The Effect of Dietary Soybean Meal with Phytase Supplementation on Digestibility and Growth of Asian Seabass Lates calcarifer

Totok Yudhiyanto1, Suminto2, Diana Rachmawati2

1) Coastal Resource Management Program, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Java, Indonesia
2) Aquaculture Study Program, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Java, Indonesia

Corresponding author: t.yudhiyanto@gmail.com
Received 1 June 2017; Accepted 10 October 2017; Available online 28 November 2017

ABSTRACT

The purpose of this research was to evaluate Asian sea bass digestibility and growth fed with phytase dietary supplementation on soybean based fish diet. The initial weight of fish was 4.99 ± 0.2 g with stocking density of 20 fishes per tanks reared in 80 L tank of sea water for 6 weeks. Completely Randomized Design of 50 % soybean meal with phytase supplementation of 0; 500; 1,000; and 1,500 FTU kg−1 and three replications was used in this trial. Chromium oxide (Cr2O3) was used as digestibility indicator. Multivariate anova and One way anova (SPSS); linear regression (Microsoft Excel) and polynomial orthogonal (Microsoft Excel and Maple) was used to analyze the data. The results of Multivariate anova showed that phytase supplementation affect total digestibility (TD) and relative growth rate (RGR) of Asian seabass significantly (P<0.01) with phytase dose 1,000 FTU. One way anova also indicated that phytase 1,000 FTU significantly affect all parameters. Apparent digestibility coefficient(ADC) phosphorus had a very significant and dominant effect to total digestibility with R2 = 0.9669 than ADC protein and ADC fat (linear regression). TD significantly affects efficiency of dietary protein (EDP)30.57 % and protein efficiency ratio (PER) 0.67 %. EDP and PER significantly effect RGR 1.56 % day−1, simultaneously. The optimal dose of phytase supplementation is 1,220 FTU.

Keywords: phytase, digestibility, growth, Latescalcarifer.

1. Introduction

Lates calcarifer (Bloch), known as Asian seabass or barramundi, is not only a predatory fish but also an economically important fish species throughout the Asia-Pacific region. Petersen et al. (2011) explained that Asian seabass is a carnivorous, so it needs high dietary protein for fast and efficient growth.

Vegetable protein sources such as soy flour are widely used as partial replacement of fish meal. Silva-Carrillo et al., (2012) stated that soy flour has high protein content and a relatively balanced amino acid profile. The use of commercial soybean meal by 30 % can replace the role of fish meal in diet of juvenile Lateolabrax japonicus (Li et al., 2014). According to Lim et al. (2011) 30% soybean meal also could replace fish meal in juvenile Takifugu rupestris. While Song et al. (2014) stated that 15 to 50 % hydrolyzed soybean meal decreased feed conversion ratio and significantly improved growth parameters on juvenile Platichthys stellatus.

Soybean meal contains anti-nutritional factors in the form of phytic acid or myo-inositol hexakis phosphate (IP6) that could not be digested by carnivorous fish. Phytic acid binds to proteins and some minerals such as phosphorus, Mg2+, Ca2+, Fe2+, and Zn2+ very strongly (Monsan and O'Donohue, 2010). Supplementation of the phytase enzyme in soybean meal not only can hydrolyze phytic acid into phosphorilated myoinositol and inorganic phosphate which is easily absorbed by fish, but also at the same time might reduce phosphorus waste introduced into aquatic environments (Muray et al., 2013; Jegannathan and Nielsen, 2013).

Previous studies have reported that phytase supplementation has significant effect on Penaeus monodon (Biswa et al., 2007), Litopenaeus vannamei (Suprayudi et al., 2012), Epinephelus fuscoguttatus (Shapawi et al., 2013a & 2013b), Morones axatilis (Hughes and Soares Jr. 1998; Papatryphon et al., 1999), and European seabass Dicentrarchus labrax (Yoo et al., 2005). Phytase supplementation treatment has never been applied to Asian seabass. The aim of this research was to evaluate Asian sea bass digestibility and
growth fed with phytase dietary supplementation on soybean based fish diet.

2. Materials and Methods

Location, time, and fish

This research was conducted for 8 weeks starting from February 2015 at Balai Besar Pengembangan Budidaya Air Payau (BBPBAP) Jepara. The initial weight of juvenile Asian seabass is 4.99 ± 0.2 g, 45 days old and it comes from one parent.

