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Effect of water hyacinth leaf flour (*Eichhornia crassipes*) fermented by *Aspergillus niger* in the commercial pellet on the growth, survival rate and blood profile of sangkuriang catfish (*Clarias gariepinus*)

Febri Ariyanto[1], Rudy Agung Nugroho[2*], Retno Aryani[3], Hetty Manurung[4], Rudianto Rudianto[2]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan Province, Indonesia.*
[2]*Animal Physiology, Development, and Molecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan Province, Indonesia.*
[3]*Animal Anatomy and Microtechnique Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan Province, Indonesia.*
[4]*Plant Physiology and Development Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan Province, Indonesia.*

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ABSTRACT

Sangkuriang catfish (*Clarias gariepinus*) is one of the aquaculture commodities with great potential to be developed in Indonesia to support the demand for feed. Efforts to reduce dependence on imported feed raw materials for fish feed is continued. Water hyacinth (*Eichhornia crasspies*) leaf which contains phytochemical compounds such as flavonoids, tannins, steroids and saponins is an alternative raw material for fish pellet component. This study aimed to determine the addition of fermented water hyacinth leaf meal in fish feed on the growth, survival rate, and blood profile of sangkuriang catfish. In total 150 fish (average initial weight 5.45 ± 0.02 g) were randomly divided into triplicates of five groups, containing 10 fishes per group. Group one is a control group, P1 (10% of non-fermented water hyacinth leaf meal), while P2, P3 and P4 were treatment groups of fish fed with 10, 15, and 20% fermented water hyacinth leaf meal by *Aspergillus niger*. At the end of the 42 days of treatment, growth, survival rate, and blood profile of all fish were determined. The results showed that the addition of 10% water hyacinth leaf meal fermented by *A. niger* had an effect on the growth of final weight, body weight gain and specific growth rate ($p < 0.05$) in comparison to all groups. However, the addition of any concentration of water hyacinth leaf meal did not affect on the feed conversion ratio and survival rate value. Further, the addition of water hyacinth leaf meal had a significant effect ($p < 0.05$) on the erythrocytes, leukocytes and hemoglobin of fish, but did not show a significant effect on the values of hematocrit and platelets. It is concluded that addition of the fermented 10% water hyacinth leaf meal in the feed has beneficial to improve the growth of sangkuriang catfish.

Introduction

African catfish or locally known as sangkuriang fish *(Clarias gariepinus)* is one of the freshwater fish commodities that has a great potential to be cultured (Marimuthu *et al*., 2019). This fish has been cultivated commercially and is growing rapidly on limited land production (Sorongan *et al*, 2019; Muchlisin *et al*., 2015). In addition, catfish can be kept with high stocking densities in line with current market demand (Simanjuntak *et al*., 2020). In addition, catfish production has increased by an average of 9.23% per year (KKP, 2021).

The culture of catfish in the community has encouraged aquaculture business actors to continue to strive for maximum production (Audila and Zulfahmi, 2021). However, this is inversely proporsional to the availability of catfish which is still not matched by the availability of feed that affects production costs (Prasekti and Irfan, 2021). Several efforts have been done to reduce dependence on

* Corresponding author.
Email address: rudyagung.nugroho@fmipa.unmul.ac.id

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imports of feed raw materials in fish diet and to find substitutes for alternative materials whose quality is cheap, easy to obtain, and has a high nutrition content (Amin *et al.*, 2020; Karina *et al.*, 2015; Yanti *et al.*, 2019). One alternative that can be used as a raw material for feed is water hyacinth *(Eichhornia crassipes).*

Water hyacinth is an aquatic plant commonly referred to as a weed and is able to reproduce very quickly. Water hyacinth is easy to adapt to its environment and is able to compete with other organisms. The rapid growth of water hyacinth can reach 3% per day. Their rapid growth can greatly disrupt activities in the waters and can result in silting and narrowing of rivers, lakes, and swamps which causes reduced oxygen content, decreased nutrients and water evaporation (Kurniawan *et al.*, 2020).

The use of water hyacinth in feed ingredients has several weaknesses, including high hemicellulose content and protein content that is difficult to digest which it can interfere with digestibility and palatability or level of preference (Rahayu, 2020). The nutritional content of water hyacinth in the form of dry matter has a crude protein content of 6.31% and crude fiber 26.61% (Putra *et al.*, 2020). Therefore, it is necessary to use an easy fermentation process using *Aspergillus niger* (Setiawan, 2013).

