



Engineering Technology of White Shrimp (*Litopenaeus vannamei*) Intensive System Culture with the Supplementation of Phytase Enzyme in the Diet

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ABSTRACT

This study aims to examine the effect of phytase enzyme in the diet on digestibility of growth of white shrimp (*Litopenaeus vannamei*) raised in intensive system culture. Research on the supplementation of phytase enzymes in *L. vannamei* diet is very important to hydrolyze phytic acid in vegetable ingredients as a source of diet protein so as to increase the efficiency of diet utilization to support growth of vaname shrimp. The shrimp used in the research had average weight of $1,19 \pm 0,06$ g/shrimp. The diet contained 38% of protein and 3200 Kcal DE/kg with various doses of phytase enzyme supplementation, namely: A (0 U/kg diet), B (500 U/kg diet), C (1000 U/kg diet) and D (1500 U/kg diet). Observed parameters were digestibility of phosphore (ADC_F), disgetibility of protein (ADC_P), efficiency of diet utilization (EFU), protein efficiency ratio (PER), diet convesion ratio (FCR) relative growth rate (RGR), and survival rate (SR). The supplementation of phytase enzyme in the diet significantly influenced ($P < 0.01$) on ADC_F , ADC_P , EFU, PER, FCR, RGR; however, it did not significantly influence on SR of white shrimp. The best treatment result was the supplementation of 1000 U/kg diet phytase enzyme (treatment C). The optimum dose of phytase enzyme in the diet was 867, 920, 923, 915, 1000 and 1000 U/kg of diet respectively for ADCF, ADCP, EFU, PER, FCR, RGR. Based on those result the supplementation of phytase enzyme can increase the growth of white shrimp.

Keywords: growth, intensive system culture, Supplementation, phytase enzyme

1. Introduction

White shrimp (*Litopenaeus vannamei*) is one of the aquaculture products Kendal District, Province of Central Java, Indonesia. The district has potential area for aquaculture with the area of 1.000.23 km² and sea shore line of 42.2 km. The aquaculture system on white shrimp in the Kendal District has not been optimum yet. One of the reasons is high price of the diet, but it is not followed by the price of the shrimp. Moreover, the share of production cost dominated total cost, it counted for 50-60%, where the contribution of frozen *L. vannamei* export value to the total value of 2016 fishery exports is more than 27% (Office of Fishery and Maritime District of Kendal, 2018). The success of white shrimp aquaculture highly depends on the efficiency of diet utlization because it can reduce cost of production. Efficiency of diet utlization has not

been optimum yet due to the diet that made of plant based ingredients.

Kumar et al. (2011) reported that the main problem of plant based diet is because it contains phytic acid. Soybean meal of 1 kg contains 3.88 g (Cao et al., 2007). Minerals that have 2 or 3 valences (Ca, Fe, Zn, Mg) can be bound by phytic acid. Then it creates complex compound that highly difficult to absorb (Baruah et al., 2007). Phytic acid can also bind protein and amino acids that can hinder diet digestibility (Ravindran et al., 2005). mineral yang memiliki 2 atau 3 valensi (Ca, Fe, Zn, Mg) dapat diikat oleh asam fitat.

Phosphore is one of macro nutrients that fish needs to grow (Jagannathan and Nielsen, 2013). Fish cannot utilize phosphore in the diet made of plant based ingredients, because fish has limited phytase enzyme (Debnath et al., 2005). About 80% of the total phosphorus

available in the plant-based diet is bound by phytic acid (Kumar et al., 2011). Therefore, fish cannot directly absorb the binded phosphore in the phytic acid, in turn it is excreted through feces. Accumulated feces will release phosphore and will put more nutrient in the aquaculture media. High phosphore content in the water can cause eutrophication that will harm the fish culture (Baruah et al., 2007).

One way to overcome the problem of phytic acid. with phytase enzyme supplementation in feed (Kumar et al., 2011), the addition of phytase enzymes in feed can be one way to overcome the problem of phytic acid. Phytase enzyme hydrolizes phytic acid; therefore, it will unbind minerals from the compound (Chung, 2001). The enzyme phytase is an enzyme that can hydrolyze phytic acid so can increase absorption of nutrients and minerals (Baruah et al., 2007). It can also hrdrolize phytic acid into inositol and phosphate acid. Beside unbinding phosphore from phytic acid, phytase enzyme can also unbind other minerals (Ravindran et al., 2005). Some studies on phytase enzyme supplementation in the diet have been done by Shapawi et al., (2013), Hassan et al. (2009), Husain et al. (2014), Bulbul et al. (2015), Danwitz et al. (2016).

