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# Growth and Digestive Enzyme Activity of *Clarias gariepinus* Fed *Myrmecodia pendens* Merr. & Perry Bulb Extract

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**Abstract.** This study was performed to evaluate the effects of dietary *Myrmecodia pendens* bulb extract (MPE) on the growth and digestive enzyme activity of the catfish *Clarias gariepinus.* Two groups with four replicates, first are control group and the other are treatment group with 1 g $kg^{-1}$ of MPE in basal diet. All fish was fed at a rate 3% of body weight for eight weeks. At the final day of study, average weekly gain (AWG), specific growth rate (SGR) and feed efficiency (FE) were evaluated, and digestive enzyme (amylase, lipase and protease) activity was determined. The results showed that after 8 weeks of feeding, AWG, SGR and FE of MPE fed fish were significantly higher than the control group, whereas amylase, lipase and protease were not affected by dietary MPE. Present results suggest that the addition of MPE in the diet of *Clarias gariepinus* is beneficial in improving growth performance and feed efficiency of fish.

## INTRODUCTION

Aquaculture was one of the most important sectors in the world, including Indonesia [1-3]. On fish production contribution in the world, aquaculture contributed about 44.1% in 2014 and this keep continues to increase every year. Indonesia itself occupies the second position with a contribution of 5.77% in fish production [4]. According to KKP [5], the level of domestic fish consumption is increasing every year. In 2020, the domestic fish consumption rate is 56.39 kg/capita/day, and it is estimated in 2021 for the national fish consumption level will increase to 58.06 kg/capita/day.

One of the fish commodities that are widely cultivated is catfish (*Clarias gariepinus*). Catfish lives in freshwater environment with high growth rates, low budget and easy to cultivate [6]. The production of aquaculture catfish continues to raise and by 2020 national aquaculture up to 1.01 million tons of catfish [5].

In an effort to increase catfish cultivation, feed with the additional plant extracts has become one of the most widely used methods, such as *Garcinia mangostana* L. [7], *Muntingia calabura* [8] and *Trifolium pratense* [9] plant extract. Beside those plants, *Myrmecodia pendens* extracts (MPE) also have potential as additional food for fish. *Myrmecodia pendens* or known as ant nest is a potential medical plant that can be found in various regions in Indonesia, especially in lowland tropical forests and open agriculture with an altitude of 600 m [10]. This plant is often used by the public as a medicine for various diseases such as ulcers, allergies and gout because of its chemical

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compounds which include flavonoids, phenolic, alkaloids, saponins, steroids and tannins which are useful as antioxidants, antibacterial and immunomodulatory effect [11].

Although there have been previous studies on the benefits of MPE in the medical and pharmacy field, research related to the growth and activity of digestive enzymes in catfish has never been done. Therefore, this study was conducted with the aim of determining growth and digestive enzyme activity of catfish after fed 1% of supplementation MPE in the diet.

## MATERIALS AND METHODS

### Fish Rearing and Analysis Site

Current research was performed in the Laboratory of Animal Physiology, Development and Molecular, Department of Biology for fish rearing. Meanwhile, digestive enzyme analysis was performed in the Laboratory of Biochemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Borneo, Indonesia.

### Extract Preparation

The *M. pendens* bulb was dried, cut, and crushed to make coarse powder. The coarse powder (100 g) was extracted using one liter of 95% ethanol for 48 hours. To obtain crude extract, the mixture of coarse powder and ethanol was filtrated (Whatman paper) and evaporated (Buchi Rotary evaporator, Buchi®, Inc.). Resulting crude extract was stored at cool temperature until being used and added to basal diet.

### Basal and Test Diet Preparation

Commercial fish pellet as Basal diet was obtained from local fish diet factory (Hi Pro Vite 781-2), containing protein (±32%), lipid (±4%), crude fiber (±5%), ash (±12%), and moisture (±12%). Meanwhile, the test diet was prepared by mixing the commercial fish pellet plus 1% of MPE and dried (60ºC) using oven for 24 h. The resulting test diet was then cooled at room temperature and stored in closed container.

### Fish and Study Setup

One-hundred sixty catfish with an average initial weight of ±6 g was obtained from fish farmers in Samarinda, East Kalimantan and acclimatized under laboratory conditions for one week. During acclimatization, the fish were fed a Basal diet. After acclimatization, the fish were randomly divided into eight tanks, containing 20 fish/tank. Eight tank were confirmed as two groups with four reply, namely the control (Control groups, four tank) and treatment group (T groups, four tanks). For 8 weeks (56 days), fish were fed with basal diet and test diet (MPE) at the rate 3% of fish total biomass three times a day.

Every two days ±30% of the water in the tank was replaced with freshwater to maintain the water quality. In addition, every 2 weeks, water quality measurements such as temperature, pH, dissolved oxygen, nitrate, nitrite and ammonia were carried using a thermometer, pH meter, dissolved oxygen meter, and sera test kit sequentially.

### Growth Performance

At the final day of study, final weight, average weekly gain (AWG), specific growth rate (SGR) and feed efficiency (FE) was evaluated to determine the growth effect of MPE. All parameters were calculated using the equation previously used by [11] as follows:

$$AWG\,(\text{g/week}) = \frac{(Wt - Wo)}{Wk} \tag{1}$$

$$SGR\,(\%\ body\ weight\ gain\ /\ day) = \frac{\left[Ln(Wt) - Ln(Wo)\right]}{day} \tag{2}$$

$$FE\ (\%) = \frac{(Wt - Wo)}{F} x\ 100\% \quad (3)$$

Note: Wt = final weight (g) and Wo = initial weight (g), Wk is the number of experimental total weeks, F is the amount of feed given (g).

