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The Addition of Guava Leaves in Feed to the Blood Glucose of Carp reared in Brackish Water and Infected with *Aeromonas hydrophila*

Pemberian Daun Jambu Biji dalam Pakan terhadap Glukosa Darah Ikan Mas yang dipelihara pada Media Air Payau dan Terinfeksi *Aeromonas hydrophila*

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ABSTRACT

Guava leaves can be utilized as feed additives and medicine for aquaculture. Limiting factors in aquaculture, including stocking density, and salinity can cause stress in fish, so they are easily infected with disease. This study was conducted to determine the effect of guava on the blood glucose of carp reared in brackish water media. This research was conducted from August to September 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The method used in this study is an experimental method using a completely randomized design with 5 (five) treatments and 3 (three) replicates, the treatments are T0 (control), T1 (1.0g), T2 (1.5g), T3 (2 g), and T4 (2.5g). Carp measuring 5.00 ± 1.00 cm with a weight of 4.00 ± 0.50 g were obtained from farmers in the Koto Panjang hydroelectric reservoir, Sumatra, Indonesia. Fish rearing was carried out for 60 days. The results showed that the provision of guava leaves in the feed affected the blood glucose levels of carp. The dose of 1.5 g/100g feed (T2) was the optimal dose in increasing the blood glucose of carp reared in brackish water media and infected with *A. hydrophila* bacteria

Keywords: Carp, Salinity, *Motile Aeromonas Septicemia*

ABSTRAK

Daun jambu biji dapat dimanfaatkan sebagai feed additive dan obat bagi pemeliharaan ikan. Faktor pembatas pada budidaya, diantaranya adalah padat tebar, dan salinitas yang mampu menimbulkan stress pada ikan , sehingga mudah terinfeksi penyakit. Penelitian untuk mengetahui efek pemberian jambu biji terhadap glukosa darah ikan mas yang pemeliharaan ikan mas pada media air payau. Penelitian ini dilakukan pada bulan Agustus s.d September 2023 di Laboratorium Bioteknologi Kelautan, Fakultas Perikanan dan Kelautan, Universitas Riau. Metode yang digunakan pada penelitian ini adalah metode eksperimen dengan menggunakan Rancangan Acak Lengkap dengan 5 (lima) perlakuan dan 3 (tiga) ulangan, adapun perlakuannya adalah T0 (kontrol), T1 (1,0g), T2 (1,5g), T3 (2 g), dan T4 (2.5g). Carp measuring 5.00±1.00 cm with a weight of 4.00 ± 0.50 g obtained from farmers in the Koto Panjang hydroelectric reservoir, Sumatra, Indonesia. Fish rearing was carried out for 60 days. Hasil penelitian menunjukkan bahwa pemberian daun jambu biji pada pakan memberikan pengaruh terhadap kadar glukosa darah ikan mas. Dosis 1,5 g/100g pakan (T2) merupakan dosis optimal dalam meningkatkan glukosa darah ikan mas yang dipelihara pada media air payau dan terinfeksi bakteri *A.hydrophila*.

Kata Kunci: Ikan mas, Salinitas, *Motil Aeromonas Septicemia*

INTRODUCTION

Guava is a plant that can be utilized as food and medicine (Díaz-de-Cerio et al., 2017). Guava contains phytochemical compounds such as essential oils, minerals, vitamins, enzymes, alkaloids, steroids, tannins, flavonoids, and saponins (Weli et al., 2019; Zou & Liu, 2023), hyperin, and guajaverin (Sobral-Souza et al., 2019). These compounds have potential as anti-bacterial, antibacterial, anti-inflammatory, and anti-cancer.

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(Naseer et al., 2018), increase the growth rate and immune response of fish (Effendi et al., 2023; Giri et al., 2015; Omitoyin et al., 2019). According to Giri et al., 2020; Hoseinifar et al., 2019) guava has been developed as a feed additive in feed to promote growth, immune response, antioxidant activity, and disease resistance.

