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Effect of Phytase Enzyme on Growth, Nutrient Digestibility and Survival Rate of Catfish *(Pangasius hypothalamus)* Fingerlings

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ABSTRACT

This study was conducted to evaluate the effect of adding phytase enzyme in diet on growth performance, nutrient digestibility, and survival rate in fingerlings of *P. hypothalamus*. Fingerlings of *P. hypothalamus* with the average weight 1.81 ± 0.06 g per fingerling, used in this study were obtained from Muntilan, Central Java. An experimental randomised complete design was used with 5 treatments and 3 repetitions. The treatments were A (0 FTU kg^{-1} diet, B (150 FTU kg^{-1} diet), C (300 FTU kg^{-1} diet), D (450 FTU kg^{-1} diet), and E (600 FTU kg^{-1} diet). The parameters to be determined include specific growth rate (SGR), efficiency of feed utilisation (EFU), protein efficiency ratio (PER), apparent digestibility coefficient protein (ADC_P), apparent digestibility coefficient phosphor (ADC_F), survival rate (SR) and water quality parameters. The experimental results significantly ($P<0.01$) affected SGR, EFU, PER, ADC_P and ADC_F. On the other hand, the had insignificant ($P>0.05$) effect on SR of *P. hypothalamus* fingerlings. Based on the results, it is concluded that optimum doses of phytase enzyme in terms of SGR, EFU, PER, ADC_P and ADC_F in the catfish (*P. hypothalamus*) are 324, 314, 300, 300 and 300 FTU kg^{-1} diet respectively.

Keywords: Catfish, nutrient digestibility, *P. hypothalamus fingerlings*, phytase enzyme, specific growth, survival rate

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INTRODUCTION

Intensive catfish *(P. hypothalamus)* aquaculture needs artificial feed that contains complete nutrients. It should also be efficient and economical. Catfish *(P. hypothalamus)* needs a complete diet which contains fat, protein, carbohydrate, vitamin and minerals. Protein is the most important

element in its diet to support growth (National Research Council [NRC], 1993). The protein in the supplemental diet can be either plant or animal based. The usage of plant-based protein is still limited, because most of them contain high fibre that is difficult to digest. Therefore, plant-based protein is not an optimal diet for the catfish. A common source of plant-based protein is soybean. According to Cao et al. (2007) plant-based protein also includes grains, such as rice and bean containing anti-nutrient compound in the form of phytate acid. They report that soybean contains 3.8% phytate acid chelates with minerals such as magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn), calcium (Ca) and protein which are beneficial for growth of plant, animal, and human. Debnath et al. (2005) found phytate acid (mio-inositol 1,2,3,4,5,6-heksakifosfat) is an anti-nutrient which obstructs nutrient absorption and decrease nutrient efficiency utilisation.

The plant-based feed will result in greater phosphorous pollution of our water. Phosphorous content in the plant-based feed cannot be utilised by the fish because there is a lack of phytase enzyme that could break down phytate acid (Cheng, & Hardy, 2003; Debnath et al., 2005; Kumar et al., 2011; Rachmawati et al., 2017). Kumar et al., (2011) explains that phytate acid is the main storage for phosphorous (P) in plant-based feed, around 80%. Phytate acid in the artificial feed would be excreted by the fish into the water. Microbes that produce phytase enzyme will decompose the phytate acid into phosphorous and release it into the water. High phosphorous content in the water will trigger eutrophication process that hinders fish growth (Baruah et al., 2004). Jegannathan and Nielsen (2013) reported that fish needs phosphorous to fuel its growth. However, phosphorous cannot be directly absorbed by the fish, because it is bound by phytate acid. In turn, it is excreted into the water. According to Jobling et al. (2002), Chung (2001) and NRC (1993) phytase enzyme can increase diet utilisation and regulate nutrient excretion (phosphorous, nitrogen, and mineral) and also hydrolise Phytate in the diet into inositol and phosphate acid. Phytase enzyme hydrolises phytate in order to breakdown minerals (Chung, 2001). Baruah et al. (2007) also explain that phytase enzyme is able to hydrolise phytate (myo-inositol hexakisphosphate) into myo-inositol mono, di, tetra and pentaphosphate and organic phosphate. Phytase enzyme will unbind phosphorous from phytate acid and also unbind other nutrient elements (Ravindran, 2000). The addition of phytase enzyme could unbind phytate from calcium, cuprum, zinc, manganese and increase nutrient absorption in intestinal system, as reported by Junqueria et al. (2011). Studies have examined the role of phytase enzyme in species, such as *Chanos chanos* (Hassan et al., 2009), *Oreochomis niloticus* (Hassan et al., 2013, Olusola & Nwanna, 2014), *Marsupenaeus japonicas* (Bulbul et al., 2015), *Psetta maxima* (Danwitz et al.,

2016), *Penaeus monodon* (Rachmawati, & Samidjan, 2016), *Channos channos* (Rachmawati et al., 2017). This study was conducted to evaluate the effect of adding phytase enzyme in diet and its optimal dose to enhance growth, nutrient digestibility, and survival rate in fingerlings of *Pangasius hypothalamus*.

