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Effects of Ant-Nest Plant (*Myrmecodia pendens*) Bulb Extract on Histology of Intestinal, Liver and Proximate Fillet of Sangkuriang Catfish (*Clarias gariepinus Var*)

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Abstract. *Myrmecodia pendens bulb extract (MPBE)* contains phytochemical compounds such as flavonoids, phenols, alkaloids, tannins and saponins that potential can be used as an additional fish feed. The purpose of this study was to determine the effects of MBPE to fish feed on the growth performance, intestine and liver histology, and proximate content of catfish (*Clarias gariepinus* Var) fillets. Fishes (initial weight 15 g) were randomly divided into several groups of triplicates, containing 20 fishes per group. The group one (K) is a control, while groups P1, P2, P3, dan P4 with the addition of 0.5; 1; 2; and 3 g/kg *M. pendens* bulb extract. Fishes were fed MPBE in the diet for 90 days and at the end of treatment fish growth was measured. Meanwhile, intestine and liver were taken out for histological analysis. The results showed, fish fed 3 g/kg *MPBE* had higher growth performance ($p<0,05$) than other groups. However, villi and depth of crypt intestine the fish fed *MPBE* was shorter than control group. The liver of the fish fed MPBE also showed significant effect on the histology of liver. Furthermore, the addition of *MPBE* to fish showed a better result of fillet proximate analysis compared to control

INTRODUCTION

Sangkuriang catfish (*Clarias gariepinus Var*) is a lot of fish consumed by people in Indonesia, so that Sangkuriang catfish is one of the six aquaculture commodities in Indonesia [1]. In addition, sangkuriang catfish is relatively resistant to diseases, has high fecundity, can easily be cultured, having a high adaptability to the environment, and economic value. Therefore, the need for catfish consumption size increasing day by day [2]. One of the efforts to increase the production of fish harvests is by providing quality feed raw materials, but can reduce feed costs so as to increase the efficiency of aquaculture [3].

Efforts to improve the growth performance of fish by adding natural ingredients such as herbal plants to fish diet [4]. The application of herbal preparations to the diet as natural feed additives is a global trend for reducing the use of chemicals that can enhance growth performance and feed utilization efficiency [5]. One of the plants extract with such potential is *Myrmecodia pendens* bulb extract (MPBE).

Myrmecodia pendens which is also known as ant-nest plant, is native plant from Papua [6]. Myrmecodia is one of the plants from the Hydnophytinae family (Rubiaceae), which is attached to other larger plants or known as

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epiphytic plants. Myrmecodia can be found in the mountains of Papua, Sumatra, and Java. The benefits of this plant can be used as herbal medicine for lowering blood glucose levels, tumors, and cancer (especially in the breast, liver, lung, ovary, and brain). In addition, this plant as an antioxidant and immunostimulant to increase immunity [7]. Myrmecodia contains several compounds, namely phenolics, flavonoids, alkaloids, saponins, tocopherols, multimineral, and polysaccharides [8].

However, the effect of feed supplementation with ant-nest plant extract on growth performance, histology of intestinal and liver, and proximate nutritional contents of catfish fillets have never been done. The aims of this research was to determine the effects of MBPE to fish feed on the growth performance, intestine and liver histology, and proximate content of catfish (*Clarias gariepinus* Var) fillets.

METHOD

Plant Materials

M. pendens bulbs were purchased from a local market. To make a powder, bulbs were dried, cut into small pieces and ground into powder. This powder was extracted using 95% ethanol for 48 hours, filtrated, evaporated at 60 ºC until all the solvent evaporated and stored in refrigerator at 10 ºC until it was used as a crude extract.

Control and Treatment Diet Preparation

The control diet was a commercial pellet and was obtained from a local commercial market (Hi Pro Vite 781-3). The control diet contained 31-33 % protein, 4-6% lipid, 3-5% crude fibre, 10-13% ash and 10% moisture. The treatment diet was prepared by adding 0,5; 1; 2; and 3 g MPBE to the control diet, repalletized using a mixer and dried with oven at 60 ºC and stored at room temperature before use.

