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Effect of Phytase on Growth Performance, Diet Utilization Efficiency and Nutrient Digestibility in Fingerlings of *Chanos chanos* (Forsskal 1775)

Diana Rachmawati[a]*, Istiyanto Samidjan[a], Maizirwan Mel[b]

[a]Department of Fisheries, Faculty of Fisheries and Marine Sciences,
Diponegoro University, Semarang, Indonesia
[b]International Islam University Malaysia, Kuala Lumpur, Selangor, Malaysia

This study evaluated the effect of phytase enzyme in supplemented diet on growth performance, diet utilization efficiency, and nutrient digestibility in *Chanos chanos* fingerlings. Fingerlings of *C. chanos* with an average body weight 3.55±0.08 g with the density one fingerling per liter were fed with four different diets supplemented with phytase enzyme: A (0 FTU kg$^-$ diet), B (500 FTU kg$^-$ diet), C (1000 FTU/kg$^-$diet), and D (1500 FTU kg$^-$ diet). The relative growth rate (RGR), feed conversion ratio (FCR), apparent digestibility coefficient protein (ADC_P), apparent digestibility coefficient phosphor (ADC_F), survival rate (SR), and water quality parameters were determined. The results obtained after feeding trials significantly (P<0.01) affected on RGR, FCR, ADC_P and ADC_F, on the other hand insignificantly (P>0.05) affected on SR of milkfish (*C. chanos*) fingerlings. Based on the results, it is concluded that optimum doses of phytase enzyme diet in terms of RGR, PER, and nutrient digestibility of milkfish (*Chanos chanos*) fingerlings ranges 983 – 1010 FTU kg$^-$ diet, respectively.

Key words: *Chanos chanos*, diet utilization efficiency, digestibility, growth performance, phytase

INTRODUCTION

Milkfish aquaculture highly depends on artificial feed. Artificial feed uses plant-based ingredients such as soy flour as a source of vegetable protein (NRC 1993). According to Kumar et al. (2011), the main challenge in using soybean meal is due to the presence of its anti-nutritional factors (ANFs) known as phytic acid (phytate) (myo inositol - 1, 2, 3, 4, 5, 6-hexakisphosphate). Studies conducted by Cao et al. (2007) supported those findings that phytate of 3.88 g · kg^{-1} diet chelates with mineral such as magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn), calcium (Ca) and protein which are very beneficial for growth of plant, animal, and human. These elements are also forming poorly soluble complexes which are difficult to decompose because of its chelating characteristics. Phytic acid reacts with protein forming phytate-protein complexes and vitamins thus decreasing their bio-availabilty and digestibility (Liu et al. 1998). Furthermore, several studies show that protein and phytate compound were barely broken down by proteolysis enzyme (Ravindran et al. 2005), even phytate can hinder pepsin, amylopsin and amylase to work properly. Moreover, phytate built protein compound which resisted proteolysis digestibility and also bind trypsin that causes lower digestibility (Hassan et al. 2009). Baruah et al. (2007) also reported that apart from minerals, phytate also forms complexes with protein and amino acids. The amino group present in the side chain of the amino acid is one of the functional groups involved in protein-phytate interaction; therefore it decreases protein digestibility. If phytate cannot break down, metal contents and protein will discharge with the feces as a waste. Bounded metal substances and protein

*Corresponding author: dianarachmawati1964@gmail.com

with phytate are very important for growth; therefore to optimize the diet it is necessary to break down the phytate. The breakdown of phytate needs an additional enzyme, phytase enzyme. The addition of phytase enzyme would help fish to digest its diet (Sajidan et al. 2004).

The more phyto-ingredients used, the more problems emerge, i.e., phosphor pollution in the water. It is due to lack absorption of phosphor in the diet. It is caused by lackness of availability of phytase enzyme (Cheng & Hardy 2004; Debnath et al. 2005). Kumar et al. (2011) explained that phytate is a place to store phosphor as much as 80% of total phosphor in the diet. Phytate that cannot be absorbed is excreted to the environment. Excreted phytate in the water is broken down by phytase enzyme available in the water and releases phosphor. High phosphor content in the water triggers eutrovication that gives negative effect on the cultivation (Baruah et al. 2007). Moreover Jagannathan & Nielsen (2013) mentioned that phosphor is micro-growth nutrient that is needed by animal including fish. Phosphor in the diet cannot directly be absorbed since it is bound by phytate; therefore, the excess of phosphor in the phytate is released to the water and in turn it is enriching nutrient in the water.