MATERIALS AND METHODS

Test Animal

Catfish Fingerlings of *Pangasius hypothalamus* with an average weight 1.81 ± 0.06 g per fingerling were obtained from aquaculture fingerlings in Muntilan, Central Java, Indonesia. The fish were selected based on their size uniformity, completeness of organs, and physical performance (Rachmawati et al., 2017). The fingerlings were first adapted to the culture media and its diet for one week. Before treatment, the fingerlings were fasted for one day to clean their digestive system. The Catfish fingerlings of *P. hypothalamus* were raised for 42 days and fed three times a day at satiation (Rachmawati & Samidjan, 2016). They were weighed weekly.

Container Research

Containers used in this study were made of happa with the dimension of 1m x 1m x 0.6m or equal to 600 l. Density for each treatment and repetition (25 fingerlings/m^3) was based on Dasuki et al. (2013).

Test Feed

The food was shaped into a pellet and dried at room temperature. It contained iso-protein (30%) and iso-energy (2720 kcal kg^{-1} diet) based on Hassan et al. (2013) and Debnath et al. (2005). Diet ingredients for artificial feed contained fish meal as source of animal protein, soybean meal as source of plant protein, corn meal, bran meal, wheat flour as source of carbohydrate, fish oil and corn oil as source of fat, mineral and vitamin mix as source of vitamin, CMC as binder, Cr_2O_3 1 % as indirect indicator to test nutrient digestibility and phytase enzyme. The soybean meal and phytase enzyme was first mixed at various doses and kept for 24 hours. It was followed by weighing of every ingredient as shown in Table 1. All the ingredients were later mixed, starting from the smallest amount to the biggest amount.

The diet treatment used proximate analysis based on the method proposed by Association of Official Analytical Chemists (AOAC, 1990). The experiment used Natuphos 5000G phytase enzyme that was produced by PT. BASF Indonesia. Natuphos 5000G form was granule which contains active materials of *myo-inositol-hexakisphosphate* β-*phosphohydrolase* (EC 3.1.3.8) which was produced by *Aspergillus niger*. Natuphos 5000G contains phytase enzyme 5.000 FTU/g. One unit of phytase activity (*Phytase Unit*/ FTU) is defined as the amount of enzyme which releases 1 micro molecule of nonorganic per minute from 0,0051 mol/l of phytase acid on pH of 5.5 and 37°C (Debnath et al., 2005). The diet composition for the study is shown in Table 1.

Table 1
Composition and proximate analysis of experimental diet

Ingredients (g)	A	B	C	D	E
Phytase enzyme	0	0.15	0.3	0.45	0.6
Fish meal	28	28	28	28	28
Soybean meal	20	20	20	20	20
Corn meal	16	16	16	16	16
Rice bran	12	12	12	12	12
Wheat flour	17	16.85	16.70	16.55	16.40
Fish oil	1	1	1	1	1
Corn oil	1	1	1	1	1
Min.Vit Mix	3.00	3.00	3.00	3.00	3.00
Cr_2O_3	1.00	1.00	1.00	1.00	1.00
CMC	1.00	1.00	1.00	1.00	1.00
Total (g)	100	100	100	100	100
Results of Proximate Analyses					
Protein (%)*	30.35	30.55	30.29	30.30	30.48
Fat (%)*	7.16	7.25	7.18	7.20	7.30
BETN (%)*	43.21	43.60	43.45	43.66	43.56
Energy (kcal)	272.28	272.57	272.48	272.36	272.50
Ratio E/P (kcal/g)	8.97	8.65	8.78	8.88	8.60

Notes. The values were calculated based on Digestible Energy (Wilson, 1982); 1 g protein equals 3.5 kcal, 1 g fat equals 8.1 kcal, and 1 g carbohydrate equals 2.5 kcal.
According De Silva (1987), the optimal E/P ratio for growth ranges from 8 kcal/g to 12kcal/g.
*Animal Nutrient Laboratory, Faculty of Husbandry and Agriculture, Diponegoro University (2016).

Research Methods

Experimental randomised complete design was used with 5 treatments and 3 repetitions. The treatments in this study entailed adding various doses of phytase enzyme, namely A (0 FTU kg^{-1} diet), B (150 FTU kg^{-1} diet), C (300 FTU kg^{-1} diet), D (450 FTU kg^{-1} diet), and E (600 FTU kg^{-1} diet). Based on Debnath et al's (2005) method, 500 FTU equals to 100 mg phytase enzyme; therefore, the doses of 150, 300, 450 and 600 FTU equals 30, 60, 90, and 120 mg phytase enzyme respectively. The dosage amount used in this study is adapted from Debnath et al. (2005) who reported the optimum dose of the phytase enzyme for growth for catfish (*Pangasius pangasius*) was 500 FTU kg^{-1} diet. To get 500 FTU of enzymatic activity, 100 mg of phytase enzyme is needed.