Making of the feed

One gram phytase contains the enzymatic activity of 5000 Phytase Units (FTU), the dosage of phytase used according to Completely Randomized Design 4 treatments and 3 replications were 0 FTU; 500 FTU equivalent to 0.1 g; 1,000 FTU equivalent to 0.2 g; And 1,500 FTU equivalent to 0.3 g (Yoo et al., 2005; Biswas et al., 2007; Suprayudi et al., 2012). For each treatment, phytase was dissolved into 100 mL water at 37 °C (Yoo et al., 2005). The solution was added to soybean meal (400 g) and incubated for 24 hours at room temperature. All feed ingredients (Table 1) were mixed and molded into 3 mm pellets then dried in an oven with a maximum temperature of 50°C (Hien et al., 2015; Danwitz et al., 2016). Pellets with 45% protein content (Ambasankar et al., 2009) were stored in the refrigerator as stock during the research.

Initial feed and fish test

Initial proximate test on feed and fish was done to determine protein and fat content (Shapawi et al., 2013a). Chromium oxide tests were performed to determine the chromium oxide content of each feed (Shapawi et al., 2013a). The initial phosphorus (P) test was performed on the feed of each treatment and several fish samples (Yoo et al., 2005).

Table 1. Composition and formulation of the feed

<table>
<thead>
<tr>
<th>Feed composition (g kg⁻¹)</th>
<th>Phytase (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>375</td>
</tr>
<tr>
<td>Head shrimp meal</td>
<td>25</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>400</td>
</tr>
<tr>
<td>Rice bran</td>
<td>85</td>
</tr>
<tr>
<td>Fish oil</td>
<td>95</td>
</tr>
<tr>
<td>Supermix a</td>
<td>10</td>
</tr>
<tr>
<td>Cr₂O₃</td>
<td>5</td>
</tr>
<tr>
<td>Binder</td>
<td>5</td>
</tr>
<tr>
<td>Phytase</td>
<td>0</td>
</tr>
<tr>
<td>Total, 1,000</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>45.79</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>14.40</td>
</tr>
<tr>
<td>NFE (%)</td>
<td>11.87</td>
</tr>
<tr>
<td>P/E (g Protein MJ⁻¹) b</td>
<td>24.70</td>
</tr>
</tbody>
</table>

a Supermix: Vit.A 200.000 IU, Vit.D 40.000 IU, Vit.E 60 mg; Vit.B1 20 mg, Vit.B2 100 mg, Vit.B12 100 mg, Vit.K 12 mg, Niacinamide 150 mg, Ca-d-Panthotenate 50 mg, Folic Acid 10 mg, Choline Chloride 2000 mg, L-Lysine 500 mg, Methionine 2.000 mg, Magnesium (Mg) 700 mg, Ferrum (Fe) 500 mg, Manganese (Mn) 1.000 mg, Cuprisulfate 10 mg, Zinc (Zn) 200 mg, Potassium Iodine 2 mg, Antioxidant a carrier ad 0,1 mg.

b Calculated based on energy coefficients for protein, fat and NFE respectively 23,6; 39,5 dan 17,2 MJ kg⁻¹ (NRC 1993 in Arredondo-Figueroa et al. 2012).
Research preparation

A total of 12 sterile tanks (four treated three replicates) volume ± 80 L was set to maintain dissolved oxygen between 4-8 mg L⁻¹. The top of the tank was covered with a net to keep the fish from jumping out. The anesthetized and weighed fish was then selected based on a weight range of 5 g (Yoo et al., 2005; De et al., 2013). Selected fish was adapted to the tank and test feed for a week (De et al., 2013).

Research Experiment

The stocking density of each tank was 20 fish (Yoo et al., 2005). The test feed was administered twice daily in satiation every morning and evening, not only to maintain the fish during the research, but also to avoid cannibalism (Ribeiro & Qin, 2016). Sampling of fish weight is done every week. Fecal samples were collected with siphon 2 hours after feeding. The sample was dried at 60 °C and stored in the freezer for further analysis (De et al., 2013). Sea water is replaced by 20% every day (De et al., 2013). Water quality control includes temperature, pH, DO and salinity. The final proximate and phosphorus test of fish and feces were performed for each treatment and replication

Fish weight sampling was done on a weekly basis. Dead fish was weighed and recorded for further analysis. The proximate analysis of feed and fish (Shapawi et al., 2013a) includes the total nitrogen (protein) measured by micro-Kjeldahl method, the fat was determined by ether extraction, ash content was determined using an oven of 600 °C for 15 hours and the energy content was determined by bomb Calorimetry. Analysis of soybean meal phosphorus was determined by spectrophotometer. The digestibility of protein, fat and phosphorus was determined by chromium oxide fecal samples (Cr₂O₃) (Yoo et al., 2005). The concentration of Cr₂O₃ was determined by Atomic Absorption Spectrophotometers (AAS).