One of the studies in phytase enzyme supplemented diet on fish growth has been conducted by Shapawi et al. (2013) found out that 30% soybean meal with the phytate enzyme 2 000 FTU · kg^{-1} diet was the optimum level for *Ephinephelus fuscoguttatus* juveniles. Moreover, Hassan et al. (2013) stated that the addition of phytate enzyme 1 000 FTU · kg^{-1} soybean meal diet can increase nutrient digestibility and performance of *Cirrhinus mrigala*. Bulbul et al. (2015) also reported that phytate enzyme supplemented diet can increase the digestibility and performance of *Marsupenaeus japonicas* shrimp.

One of the solutions to solve the binding phosphor is to add exogenous enzyme (Chung 2001). According Jobling et al. (2002) and NRC (1993) phytate can be reduced by adding phytase enzyme in the diet. Phytase enzyme can increase diet utilization and regulate nutrient excretion (phosphor, nitrogen, and mineral) and also hydrolize Phytate in the diet into inositol and phosphate acid. Phytase enzyme hydrolizes phytate in order to breakdown the minerals (Chung 2001). Baruah et al. (2007) also explained that phytase enzyme is able to hydrolize phytate (myo-inositol hexakisphosphate) into myo-inositol mono, di, tetra and pentaphosphate and organic phosphate. Phytase enzyme will unbind phosphor from phytate acid and also unbind other nutrient elements (Ravindran 2000). Some species that have been studied about phytase enzyme were *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).

The objectives of the study are to evaluate effects of phytase enzyme in supplemented diet on growth performance, diet utilization efficiency and nutrient digestibility in fingerlings of *Chanos chanos*.

MATERIALS AND METHODS

Test Animal

The milkfish fingerlings of *Chanos chanos* were used in this study and obtained from Center for Brackish Water Aquaculture, Jepara, Central Java. Fingerlings of *Chanos chanos* with the initial average weight of 3.55 g ± 0.08 g have been raised for 42 d. The examination of growth was done every week. The fingerlings used in this study were healthy individuals and had uniform size as suggested by Rachmawati & Samidjan (2016).

Experimental Tank

The fingerlings were raised in rectangular fiberglass containers with the dimension of 1.0 m × 3.0 m × 1.0 m which were equipped with aeration and water circulation. The fingerlings test fish maintained a closed recirculation system to stabilize the water quality in order to remain in the optimum range. Twenty five percent (25%) of water volume was replenished daily. To keep the containers clean, the feces of the animals were syphoned every day before feeding. The media was brackish water with the salinity of 15 mg · L^{-1}. The fingerling treatment was placed in the containers with the density 1 fingerling · L^{-1} media and was feds *ad satiation* (Hassan et al. 2009). The fingerlings were sampled and scaled the weight every week. The feces was syphoned two hours after feeding in order to keep the water media cleaned and viable to raise the fish.

Test feed

Feeding experiments used in this study were made in the form of pellet with the isoprotein content of 30 % and isoenergy of 301 kcal (1 kcal = 4 186.8 J) (Hassan et al. 2009). The diet contained fish meal, soybean meal, corn meal, rice bran, wheat flour, fish oil, corn oil, vitamin mix, and mineral mix, and Cr_2O_3 0.5% as an indicator of feed digestibility (NRC 1993) and phytase enzyme. Preparation on feed treatment done in this study consisted of proximate test for feed treatment (AOAC 1990), 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 in granular form which contains active materials of *myo-inositol-hexakisphosphate* β-*phosphohydrolase* (EC 3.1.3.8) produced by *Aspergillus niger*. Natuphos 5000G

Table 1. Composition of experimental diets.