Data

Data collected during this study were Specific Growth Rate (SGR) using Steffens's method (1989), Efficiency of Feed utilization (EFU), and Protein Efficiency Ratio (PER), according to Tacon (1987), apparent digestibility content protein ($ADC_{P)}$ and apparent digestibility content phosphor (ADC_F) according to Fenucci (1981), and Survival Rate (SR) according to NRC (1993). The chromic oxide levels in feeds and faeces were analysed using a modified colorimetric method (Fenucci, 1981). The levels were measured with a spectrophotometer (540 nm) (Shimadzu UV-2102 PC, UV-visible Scanning Spectrophotometer) after perchloric acid oxidation and forming a coloured complex with diphenylcarbazide (DPC). Samples were analysed to determine phosphorous (P) concentrations by flame atomic absorption spectrophotometry on a Shimadzu AA6800 (Shimadzu, Japan). Variables of water quality that were tested were pH (Jenway 3510), DO (Jenway 970), temperature and Ammoniac (HANNA: HI. 8633). Aerator to recirculate the water was placed in every container. The parameters were measured by the following equations:

$$\left[SGR = \ln\left(\frac{\text{Final weight} - \text{Initial weight}}{\text{Time experiment}}\right) \text{x } 100\%\right] \quad [1]$$

$$\left[EFU = \frac{(\text{Final weight} - \text{Initial weight})}{\text{The amount of feed consumed}} \text{ x } 100\%\right] \quad [2]$$

$$\left[PER = \frac{\text{The amount of feed consumed}}{(\text{Final weight} + \text{Total weight fish deat}) - \text{Initial weight}} \; x \; 100\%\right] \quad [3]$$

$$\left[ADCp = 100 \text{ x}\left\{\frac{\%\ Cr2O3 \text{ in the feed}}{\%\ Cr2O3 \text{ in the feces}} \text{x} \frac{\%\ \text{protein in the feces}}{\%\ \text{protein in the feed}}\right\}\right] \quad [4]$$

$$\left[ADCf = 100 \; x\left\{\frac{\%\ Cr2O3 \text{ in the feed}}{\%\ Cr2O3 \text{ in the feces}} x \frac{\%\ \text{fosfor in the feces}}{\%\ \text{fosfor in the feed}}\right\}\right] \quad [5]$$

$$\left[\text{Survival } (\%) = \frac{(\text{Initial count} - \text{Final count})}{\text{Initial count}} \; x \; 100\%\right] \quad [6]$$

Statistical Analysis

Analysis of Variance (ANOVA) was used to analyse data after conducting tests of normality, homogeneity, and additives. If the ANOVA shows significant ($P<0.05$) or very significant ($P<0.01$) results, Duncan test are conducted to find out whether the means are different (Steel et al., 1993). To find out the optimal dose of the enzyme, polynomial orthogonal test using Minitab version 17.0 and Maple version 12.0 was conducted.

RESULTS AND DISCUSSION

The results of study on *P. hypothalamus* fingerlings for specific growth rate (SGR), efficiency of feed utilization (EFU), protein efficiency ratio (PER), apparent digestibility content protein ($ADC_{P)}$,

apparent digestibility content phosphorous (ADC_f), survival rate (SR) *Catfish (P. hypothalamus)* and phytate acid content artificial feed, faeces and decreased phytate acid are shown in Table 2.

The addition of phytase enzyme on the diet significantly ($p<0.01$) increased the specific growth rate (SGR) of the catfish (*P. hypothalamus*) fingerlings as shown in Table 2. Similar studies were also conducted by Debnath et al. (2005), Tahoun et al. (2009), Olusola and Nwanna (2014) and Danwitz et al. (2016). The increase in specific growth rate is due adding of phytase enzyme that was able to break down the phytate acid anti-nutrient. The contents of phytate acid in the diet are A (0.64%), B (0.63%), C (0.58%), D (0.68) and E (0.69%), as shown in Table 2. The existence of phytate acid can hamper the growth, as reported by NCR (1993) which stated that 0.5% phytate acid in the diet can reduce growth and diet efficiency for rainbow trout (*O. mysskis*). Tacon (1987) reported that 2.58% phytate acid can reduce growth, diet efficiency, protein efficiency ratio and cause mortality.

