02 – 0.37	0.02 – 0.25	0.02 – 0.25	0.02 – 0.18	< 1 (Jianguang,1997)

The water quality in this study was following the life needs of snakehead fish. The water temperature during the study ranged from 25.1 to 29.9°C, according to Rukmini (2013), the optimal temperature for the development of snakehead fish life ranged from 25 to 30°C. The pH of the water ranged from 6.1 to 7.6 and dissolved oxygen in snakehead fish rearing water during the study ranged from 4.01 to 4.90 mg/L. According to Muflikhah *et al*. (2008), a good pH for the rearing of snakehead fish fry ranged from 4-9 and dissolved oxygen at least 3 mg/L. Ammonia content 0.02 – 0.37 mg/L. According to Jianguang (1997), the good ammonia content for snakehead fish is less than 1 mg/L.

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses significantly affected growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead. However, it has no significant effect on the survival of snakehead. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg/100 g of vegetable ingredients (P3).

CONFLICT OF INTEREST

The authors declares there is no conflict of interest.

AUTHOR CONTRIBUTION

The contribution of each author is a follows; Rinda Julita Fahlevie collecting and analysis data, Mohamad Amin participated in conception, experimental design, drafting, and manuscript preparation, Retno Cahya Mukti drafted the manuscript and revision.

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LETTER OF ACCEPTANCE

August 23rd, 2023

Dear Mohamad Amin,

We are pleased to inform that the following paper:

ID #	Authors	Title
34076	Rinda Julita Fahlevie, Mohamad Amin and Retno Cahya Mukti	The Effect of Additional Phytase Enzymes with Different Dosages on Feed to Growth, Feed Efficiency, and Survival of Snakehead Fish (*Channa striata*)

has been **ACCEPTED** in our journal and will be published in **Journal of Aquaculture and Fish Health Volume 12 No. 3 (2023).**

Thank you for choosing to publish in our journal.

Kindest Regards,

Luthfiana Aprilianita Sari, S.Pi., M.Si.
Chief Editor - Journal of Aquaculture and Fish Health

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The Effect of Additional Phytase Enzymes with Different Dosages on Feed to Growth, Feed Efficiency, and Survival of Snakehead Fish (*Channa striata*)

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Feed, Phytase enzymes, Phytic acid, Snakehead

Abstract

Snakehead (*Channa striata*) is a type of freshwater fish. Artificial feed generally contains animal and vegetable protein sources. The use of vegetable protein in snakehead has been carried out. However, it is still not optimal due to the presence of phytic acid in vegetable materials which makes fish unable to digest food properly. Therefore, it is necessary to add a phytase enzyme to hydrolyze phytic acid. This study aims to determine the effect of adding phytase with different doses to artificial feed on the growth, protein efficiency ratio, feed efficiency, and survival of snakeheads. This study used a completely randomized design (CRD) which consisted of four treatments and three replications, namely P0 (without the addition of phytase), P1 (addition of phytase 30 mg/100 g vegetable material), P2 (addition of phytase 50 mg/100 g vegetable material), P3 (addition of phytase 70 mg/100 g vegetable material). The size of the fish used in this study was 3-4 cm, with a stocking density of 2 fish/L fish. The rearing of the study was 30 days. The results showed that the addition of phytase 70 mg/100g vegetable material (P3) was the best result with absolute weight growth (1.49 g), absolute length growth (2.48 cm), feed efficiency (79.44%), protein efficiency ratio (2.03%), and survival (100%).

INTRODUCTION

Snakehead *(C. striata)* is one type of freshwater fish that is also one of the most popular consumption fish in Southeast Asia (Song *et al.*, 2013). Natural catches still dominate the availability of snakeheads. Snakehead can be cultivated with artificial feeding. The main obstacle in the cultivation of snakehead is maintaining a low life and relatively slow growth (Triyanto *et al.,* 2020).

Based on Supandi *et al*. (2016), snakehead-fed artificial feed with a protein content of 40% resulted in the best growth. Snakehead is a carnivorous fish that uses more animal protein. The use of vegetable protein in snakehead has been carried out but still needs to be optimal due to the presence of phytic acid in the vegetable material. One of the vegetable ingredients used is soybean meal and bran. Soybean meal is one of the feed in-

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gredients that contain high protein. However, soy meal also contains minerals combined with phytic acid, making it difficult for the body to absorb and bran. According to Rasyid (2017), the phytic acid content in soybean meal reaches 0.605mg/g. While the bran contains about 0.370 mg/g phytic acid. Phytic acid is the primary source of phosphorus (P) in these raw vegetable materials (Amin *et al.,* 2011). Under natural conditions, this phytic acid will form bonds with minerals (Ca, Mg, and Fe) and proteins so that minerals and proteins cannot be digested by the fish's body, resulting in lower absorption. As a result, soybean meal and bran utilization could be more optimal.

