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Evaluation of pollard substitution with water hyacinth *Eichhornia crassipes* on enzyme activity and growth performance of tilapia *Oreochromis niloticus*

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Abstract

The present study was to subtitute pollard as one of raw materials in fish feed production obtained from the import with other raw material. One potential alternative raw material is water hyacinth *Eichhornia crassipes*. This research was conducted with 5 treatments and 3 replications namely feed with substitution of pollard with *E. crassipes* at 0%, 25%, 50%, 75% and 100%. On the test of growth performance, tilapias (*Oreochromis niloticus*) with the initial weight of 6.55 ± 0.02 g $fish^{-1}$ were maintained for 60 days. On digestibility test, treatment feeds were added with 0.6% Cr_2O_3 and fed to tilapia with a weight of 23.70 ± 0.48 g $fish^{-1}$. The results show that tilapia fed with 50% replacement rate of pollard yielded similar relative growth, feed efficiency, protein retention and protein digestibility with 0% treatment. The activities of protease, amylase and cellulase decreased due to the increase in water hyacinth in the feed. In conclusion, water hyacinth flour can be used up to 50% as a pollard substitution with 12.5% of material quantity in the diet of tilapia

Keywords: Eichhornia crassipes, enzyme activity, growth, tilapia

1. Introduction

Culture feed is the major part of production cost in aquaculture activities. In general, feed cost contributes approximately 60-70% of the total production cost in semi-intensive and intensive aquaculture activities (Singh *et al*. 2006) [15]. The availability and price of imported raw materials are highly dependent on producer countries. The rarely of raw materials leads to higher prices, it is also caused by Dollar exchange rate. Therefore, an alternative raw material to reduce dependence on imported raw materials is needed. One of the materials that could potentially be used as feed ingredients is water hyacinth.

Eichhornia crassipes has the ability to adapt from extreme changes in water conditions, nutrient levels, pH, temperature and water level (Sotolu 2012) [17]. Based on the chemical analysis, this plant contains 80.1% organic material, 12.4% protein, amino acids, lysine and methionine, 19.9% crude fiber and 4.72% fat (Chang *et al*. 2013) [2]. A rapid growth of water hyacinth is often considered as a weed in the water because it can cover water surface. Therefore, water hyacinth is utilized not only for reducing the use of imported raw materials but also for reducing waste waters.

The use of water hyacinth in the fish feed will affect the fish growth. Sotolu and Sule (2011) [18] reported that the utilization of water hyacinth flour in replacing soybean meal amounted to 22.91% in catfish showed significantly different fish growth. Utilization of vegetable raw materials may affect the activity of the protease of fish (Liao *et al.* 2015) [8]. Manufacture of plant-based fish feed should be focused on its optimal level in the feed for effective utilization (Francis *et al*. 2001) [3].

This research used *E. crassipes* obtained from Sukabumi (West Java) contained 11.91% protein. The protein content was not much different from pollard (14.54%) which is an imported product. Therefore, this study was performed to evaluate the utilization of *E. crassipes* in replacing pollard on enzyme activity, growth performance, and feed digestibility test.

2. Materials and Methods
2.1 Experimental Design
In this study, a completely randomized design was applied consisted of five treatments with three replications. The study was divided into two stages, namely fish maintenance for growth test and for digestibility test of feed treatments. The treatment in this study was pollard substitution with *E. crassipes* in feed at 0, 25, 50, 75 and 100%.

2.2 Experimental Diets
Parts of *E. crassipes* which were ground were the leaves and stems. *E. crassipes* were dried in oven at 60 °C for 24 hours, then mashed up into powder with a grinding machine. Results of the proximate analysis of *E. crassipes* flour were 11.91% protein, 0.65% fat, 22.58% ash, 30.05% crude fiber and 34.81% Nitrogen Free Extract (NFE). Tested feeds were made in accordance with the formulation. Raw materials were mixed by using mixer which was suitable for formulation composition in each treatment (Table 1). Protein for experimental diet was made in the range of 30%. Once mixed, feed were pelleted with a size of 1 mm and then dried using oven at 60 °C for 12 hours. On the test of feed digestibility, chromium oxide (Cr_2O_3) was used as much as 0.6% as an indicator.