Table 2
The values of SGR, EFU, PER, ADC_P ACD_F SR of *Catfish* (P. hypothalamus) *and phytate acid content in artificial feed, faeces and decreased phytate acid*

Data	Treatment				
	A	B	C	D	E
SGR %/day)	3.07±0.37^{c}	3.38±0.28^{bc}	4.14±0.17^{a}	3.85±0.11^{ab}	3.80±0.15^{ab}
EFU (%)	51.80±4.60^{b}	58.43±5.15^{ab}	66.97±1.42^{a}	66.61±1.72^{a}	65.86±2.93^{a}
PER	1.73±0.16^{b}	1.95±0.17^{ab}	2.23±0.05^{a}	2.22±0.06^{a}	2.20±0.10^{a}
ADC_P (%)	75.27±0.02^{c}	79.65±0.05^{bc}	81.93±0.05^{a}	77.35±0.04^{ab}	75.35±0.04^{c}
ADC_F (%)	71.37±0.03^{c}	73.64±0.04^{bc}	74.89±0.06^{a}	72.53±0.02^{ab}	71.32±0.04^{c}
SR (%)	82,67±4,62^{a}	85,33±2,31^{a}	85,33±2,31^{a}	86,67±2,31^{a}	86,67±2.31^{a}
Phytate acid Artificial Feeds (%)	0,64	0,63	0,58	0,68	0,69
Phytate acid Fish Faeces (%)	0,54	0,47	0,29	0,46	0,52
Phytate Acids Decrease (%)	0,1	0,16	0,29	0,22	0,17

Note. The values with the same superscripts in the column show that there was no difference.

The contents of phytate acid in the faeces A (0.54%), B (0.47%), C (0.29%), D (0.46%) and E (0.52%) as shown in Table 2. The decrease in the phytate acid were A (0.1%), B (0.16%), C (0.29%), D (0.22%) and E (0.17%) respectively. The decrease in phytate acid shows that it has been broken down by the phytase enzyme.

Cao et al. (2007) reported the breakdown of phytate acid can increase the absorption of nutrient. The hydrolysis reaction was thought to decrease phytate acid and unbind protein and minerals. According to Wang et al. (2009), the breakdown of the bond can increase the activities of trypsinogen and become trypsin enzyme that breakdown protein becoming amino acids. Moreover, it can unbind phytate and multivalent cation. The breakdown of these elements cause protein and minerals readily available to dissolve in the body of the fish.

The highest SGR values were obtained from C treatment (300 FTU kg^{-1} diet) that was 4.14 %/day, while the lowest was obtained from A treatment that was 3.07 %/day. The study shows that the C treatment is the best. The 300 FTU^{-1} kg diet dose is effective in unbind phosphorous, protein, and mineral from soybean meal, as shown by Tahoun et al. (2009) that unbinding of complex compound is able to ease phosphorous, protein, and mineral absorption and it can increase growth. The lowest SGR was due to no activity of phytase enzyme to break down phytate acid. This was supported by Rachmawati and Samidjan (2016), who found that treatment without additional phytase enzyme was not able to hydrolyse phytate acid from the diet; therefore, protein and mineral could not be utilised. The equation from orthogonal test is cubical equation as $Y = -5{,}679x^3 - 0{,}254x^2 + 3{,}8468x + 3{,}1916$, $R^2 = 0{,}9396$ (Figure 1). The optimum dose of the phytase enzyme in the diet was 324 FTU kg^{-1} diet with the maximum value of SGR 4.21 %/day.

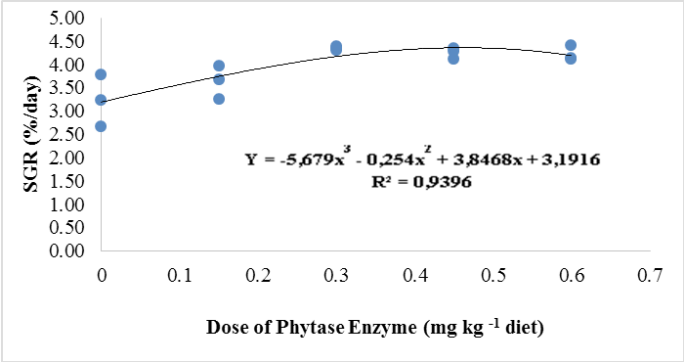

Figure 1. SGR polynomial orthogonal (%/day) of catfish *(P. hypothalamus)* fingerlings

The analysis covariance results show that the addition of various doses of phytase enzyme had significant effect ($P<0.01$) on efficiency of feed utilisation (EFU) of the catfish (*P. hypothalamus*). Phytase enzyme therefore could increase effectiveness and efficiency of energy usage for metabolism. The highest EFU in the catfish (*P. hypothalamus*) that was treated by adding phytase enzyme was C treatment (300 FTUkg^{-1} diet) was 66.97%, while the lowest EFU was A treatment

which was 51.80%. Further, it was found phytase enzyme dose of 300 FTU kg^{-1} diet was effective to improve energy retention, as Olukosi (2008) reported that adding enzyme could optimise nutrient absorption in the intestinal system. Meanwhile no addition of phytase enzyme in the supplemental diet brought about the existence of anti-nutrient to keep EFU low. Rachmawati and Samidjan (2016) found that anti-nutrient hampered nutrient absorption and utilisation. Orthogonal test resulted in the cubical response as $Y = -90{,}37x^3 + 3{,}3968x^2 + 53{,}962x + 45{,}589$, $R^2 = 0{,}8985$ (Figure 2). The optimum dose of the phytase enzyme in the diet on the EFU was 314 FTU kg^{-1} diet with the maximum value of EFU 67.7 %.