Fish Preparation and Experimental Setup

Three-hundred sangkuriang catfish (average initial weight ± 15 g) were obtained from local breeding fish farm in Samarinda, East Kalimantan, Indonesia. All fish were acclimated at the Animal Physiology, Development, and Molecular Laboratory, Mulawarman University, East Kalimantan for five days. The fish were then randomly distributed into five groups (K, P1, P2, P3, and P4) with triplicate groups of 15 fishes per replicate group. For 90 days, the fish were fed with various concentrations of MPBE or the control group at a rate of 3% of body weight three times per day. Temperature, pH, total ammonia nitrate, nitrate, and dissolved oxygen (DO) of the water in experimental tanks were also checked weekly. Siphoning was carried out weekly to remove uneaten food and faeces before renewing the water, forty percent of water volume was replaced with fresh water.

Growth and Feed Utilization Parameters

At the end of 90 days feeding trial, biomass weight, average weekly gain (AWG), body weight gain (BWG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), and feed efficiency (FE), was measured to determine the growth and feed utilization of fish fed with various concentrations of MPBE. All growth parameters were calculated by using equation previously used by [9] as follow,

BWG (g) = final weight (g) - initial weight (g);
AWG (g) = BWG (g)/ number of weeks
DWG (g) = BWG x 7 (number of weeks)/ t
SGR (%) = 100 [Ln final weight (g) – Ln initial weight (g)]/the experimental period (day)
FE (%) = [(Final fish weight (g) + dead fish weight (g)) – initial weight/Feed consumed (g dry weight] x 100
FCR (%) = Feed intake/weight gain

Where t is trial time (days).

Intestine and Liver Histology

After biomass measurement, two fish from each tank were dissected and the liver, and intestines were taken out for histological analysis in the Animal Anatomy and Microtechnique Laboratory, Mulawarman University, East Kalimantan. The liver and intestine were fixed in Bouin's fixative solution and then transferred to 70% ethanol after 24 hours. These samples were routinely processed into paraffin wax for histology, sectioned at 5 μm (to the intestines sliced crosswise), and stained with hematoxylin and eosin. Slides examined under a light microscope (Zeiss Primo Star). Measurements of villi length and depth of the crypt were analysed using Fiji ImageJ software. Measurements of liver histology damage using the Mordue scoring method (Table 1).

TABLE 1. Hepatocyte histopathological grade study score (Mordue, 2001)

Score 0	One field of view was not found degeneration and necrosis in the observed part
Score 1	One field of view was 1-20% found degeneration and necrosis in the observed part
Score 2	One field of view was 21-50% found degeneration and necrosis in the observed part
Score 3	One field of view was 51-75% found degeneration and necrosis in the observed part
Score 4	One field of view more than was 75% found degeneration and necrosis in the observed part

Proximate Chemical Analysis

The proximate chemical analyses of fish body were conducted for protein, lipid, moisture, crude fibre, and ash according to the standard methods of SNI (1992). Whole the sample was cleaned using running water, then weighed before cleaning entrails. The head and fins of the fish were removed then filleted so that the meat was separated from the bones. Protein content tested using the micro-Kjeldahl method to determine total content nitrogen in the sample. Fat content tested using the Soxhlet extraction method with ether solvent. Water content is tested with drying the sample using an oven at temperature of 70 ºC until the weight was obtained constant, while the ash content was tested with dried in oven at 105 ºC for an hour to remove organic to constant weight is obtained.

Statistical Analysis

Data were expressed as means ± standard error (SE) and were analysed using SPSS version22. Growth performance and feed efficiency data were analyzed using One way- Anova followed by Duncan test to determine the level of significance between treatment and control groups with confidence level of 95%. The histological data were analyzed using Kruskal Wallis test followed by Mann Whitney test to determine the level of significance between treatment and control groups.

RESULT AND DISCUSSION

Growth Performance and Feed Efficiency

The result showed that the growth parameters and feed efficiency of catfish fed diets with addition different levels of MPBE for 90 days are presented in Table 2. Our result exhibited that the growth of fish fed MPBE in the diet was significantly higher ($P<0.05$) compared to the control group, as shown in the final weight, BWG, AWG, amd DWG. However, SGR, FE, and FCR values of the experimental groups were not significant. Even though, the treatment groups had a higher value than the control group.

