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THE EFFECT OF ADDITION CINNAMON LEAF EXTRACT IN FEED ON LIVER FAT CONTENTS AND HEALTH OF CATFISH

Pengaruh Penambahan Ekstrak Daun Kayu Manis dalam Pakan Terhadap Kadar Lemak Hati dan Kesehatan Ikan Patin

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ABSTRACT

Cinnamon leaves contain active compounds such as polyphenols and flavonoids which have antioxidant properties and play a role in lipid metabolism processes. This active substance is known to reduce liver fat levels and has the potential to increase the immune response in catfish. This research aims to determine the effect of adding cinnamon leaf extract to feed on liver fat levels and the health of catfish. Cinnamon leaf extract was added to the feed at five different doses, namely 0 g/kg (control), 0.5 g/kg, 1 g/kg, 2 g/kg, and 4 g/kg feed. The results of the study showed that the addition of cinnamon leaf extract to the feed reduced the fat content in the liver of catfish as seen from the decrease in fat degeneration in the liver of catfish compared to the control. Total erythrocytes, hemoglobin levels and hematocrit levels of fish treated with the addition of cinnamon leaf extract to the feed were lower and significantly different ($P<0.05$) compared to controls, but total leukocytes were not significantly different between treatment and control.

Keywords: Blood, Catfish, Cinnamon, Liver

ABSTRAK

Daun kayu manis mengandung senyawa aktif seperti polifenol dan flavonoid yang memiliki sifat antioksidan dan berperan dalam proses metabolisme lipid. Zat aktif ini diketahui dapat menurunkan kadar lemak hati dan berpotensi meningkatkan respon imun pada ikan patin. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan ekstrak daun kayu manis dalam pakan terhadap kadar lemak hati dan kesehatan ikan patin. Ekstrak daun kayu manis ditambahkan ke dalam pakan dengan lima dosis berbeda, yaitu 0 g/kg (kontrol), 0,5 g/kg, 1 g/kg, 2 g/kg, dan 4 g/kg pakan. Hasil penelitian menunjukkan bahwa penambahan ekstrak daun kayu manis pada pakan menurunkan kadar lemak hati ikan patin yang terlihat dari semakin menurunnya degenerasi lemak di hati ikan patin dibandingkan kontrol. Total eritrosit, kadar

hemoglobin, dan kadar hematokrit ikan yang diberi perlakuan penambahan ekstrak daun kayu manis pada pakan lebih rendah dan berbeda nyata (P<0,05) dibandingkan kontrol namun untuk total leukosit tidak berbeda nyata antara perlakuan dan kontrol.

Kata Kunci: Darah, Hati, Kayu Manis, Patin

INTRODUCTION

Catfish (*Pangasianodon hypophthalmus*) is one of the aquaculture commodities that has high economic value, both in Indonesia and in the global market. Vietnam is the largest producer of catfish in the world, contributing part of global production. However, catfish exports from Indonesia are still limited, with the majority of production intended for the domestic market (Ramadhan et al., 2016). One of the challenges in catfish cultivation is the high fat content in the liver and meat, which can affect fish growth and the quality of the meat produced (Cheng et al., 2014). One alternative feed additive that can help reduce fat accumulation, increase growth, and improve the quality of catfish meat is the cinnamon plant.

Cinnamon (*Cinnamomum burmannii*) is one of the natural ingredients that has the potential to be used as a feed additive, especially the skin and leaves. Cinnamon plants, both leaves and skin, contain active compounds such as polyphenols and flavonoids which are antioxidants and play a role in lipid metabolism. Bioactive components in polyphenols have insulin-like activity (insulin mimetic), which plays a role in lipid metabolism in adipose tissue, liver, and meat (Kazeem et al., 2016; Mollazadeh et al., 2016).

Research by Setiawati et al., (2016) showed that the addition of cinnamon leaf extract in feed can reduce fat levels in meat and reduce cholesterol and triglycerides in catfish. Rolin et al., (2015) also showed that the use of cinnamon leaf extract in feed can increase protein digestibility and retention in catfish, thereby increasing the efficiency of nutrient utilization and supporting better fish growth. Other studies have also confirmed the effectiveness of cinnamon in improving the quality of catfish meat (Laheng et al., 2016; Tartila et al., 2023) and fish growth (Dedi et al., 2016 in carp; Amer et al., 2018 in tilapia; Zhou et al., 2020 in grass carp; Ravardshiri et al., 2021 in rainbow trout). In addition, cinnamon leaves are known to have the potential to increase the immune response of fish, as reported by Safratilofa et al. (2015) in catfish and Abdel-Tawwab et al. (2018) in tilapia infected with *Aeromonas hydrophila*, and Lestari et al. (2018) in tilapia infected with *Streptococcus agalactiae*. Recent studies have shown that cinnamon bark has antibacterial and immunostimulant properties that can strengthen non-specific immune responses in jambal siam fish (Tanjung et al., 2023).