There is no information found in the study of phytase enzyme supplementation in the diet or the growth of *L. vannamei* so this study is indispensable. This study aims to examine the effect of phytase enzyme in the diet on digestibility of growth of white shrimp (*Litopenaeus vannamei*) raised in intensive system culture.

2. Materials and methods

Shrimp Sample

White shrimp (*L. vannamei*) used in the study has average weight of 1.19 ± 0.06 g/shrimp. The shrimp was obtained from Brackish Water Aquaculture Center, Situbondo, East Java, Indonesia. The sample shrimp was selected based on the size uniformity, organ completeness, and health (Rachmawati and Samidjan, 2016). Then the shrimp was adapted to the media culture environment and the diet in 7 (seven) days. The weith gain was measured every week for 60 days to determine the effect of phytase enzyme on the growth of *L. vannamei*.

Diet Preparation

Diet that was used in the study contained 40% protein and 3600 Kcal DE/kg energy according to energy requirements for of *L. vannamei*. (Suprayudi et al., 2012). The ingredients of the diet were fish meal, soybean meal, cornflour, rice bran, wheatflour, fish oil, corn oil, minerals and vitamine mix (aquamine), CMC, Cr_2O_3 as indirect indicataor of diet digestibility as much as 1% (NRC, 1993) and phytase enzyme. The enzyme used in the study was Natuphos[®] 5000 that was produced by PT.BASF Indonesia. Proxymate analysis, based on (AOAC, 1990), was used to identify nutrient contents. The diet composition and the results of proxymate analysis were shown in the Table 1.

Table 1. The Diet Composition for White Shrimp (*L. vannamei*)

| Ingredients | Treatment | | | |
|-----------------------------------|-----------|--------|--------|--------|
| | A | B | C | D |
| Phytase Enzyme (g) | 0 | 0.1 | 0.2 | 0.3 |
| Fish Meal | 28 | 28 | 28 | 27.9 |
| Soybean Meal | 37.8 | 37.8 | 37.8 | 37.8 |
| Cornflour | 6.5 | 6.5 | 6.5 | 6.5 |
| Rice Bran | 7.9 | 7.9 | 7.9 | 7.9 |
| Wheatflour | 11.8 | 11.7 | 11.6 | 11.5 |
| Fish Oil | 3 | 3 | 3 | 3 |
| Corn Oil | 2 | 2 | 2 | 1.99 |
| Vit Min Mix | 1 | 1 | 1 | 1 |
| CMC | 1 | 1 | 1 | 1 |
| Cr_2O_3 | 1 | 1 | 1 | 1 |
| Total | 100 | 100 | 100 | 100 |
| The Results of Proxymate Analysis | | | | |
| Protein (%)* | 40.25 | 40.28 | 40.32 | 40.26 |
| Fat (%)* | 9.47 | 9.58 | 9.75 | 9.63 |
| BETN (%)* | 40.12 | 40.12 | 40.18 | 40.21 |
| Energy (Kcal)* | 306.42 | 306.27 | 306.32 | 306.19 |
| Ratio E/P | 9.12 | 9.23 | 9.18 | 9.23 |

a. Based on Wilson (1987) : Digestible Energy for 1 g protein equals 3.5 kcal/g, 1 g fat equals 8.1 kcal/g, and 1 g carbohydrate equals 2.5 kcal/g

b. Based De Silva (1987) : value of E/D for optimum growth is 8-12 Kcal/g

c. * Laboratory for Animal Nutrient, Faculty of Animal Husbandry and Agriculture, Diponegoro University (2017)

Research method used experiment with Completely Randomized Design, 4 (four) treatments and 4 (four) repetitions. The treatments in this study were different doses of phytase enzymes, namely A (0 U/kg diet), B (500 U/kg diet), C (1000 U/kg diet), and D (1500 U/kg diet). The levels of dose of phytase enzyme supplementation based on Suprayudi et al. (2012) and Rachmawati and Samidjan (2016) study, but they were modified. The shrimp was fed 4 (four) times daily with the diet dose of 5% of the weight.

Preparation for Intensive Culture System

The study was conducted in Kendal District, Central Java, Indonesia. The study utilized media culture with the total area of 6000 m² divided into 12 ponds, each has area of 500 m². The bottom of the pond was covered by plastic mulch. Then the pond was filled with the saline water the depth of 1.5 m, obtained from reservoir pond with. Each pond was installed 4 (four) paddle wheels. One week after the pond filled with saline water, white shrimp juveniles were spread in each pond with the density of 80 shrimp/m².