Table 1: Formulation and proximate composition of the experimental diets

Ingredients	Treatment of pollard substitution with *E. crassipes*				
	0%	25%	50%	75%	100%
Fish meal	13.00	13.00	13.00	13.00	13.00
Meat bone meal	20.00	20.00	20.00	20.00	20.00
Soybean meal	23.00	23.00	23.00	24.00	24.50
Bran	10.00	9.00	8.50	6.00	4.50
Pollard	25.00	18.75	12.5	6.25	0.00
E. crassipes Flour	0.00	6.25	12.5	18.75	25.00
Fish Oil	1.00	1.50	1.75	2.50	3.00
Corn Oil	1.00	1.50	1.75	2.50	3.00
Premix	5.00	5.00	5.00	5.00	5.00
Binder (CMC)	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Protein	30.26	30.41	30.35	30.31	30.64
Lipid	7.28	8.33	8.56	10.07	10.75
Ash	13.39	18.37	20.07	20.47	20.32
Fiber	3.47	7.76	8.71	10.91	13.06
NFE	45.01	35.14	32.31	28.24	25.22
GE (kkal kg^{-1})	4243.74	3945.85	3848.23	3821.17	3780.40
C/P ratio	14.24	12.98	12.68	12.61	12.34

Description: NFE = Nitrogen free extract; GE = Gross Energy; 1 g protein = 5.6 kkal, 1 g lipid = 9.4 kkal, 1 g carbohydrate/ NFE = 4.1 kkal (Watanabe 1988) [22]

2.3 Fish Rearing for Growth Test
The container used was aquarium of 60 cm × 50 cm × 40 cm. The fish samples used were tilapia *Oreochromis niloticus* with the initial weight of 6.55 ± 0.02 g $fish^{-1}$. Tilapia were acclimatized in advance for seven days before they were used in experiments. The fish were reared at density of 10 fish per aquarium and fed with tested feed as at satiation for 60 days. Feeding frequency was done three times a day namely at 8 am, 12 pm, and 4 pm. During the maintenance period, 50% water exchange was performed every three days. Water quality parameters measured were temperature, pH, DO, and ammonia. The ranges of water quality values are listed as follows: water temperature of 26.1-28 °C, dissolved oxygen (DO) of 6.1-6.7 mg L^{-1}, pH of 7.7-7.9 and total ammonia of 0.15-0.22 mg L^{-1}.

2.4 Fish Digestibility
Digestibility test was performed on tilapia with weight of 23.70 ± 0.48 g $fish^{-1}$ stocked in 60 cm × 40 cm × 40 cm of aquarium with a density of 10 fish per aquarium. The fecal collection was done in day 5 after the fish were given feed containing Cr_2O_3. *Faeces* were collected every day, 30 minutes after feeding for 20 days.

2.5 Test Parameters
Test parameters in the study included the amount of feed intake, feed efficiency, daily growth rate, survival rate, protein and total digestibility based on the equation stated by Halver and Hardy (2002) [4]. Fat and protein retention were based on the equation stated by Takeuchi (1988) [20]. Protein efficiency ratio was done by the method of Webster and Lim (2002) [23]. The enzyme activity analyzed included amylase, protease, and cellulase. The enzyme activity was performed on the intestine segment and observed after 60 days of maintenance. Amylase activity was measured by the method of Worthington (1993) [24], protease activity was measured by the method of Bradford (1976) [1], and cellulase activity was measured by the method of Omar and Kader (1998) [7]. The data obtained were tabulated using Microsoft Office Excel 2013, and analysis of variance (ANOVA) was done using SPSS ver. 21.0. If treatment was significantly different, Duncan test was further performed.

3. Results
3.1 Growth Performance and Digestibility
The results of the growth performance of tilapia fed with different levels of *E. crassipes* are shown in Table 2. The use of *E. crassipes* as a pollard substitute had a significant effect on the final biomass (Bt), protein digestibility (PD), total digestibility (TD) protein retention (PR), lipid retention (LR), protein efficiency ratio (PER), daily growth rate (DGR) and feed efficiency (FE) (p<0.05). However, the amount of feed consumption (FC) and the survival rate (SR) had not significant effect on each treatment (p>0.05).

