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EFFECTS OF DIETARY SUPPLEMENTATION OF *Archidendron pauciflorum* PEEL ON BLOOD PROFILE OF *Pangasianodon hypophthalmus*

Pengaruh Pemberian Ekstrak Kulit Jengkol pada Pakan Terhadap Profil Darah Ikan Patin Siam (*Pangasius hypophthalmus*)

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ABSTRACT

Pathogenic bacteria are one of the main challenges in the cultivation of *Pangasianodon hypophthalmus* because they can cause organ damage and even death. The utilisation of natural ingredients such as *Archidendron pauciflorum* peel extract is one alternative to improve fish health in an environmentally friendly manner. This study aimed to evaluate the effect of adding *Archidendron pauciflorum* peel extract in feed on haematological parameters of *Pangasianodon hypophthalmus*. A total of 160 test fish were divided into four treatments with extract doses of 0% (control), 0.01%, 0.02%, and 0.03%, for 21 days. Parameters observed included total erythrocytes, haemoglobin levels, and haematocrit values. The results showed that *Archidendron pauciflorum* peel extract significantly increased haemoglobin levels and haematocrit values ($P<0.05$), especially at the 0.03% dose, compared to the control. Total erythrocytes showed a decrease on day 21 in all extract treatments, presumably due to the content of bioactive compounds (tannins, flavonoids, and saponins) in high doses. Bioactive compounds in *Archidendron pauciflorum* peel extract such as flavonoids, saponins, and tannins are known to play a role in improving haematological parameters, but their use needs to pay attention to the appropriate dose so as not to cause negative effects.

Keywords: *Archidendron pauciflorum* Peel Extract; Flavonoids; Haematology; Saponins; Tannins.

ABSTRAK

Bakteri patogen merupakan salah satu tantangan utama dalam budidaya ikan patin siam (*Pangasianodon hypophthalmus*) karena dapat menyebabkan kerusakan organ hingga kematian. Pemanfaatan bahan alami seperti ekstrak kulit jengkol (*Archidendron pauciflorum*) merupakan salah satu alternatif untuk meningkatkan kesehatan ikan secara ramah lingkungan. Penelitian ini bertujuan untuk mengevaluasi pengaruh penambahan ekstrak kulit jengkol dalam pakan terhadap parameter hematologi ikan patin siam. Sebanyak 160 ekor ikan uji dibagi ke

dalam empat perlakuan dengan dosis ekstrak 0% (kontrol), 0,01%, 0,02%, dan 0,03%, selama 21 hari. Parameter yang diamati meliputi total eritrosit, kadar hemoglobin, dan nilai hematokrit. Hasil penelitian menunjukkan bahwa pemberian ekstrak kulit jengkol meningkatkan kadar hemoglobin dan nilai hematokrit secara signifikan (P<0,05), terutama pada dosis 0,03%, dibandingkan dengan kontrol. Total eritrosit menunjukkan penurunan pada hari ke-21 pada semua perlakuan ekstrak, diduga akibat kandungan senyawa bioaktif (tanin, flavonoid, dan saponin) dalam dosis tinggi. Senyawa bioaktif dalam ekstrak kulit jengkol seperti flavonoid, saponin, dan tanin diketahui berperan dalam peningkatan parameter hematologi, namun penggunaannya perlu memperhatikan dosis yang sesuai sehingga tidak menimbulkan efek negatif.

Kata Kunci: Ekstrak Kulit Jengkol; Flavonoid; Hematologi; *Pangasianodon hypophthalmus*; Saponin; Tanin.

INTRODUCTION

Pathogenic bacteria pose a significant threat to the cultivation of Pangasianodon hypophthalmus. Several studies have identified the main pathogenic bacteria affecting this species. These pathogenic bacteria include Aeromonas hydrophila, Edwardsiella ictaluri, and Pseudomonas sp. (Parven et al., 2020; Abedin et al., 2021). These pathogen attacks are known to cause tissue necrosis, liver and kidney damage, and high mortality (Setiyaningsih et al., 2017).

The use of natural ingredients as feed additives in aquaculture has become a focus of research to improve fish health and productivity (Emeka et al., 2014). Natural ingredients are known to be used as alternatives for preventing and treating fish diseases (Ariyanti et al., 2022) and enhancing immune responses and growth (Pramono et al., 2023). The use of natural ingredients is an environmentally friendly and cost-effective alternative (Caipang et al., 2019). One potential ingredient is jengkol (Archidendron pauciflorum) skin extract, which is known to contain bioactive compounds such as flavonoids and tannins (Alfauzi et al., 2022) with antimicrobial and antioxidant properties. Research by Rosmawaty et al. (2016) showed that the use of jengkol skin extract in gourami (Osphronemus gouramy) feed can increase the fry's immunity against A. hydrophila bacterial infection.