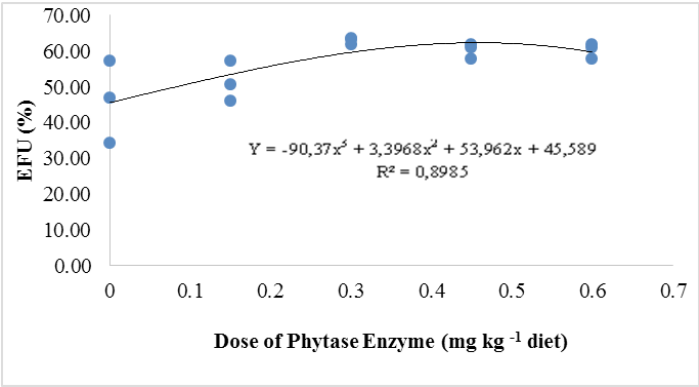

Figure 2. EFU polynomial orthogonal (%) of catfish *(P. hypothalamus)* fingerlings

Protein efficiency ratio measures additional weight of the fish due to 1 g protein consumption (Tacon, 1987). The results of analysis covariance (Table 2) show that the additional various doses of phytase enzyme in the diet had a significant effect ($P<0.01$) on the protein efficiency ratio (PER) of catfish (*P. hypothalamus*) fingerlings. It was thought that the addition of phytase enzyme was able to increase phytate acid solvability; therefore, the nutrient that was bound by the phytate acid can be absorbed by intestinal system and increase protein digestibility (Hassan et al., 2013). The highest PER was obtained from the C treatment (300 mg kg^{-1} diet) that was 2.23, and followed by D and E, B and A treatment with the values of 2.22, 1.95, and 1.73 respectively. The high PER in treatment C (300 FTU kg^{-1} diet) among other treatments was due to the ability of the phytase enzyme to reduce and breakdown phytate acid enzyme and unbind the bond of protein and minerals with the phytate acid. In turn, it increases amino acid solvability, therefore it was easier to be digested by intestinal system and leading to increase in biomass. Meanwhile the low PER in treatment A (0 FTU kg^{-1} diet) was due to no activity on phytase on the supplemental

diet. According to Olusola and Nwanna (2014) and Rachmawati et al. (2017), the phytate acid existence in the supplemental diet hampered the absorption of protein. The orthogonal test resulted in the cubical response as $Y = -3{,}2099x^3 + 0{,}2857x^2 + 1{,}7675x + 1{,}5219$, $R^2 = 0{,}8983$ (Figure 3). The optimum dose of the phytase enzyme in the diet on the PER was 300 FTU kg^{-1} diet with the maximum value of PER 2.23.

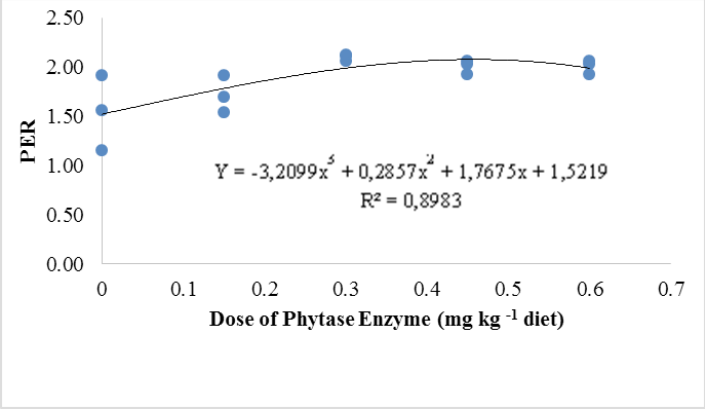

Figure 3. PER polynomial orthogonal of catfish *(P. hypothalamus)* fingerlings

Digestibility is an important indicator on the effectiveness of the diet. If digestibility of the diet is low, it means that the fish cannot optimally utilise the diet. Factors that affect digestibility are chemical and physical characteristics of the diet, types of the diet, nutrient contents, digestive enzyme in the fish, size of the fish, chemical and physical characteristics of the water (NRC, 1993). The results show that the effect of various doses in the supplemented diet was very significant ($P<0.01$) on apparent digestibility content of the protein (ADC_P) and on apparent digestibility content of phosphorus fosfor (ADC_F) in the catfish (*P. hypothalamus*). The C treatment (300 FTU kg^{-1} diet) showed the highest ADC_P and ADC_F with the values of 81.93% and 74.89% respectively. *Peniophora lycii* fungi hydrolises phytate acid in the intestine. *Peniophora lycii* can synthesise protein by obtaining carbon from carbohydrate (glucosamaltose and sucrose) nitrogen from organic source or inorganic carbon from minerals (Vandenberg et al., 2011). The existence of the micro-organism in the diet can improve quality, absorption of nutrients and digestibility of the diet (Sajjadi & Carter, 2004). They reported that phytase enzyme can unbind anti-nutrient in the diet, such as phytate acid, non-starch poly-saccharide, and trypsin inhibitor, and thus, improving nutrient digestibility. Rachmawati and Samidjan (2016) also suggested that raw protein and

