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Effect of The Phytase Enzyme Addition in The Artificial Feed on Digestibility of Feed, Feed Conversion Ratio and Growth of Gift Tilapia Saline Fish (*Oreochromis niloticus*) Nursery Stadia I

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Abstract. The purpose of this study was to determine the effect of adding the phytase enzyme in the artificial feed on digestibility of feed, feed conversion ratio and growth of gift tilapia saline fish (*Oreochromis niloticus*) nursery stadia I. The fish samples in this study used gift tilapia saline fish (*O. niloticus*) with an average weight of 0,62 ± 0,008 g/fish and the stocking density of 1 fish^{1} L. Experimental method used in this study was completely randomized design with 4 treatments and 3 repetitions. The treatments were by adding phytase enzyme in artificial feed with the different level of doses those were A (0 FTU kg^{1} feed), B (500 FTU kg^{1} feed), C (1000 FTU kg^{1} feed) and D (1500 FTU kg^{1} feed). The results show that the addition of phytase enzyme was significantly ($P<0.01$) affected on apparent digestibility coefficient of protein (ADC_P), apparent digestibility coefficient of Phospor (ADC_F), feed conversion ratio (FCR), protein efficiency ratio (PER), and relative growth rate (RGR), on the other hand it insignificantly ($P>0.05$) affected on Survival Rate (SR) of gift tilapia saline fish. The optimum doses of phytase enzyme on RGR, FCR, PER, ADC_P and ADC_F of gift tilapia saline fish ranged from 1060 to 1100 FTU kg^{-1} feed.

Keywords : artificial feed, phytase enzyme, digestibility of feed, feed conversion ratio, gift tilapia saline fish (*Oreochromis niloticus*)

1. Introduction

Gift tilapia saline fish (*Oreochromis niloticus*) has several advantages, such as the fish can be grown in the used shrimp pond which has high salinity (0.5-30 ppt), easily cultivated, easily adapted, fast growth, able to eat any kind of feed (omnivores), and has high adaptability to various conditions. The intensive cultivation of the fish highly depends on the artificial (commercial) feed. Commercial feed currently still uses plant based ingredients such as soybean meal, as source of protein. Baruah *et al* [1] reported that the soybean meal has been commonly used in the artificial feed; however, it contains

anti-nutrient that can reduce the benefits of using plant based artificial feed. One of the anti-nutrient is Phytate acid [2]. Cao *et al* [3] reported that every kilogram of soybean meal contains Phytate acid 3.88 g. Phytate acid will bind minerals that have 2 or 3 valence (calcium, ion, zinc, and magnecium) to form complex that is difficult to absorb [4]. Besides bonding with minerals, Phytate acid also binds with protein and amino acid to cause decreasing in digestibility [5]. The digestibility of feed also depends on physical and chemical factors, types of feed, nutrient content, type and amount of digestive enzyme, and size and age of fish [6].

Moreover, an increase of plant based feed use creates another problem, that it produces phosphor pollutant into the water. Phosphor in the plant based feed cannot fully be utilized by the fish due to lack of phytase enzyme that decomposes Phytate acid [7][8][9][10]. Kumar *et al* [2] explained that phytate acid bound 80% phophor of the total phosphor available in the plant based feed. Phytate acid in the artificial feed is excreted along with feces into the environment, then it is degraded by microbe that produces phytase enzyme and the phosphor is released into the water. High phosphor concentration in the water will trigger eutrofication that hinders the cultivation of the fish [1]. Jagannathan and Nielsen [11] stated that phosphor is macro nutrient that is needed by animal including fish.

One of the solutions to solve the problem is by adding exogenous phytase enzyme [12] [13][14]. The addition of phytase enzyme into the artificial feed can increase nutrient absorption by the fish and regulate nutrient excretion (phosphor, nitrogen, and minerals) and hydrolyze phytate acid becoming inositol and phosphate acid [12]. Baruah *et al* [1] explained that the enzyme can hydrolyze phytate acid (mio-inositol hexahisphosphate) becoming mio-inositol mono, di, tetra and pentaphosphate and organic phosphate. Besides extracting phosphor from phytate acid, the enzyme can release other nutrients that are bound by phytate complex [5]. Moreover, Vielma *et al* [15] explained that the addition of phytase enzyme into the feed containing 50% soy meal can increase protein and phosphor digestibility of rainbow trout (*Oncorhynchus myskiss* Walbaum).