A. niger is one of the microbial species that does not produce mycotoxins compared to other microbes (Maryanty *et al.*, 2010). In addition, *A. niger* can produce enzymes that can help the digestive process such as cellulase, amylase, protease, phytase and mananase which make it easier for livestock feed to be digested (Sukaryani *et al.*, 2021).

The previous study by Raudah *et al.* (2018) used *A. niger* as a fermenter on the leaf plant of Lamtoro Gung *(Leucaena leucocephala)* in the feed of Siamese catfish *(Pangasius hypophthalmus)*. Meanwhile, Pane *et al.* (2018) added water hyacinth leaf meal in the manufacture of feed with concentration of 10% which is the optimumt concentration on the growth and survival or survival of sangkuriang catfish fry *(C. gariepinus*). Further, Syaraswati *et al.* (2022) performed an addition of 10% water hyacinth leaf meal fermented by *A. niger* in the feed which contains flavonoid compounds, resulting high survival rates for the growth of nilem fish *(Osteochilus hasselti)* fry. In addition, the effects of ginger powder *(Zingiber officinale)* 3.75 g/kg feed containing flavonoid compounds affected on the blood profile of catfish *(Pangasius* sp.*)* (Fajriyani *et al.*, 2017).

Though several studies have been performed to determine the effects of the water hyacinth meal on the feed of fish, limited study on the effects of fermented water hyacinth meal on the sangkuriang catfish. Present research was conducted to determine the effects of fermented water hyacinth leaf meal in feed on the growth, survival, and blood profile of sangkuriang catfish.

Materials and Methods

Fish preparation

In total of 150 sangkuriang catfish with an initial average weight of 5.45 ± 0.02 g, 45 days old was provided from local farmer at Samarinda. All fish was fed a commercial pellet feed (Hi-Pro-Vite 781-2) Made in Indonesia, during acclimatization (7 Days).

After acclimatization, all fish were randomly stocked into triplicates of five tarpaulin ponds, containing 10 fish per pond. Ponds were labelled as (K) control/commercial pellet (Hi-Pro-Vite 781-2) group, (P1) water hyacinth leaf meal without fermentation (10%) + Pellet 90%; (P2) fermented water hyacinth leaf meal 10% + Pellet 90%; (P3) fermented water hyacinth leaf meal 15% + Pellet 85%, and (P4) fermented water hyacinth leaf meal 20% + Pellet 80% (Pane *et al.*, 2018).

Water hyacinth leafs meal fermentation

Water hyacinth leaves were cut, washed and finely chopped. The water hyacinth leaves were dried under the sun for 4 days. After that, the water hyacinth leaves were milled and sifted. The water hyacinth leaf meal was ready to be fermented.

The water hyacinth leaf meal was added with water at ratio 2:1.5 (Raudah *et al.*, 2018). The mixture was steamed for 15 minutes to kill pathogens that can interfere fermentation process. Further, the mixture was cooled and inoculated with *A. niger* powder at a dose of 2%, put into a plastic that had been perforated to obtain aerobic conditions. The fermentation process was done for 72 h at room temperature. After 72 h, the fermented product was re-steamed for 15 minutes to stop the fermentation process. The fermented product was dried and kept into the plastic bag and store in 4 °C until its used. The fermented water hyacinth leaf meal was also measured for fiber analysis. Based on our results, the fiber content of water hyacinth leaf meal before fermentation was 38.05±0.43%, while after fermentation was 7.78±1.87%

The dry fermented water hyacinth leaf meal was mixed with commercial feed with the ratio as describe previously. After added with 1000 mL of distilled water, all the raw materials were mixed, then the dough was re-pellet using pelletizer. The pellet was then dried using an oven at 60°C or for 17 hours.

Fish rearing

Fish were reared for 42 days, fed with commercial feed and fermented water hyacinth leaf meal two times per day at satiation rate. Ponds siphoning was carried 2 times per week to remove faeces and food residue and maintained water quality.

