JPPIPA 10(5) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

The Effect of Papaya Leaf Infusion on Nile Tilapia Infected with *Aeromonas hydrophila*

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Received: January 05, 2024
Revised: March 27, 2024
Accepted: May 25, 2024
Published: May 31, 2024

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DOI: 10.29303/jppipa.v10i5.6852

Abstract: This study aimed to determine the effectiveness of papaya leaf infusion against the weight gain of Nile tilapia infected with *Aeromonas hydrophila* and identify the active compounds contained therein.This study used a complete randomized design. The treatment consists of 5 treatments: treatment K (control), treatment A (25 ppt), treatment B (50 ppt), treatment C (75 ppt), and treatment D (100 ppt). This research includes several stages, the first being to infect tilapia with *A. hydophila*. The second stage is to mix papaya leaf infusion into the feed and observe behaviour, clinical symptoms, weight gain, water quality parameters, and phytochemical analysis. Results showed that treatment A showed the highest value for weight gain. Active compounds in papaya leaf infusion are alkaloids, flavonoids, terpenoids/steroids, glycosides, saponins, and tannins. Papaya leaf infusion increased the weight gain of Nile tilapia infected with *A. hydrophila*.

Keywords: Disease; Nile tilapia; Phytochemicals; Weight gain

Introduction

World fish consumption increased from 1960 to 2015. Per capita consumption of fish in the 1960s was around 9.90 kg and became 14.40 kg in the 1990s. Total fish consumption increased to 19.70 kg in 2013 and is estimated to exceed 20 kg from 2014 to 2015 (FAO, 2016). Capture fisheries cannot meet the protein needs of fish as the number of human populations increases globally. Aquaculture is a solution to meeting global fish protein needs (Abd El-Hack et al., 2022). Aquaculture is essential in the industrial world because the production period is fast (Zaher et al., 2021; Sherif & Kassab, 2023).

Aquaculture includes the cultivation of freshwater fish and saltwater fish under controlled and semi-controlled conditions (Abd El-Hack et al., 2022). Fish is a source of protein production (Zaher et al., 2021; Youssef et al., 2023), vitamins and minerals essential for humans (Qu et al., 2022). Nile tilapia is one type of fish suitable for cultivation (Abd El-Hack et al., 2022), lives in freshwater (Ngamkala et al., 2020; Amer et al., 2022); Kerigano et al., 2023) and is originally from Africa (Nivelle et al., 2019). The advantages of Nile tilapia are high market demand, low production costs, ability to live in different environments, ability to live in low dissolved oxygen conditions, ability to live at high ammonia levels (Abd El-Hack et al., 2022), disease resistance (El-Garawani et al., 2022), easy to reproduce (Ahmed et al., 2023), fast growth (Levia et al., 2021) and cultivated extensively and intensively (Kerigano et al., 2023). Nile tilapia is farmed globally by various countries (Shen et al., 2021; Sherif & Kassab, 2023) and placed third in the world (FAO, 2020).

Aquaculture has health constraints due to diseases caused by pathogenic bacteria, such as the *Aeromonas hydrophila* (Kerigano et al., 2023). *A. hydrophila* is a facultative, gram-negative anaerobic bacterium with a broad spectrum of warm-blooded and cold-blooded animals to humans (Kerigano et al., 2023). *A. hydrophila* bacteria is the causative agent of water infection and the

How to Cite:
Puspitasari, D., Jeki, & Zahar, I. (2024). The Effect of Papaya Leaf Infusion on Nile Tilapia Infected with Aeromonas hydrophila. *Jurnal Penelitian Pendidikan IPA*, *10*(5), 2416–2422. https://doi.org/10.29303/jppipa.v10i5.6852

leading cause of Motile Aeromonas Septicemia (MAS) (Ma et al., 2020). In addition, it is resistant to certain antibiotics (Saleh et al., 2021).