Table 2: Growth performance and digestibility of tilapia fed with substitution feed of pollard with *Eichhornia crassipes* at 0, 25, 50, 75, 100%

Parameters	Pollard substitution treatment with *Eichhornia crassipes*				
	0%	25%	50%	75%	100%
B_0 (g)	98.44±0.08^{a}	98.42±0.16^{a}	98.34±0.10^{a}	98.26±0.09^{a}	98.15±0.09^{a}
Bt_{60} (g)	243.12±3.31^{a}	241.10±2.35^{a}	240.70±0.80^{a}	228.70±1.02^{b}	222.65±4.92^{b}
FC (g)	317.87±7.41^{a}	317.39±3.48^{a}	318.82±2.76^{a}	318.44±8.64^{a}	308.52±7.25^{a}
TD (%)	45.78±1.80^{a}	38.30±5.61^{b}	38.22±4.45^{b}	30.64±3.04^{c}	28.06±1.79^{c}
DP (%)	83.09±5.22^{a}	82.94±1.62^{a}	83.33±1.44^{a}	74.76±4.33^{b}	75.47±1.30^{b}
PR (%)	27.65±3.97^{a}	26.78±5.53^{ab}	26.48±0.34^{ab}	23.04±0.62^{ab}	20.96±2.36^{b}
LR (%)	41.05±6.07^{a}	31.26±1.04^{b}	30.96±3.11^{b}	23.17±0.45^{c}	23.07±3.01^{c}
PER (%)	1.59±0.01^{a}	1.59±0.04^{a}	1.60±0.10^{a}	1.46±0.04^{b}	1.43±0.06^{b}
DGR (% day^{-1})	2.21±0.03^{a}	2.19±0.02^{a}	2.19±0.01^{a}	2.11±0.01^{b}	2.06±0.05^{c}
FE (%)	45.51±0.16^{a}	44.96±0.90^{a}	44.65±0.84^{a}	40.98±0.84^{b}	40.32±1.41^{b}
SR (%)	95.33±4.04^{a}	97.67±4.04^{a}	95.33±4.04^{a}	95.33±4.04^{a}	95.33±4.04^{a}

3.2 Enzyme Activity (Protease, Amilase, Cellulase)

Results of enzyme activities in intestinal segments of tilapia fed with *E. crassipes* consisted of protease, amylase, and cellulase. The results of protease enzyme activity in tilapia significantly different on each treatment (Figure 1). Protease activity in treatment of 25-100% decreased by 2.59-6.56% of control (p<0.05).

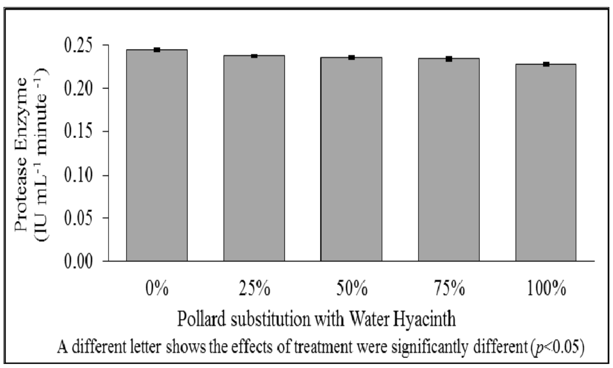

Fig 1: Activities of protease in tilapia fed with different levels of *E. Crassipes*

Figure 2 show the results of amylase enzyme activity in tilapia. Treament of 25% and 50% not significantly different of control (p>0.05). Subtitute pollard with 75-100% *E. crassipes* decreased amylase activity by 3.59 to 4.23% of control.

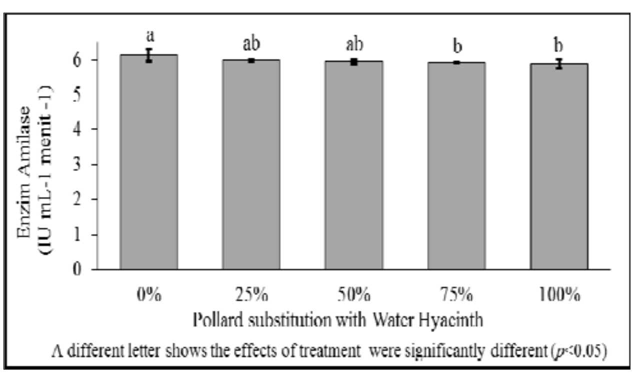

Fig 2: Activities of amylase in tilapia fed with different levels of *E. Crassipes*

The results of cellulase enzyme activity in tilapia shown in Figure 3. Cellulase enzyme in the treatment of 25% not significantly different of control. Pollard subtitution with *E. crassipes* in the treatment of 50-100% decreased by 1.03% (p<0.05).