Fish parameters measurement

Fish biomass determination was carried out every 2 weeks. Measurement of fish biomass was performed by taking the total number of fish in the pond for each replication using a net. The fish were weighed using a digital scale (HC C100001 Electronic Analytical Balance, China). Fish was also determined for Body Weight Gain (BWG), Specific growth rate (SGR), Feed Conversion ratio (FCR), and Survival Rate (SR) using equation below:

$$BWG = Wt - Wo$$

(Suci *et al.*, 2020)

Where, BWG = Body weight gain (g); Wt = Fish weight at the end of the research (g), Wo = Fish weight at the beginning of the research (g).

$$SGR = \frac{[\ln Wt - \ln Wo]}{t} \times 100\%$$

(Setiawati *et al.*, 2013)

Where, SGR = Spesific growth rate (%), Wt = Fish weight at the end of the research (g), Wo = Fish weight at the beginning of the research (g), t= Experiment duration (days).

$$FCR = \frac{F}{W_t - W_0}$$

(Anis *et al.*, 2019)

Where, FCR = Feed Conversion Ratio (g), F = Total of Feed Consumed (g), Wt = Fish Weight at the end of the research (g), Wo= Fish weight at the beginning of the research(g).

$$SR(\%) = \frac{Nt}{No} \times 100\%$$

(Tamba *et al.*, 2020)

Where, SR = Survival Rate (%), Nt = Total Fish at the end of the research, No=Total Fish at the beginning of the research

Fish blood sampling

Fish blood sampling was done by taking out the fish blood through the dorsal aorta, placing a syringe between the anus and anal fin. The collected blood was put into a tube containing 0.5 mL of 0.2% EDTA.

Hematology analysis

Hematology profile analysis of collected fish blood was carried out to determine the health condition of fish using a hematology analyzer (Wincom HA-Y7021, China). The blood profiles data were White Blood Cell Count (WBC), Red Blood Cell Count (RBC), platelets/platelets (Plt), hemoglobin (Hb) and hematocrit (HCT). Observation of fish blood profile was performed at the end of the final day of the study. The hematology analysis was not performed at the beginning because of the study was used post experiment analysis which was compare the effects of the water hyacinth at the end of the experiment. This was also used to minimize stress of fish.

Water quality measurement

Water quality measurements such as temperature, pH, DO, TAN, and nitrite were checked once per week.

Data analysis

The result data such as BWG, FCR, SR, and blood profiles were analyzed using SPSS statistic 22 and presented as Mean ± standard error. Meanwhile water quality data, namely pH, DO (Dissolved Oxygen), TAN (Total Ammonia Nitrogen), and Nitrite were measured once a week. To determined significantly difference, Duncan Multiple Range Test with a significance level of $p<0.05$ was applied.

Results

The results showed that the water hyacinth leaves gave a significant effect on the growth performance ($P<0.05$), but it was not gave a significant effect on the feed conversion ratio and survival rates ($P>0.05$). Where the best growth performance was recorded at fish fed on 10% fermented water hyacinth leaf meal + 90% Pellet. This value was significantly different from other treatments (Table 1).

Blood profile parameters

The water hyacinth leaf meal gave a significant effect on the number of leukocytes, erythrocytes, and platelets in the blood of catfish ($p<0.05$). However, it was not revealed a significant effect on the hemoglobin ($p>0.05$). The lowest Leukocyte and Platelets, and also the highest Erythrocyte and Hematocrit cells were found at treatment P2 (fermented water hyacinth leaf meal 10% + Pellet 90%). But, these values were not different significantly with P3 and P4 (Table 2).

Water quality sangkuriang catfish

The water quality in the rearing pond was still within the range below the water quality standard for catfish and is categorized as suitable for use as a rearing place (Table 3).

Table 1. Growth of Sangkuriang Catfish *(Clarias gariepinus)* fed various levels of fermented and unfermented water hyacinth *(Eichhornia crasspies)* leaf meal for 42 days

Parameters	K	P1	P2	P3	P4
Final Weight (g)	138.28 ± 6.49[b]	94.20 ± 8.10[a]	167.01 ± 2.85[c]	126.74 ± 6.31[b]	122.13 ± 7.20[b]

Parameters	K	P1	P2	P3	P4
BWG (g)	85.62 ± 4.71^{b}	42.83 ± 8.17^{a}	116.63 ± 1.25^{c}	75.17 ± 5.20^{b}	70.98 ± 5.50^{b}
SGR (%)	10.57 ± 0.13^{a}	8.86 ± 0.42^{b}	11.33 ± 0.26^{c}	10.27 ± 0.16^{a}	10.13 ± 0.16^{a}
FCR	3.06 ± 0.27^{a}	4.09 ± 0.34^{a}	2.44 ± 0.58^{a}	3.01 ± 0.45^{a}	3.23 ± 0.06^{a}
SR (%)	80.00 ± 0.00^{a}	80.00 ± 5.77^{a}	86.66 ± 3.33^{a}	86.66 ± 3.33^{a}	86.66 ± 8.81^{a}