Water Quality Observation

Parameters included ADC_F, ADC_P, EFU, PER, FCR, RGR, SR, and water quality. Fenucci (1981) method was used to measure ADC_F and ADC_P. Tacon (1987) method was used to measure EFU, PER, and FCR. NRC,

(1993) method was used to measure RGR and SR.

Observed Parameters

Physical and chemical water quality parameters consisted temperature measured by HANNA: HI. 8633, pH measured by Jenway 3510, dissolved oxygen measured by Jenway 970 and ammoniac measured by (APHA, 2005). Water salinity was measured everyday and calculated by (ATAGO S-10, Japan).

Data Analysis

Data Analysis used analysis covariance (ANOVA). If the result had significant effect ($P < 0.05$) or high significant effect ($P < 0.01$), the next step was analyzed using Duncan test to find mean value of the treatment. No needs to write Polynomial orthogonal was used to find optimum dose (Steel et al., 1993). Water quality was descriptively explained.

3. Results and Discussion

Result

The supplementation of phytase enzyme in the diet had very significant effect ($P < 0.01$) on ADC_F, ADC_P, EFU, PER, FCR, RGR; however, it had insignificant effect on SR. *L. vannamei* were shown in the Table 2.

Table 2. Phosphore Digestibility (ADC_F), Protein Digestibility (ADC_P), Efficiency Of Diet Utilization (EFU), Protein Efficiency Ratio (PER), Diet Conversion Ratio (FCR), And Relative Growth Rate (RGR) and Survival Rate (SR) of *L. vannamei*

| Data | Treatments | | | |
|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | A | B | C | D |
| ADC _F (%) | 58.03±0.21 ^d | 67.98±0.23 ^c | 79.98±0.37 ^a | 71.60±0.32 ^b |
| ADC _P (%) | 60.43±1.78 ^d | 70.98±2.24 ^c | 88.65±1.89 ^a | 73.29±2.15 ^b |
| EFU (%) | 53.50±0.23 ^d | 62.54±2.19 ^c | 79.98±2.21 ^a | 67.75±2.20 ^b |
| PER | 1.82±0.21 ^d | 2.02±0.23 ^c | 3.25±0.20 ^a | 2.45±0.23 ^b |
| FCR | 2.64±0.15 ^c | 2.34±0.23 ^b | 1.78±0.08 ^a | 2.02±0.12 ^b |
| RGR (%) | 1.89±0.01 ^d | 2.65±0.06 ^c | 3.05±0.03 ^a | 2.80±0.08 ^b |
| SR (%) | 96.33±3.32 ^a | 90.00±2.53 ^a | 93.33±2.32 ^a | 90.00±2.35 ^a |
| Phytic Acid of Diet (%) | 0.73 | 0.68 | 0.58 | 0.63 |
| Phytic Acid of Feces (%) | 0.64 | 0.57 | 0.29 | 0.46 |
| Phytic Acid Decrease (%) | 0.09 | 0.11 | 0.29 | 0.17 |

Note: The Values with the same superscripts in the column show insignificant

Table 3. Water Quality Parameters

| Parameter | Value Ranges | | | | References |
|------------------|--------------|-----------|-----------|-----------|------------------------|
| | A | B | C | D | |
| Temperature (°C) | 28-30 | 28-30.5 | 28-30 | 28.5-30.5 | 27.5-31.5 ^a |
| DO (mg/l) | 3-4 | 3.5-4.5 | 3.5-4.2 | 3.5-4.5 | 3.2-4.2 ^a |
| Salinity (ppt) | 29-34 | 30-35 | 28-33 | 29-35 | 4-35 ^a |
| pH | 7.5-8.5 | 7.7-8.2 | 7.5-8.2 | 7.7-8.5 | 7.6-8.6 ^a |
| Ammoniac (ppm) | 0.01-0.23 | 0.01-0.38 | 0.01-0.22 | 0.01-0.31 | <1 ^a |

^a Soundarapandian *et al.* (2010)

Table 3. show that in the temperature between 28 °C and 30.5 °C, DO, salinity, pH, ammoniac were 3-4.5 mg/l, 28-35 ppt, 7.5-8.5 and 0.01-0.013 ppm respectively. The water quality was suitable for white shrimp culture (Soundarapandian *et al.*, 2010).