Hematological profiles are important indicators in assessing fish health status, including erythrocyte count, hemoglobin levels, hematocrit values, and total leukocytes (Docan et al., 2018). Changes in hematological parameters can reflect the fish's immune response to infection or environmental stress (Satkar et al., 2024). A study by Tanjung et al. (2023) indicated that adding cinnamon bark (Cinnamomum burmannii) solution to the feed of Siamese catfish (Pangasianodon hypophthalmus) can improve the hematological profile and resistance to A. hydrophila infection.

Research on the specific effects of jengkol peel extract on the hematological profile of Siamese catfish is still limited. Based on the potential bioactive compounds in jengkol peel and positive results in other fish species, this study aims to explore the effect of adding jengkol peel extract to feed on the hematological profile of Siamese catfish. It is hoped that the results of this study can provide alternative strategies for improving the health and productivity of Siamese catfish cultivation through the use of locally available natural ingredients.

RESEARCH METHODS

Tools and Materials

The tools used in this study included a 1 ml syringe, a hemocytometer, a hematocrit tube, a pipette, a centrifuge, an Eppendorf tube, and a Sahli pipette. The materials used in this study included HCl, blood samples, and jengkol skin extract.

Place and Time

This research was conducted in November-December 2024 at the Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University, using 160 catfish (P. hypophthalmus) with a length of 12 cm and an average weight of ± 14.1 grams. The fish were kept for 21 days in a 30x50x50 cm fiber tank with a density of 25 fish per tank. The method used in this study was a Completely Randomized Design with four treatments and four replications. The treatments used included: Feeding without additional jengkol skin extract (P0), Feeding with the addition of 0.01% jengkol skin extract (P1), Feeding with the addition of 0.02% jengkol skin extract (P2), and Feeding with the addition of 0.03% jengkol skin extract (P3) (Shite et al., 2023).

Making jengkol skin extract

Jengkol skin extract is made using the maceration method. Approximately 1 kg of jengkol skin is washed thoroughly and then drained. The jengkol skin is then dried in the sun. Drying is carried out for several days, until the jengkol skin is completely dry and can be easily broken by hand. The dried jengkol skin is then made into powder (simplicia) by crushing it with a grinder, resulting in approximately 125 grams of simplicia. The simplicia is ready to be macerated with a ratio of 1:5 (one part simplicia in 5 parts solvent). It is macerated with 96% ethanol solvent until completely submerged for approximately 24 hours, then filtered with flannel cloth. The residue is macerated again in the same way, up to three times. The extract or filtrate from the maceration is collected together and evaporated to separate the solvent. Evaporation is carried out using a water bath until a thick jengkol skin extract is obtained (Dewi, 2010).

Feed Preparation Treatment

The feed used was floating pellets brand 781-1 with a protein content of 33%. Mixing jengkol skin extract in the feed by spraying. 400 grams of feed was divided into 100 grams each and then sprayed with a predetermined dose, namely P1 = (feed + 0.01% jengkol skin extract), P2 = (feed + 0.02% jengkol skin extract), P3 = (feed + 0.03% jengkol skin extract) (Shite et al., 2023). In the P0 (control) treatment, the feed was only sprayed with 50 mL of 96% ethanol without the addition of jengkol skin extract. The feed that had been sprayed with the extract was then allowed to dry.

Observation parameters

Total Erythrocytes

Total erythrocytes were counted using the method according to Blaxhall and Daisley (1973). Blood samples were drawn with a pipette graduated to 0.5, then Hayem's solution was drawn up to 101, shaken to mix homogeneously. The first drop was discarded, and the next drop was placed into the hemocytometer and covered with a coverslip. Counting was carried out on 5 small squares of the hemocytometer.