Phytic acid can be reduced by heating, but heating can damage the feed's nutrients. Another way to reduce phytic acid is to use phytase enzymes which can hydrolyze phytic acid gradually into its derivative compounds, which can be dissolved and absorbed in the digestive system (Sari and Ginting, 2012). Winata *et al.* (2018) stated that the phytase enzyme could break down phytic acid into phosphoric acid in the feed so that the nutrients in the fish body are absorbed optimally. Several studies on the addition of phytase enzymes in fish-made feed, among others, in white snapper with the addition of phytase enzymes at a dose of 1.5 g/kg of feed resulted in the highest feed efficiency value of 20.41% (Panjaitan *et al.,* 2019).

The addition of phytase enzymes to an artificial feed of 500 mg/kg resulted in a feed utilization efficiency of 84.55% in catfish (Kosim *et al*., 2016). Adding the phytase enzyme 1000 mg/kg of feed suppresses the efficiency of maximum feed utilization by 28.5% against grouper duck (Zulaeha *et al*., 2015). Adding the phytase enzyme 1,200 mg/kg of feed produced a relative growth rate and optimal feed utilization efficiency of 11.9%/day and 67.5% for tilapia (Restianti *et al*., 2016). Based on the description above, the dose of the phytase enzyme in snakehead feed has yet to be discovered. Therefore, it is necessary to research the addition of phytase enzymes to feed to determine its effect on feed utilization, growth, and survival rate of snakeheads.

METHODOLOGY
Ethical Approval

Not applicable.

Place and Time

This research was conducted at the Fisheries Basic Laboratory, Aquaculture Study Program, Faculty of Agriculture, Sriwijaya University. Feed proximate analysis was carried out at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Dissolved phosphorus analysis of feed was carried out at the Palembang Industrial Center. This research was conducted in July – August 2021.

Research Materials

The materials used in this study were snakehead size 3-4 cm, a fish meal with a protein content of 62.65%, soybean meal with a protein content of 37%, tapioca with a protein content of 0.5%, bran with a protein content of 13%, fish oil, corn oil, premix, phytase enzyme Bio-Phytase 5000®, and potassium permanganate. The tools used in this research are aquarium measuring 25 x 25 x 25 cm, pH meter (ATC digital tester®) with an accuracy of 0.1 pH units, thermometer (Oxygen meter®) with an accuracy of 0.1°C, DO meter (Oxygen meter®) with an accuracy of 0.01 mg/L, spectrophotometer (Thermo scientific Genesys 150®) (Oxygen meter®) with an accuracy of 0.001mg/L, UV-VIS spectrophotometer (Unico®) with a wavelength of 200-750 nm, a digital scale (Mini Digital Platform Scale I-2000®) with an accuracy of 0.01 g, centrifuge (Backman coulter®) with speed control ±50 rpm, measuring cup with an accuracy 1 mL, blender (Miyako BL-152 GF®) with a capacity 1,5 L, sieve with size 40-60 mesh, basin with volume 10 L, pellet machine (Mincer Manual

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VIPOO-A12®) with a capacity of 5 kg, aerator (Amara®), and oven (Memret®) with maximum temperature ±70 °C.

Research Design

This study used a completely randomized design (CRD) with four treatments and three replications. The feed is made with isoprotein by 38%. The treatment in this study refers to the study of Amin *et al.* (2011). The treatments included P0 (feed not added with phytase enzymes); P1 (feed added with phytase enzymes at a dose of 30 mg/100g of vegetable ingredients); P2 (feed added with phytase enzymes at a dose of 50 mg/ 100g of vegetable ingredients); and P3 (feed added with phytase enzymes at a dose of 70 mg/100g of vegetable ingredients).

Work Procedure

Preparation and Preparation of Experimental Feed

The feed formulations used are presented in Table 1.

Table 1. The feed formulations used.

