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GROWTH PERFORMANCE AND ENZYME ACTIVITIES IN CATFISH [*Pangasianodon hypophthalmus*] FED WITH WATER HYACINTH-BASED DIET

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ABSTRACT

An alternative subtitution of pollard as an imported feed ingredient is a neccesity and one of the potential ingredients is water hyacinth (*Eichhornia crassipes*). This study was conducted to evaluate growth performance and enzyme activity in catfish (*Pangasianodon hypophthalmus*). Diet with five different levels of water hyacinth subtitute of pollard (0%, 25%, 50%, 75%, and 100%) were fed to catfish for 60 days. Seventy (70) catfish fry with average initial body weight of 2.45±0.15 g were maintained in 100cm x 80cm x 60cm aquaria. Fish fed at satiation level three times daily at 8 am, 12 pm, and 4 pm. With dietary of 25% water hyacinth, growth performance and protease activity similar to 0% treatment. Feed intake, protein digestibility, feed efficiency, protein efficiency ratio, and protease and amylase enzyme activities decreased ($p<0.05$) in those fed with more than 25% water hyacinth. Catfish fed 25% water hyacinth showed significantly ($p<0.05$) higher daily growth rate, feed efficiency and protein digestibility than those with other treatments. Based on the growth performance and enzyme activity, we can conclude that the optimum dietary level of water hyacinth subtitute pollard for fry catfish is 25%.

Keywords: catfish, growth performance, pollard, water hyacinth

INTRODUCTION

Feed is the main component to meet the energy needs of the fish. Pollard has a high digestibility and bioavailability as carbohydrate source in fish feed (Suprayudi *et al.* 2010). Munir *et al.* (2015) report that pollard contains 53.40% NFE, 14.78% protein, 7.77% lipid, 9.78% crude fibre, and 4.34% ash, however an alternative subtitution of pollard as an imported feed ingredient is a neccesity. Research on the use of ingredients of fish feed has been done; *Ulva lactuca*, *Havea brasiliensis*, *lemna, singgle cell protein* (Mahasu 2016; Inara 2011; Bag *et al.* 2011; Utomo *et al.* 2007). The research among others, focused on the efforts of using local ingredients and creating efficiency of feed cost. One of the potential ingredients to substitute pollard in fish feed is water hyacinth (*Eichhornia crassipes*). Water hyacinth has lower cost than pollard, water hyacinth cost Rp. 2000/kg while pollard cost Rp. 4800/kg and changes with time base on the Dollar rate.

Water hyacinth is a South American native plant that has spread to more than 50 countries, including Indonesia. Water hyacinth is one of the aquatic plants that grow in abundance in rivers, rice fields, and lakes or dams. Its characteristics include rapid growth and the ability to compete with other aquatic plants (Shu *et al.* 2014). Water hyacinth can cover the surface of water bodies, thereby reducing the amount of sunlight and air penetrating into the water. In addition, the plant can cause shallowing and increase evaporation due to evapo-transpiration. In a dense population, water hyacinth can impede water flow and block irrigation channels (Sotolu & Sule 2011). Therefore, the presence of water hyacinth in water bodies is more often regarded as harmful weeds that can negatively impact the aquatic ecosystem (Tellez *et al.* 2008).

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In previous study, water hyacinth has been used as fish feed in tilapia (Khalil *et al.* 2015; Bag *et al.* 2011; Hontiveros *et al.* 2015; Muchtaromah *et al.* 2012), *Cyprinus carpio* (Mohapatra 2015), *Clarias gariepinus* (Sotolu & Sule 2011), and *Labeo rohita* (Saha & Ray 2011). The use of water hyacinth flour as a substitute of fish meal for *Clarias gariepinus* feed for 26.72% and 31.63% showed a lower digestibility rate (Sotolu & Sule 2011). However, according to Sotolu and Sule (2011), it is still possible to use the plant for fish feed. Hence, water hyacinth was chosen to be the raw material of fish feed in this research. Furthermore, chemical analysis of water hyacinth shows that the plant contains nutrients similar to that of pollard, 30.81% NFE, 12.40% protein, 4.72% lipid with digestibility of water hyacinth leaves reaching up to76.4% (Chang *et al.* 2013; Hontiveros & Serrano 2015).

