Omni-Akuatika, 13 (2): 144 – 154, 2017
ISSN: 1858-3873 print / 2476-9347 online

Research Article

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

Totok Yudhiyanto[1,*], Suminto[2], Diana Rachmawati[2]

[1)] *Coastal Resource Management Program, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Jawa, Indonesia*
[2)] *Aquaculture Study Program, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Jawa, 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 (Cr_2O_3) 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 alsoan economically important fish species througout 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 couldreplace fish meal in juvenile *Takifuguru bripes*. 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, Mg^{2+}, Ca^{2+}, Fe^{2+}, and Zn^{2+} 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 (Murray et al., 2013; Jegannathan and Nielsen, 2013).

Previous studies have reported that phytase supplementation has significant effect on *Penaeus monodon* (Biswas 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 aimof this research was to evaluate Asian sea bass digestibility and

http://dx.doi.org/10.20884/1.oa.2017.13.2.263

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

Feed composition ($g\ kg^{-1}$)	Phytase ($g\ kg^{-1}$)			
	0	0.1	0.2	0.3
Fish meal	375	375	375	375
Head shrimp meal	25	25	25	25
Soybean meal	400	400	400	400
Rice bran	85	85	85	85
Fish oil	95	95	95	95
Supermix[a]	10	10	10	10
Cr_2O_3	5	5	5	5
Binder	5	4.9	4.8	4.7
Phytase	0	0.1	0.2	0.3
Total	1,000	1,000	1,000	1,000
Protein (%)	45.79	45.15	45.72	45.99
Fat(%)	14.40	13.96	14.32	14.56
NFE (%)	11.87	11.78	11.83	11.37
P/E (g Protein MJ^{-1}) [b]	24.70	24.81	24.74	24.78

[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 wasset 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 wasthen selected based on a weight range of 5 g (Yoo et al., 2005; De et al., 2013). Selected fish wasadapted 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 feceswere 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 wasdetermined using an oven of 600 °C for 15 hours and the energy content was determined by bomb Calorimetry. Analysis of soybean meal phosphoruswas determined by spectrophotometer. The digestibility of protein, fat and phosphorus was determined by chromium oxide fecal samples (Cr_2O_3) (Yoo et al., 2005). The concentration of $Cr_2O_3$was determined by Atomic Absorption Spectrophotometers (AAS).

Digestibility parameters

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

Growth parameters

- Relative Growth Rate(RGR) (Takeuchi, 1988):

  $RGR = [W_t – W_0 / W_0 x t] x 100\%$

  Information :

  W_t = final weight
  W_0= initial weight
  t = rearing time

- Efficiency of dietary protein(EDP) (Takeuchi, 1988):

  $EDP = \{[(W_t + D) – W_0]/F\} x 100\%$

  Information:

  D = dead fish weight
  F = total feed consumed

- Protein Efficiency Ratio(PER) (Maldonado-García, 2012):

  $PER = W_f – W_0 (g)/ Pi$

  Information :
  Pi = 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 :

$\% Cr_d$ = % Cr diet	$\% N_d$ = % feed nutrition
$\% Cr_f$ = %Cr faeces	$\% N_f$ = % faeces nutrition
N = protein, fat,orphosphorus	

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 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, whereTD, 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 phytasedose. The Levene homogeneity test showed that both TD and RGR were homogeneous (P> 0.05). The results of the manova test showed that the phytasedose 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 on TD (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)