A. hydrophila is known to be the cause of mass death in aquacultures such as Nile tilapia farming (Sherif & Kassab, 2023), catfish (Kusdarwati et al., 2018) and vaname/whiteleg shrimp (Zhou et al., 2019). It is commonly found in fresh waters (Ngamkala et al., 2020) and becomes a pathogen when fish are stressed and water quality is poor (Rozi et al., 2022). The mortality rate of fish caused by *A. hydrophila* reaches 80-100% of fish (Kusdarwati et al., 2018; Rozi et al., 2018).

Nile tilapia infected with *A. hydrophila* have symptoms such as decreased appetite, haemorrhage, and damaged fins (Rozi et al., 2022). The solution used is to use herbal ingredients. Herbal ingredients that have the potential to be antibacterial and able to fight *A. hydrophila* have an essential role in aquaculture activities (Zhou et al., 2019). In addition, herbal ingredients containing papain enzyme can break down protein so the body quickly absorbs it (Hamid et al., 2022). One example of an herbal ingredient with papain content is papaya leaves (Candra et al., 2017).

Papaya leaves are natural ingredients used in medicine (Devmurari et al., 2021; Rifaath, 2022) and have the potential to be antibacterial (Sharma et al., 2022) because they contain phenols (Hariono et al., 2021). In addition, it also contains papain, which can stimulate growth (Hamid et al., 2022). This study aimed to determine the effectiveness of papaya leaves on Nile tilapia weight gain, and identify the active compounds.

Method

Research Design

This research is an experimental laboratory and uses a posttest-only control group design approach (comparing two groups, namely the control group and the treatment group). The experimental design was completely randomized, with five treatments and three replications.

Making Papaya Leaf Infusion

Papaya leaves are washed and then dried. After that, the leaves are mashed and boiled at a temperature of 90 ^{0}C for 15 minutes (DirJen POM, 2000). The doses used in this study were 25 ppt, 50 ppt, 75 ppt, and 100 ppt.

Nile Tilapia Infection Procedure

Nile tilapia were infected with *A. hydrophila* with a bacteria density of 1 x 10^8 CFU/mL (Indriani et al., 2014). Nile Tilapia was injected with *A. hydrophila* at a dose of 0.1 mL in the intramuscular section. Then, the Nile tilapia is transferred into a maintenance aquarium given aeration. Next, an infusion of papaya leaves that have been mixed into fish pellets is given at a dose of 5% of the body weight of the fish. Observation was carried out for 14 days. The size of Nile tilapia is 7-9 cm. *A. hydrophila* bacteria come from the Fish Quarantine Station for Quality Control and Safety of Fishery Products in Tanjung Balai.

Observation of Clinical Symptoms

The symptoms observed are behavioral observations and physical symptoms of Nile tilapia infected with *A. hydrophila.*

Observation of Weight Gain

The weight gain of tilapia is calculated using the formula according to Effendie (1979).

Weight gain (WG) = final weight – initial weight

Water Quality Parameters

Water quality parameters include temperature, dissolved oxygen, and pH. Observation time is every week.

Phytochemical Analysis

The active compounds analyzed are alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids. At the same time, the quantitative analysis is flavonoids. Phytochemical and flavonoid level analyses were conducted at the Pharmaceutical Biology Laboratory, University of Sumatera Utara.

Data Analysis

The research data were analyzed descriptively and using ANOVA. The data analyzed descriptively in tables and graphs are weight gain, water quality parameters, phytochemical analysis, and test flavonoid quantification. The data analyzed statistically using ANOVA is Nile tilapia weight gain.

Result and Discussion

Result

Behaviour and Clinical symptoms

The behavior of Nile tilapia infected with *A. hydrophila* is irregular swimming, slow feeding response, and staying on one side. The clinical symptom of Nile tilapia is haemorrhage on the surface of its body, as shown in Figure 1.

Figure 1. Nile tilapia with haemorrhage

Weight Gain

The weight gain of Nile tilapia is shown in Fig. 1. The highest value of Nile tilapia weight gain was in treatment A, and the lowest value was in treatment D. Treatment A has a weight gain value of 1.5 grams, and treatment D weighs 1.25 grams. The results of ANOVA papaya leaf infusion showed that the F_{values} (6.654) > F_{table} (3.48), which means that papaya leaf infusion water affects the weight gain of Nile tilapia.