Digestibility parameters

- Total Digestibility (TD) (Cho et al., 1985):
  \[TD = 100 - [(\%Cr_d / \%Cr_f) \times 100]\]
- Apparent Digestibility Coefficient (ADC) (Hughes and Soares, Jr., 1998):
  \[ADC = 100 - [(\%Cr_d \times \%N_d)/(\%Cr_f \times \%N_f) \times 100]\]

Growth parameters

- Relative Growth Rate (RGR) (Takeuchi, 1988):
  \[RGR = \frac{W_f - W_0}{W_0 \times t} \times 100\%
  Information:
  \[W_f = \text{final weight}
  \[W_0 = \text{initial weight}
  \[t = \text{rearing time}

- Efficiency of dietary protein (EDP) (Takeuchi, 1988):
  \[EDP = \frac{([W_f + D] - W_0)/F} \times 100\%
  Information:
  \[D = \text{dead fish weight}
  \[F = \text{total feed consumed}

- Protein Efficiency Ratio (PER) (Maldonado-García, 2012):
  \[PER = \frac{W_f - W_0}{\text{g}/\text{Pi}}
  Information:
  \[\text{Pi} = \text{total feed consumed multiplied by percentage of feed protein}

Data analysis

Analysis of Variance (Manova) was used to analyze the effect of independent variables of dose enzyme phytase treatment on dependent variables of TD (digestibility) and RGR (growth). One way anova was also performed to examine the effect of phytase treatment on all parameters.

Information:

\[\% \text{Cr}_d = \% \text{Cr diet}
\[\% \text{Cr}_f = \% \text{Cr faeces}
\[\% \text{N}_d = \% \text{feed diet}
\[\% \text{N}_f = \% \text{faeces faeces}
\[N = \text{protein, fat, or phosphor us}

The Shapiro-Wilk normality test, Levene Homogeneity test, and Tukey Additivity test were performed before the analysis of manova and one way anova. If there is significant difference of 95 % (P = 0.05) or very significant difference 99 % (P = 0.01), Tukey HSD multiple comparison test is used to determine the difference of median value between treatment. Multiple linear regression analysis was performed between independent variables of ADC protein, ADC fat and ADC phosphorus to dependent variable of TD. Simple linear...
regression analysis was performed between independent variables of TD on dependent variable of EDP and PER. The last multiple linear regression analysis is between the independent variables of EDP and PER against the dependent variable of RGR. Analysis of manova and one way anova processed with SPSS, while regression analysis processed with Microsoft Excel. Regression analysis of orthogonal polynomials (Microsoft Excel) was used to determine the equation of phytase treatment to the parameter of digestion and growth. The orthogonal polynomial regression equation was processed by Maple to determine the optimum value of the phytase dose. The water quality data of dissolved oxygen (DO), pH, temperature and salinity was taken daily and analyzed descriptively.

3. Result and Discussion

Parameters data

This research applied 50% soybean meal meal and 50% fish meal. Phytase enzyme with several doses is used as a treatment to analyze the effect of reinforcement to the digestibility of feed by Asian seabass. Table 2 shows the final results data of all parameters based on each treatment and replication, where TD, ADC proteins, ADC fat and ADC phosphorus are digestibility parameters, EDP and PER are protein representations utilized by Asian seabass during the research, while RGR is a parameter growth. One way anova analysis results in all parameters showed that the dose of phytase of 1,000 FTU was significantly different and resulted in higher values than other phytase doses (Table 2).

**Phytase effect on digestibility and growth**

Initial stage to analyze the effect of phytase enzyme treatment on digestibility and growth is manova analysis between dependent variable of TD and RGR to independent variable of phytase dose. The Levene homogeneity test showed that both TD and RGR were homogeneous (P> 0.05). The results of the manova test showed that the phytase dose had a very significant effect (P<0.01) on TD and RGR. Tukey HSD multiple comparisons test for TD and RGR showed that the dose of 1,000 FTU was higher and significantly different than other doses.