Hematocrit value

The hematocrit value is calculated using the Anderson (1993) method. Blood samples are drawn into a microhematocrit tube until it reaches ¾ of the tube. The end of the tube is closed with crytoseal to a depth of approximately 1 cm, forming a crytoseal or wax plug. The microhematocrit tube containing the blood is centrifuged at 3000 rpm for 5 minutes. Hematocrit levels are measured by comparing the volume of red blood cell solids to the total blood volume using the hematocrit scale.

$$\text{Hematocrit Level} = \frac{\text{Length of the volume of red blood cells that settle}}{\text{total length of blood volume in the tube}} \times 100\%$$

Hemoglobin Level

Hemoglobin levels were measured using the Wedemeyer and Yasutake (1977) method. A Sahli tube was filled with 0.1 N HCl solution up to 10 (the bottom scale line on the Sahliometer). The tube was placed between two tubes with standard colors. Fish blood was taken from an Eppendorf tube with a Sahli pipette in the amount of 0.02 mL. The tip of the pipette was cleaned, the blood was placed into the Sahli tube and left for 3 minutes. Aquadest was added with a dropper pipette little by little while stirring with a stirring glass until the color exactly matched the standard color. Hemoglobin levels were expressed in g/dL.

RESULT

Total Erythrocytes

Based on the observation results, it can be seen that the total erythrocytes of Siamese fighting fish (P. hypophthalmus) on the 7th, 14th, and 21st days showed results that were not significantly different in each treatment. The total erythrocytes in the 0.03% dose treatment gave the highest results on the 7th, 14th, and 21st days, but were not significantly different (P>0.05) from the 0.02% and 0.01% dose treatments. The total erythrocytes in all treatments ranged from 20.9 to 36.6 x 106 cells/mm3. The total erythrocytes of Siamese fighting fish (P. hypophthalmus) during subsequent observations are presented in Figure 1.

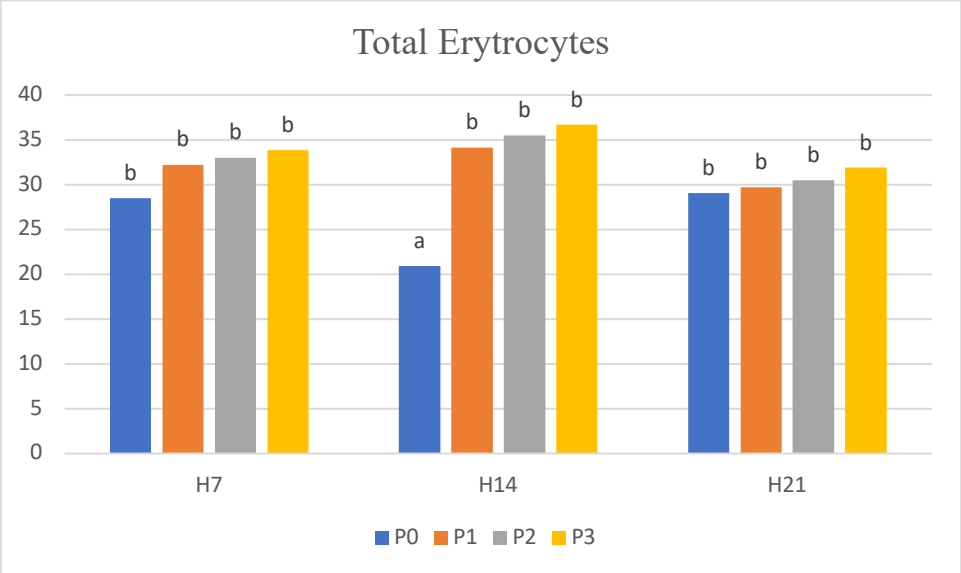

Figure 1. Graph of Total Erythrocytes During the Maintenance Period

Based on the observation results, it can be seen that the 0.03% dose treatment has the highest effect on the number of erythrocytes on the 7th, 14th and 21st day of maintenance. The erythrocyte value increases along with the increasing dose of treatment given. All treatments provide higher erythrocyte values compared to the control.

Hemoglobin levels

Based on the observation results, it can be seen that the 0.03% dose treatment gave the highest hemoglobin levels on days 7, 14, and 21 and was significantly different across all treatments (P<0.05). The treatment of jengkol skin extract showed higher hemoglobin levels compared to the control (without jengkol skin extract). The results showed that the hemoglobin levels in the study ranged from 7.3 to 12.4 g/dL. Hemoglobin levels during the subsequent study are presented in Figure 2.