The objective of the study is to find the effects of the phytase enzyme addition into the artificial feed on feed digestibility, phosphor digestibility, feed conversion ratio, and growth of tilapia gifts saline.

2. Materials and Methods

The Gift tilapia saline fish (*Oreochromis niloticus*) with the average weight 0.62 ± 0.008 g were used in this study and obtained from Center for Brackish Water Aquaculture, Jepara, Central Java. The cultivation density for every treatment and repetition was one fish per liter [10]. The fish used in the study have been purposedly selected and healthy, no deformity, uniform in size and weight [6].

The feed used in this study was phytase enzyme supplemented feed in the form of pellet and with the protein content of 30%. The treatments were with the doses of A (0 FTU kg^{-1} feed), B (500 FTU kg^{-1} feed), C (1000 FTU kg^{-1} feed), and D (1500 FTU kg^{-1} feed). The feed contained fish meal as a source of animal protein; soybean meal as a source of plant based protein; corn meal, rice bran, wheat flour as sources of carbohydrate; fish oil and corn oil as sources of fat, vitamin mix and mineral mix as sources of vitamin and mineral; CMC as a binder; phytase enzyme as an unbinder of phytate acid; and Cr_2O_3 as much as 0.5-1%, these were used as indicators of feed digestibility [14].

Preparation on feed treatment done in this study consisted of proximate test for feed treatment [16], calculating feed treatment, and manufacturing feed treatment. The formulated feed ingredients and proximate analysis can be seen in Table 1. Phytase enzyme was Natuphos 5000G 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 of pythase enzyme 5.000 FTU/g. One unit of phytase activity (*Phytase Unit*/FTU) was defined as the amount of enzyme which release 1 micro molecule of non organic per minutes from 0,0051 mol/l of phytase acid on pH of 5,5 and 37^{o}C [17]. To get 500 FTU of enzymatic activity needs 100 mg of phytase enzyme.

The procedure to prepare feeding experiment was first to dissolve an appropriate dose of phytase enzyme into warm water (45° C) and then mix with soybean meal evenly. The mixture was stored in the air sealed container for around 24 h [18]. Artificial feed made by mixing the least amount of the ingredients first and gradually adding and mixing the bigger amount of the ingredients except fat source (corn oil and fish oil) was added after all ingredients have been mixed. The evenly mixture of the artificial feed was formed into granules with the diameter of 1 mm to 2 mm. Then the artificial feed was dried in the oven with temperature of 40° C [14].

Table 1. Composition and Proximate Analysis in the Artificial Feed

Ingredients	Composition			
	A	B	C	D
Phytate enzyme (FTU)	0	0.1	0.2	0.3
Fish meal	25	25	25	25
Soybean meal	26	26	26	26
Corn meal	13	13	13	13
Rice bran	14	14	14	14
Wheat flour	13.5	13.4	13.3	13.2
Fish oil	2	2	2	2
Corn oil	2	2	2	2
Min.Vit	3	3	3	3
Cr_2O_3	0.5	0.5	0.5	0.5
CMC	1	1	1	1
Total (g)	100	100	100	100
Results of Proximate Analyses				
Protein (%) (*)	30.46	30.41	30.69	30.27
Fat (%) (*)	9.35	9.46	9.34	9.45
BETN (Extract without Nitrogen) (%) (*)	41.70	41.40	41.34	41.73
Energy (kkal)	286.62	286.55	286.42	286.81
Ratio E/P	9.41	9.42	9.33	9.48

Notes:

a.The values were calculated based Digestible Energy [19] for 1 g protein equals 3.5 kcal, 1 g fat equals 8.1 kcal, and 1 g carbohydrate equals 2.5 kcal.

b. According [20], the optimal E/P ratio for growth ranges from 8 kcal/g to 12kcal/g.