Ingredients (g)	Treatment			
	A	B	C	D
Phytate enzyme	0	0.1	0.2	0.3
Fish meal	20	20	20	20
Soybean meal	30	30	30	30
Corn meal	15	15	15	15
Rice bran	12	12	12	12
Wheat flour	18	18	18	18
Fish oil	1.50	1.50	1.50	1.50
Corn oil	0.50	0.50	0.50	0.50
Min.Vit	2.00	1.50	1.00	0.70
CMC	0.50	0.50	0.50	0.30
Cr_2O_3	0.50	0.50	0.50	0.50
Total	100	100	100	100
Results of Proximate Analyses				
Protein (%)*	30.17	30.27	30.29	30.50
Fat (%)*	7.87	8.40	8,99	8,73
BETN (%)*	35.22	35.36	34.8	32.73
Energy (kcal)	301.34	301.82	301.19	301.98
Ratio E/P (cal · g^{-1})	9.98	9.97	9.97	9.90

contains 5.000 FTU/g of pythase enzyme. One unit of phytase activity (*Phytase Unit*/FTU) was defined as the amount of enzyme which release one micro molecule of non-organic per minutes from 0,0051 mol/l of phytase acid on pH of 5,5 and 37°C (BASF 2010). To get 500 FTU of enzymatic activity needs 100 mg of phytase enzyme.

In preparing the feeds for the experiment, an appropriate dose of phytase enzyme was disselved first into warm water (45°C), and then was mixed evenly with soybean meal. The mixture was stored in the airsealed container for around 24 h (Fox et al. 2006). The artificial feed was prepared by mixing the least amount of the ingredients first; then the more voluminous ingrediets were gradually added and mixed. The corn oil and fish oil were added last to the mixture. After this, granules with the diameter of 1 mm to 2mm were formed from the mixture. Lastly, the artificial feed was dried in the oven with temperature of 40°C (NRC 1993).

Feeding Trial

The experiments were conducted at Center for Brackish Water Aquaculture, Jepara, Central Java using Complete Random Design with four treatments and each treatment repeated three times. Phytase enzyme supplemented diet was used in the experiment with the doses of A (0 FTU · kg^{-1} diet), B (500 FTU · kg^{-1} diet), C (1 000 FTU · kg^{-1} diet), and D (1 500 FTU · kg^{-1} diet). The enzyme doses used in this study were the modified results from the study by Hassan et al. (2009). The results suggested that the phytate enzyme as much as 1 000 FTU · kg^{-1} diet was the optimum level for the growth of milkfish (*Chanos chanos*) with the average weight of 9 g · $fingerlings^{-1}$.

The differences between this study and Hassan's study were the use of similar feed formula of 20% fish meal and 30% soy meal which each formula used four different dosages of phytase enzyme (0 FTU kg^{-} diet, 500 FTU kg^{-} diet, 1000 FTU kg^{-} diet and 1500 FTU kg^{-} diet) (Table 1) while in Hassan's study there was four different feed formula with different dosages of phytase enzyme: A (61.35% of fishmeal), B (61.35% of soybean meal and 500 FTU kg^{-} diet of phytase enzyme), C (61.35% of soybean meal and 1000 FTU kg^{-} diet of phytase enzyme) and D (61.35% of soybean meal and 1500 FTU kg^{-} diet of phytase enzyme).

Parameter of Observation

The parameters observed include RGR and FCR, suggested by Tacon (1995), ADC_P and ADC_F according to Fenucci (1981), and Survival Rate (SR) according to NRC (1993). The pH (Jenway 3510), DO (Jenway 970), temperature and Ammoniac (HANNA: HI. 8633) of water were determined. Aerator was placed in every container to recirculate the water. The chromic oxide levels in diets and feces were analyzed 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 colored complex with diphenylcarbazide (DPC). Samples were analyzed to determine phosphorus (P) concentrations by flame atomic absorption spectrophotometry on a Shimadzu AA6800 (Shimadzu, Japan).

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

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

$$ADC_P : 100 \left\{ \frac{\%\ Cr_2O_3 \text{ in the diet}}{\%\ Cr_2O_3 \text{ in the feces}} \times \frac{\%\ \text{protein in the feces}}{\%\ \text{protein in the diet}} \right\}$$

$$ADC_F : 100 \left\{ \frac{\%\ Cr_2O_3 \text{ in the diet}}{\%\ Cr_2O_3 \text{ in the feces}} \times \frac{\%\ \text{fosfor in the feces}}{\%\ \text{fosfor in the diet}} \right\}$$

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

Statistical Analysis

Experimental design used Completely Randomized Design (CRD) with four dosages of phytase enzyme content as a treatment and each treatment repeated three times. Effect of the treatments was tested using an analysis

of variance (ANOVA) which was then used to analyze the data. Before analyzing, the normality, additivity, and homogeneity of the data were first tested. If the analysis of variance was significant (p<0.05) or highly significant (p<0.01), Duncan test was conducted to find out the mean of the treatment (Steel et al. 1996). To determine optimal dose of phytase enzyme, polynomial orthogonal test was conducted using SAS9 and Maple12. Water quality data were descriptively analyzed.

