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Supplementation of Fermented Moringa Leaves in Feed on Blood Glucose of Striped Catfish (*Pangasianodon hypophthalmus*) Infected with *Aeromonas hydrophila*

Penambahan Daun Kelor Fermentasi pada Pakan terhadap Glukosa Darah Ikan Patin (*Pangasianodon hypophthalmus*) yang terinfeksi *Aeromonas hydrophila*

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ABSTRACT

Moringa leaves can be used as an herbal plant in fish growth to prevent and treat bacterial infections. This study aims to determine the effect of fermented moringa leaves through feed on blood glucose in striped catfish (*Pangasianodon hypophthalmus*). This research was conducted from September to November 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Riau University. The method used is an experimental method using a completely randomized design with 4 (four) treatments and 3 (three) replicates; the treatment dose of fermented moringa leaves is T0 (control), T1 (10 g/kg feed), T2 (15 g/kg feed), and P3 (20 g/kg feed). The catfish measured 5.00 ± 1.00 cm and weighed 4.00 ± 0.50 g, reared in an 80 L tank at a stocking density of 1 fish / 4 L water. The maintenance was carried out for 60 days, and blood glucose observations were made at the beginning (H-1), day 30 (H-30), day 60 (H-60), and post-challenge test (H-75). The results showed that adding fermented moringa leaves to the diet did not affect the blood glucose of the catfish ($p>0.05$). This indicates that moringa leaves can keep the fish in normal condition after being challenged with *Aeromonas hydrophila* bacteria.

Keywords: Stress response, Fermentation, Moringa leaf, *Motile Aeromonas Septicemia*

ABSTRAK

Daun kelor dapat dimanfaatkan sebagai tanaman herbal pada pertumbuhan ikan dalam pencegahan dan pengobatan infeksi bakteri. Penelitian ini bertujuan untuk mengetahui pengaruh daun kelor fermentasi melalui pakan terhadap glukosa darah ikan patin (*pangasianodon hypophthalmus*). Penelitian ini dilaksanakan pada bulan September-November 2023 di Laboratorium Bioteknologi Kelautan Fakultas Perikanan dan Kelautan Universitas Riau. Metode yang digunakan adalah metode eksperimen dengan menggunakan rancangan acak lengkap 4 (empat) perlakuan dan 3 (tiga) ulangan, perlakuan dosis daun kelor fermentasi adalah P0 (kontrol), P1 (10 g/kg pakan), P2 (15 g/kg pakan), dan P3 (20 g/kg pakan). Ikan patin yang digunakan berukuran 5,00±1,00 cm dan bobot 4,00±0,50g, dipelihara pada wadah bervolume 80L dengan padat tebar 1 ekor/4L air. Pemeliharaan dilaksanakan selama 60 hari dan pengamatan glukosa darah dilakukan pada awal (H-1), hari ke-30 (H-30), hari ke-60 (H-60), and pascaujitantang (H-75). Hasil penelitian menunjukkan bahwa pemberian daun kelor fermentasi pada pakan tidak memberikan pengaruh terhadap glukosa darah ikan patin ($p>0,05$). Hal ini menunjukkan bahwa pemberian daun kelor dapat menjaga ikan tetap berada pada kondisi normal pascaujitantang dengan bakteri *Aeromonas hydrophila*.

Kata Kunci: Respon stress, Fermentasi, Daun kelor, *Motile Aeromonas Septicemia*.

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INTRODUCTION

Herbs have often been used for traditional medicine, especially in rural communities (Rahayu et al., 2020). Besides being used in humans, herbal ingredients are also used in improving the growth and immunity of livestock (Haniarti et al., 2019) and aquatic animals to improve growth, immunity, and treatment of infectious diseases (Awad & Awaad 2017).