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Containers used in this study were made of plastic with the volume of 25 l as many as 12 buckets. The buckets were first cleaned with calium permanganat to sterilize the containers from bacteria. After cleansing, the buckets were cleaned with water and then dried off. The buckets were equipped with sand filter and filled with water that has 20 ppt salinity. To provide enough oxygen during experiment was put blower. The fingerlings were raised in recalculated water in order to maintain water quality in optimum range. Finally the buckets were covered with plastic sheet to prevent fish from jumping out and to maintain temperature. The study was started by scaling the weight of the fish, and then the fish were put in the cultivating containers for 42 days. The fish were fed 3 times a day at satiation and fed in the morning at 06:00, in the noon at 12:00, and afternoon at 16:00. The fish were sampled and scaled the weight every week. The feces were siphoned one hour before feeding in order to keep the water media cleaned and viable to raise the fish.

Parameters include Relative Growth Rate (RGR), Protein Efficiency Ratio (PER), Feed Conversion Ratio (FCR) according to [21], Apparent Digestibility Coefficient of Protein (ADC_P) and Apparent Digestibility Coefficient of Phosphor (ADC_F) according to [22], and Survival Rate (SR) according to [14]. The chromic oxide levels in feeds and feces were analyzed using a modified colorimetric method [22]. The levels were measured with a spectrophotometer (540 nm) (Shimadzu UV-2102 PC, UV-visible Scanning Spectrophotometer) after perchloric acid oxidation and forming a colored complex with diphenylcarbazide (DPC). Samples were analyzed to determine phosphorous (P) concentrations by flame atomic absorption spectrophotometer 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 recalculated the water was placed in every container.

$$RGR : \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight x Time experiment}} \times 100\% \quad (1)$$

$$FCR : \frac{\text{The amount of feed consumed}}{(\text{Final weight} + \text{Total weight fish death}) - \text{Initial weight})} \times 100\ \% \quad (2)$$

$$PER : \frac{\text{Final weight} - \text{Initial weight}}{\text{The amount of feed consumed x Protein content of feed}} \times 100\% \quad (3)$$

$$ADC_P : 100\% \left\{ \frac{Cr_2O_3 \text{ in the feed}}{\%\ Cr_2O_3 \text{ in the feces}} \times \frac{\%\ \text{protein in the feces}}{\%\ \text{protein in the feed}} \right\} \quad (4)$$

$$ADC_F : 100\% \left\{ \frac{Cr_2O_3 \text{ in the feed}}{\%\ Cr_2O_3 \text{ in the feces}} \times \frac{\%\ \text{fosfor in the feces}}{\%\ \text{fosfor in the feed}} \right\} \quad (5)$$

$$SR : \frac{\text{Final count}}{\text{Initial count}} \times 100\% \quad (6)$$

Analysis of variance (ANOVA) was used to analyze the data. Before analyzing, the data were first tested the normality, additively, and homogenity. The test was to make sure that the data were normal, homogen, and additive property. If the analysis of variance was significant ($p<0.05$) or highly significant ($p<0.01$), Duncan test was conducted to find out the difference of the treatmens, while water quality data were descriptively analyzed. To determine optimal dose of phytase enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12 [23].

3. Results and Discussion

The results of study on Tilapia Gift Saline Fish (*O.niloticus*) for RGR, FCR, PER, ADC_P, ADC_F, and SR were shown in the Table 2.

Table 2. The Values of RGR, FCR, PER, ADC_P, ADC_F, and SR Tilapia Gift Saline Fish

Data	Treatment			
	A	B	C	D
RGR %/day)	$9,46\pm0,40^{bc}$	$10,62\pm0,69^{b}$	$12,26\pm0,32^{a}$	$9,97\pm0,61^{bc}$
FCR	$2,65\pm0,10^{a}$	$2,36\pm0,24^{b}$	$1,75\pm0,07^{c}$	$2,30\pm0,22^{ab}$
PER	$0,90\pm0,04^{b}$	$1,19\pm0,23^{b}$	$1,95\pm0,05^{a}$	$1,24\pm0,25^{b}$
ADC_P (%)	75.47 ± 0.02^{c}	79.65 ± 0.05^{bc}	83.93 ± 0.05^{a}	77.65 ± 0.04^{ab}
ADC_F (%)	71.57 ± 0.03^{c}	74.64 ± 0.04^{bc}	78.89 ± 0.06^{a}	72.23 ± 0.02^{ab}
SR (%)	$80,00\pm5,00^{a}$	$88,33\pm2,89^{a}$	$88,67\pm2,89^{a}$	$83,\pm2,89^{a}$