RESULTS AND DISCUSSION

The addition of phytase enzyme on the diet significantly (p<0.01) increased the relative growth rate of milkfish fingerlings as shown in the Table 2. The results gave evidence that supplemented diet with the phytase enzyme could hydrolyze protein that was bind by phytate into amino acid. This amino acid was readily digested and could provide energy to grow (Amoah et al. 2011; Haghbayan & Mehdi 2015).

The addition of phytase enzyme on the feed 1000 FTU kg^- diet significantly increased the growth of milkfish fingerlings compared to the addition of 500 and 1500 FTU phytase enzyme per kg^- diet. It can be concluded that the addition of 1000 FTU phytase enzyme on the every kg feed could reduce antinutrients or phytate on soybean meal (Liu et al. 1998). Yu & Wang (2000) also reported that the addition of phytase enzyme 1000 FTU kg^- diet could increase average weight of crucian carp *Carassius carassius* by 25 percent. The same results were also found in carp (Schaefer et al. 1995), African catfish (Weerd et al. 1999), striped bass (Papatryphon et al. 1999), rainbow trout (Vielma et al. 2002), Atlantic salmon (Sajjadi & Carter 2004), Korean *Sebastes schlegeli* (Yoo et al. 2005).

The results of the phytate contents in the diet on the A, B, C, and D treatments were 0.75%, 0.72%, 0.70%, and 0.68%, respectively. The results of the phytate contents in the feces on the A, B, C, and D treatments were 0.60%, 0.55%, 0.38%, and 0.46%, respectively. Therefore, the decrease of phytate can be calculated and the decreases were 0,15%, 0,17%, 0,32%, and 0,22% on the A, B, and D treatments respectively. It shows that the phytase enzyme has broken down the phytate (Hassan et al. 2013). When the diet contained phytate, it could decrease the growth, as reported by NRC (1993). It was due to the strong activity of phytate on hindering and reducing the balance of protein and growth. Moreover, NRC (1993) reported that the phytate content 0.5% in the diet could decrease the growth and feed efficiency for rainbow trout (*O. misskis*). Tacon (1995) also suggested that 2.58% phytate in the diet could decrease growth, feed efficiency, protein efficiency, and also cause mortality. Alvi (1994) also mentioned that the growth of *Labeo rohita* significantly decreased when the diet contained more than 1% phytate acid; otherwise the growth would increase if there was addition of phytase in the diet. The results of orthogonal polynomial test on the relationship of phytase enzyme in the diet and the relative growth rate (Figure 1) had cubical pattern with the equation $Y = -1.481x^3 + 1.404x^2 + 1.6736x + 1.950$, R^2=0.92. The optimum dose of the phytase enzyme in the diet on the relative growth rate was 1010 FTU kg^- diet with the maximum relative growth rate of 3.55% per day.

The addition of phytase enzyme significantly (P<0.01) affected on the feed conversion ratio of milkfish fingerlings. Table 2 shows that the diet treatment of B, C, and D had higher feed conversion rate than that of diet A treatment (0 FTU kg^- diet). It was suggested that phytase enzyme can break down phytate, losing the binding between phytate acid and protein and minerals compound. It would positively increase activity to convert trypsinogen into trypsine enzyme which broke down protein into amino acids. It can increase feed utilization and reduce feed conversion ratio. Wang et al. (2009) also reported that the addition of phytase enzyme the soybean diet for rainbow trout also made feed conversion ratio better. The same result was also found for *L. rohita* (Baruah et al.

Table 2. Relative growth rate (RGR), feed conversion ratio (FCR), apparent digestibility content % protein digestibility (ADC_p), apparent digestibility content % phosphor digestibility (ADC_f), and survival rate of milkfish (*C. chanos*).