Discussion

The results showed that a dose of 500-1500 U/kg of diet could increase phosphore digesibility (ADC_F), as shown in Table 2. This increase was due to an increase in phytic acid hydroxicity caused by phytase enzyme supplementation. The results of this study are in accordance with Baruah *et al.* (2007) and Ravindran (2005) which states that phytase enzymes can hydrolyze phytic acid into inositol and phosphate acids so can increase phosphorus absorption.

The dose of 1000 U/kg diet (treatment C) phytase enzyme gave the highest phosphore digestibility. The same result was also reported by Storebakken *et al.* (1998) that teh dose of 1000 U/kg diet phytase enzyme was able to improve the phosphore digestibility of *rainbow trout* (*O. myskiss*). Rachmawati and Samidjan (2016) and Rachmawati *et al.* (2018) also reported the same results on *Penaeus monodon* and *Chanos chanos* studies.

The relationship of phytase enzyme supplementation and ADC_F based on Polynomial Orthogonal test has a quadratic relationship. The equation was $Y = -0.00003x^2 + 0.046x + 57.271$ and $R^2 = 0.95$, from this equation can be found the optimum dose of phytase enzyme on ADC_F and $R^2 = 0.95$ states that 95% ADC_F is influenced by the supplementation of phytase enzymes in diet while environmental factors only affect 5% (Fig 1). The optimum dose of the study on ADC_F was 867 U/kg diet with the value of 74.09%.

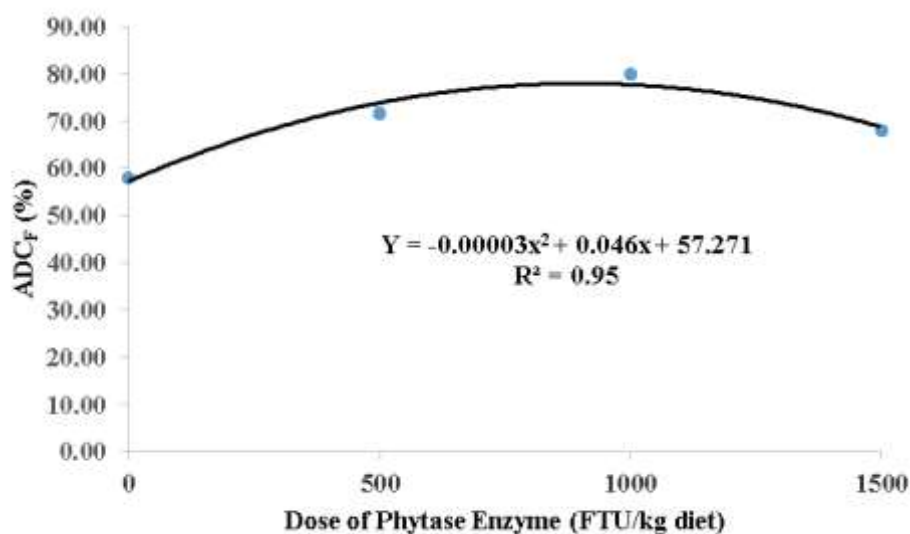


Figure. 1. Relationship Graph between phytase enzyme and ADC_F of *L. vannamei*

The supplementation of phytase enzyme with the doses of 500-1500 U/kg diet has higher increase of ADC_p than those of 0 U/kg diet dose (Table 2). It was suggested that phytic acid has been unbinded by the phytase enzyme; therefore, protein can be readily digested. Similar result was reported by Carter and Sajjadi (2011) that the supplementation of phytase enzyme in the diet can have Atlantic salmon (*Salmo solar*) increased in the protein absorption. Phytase enzyme supplementation in the diet can break down phytine-protein compound (Baruah et al., 2007). Similar results have been obtained from the studies on *Labeo rohita* (Baruah et al., 2007), *Channos channos* (Hassan et al., 2009; Rachmawati et al., 2017), *Penaeus monodon* (Rachmawati and Samidjan, 2016), Tilapia Saline (Rachmawati et al., 2018) and *Cyprinus carpio* (Rachmawati and Samidjan, 2016).

This study show that the phytase enzyme supplementation in the plant based diet can increase the digestibility of phosphore and

protein. The result was also supported by the study (Phromkunthong et al., 2010). They found that the phytase enzyme supplementation can increase the digestibility of plant based diet as protein source. Phytase enzyme can break down anti-nutrient in the diet to increase diet digestibility and diet nutrients (Cao et al., 2007).