Note: The Values with the same superscripts in the column show that there was no difference

Table 2. shows that the addition of phytase enzyme in the artificial feed significantly ($p<0.01$) increased the growth of Gift Tilapia Saline. The results gave evidence that supplemented feed with the phytase enzyme could hydrolyze protein that was bind by phytate acid into amino acid. This amino acid was readily digested and could provide energy to grow [24][25]. According to Chung [12] the addition of phytase enzyme into the artificial feed can increase nutrient absorption by the fish and regulate nutrient excretion (phosphor, nitrogen, and minerals) and hydrolyze phytate acid becoming inositol and phosphate acid. The decomposition of phytate acid makes easier to methabolize protein and mineral compounds. The addition of the enzyme were also reported to increase the weight of *Marsupenaeus japonicus* [26], *Psetta maxima* L. [27], *Panaeus monodon* [6], and *Channos channos* [10].

Table 2 also shows that the addition of phytase enzyme in the artificial feed of 1000 FTU kg^{-1} feed significantly increased the growth of Gift Tilapia Saline compared to the addition of 500 and 1500 FTU kg^{-1} feed. The highest relative growth was obtained in the C treatment (12.26%). It can be concluded that the addition of 1000 FTU phytase on the every kg feed could reduce antinutrients or phytate acid on soybean meal. This finding was supported by Masumoto [6] that the dose of 1000 FTU kg^{-1} feed was the optimal dose to increase growth of *Penaeus monodon*. Yu FN and Wang [28] also reported that the addition of phytase 1000 FTU kg^{-1} feed could increase average weight of crucian carp *Carassius carassius* by 25 percent. Moreover Rachmawati *et al* [10] reported that the enzyme addition of 1000 FTU kg^{-1} feed can increase the weight of *Channos channos*. The same results were also found in carp [29], African catfish [30], striped bass [31], rainbow trout [15], Atlantic salmon [32], Korean Sebastes schlegeli [33].

The lowest growth was obtained in the A treatment as much as 9.46% per day. It was thought due to lack of inositol in the Gift Tillapia Saline, as reported by [14] that when the feed contained phytate acid, it could decrease fish' appetite, the growth, and cause anemia. The deficiency of inositol indicated that phytate acid has not been hydrolized into inositol and phosphate acid as reported by [12] [1]. They explained that the enzyme can hydrolize phytate acid (mio-inositol hexahisphosphate) becoming mio-inositol mono, di, tetra and pentaphosphate and organic phosphate. Moreover, phytate acid can obstruct the decomposition of complex mineral, therefore the availability of minerals, especially phosphore, cannot fullfil the need of Gift Tillapia Saline and in turn it reduce the growth of the fish. NRC [14] stated that phosphor is an important factor in the process of muscle constraction, bone development, and phosphate development that is needed to transform energy. Baruah *et al* [1] and Fox *et al* [18] reported that an increase on growth, raw protein coefficient, sulphur, phosphor total, and phytate phosphor have happened in rainbow trout which was given plant based feed with addition of phytase enzyme.

The results of orthogonal polynomial test on the relationship of phytase enzyme in the artificial feed and the relative growth (Figure 1) had cubical pattern with the equation, $Y = -5.8844x^3 + 9.7867x^2 - 1.1022x + 9.46$, $R^2=0.86$. The optimum dose of the phytase enzyme in the feed on the relative growth was 1060 FTU kg^{-1} feed with the maximum relative growth of 12.28% per day.