Moringa is a widely available herbal plant with several industrial and medicinal uses (Saini et al., 2016). Moringa leaves, fruits, bark, and roots have high nutritional value and are immunostimulant, anti-inflammatory, antiulcer, anti-bacterial, hypoglycemic, anti-hypertensive, and hepatoprotective (Kazeem, 2017). According to Leone et al. (2015), leaves are the most widely used part because they are rich in vitamins and other secondary metabolites. According to Lin et al. (2018), Moringa leaves contain 27% protein, vitamin C, A, Ca, Fe, phosphorus, and Zn. It also contains tannins, flavonoids, alkaloids, steroids, saponins, carotenoids, phenols, and terpenoids. Moringa leaves fermented using *Rhizopus* sp will be broken down into more straightforward and easily digestible compounds, such as proteins, into simpler amino acids (Suprihartini, 2021). In several studies, Moringa leaves have been used as a feed supplement to increase fish growth and immunity (Kazeem, 2017; Puycha, 2017; Nyadjeu et al., 2020; Windarti et al., 2023). In addition, Moringa leaves are also used as anti-parasites and anti-bacterial (Nafiqoh et al., 2020; Hodar et al., 2021).

The utilization of Moringa leaves in fish growth, disease prevention, and treatment of bacterial infections has been widely carried out, among others, on catfish (Ukenye et al., 2023), striped catfish (Saputra et al., 2020; Windarti et al., 2023), and tilapia (Subryana et al., 2020). Striped catfish is one of the freshwater fish with high economic value (Kurniawan et al., 2020; Syawal et al., 2020; Windarti et al., 2021), fast growth, high adaptability to environmental changes (Akter et al., 2021; Chowdhury & Roy, 2020), disease resistance (Abdel-Latif, 2022), and high market potential (Ali et al., 2018; Abd-Elaziz et al., 2023).

Intensive patin fish farming with high stocking density can cause stress to the fish, which induces an increase in blood glucose. This can cause growth inhibition and is easily infected with diseases, including *Aeromonas hydrophila* bacterial infection, which causes Motile Aeromonas Septicemia (MAS). Blood glucose is related to fish health conditions, and stressful conditions can cause changes in blood glucose levels (Hanafi et al., 2023). Based on this background, this study aims to determine the effect of fermented Moringa leaves added to feed on the blood glucose of striped catfish infected with *A. hydrophila*.

MATERIALS AND METHOD

Time and place of research

This research was conducted from September to November 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Experimental design

The method used in this research is an experimental method using a completely randomized design with 4 (four) treatments and 3 (three) replicates. The treatment in this study refers to the results of research by Windarti et al. (2023); the treatments are as follows:

P0 : Commercial feed without the addition of fermented Moringa leaves (Control)
P1 : Add fermented moringa leaves at a dose of 10 g/kg feed.
P2 : Add fermented moringa leaves at a dose of 15 g/kg feed.
P3 : Add fermented moringa leaves at a dose of 20 g/kg feed.

Feed preparation

The stages of making feed with fermented moringa leaves are as follows: In the first step, moringa leaves obtained from one of the traditional markets in Pekanbaru City were separated from the stalks and washed using running water. Next, they were boiled until they wilted, aiming to remove anti-nutrient substances. Then, the moringa leaves were removed, drained, and placed on a tray. Fermentation of moringa leaves uses *Rhizopus* sp fungus with the trademark Raprima Tempe Yeast, Indonesia, which is weighed as much as 1 g and then mixed in 1 kg of moringa leaves until homogeneous. After that, it was put into a plastic seal measuring 10x15 cm with air holes evenly distributed throughout it. The dough is placed on a tray, stored in a dark place, and covered with a cloth. Fermentation lasts 2-3 days until white mold (*mycelium*) grows, like tempeh. Then, the fermented moringa

is steamed for 15 minutes, removed, and cooled. After cooling, the fermented moringa is cut thinly and dried in the sun. After drying, fermented moringa is pulverized using a blender and filtered using a sieve, and the results are obtained like flour.