The utilization of nutrients in fish feed is closely linked to enzyme activity in fish. The use of different feeds in fish can affect their enzyme activities. Marzuqi (2015) revealed that milkfish given different contents of carbohydrates experience different amylase enzyme activities in line with the increasing contents of carbohydrates in the feed. According to Tibin *et al.* (2012) the use of water hyacinth indicated a decrease in feed utilization in tilapia fish. There is still gap in knowledge about the use of water hyacinth as a source of plant material in catfish feed. Thus, the present research was carried out to evaluate the use of water hyacinth flour in fish feed as a source of carbohydrate in the growth performance and enzyme activity of catfish *Pangasianodon hypophthalmus*, an important farmed fish in Southeast Asia.

MATERIALS AND METHODS

Water Hyacinth Flour

The water hyacinth flour made from leaves and stems used in this research was taken from the Sukabumi Centre for Freshwater Aquaculture. The water hyacinth was first cleaned and then dried in oven at 60 ^{0}C for 24 hours. The dried water hyacinth was then grinded into flour. Before being used as the ingredient for the feed, a test of heavy metal content was conducted to determine the presence of lead (Pb), cadmium (Cd) and mercury (Hg). The heavy metal content analysis was done using the Atomic Absorption Spectrophotometry method (APHA 2012). The test results showed that no heavy metal contents of lead, cadmium, and mercury were detected in the water hyacinth flour.

Experimental Diets

The diet containing five different levels of water hyacinth (0%, 25%, 50%, 75% and 100%) as pollard substitute. The premix used were commercial product called Lagantor from Kalbe.

Table 1 Composition and nutrients contained of the experimental diets (% kg^{-1} dry matter)

Feed ingredient	Level of pollard subtitution with water hyacinth (%)				
	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
Water Hyacinth 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[1]	45.01	35.14	32.31	28.24	25.22
GE[2] (kcal kg^{-1})	4243.74	3945.85	3848.23	3821.17	3780.40

Note: [1]Nitrogen Free Extract
[2]*Gross Energy,* 1 g protein = 5.6 kcal, 1 g lipid = 9.4 kcal, 1 g NFE = 4.1 kcal (Takeuchi 1988).

The ingredients used were weighed according to their composition and mixed in a mixing machine. After getting evenly mixed, the feed was moulded and heated in the oven at 60^0C for 4 hours. The composition of the feed and the nutrients contained in it are presented in Table 1.

Fish Rearing

The catfish *Pangasianodon hypopthalmus* used originated from Cibanteng, Bogor, Indonesia. The fish were maintained in the Aquatic Laboratory of the Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). They were reared in 100cm x 80cm x 60cm aquaria. The aquaria were cleaned and disinfected using 30 ppm chlorine before use. Each of the aquaria was filled with 200L of water. Fish acclimatization was done in two weeks before the rearing period. During the acclimatization, the fish were fed with 50% treatment feed at satiation level. After acclimatization, the fish were weighed, and the average weight was 2.45±0.15 g. The fish were then placed randomly into the 15 aquaria with a stocking density of 70 fishes/aquaria.

The rearing of the fish was done for 60 days. Feeding was done at satiation for three times daily at 8 am, 12 pm, and 4 pm. The growth was measured by sampling every fifteen days. Every three days 70% of the water was replaced to maintain the quality of the water during the rearing. Each aquaria was equipped with aeration and heater regulated at 28^0C. The temperature of the water was measured daily, while the water quality was measured three times during the farming process, with parameters including dissolved oxygen, pH, and ammonia. The water quality during farming was at the optimum range for catfish, with a temperature ranging from 28–29^0C, dissolved oxygen of 5.6–6.3 mg L^{-1}, pH 7.6–7.8 and ammonia 0.0005–0.02 mg L^{-1}.