The relationship of phytase enzyme supplementation and ADC_p based on Polynomial Orthogonal test has a quadratic relationship. The equation was Polynomial orthogonal test show that the relationship between phytase enzyme and ADC_p was in quadratic form. The equation was as follow : $Y = - 0.00003x^2 + 0.0552x + 58.654$ and $R^2 = 0.84$, from this equation can be found the optimum dose of phytase enzyme on ADC_p and $R^2 = 0.84$ states that 84 % ADC_p is influenced by the supplementation of phytase enzymes in diet while environmental factors only affect 16 % (Fig 2). The optimum dose for ADC_p was 920 U/kg diet with the value of 80.00% ADC_p.

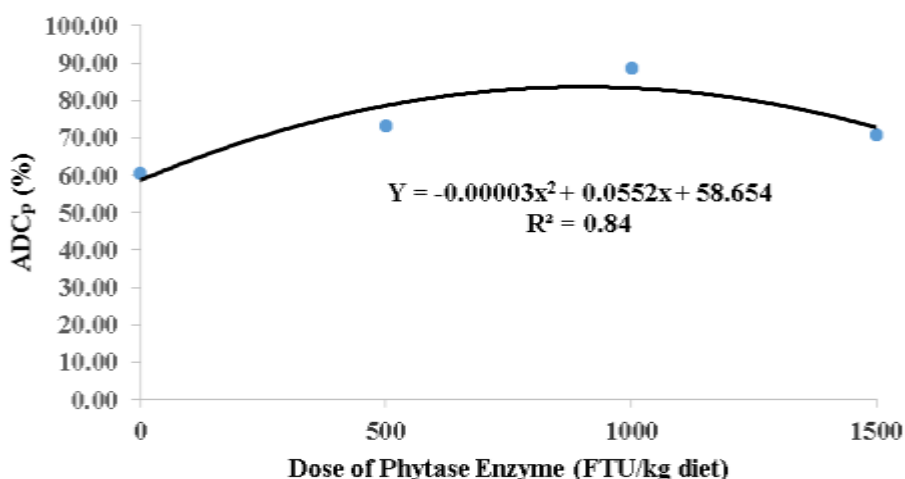


Figure. 2. The relationship between phytase enzyme and ADC_p of *L. vannamei*

The result of various doses of phytase enzyme on diet utilization was highly significant (P<0.001). Supplementation of phytase enzyme was able to improve efficiency of diet utilization in white shrimp. Wang et al., (2009) finding supported the result that phytase enzyme can increase efficiency of diet utilization in the digestible system.

Table 2, show that the highest efficiency of diet utilization (EFU) was treatment C (1000 U/kg diet phytase enzyme supplementation) with the value of 79.98% EFU, while the lowest value was obtained from the treatment A (0 U/kg diet phytase enzyme supplementation) with the value of 53.50% of EFU. The dose of 1000

U/kg diet phytase enzyme supplementation was suggested that the dose was just the right dose to hydrolize phytic acid into readily available nutrient to absorb. The finding was supported by Chung (2001) study that he suggested the phytase acid can increase efficiency of diet utilization. Meanwhile, the lowest EFU was due to shrimp cannot utilize the nutrient since it has still bound by phytic acid. The similar result was also found by Rachmawati et al. (2017), Rachmawati et al. (2018), Rachmawati and Samidjan (2018) and Qinghui, and Xie (2005)..

The relationship of phytase enzyme supplementation and EFU based on Polynomial

Orthogonal test has a quadratic relationship. The equation was Polynomial orthogonal test on the relationship between the supplementation of phytase enzyme and EFU resulted in quadratic equation as follow: $Y = -0.00003x^2 + 0.0554x + 52.118$ and $R^2 = 0.89$, from this equation can be found the optimum dose of phytase enzyme on

EFU and $R^2 = 0.89$ states that 89 % EFU is influenced by the supplementation of phytase enzymes in diet while environmental factors only affect 11 % (Fig 3). The optimum dose of phytase enzyme supplementation was 923 U/kg diet with the value of 77.69% EFU.