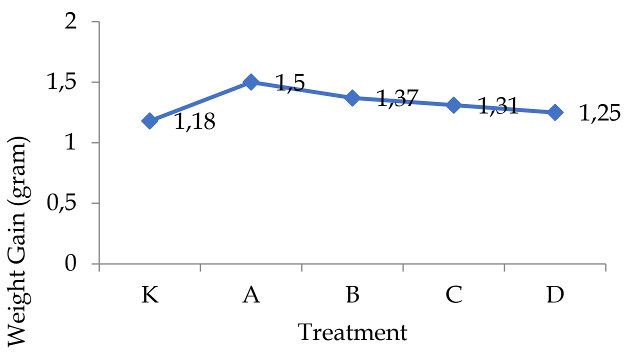

Figure 2. Weight gain of Nile tilapia

Water Quality Parameters

Water quality parameters from this study are shown in Table 1. The temperature ranges from 26 ^{0}C to 29 ^{0}C, pH values are between 6.82 and 7.7, and dissolved oxygen (DO) values range from 4.6 Mg/L to 7.1 Mg/L.

Table 1. Value of Water Quality Control

Treatment	Range Value		
	Temperature (^{0}C)	pH	DO (Mg/L)
K	26 – 29	6.82 – 7.1	4.7 – 6.6
A	26 – 29	6.90 – 7.1	4.6 – 6.8
B	26 – 29	6.90 – 7.5	4.7 – 6.9
C	26 – 29	6.85 – 7.7	4.7 – 7.1
D	26 – 29	6.92 – 7.2	4.8 – 7.0

Phytochemical Analysis Result

The results of this study's phytochemical analysis are presented in Table 2, and the quantitative analysis of flavonoids is presented in Table 3. The active ingredients of papaya leaf infusion are alkaloids, flavonoids, terpenoids/steroids, glycosides, saponins, and tannins. The flavonoid infusion of papaya leaves ranges from 16,940 QE/g Extract to 34,084 QE/g Extract.

Table 2. Result Test of Phytochemical

Class of phytochemical	Description
Alkaloids	+
Flavonoids	+
Terpenoids/Steroids	+
Glycosides	+
Saponin	+
Tannins	+

Table 3. Result of Flavonoids Analysis

Treatment	Value (QE/g Extract)
A	16.94
B	19.00
C	23.85
D	34.08

Discussion

The behaviour of Nile tilapia infected with *A. hydrophila* from day 1 to day 4 is abnormal swimming, slow feeding response, and staying on one side. The clinical symptom of fish is hemorrhage on the body surface. The research results on gourami infected with *A. hydrophila* showed the same symptoms as in this study, which is a slow feeding response (Rozi et al., 2022) fish stay on one side and haemorrhage (Murwantoko et al., 2013). *A. hydrophila* produces enzymes and toxins. The toxins produced by *A. hydrophila* are hemolysine, enterotoxins, and cytotoxins. Hemolysin toxin damages red blood cells, causing cells to come out of blood vessels and resulting in a red colour on Nile tilapia skin. The working of this toxin is related to the specific interaction between receptor cells and hemolysin, resulting in injury to the fish body (Rozi et al., 2022).

Extracellular toxins have two regions determining virulence. They are the active region as the leading cause of infection in cells, and the attachment region is the place of attachment of toxins to specific receptor cells. Hemolysin and enzymes work together to open up tissues on the skin and scales of fish. The invasion of pathogenic bacteria into the fish body begins with the attachment of bacteria to the skin and firmly adheres to the scales protected by the substance chitin. *A. hydrophila* produces the enzyme chitinase to degrade the chitin layer and facilitate penetration by bacteria. In addition, these bacteria produce the enzyme lecithinase, aiding the entry of bacteria into the bloodstream of fish (Rozi et al., 2022).