Currently, the use of guava leaves in the prevention of bacterial infections has been widely carried out, including in mujair (Gobi et al., 2016), tilapia (Omitoyin et al., 2019), striped catfish (Nhu et al., 2020), and carp (Effendi et al., 2023). Carp is a freshwater fish that is widely cultivated because it has several advantages, including high nutritional and economic value (Abdel-Latif et al., 2020), and tolerance to salinity (Mubarik et al., 2019; Effendi et al., 2022). Intensive cultivation with high stocking densities can cause fish to be easily stressed, so they are easily infected with diseases such as *Motile Aeromonas Septicemia* (MAS).

According to Pratama et al. (2022), increasing stocking density will cause stress that induces high blood glucose levels, further disrupting growth and even causing death. Blood glucose levels are related to the health condition of fish, stressful conditions can reduce blood glucose levels (Hanafi et al., 2023). In addition, salinity is also a limiting factor in fish farming, as it affects osmoregulatory capacity which causes several physiological problems and fish health status (Saravanan et al., 2018). Therefore, this study aims to determine the effect of guava feeding on the blood glucose of carp reared in brackish water media.

MATERIALS AND METHOD

Time and Place

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

Method

The method used in this research is an experimental method using a Completely Randomised Design with 5 (five) treatments and 3 (three) replicates. The treatment in this study refers to the results of research by Effendi et al. (2023), the treatments are as follows:

P0 : Without the addition of guava leaves (control)
P1 : Addition of guava leaves at a dose of 1.0 g/100 g feed
P2 : Addition of guava leaves at a dose of 1.5 g/100 g feed
P3 : Addition of guava leaves at a dose of 2.0 g/100 g feed
P4 : Addition of guava leaves at a dose of 2.5 g/100 g feed

Fish rearing

Carp measuring 5.00 ± 1.00 cm with a weight of 4.00 ± 0.50 g were obtained from farmers in the Koto Panjang hydroelectric reservoir, Sumatra, Indonesia. The fish were adapted to brackish water media (salinity 5 ppt) for 7 days and fed daily with commercial feed. The rearing container used was a black tank with a diameter of 1 m and a water volume of 80 L, with a stocking density of 1 fish/4L of water. Feeding was carried out 3 times, namely at 08.00 AM, 01.00 PM, and 05.00 PM, with a dose of 5% of body weight. Fish rearing was carried out for 60 days.

Feed preparation

Preparation and manufacture of feed containing phytoimmunostimulants referred to Effendi et al. (2022). Thus, guava leaves used were obtained from plants that grow around the area of the Faculty of Fisheries and Marine, Universitas Riau. These leaves were first air-dried at room temperature (28-30^{o}C) for two days until the leaves were dry. Next, the dry leaves are crushed using a blender and sifted until you get guava leaf flour. Then the guava leaf flour is mixed into the commercial fish feed pellet Hi-Pro-Vite 781-2. This mixture is stirred until homogeneous and molded into fish feed using a pellet machine and air-dried until it becomes fish pellets.

Challenge test

The challenge test was carried out after 60 days of rearing. The bacteria used were obtained from the collection of the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The bacterial density used was 10^8 CFU/mL which infected the fish via an intramuscular injection method of 0.1 mL.

Blood glucose observation

Fish blood samples were taken 3 times, namely at the beginning (Day 1), day 60 (Day 60), and after the challenge test (Day 75). Blood collection is carried out by anesthetizing the fish first using cold temperatures (±8^o C) (Gul et al., 2012; Witeska et al., 2022). After that, blood was drawn from the caudal vein using a syringe (1 mL). Next, the blood was stored in an Eppendorf tube for observation of the blood glucose.

The glucose test kit used is the *GlucoDr* brand with a range of 20-600 mg/dL. Glucose testing is done in the morning before the fish is given feed. Fish blood obtained is dripped onto a strip that has been 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. The data were analyzed statistically using the SPSS version 26 application. The data were analysed using *One Way Anova* and homogeneity was observed. If the results of the analysis showed an effect, then further tests using Student Newman Keuls (SNK).