At the end of the rearing period, the fish biomass in each of the aquaria was measured. The parameters of survival rate, the amount of feed intake, feed efficiency, and daily growth rate were calculated based on Halver and Hardy (2002); protein efficiency ratio was calculated based on Webster and Lim (2002); protein and lipid retention were calculated based on Takeuchi (1988). After the final weighing, three pieces of fish were randomly taken from each aquaria for a proximate analysis. Water content analysis was done by drying the sample in an oven at the temperature of 105–110^0C for 6 hours; analysis of crude fibre was carried out with dissolving method using strong acids and bases and heating; protein analysis with Kjeldahl method, lipid analysis of dried samples with Soxhlet method, lipid analysis of wet samples with Folch method, and ash content analysis by heating sample in a furnace at 600^0C (Takeuchi 1988).

Enzyme Activity Analysis

The enzyme activities analysed included those of amylase, protease, and cellulase. The enzyme activities were observed at the end of the experiment. Amylase enzyme activity was measured by the method of Worthington (1993). Cellulase enzyme activity was measured by the method of Kader and Omar (1998). Protease enzyme activity was measured using Bradford's method (Bradford 1976).

Digestibility Test

The effect of water hyacinth used in feed digestibility was measured using the indirect method by adding chromium oxide Cr_2O_3 as an indicator in the feed. Digestibility test was conducted after growth performance tests using the same fish. The fish were fed three times a day at satiation at 8 am, 12pm, and 4 pm. Faecal wastes were first to be collected on the fourth day after giving the feed containing Cr_2O_3. The faeces were collected 30 minutes after feeding. The collected faeces from each aquaria were stored in a freezer. The collection of the faeces was done for 25 days. Once collected, the faeces were dried in the oven at 110^0C for 4-6 hours. The faeces sample was analysed for its Cr_2O_3 and protein contents. The total digestibility value and protein digestibility were calculated using formula of Takeuchi (1988).

Statistical Analysis

The design employed in this research was the completely randomized design with five treatments and three replications. Data were analysed using SPSS software ver. 22.0. To find the effects of treatments, data were tested with ANOVA and followed by Duncan test with a 95% confidence level. The difference between treatments was found with a significance value of $p<0.05$.

RESULTS AND DISCUSSION

Growth Performance

The results showed that the use of water hyacinth of more than 25% in diet caused a decrease in feed intake, daily growth rate, protein digestibility, protein efficiency ratio, and daily growth rate. Lipid retention and protein retention in the 75% and 100% treatments were lower compared to those in other treatments, where in the 100% treatment, the retention rates were 7.85% and 19.87%, respectively (Table 2).

The use of water hyacinth in fish feed at certain levels gave different results in the feed intake (FI) of the catfish. In this research, the use of more than 25% water hyacinth in feed caused a decreased in FI. The decreases of FI in the 50%, 75% and 100% treatments compared to 0% treatment were 11.83%, 38.89%, and 45.88% respectively. In tilapia, the use of water hyacinth in feed did not affect feed consumption, indicating that tilapia could accept feed containing water hyacinth better than *Pangasianodon hypopthalmus* (Hontiveros *et al.* 2015). The difference in the feed intake levels in each treatment was assumed to be caused by a difference in the feed palatability. Palatability is a response to certain feed, which is influenced, among others, by feed characteristics. In addition, the acceptance of fish to raw material is different for each species (Setiawati *et al.* 2014). Venero *et al.* (2008) mentioned that the use of plant materials can reduce feed acceptability and feed palatability. Palatability is linked to attractability that will influence responses in feed searching, intake, and ingestion or acceptability (Inara 2011). The decreased rate of FI was followed by a decreased in digestibility rate. Digestibility is the process of breaking down feed into a simpler form that is readily absorbed by intestinal walls and into the blood vessel system. There was no difference in the total digestibility of feed in all treatments ($p>0.05$). This is in contrast to protein digestibility that experienced a decrease in line with the increasing amount of water hyacinth in feed ($p<0.05$). Diet containing 0% and 25% water hyacinth showed similiar result of 82%, whilst in