TABLE 2. Growth performance and feed efficiency of *Clarias gariepinus Var* fed of *Myrmecodia pendens* bulb extract (MPBE) for 90 days

Parameter	Groups				
	Control	P1	P2	P3	P4
Initial weight (g/fish)	15.621 ± 0.108[a]	15.556 ± 0.195[a]	15.428 ± 0.105[a]	15.240 ± 0.363[a]	15.164 ± 0.214[a]
Final weight (g/fish)	35.341 ± 0.139[a]	34.948 ± 0.591[a]	36.281 ± 0.979[ab]	37.399 ± 1.212[b]	37.722 ± 0.539[b]
BWG (g/fish)	19.720 ± 0.124[ab]	19.392 ± 0.441[a]	20.853 ± 0.876[bc]	22.159 ± 1.093[cd]	22.558 ± 0.698[d]
AWG (g/fish)	1.729 ± 0.010[ab]	1.701 ± 0.038[a]	1.829 ± 0.076[bc]	1.943 ± 0.095[cd]	1.978 ± 0.061[d]
DWG (g/fish)	0.246 ± 0.001[ab]	0.242 ± 0.005[a]	0.260 ± 0.010[bc]	0.282 ± 0.013[cd]	0.282 ± 0.008[d]
SGR (%)	1.020 ± 0.007[a]	1.011 ± 0.017[a]	1.068 ± 0.025[a]	1.121 ± 0.374[a]	1.139 ± 0.032[a]
FE (%)	78.902 ± 0.838[a]	77.908 ± 1.320[a]	84.462 ± 2.988[a]	90.895 ± 4.634[a]	93.000 ± 4.046[a]
FCR (%)	1.267 ± 0.013[a]	1.283 ± 0.021[a]	1.184 ± 0.041[a]	1.102 ± 0.054[a]	1.076 ± 0.461[a]

Note, Control (group of fish without *Myrmecodia pendens* bulb extract in the diet), P1 (group of fish with 0,5 g *Myrmecodia pendens* bulb extract in the diet), P2 (group of fish with 1 g *Myrmecodia pendens* bulb extract in the diet), P3 (group of fish with 2 g *Myrmecodia pendens* bulb extract in the diet), P4 (group of fish with 3 g *Myrmecodia pendens* bulb extract in the diet). Mean±Standard error (SE) followed by different letter superscript (a,b,c,d) at the same row show a significant difference ($P < 0.05$). BWG= Body weight gain, AWG= Average weekly gain, DWG = Daily weight gain, SGR= Specific growth rate, FE= Feed efficiency, FCR= Feed conversion ratio.

Effect of Treatment on Intestinal and Liver Histology and Histomorphometry

The results of the average analysis of the height villi and depth of crypt of sangkuriang catfish in Table 3 that the addition of MPBE to sangkuriang catfish diet significantly different (P<0,05). The highest villi was in the Control (437.28 ± 7.53 µm) and the shortest in the P3 (148.03 ± 11.73 µm). Meanwhile the highest crypt depth was found in the Control (65.08± 2.26 µm) and the shortest crypt depth in P1 (16.52 ± 1.01 µm). This shows that MPBE can affect villi height and depth of crypt intestine.

TABLE 3. Histomorphometry of the intestinal average of *Clarias gariepinus Var fed diet of Myrmecodia pendens bulb extract*

Parameters	Groups				
	Control	P1	P2	P3	P4
Villi Height (µm)	437.28 ± 7.53[a]	252.52 ± 18.50[c]	211.20 ± 7.02[d]	148.03± 11.73[e]	352.27 ± 25.90[b]
Depth of Crypt (µm)	65.08± 2.26[a]	16.52 ± 1.01[e]	27.86 ± 2.64[c]	20.42 ± 0.61[d]	52.04± 4.27[b]

Description, Control (without adding MPBE), P1 (treatment with the addition of 0.5 g/kg MPBE), P2 (treatment with the addition of 1 g/kg MPBE), P3 (treatment with the addition of 2 g/kg MPBE), P4 (treatment with the addition of 3 g/kg MPBE). , Results are indicated by Mean ± SE. Numbers followed by different letter superscripts (a, b, c, d, and e) in the same row showed significant differences (P<0.05).