Studies on the effect of adding cinnamon leaf extract on liver fat levels and catfish health are still needed. Liver fat levels are known through liver histology observations, and fish health is known through blood picture observations. Liver histology can provide important information about the condition of the organ, such as fatty degeneration/fat accumulation. Meanwhile, blood picture parameters, such as hemoglobin levels, hematocrit, total leukocytes, and total erythrocytes play a role in assessing the health status and immune response of fish. This study aims to evaluate the effect of adding cinnamon leaf extract to feed on liver fat levels and catfish health. It is hoped that the addition of this extract can reduce liver fat levels and improve catfish health, thus impacting better fish growth and quality.

METHODS

Tools and Materials

This research was conducted at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The tools used were: aquarium, scales, fish dissection tools, microscope, scale pipette, haemocytometer, cover glass, Sahlinometer tube, Eppendorf tube, Sahli pipette, dropper

pipette, microhematocrit, crytoceal, centrifuge, while the materials used were: catfish seeds, commercial feed, cinnamon leaf extract, 10% Neutral Buffer Formalin (BNF) solution, hematoxylin-eosin dye, Hayem's solution, Turk's solution, 0.1 N HCI solution, and distilled water.

Research Design

This research used a completely randomized design (CRD) with five treatments and three replications. The treatment in this study refers to the research of Setiawati et al., (2016), namely: control (without the addition of cinnamon leaf extract), the addition of cinnamon leaf extract (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed). The cinnamon leaf extract used was obtained from the Experimental Garden of the Medicinal and Aromatic Plants Research Institute (BALITTRO) in Cimanggu, Bogor.

Research Procedure

1. Preparation of Test Feed and Maintenance

The preparation of test feed and maintenance was carried out referring to the research of Setiawati et al., (2016), namely by using commercial feed that has a protein content of 31%. The test fish used were catfish seeds (*Pangasianodon hypophthalmus*) measuring 3 inches and an average weight of 7.43 ± 0.01 g. Maintenance was carried out in 15 aquariums measuring 100 × 40 × 50 cm^3 with a water volume of 160 L, with a density of 30 fish per aquarium. The study lasted for 60 days, with feeding three times a day at 08.00, 12.00, and 16.00 WIB. At the end of the maintenance, 4 fish from each replication were taken for liver histology testing and fish blood examination.

2. Liver Histology Observation

Histology observation was carried out to observe the fatty degeneration found in the catfish liver. The liver was fixed in 10% Neutral Buffer Formalin (BNF) solution. After the fixation process, histology preparations were made using the Mayer Bennett hematoxylin-eosin phloxine staining method (Bell & Lighner, 1988). Observations were made under a microscope with a magnification of 400 times.

3. Blood Picture Observation

• Total Erythrocytes

Blood samples were taken using a pipette containing red stirring beads until reaching a scale of 0.5, then Hayem's solution was added to a scale of 101. The mixture of blood and Hayem's solution was homogenized by shaking the pipette for 3-5 minutes. The first two drops of solution were discarded, then the remaining solution was dripped onto a hemocytometer that had been covered with a cover glass. Red blood cell counting was carried out using a microscope with a magnification of 400x in five small boxes of the hemocytometer. The total number of erythrocytes was calculated using the Blaxhall & Daisley method (1973) with the formula:

$$\sum \text{erythrocytes} = \text{average } \sum \text{counted cells} \times \frac{1}{\text{large box volume}} \times \text{thinner}$$

• Total Leukocytes

Blood was taken using a white bead pipette up to a scale of 0.5, then diluted with Turk's solution until it reached a maximum scale of 11. Both ends of the pipette were closed parallel, then shaken for 3-5 minutes so that the blood and Turk's solution were mixed evenly. The first drop of solution was discarded, while the next drop was dropped on a haemocytometer that had been covered with a glass object on the concave part. Leukocyte counting was carried out under a microscope with a magnification of 400x in four large boxes of the haemocytometer. The total number of leukocytes was calculated using the Blaxhall & Daisley method (1973) with the formula:

$$\sum \text{leukocytes} = \text{average } \sum \text{counted cells x} \frac{1}{\text{large box volume}} \text{x thinner}$$

• Hemoglobin Level

Hemoglobin levels were measured using the Sahli method (Wedemeyer & Yasutake, 1977). The Sahlinometer tube was filled with 0.1 N HCl solution to the bottom scale line, then placed between two tubes with standard colors. Fish blood as much as 0.02 ml was taken from the Eppendorf tube using a Sahli pipette, then put into the Sahli tube, and left for three minutes after the tip of the pipette was cleaned. Furthermore, distilled water was added gradually using a dropper while stirring until the color of the solution matched the standard color. Hemoglobin levels were expressed as a percentage (%).