Data	Treatment			
	A	B	C	D
RGR (% · d^{-1})	1.95 ± 0.04^c	2.95 ± 0.29^b	3.55 ± 0.21^a	2.62 ± 0.19^b
FCR	2.92 ± 0.13^a	2.36 ± 0.19^b	1.84 ± 0.12^c	2.54 ±0.20^{ab}
ADC_P (%)	75.27 ± 0.02	79.65 ± 0.05	81.93 ± 0.05	77.35 ± 0.04
ADC_F (%)	82.03 ± 0.21	87.98 ± 0.23	88.98 ± 0.37	88.60 ± 0.32
Survival Rate (%)	86.87 ± 0.02^a	83.33 ± 0.02^a	86.67 ± 0.02^a	80.00 ± 0.02^a

Note: Value ± SD with the same superscript in the column indicated insignificant

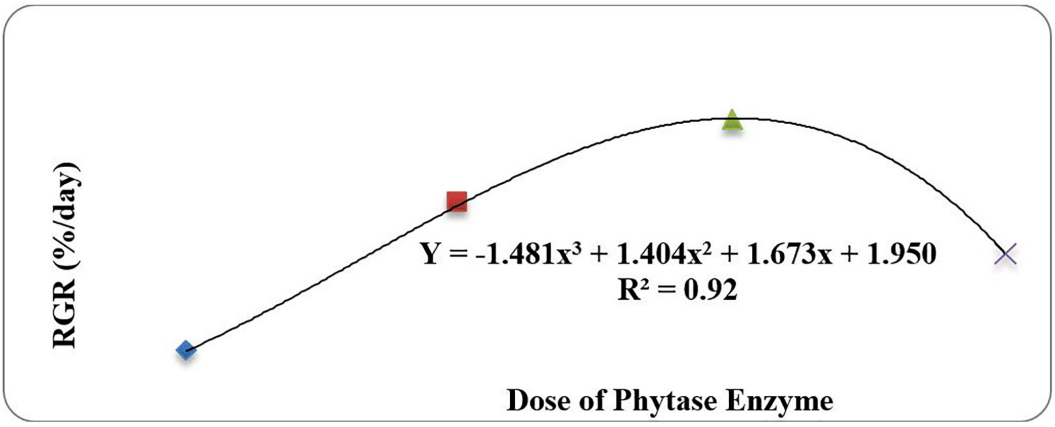

Figure 1. Graph of orthogonal polynomial RGR of milkfish fingerlings (*C. chanos*).

2007). C treatment (1000 FTU kg^{-} diet) resulted in the lowest feed conversion ratio among other treatments, B (500 FTU kg^{-} diet), D (1500 FTU kg^{-} diet), and A (0 FTU kg^{-} diet). It was suggested that the addition of phytase enzyme could increase the efficiency in diet utilization and make feed conversion ratio low. Li & Robinson (1997) studied on the addition of 250 units or more microbial phytase enzyme in the diet. 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 diet. The relationship between phytase enzyme in diet and the feed conversion ratio based on the orthogonal polynomial test, as shown in the Figure 2, was cubical. The equation was $Y= 0.069x^3 + 0.415 x^2 – 0.989x + 1.127$ and $R^2= 0.98$. The optimum dose of the phytase enzyme in the diet on the feed conversion ratio was 1000 FTU kg^{-} diet with the maximum value of feed conversion ratio 1.62.

Table 2 shows that the addition of phytase enzyme 500-1000 FTU kg^{-} diet could increase the apparent digestibility coefficient protein. Storebakken et al. (1998) has already reported that the addition of phytase enzyme increased protein digestibility and protein retention. These results were also confirmed by Debnath et al. (2005) that the addition of phytase enzyme significantly increased protein utilization and digestibility on Atlantic salmon, otherwise they had low protein utilization and digestibility. Hunter (2002) also found that the addition of phytase enzyme significantly increased protein digestibility from 84.5% to 87.7%. Similar results were found on carp (Vielma et al. 2002), rainbow trout (Sugiura et al. 1999; Forster et al. 1999), *Labeo rohita* (Hussain et al. 2014). Vielma et al. (2002), and Baruah et al. (2004) also reported that addition of phytase enzyme in the plant diet increased protein digestibility due to breaking down of phytin-protein compound. They found that phytase enzyme could break down anti nutrients in the diet, such as phytate acid, non-starch polysaccharide, and trypsin inhibitor. It could also increase feed digestibility. The relationship between phytase enzyme and the protein digestibility on the orthogonal polynomial test, as shown in the Figure 3, was cubical. The equation was $Y= -6.36x^3 + 5.353x^2 + 7.666x + 75.72$ and $R^2= 0.99$. $R^2= 0.99$. The optimum dose of the phytase enzyme in the diet on the digestibility protein was 983 FTU kg^{-} diet with the maximum value of protein digestibility 81.00 %.