Histological figure of the intestine catfish fed diets with addition different levels of MPBE from each treatment namely control, P1, P2, P3, and P4 can be seen in Fig 1, 2, 3, 4 and 5.

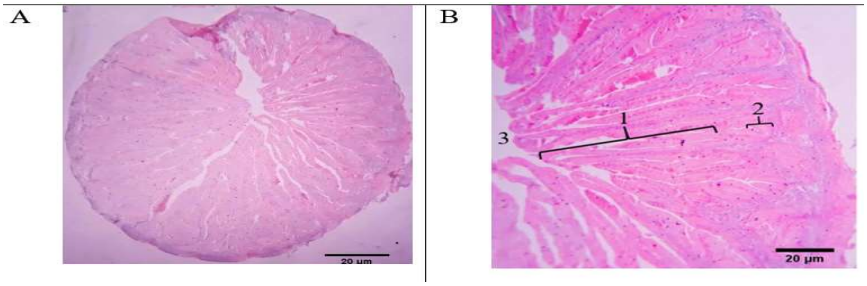

FIGURE 1. Intestinal Histology of *Clarias gariepinus Var* in Control.
A. Magnification 10X10; B 40x10. 1. Villi height; 2. Depth of Crypt; 3. Lumen.

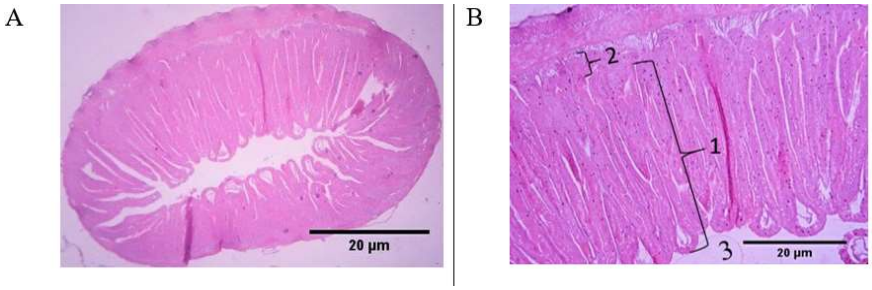

FIGURE 2. Intestinal Histology of *Clarias gariepinus Var* in P1 (with addition of 0,5 g MPBE).
A. Magnification 10X10; B 40x10. 1. Villi height; 2. Depth of Crypt; 3. Lumen.

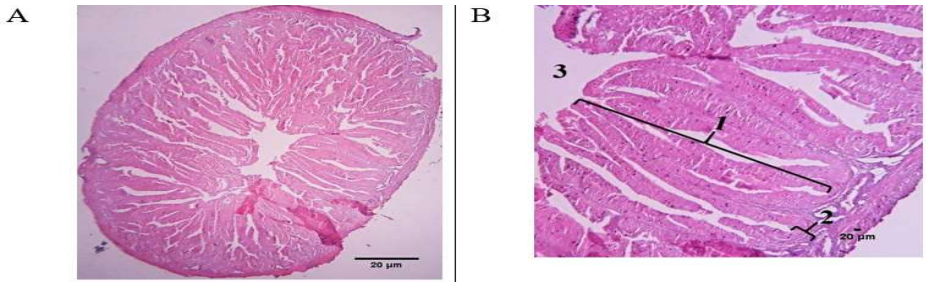

FIGURE 3. Intestinal Histology of *Clarias gariepinus Var* in P2 (with addition of 1 g MPBE).
A. Magnification 10X10; B 40x10: 1. Villi height; 2. Depth of Crypt; 3. Lumen.