**The main parameter that affect total digestibility**

Multiple linear regression analysis between independent variables of ADC Protein, ADC Fat and ADC Phosphorus against independent variables of TD showed that all of them simultaneously had a significant effect on TD. But individually, the regression results show that only the ADC phosphorus has a dominant and significant effect (P <0.01). The ADC phosphorus regression equation for TD (Figure 1) is $y = 1.2174x - 55.612$, $R^2 = 0.9669$. It means that the percentage effect of phosphorus ADC on TD is 96.7%, while the rest is influenced by ADC protein and ADC fat but both have no significant effect.

### Table 2. Parameter measured of total digestibility (TD), apparent digestibility coefficient (ADC) protein, ADC fat, ADC phosphorus, relative growth rate (RGR), efficiency of dietary protein (EDP) and protein efficiency ratio (PER)

<table>
<thead>
<tr>
<th>Treatment (FTU)</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TD (%)</td>
</tr>
<tr>
<td>0</td>
<td>23.57±1.8(^b)</td>
</tr>
<tr>
<td>500</td>
<td>26.89±2.7(^a)</td>
</tr>
<tr>
<td>1,000</td>
<td>30.85±2.1(^a)</td>
</tr>
<tr>
<td>1,500</td>
<td>29.93±1.2(^a)</td>
</tr>
</tbody>
</table>

**Note**: different superscript indicates a statistical different (P<0.05); data analyzed with one way anova and duncan multiple range test (SPSS)
One way ANOVA analysis (Table 2) showed that the dose of phytase 1,000 FTU resulted in the highest phosphorus ADC value of 71.58% but not significantly different from the 1,500 FTU dose of 69.63%. Phosphorus digestibility at 500 FTU is 67.67% not significantly different with 1,500 FTU and 0 FTU (65.22%). However, 0 FTU produced significantly different phosphorus digestibility than the 1,000 and 1,500 FTU dose.

The quadratic equation $y = -4.4142x^2 + 10.76x + 63.95$ and $R^2 = 0.9923$ is seen from the Figure 2 orthogonal polynomial graph for phosphorus digestibility. The calculation of the optimum dose of phytase for the ADC phosphorus is 1,220 FTU. The optimum phytase dose in this research was based on the significant and dominant influences of significant phosphorus ADCs against total digestibility (TD). According to Zhu et al. (2015) significant reductions in phytic acid by the phytase enzyme in catfish Ictalurus punctatus stomach in line with the absorption of phosphorus in the intestinal tract significantly. The addition of the 1,500 FTU phytase in this study showed an increase in phosphorus ADC not higher than the 1,000 FTU. A similar pattern is shown by red sea bream, the higher the dose of phytase does not increase the phosphorus digestibility but tends to decrease, the results may be due to higher phytase doses affecting the absorption process and the digestibility of the phosphorus in the digestive tract of fish (Biswa et al., 2007).

<table>
<thead>
<tr>
<th>Feed</th>
<th>Phosphorus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 FTU</td>
<td>0.74</td>
</tr>
<tr>
<td>500 FTU</td>
<td>0.78</td>
</tr>
<tr>
<td>1,000 FTU</td>
<td>0.83</td>
</tr>
<tr>
<td>1,500 FTU</td>
<td>0.77</td>
</tr>
</tbody>
</table>

The results of the phosphorus test in the test feed (Table 3) showed a gradual increase as the addition of phytase dose, the highest phosphorus content was 0.83% (1,000 FTU). The decrease of phytic acid level due to hydrolysis process by phytase is suspected to cause an increase in phosphorus digestibility in the test feed. The phytasehydrolysis of phytic acid liberates previously strongly bonded minerals such as phosphorus. Kumar et al. (2011) explained that phytic acid reacts with important minerals such as phosphorus in the feed forming very strong bonds that can not be digested by the upper intestine as well as by the next gastrointestinal tract. Papatryphon et al. (1999) stated that the addition of 1,000 FTU...
Phytase gives a phosphorus absorption of 67.1% for *Morone saxatilis*. The phytase dose of 1,000 FTU on feed of *Paralichthys olivaceous* based on 40% soybean meal resulted in the highest phosphorus digestibility significantly compared to other test feed of approximately 60% (Pham et al., 2008). Suprayudi et al. (2012) suggests that the addition of 1,000 FTU phytase can increase the phosphorus digestibility of white shrimp Litopenaeus vannamei by up to 88%.