Description	Treatment (g/1000 g)			
Material (g)	P0	P1	P2	P3
Fish meal	375	375	375	375
Soybean meal	402	402	402	402
Tapioca	44	43.84	43.73	43.62
Rice Bran	144	144	144	144
Fish oil	2.5	2.5	2.5	2.5
Corn oil	2.5	2.5	2.5	2.5
Premix	30	30	30	30
Phytase enzyme	0	0.16	0.27	0.38
Total (g)	1000	1000	1000	1000
Nutrient components (%)				
Protein (%)	37.00	37.64	38.68	39.16
Phytic Acid (%) *	2.5	2.5	2.5	2.5
lipid (%)	2,65	3.40	1.56	1.65
Nitrogen free-extract (NFE) (%)	31.69	31.68	30.88	29.86
Ash (%)	13.85	14.62	14.40	15.42
Water (%)	7.91	6.84	9.67	9.16
Crude fiber (%)	6.90	5.83	4.81	4.75

Note: *Phytic acid content: 3.88% soybean meal (Cao *et al.*, 2007); bran 6.9% (Sumiati *et al.*, 2001).

Prepare all the materials used and the feed raw materials are weighed according to the formulation. Next, the soybean meal and rice bran are mixed and stirred until homogeneous. The material is stirred again until evenly distributed, and the phytase enzyme is added according to the treatment dose. Then incubated for two hours at 37°C (Matsumoto *et al.*, 2001), the material mixture was used as the building blocks of feed.

Making feed is done by mixing all the ingredients from the fewest to the most until all the ingredients are evenly and homogeneously mixed. After the ingredients are well mixed, add warm water until smooth. Dough feed that has been printed using a feed printing machine. Feed that has been printed, and dried in the sun to dry. After the feed is dry, it is put into a container and labeled with the treatment dose.

Preparation of Test Fish Rearing Media

The container used to maintain the snakehead is an aquarium measuring 25 x 25 x 25 cm. Before the aquarium was used,

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it was cleaned and disinfected using potassium permanganate with a concentration of 1.2 g/L. Then the aquarium was rinsed and dried. After the aquarium is clean, the aquarium is filled with 10 L of water and an aeration hose is connected to the aerator, left overnight, and then labeled according to treatment (Zainuri *et al.*, 2017).

Fish Rearing

The snakehead was 3-4 cm in size, with a stocking density of 2 fish/L (Zainuri *et al.*, 2017). Fish were acclimatized first for three days to adjust to the new environment and were still given treatment feed without the addition of phytase enzymes with the method *at satiation* and given three times a day, namely at 08.00 am, 12.00 am, and 4.00 pm (Hidayat *et al.*, 2013). The adapted fish were weighed, and their body length was measured first as initial data. Fish were kept for 30 days. During rearing, the fish were fed with a frequency of three times a day (08.00 am, 12.00 am, and 4.00 pm) *at satiation*. Sampling was carried out every ten days during rearing, and sampling was carried out every three days.

Parameters

Dissolved Phosphorus Content Test

Testing methods phosphorus dissolved from the feed by Masumoto *et al.* (2001), namely vegetable material and the enzyme phytase, which was mixed and incubated at 37 °C for 2 hours was taken as much as 50 mg and added 5 mL of distilled water, then centrifuged at a speed of 1700 rpm for 10 minutes. The supernatant formed was used to measure dissolved P using a spectrophotometer.

Absolute Length Growth

The absolute length growth of the snakehead is calculated as follows (Extrada *et al.*, 2013):

$L = L_t - L_0$

Where:

L = growth in absolute length (cm)
L_t = final length (cm)
L_0 = initial length (cm)

Absolute Weight Growth

The absolute weight growth of the snakehead is calculated using the formula below (Extrada *et al.*, 2013):

$W = W_t - W_0$

Where:

W = absolute weight growth (g)
W_t = final weight (g)
W_0 = initial weight (g)

Feed Efficiency

Feed efficiency is calculated using the formula according to NRC (2011):

$FE = \frac{(Wt+D)-Wo}{f} x100\%$

Where:

FE = Feed efficiency (%)
W_t = Final fish biomass (g)
W_0 = Initial fish biomass (g)
D = Dead fish biomass (g)
F = Feed consumed (g)

Protein Efficiency Ratio

Calculation of protein efficiency ratio (PER) using the formula according to Hardy and Barrows (2002):

$PER = \frac{Wt-Wo}{Pi}$

Where:

W_t = Final fish biomass (g)
W_0 = Initial fish biomass (g)
P_i = feed protein consumed (g)

Survival Rate

The survival rate of snakehead is calculated using the formula according to Wang *et al.* (2018):

$SR = \frac{Nt}{No} \times 100\%$

Where:

SR = survival rate (%)
N_t = final number of fish
N_0 = initial number of fish

Water Quality

The water quality of the snakehead rearing media measured included temperature, dissolved oxygen, pH, and ammonia. Temperature (°C) was measured every day and dissolved oxygen (mg/L) and pH were measured once every ten days. Ammonia was measured at the beginning and end of rearing.