From day 5 to day 6, the feeding response of Nile tilapia is still slow. On the 7th day, the Nile tilapia responded when fed and began swimming normally. Nile tilapia already shows symptoms such as swimming normally and, when fed, immediately gives a response on the 10th day. The condition of the fish becomes normal, and the clinical symptoms of tilapia disappear allegedly due to the phytochemical content of papaya leaves. The results of the phytochemical analysis show that papaya leaf infusion contains alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids. The analysis of flavonoid quantitative showed that the highest value was found in treatment D of 34,084 QE/g Extract, and the lowest was found in treatment A of 16,940 QE/g Extract.

Flavonoids have anti-inflammatory (Zaragozá et al., 2020; Adinew et al., 2021) and antibacterial potential (Chotimah et al., 2020; Hariono et al., 2021; Sychrová et al., 2022; Gerrine et al., 2023; Winda et al., 2023). Flavonoid compounds work by inhibiting the formation of nucleic acids (Chotimah et al., 2020). The B ring inhibits the synthesis of nucleic acids in the process of intercalation. The hydroxyl group in the flavonoid structure modifies organic components, making them toxic to bacteria (Safitri et al., 2021).

Nile tilapia weight gain was 1.5 grams in treatment A and the lowest value in the control treatment. Treatments A, B, C, and D had higher values when compared to controls. Papaya leaf infusion contains papain enzyme and stimulates Nile tilapia growth (Hamid et al., 2022). *Papain* is an enzyme derived from papaya sap containing protein-breaking or proteolytic enzymes (Candra et al., 2017). Protease enzymes are essential in breaking down protein macromolecules into simpler compounds (Tacias-Pascacio et al., 2021). Meanwhile, lipase and amylase play a role in breaking down lipids and carbohydrates. Using papain enzymes in the substrate, like proteins, lipids, and carbohydrates in fish pellets, causes these macromolecules to decompose before consumption (Ihsan & Mahsul, 2018) This process facilitates the digestion and absorption of food nutrients in the body to increase growth (Candra et al., 2017) and affects the efficiency of feed used by fish (Rachmawati & Prihanto, 2019).

Weight gain in treatment D is 1.25 grams, which is smaller than treatment A. The papain enzyme content mixed into fish feed is in excess, interfering with fish growth (Salam et al., 2020). Enzymes have an optimum limit and catalyze specifically on one substrate and will be inactive if no substrate is present. The adverse effects of adding protease enzymes that exceed the needs of fish are inhibiting metabolism and can affect growth and survival. The addition of excessive protein hydrolysis is thought to affect the regulation of trypsin synthesis and secretion (Rachmawati & Prihanto, 2019). The content of phytochemicals in tannins affects the absorption of nutrients carried out by the body's digestive organs. Treatment D has the highest dose and tannin value compared to other treatments. A high amount of tannins can reduce the rate of absorption of nutrients by the digestive organs because it inhibits metabolism in the body (Hamid et al., 2022).

The water quality values are temperatures ranging from 26 ^{0}C – 29 ^{0}C, pH values ranging from 6.82 – 7.01, and dissolved oxygen values ranging from 4.6 Mg/ L – 7.1 Mg / L. The temperature range optimal for Nile tilapia survival is 25 ^{0}C – 32 ^{0}C, with a pH of 6.5 – 8.5 and a dissolved oxygen value of ≥ 3 Mg / L (SNI 7550, 2009). The maintenance water quality in this study is still normal and supports Nile tilapia's life.

Conclusion

Papaya leaf infusion increased the weight gain of Nile tilapia infected with *A. hydrophila*. The phytochemical content of papaya leaf infusion includes alkaloids, flavonoids, terpenoids/steroids, glycosides, saponins, and tannins.

Author Contributions

Author contributions are writing draft, review and editing, D.P.; resources, J; methodology and data analysis, I. Z. All authors have read and agreed to the published version of the manuscript.

Funding

No external funding.

Conflicts of Interest

No conflict interest.

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