Amino acid content in the test feed has increased with increasing levels of phytase enzyme. Lysine increased consecutively from feed without phytase to feed with a phytase content of 1500 FTU of 3.30%, 3.36%, 3.53% and 3.73%. While methionine also experienced the same trend, respectively 2.25%, 2.31%, 2.50% and 2.73%. According to Boonyaratpalin & Williams (2002) in Webster and Lim (2002) essential amino acids required by Asian seabass are methionine 2.24%, lysine 4.5%-5.2%, arginine and tryptophan 0.5%. Murillo-Gurrea et al. (2001) added that Asian seabass requires 4.5% lysine and 3.8% arginine of the total feed protein. In this research no amino acids were added and it appears that the amino acid content in the test feed did not achieve the recommended Asian seabass requirement of 4.5% for lysine (Boonyaratpalin & Williams (2002) in Webster and Lim (2002); Murillo-Gurrea et al. (2001)). While the level of methionine in the feed has been above the recommendation needs of Asian seabass. Insufficient amino acid levels in the test feed are thought to cause low ADC protein (Table 2) ranging from 62 to 71%

Bulbul et al. (2015) explained that methionine and lysine are limiting factors of amino acids from vegetable protein so that an additional amino acid from outside the feed protein is required to meet the fish's needs. Bulbul et al. (2015) added amino acid crystals (methionine and lysine) as well as phytase enzymes into the shrimp feed of *Marsupenaeus japonicas* kuruma shrimp made from 60% vegetable meal produced significant growth. An increase in the use of soybean meal by up to 40% in *Channa striata* and *C. micropterus* was obtained by addition of amino acid crystals (lysine, methionine and threonine) to feeds given phytase (Hien et al., 2015). Vandenbergen et al. (2011) also stated the same for *Oncorhynchus mykiss* rainbow trout.

The protein digestibility value in this study only ranged from 63.79% (0 FTU) to a high of 70.98% (1,000 FTU), which means an increase in protein digestibility of 7.19%. While the increase in levels of 500 and 1,500 FTU respectively are 3.08% and 6.44%. Danwitz et al. (2016) stated that the increase in protein digestibility occurs due to reduction in phytic acid levels in the digestive tract of fish due to the addition of phytase in the feed. Phytase does not hydrolyze proteins, but only releases protein bonds from phytic acid, so digestive enzymes work well and effective for treating liberated proteins (Kies et al 2006).

Suprayudi et al. (2012) showed that phytase hydrolyzes minerals and proteins bound by phytic acid into free nutrients to be digested by the digestive tract of fish so that protein digestibility increases. Morales et al. (2011) adds that the presence of phytic acid bonds significantly reduces the performance of protease enzymes in fish stomach and intestine. The 1,000 ft dose of FTU gives the highest protein digestibility compared to other doses, suggesting that the dosage works better.

**Effect of total digestibility on EDP and PER**

Linear regression analysis of independent variables of TD to dependent variable of EDP and PER showed that TD had significant effect (P < 0.01) to EDP. One way anova (Table 2) showed that the 1,000 FTU treatment resulted in an EDP of 30.57% and significantly different from other doses. The EDP values of the 500 and 1,500 FTU treatments did not differ significantly, respectively 23.56 and 24.80%. While the dose of 0 FTU showed the lowest EDP value of 17.08%.

The next linear regression analysis was between the independent variables of the TD to the dependent variable PER, with the result of TD giving a very significant effect (P < 0.01) to PER. Based on one way anova (Table 2), the dose of 1,000 FTU shows the highest PER value of 0.67% and is significantly different from other doses. The non-significant difference for PER occurs between the doses of 500 FTU and 1,500 FTU, 0.52% and 0.54% respectively. While the dose of 0 FTU showed the lowest PER of 0.37% and significantly different from other doses.

**Effect of EDP and PER on RGR**

The last stage to analyze the growth of Asian seabass is multiple linear regression analysis between independent variables of EDP and PER on dependent variable RGR. The results of multiple linear regression analysis showed that the increase of EDP and PER values simultaneously had a very significant effect (P < 0.01) on the growth of Asian seabass which is represented by RGR value. The graphic of EDP regression equation to RGR (Figure 3) shows the equation $y = 0.0516x - 0.1477$; $R^2 = 0.7389$. While the graphic of PER regression equation to RGR (Figure 4) shows the equation $y = 2.3593x - 0.1497$; $R^2 = 0.7298$. 
Total feed digestibility has significant effect on protein utilization value i.e. EDP and PER. The EDP value increased by 13.49% at a dose of 1,000 FTU higher than the increase in EDP values at other doses i.e. 7.72% (1,500 FTU) and 6.48% (500 FTU), respectively. The dose of 1,000 FTU produces the highest EDP may be due to white snapper is able to utilize proteins that are freed from phytic acid more efficiently. As shown in the digestibility parameters that the dose of 1,000 FTU gives the best results among other doses, so that with higher digestibility values will be followed by increased absorption of fish to feed proteins as represented by EDP values. Increased levels of amino acids in the test feed along with increased levels of phytase enzyme supposedly provide an increase in EDP values.