total protein digestibility depends on the ability of the fish to absorb the nutrients. The increase in diet digestibility was followed by the increase of PER (2.23) and EFU (66.97%). Therefore, it had a positive effect on specific growth (4.14%/day).

Digestibility test, as displayed in Table 2, shows that the addition of phytase enzyme of 150-450 FTU kg^{-1} diet boosts ADC_P and ADC_F. Similar finding was also reported by Storebakken et al. (1998) that the addition of enzyme in the diet increased protein digestibility and retention. Debnath et al. (2005) also found that Atlantic salmon increased its digestibility and retention of protein if phytase enzyme was added to its diet. Meanwhile, without addition of phytase enzyme, resulted in low digestibility and retention of protein. Hunter (2001) found that the addition of enzyme increased protein digestibility from 84.5% to 87.7%. Similar results were found in the species of carp (Vielma et al., 2004), rainbow trout (Sugiura et al., 2001; Forster et al., 1999), Labeo rohita (Marjan et al., 2014). Other researchers, Baruah et al. (2004), Rachmawati and Samidjan (2016), and Rachmawati et al. (2017) observed that the addition of phytase enzyme in the diet made of plant ingredients increased protein digestibility due to the breakdown of phytin-protein complex. Cao et al. (2007) also reported that phytase enzyme can unbind anti-nutrient in the diet, such as phytate acid, non-starch polysaccharide, and trypsin inhibitor, and it also improved nutrient digestibility. The equation resulting from orthogonal test is $Y = 115.88x^3 – 166.63x^2 + 58.429x + 75.051$, $R^2 = 0.9396$ (Figure 4). The optimum dose of the phytase enzyme in the diet on the ACD_P was 300 FTU kg^- diet with the maximum value of ADC_P 81.92 %.

The equation resulting from orthogonal test is $Y = 14.815x^3 – 49.354x^2 + 24.19x + 71.314$, $R^2 = 0.85$ (Figure 5). The optimum dose of the phytase enzyme in the diet on the ACD_F was 300 FTU kg^- diet with the maximum value of ADC_F 74.89 %.

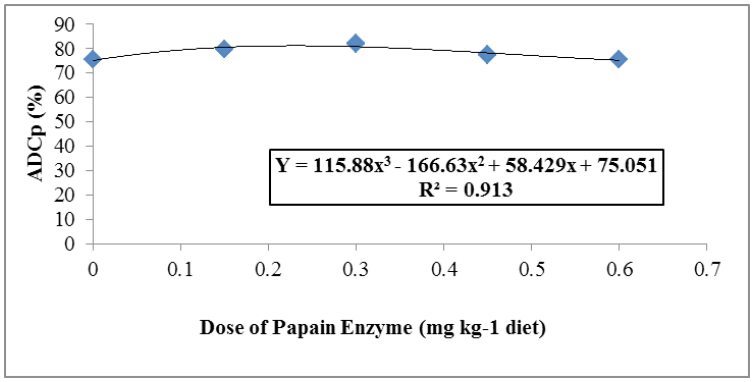

Figure 4. ADC_P polynomial orthogonal (%) of catfish *(P. hypothalamus)* fingerlings

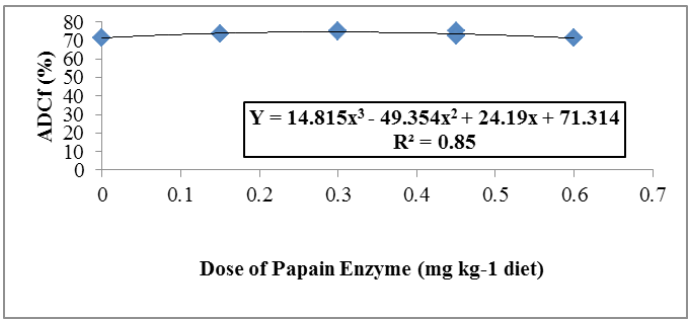

Figure 5. ADC_F polynomial orthogonal (%) of catfish *(P. hypothalamus)* fingerlings

The ANOVA results show that the addition of various doses of phytase enzyme has no significant effect (P>0.05) on SR in the catfish (*P. hypothalamus*). The same results were reported by Hassan et al. (2009), Hassan et al. (2013), Olusola and Nwanna (2014), Bulbul et al. (2015), Danwitz et al. (2016), Rachmawati and Samidjan (2016), and Rachmawati et al. (2017). As reported by Yakuputiyage (2013), diet alone is not a factor to determine SR, as the latter is determined by fish handling and media quality. Robinson et al. (2002) explained the addition of phytase enzyme did not significantly influence the SR. Mortality during study was caused by abiotic factors (Stickey, 1979), such as environment, handling, population density, competitor, disease, age, and predators.