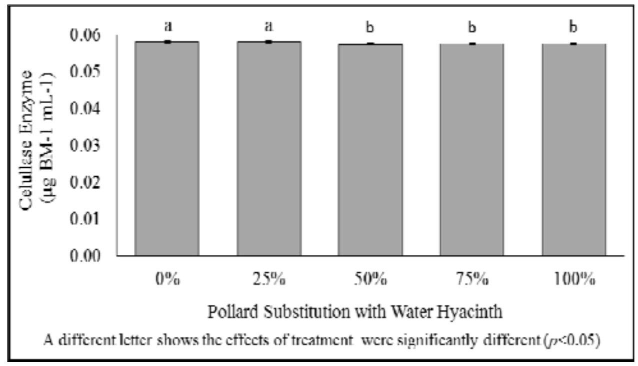

Fig 3: Activities of cellulase in tilapia fed with different levels of *E. Crassipes*

4. Discussion

Total feed consumption obtained on each level of substitution did not different (p>0.05). This is because the acceptability of tilapia to the feed palatability was similar. The study conducted by Hontiveros *et al*. (2015) [6] showed that replacement of soybean meal with E. crassipes resulted in the same amount of feed consumption in tilapia fry until 40% of replacement. The amount of feed consumption will determine how much food to be digested and nutrients that can be absorbed by fish. Digestibility showed the total composition of nutrients that can be absorbed and used for growth as well as metabolism results discarded (NRC 2011) [11]. Information of digestibility value of the feed is necessary to maximize the nutritional and metabolic needs disposed (Zhou *et al*. 2004) [25]. Protein digestibility value decreased in the treatment of 75-100% and total digestibility decreased from 16.34 to 38.70% at 25-100% of substitution pollard with E. crassipes. This condition is consistent with research conducted by Sotolu (2010) [16] which showed that replacement of soybean meal with E. crassipes in catfish reduced coefficient value of digestibility of energy and protein. The reduced digestibility of fish might be due to the asha and fibre content of feed. The higher pollard subtitution with E.crassipes causes the higher ash content of feed (Table 1). The high consumption of ash content in the feed will lead to the decrease in absorption of nutrients, resulted in decrease in fish growth (Sugiura *et al*. 1998) [19]. The use of high fiber in the diet may lower the growth as a result of a decrease feed digestibility (Mohapatra 2015) [10].

The ability of fish to digest the feed highly depends on the completeness of the digestive organs and the availability of digestive enzymes. The existence of enzymes in the digestive tract of fish affects feed digestibility (Liao *et al*. 2015) [8]. Protease activity in treatment of 25-100% decreased by 2.59-

6.56% of controls (Figure 1). This might be due to a content of tannins in the E. crassipes. Tannin is able to inhibit protease and other digestive enzyme activities as well as can bind proteins (Francis *et al.* 2001) [3]. The activity of amylase in the treatment of 75-100% decreased by 3.59 to 4.23% of controls (Figure 2). These results are consistent with research conducted by Marzuqi (2015) [9] which showed that the high use of carbohydrates in the feed lead to the increase in amylase activity. The activity of cellulase in the treatment of 50-100% decreased by 1.03% (Figure 3). The high fiber in the diet generates a higher cellulose, resulted in a decrease of action of the enzymes. The high use of E. crassipes yielded the decrease in the enzyme activity of tilapia.