Treatment (FTU)	Parameters						
	TD (%)	ADC Protein (%)	ADC Fat (%)	ADC Phosphorus (%)	RGR (%)	EDP (%)	PER
0	23.57±1.8^{b}	63.80±0.8^{c}	75.51±2.1^{a}	65.22±1.4^{c}	0,78±0,2^{c}	17,08±0,3^{c}	0,37±0,0^{c}
500	26.89±2.7^{ab}	65.40±1.3^{b}	71.81±1.4^{b}	67.67±1.0^{bc}	0,96±0,1^{bc}	23,56±2,0^{b}	0,52±0,0^{b}
1,000	30.85±2.1^{a}	70.98±0.8^{a}	63.92±2.1^{c}	71.58±1.8^{a}	1,56±0,0^{a}	30,57±2,4^{a}	0,67±0,0^{a}
1,500	29.93±1.2^{a}	70.23±0.5^{a}	74.15±1.2^{ab}	69.63±1.1^{ab}	1,06±0,0^{b}	24,80±2,9^{b}	0,54±0,1^{b}

Note : different superscript indicates a statistical different (P<0,05); data analyzed with one way anova and duncan multiple range test (SPSS)

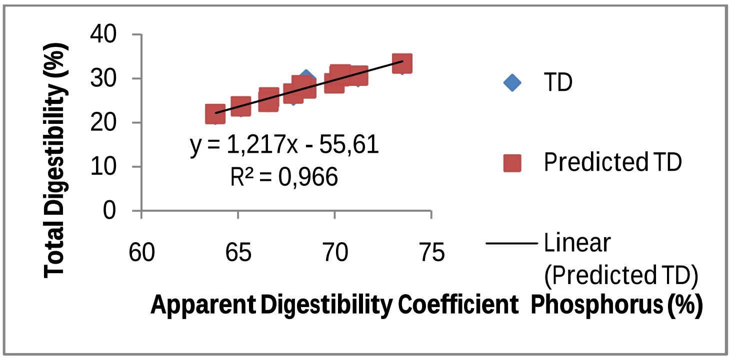

Figure 1.Linear regression analysis graphic of ADC phosphorusonTD

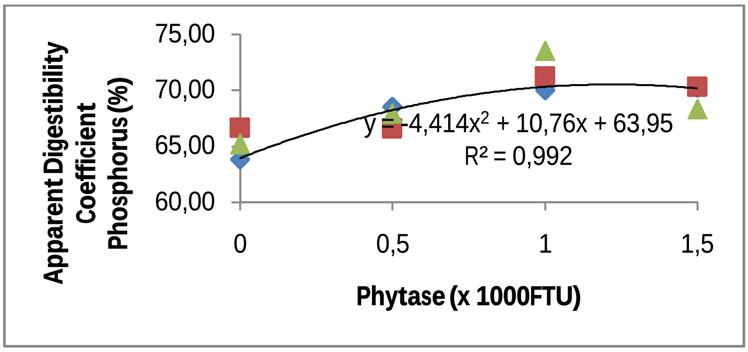

Figure2. Polynomial orthogonal graphic of ADC phosphorus

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 = -4.4142x2 + 10.76x + 63.955 and R^2 = 0.9923 is seen from the Figure 2 orthogonal polynomial graph for phosphorus digestibility. The calculation of the optimum dose of phytasefor 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 *Ictaluruspunctatus* 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 (Biswas et al., 2007).

Table3.Phosphorus level on test

Feed	Phosphorus (%)
0 FTU	0.74
500 FTU	0.78
1,000 FTU	0.83
1,500 FTU	0.77

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 olivaceus* based on 40 % soybean mealresulted 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 Boonyarat palin & Williams (2002) in Webster and Lim (2002) essential amino acids required by Asian seabass are methionine 2.24 %, lysine 4.5 - 5.2%, 3.8% 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 and 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 *Channastriata* and *C. micropeltes* was obtained by addition of amino acid crystals (lysine, methionine and threonine) to feeds given phytase (Hien et al., 2015).Vandenberg 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 $y = 2,3593x - 0,1497$; $R^2 = 0,7298$.