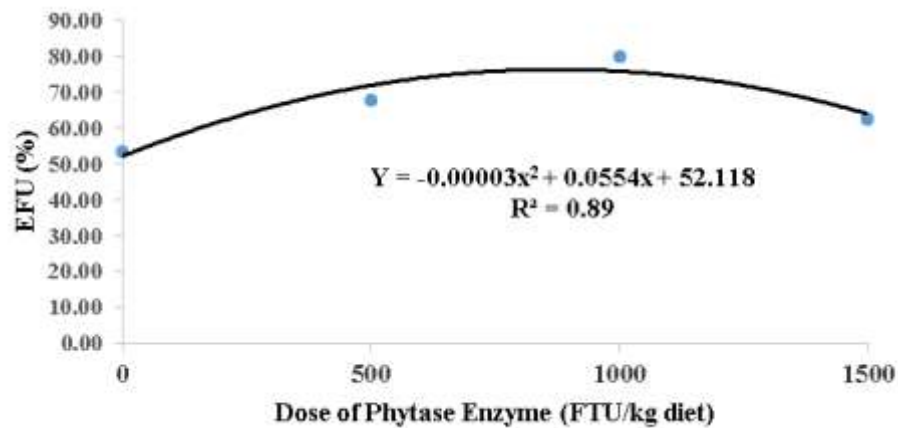


Figure. 3. The relationship Graph between phytase enzyme and EFU of *L. vannamei*

Treatment C (1000 U/kg diet phytase enzyme supplementation) gave highest PER with the value of 3.25, while the lowest protein efficiency ratio was obtained from Treatment A (0 U/kg diet phytase enzyme supplementation) with the value of 1.82. The highest value of protein efficiency was suggested because of the result of effective dose to break down the binding of phytic acid with protein and minerals compound; therefore, it made digesting enzymes readily available. Tawwab (2012) also reported that hydrolysis of phytic acid due to phytase enzyme can increase protein absorption. The increase of protein efficiency due to the supplementation of phytase enzyme in the diet

was also reported by Vielma et al. (2004) and Suguira et al. (2001).

The relationship of the supplementation of phytase enzyme and protein efficiency ratio was indicated by quadratic equation as follows: $Y = -0.000002x^2 + 0.0031x + 1.71$ and $R^2 = 0.80$, from this equation can be found the optimum dose of phytase enzyme on PER and $R^2 = 0.80$ states that 80 % PER is influenced by the supplementation of phytase enzymes in diet while environmental factors only affect 20 % (Fig 4). The optimum dose of phytase enzyme supplementation was 915 U/kg diet with the value of 3.02 PER.

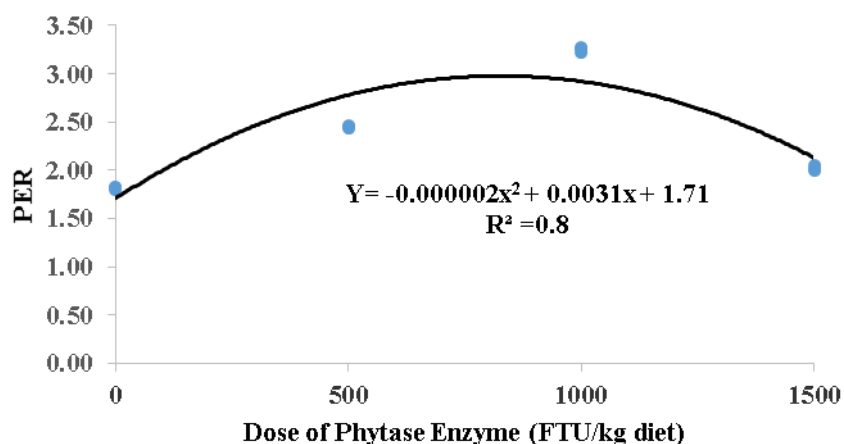


Figure 4. The relationship Graph between phytase enzyme and PER of *L. vannamei*

The doses of phytase enzyme of 500 U/kg diet, 1000 U/kg diet and 1500 U/kg diet (Treatments B, C and D) gave lower FCR than that of 0 U/kg diet dose (Treatment A). They were suggested that the phytase enzyme can catalyze the break down of phytic acid; therefore, it unbound the compound of phytic acid and protein and minerals into trypsin enzyme which can break down protein into amino acids. In turn, it increased efficiency of diet utilization and lowered diet conversion. Tahoun *et al.* (2009) stated that the lower the efficiency of diet conversion the better diet utilization. Other studies which had similar results were done in *Rainbow trout* (Wang *et al.*, 2009) and Labeo Rohita (Baruah *et al.*, 2007).

The lowest ratio of FCR was obtained from Treatment C (1000 U/kg diet) with the value of 1.78, followed by treatments D, B, and A with the values of 2.02, 2.34, and 2.64 respectively. The dose of 1000 U/kg diet phytase enzyme was suggested the right dose

of enzyme to increase efficiency of diet utilization. Rachmawati and Samidjan (2016), Rachmawati *et al.* (2017), Rachmawati *et al.* (2018), and Rachmawati and Samidjan (2018) found the same result in his study that phytase enzyme has an important role in increasing efficiency of diet utilization and can make diet conversion ratio lower than that of without phytase enzyme.