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Data Analysis

The data obtained were analyzed using analysis of variance at a 95% confidence interval. If there is a significant difference, it is continued with the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Dissolved phosphorus was measured to determine the action of enzymes *in vitro*. The content of dissolved phosphorus in the feed of each treatment is presented in Table 2.

Table 2. Dissolved phosphorus content in feed at each treatment.

Treatment	Dissolved Phosphorus (mg/L)
P0	3.87
P1	4.57
P2	5.12
P3	5.26

Based on Table 2, it is known that the highest dissolved phosphorus was found in P3 (addition of phytase enzyme 70 mg/100 g of vegetable matter), and the lowest was found in P0 (without the addition of phytase enzyme). Phytic acid in the feed can be hydrolyzed by phytase, which is added to it. The amount of dissolved phosphorus in the feed provides evidence of this. Phytase performs better at hydrolyzing phytic acid the higher the dissolved phosphorus content indicates. The findings demonstrated that the P3 treatment, which had a dose of 70 mg/100 g of vegetable ingredients, led to higher levels of dissolved phosphorus, whereas the P0 treatment had the lowest levels of phosphorus production (treatment without phytase enzyme).

Based on Amin *et al.* (2011), feeding with the addition of the phytase enzyme increased the mineral P in the catfish's body. The fish body needs mineral P for metabolic activities. Increased metabolic processes in the fish's body will spur fish to consume more feed, and fish growth will increase. The results showed that the feed given the phytase enzyme at a dose of 70 mg/100 g of vegetable matter gave the highest growth compared to other treatments. According to Khotimah (2020), the more dissolved phosphorus increases, the more phosphorus is absorbed, and less is wasted as feces. It can be seen from the results of the study that the higher the dissolved phosphorus, the higher the growth. High growth indicates an efficient use of feed.

Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing are presented in Table 3.

Table 3. Data on average growth in length and absolute weight, feed efficiency, protein efficiency ratio, and survival of snakehead during rearing.

Parameters	Treatments			
	P0	P1	P2	P3
L (cm)	2.25 ± 0.02 [a]	2.31 ± 0.04 [a]	2.34 ± 0.04 [a]	2.48 ± 0.08 [b]
W (g)	1.12 ± 0.02 [a]	1.39 ± 0.05 [b]	1.42 ± 0.02 [b]	1.49 ± 0.04 [c]
FE (%)	62.13 ± 7.06 [a]	74.36 ± 3.32 [b]	75.18 ± 0.93 [b]	79.44 ± 0.99 [b]
PER	1.68 ± 0.19 [a]	1.98 ± 0.09 [b]	1.94 ± 0.02 [b]	2.03 ± 0.03 [b]
SR (%)	91.67 ± 7.64	100 ± 0.00	100 ± 0.00	100 ± 0.00

Note: The numbers in the same row, followed by different superscript letters, show significantly different effects on the 95% LSD test.

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Based on the analysis of variance, it was shown that the addition of phytase enzyme with different doses had a significant effect on the absolute grown significantly affects efficiency of snakehead. The Least Significant Difference Test (LSD) showed that the absolute length growth in P3 was significantly higher than in the other treatments. Likewise, the absolute weight growth in P3 was significantly higher than in other treatments, and the feed efficiency value showed that P3 was significantly higher than P0 but not significantly different from P1 and P2.

Feed efficiency is the percentage of feed utilization to produce fish growth. The higher the feed efficiency value indicates that fish use, the more efficient the feed for growth (Amin *et al.,* 2020). Many factors, including feed quality, influence the feed efficiency. According to Isnawati *et al.* (2015), the feed eaten by fish will be processed in the body, and the nutritional elements or nutrients will be absorbed to be used to build tissues so that growth occurs. The increase in the efficiency value of feed utilization indicates that the feed consumed has good quality so that it can be utilized efficiently. Based on the study's results, the highest feed efficiency in the P3 treatment at 79.44%.