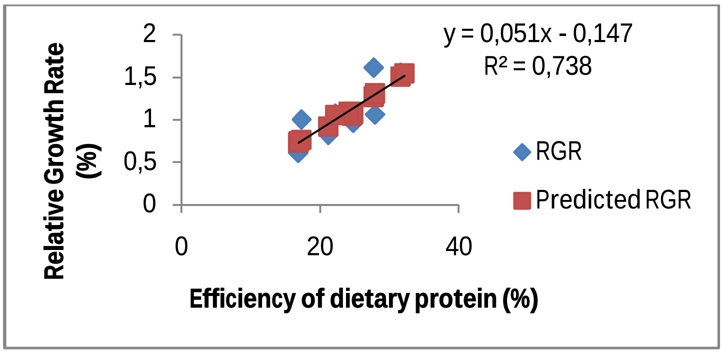

Figure 3. Linear regression analysis graphic ofEDP on RGR

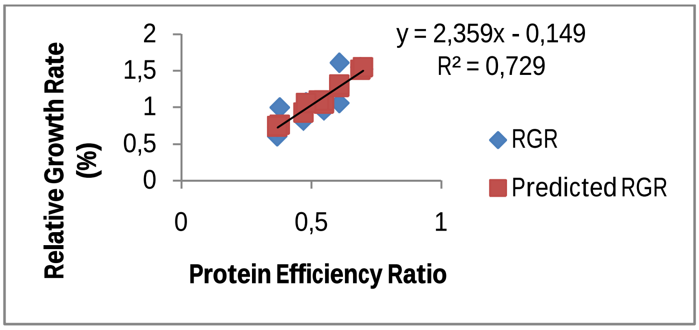

Figure 4.Linear regression analysis graphic ofPER on RGR

EDP

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 ie 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.

Phytase 1,000 FTU supplementation in Japanese seabass *Lateolabraxjaponicus* feed produced a high feed efficiency value of 97% (Ai et al., 2007). Fortes-Silva et al. (2011) stated that the value of feed efficiency increased to 56% for *Dicentrarchuslabrax* fish with the addition of 1,500 FTU phytase. The incomplete profile of amino acids to meet the needs of Asian seabassin this study allegedly led to low EDP value although fermentation process of soybean meal with phytasewas done before pellet making.

PER

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 showed 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 phyase resulted inthe 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 ^{o}Cis 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 incomplete amino acid profile in the feed is thought to cause lower digestibility and then impact on slow growth of Asian seabass. Low phosphorus digestibility is also thought to affect the growth of Asian seabass, the main function of phosphorus for fish is as an important mineral growth, bone formation and reproduction (Cao et al., 2007).

Zhu et al. (2014) stated that a dose of 1,000 FTU was able to provide the growth performance of juvenile *Pelteobagrus fulvidraco* significantly. Whereas in subsequent studies in the same fish with 500 FTU,phytase did not give significant growth (Zhu et al., 2015). Danwitz et al. (2016) added that phytase supplementation with a dose of 1,000 FTU could significantly increase fish growth compared to diet without phytase supplementation in *Psetta maxima*.

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.

Table 4. Water quality data during the research.

Parameter	Scale	Result	Optimum range	Reference
Temperature	°C	27,1 – 28,9	26 – 32°C	Thirunavukkarasu et al. (2009)
pH		7,31 – 7,75	7,0 -8,2	Thirunavukkarasu et al. (2009)
DO	mg L^{-1}	5,41 - 6,99	4 – 8 mg L^{-1}	Ignatius (2009)
Salinity	‰	30 – 31	28 – 33‰	Ignatius (2009)

4. Conclusion

Based on the result of this research, it couldbe 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.

References

Ai, Q., Mai, K., Zhang, W., Xu, W., Tan, B., Zhang, C., Li, H. 2007. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. Comparative Biochemistry and Physiology Part A 147: 502–508.

Ambasankar, K., Ahmad Ali, S., Syamadayal, J. 2009. Nutritional Requirements of Asian Seabass, *Lates calcarifer*. In: Course manual: National Training on Cage Culture (Ed. Imelda Joseph et al.), Central Marine Fisheries Research Institute. December 14-23, 2009. Cochin. pp. 60-65.