The relationship between the supplementation of phytase enzyme and protein efficiency ratio was indicated by quadratic equation as follows : $Y = 0.000001x^2 + 0.002x + 2.6655$ and $R^2 = 0.97$, from this equation can be found the optimum dose of phytase enzyme on FCR and $R^2 = 0.97$ states that 97 % FCR is influenced by the supplementation of phytase enzymes in diet while environmental factors only affect 3 % (Fig 5). The optimum dose of phytase enzyme for FCR was 1000 U/kg diet with the value of 1.78.

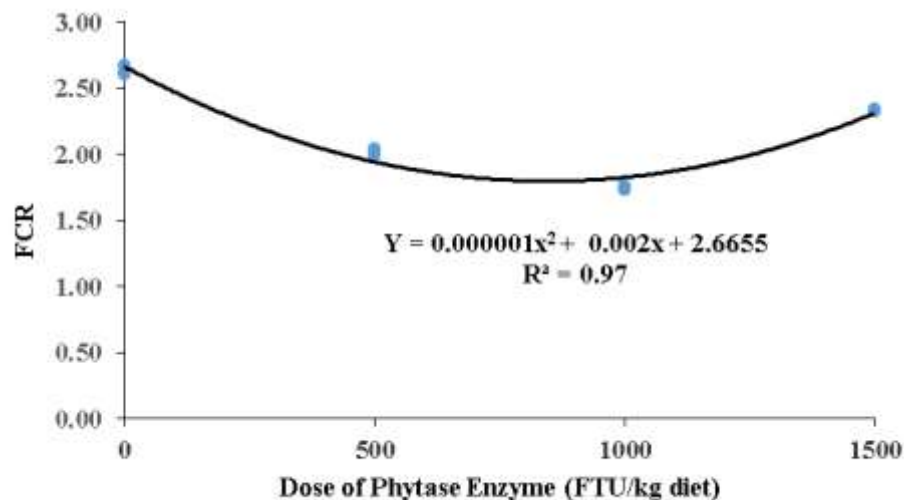


Figure 5. The relationship Graph between phytase enzyme and FCR of *L. vannamei*

The results show that the phytase enzyme supplementation in the diet significantly ($P < 0.01$) influenced the growth of white shrimp. It can be proven by comparing the content of phytic acid in the diet and feces. The contents of phytic acid in the feces were lower than those in the diet by as much 0.09%, 0.11%, 0.30%, 0.17% as in the treatments of A, B, C, and D respectively, as shown in the Table 2. The results of the laboratory test on phytic acid content in Treatment A (0 U/kg diet), B (500 U/kg diet), C (1000 U/kg diet), D (1500 U/kg diet) were 0.73%, 0.68%, 0.58%, and 0.63% respectively; while the contents of phytic acid in the feces were 0.64%, 0.57%, 0.29%, and 0.46% for treatments A, B, C, and D.

Those data show that diet containing phytic acid can influence the growth of white shrimp. This instance was supported by NRC (1993), it reported that diet containing 0.5% phytic acid can reduce growth and diet efficiency in rainbow trout (*O. mysskis*). The supplementation of phytase enzyme can unbind protein from phytic acid that increase the growth of fish (Amoah *et al.*, 2011; Haghbayan, and Mehrgan, 2015). Similar results were also reported in *Oreochromis niloticus* (Tahoun *et al.*, 2009), *Epinephelus fuscoguttatus* (Shapawi *et al.*, (2013), *Marsupenaes japonicus* (Bulbul *et al.*, 2015), *Psetta maxima* L. (Danswitz *et al.*, 2016), *Penaeus monodon* (Rachmawati and Samidjan, (2016), *Chanos chanos* (Rachmawati *et al.*, 2017), *Tilapia saline* (Rachmawati *et al.*,

2018) and *Cyprinus carpio* (Rachmawati and Samidjan, 2018).