• Hematocrit Level

Blood was taken using a capillary tube (microhematocrit) until it reached ¾ of the length of the tube, then one end of the tube was closed with crytoceal. The microhematocrit tube containing blood was centrifuged at 3000 rpm for 5 minutes until blood sediment formed. The hematocrit level was calculated by comparing the length of the blood sediment (a) with the total length of the blood in the tube (b). Hematocrit levels are calculated based on the Anderson & Siwicki (1993) method using the following formula:

$$\text{Hematocrit Level (\%)} = \frac{a}{b} x\ 100$$

Data Analysis

The histological data of the liver organ were analyzed descriptively by observing cells undergoing fatty degeneration. The blood picture data were tabulated and analyzed using ANOVA with the SPSS program. If there was a significant difference between treatments, further testing was carried out using the Duncan test.

RESULTS

Liver Histology

Figure 1 shows a histological image of the catfish liver under a microscope with a magnification of 400x. In the control treatment (without the addition of cinnamon leaf extract), a lot of fatty degeneration was found in the liver, while in the treatment of adding cinnamon leaf extract (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg of feed) it was seen that the higher the dose of cinnamon leaf extract added to the feed, the less fatty degeneration was found in the liver.

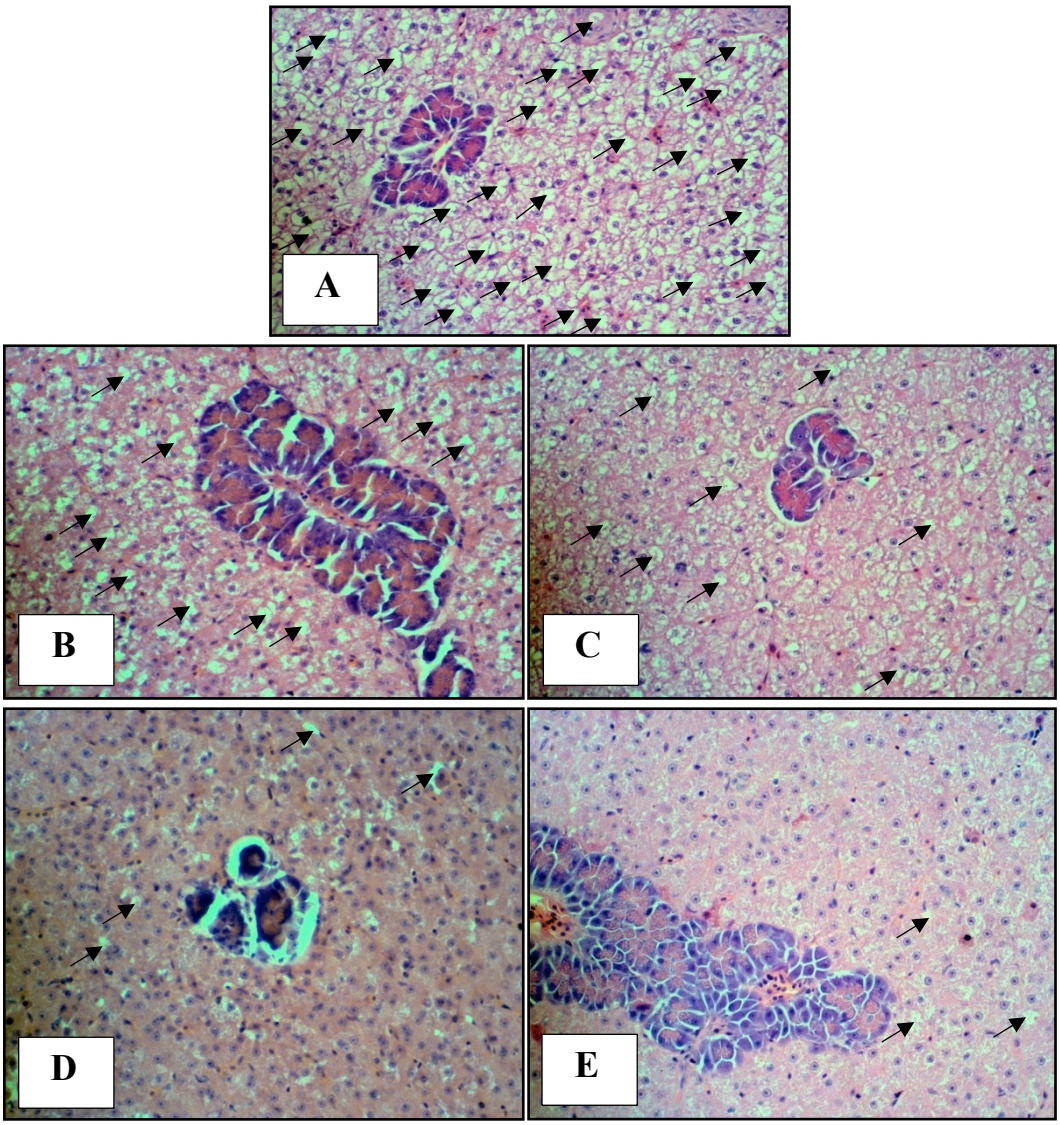

Figure 1. Histology of the Liver Organ of Catfish After Being Given Treatment Feed for 60 Days (Magnification 400x) (A) Control (Without Adding Cinnamon Leaf Extract, (B) Adding Cinnamon Leaf Extract 0.5 g/kg Feed, (C) Adding Cinnamon Leaf Extract 1 g/kg Feed, (D) Adding Cinnamon Leaf Extract 2 g/kg Feed, and (E) Adding Cinnamon Leaf Extract 4 g/kg Feed.

Blood Picture

The blood picture (total erythrocytes, total leukocytes, hemoglobin levels and hematocrit levels) of catfish during maintenance is shown in Table 1.

Table 1. Blood Picture of Catfish After Being Given Maintenance Treatment for 60 Days

Test Parameters	Treatment				