Biswas, A.K., Kaku, H., Ji, S.C., Seoka, M., Takii, K. 2007. Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. Aquaculture 267: 284 – 291.

Boonyaratpalin, M., Williams, K. 2002. Asian Sea Bass, *Lates Calcarifer*. *in* Webster, C.D., and C.E. Lim (Eds). 2002. Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI Publishing.pp. 40-50.

Bulbul, M., Abdul Kader, M.D., Ambak, A.A., Hossain, M.S., Ishikawa, M., Koshio, S. 2015. Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, *Marsupenaeus japonicus*. Aquaculture 438: 98–104.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luoa, Z., Li, D. 2007. Review : Application of Microbial Phytase in Fish Feed. Enzyme and Microbial Technology.11 p.

Cho, C.Y., Cowey, C.B., Watanabe, R. 1985. Finfish Nutrition in Asia : Methodological Approaches to Research and Development. International Development Research Centre. Ottawa. 154 pp.

Cuzon, G., Guttierez, D., Da Silva, A., Castillo A., de los Santos, N., Chiappa, X.,Gaxiola, G. 2011. Contribution to Protein/Energy Requirement and Metabolic Aspects for Two Carnivorous Species *Lates Calcarifer, Epinephelus Morio* Juveniles With Reference to Trout. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Hernández-Hernández, L. (Eds), Avances en Nutrición Acuícola XI - Memorias del Décimo Primer Simposio Internacional de NutriciónAcuícola, 23-25 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-775-4.Universidad Autónoma de Nuevo León, Monterrey, México, pp. 224-235.

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

De, D., Ghoshal, T. K., Raja, R. A., Kumar, S. 2013. Growth performance, nutrient digestibility and digestive enzyme activity in Asian seabass, *Lates calcarifer* juveniles fed diets supplemented with cellulolytic and amylolytic gut bacteria isolated from brackish water fish. Aquaculture Research: 1–11.

Fortes-Silva, R., F.J. Sánchez-Vázquez, F.J. Martínez. 2011. Effects of pretreating a plant-based diet with phytase on diet selection and nutrient utilization in European sea bass. Aquaculture 319: 417–422.

Hassaan, M.S., Soltan, M.A., Agouz, H.M., Badr, A.M. 2013. Influences of calcium/phosphorus ratio on supplemental microbial phytase e□ciency for nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Research 39: 205–213.

Hien, T.T.T., Be, T.T., Lee, C.M., Bengtson, D.A. 2015. Development of formulated diets for snakehead (*Channa striata* and *Channa micropeltes*): Can phytase and taurine supplementation increase use of soybean meal to replace fish meal?. Aquaculture 448: 334 – 340.

Hughes, K.P., Soares, J.H. 1998. Efficacy of Phytase on Phosphorus Utilization in Practical Diets Fed to Striped Bass *Moronesaxatilis*. Aquaculture Nutrition 4: 133-140.

Ignatius, B. 2009. Grow Out Culture of Seabass in Cages. *In*: *Course manual: National Training on Cage Culture* (Ed. Imelda Joseph et al.), Central Marine Fisheries Research Institute. December 14-23, 2009. Cochin. pp. 99-101.

Jegannathan, K.R., Nielsen, P.H. 2013. Environmental Assessment of Enzyme Use in Industrial Production - A Literature Review. Journal of Cleaner Production 42 : 228-240.

Kies, A.K., De Jonge, L.H., Jongbloed, A.W., Kemm, P.A. 2006. Interaction between Protein, Phytate, and Microbial Phytase. In Vitro Studies.J. Agric. Food Chem. 2006, 54.Journal of Agricultural and Food Chemistry, pp, 1753-1758.

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

Li, Y., Ai, Q., Mai, K., Xu, W., Deng, J., Cheng, Z. 2014. Comparison of high-protein soybean meal and commercial soybean meal partly replacing fish meal on the activities of digestive enzymes and aminotransferases in juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828). Aquaculture Research 45: 1051–1060.