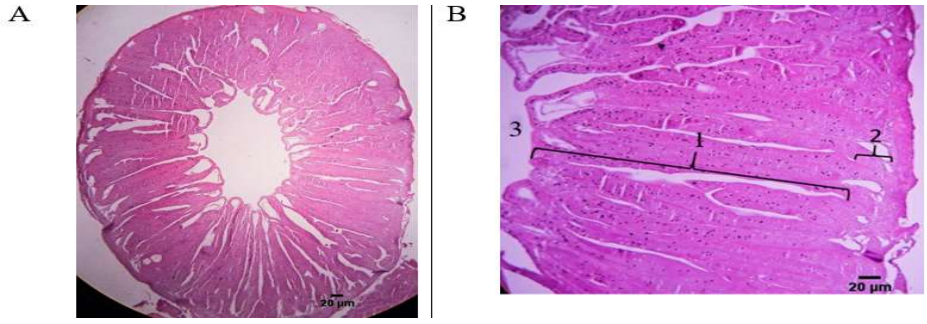

FIGURE 4. Intestinal Histology of *Clarias gariepinus Var* in P3 (with addition of 2 g MPBE).
A. Magnification 10X10; B 40x10: 1. Villi height; 2. Depth of Crypt; 3. Lumen.

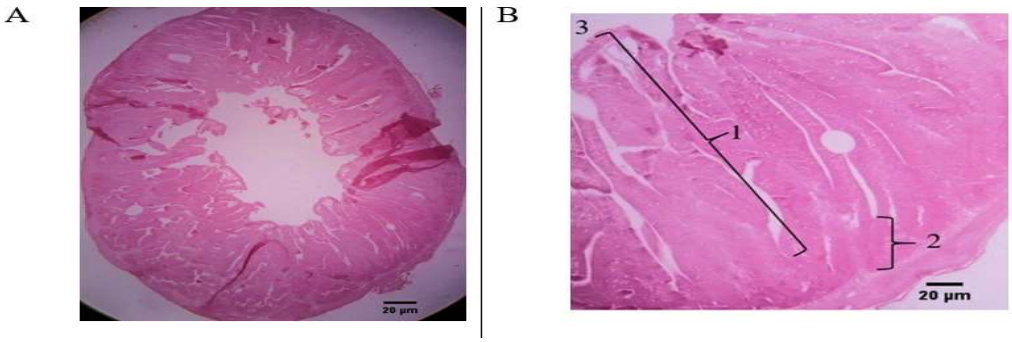

FIGURE 5. Intestinal Histology of *Clarias gariepinus Var* in P3 (with addition of 3 g MPBE). A. Magnification 10X10; B 40x10: 1. Villi height; 2. Depth of Crypt; 3. Lumen.