The resulting apparent digestibility coefficient phosphor treatment A is lower than treatment B, C and D. The addition of phytase enzyme from 500 units of phytase to 1000 units of phytase enzyme FTU kg^{-} diet brought about increasing phosphor digestibility. It was due to hydrolizing phytate by phytase enzyme; therefore, milkfish were easily able to digest the phosphor. This finding was in line with the finding of Debnath et al. (2005) that the addition of phytase enzyme as much as 1000 and 2000 units kg^{-} diet can increase phosphor digestibility in the *Pangasius pangasius*. Phytate is an anti-nutrient compound that is usually found in the phyto-diet such as soybean which is as a main source of protein in the diet. Oliva-Teles et al. (1997) stated that around 60-70% phosphor in the soybean meal as phytate cannot be digested by fish. Morover, Galtin III et al. (2007) stated that the digestibility of phosphor in the phyto-protein was very low, just around 0-20%. In this study, the addition of phytase enzyme as much as 500-1000 FTU kg^{-} diet (B and C treatment) can increase phosphor digestibility compared to A treatment (phytase enzyme 0 FTU kg^{-} diet). Similar result found by Lanari et al. (1998) that the addition of 1000 units of phytase enzyme per kg diet increased phosphor digestibility in the rainbow trout (*O. myskiss*). Oliva-Teles et al. (1997) studied that seabass juveniles (*Diencetrarchus labrax*) can digest phosphor higher when as much as 1,000-2,000 units of phytase enzyme was added into the diet. The relationship between phytase enzyme and the phosphor

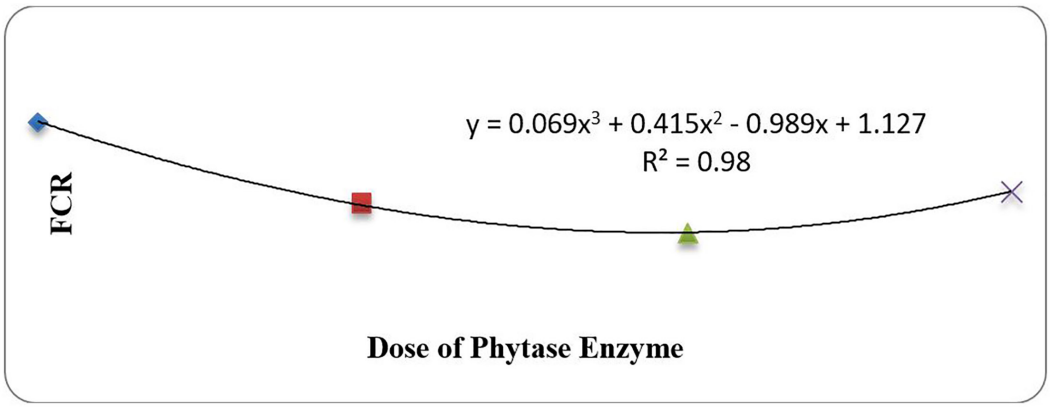

Figure 2. Graph of orthogonal polynomial FCR of milkfish fingerlings (*C. chanos*).