	K (0 g/kg)	P1(0.5 g/kg)	P2 (1 g/kg)	P3 (2 g/kg)	P4 (4 g/kg)
Total Erythrocytes (x 10^6 cells/mm^3)	2.39±0.38 ^{a}	1.78±0.05 ^{b}	1.85±0.05 ^{b}	1.87±0.12 ^{b}	1.40±0.02 ^{c}
Total Leukocytes (x 10^5 cells/mm^3)	8.59±0.54 ^{a}	8.45±0.31 ^{ab}	7.86±0.42 ^{ab}	7.57±0.48 ^{ab}	8.59±0.34 ^{a}

Test Parameters	Treatment				
	K (0 g/kg)	P1(0.5 g/kg)	P2 (1 g/kg)	P3 (2 g/kg)	P4 (4 g/kg)
Hb (g %)	11.60±0.40 [a]	7.10±1.10 [bc]	7.53±0.31 [bc]	8.13±0.42 [b]	6.80±0.20 [c]
Ht (%)	39.26±0.36[a]	37.54±1.69 [ab]	36.00±0.00 [bc]	34.95±0.90 [c]	31.21±1.03 [d]

The results showed that after 60 days of maintenance there was no significant difference (P>0.05) in the total leukocyte parameters in both the control treatment (without the addition of cinnamon leaf extract) and the cinnamon leaf extract addition treatment (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed). The highest total erythrocyte parameters, hemoglobin levels, hematocrit levels were found in the control treatment, which were 2.39±0.38 x 10^6 cells/mm^3; 11.60 g %; 39.26±0.36% and were significantly different from the cinnamon leaf extract addition treatment (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed). The cinnamon leaf extract addition treatment with a dose of 4 g/kg feed had the lowest total erythrocytes, hemoglobin levels, and hematocrit levels compared to the control and other extract addition treatments.

DISCUSSION

The liver is a vital organ that plays an important role in the metabolism of various substances in the body, including as a place to store fat and various other toxic compounds (Huda et al., 2017). Liver histology was carried out to determine the condition of the catfish liver with or without the addition of cinnamon leaf extract (control). The results of observations under a microscope with a magnification of 400x showed that in the control there was a lot of fatty degeneration in the liver, while in the treatment (addition of cinnamon leaf extract 0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed) it was seen that the higher the dose of cinnamon leaf extract added to the feed, the less fatty degeneration was found in the liver.