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

Soybean Meal in Diets for Tiger Puffer, *Takifuguru bripes*. Aquaculture 313: 165–170.

Lim, S.-J., Lee, K-J. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquculture 290: 283 – 289.

Maldonado-García, M., J. Rodríguez-Romero, M. Reyes-Becerril, C.A. Álvarez-González, R. Civera-Cerecedo, M. Spanopoulos. 2012. Effect of Varying Dietary Protein Levels on Growth, Feeding Efficiency, and Proximate Composition of Yellow Snapper *Lutjanus argentiventris* (Peters, 1869). Latin American Journal of Aquatic Research 40(4): 1017-1025.

Monsan, P., M.J. O'Donohue. 2010. Industrial Biotechnology in the Food and Feed Sector. *In* :Soetaert, W. and E.J. Vandamme. 2010. *Industrial Biotechnology Sustainable Growth and Economic Success (Eds)*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. Germany. pp. 370-371.

Morales, G.A., Márquez, L., Saenz de Rodrigañez, M., Bermúdez, L., Robles, R., Moyano, F.J. 2014. Effect of phytase supplementation of a plant-based diet on phosphorus and nitrogen bioavailability in sea bream *Sparusaurata*. Aquaculture Nutrition 20: 172-182.

Morales, G.A., Moyano, F-J., Marquez, L. 2011. In Vitro Assessment of the Effects of Phytate and Phytase on Nitrogen and Phosphorus Bioaccessibility within Fish Digestive Tract. Animal Feed Science and Technology 170: 209– 221.

Murillo-Gurrea, D. P., Coloso, R.M., Borlongan, I.G., Serrano Jr, A.E. 2001.Lysine and arginine requirements of juvenile Asian sea bass (*Lates calcarifer*). Journal of Appllied Ichthyology 17 : 49-53.

Murray, P.M., Moane, S., Collins, C., Beletskaya., T., Thomas, O.P., Duarte, A.W.F., Nobre, F.S., Owoyemi, I.O., Pagnocca, F.C., Sette, L.D., McHugh, E. Causse, E., Perez-Lopez, P., Feijoo, G., Moreira, M.T., Rubiolo, J., Leiro, M., Botana, L.M., Pinteus, S., Alves, C., Horta, A., Pedrosa, R., Jeffryes, C., Agathos, S.N., Allewaert, C., Verween, A., Vyverman, W., Laptev, I., Sineoky, S., Bisio, A., Manconi, R., Ledda, F., Marchi, M., Pronzato, R., Walsh, D.J. 2013. Sustainable Production of Biologically Active Molecules of Marine Based Origin. New Biotechnology 30(6): 839-850.

Papatryphon, E., Howell, R.A., Soares Jr, J.H. 1999. Growth and Mineral Absorption by Striped Bass *Morones axatilis* Fed a Plant Feedstuff Based Diet Supplemented with Phytase. Journal of the World Aquaculture Society 30(2): 161-173.

Petersen, E.H., Phuong, T-H., Ng.K.,Dat, V.A., Tuan, L.V., Truc. 2011. Bioeconomics of Asian seabass, *Lates calcarifer*, culture in Vietnam. In : ACE Discussion Paper.2011/3.15 p.

Pham, M.A., Lee, K-J., Dang, T.M., Lim, S-J., Ko, G-Y., Eo, J., Oh, D-H. 2008. Improved Apparent Digestibility Coefficient of Protein and Phosphorus by Supplementation of Microbial Phytase in Diets Containing Cottonseed and Soybean Meal for Juvenile Olive Flounder (*Paralichthys olivaceus*).Asian-Australian Journal Animal Sciences 21(9): 1367-1375.