The protein efficiency (PER) ratio in this study ranged from 0.37 to 0.67. The addition of 1000 FTU phytase gives the highest PER value of 0.67. Increases in PER value with the addition of phytase are 0.13 (500 FTU), 0.3 (1,000 FTU) and 0.17 (1,500 FTU), respectively. The low PER value in this research is thought to be influenced by the lack of amino acid levels in the test feed. Rachmawati and Hutabarat (2006) proves that the addition of phytase can improve the protein efficiency ratio in tiger grouper feed, in which 1,000 FTU produce PER 0.90. In addition, high levels of fiber in the feed are also suspected to limit the efficiency of proteins that can be absorbed by fish.

A ratio of fish and soybean meal 50:50 was also thought to cause a low PER value. Although the PER value in this research is low, phytase of 1,000 FTU shows the best results among other treatments, this is supported by the following studies. Hassaan et al. (2013) reported that PER tilapia was significantly higher at 1.84% (1,000 FTU). The protein-based feed of soybean meal with 1,000 FTU phytase resulted in the PER that not significantly different than that of 100% fish meal, 2.24% and 2.32% respectively (Hien et al., 2015). Based on Lim & Lee (2009) phytase 1,000 FTU on feed of parrot Oplegnathus fasciatus shows
higher PER value which is 1.32% but not significantly different than 100% fish meal.

**RGR**

Supplementation of the 1,000 FTU enzyme in the test feed gave a significant percentage of growth rate of 1.56 % compared to the other three treatments. Increasing the growth rate in Asian seabass with a feed of FTU 1000 is possible because the fish is able to perform metabolism process more efficiently than fish on other treatments. The utilization of energy by fish in the 1,000 FTU dose is possible higher, resulting in greater growth than other treatments. According Cuzon et al (2011) optimal energy requirement for growth of white snapper at water temperature 28 °C is 17 kJ g⁻¹.

The increase of RGR value compared to phytase 0 FTU is 0.18 % (500 FTU), 0.78 % (1,000 FTU) and 0.28 % (1,500 FTU). The highest RGR value is indicated by the dose of phytase 1,000 FTU of 1.56 %. The result of linear regression analysis showed that EDP and PER significantly influenced RGR value. Low EDP and PER values directly affect the relative growth rate of Asian seabass. The highest RGR value is indicated by the dose of 1,000 FTU and 0.28 %.

4. Conclusion

Based on the result of this research, it could be concluded that phytase enzyme supplementation of 1,000 FTU on soybean meal based feed has highly significant effect to digestibility and growth of Asian seabass. Apparent digestibility coefficient (ADC) of phosphorus has significant and dominant effect on total digestibility (TD). The optimum dose of phytase enzyme supplementation is 1,220 FTU.

Table 4. Water quality data during the research.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Scale</th>
<th>Result</th>
<th>Optimum range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>27.1 – 28.9</td>
<td>26 – 32°C</td>
<td>Thirunavukkarasu et al. (2009)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.31 – 7.75</td>
<td>7.0 - 8.2</td>
<td>Thirunavukkarasu et al. (2009)</td>
</tr>
<tr>
<td>DO</td>
<td>mg L⁻¹</td>
<td>5.41 - 6.99</td>
<td>4 – 8 mg L⁻¹</td>
<td>Ignatius (2009)</td>
</tr>
<tr>
<td>Salinity</td>
<td>‰</td>
<td>30 – 31</td>
<td>28 – 33‰</td>
<td>Ignatius (2009)</td>
</tr>
</tbody>
</table>

**Water quality**

Water quality during the research is still considered to meet the requirements and survival of Asian seabass. Water quality observation during the research can be seen in table 4.

**References**


Lim, S-J., Kim, S-S., Ko, G-Y., Song, J6W., Oh, D-H., Kim, J-D., Kim, J-U., Lee, K-J. 2011. Fish Meal Replacement by