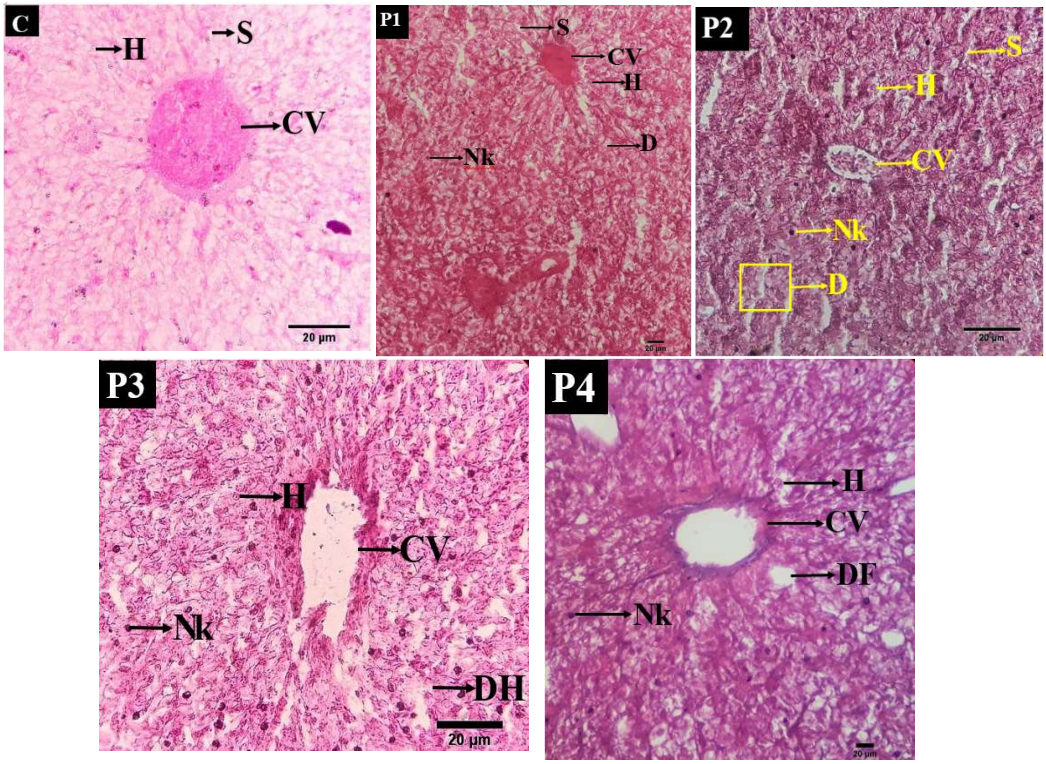

FIGURE 6. Liver histology of *Clarias gariepinus Var* fed of *Myrmecodia pendens* bulb extract (MPBE) (Paraffin Method and HE staining, Magnification 400x). C=Control group; P1 0,5/kg MPBE; P2 1/kg MPBE; P3 2/kg MPBE; P4 3/kg MPBE. Sinusoid (S), Hepatocyte (H), Central vein (CV). The damage that occurs are necrosis (Nk), degeneration (D), hydropic degeneration (DH), and Fatty degeneration (DF).

TABLE 4. The level of Liver damage of *Clarias gariepinus Var* fed diet of *Myrmecodia pendens* bulb extract

Parameters	Groups				
	Control	P1	P2	P3	P4
Degeneration	0.80 ± 0.37^{a}	1.00 ± 0.31^{a}	2.00 ± 0.54^{ab}	2.40 ± 0.67^{ab}	3.60 ± 0.24^{b}
Necrosis	1.20 ± 0.37^{a}	1.40 ± 0.50^{a}	2.20 ± 0.58^{ab}	3.00 ± 0.31^{b}	3.40 ± 0.37^{b}

Description, Control (without adding MPBE), P1 (treatment with the addition of 0.5 g/kg MPBE), P2 (treatment with the addition of 1 g/kg MPBE), P3 (treatment with the addition of 2 g/kg MPBE), P4 (treatment with the addition of 3 g/kg MPBE), Results are indicated by Mean ± SE. Numbers followed by different letter superscripts (a, b, c, d, and e) in the same row showed significant differences ($P<0.05$).

Table 4 presented the level of liver damage caused by the addition of MPBE on sangkuriang catfish. At the higher concentration MPBE (P4) showed significantly compared the control. As can be seen in Table 4, there was a significant higher in the number of degeneration (3.60 ± 0.24) and necrosis (3.40 ± 0.37) in P4. Meanwhile, fish in the control had low level of liver damage in the number of degeneration (0.08 ± 0.37) and necrosis (1.20 ± 0.37). That showed additional of MPBE can affect to liver damage of sangkuriang catfish.

Effect of Treatment on Proximate

Table 5. Proximate Analysis of *Clarias gariepinus Var* fed diet of *Myrmecodia pendens* bulb extract

Group	***Proximate Analysis (%)***				
	Water	*Protein*	*Ash*	*Crude Fiber*	*Fat*
C	75.29	19.72	5.16	0.48	0.35
P1	75.94	20.83	5.15	0.07	0.6
P2	76.34	18.25	5.3	0.66	0.45
P3	76.47	18.77	4.8	0.07	0.4
P4	76.7	17.65	5.34	0.19	0.6
Reference ***(Estel et al., 2012)***	74-85%	12-22%	0.8-2%	0.8-2%	0,4-5.70%

Based on the table 5, as can be seen that the catfish fillet with addition of Myrmecodia pendens showed the average water content was quite good with a percentage 74-76%. Protein classified as good with a percentage 12-22%. In other hand, the fiber content classified as good except in P1 and P3 groups which are below the standard 0.8-2% and the fat contain of catfish fillets treatment groups as classified better than control which had a lower percentage. Although, ash content of catfish fillets classified as not good because it exceeds the standard with a percentage 2%.