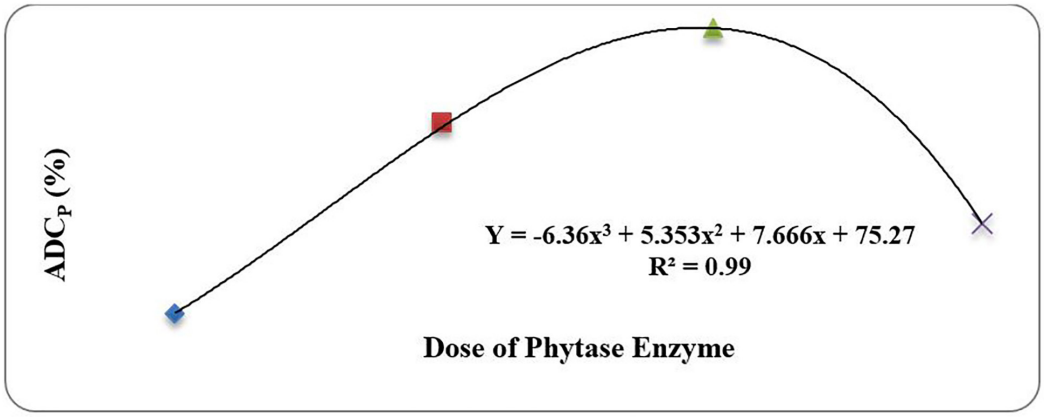

Figure 3. Graph of orthogonal polynomial ADCP of milkfish fingerlings (*C. chanos*).

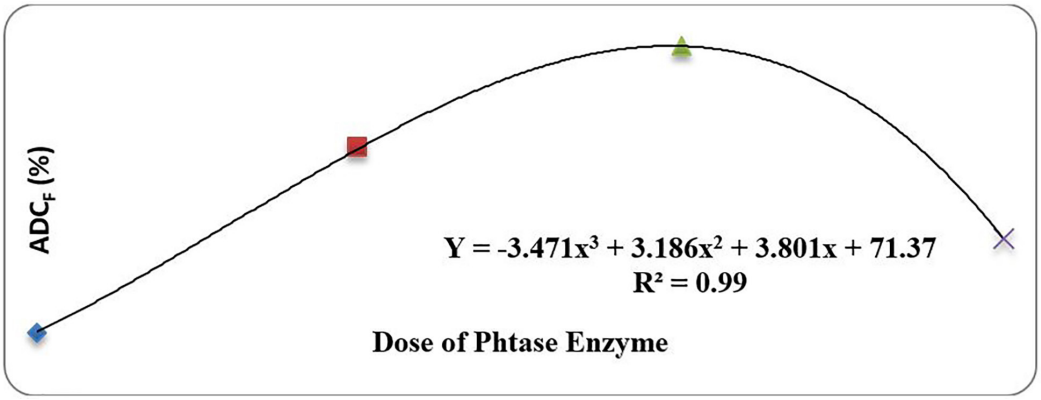

Figure 4. Graph of orthogonal polynomial ADCF of milkfish fingerlings (*C. chanos*).

digestibility based on the orthogonal polynomial test, as shown in the Figure 4, was cubical. The equation was $Y= -3.471x^3 + 3.186x^2 + 3.801x + 71.37$ and $R^2= 0.99$. The optimum dose of the phytase enzyme in the diet on the phosphor digestibility was 983 FTU kg$^-$ diet with the maximum value of digestibility total protein 88.15%.

The addition of phytase enzyme in the diet insignificantly affected on survival rate of milkfish fingerlings, as shown in the Table 2. The result was in line with the Robinson et al. (2002) finding that survival rate was insignificantly affected by addition of phytase enzyme in the diet. 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 diet (Hepher 1988).

The water quality during the research was suitable for cultivating the fingerlings of *Chanos chanos*. The measurement of the observed parameters of water during cultivation of *Chanos chanos* fingerlings can be seen in the Table 3.

Table 3. Parameters of water quality for cultivation of milkfish (*C. chanos*).

Treatment	Variables			
	Temperature (^{0}C)	pH	DO (mg/L)	Ammoniac (mg/L)
A	25.0 - 28.2	8.53 - 8.85	4.51 - 4.81	0.15 - 0.5
B	25.1 - 28.0	8.64 - 8.77	4.05 - 4.63	0.15 - 0.5
C	25.2 - 28.3	8.70 - 8.79	4.04 - 4.62	0.15 - 0.5
D	25.1 - 28.1	8.56 - 8.81	4.00 - 4.63	0.15 - 0.5
Feasibility	25 - 32 *	7-9*	4-6*	<1 *

CONCLUSIONS

The addition of phytase enzyme in the diet significantly affected on the relative growth rate, feed convertion ratio, apparent digestibility coefficient protein digestibility); otherwise it insignificantly affected on survival rate of milkfish fingerlings (*C. Chanos*). The optimal doses of phytase enzyme on growth performance, diet utilization efficiency and nutrient digestibility in fingerlings of (*Chanos chanos)* ranges from 983 – 1010 FTU kg$^-$ diet.

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