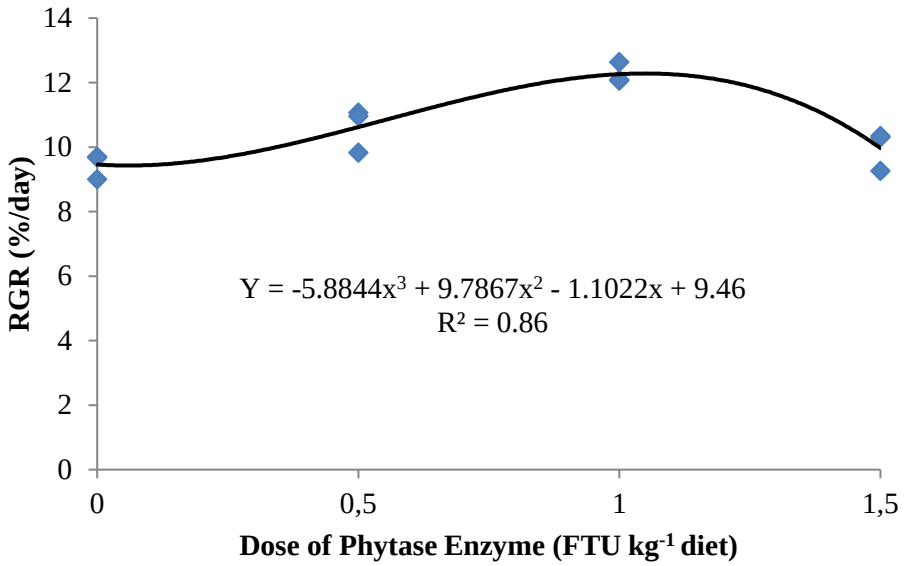

Figure 1. Graph of the Relationship between Phytase Enzyme Addition in the Artificial Feed and RGR of Gift Tillapia Saline (*O. niloticus*)

According to Stickney [34] feed conversion can be found by comparing between the amount of feed and the weight addition of the fish and the weight of dead fish during study. The less feed conversion the more efficient of feed utilization. Table 2 shows that artificial feed with the addition of phytase enzyme (B, C, and D treatments) has feed conversion ratio lower than the artificial feed without the addition of phytase enzyme (A treatment). It was suggested that phytase enzyme can catalyze phytate acid decomposition. Hydrolized reaction was able to unbind between phytate acid and protein and mineral complex that increased trypsinogen activity into trysin enzyme. The trysin enzyme can decompose protein into amino acids, therefore feed utilization efficiency became maximal and feed conversion ratio became low. The similar results of study have been done by [17] in the rainbow trout. Wang *et al* [35] also reported that artificial feed with phytase enzyme addition can improve feed conversion ratio in the rainbow trout. The same result was also found for *L. Rohita* [4].

The addition of phytase enzyme significantly ($P<0.01$) affected on the feed conversion ratio in the Gift Tilapia Saline *(O. niloticus)* (Tabel 2). The results shows that the highest feed conversion ration happened in the A treatment as much as 2.65, while the lowest was in the C treatment as much as 1.75. The C treatment (1000 FTU kg^{-1} feed) resulted in the lowest feed conversion among other treatments, B (500 FTU kg^{-1} feed), D (1500 FTU kg^{-1} feed), and A (0 FTU kg^{-1} feed). It was suggested that the addition of phytase enzyme could increase the efficiency in feed utilization and make feed conversion ratio low. Li and Robinson [36] studied on the addition of 250 units or more microbial phytase in the feed. The results show that there were higher feed consumption, higher weight gain, and lower feed conversion ratio than those without additional microbial phytase in the feed. Phytase enzyme in the feed had very important role since it could increase feed utilization [17].

The relationship between phyta.se enzyme and feed conversion ratio based on the orthogonal polynomial test, as shown in the Figure 2, was cubical. The equation was $Y = 1.9289x^3 – 3.4933x^2 + 0.6644x + 2.6533$, $R^2= 0.84$. The optimum dose of the phytase enzyme in the feed on the feed conversion ratio was 1100 FTU kg^{-1} diet with the maximum value of feed conversion ratio 1.72.