## Digestive Enzyme Activity

To determine the digestive enzyme activity (amylase, lipase and protease) of catfish fed 1% MPE in the diet, all fish were sacrificed, and fish intestine were taken out. Crude extract of the enzyme was made by homogenizing fish intestines with 0.5 mL of 0.2 M phosphate buffer (pH 7.4). The homogenized results were then centrifuged at 15,000 rpm 4ºC for 20 minutes using a centrifuge (Beckman Coulter AllegraTM X-22R) and the supernatants were used as crude extract for enzyme analysis.

The activity of the amylase enzyme was analyzed using the method proposed by [12]. One unit enzyme amylase activity was calculated as the amount of amylase required to hydrolyze mg maltose per minute per mL of enzyme extract. Meanwhile, the lipase activity was analyzed using the [13] method. One lipase unit per mL is expressed by the number of lipase enzymes that can release 1 mol of free fatty acids per minute. The activity of the protease enzyme was analyzed using the method of [14]. One unit of protease enzyme activity was determined as the amount of protease required to hydrolyze mg protein per minute per mL of enzyme extract.

## Data Analysis

The t-test (Significantly levels of $P<0.05$) was performed to determine the significant difference between groups using software SPSS 24 (SPSS, inc., USA) was used to analyze the data. Resulting analysis data was represented as means standard error (SE).

# RESULTS AND DISCUSSION

## Results

The growth parameter results such as AWG, SGR, and FE are presented in Table 1. Based on these data, it is known that fish in group P with the addition of 1% ant nest bulb extract had a significant effect on growth performance in fish. ($p<0.05$). The fish in the P group showed significantly highest average weekly gain (2.73 g), specific growth rate (2.64 %) and feed efficiency (96.3 %) compared to control group.

**TABLE 1.** Growth of Catfish (*Clarias gariepinus*) fed 1% of Myrmecodia pendens bulb extract in the diet for eight weeks

| Parameter | Control | P |
|---|---|---|
| Initial Weight (g) | 6.42±0.03[a] | 6.40±0.11[a] |
| Final Weight (g) | 19.94±3.63[a] | 28.26±3.48[b] |
| AWG (g) | 1.68±0.45[a] | 2.73±0.42[b] |
| SGR (%) | 2.00±0.31[a] | 2.64±0.20[b] |
| FE (%) | 63.61±13.50[a] | 96.30±14.84[b] |

Description: Mean±SE followed by different superscript letters (a,b) on the same row indicated significant difference ($p<0,05$).

Present finding is consistent with previous study, confirming that the addition of MPE in the diet increase the growth performance of catfish fed *Myrmecodia* bulb extract in their diet [15]. Furthermore, based on Table 2, fish fed 1% *Myrmecodia pendens* bulb extract in diet has a higher digestive enzyme activity than control group. Nevertheless, there is no significant differences ($p>0.05$) on digestive enzyme (amylase, lipase and protease) activity of *Clarias gariepinus* between control and test group.

**TABLE 2.** Digestive Enzyme Activity of *Clarias gariepinus* fed 1% of Myrmecodia pendens bulb extract in the diet for eight weeks

| Parameters | Activity Value (U/mL) | |
|---|---|---|
| | Control | P |
| Amylase | 1393.6±110.6[a] | 1554.0±90.7[a] |
| Lipase | 0.27±0.02[a] | 0.33±0.00[a] |
| Protease | 1760.6±147.5[a] | 1877.3±165.8[a] |

Description: Mean±SE followed by same letters (a) on the same row indicated have no significant difference ($p$>0,05).

## Discussion

The inclusion of plant extracts in aquaculture fields, especially for fish culture has been performed in several study [16-19]. Our findings revealed that fish fed 1% of MPE in the diet has higher average weekly gain, specific growth rate and feed efficiency than fish in the control diet. This may be due to the phytochemical contain such as flavonoids, phenolic and alkaloids in the MPE which might improve antioxidants capacity, antibacterial, growth boosters and enhance immunomodulatory that can affect on the fish growth [11]. Present research is in line with the past research from Nugroho et al. [15] revealed that flavonoid and phenolic from *Myrmecodia tuberosa* at 0.5-1% concentration increased the growth of *Pangasianodon hypophthalmus*. Similar study also mentioned that dietary *Andrographis paniculata* leaf extract which contains phytochemical compounds improved the growth of *Pangasianodon hypophthalmus* [20].

Furthermore, the digestive enzyme activities (amylase, lipase and protease) of *Clarias gariepinus* fed 1% MPE showed no significant differences (Table 2). Current results is in contrast with past study mentioned the application of 1% *Lupinus perennis* extract in the diet enhanced the digestive enzyme activity of rainbow trout (*Oncorhynchus mykiss*, Walbaum) [21]. Present results might due to the level of addition has no adequate to enhance the digestive metabolism system of catfish or antinutritional compound such as saponin that can be found in the plant extract [22].

## CONCLUSION

In conclusion, these findings showed that dietary with the addition of 1% *Myrmecodia pendens* extract in the diet is useful in enhancing growth performance and feed efficiency of catfish. However, Digestive enzyme is not affected by inclusion of 1% of *Myrmecodia pendens* extract in the diet. Further study needs to be performed to determine the histological and molecular aspects of catfish fed *Myrmecodia pendens* extract in their diet.

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