Note: K (Control), P1 (Treatment with the addition of 10% water hyacinth leaf meal without fermentation), P2-P4 (Treatment with the addition of 10, 15, and 20% of fermented water hyacinth leaf meal. All data shows as mean±standard error. The mean followed by different superscript letters (a, b, c) in the same row indicates a significant difference ($p < 0.05$). BWG (Body Weight Gain), SGR (Specific Growth Rate), FCR (Feed Conversion Ratio), SR (Survival Rate)

Table 2. Blood Profile of sangkuriang catfish *(Clarias gariepinus)* fed various levels of fermented and unfermented Water hyacinth *(Eichhornia crasspies)* leaf meal for 42 days.

Parameters	Control	P1	P2	P3	P4
Leukocyte (10^3/µL)	0.95 ± 0.03^{a}	0.42 ± 0.04^{a}	5.69 ± 0.41^{b}	7.20 ± 2.64^{b}	6.35 ± 0.41^{b}
Erythrocyte (10^6/µL)	0.25 ± 0.19^{b}	0.04 ± 0.04^{a}	0.76 ± 0.09^{c}	0.55 ± 0.03^{c}	0.76 ± 0.09^{c}
Hemoglobin (g/dL)	3.96 ± 0.23^{a}	2.86 ± 0.03^{b}	2.46 ± 0.03^{b}	2.30 ± 0.17^{b}	2.46 ± 0.03^{b}
Hematocrit (%)	3.73 ± 3.08^{b}	0.46 ± 0.03^{a}	10.76 ± 1.02^{c}	8.20 ± 1.03^{c}	10.56 ± 1.02^{c}
Platelets (10^3/µl)	21.00 ± 15.01^{b}	20.66 ± 0.33^{b}	4.33 ± 0.33^{a}	7.00 ± 1.73^{a}	5.33 ± 1.45^{a}

Note: K (Control), P1 (Treatment with the addition of 10% water hyacinth leaf meal without fermentation), P2-P4 (Treatment with the addition of 10; 15, and 20% of fermented water hyacinth leaf meal. All data shows as mean±standard error. The mean followed by different superscript letters (a, b, c) in the same row indicates a significant difference ($p < 0.05$).

Table 3. Water quality parameters during the study

Parameters	Control	P1	P2	P3	P4	*BSN
Temperature (°C)	27.41±0.006	27.38±0.003	27.41±0.006	27.38±0.003	27.38±0.003	25-30
pH	7.57±0.009	7.50±0.003	7.57±0.009	7.50±0.003	7.50±0.003	6.5-8.5
DO (mg/L)	6.00±0.033	6.90±0.033	6.00±0.033	6.90±0.033	6.90±0.033	>4.0
TAN (mg/L)	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	<1
Nitrite (mg/L)	0.01±0.003	0.01±0.00	0 - 0.01±0.00	0.01±0.00	0.01±0.00	<1

Note: K (Control), P1 (Treatment with the addition of 10% water hyacinth leaf meal without fermentation), P2-P4 (Treatment with the addition of 10; 15, and 20% of fermented water hyacinth leaf meal. *(BSN) National Standardization Agency Quality Standard, 2014. Tolerance limit for the maintenance of sangkuriang catfish.

Discussion

Growth performance

Present study showed that, the highest BWG (116.63 g) and SGR (11.33%) were found in fish fed 10% of fermented water hyacinth leaf meal groups. The addition of water hyacinth leaf meal with a concentration of 10% in the feed was able to improve the growth performance of catfish. This is in line with the past study performed by Wijaya *et al.* (2015) stated that water hyacinth leaves contain several plant phytochemical compounds such as flavonoids, tannins, steroids and saponins that are beneficial in growth. According to Zhai and Liu (2013), fish fed flavonoid compounds can increase the average final weight and BWG of the fish.

Further, Indariyanti & Rakhmawati (2013) revealed that *A. niger* produces cellulase and xylanase enzymes which will remodel cellulose into cellulose and eventually become glucose. This is in accordance with the research of Ikhwanuddin *et al.* (2018) fermented bran by *A. niger* as a feed raw material can increase the value of protein digestibility and growth in tilapia *(Oreochromis niloticus)* compared to unfermented feed.