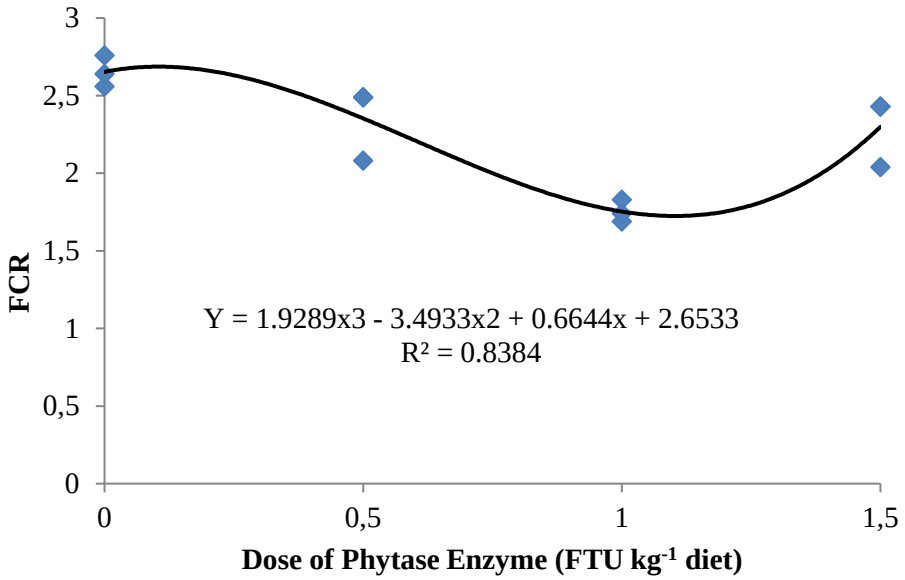

Figure 2. Graph of the Relationship between Phytase Enzyme Addition in the Artificial Feed and FCR of Gift Tillapia Saline (*O. niloticus*)

The addition of phytase enzyme in the feed significantly ($P<0.01$) affected Protein Efficiency Ratio on the Gift Tilapia Saline (*O. niloticus*) (Table 2). The highest protein efficiency ratio reached at C treatment with the value of 1.95, while the lowest was the A treatment with the value 0.90. The C treatment that gave the highest result suggested that the enzyme dose was very effective to reduce and break down the phytate acid, to dissociate between phytate acid and protein and minerals, therefore it affected digestibility enzymes to break down protein into amino acids. Li and Robinson [36] stated that the higher the protein conversion ratio indicates better efficient feed because the fish can utilize protein better. Maximum absorption of protein was due to phytase enzyme that break down phytate acid [37]. It also caused digestibility of phosphor to increase and anti-nutrient to decrease [39]. The breakdown of phytate acid provided favourable situation for the fish to absorb protein. The addition of phytase enzyme that could increase the utilization of protein has been reported by [39][40].

The results of orthogonal polynomial test (Figure 3) shows the relationship between phytase enzyme in the artificial feed and protein efficiency ratio. It had cubical relationship with the equation $Y = -2.6133x^3 + 4.88x^2 – 1.22x + 0.9033$, $R^2=0.88$. The optimum dose of the phytase enzyme in the artificial feed for the protein efficiency ratio was 1100 FTU kg^{-1} diet with the maximum value of the protein efficiency ratio of 1.98.

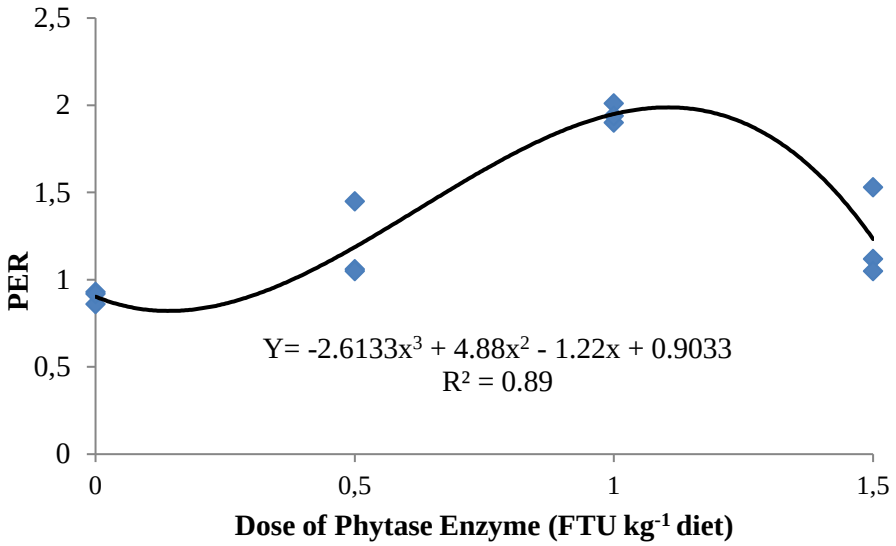

Figure 3. Graph of the Relationship between Phytase Enzyme Addition in the Artificial Feed and PER of Gift Tillapia Saline (*O. niloticus*)