Moringa flour is mixed with commercial feed according to a predetermined dose using tapioca flour dissolved in boiling water, and tapioca flour is used as an adhesive. After that, the fermented moringa and tapioca starch mixture is coated on the feed. Next, the feed is dried in the sun at room temperature until dry, and then the feed can be given to the fish.

Fish Maintenance

The striped catfish measured 5.00 ± 1.00 cm in length, weighed 4.00 ± 0.50 g and had clinical symptoms such as active movement, no wounds, and high appetite. The test fish were adapted for seven days in 2x1 m fiber tanks and fed with commercial feed. The rearing container used was a black container with a diameter of 1 m and a water volume of 80 L, and the stocking density used was 1 fish/4L of water. Maintenance was carried out for 60 days with feeding twice, at 09:00 and 17:00, and a dose of 5% of body weight.

Challenge Test with *A.hydrophila* Bacteria

The challenge test was conducted after 60 days of rearing using *A. hydrophila* bacteria obtained from the collection of the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The density of bacteria used was 10^8 CFU/mL, infected to fish through an intramuscular injection method of 0.1 mL/fish and maintained for 14 days.

Blood glucose observation

Fish blood sampling was carried out three times, namely at the beginning (day 1), day 30 (day 30), and after the challenge test (day 75). Blood collection is done by first anesthetizing the fish at a cold temperature (±8^o C) (Witeska et al., 2015; Gul et al., 2012; Effendi et al., 2023). After that, blood was taken from the caudal vein using a syringe (1 mL). Furthermore, the blood was stored in Eppendorf tubes to be observed for blood glucose levels. The glucose test kit was GlucoDr brand with a 20-600 mg/dL range. Glucose testing was done in the morning before the fish were fed. Fish blood obtained is dripped on a strip installed on the GlucoDr tool. The tool will immediately read the blood glucose level of the fish and display it on the GlucoDr screen (Eames et al., 2010).

Data analysis

Data obtained from blood glucose measurements were collected and tabulated into tables. Data were analyzed statistically using SPSS version 26. Data were analyzed using One Way Anova and observed for homogeneity. If the analysis results showed an effect, further testing was carried out using Student Newman Keuls (SNK)

RESULT AND DISCUSSION

At the beginning of rearing, blood glucose ranged from 39-42 mg/dL. Observations of the blood glucose of catfish during 60 days of maintenance and post-challenge test can be seen in Figure 1.

Blood glucose levels of catfish during 60 days of maintenance ranged from 41.00 to 69.33%; this indicates that the feed's nutritional content can meet nutrient needs during fish maintenance. Feed containing complex carbohydrates can affect blood glucose levels. According to Lal et al. (2021), complex carbohydrates provide a slower and more stable increase in blood glucose. This can help maintain more consistent insulin regulation and play a role in lowering blood glucose levels and increasing glucose uptake by body cells. At the same time, high-protein feed can impact glucose metabolism. In addition, high protein intake can trigger the secretion of insulin hormone in fish (Li et al. 2021).

After the challenge using *A. hydrophila* bacteria, blood glucose levels of catfish increased to 59-94 mg/dL in the treatment given the addition of fermented moringa leaves, and the value is still within the normal range. This indicates that adding fermented moringa leaves can maintain fish blood glucose in the normal range or make it suitable for fish. Meanwhile, the control treatment (T0) could not be observed because mortality reached 100%. Blood glucose is one of the important components in the blood, serving as the primary fuel source and essential substrate for cell metabolism, especially brain cells. Glucose levels increase due to secondary effects of stress

(Malini et al., 2016). Glucose levels in fish can be influenced by several factors such as activity level, type of food consumed, and water environment conditions. Fish generally produce glucose by metabolizing carbohydrates contained in the food they consume. Fish blood glucose levels under normal conditions range from 40-90 mg/dL (Nasichah et al., 2016) and 41-150 mg/dL (Hartanti et al., 2013).