CONCLUSION

This study has shown adding phytase enzyme in artificial feed significantly increased specific growth rate and nutrient digestibility in catfish (*P. hypothalamus*). The optimal doses of phytase enzyme diet in terms of SGR, EFU, PER, ADC_P and ADC_F in the catfish (*P. hypothalamus*) were 324, 314, 300, 300 and 300 FTU kg^{-1} diet respectively.

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REFERENCES

Association of Official Analytical Chemists. (1990). O*fficial methods of analysis of AOAC Internationa*l (15th Ed.). Washington, DC.

Baruah, K., Sahhu, N. P., Pal, A. K., & Debnath, D. (2004). Dietary phytase: an ideal approach for a cost effective and low-polluting aqua feed. *NAGA World Fish Center Quarterly, 27*(3&4), 15-19.

Baruah, K., Sahhu, N. P., Pal, A. K., Debnath, D., & Mukherjee, S. C. (2007). Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-Optimal protein level. *Aquaculture Research*, *38*(2), 109-120.

Bulbul, M., Kader, M. A., Ambak, M. A., Hossain, M. S., Ishikawa, M., & Koshio, S. (2015). Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for Kuruma Shrimp, *Marsupenaeus japonicus*. *Aquaculture Elsevier*, *438*, 98-104.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., & Li, D. (2007). *Application of Microbial Phytase in Fish Feed, 40*(4), 497-507.

Cheng, Z. J., & Hardy, R. W. (2003). Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 218*(1-4), 501–514.

Chung, T. K. (2001). *Sustaining livestock production and environment.* Singapore: Food and Agriculture Asia Pacific Development.

Danwitz, A. Von, van Bussel, C. G. J., Klatt, S. F., & Schulz, C. (2016). Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (*Psetta maxima* L.). *Aquaculture Elsevier*, *450*, 405-411.

Dasuki, A., Auta, J., & Oniye, S. J. (2013). Effect of stocking density on production of *Clarias gariepinus* (Tuegels) in floating bamboo cages at Kubanni Reservoir, Zaria, Nigeria. *Pure and Applied Sciences, 6*(1), 112-117. ISSN: 2006-6996.

Debnath, D., Pal, A. K., Sahu, N. P., Yengkokpam, S., & Mukherjee, S. C. (2005). Effects of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. *Aquaculture Research*, *36*(2), 180-187.

De Silva, S. S. (1987). Finfish nutrition research in Asia. In *Proceeding of the Second Asian Fish Nutrition Network Meeting* (Vol. 128). Heinemann, Asia, Singapore.

Fenucci, J. L. (1981). Studies on the nutrition of marine shrimp of the *Penaeus*. (Ph.D. Thesis). University of Houston, USA. Retrieved from https://elibrary.ru/item.asp?id=7341293

Forster, I., Higgs, D. A., Dosanjh, B. S., & Rowshandeli, M. (1999). Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11ºC fresh water. *Aquaculture*, *179*(1), 109–125.

Hassan, S., Altaff, K., & Satyanarayana, T. (2009). Use of soybean meal supplemented with cell bound phytase for replacement of fish meal in the diet of juvenile milkfish, *Chanos chanos*. *Pakistan Journal of Nutrition, 8*(4), 341–344.

Hassan, M. S., Soltan, M. A., Agouz, H. M., & Badr, A. M. (2013). Influences of calcium/phosphorous ratio on supplemental microbial phytase efficiency for Nile tilapia. *Aquatic Research*, *39*(3), 205–213.

Hunter, B. T. (2001). The downside of soybean consumption. *NOHA NEWS*, *26*(4), 3.

Jegannathan, K. R., & Nielsen, P. H. (2013). Environmental assessment of enzyme use in industrial production – a literature review. *Journal of cleaner production, 42*, 228–240.

Jobling, M., Gomes, E., & Diaz, J. (2002). Feed types manufacture and ingredients. In D. Houlihan, T. Boujard, & M. Jobling (Eds), *Food Intake in Fish* (pp. 25–48). Oxford, UK: Blackwell Science.

Junqueira, O. M., Duarte, K. F., Assuena, V., Filardi, R. S., Laurentis, A. C., & Praes, M. F. F. M. (2011). Effects of phytase supplementation on performance, bone densitometry and carcass yield in broiler chicks. *Animal Science, 33*(3), 301–307.