Rachmawati, D., Hutabarat, J. 2006. Efek Ronozyme P dalam Pakan Buatan Terhadap Pemanfaatan Efek Ronozyme P dalam Pakan Buatan Terhadap Pemanfaatan Pakandan Pertumbuhan Ikan Kerapu Macan (*Epinephelus fuscoguttatus*). Ilmu Kelautan 11 (4), 193 – 200.

Ribeiro, F.F., Qin, J.G. 2016. Bioenergetics of cannibalism in juvenile barramundi *Lates calcarifer* (Bloch): exploring growth advantage of fish fed live prey and formulated diet. Aquaculture Research 47: 2324–2333.

Shapawi, R., Ebi, I., Yong, A. 2013a.Soybean Meal as a Source of Protein in Formulated Diets for Tiger Grouper, *Epinephelus fuscoguttatus* Juvenile. Part I: Effects on Growth, Survival, Feed Utilization and Body Compositions. Agricultural Sciences 4 (7): 317-323.

Shapawi, R., Ebi, I., Yong., Chong, M., Chee, L.K., Sade, A. 2013b. Soybean Meal as a Source of Protein in Formulated Diets for Tiger Grouper, *Epinephelus fuscoguttatus* Juvenile. Part II: Improving Diet Performances with Phytase Supplementation. Agricultural Sciences 4 (6A): 19-24.

Silva-Carrillo, Y., Hernández, C., Hardy, R.W., González-Rodríguez, B., Castillo-Vargasmachuca, S. 2012. The Effect of Substituting Fish Meal with Soybean Meal on Growth, Feed Efficiency, Body Composition and Blood Chemistry in Juvenile Spotted Rose Snapper *Lutjanus guttatus* (Steindachner, 1869). Aquaculture 364-365 : 180-185.

Song, Z., Li, H., Wang, J., Li, P., Sun, Y., Zhang, L. 2014. Effects of Fishmeal Replacement with Soy Protein Hydrolysates on Growth Performance, Blood Biochemistry, Gastrointestinal Digestion and Muscle Composition of Juvenile Starry Flounder (*Platichthys stellatus*). Aquaculture 426–427 : 96–104.

Suprayudi M.A., Harianto, D., Jusadi, D. 2012. Kecernaan Pakandan Pertumbuhan Udang Putih *Litopenaeus vannamei* Diberi Pakan Mengandung Enzim Fitase Berbeda. Jurnal Akuakultur Indonesia 11 (2):103-108.

Takeuchi, T. 1988. Laboratory work, chemical evaluation of dietary nutrients.*In*: Watanabe, T., editor. *Fish Nutrition and Mariculture. JICA Textbook, the General Aquaculture Course*. Department of Aquatic Biosciences, Tokyo University of Fisheries. Tokyo. pp 179-233.

Thirunavukkarasu, A. R., Kailasam, M.,Sundaray, J. K. 2009.Success in Hatchery Development of Seabass and Its Potential for Commercial cage Culture in India.*In*: Course manual: *National Training on Cage Culture* (Ed. Imelda Joseph et al.). Central Marine Fisheries Research Institute. Cochin. pp. 71-80.

Vandenberg, G.W., Scott, S.L. Sarker, P.K. Dallaire, V., de la Noüe, J. 2011. Encapsulation of microbial phytase: Effects on phosphorus Bioavailability in rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology 169: 230–243.

Yoo, G.-Y., Wang, X., Choi, S., Han, K.,.Kang, J-C., Bai;, S.C. 2005. Dietary Microbial Phytase Increased the Phosphorus Digestibility in Juvenile Korean Rockfish *Sebastesschlegeli* Fed Diets Containing Soybean Meal. Aquaculture 243: 315 – 322.

Zhu, W.X., Zhong, G.F. ., Hua, X.M., Ju, M., Chen, X.M., Du, Z.Y. 2015. The optimum active site of acidic phytase in the gastrointestinal tract of channel fish (*Ictalurus punctatus*) *in vitro* and *in vivo* studies. Aquaculture Nutrition: 1-9.