However, feed efficiency was high because, according to Craig and Helfrich (2017), the value of good feed efficiency was more than 50%. Mukti *et al*. (2021) state the higher the feed efficiency value, the more efficient the feed is for its growth. On the other hand, the P0 treatment had the lowest feed utilization efficiency value, and it was suspected that in the P0 treatment, the phytic acid content in the test feed did not decompose because it did not contain phytase enzymes, so the availability of P was thought to be insufficient so that its growth was low. In addition to releasing phosphorus, phytase enzymes are thought to be able to release proteins bound in phytic acid.

The variance analysis showed that adding phytase enzymes with different doses significantly affected the protein efficiency ratio of snakehead feed. The Least Significant Difference Test results showed that the protein efficiency ratio in P3 was significantly higher than P0 but not significantly different than P1 and P2. The variance analysis showed that adding phytase enzymes with different doses in the feed did not significantly affect the survival of snakeheads.

Indicators of protein utilization by fish for growth can be seen in the efficiency of the protein feed consumed. The results showed that adding phytase enzymes to feed, especially at P3 (70 mg/ 100 g vegetable matter), caused the highest ratio of feed protein efficiency in snakehead. The high value of the protein efficiency ratio can be caused by the phytase enzyme in the feed, which can reduce and decompose phytic acid and break the bonds between phytic acid and protein and mineral complexes, thus giving an effect on digestive enzymes, especially protein-breaking enzymes in breaking down protein into amino acids. (Rachmawati and Samidjan, 2014).

According to Bhilave *et al.* (2012), the protein efficiency ratio determines protein's effectiveness by measuring weight gain in grams per gram of protein consumed. Based on this study's results, the lowest protein was found in treatment P0 at 37.00%, and the highest protein was found in treatment P3, which was 39.16%. Snakehead-fed feed without adding phytase enzyme (P0) caused lower growth, feed efficiency, and feed protein efficiency ratio. This is presumably because the phytic acid in the feed cannot be decomposed. As a result, the nutrients bound to the phytic acid cannot be absorbed and utilized by fish and will even be wasted as feces. Amin *et al*. (2011) stated that fish cannot digest phytic acid, so it will be released into the waters.

The variance analysis showed that adding phytase with different doses in the feed had no significant effect on the survival of the snakehead. The survival of fish

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is influenced by several factors, one of which is water quality during rearing. This study's water quality followed the life needs of snakehead. Data on water quality during rearing is presented in Table 4. This study's water quality followed the needs of snakehead fish.

Table 4. Data on water quality during rearing.

Parameters	Treatments			
	P0	P1	P2	P3
Temperature (°C)	25.1 – 29.9	25.1 – 29.7	25.2 – 2.,9	25.2 – 29.7
pH	6.3 – 7.4	6.2 –7.6	6.1 – 7.5	6.1 – 7.5
Dissolved oxygen (mg/L)	4.19 – 4.81	4.15 – 4.74	4.08 – 4.90	4.01 – 4.82
Ammonia (mg/L)	0.02 – 0.37	0.02 – 0.25	0.02 – 0.25	0.02 – 0.18

The water quality in this study was following the life needs of snakehead fish. The water temperature during the study ranged from 25.1 to 29.9 °C, according to Rukmini (2013), the optimal temperature for the development of snakehead fish life ranged from 25 to 30 °C. The pH of the water ranged from 6.1 to 7.6 and dissolved oxygen in snakehead fish-rearing water during the study ranged from 4.01 to 4.90 mg/L. According to Muflikhah *et al.* (2008), a good pH for the rearing of snakehead fish fry ranged from 4-9 and dissolved oxygen at least 3 mg/L. Ammonia content 0.02 – 0.37 mg/L. According to Qin *et al.* (1997), the good ammonia content for snakehead fish is less than 1 mg/L.

CONCLUSION

The addition of phytase enzyme in artificial feed with different doses significantly affected the growth in length, weight, feed efficiency, and protein efficiency ratio of snakehead. However, it has no significant effect on the survival of snakeheads. The best treatment in this study was the addition of the phytase enzyme at a dose of 70 mg/100 g of vegetable ingredients (P3).

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTION

The contribution of each author is as follows: Rinda Julita Fahlevie collected and analyzed data, Mohamad Amin participated in conception, experimental design, drafting, and manuscript preparation, and Retno Cahya Mukti drafted the manuscript and revision.

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