Meanwhile, present study found that there was no significant difference ($p>0.05$) on the FCR between control and all treatment groups. Widyastuti *et al.* (2010) stated that the smaller the FCR value, the higher the quality of feed and vice versa, while Marzuqi and Anjusary (2013) added that the digestibility of fish itself is influenced by several factors, namely the age of the fish, the nutritional level of the feed and the water temperature.

Furthermore, the results of the SR during the 42-day study showed no significantly different and SR was in the range between 80.00 and 86.66%. This range is supported by previous study by Mulyani *et al.* (2014) stating that the survival rate of more than 50% is classified as good, 30-50% is classified as moderate and less than 30% is classified as not good. The current study is also in concomitant with Syaraswati *et al.* (2022) and Kurniawan *et al.* (2022) study, revealed that the use of fermented or unfermented water hyacinth leaf meal did not affect on the survival rate of nilem *(Osteochilus hasselti)* fish and baung fish *(Hemibagrus nemurus)*.

Blood profiles

The number of erythrocyte of fish fed fermented water hyacinth leaf meal was higher than the control group and the unfermented treatment group. The increase in the number of erythrocytes was due to the flavonoid compounds which is found in water hyacinth leaves that might be able to improve the health of catfish. These results are in line with the research of Zissalwa *et al.* (2020), who tested the effect of mangrove leaf extract on the erythrocyte profile parameters of jambal siam *(Pangasius hypophthalmus)* fish. It is found that feeding of mangrove leaf extract containing flavonoid compounds can increase the number of erythrocytes. Putri *et al.* (2013) also stated that the number of erythrocytes describe the condition of the fish's body which is indicate as the fish's body defense against pathogenic bacteria.

Similar to erythrocyte, the leukocyte and hematocrit parameter indicated significantly different. Present results are in concomitant with the research of Kanani *et al.* (2014), which tested the effect of ginger extract supplementation on the hematological parameters of Huso huso fish. It is revealed that feeding from plants containing flavonoid compounds can increase the percentage of hematocrit in fish.

On the other hand, the number of hemoglobin showed fish group fed with fermented water hyacinth leaf meal was lower than the fish both in control group and the unfermented treatment group. This is presumably due to the presence of saponin compounds in water hyacinth leaf meal (Lata and Venapani, 2010). Current result is in accordance with the previous study of Lestari *et al.* (2018) stated that the effect of adding cinnamon leaf powder *(Cinnamomum burmannii)* to the fish feed affected on the blood profile (hemoglobin level) of tilapia *(Oreochromis niloticus)* infected with *Streptococcus agalactiae*. It is also known that hemoglobin level can be decreased due to the presence of saponin compounds found in cinnamon leaf meal. Further, a low number of hemoglobin causes metabolism to be hampered and the energy produced becomes less (Hardi *et al.*, 2011).

The results showed that the platelet of fish fed fermented water hyacinth leaf meal was lower than either the control group or the unfermented treatment group. These results are supported by past research of Puspitowati *et al.* (2022), mentioned that the leukocyte of jambal siam fish *(Pangasianodon hypophthalmus)* had decreased after fed with fermented papaya leaf solution *(Carica papaya)*. According to Santoso *et al.* (2013), platelets play a part in the blood clotting process and work to stop fluid loss from infections on the body's surface. To aid in blood clotting and stop further bleeding, platelets are formed. The increase in platelet levels in fish blood is an indicator that fish are in the process of healing wounds (Salim *et al.*, 2016).

In addition, water quality measurement during the study showed a suitable temperature (27.38-27.41 °C), pH (7.50 - 7.57), DO (6.00 - 6.90 mg/L), TAN (0.1 mg/L) and nitrite (0.01 mg/L)for the growth of catfish. According to Pratama *et al.* (2016) the optimal temperature for catfish is around 20 - 30 °C. Meanwhile, the pH, DO, TAN, and nitrite obtained in the rearing pond for 42 days ranged was in the range tolerance as suggested by the National Standardization Agency (2014), which is 6.5-8.5 (pH), > 4 mg/L (DO), < 1.0 mg/L (TAN), < 1.0 mg/L (Nitrite).

Conclusions

Based on the results of the study, it can be concluded that, adding fermented 10% water hyacinth leaf meal in the feed has beneficial to improve the growth of sangkuriang catfish. The fermented water hyacinth leaf flour in feed shows a significant effect on the number of blood profiles of catfish.

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