The highest relative growth rate (RGR) of white shrimp happened in the Treatment C (1000 U/kg diet phytase enzyme supplementation) with the value of 3.05%, followed by Treatments D, B, and A with the value of 2.80%, 2.65%, and 1.89% respectively. The dose of (1000 U/kg diet phytase enzyme supplementation) was suggested the right dose to reduce phytic acid in the food. It was shown in the Table 2. the dose gave the biggest decrease in phytic acid as much as 0.29%, compared to other treatments. The similar result of study on *Penaeus monodon* was discovered by Rachmawati and Samidjan (2016). Moreover, Yu and Wang (2000) found that the dose of 1000 U/kg diet phytase enzyme supplementation can increase weight by 25% in crucian carp *Carassius carassius*, as Rachmawati et al. (2017), Rachmawati et al. (2018), Rachmawati dan Samidjan (2018) and Hassan et al. (2009) did in *Chanos chanos*.

The lowest RGR of white shrimp was found in the treatment A (0 U/kg diet) with the value of 1.89%. This was because of lack of phytase enzyme; therefore, the nutrients were not readily able to absorb. In turn, the Treatment A had the lowest decrease of phytic acid content as much as 0.09%. The lack of

enzyme caused phytic acid not able to break down into inositol and phosphate acid and would deprive white shrimp from inositol to grow. The effect of inositol deficiency in the fish was report by (NRC, 1993). It said that inositol deficiency can reduce appetite, slow bowel movement, cause anemia, and slow growth. The inositol deficiency also hampered mineral compound break down so it made shortage of phosphor availability. Phosphor is an important element to transform energy (NRC, 1993). The similar results were reported by Shapawi et al. (2013), Rachmawati and Samidjan (2016), Rachmawati et al. (2017), Rachmawati et al. (2018), Rachmawati and Samidjan (2018).

The relationship of phytase enzyme and RGR polynomial orthogonal resulted in quadratic pattern as follows $Y = -0.000001x^2 + 0.0025x + 1.8905$ and $R^2 = 0.99$, from this equation can be found the optimum dose of phytase enzyme on RGR and $R^2 = 0.99$ states that 99 % RGR is influenced by the supplementation of phytase enzymes in diet while environmental factors only affect 1 % (Fig 6). The optimum dose of phytase enzyme on RGR was 1000 U/kg diet with the value of 3.05%.

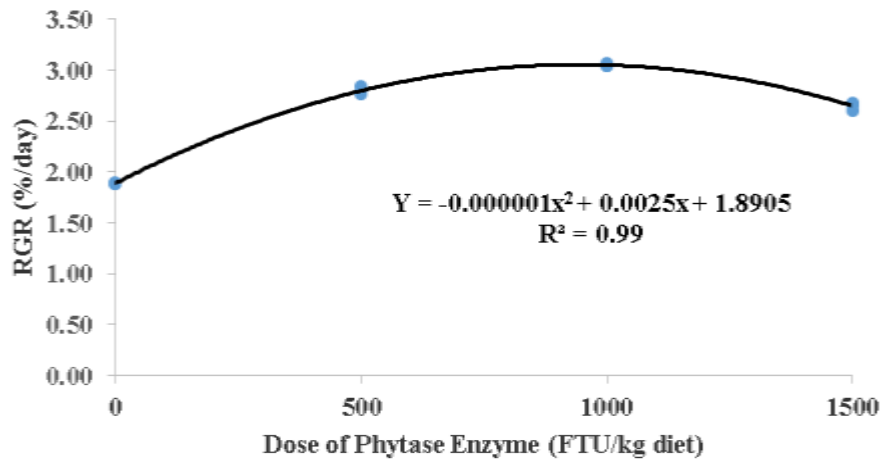


Figure 6. Relationship Graph between phytase enzyme and RGR of *L. vannamei*

The supplementation of phytase enzyme in the diet was insignificant ($P > 0.05$) on the survival rate of white shrimp, although it had significant effect on diet digestibility, efficiency of diet utilization, and growth. This phenomenon was also reported by Yakuputiyage (2013). Moreover, Yakuputiyage (2013) suggested that survival rate was

influenced by initial treatment and quality of culture media. The similar results were reported by Yoo et al. (2005), Debnath (2005), Baruah et al. (2007), Tahoun et al. (2009), Hassan et al. (2009), Bulbul et al. (2015), Danwitz et al. (2016), Husain et al. (2014), Rachmawati and Samidjan, (2016), Rachmawati et al. (2017).

4. Conclusion

Conclusions from the results of this study that phytase enzyme supplementation in diets with different doses can increase the growth of *L. vannamei* in the intensive system aquaculture. The best treatment result was the supplementation of 1000 U/kg diet phytase enzyme in the diet. The optimum doses of phytase enzyme in the diet of 1000 U/kg of diet produces *L. vannamei* growth up to 3.05% / day.

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