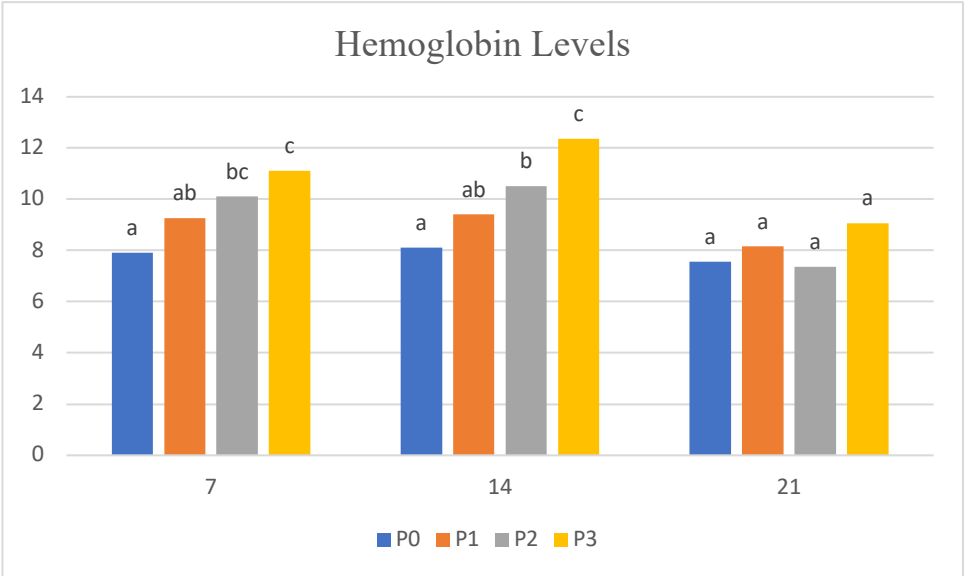

Figure 2. Graph of Hemoglobin Levels During the Maintenance Period

Observations showed that the 0.01%, 0.02%, and 0.03% doses produced higher hemoglobin values than the control. The increase in hemoglobin values was proportional to the increase in the dose given. Observations on the 21st day showed a different trend from the previous observations, where the 0.02% dose treatment produced lower hemoglobin values than the 0.01% dose treatment.

Hematocrit

Based on the observation results, it can be seen that the hematocrit value of the jengkol skin extract treatment was higher than the control (without jengkol skin extract) on the 7th and 14th day of observation. The highest hematocrit value during the treatment was produced at a dose of 0.03% compared to the control (not significantly different P>0.05). The results of the study showed that the hematocrit value of the study ranged from 26.3 - 39.1%. The subsequent hematocrit value can be seen in Figure 3.

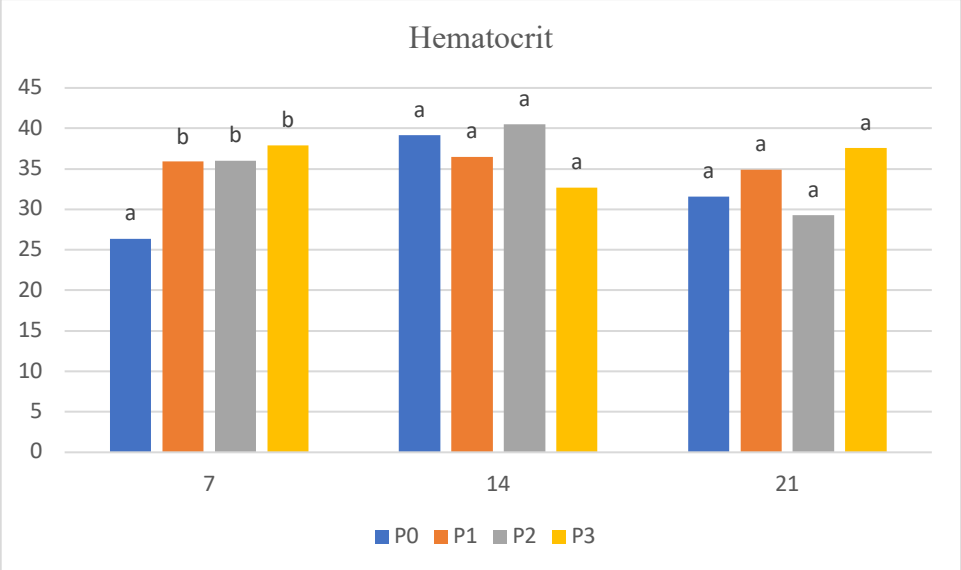

Figure 3. Hematocrit Value During Maintenance Period

Treatment with jengkol peel extract at different doses showed different hematocrit values. Increasing the treatment dose resulted in an increase in hematocrit values, except on day 21 of maintenance, where the 0.01% dose resulted in a higher hematocrit value than the 0.02% dose. At the end of the maintenance period, the 0.01% and 0.03% treatments produced higher hematocrit values than the control, while the 0.02% dose treatment produced a lower hematocrit value than the control.