Tabel 2 Final biomass (Bt), feed intake (FI), total digestibility (TD), protein digestibility (PD), protein retention (PR), lipid retention (LR), protein efficiency ratio (PER), daily growth rate (DGR), feed efficiency (FE), survival rate (SR) of catfish (*Pangasianodon hypophthalmus*) fed with feed containing water hyacinth

Parameter	Treatments of water hyacinth flour substitution				
	0%	25%	50%	75%	100%
Bt_{60} (g)	563.39±3.69^a	558.33±4.82^a	408.17±3.89^b	262.83±1.68^c	239.62±5.24^d
FI (g)	599.26±8.23^a	585.62±11.88^a	528.35±6.94^b	366.21±5.38^c	324.31±10.61^d
TD (%)	41.19±0.58^a	41.26±5.47^a	42.07±6.39^a	41.03±2.13^a	42.91±0.14^a
PD (%)	82.09±0.21^a	82.56±1.50^a	79.92±1.43^b	74.57±1.31^c	75.96±0.49^c
PER	2.30±0.07^a	2.37±0.03^a	1.63±0.04^b	0.96±0.06^c	0.81±0.06^d
PR (%)	27.30±4.37^a	25.93±1.36^a	22.02±7.84^a	7.79±2.51^b	7.83±5.19^b
LR (%)	47.46±7.68^a	46.54±1.67^a	40.93±6.84^a	18.35±6.79^b	19.67±1.37^b
DGR (%day^{-1})	2.03±0.07^a	2.03±0.03^a	1.48±1.11^b	0.75±0.03^c	0.61±0.03^d
FE (%)	65.69±1.95^a	67.01±0.85^a	45.42±1.31^b	27.00±1.75^c	22.81±1.45^d
SR (%)	98.10±3.30^a	97.62±0.82^a	98.57±1.43^a	97.62±2.18^a	97.14±3.78^a

Note: *) The values in the same rows with different superscript letters indicate significant differences ($p<0.05$).

the 50%, 75%, and 100% treatments, it decreased to 79.92%, 74.57%, and 75.96% respectively. The factors affecting digestibility are, among others, the treatments before and after feed making, material sources, particle size, fish size, and non-protein components in the feed (Usman 2002). The decrease in protein digestibility value of 50-100% treatment in this study were caused by the increasing amount of crude fibre that can disturb feed utilization and growth. Mohapatra (2015) reports that the use of water hyacinth significantly increases the crude fibre and affects the feed utilization of *Cyprinus*. In the current study, the crude fibre content of 0–100% treatments were at the range of 3.47–13.06%. The higher the fibre content in the feed, the lower digestibility of protein. This result is in accordance with the report of Suprayudi *et al.* (2010).