Feeds which have been consumed and digested contained nutrient that can be utilized that later is stored in the fish body. Values of protein efficiency ratio are influenced by the ability of fish to digest the feed. Protein efficiency ratio in the treatment of 25% and 50% were not significantly different from the control, whereas treatment of 75-100% decreased from 8.17 to 10.07%. According to Sotolu (2010) [16] replacement of soybean meal with E. crassipes flour and E. crassipes leaf flour reduced the value of protein efficiency ratio, this could be due to the high crude in fiber E. crassipes so that the ability of fish to digest protein decreased. The decrease in nutrient absorption affects protein and lipid retention of fish. Protein and lipid retentions are the percentages of protein and lipid eaten by fish and stored in the fish body during the experiment (Halver and Hardy 2002) [4]. Protein retention of fish was significantly different ($p<0.05$) in the treatment of 100% pollard substitution with hyacinth. This is in line with research conducted by Sotolu and Sule (2011) [18] which showed that the administration of E. crassipes in catfish lowered protein retention of fish. The presence of anti-nutritive substances in the E. crassipes lead to difficulty for fish to digest the nutrients contained in the feed. According to Hontiveros and Serrano (2015) [5], E. crassipes contains anti-nutritive substances and crude fibers which become limiting factor in its utilization.

Lipid and protein retention are the percentage of lipid and protein eaten by the fish and can be stored in the fish body during the experiment (Halver and Hardy 2002) [4]. The higher the administration of E. crassipes to the fish, the lower the lipid retention of fish in research. This is similar to the research conducted by Hontiveros *et al*. (2015) [6] where the higher the E. crassipes replaced soybean meal in tilapia fish, the lower the lipid retention value produced. It can be caused by the reason that higher administration of E. crassipes in the feed resulted in lower level of NFE (nitrogen free extract) in the feed (Table 1). The fish body needs lipid to be stored as structural fat. Research performed by Tibbetts *et al*. (2005) [21] in haddock juvenile also showed a similar pattern, namely the higher the administration of carbohydrates in the feed, the higher the lipogenesis process so that the lipid retention was also increasing.

The use of E. crassipes to substitute pollard up to 50% yielded not significantly different biomass and daily growth rate of fish compared with control ($p>0.05$). Although the E. crassipes has a limiting essential amino acid, yet it has an essential amino acid index value of 0.88. According to Peñaflorida (1989) [12], criteria for the classification of a good protein source namely the value of EAAI is greater than or equal to 0.9. Adequate protein source has a value of 0.8 while the inadequate one has a value below 0.7. This indicates that the hyacinth was quite good as a protein source. Therefore, the use of E. crassipes had no effect on the survival rate of fish ($p>0.05$). This is in line with the results of Sotolu (2010) [16] and Sotolu and Sule (2011) [18] which showed that the use of E. crassipes in catfish had no impact on survival rate.

The growth of fish declined at 75% and 100% substitution pollard with E. crassipes. Similar thing happened to the research conducted by Mohapatra (2015) [10] which showed that the use of hyacinth to replace fishmeal in carp resulted in decrease in fish growth along with the higher use of E. crassipes in the feed. The decrease in growth could be caused by anti-nutritive substances that affect the growth. The anti-nutritive substances contained in the E. crassipes are Tannins and phytic acid. The contents of Tannins and phytic acid in the E. crassipes leaf meal amounted to 0.98% and 0,42%, respectively (Saha and Ray 2011) [14]. Tannins can inhibit the activities of protease and other digestive enzymes activity because it can bind to proteins (Francis *et al*. 2001) [3].

Raw material such as E. crassipes has a limiting amino acids namely methionine and lysine (Hontiveros and Serrano 2015) [5]. The amino acid profile of E. crassipes has a shortage of methionine and lysine which might interfere with protein utilization for growth. Deficiency of essential amino acids can inhibit fish growth. Some species of fish showed that deficiency of methionine can cause illness because this amino acid is not only associated with protein but is also used for a synthesis of other compounds (NRC 2011) [10]. Fish need feed ingredients that provide a combination of essential amino acids (Pratama *et al.* 2015) [13]. Feed efficiency value in the treatment of 75% and 100% decreased by 9.9% and 11.4%. This is consistent with research conducted by Sotolu and Sule (2011) [18] which showed that the use of E. crassipes in catfish resulted in significant effect on feed efficiency value where efficiency value decreased with the addition of E. crassipes. Based on the results of feed efficiency, substitution of pollard with E. crassipes was effective until a replacement by 50%.

5. Conclusion

Substitution of pollard with E. crassipes of 0-100% gave significantly different results on enzyme activity, growth performance and digestibility of tilapia. The enzyme activity declined with high use of E. crassipes in the feed. The effective use of E. crassipes Echhornia crassipes flour as pollard substitution was up to 50% or 12.5% in the diet of tilapia Oreochromis niloticus.

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