DISCUSSION

This study showed that effect of addition Myrmecodia pendens bulb extract on *Clarias gariepinus* on the growth performance with high significant difference ($P<0.05$) when compared with the control. Highest value found on the addition of 3 g/kg Myrmecodia pendens bulb extract in P4 group. Increase in growth performance obtained in this study can be caused by the phytochemical content of Myrmecodia. According to [7] Myrmecodia contained phytochemical such as flavonoids, phenols, tannins, saponins, steroids and terpenoids. This is in line with statement of [10] that fishes are fed a diet containing flavonoid compounds, and quercetin can increase mean values for final fish weight, SGR and condition factors were compared with control groups. It was reported that flavonoids contain powerful antioxidants which increases the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and reduce malondialdehyde levels [11]. Beside flavonoids, the content of compounds tannins and saponins to improve fish growth performance have also been widely reported. Based on [5, 11] that

saponins and tannins compounds can show increased growth performance, efficiency utilization of feed and SGR of fish through increased feed intake and enzyme activity digestion compared to control group.

Absorption that occurs in the intestinal villi is faster than the stomach because the small intestinal epithelium is much wider than the gastric epithelium [12]. The results showed that the groups of fish that had the highest villi and depth of crypt was the control groups. The addition of Myrmecodia to fish fed can causes changes in the intestinal structure. As can be seen in (Fig. 1) was group C had normal villous structure and no damage as well as group P1 (Fig.2) but the villi height was shorter than group C. In group P2 (Fig. 3) had fewer villi than C and P1 groups and also thinning of the submucosa layer. Then the worst damage occurred in P3 and P4 groups which were characterized by an irregular arrangement of villi, increased lumen width, and thinning of the mucosa and submucosa layers. According to [13], the thinning of the mucosa is caused by inflammation, which is characterized by the infiltration of neutrophils into the lamina propria and possibly this causes the villi to become blunt, shortened and damaged. It is consistent in this study that the damaged villi become blunted, the boundaries between the villous walls have been distinguished, and the lumen widening occurs. [14] stated that flavonoids and tannins compounds can support fish growth, if used excessively and for a long time they become antinutrients, causing protein denaturation, cell permeability and capable of damaging cell walls. In addition, it can be seen that the fish intestines in the P4 groups have a large lumen width compared to the other groups (Fig. 5). The increase in the villi of the P4 groups probably occurred closely with the width of the lumen in the intestine. According to [15] that the lumen diameter in the duodenum is closely related to the increase in the rate of cell turnover, the height of the villi and the increase in the depth of the crypt of the mucosa layer. Meanwhile, some other signs that cause inflammation in the intestines are the intestinal villi become longer, the intestinal wall thickens, and the amount of lymphatic tissue becomes more numerous. One of the causes of irritation is the toxic effect of the herbal plants used [12]. These contents are included in bioactive compounds that have high antioxidants, but can be toxic if given high doses and for a long time [14].