The end products of the consumed and digested feed are nutrients that can be used and then stored in the fish body. The levels of protein and lipid retentions in the 0–50% treatments were the same, and they declined in the 75% and 100% treatments. The different protein and lipid retention levels were caused by differences in the amount of feed intake and feed digestibility value. Carbohydrate, protein, and lipid are the three components that contribute the highest amount of energy for the fish. If low amounts of nutrients are absorbed, their potential to be stored in the fish will also be low, because the nutrients will be first used for the fish activities. This also applies to growth: if a small amount of energy is received by the fish, then the energy for its growth will be even much less. The use of certain levels of water hyacinth in feed gave positive effect on the growth of *Cyprinus* (Mohapatra 2015). In this research, the use of more than 25% water hyacinth negatively impacted the growth of the catfish. Compared to 0% treatment, the percentages of daily growth in the 50%, 75% and 100% treatments decreased. The decreased of daily growth in the 50%, 75%, and 100% treatments were 27.1% day^{-1}, 55.6% day^{-1}, and 69.9% day^{-1}, respectively. The lowering growth rate in the fish can be attributed to the unfulfilled needs of nutrients in the fish and the inability of the fish to use the energy and materials in their feed (Usman 2002). Feed intake in the 0% and 25% treatments were better than that in the other treatments, hence the amount of the ingested feed was greater in the 0% and 25% treatments. The difference presumably caused more energy to be received in 0% and 25% treatments, resulting in better growth. In this research, the crude fibre content in the feed increased along with the increasing proportion of water hyacinth flour in the feed. The crude fibre derived from vegetable materials is in general difficult to be digested by fish. In addition, one of the components of cell walls in plants is cellulose that is intractable (Inara 2011). The better growth in the 0% and 25% treatments reflects feed efficiency value. This value indicates that the anti-nutritional substances and the high fibre content in the 0% and 25% treatments did not disturb the use of feed by the catfish. The survival rate ranged from 97.14–98.57%, indicating that water hyacinth did have any harmful effects on the fish.

Enzymes Activities

Similar protease enzyme activities were found in the 0% and 25% treatments, thus have different result compared to the 50%-100% treatments (Fig. 1). Similar cellulase enzyme activities were found in all treatments (Fig. 2). Different amylase enzyme activities were observed in all treatment (Fig. 3).

Enzymes have an important role in digestion process. The protease enzyme activities in the 0% and 25% treatments were greater than those in the 50–100% treatments. The declining protease enzyme activities in the 50–100% treatments were assumed to be caused by the anti-nutritional substances in the feed. Water hyacinth contains anti-nutritional substances such as tannin and phytic acid of 0.98% and 0.42%, respectively. Anti-nutritional substances such as tannin can affect growth and enzyme profiles of fish. Tannin impedes protease enzyme activity and therefore can reduce protein digestibility, while phytic acid can reduce protein and mineral bioavailability (Saha & Ray 2011). In addition, the declining enzyme activities can also be caused by the amount of the substrates present in the feed intake. In this study, fish fed diet containing 50–100% water hyacinth showed lower feed intake than those fed with 0% and 25% water hyacinth, which was directly proportional to the amount of substrates for the enzyme in their digestive systems. The higher feed intake and protein digestibility in the 0% and 25% treatments compared to those in other treatments had an impact on the high value of protein

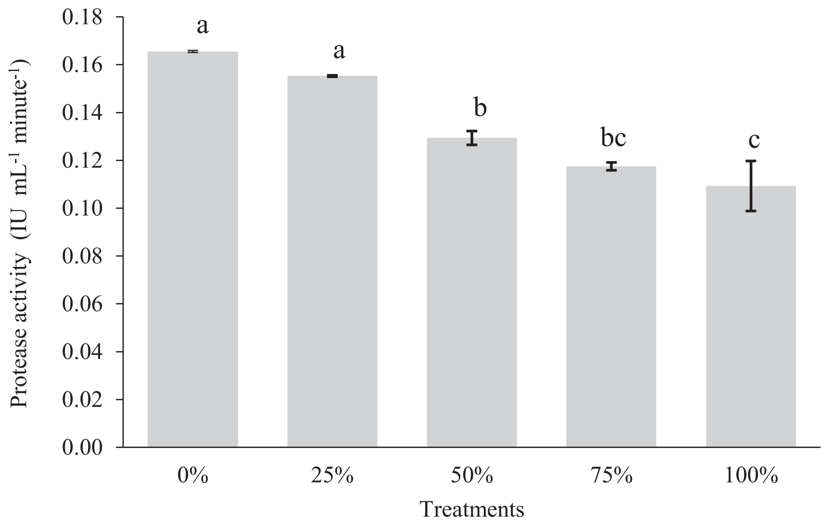

Figure 1 Protease enzyme activities in catfish fed with feed containing different levels of water hyacinth