The addition of phytase enzyme significantly ($P<0.01$) affected on the digestibility of protein and the digestibility of phosphor on the Gift Tilapia Saline (*O. niloticus*). Table 2 shows that the addition of phytase enzyme 500-1000 FTU kg^{-1} feed could increase the digestibility protein and the digestibility of phosphor. Storebakken *et al* [40] has already reported that the addition of phytase enzyme increased protein digestibility and protein retention. These results were also confirmed by Debnath *et al* [9] that the addition of phytase enzyme significantly increased protein utilization and digestibility on Atlantic salmon, otherwise they had low protein utilization and digestibility. Hunter [17] also found that the addition of phytase enzyme significantly increased protein digestibility from 84.5% to 87.7%. Similar results were found on carp [15], rainbow trout [40][41], Labeo rohita [42]. Kornegay and Qian [43] and Baruah *et al* [1] also reported that addition of phytase enzyme in the plant based feed increased protein digestibility due to breaking down of phytin-protein compound. The addition of phytase enzyme of 1000 FTU kg^{-1} feed (C treatment) was the optimal dose to disintegrate the anti-nutrient and increase feed digestibility as in the [17] findings. He found that phytase enzyme can break down anti nutrients in the feed, such as phytate acid, non-starch polysaccharide, and trypsin inhibitor. It could also increase feed digestibility.

The results of orthogonal polynomial test (Figure 4) shows the relationship between phytase enzyme in the artificial feed and ADC_P was cubical, with the equation $Y = -22.20x^3 + 36.64x^2 + 2.545x + 73.53$, $R^2 = 0.99$. The optimum dose of the phytase enzyme in the artificial feed for the protein efficiency ratio was 1060 FTU kg^{-1} feed with the maximum value of the protein efficiency ratio of 85.56%.

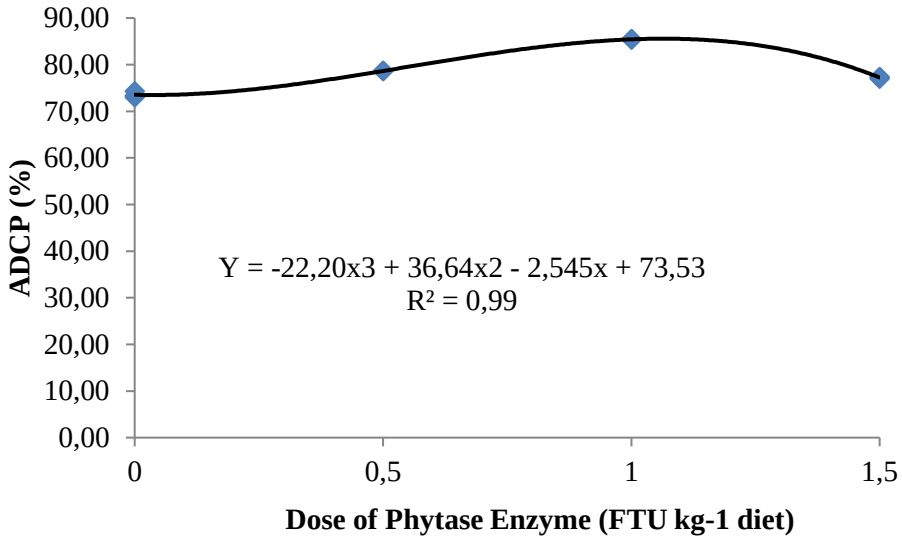

Figure 4. Graph of the Relationship between Phytase Enzyme Addition in the Artificial Feed and ADCp of Gift Tillapia Saline (*O. niloticus*)

The results of orthogonal polynomial test (Figure 5) shows the relationship between phytase enzyme in the artificial feed and ADC_F was cubical, with the equation $Y = -26.627x^3 + 49.753x^2 – 11.71x+72.213$, $R^2 = 0.99$. The optimum dose of the phytase enzyme in the artificial feed for the protein efficiency ratio was 1070 FTU kg^{-1} feed with the maximum value of the protein efficiency ratio of 81.80%.