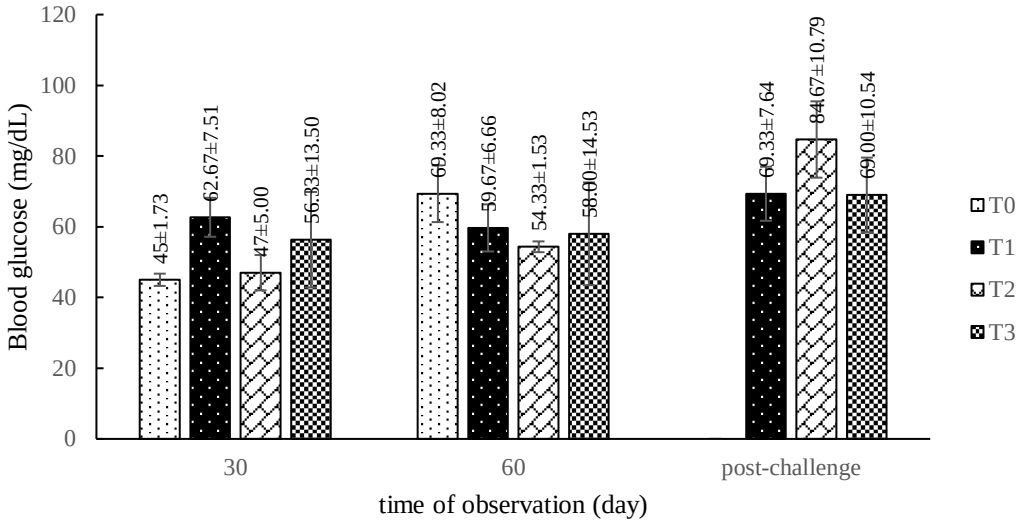

Figure 1. Blood glucose observation of Striped catfish

Compounds contained in moringa leaves, such as flavonoids, can maintain the condition of fish blood glucose. According to Hanhineva et al. (2010), flavonoid compounds trigger the release of glucose from the liver, activate insulin receptors, increase insulin receptors and glucose absorption in insulin tissues, and gene expression. Imani et al. (2012) flavonoid administration significantly reduced blood glucose levels in freshwater fish, inhibiting glucose absorption in the intestine and adjusting its levels in fish blood (Nazeri et al., 2017). In addition to flavonoids, tannin compounds in Moringa leaves play a role in reducing blood glucose levels due to natural antioxidant compounds in plants that can inhibit free radicals to reduce insulin resistance (Rizki et al., 2020).

Another critical factor in the body is the natural hormone produced by the pancreas. The pancreas releases the hormone insulin in the body by signaling fat cells, muscle cells, and the liver to take glucose from the blood and convert glucose into energy, so the development of insulin resistance is the reduced ability of skeletal muscle to adjust easily between glucose and fatty acid oxidation in response to homeostatic signals. In skeletal muscle cells, insulin-stimulated glucose transport depends on several signaling components that decrease glucose transporters' translocation (Ayeleso et al., 2018).

Fish under stress will need much energy to survive, so it will stimulate the flow of glucose into the blood. Blood glucose is a picture of the stress response due to the release of the hormone cortisol in the hypothalamus through the bloodstream to the liver to break down glycogen into glucose so that blood glucose increases (Porchase et al., 2009). According to Zakharova et al. (2021), the mechanism of change is by the receptor organ. Then, the information is conveyed to the hypothalamus part of the brain through the nervous system. Furthermore, chromaffin cells receive orders through sympathetic nerve fibers to secrete the hormone catecholamine. This hormone will activate enzymes involved in the catabolism of liver and muscle glycogen stores and suppress the hormone insulin secretion, so blood glucose increases (Nirmalan & Nirmalan, 2020).

CONCLUSION

Based on the study's results, it can be concluded that adding fermented moringa leaves with different doses through feed does not affect the blood glucose level of striped catfish. In general, the provision of fermented moringa leaves was able to maintain the blood glucose levels of striped catfish in normal conditions before and after the challenge test with *A. hydrophila* bacteria.

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