DISCUSSION

Administration of jengkol peel extract treatment showed a decrease in total erythrocytes on day 21 (dose 0.1%; 0.2% and 0.3%). The decrease in total erythrocytes on the 21st day is thought to be the result of a response to the tannins contained in jengkol extract. The tannin, flavonoid and saponin content is known to support fish health because it has antimicrobial and antioxidant properties. According to Zizzalwa et al., 2020 and Ridwan et al., 2020, jengkol peel extract is known to contain flavonoids, saponins and tannins, where these bioactive compounds have an important role in increasing erythrocyte, hemoglobin and hematocrit levels, however the presence of these substances in high amounts can have a bad effect such as decreasing erythrocyte values.

Administration of jengkol peel extract showed an influence on hemoglobin levels during treatment. The hemoglobin level values at treatment doses of 0.01%, 0.02% and 0.03% were higher when compared to the control (without administration of jengkol peel extract). The 0.03% dose treatment showed the highest hemoglobin levels during the treatment. High hemoglobin levels indicate the blood's ability to transport oxygen. The increase in hemoglobin levels in treatments P1, P2, and P3 indicates that jengkol peel extract can be used to increase blood hemoglobin levels. According to Nugroho et al., 2016, flavonoids function as antioxidants that protect red blood cells from oxidative damage, thereby increasing blood hemoglobin levels. Research by Cerlina et al., (2020) showed that administering flavonoid-rich bay leaf extract to African catfish (Clarias gariepinus) infected with Aeromonas hydrophila significantly increased hemoglobin levels.

Administration of jengkol peel extract is known to increase hematocrit values. The increase in hematocrit values at doses of 0.01%, 0.02%, and 0.03% may be due to the active compounds contained in jengkol peel extract. Jengkol peel extract is known to contain flavonoids that can increase blood production. According to Caipang (2020) and Ahmadifar et

al., 2020, tannins, flavonoids, and saponins are known to support fish health due to their antimicrobial and antioxidant properties. These compounds exhibit antimicrobial and antioxidant properties that can improve health by enhancing the immune response (Rathod et al., 2021).

Saponins are immunostimulants that can stimulate red blood cell production. Ninggolan et al., (2021) reported that supplementing moringa leaf flour containing saponins in catfish (Clarias gariepinus) feed improved non-specific immune responses and fish growth performance. Saponins are known to be used as alternative ingredients to improve fish health and disease resistance (Elumalai et al., 2017). Saponins can boost immunity, reduce stress levels, and increase antioxidant activity in fish (Mehana et al., 2015).

The use of compounds containing tannins, flavonoids, and saponins in high amounts can have adverse effects on fish health. The presence of tannins in excessive doses can absorb iron in the blood, resulting in a decrease in the number of red blood cells. Giving tannin compounds in excessive doses can condense and cause oxidative damage to the liver and intestines of fish (Kang et al., 2023). Excessively high concentrations of saponins are suspected to cause blood hemolysis (Pasaribu et al., 2023). Overall, the optimal combination of saponins, tannins, and flavonoids from jengkol skin extract in feed can contribute positively to improving hematological parameters in fish, but the correct dosage and the needs of the fish species must be considered.

CONCLUSION

The results of this study indicate that the addition of jengkol peel extract (Archidendron pauciflorum) to the feed of Siamese catfish (P. hypophthalmus) significantly increased hematological parameters, particularly hemoglobin levels and hematocrit values, compared to the control. This increase is thought to be due to bioactive compounds such as flavonoids, saponins, and tannins, which have antimicrobial and antioxidant properties. However, the administration of jengkol peel extract at high doses tends to cause a decrease in total erythrocytes. This decrease in total erythrocytes is thought to be related to the side effects of excessive amounts of tannins. Therefore, this study confirms that the use of jengkol peel extract as a feed additive has the potential to improve the health of Siamese catfish, but optimal dosages need to be considered to avoid negative effects. The results of this study can serve as a reference for developing strategies for utilizing natural ingredients in more sustainable Siamese catfish cultivation.

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