Kumar, V., Sinha, A. K., Makkar, H. P. S., De Boeck, G., & Becker, K. (2011). Phytate and phytase in fish nutrition. *Journal of Animal Physiology and Animal Nutrition, 96*(3), 335–364.

Marjan, S., Afzal, M., Shah, S. Z. H., Hussain, S. M., & Mubarik, M. S. (2014). Role of phytase supplementation in improving mineral digestibility of dry bread meal based diet fed to *Labeo rohita* fingerlings. *Pakistan Journal of Life and Social Science, 12*(3), 150–154.

National Research Council (1993). *Nutrient requirements of fish*. Washington, DC: The National Academy Press.

Olukosi, O. A. (2008). Biochemistry of phytate and phytases: Applications in monogastric nutrition. *Experimental Biology*, *24*(2), 58-63.

Olusola, S. E., & Nwanna, L. C. (2014). Growth performance of Nile tilapia (*Oreochromis niloticus*) fed processed soybean meal based diets supplemented with phytase. *International Journal of Aquaculture, 4*(08), 48-54.

Rachmawati, D., & Samidjan, I. (2016). Effect of phytase enzyme on growth boost in the artificial feed made of plant protein to shorten production time of giant tiger prawn [*Penaeus monodon*, (Fabricus 1798)]. *Aquatic Procedia*, *7*(016), 46 – 53.

Rachmawati, D., Istiyanto, S., & Maizirwan, M. (2017). Effect of phytase on growth performance, diet utilization efficiency and nutrient digestibility in fingerlings of *Chanos chanos* (Forsskal 1775). *Philippine Journal of Science*, *146*(3), 237–245.

Ravindran, V. (2000). *Effect of Natuphos Phytase on the bioavailability of protein and amino acids – a review* (pp. 1-10.). Palmerston North, New Zealand: Monogastric Research Center Institute of Food Nutrition and Human Health Massey University

Robinson, E. H., Li, M. H., & Manning, B. B. (2002). Comparison of microbial phytase and dicalcium phosphate for growth and bone mineralization of pond-raised channel catfish, *Ictalurus punctatus*. *Journal of Applied Aquaculture, 12*(3), 81–88.

Sajjadi, M., & Carter, C. G. (2004). Effects of phytate acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar*, L.). *Aquaculture Nutrition, 10*(2), 135-142.

Steel, R. G. D., Torrie, J. H., & Dickey, D. A. (1993). *Principles and procedures of Statistics* (3rd Ed.). New York, USA: McGraw Hill International Book Company, Inc.

Steffens, W. (1989). *Principles of fish nutrition*. London, England: Elis Horwood Limited.

Stickey, R.R. (1979). *Principle of warm water aquaculture*. New York: John Willey and Sons.

Storebakken, T., Shearer, K. D., & Roem, A. J. (1998). Availability of protein, phosphorus and other elements in fishmeal, soy-protein concentrate and hytase-treated soy-protein concentrate-based diets to Atlantic salmon, *Salmo salar*. *Aquaculture*, *161*(1), 365–379.

Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M., & Hardy, R. W. (2001). Availability of phosphorus and trace elements in low phytate varieties of barley and corn for rainbow trout *Oncorhynchus mykiss*. *Aquaculture, 170*(3-4), 285–296.

Tahoun, A. M., Abo-State, H. A., & Hamouda, Y. A. (2009). Effect of adding commercial phytase to DDGS based diets on the performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) Fingerlings. *Agriculture and Environment Science*, *5*(4), 550-555.

Tacon, A. G. (1987). *The nutrition and feeding of farmed fish and shrimp-A training manual*. Brazil: FAO of the United Nations.

Vandenberg, G. W., Scott, S. L., Sarker, P. K., Dallaire, V., & De La Noue, J. (2011). Encapsulation of microbial phytase: Effects on phosphorous bioavailability in rainbow trout (*Oncorhynchus mykiss*). *Agriculture and Agri-Food*, *169*(3-4), 230-243.

Vielma, J., Ruohonen, K., Gabauand, J., & Vogel, K. (2004). Top-spraying soybean mealbased diets with phytase improves protein and mineral digestibility but not lysine utilization in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, *35*(10), 955–964.

Wang, F., Yang, Y. H., Han, Z. Z., Dong, H. W., Yang, C. H., & You, Z. Y. (2009). Effects of pretreatment of soybean meal and phytase sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture International, 17*(2), 143-157.

Wilson, R. P. (1982). Energy relationships in catfish diets. In R.R. Stickney and R. T. Lovell (Eds.), *Nutrition and Feeding of Channel Catfish* (pp. 193-201). Southern Cooperative Series.

Yakuputiyage, A. (2013). On-Farm feeding and feed management Strategies in tropical aquaculture. In M. R. Hasan & M. B. New, (Eds.), *On-Farm Feeding and Feed Management in Aquaculture* (pp. 361-376). FAO Fisheries and Aquaculture Technical Paper.