Based on the observation, increased concentration of *Myrmecodia pendens* bulb extract relates to the histological conditions in all groups (Fig. 6). The amount of damage in each group was the averaged and presented in Table 4. In this research, all of the hepatocyte shown there were damage, but most of the normal cells was observed in control groups. According to research conducted by [16], if the liver score of catfish is below number 1, the damage that occurs to the hepatocytes of catfish is categorized as normal. The liver of the control groups that had necrosis and degeneration were not included in the pathological events, because necrosis and degeneration can occur under normal circumstances. According to [17] as for the occurrence of catfish liver damage in the control group was probably due to the use of catfish that is not Specific Pathogen Free (SPF) so that it suffers infections or disorders such as parasitic infestations. In other hand, and then severe condition was found in P4 groups. This shows that increasing the concentration used causing more damage. (Fig. 6) showed that P1 groups had focal necrosis (mild) with morphological changes that look like pyknosis characterized by the occurrence of clumping of chromatin and nucleus so that cells appear denser and more colorful dark. [18] Necrosis is the death of tissue cells while the individual is still alive, that cause namely strong toxics (such as viruses' infections and metabolic disorders (in metabolism proteins). In contrast to P2 groups also experienced many liver structure cells were damage in the form of hydropic degeneration, fatty degeneration and necrosis, but the numbers were lower than P3 and P4 groups (Table 4). As can be seen (Fig. 6), that P3 groups had hydropic degeneration damage occurred. This is possible because of the effect of plant phytochemicals contained in *Myrmecodia pendens* bulb extract that enter the liver, causing necrosis and degeneration of the liver tissue. According to [16] swelling occurs due to manifestations of fluid accumulation that excessive due to the failure of cells to maintain homeostasis. Fat degeneration is a continuation of hydropic degeneration that has undergone irreversible damage [17]. As seen in the P4 groups (Fig. 6) that the accumulation of fat contained in the cytoplasm of the liver cells to form vacuoles of various shapes. According to [19-20] this is due that chemical compounds in several plants such as alkaloids in can cause the percentage of liver damage likely necrosis and degeneration. [21] found that *Myrmecodia pendens* is believed to be able to produce the proliferation of new liver cells so that they can improve liver cell repair although not yet known specifically. This result is similar to [22] who reported the test acute toxicity with a dose of 3750 mg/kg water extract of Myrmecodia in the liver histology of mice is not cause disturbances that causes disease in animals although it causes necrosis and abnormal vacuolization with cell infiltration macrophages and neutrophils, but no dose induced deaths during the study period. It is suspected that plant water extracts are still safe for developed as a medicinal raw material.

Catfish is one type of aquaculture commodity that contains vitamins, proteins, minerals, and low content of unsaturated fat and carbohydrates [23]. According to [1], the composition of feed can affect the proximate value of fish. Based on the results showed that the highest water content is found in group P4 with a percentage of 76.7% and the lowest in C group with a percentage of 75.29%. The water content in the catfish fillet shows water stability in the

fish's living environment [24]. In other hand, protein content of P1 group catfish fillets was higher (20.83%) than that of P4 group with a low percentage of 17.65%. Based on [25] fish in absorbing protein depends on the ability to absorb and convert nutrients properly from the feed and environment, so in his research the use of plant material as a substitute for feed can affect growth, nutrient absorption and proximate value of *C. carpio* fillets. The highest ash content in catfish fillets was the P4 group with a percentage of 5.34% and the lowest in the P3 group with a percentage of 4.8%. Whereas the ash content in this study is higher than the value of the content in the study [26] namely around 0.8-2%. This is a possibility due to the addition of *Myrmecodia pendens bulb extract* that the amount of minerals which can enter the fish was high. According to [27] the ash content closely related to the mineral content, purity and cleanliness contained in a material. While the Myrmecodia had mineral content in units of mg/100 g, namely calcium (0.37), sodium (68.58), potassium (3.61), zincs (1.36), irons (29.24), phosphorus (0.99), and magnesium (1.50) [28]. The fiber content the highest in the P2 group with a percentage of 0.66% and the lowest in the P1 and P2 groups with a percentage of 0.07%. Based on [29] excessive crud fiber resulting in decreased digestibility, decreased absorption, and increased waste metabolism. The highest fat content of catfish fillets in P1 and P4 groups with same percentage 0.6% and the lowest in group C with a percentage of 0.35%. According to [26] the fat content of fish fillets can be categorized as lean (<2% fat), low fat (2-4%), moderate fat (4-8%) and fatty (>8%) and based on the value of fat content in this research included in the category without fat (<2%).

CONCLUSION

This study indicated that fish fed 3 g/kg *MPBE* had higher growth performance ($P<0,05$) than other groups. However, villi and depth of crypt intestine the fish fed *MPBE* was shorter than control group. The liver of the fish fed MPBE also showed significant effect on the histology of liver. Furthermore, the addition of *MPBE* to fish showed a better result of fillet proximate analysis compared to control.

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