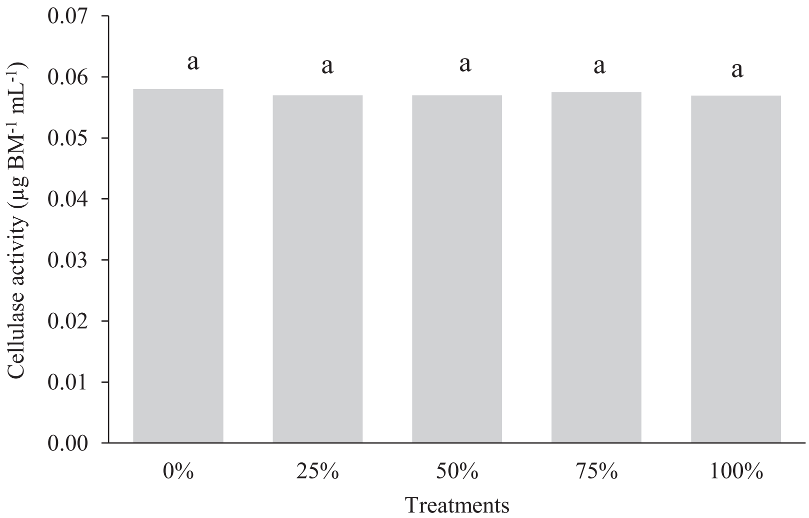

Figure 2 Cellulase enzyme activities in catfish fed with feed containing different levels of water hyacinth

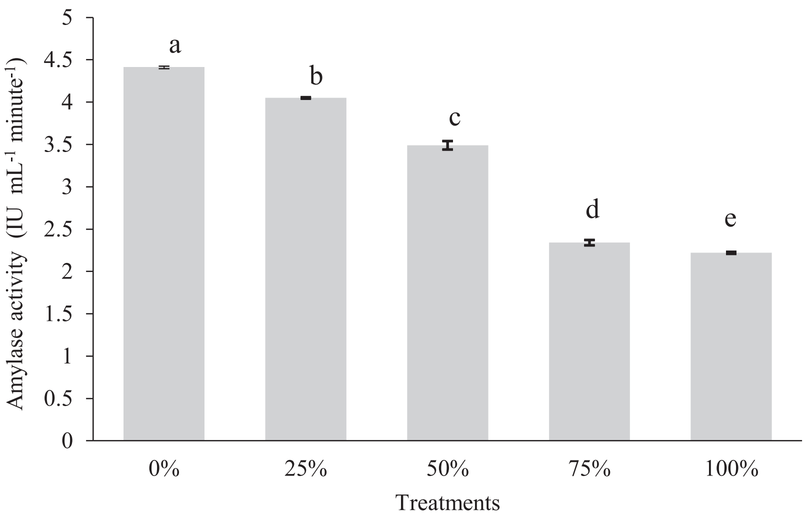

Figure 3 Amylase enzyme activities in catfish fed with feed containing different levels of water hyacinth

efficiency ratio in these treatments, namely 2.30 and 2.37, respectively.

The cellulase enzyme activities observed were not different in all treatments. This is attributed to the low amount of cellulase enzyme in the fish that worked maximally in all treatments, although there were differences in the amounts of feed intake and water hyacinth in each feed. The amylase enzyme activities were found to be different in all treatments. The amylase enzyme activities in the 50–100% treatments were lower than those in other treatments. Amylase enzymes break down starch in feed. The amylase enzyme activites decreased with increasing level of water hyacinth assumed to be caused by the low carbohydrate content as the level of water hyacinth increased.

CONCLUSION

The *Pangasianodon hypopthalmus* fed diet containing 25% water hyacinth subtitute pollard showed similar growth performance and protease activity with 0% treatment. The use of water hyacinth of more than 25% showed lower growth performance than other treatments. Water hyacinth flour can be used for up to 25% or 6.25% in the diet of *Pangasianodon hypopthalmus*.

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