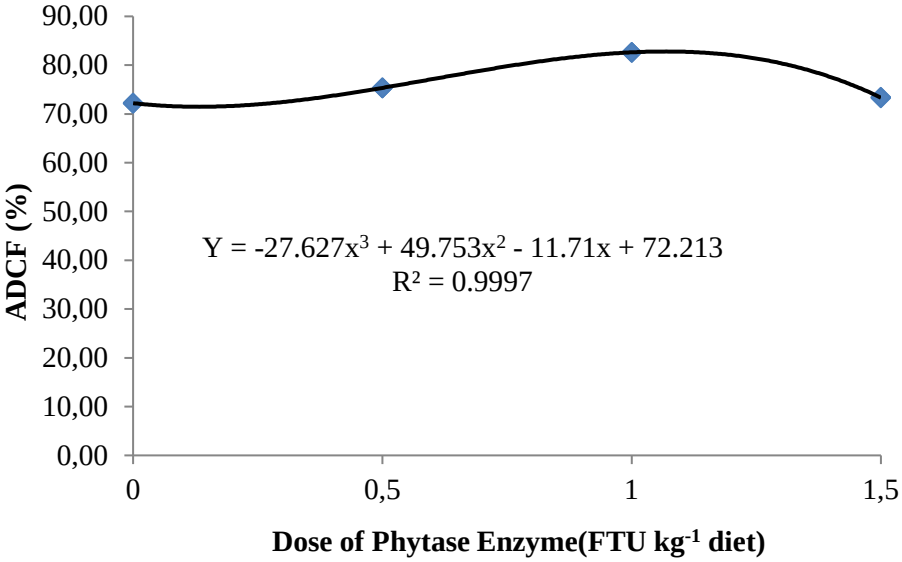

Figure 5. Graph of the Relationship between Phytase Enzyme Addition in the Artificial Feed and ADC_F of Gift Tillapia Saline (*O. niloticus*)

The addition of phytase enzyme in the artificial feed insignificantly affected on survival rate of Gift Tilapia Saline, as shown in the Table 2. The result was in line with the Li Robinson and Manning [44] finding that survival rate was insignificantly affected by addition of phytase enzyme in the feed. The survival rate was affected by internal factors such as gender, heredity, age, reproduction, disease

resistance and external factors such as water quality, density, number and composition of amino acid in the feed [45]. Water of quality during the research still on condition that overpass to the cultivation of Gift Tillapia Saline. The measurement of water parameter during cultivation Gift Tilapia Saline can be seen in the Table 3.

Table 3. Parameters of Water Quality for Gift Tillapia Saline (*O. niloticus*) Cultivation

Treatment	Water Quality			
	Temperature (^{0}C)	pH	DO (mg/l)	NH_3 (%)
A	26 – 33	7.50 – 7.85	3.30 – 3.55	0.0072 – 0.0074
B	26 – 33	7.50 – 7.82	3.24 – 3.48	0.0072 – 0.0074
C	26 – 33	7.50 – 7.81	3.28 – 3.58	0.0072 – 0.0074
D	26 – 33	7.50 – 7.81	3.32 – 3.53	0.0072 – 0.0074
Feasibility	14-38*	6.5 – 8.5*	>2*	<0.1*

Note : * Rachmawati *et al*. (2017)

4. Conclusion

The addition of phytase enzyme in the artificial feed significantly increased on the growth rate, protein efficiency ratio, protein and phosphor digestibility, and decreased feed coversion ratio, otherwise it insignificantly affected on survival rate of the Gift Tillapia Saline (*O. niloticus*). The optimal doses of phytase enzyme in the artificial feed on RGR, FCR, PER, ADC_P and ADC_F of gift tilapia saline fish ranged from 1060 to 1100 FTU kg^{-1} feed.

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