RESULT AND DISCUSSION

According to Singh et al. (2019), carp have tolerance to salinity up to 15 ppt. Carp rearing in brackish water media (5 ppt) produces fish with high protein content and better meat quality and indicates fish in a healthy condition. Based on this, the carp rearing in this study used a salinity of 5 ppt. When using salinities >5 ppt, it can cause increased osmoregulation which results in feed consumption and growth rates. According to Saravanan et al. (2018); Tang et al. (2020), hypersalinity can lead to increased osmoregulation as an effort to adapt fish to the stress caused, thus requiring high-energy fish and causing low consumption and growth rates of fish.

The blood glucose of carp during 60 treatments ranged from 52.33-86.67%, while the post-challenge test with *A. hydrophila* bacteria increased in several treatments, including P1 and P2, namely 63.33% and 108.33%. P0 could not be observed, because mortality reached 100% (Figure 1)

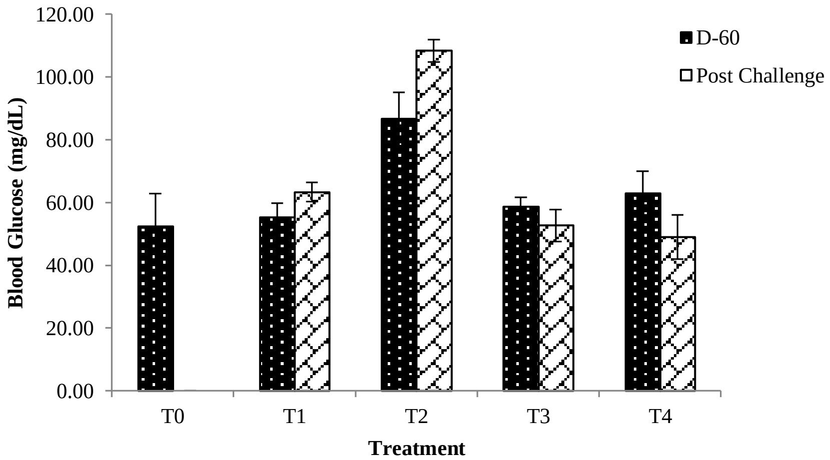

Figure 1. Carp blood glucose observation

Blood glucose levels of carp during rearing ranged from 49-108%, this range is still in the normal range. Normal carp glucose levels range from 36-90 mg/dL (Athanasopoulus et al. 2018); to 94-112 mg/dL (Kesbic et al., 2020). In general, the blood glucose concentration of carp during rearing indicates that the fish are in good condition.

The content of secondary metabolite compounds contained in guava leaves, such as flavonoids, maintains the blood glucose condition of fish. 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 has been shown to significantly reduce blood glucose levels in freshwater fish, with the mechanism of inhibiting glucose absorption in the intestine and adjusting its levels in fish blood (Nazeri et al., 2017).

Furthermore, the nutritional content of the feed has fulfilled the nutritional needs required by the fish. According to Hasan (2012), a lack of protein, fat, and carbohydrates can cause a decrease in blood glucose levels in fish, which will trigger the process of glycolysis and/or gluconeogenesis, namely breaking down liver glycogen into glucose or catabolizing protein and fat to maintain blood glucose levels in normal conditions.

The increase in carp blood glucose after the challenge test is due to the fish's immune response to infection with *A. hydrophila* bacteria. When stressed fish will need a lot of energy to survive so that it will stimulate the flow of glucose into the blood. Blood glucose is a description of the stress response as a result of 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).

In addition, the nutrients contained in the feed fulfill the nutritional needs of the fish. Increasing blood glucose values indicate that the flow of glucose into the blood is greater than the intake of blood glucose into cells. Blood glucose level is the result of a momentary balance between the rate of glucose absorption from the digestive tract into the bloodstream and the rate of blood glucose entry into cells in the process of carbohydrate metabolism. Excess glucose will be stored in the form of glycogen and the excess will be converted into triglycerides. High triglyceride levels are due to the endogenous synthesis of triglycerides from glucose (lipogenesis) resulting from the mobilization of liver glycogen and free fatty acids transported from adipose tissue to the liver (Arifin et al., 2016).

CONCLUSION

Based on the results of the study it can be concluded that the dose of 1.5 g/100g feed is the optimal dose for increasing the blood glucose of carp reared in brackish water media and infected with *A. hydrophila* bacteria.

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