Fatty degeneration is a condition in which fat accumulates in the cytoplasm of cells (Mudiana et al., 2023). This condition generally occurs in parenchymatous cells, especially in the liver, renal tubules, and heart (Berata et al., 2020). Microscopically, this fatty degeneration looks like an empty sphere that does not absorb Hematoxylin-Eosin (HE) dye, characterized by the appearance of vacuoles of varying sizes, and in some cases, fat in the cytoplasm can push the nucleus to the edge (Adikara et al., 2013). Fatty degeneration can be caused by exposure to toxic substances, nutritional disorders, and the aging process (Fahmi et al., 2015). In addition, fatty degeneration can also occur due to disorders of fat metabolism, such as mitochondrial dysfunction or hypoxia which inhibits fat oxidation in cells (Sijid et al., 2020).

The results of this study are in line with the research of Setiawati et al., (2016) which showed a decrease in fat levels in the liver of catfish along with the increasing dose of cinnamon leaf extract added to the feed. It is suspected that the bioactive components in cinnamon leaf extract which have insulin-like activity (insulin mimetic) work effectively in the liver. Fish treated with cinnamon leaf extract (0.5–4 g/kg feed) showed a lower hepatosomatic index (HSI) compared to the control group (P<0.05). A low HSI value indicates minimal fat accumulation in the liver, because most of the fat has been utilized as an energy source (Craig et al., 2006; Ighwela et al., 2014). The results of Wahyudi et al., (2023) also showed something similar, namely the control treatment had more lipid droplets than other treatments.

Based on the results of the blood picture test of catfish after 60 days of maintenance, it was found that there was no significant difference (P>0.05) in the total leukocyte parameters in both the control treatment and the cinnamon leaf extract addition treatment (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed). The highest total erythrocyte parameters, hemoglobin levels, and hematocrit levels were found in the control treatment, which was 2.39±0.38 x 10^6 cells/mm^3; 11.60 g %; 39.26±0.36% and significantly different from the treatment of cinnamon leaf extract addition (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed). The treatment of cinnamon leaf extract addition

with a dose of 4 g/kg feed had the lowest total erythrocytes, hemoglobin levels, and hematocrit levels compared to the control or other extract dose addition treatments.

The low total erythrocytes and hemoglobin levels in the cinnamon leaf extract treatment (0.5 g/kg: 1 g/kg; 2 g/kg; 4 g/kg feed) compared to the control are thought to be caused by the high tannin and saponin content in the cinnamon leaf extract (Rosmawaty et al., 2016). The higher the dose of cinnamon leaf extract added to the feed, the more toxic it is to fish. Saponin compounds are thought to be able to lyse red blood cells, resulting in a decrease in total erythrocytes. This is in line with research by Ubaidillah et al., (2018), which states that the higher the dose of cinnamon leaf powder, the lower the survival rate of tilapia. The high tannin and saponin content in cinnamon leaves can produce toxic substances that inhibit fish growth and are at risk of causing death. Lysed erythrocytes experience damage to their membranes and hemoglobin, which causes a decrease in hemoglobin levels (Safratilofa et al., 2015). Hemoglobin plays a role in binding oxygen needed in the catabolism process to produce energy. The ability of blood to bind oxygen depends on the amount of hemoglobin in red blood cells. A decrease in hemoglobin levels due to the addition of cinnamon leaf extract causes inhibition of metabolism and reduced energy production, which has an impact on slow fish growth. In addition, a decrease in the number of erythrocytes reduces the supply of nutrients to cells, tissues, and organs, thus further inhibiting the metabolic process (Harikrishnan et al. 2010).

Hematocrit levels are directly related to hemoglobin levels, so a decrease in hematocrit will be followed by a decrease in hemoglobin, and vice versa. A decrease in hematocrit levels in catfish fed with additional cinnamon leaf extract causes metabolism to be inhibited, so that growth will be lower compared to the control. This is in accordance with the results of research by Rolin et al., (2015) in the treatment of adding cinnamon leaf extract at a dose of 4 g/kg of feed, the growth rate of fish decreased significantly ($P < 0.05$). This decrease is thought to be caused by the presence of antinutrients in the feed. The presence of flavonoids, tannins, and calcium oxalate in cinnamon leaves can inhibit the absorption of nutrients important for growth. Tannins can interfere with digestion by binding to digestive enzymes or forming complexes with feed components such as proteins and minerals (Liener 1989 in NRC 2011). Similarly, calcium oxalate can affect the utilization of minerals in the body (Francis et al., 2001), thus impacting on decreasing fish growth.

CONCLUSION

The addition of cinnamon leaf extract to the feed reduced the level of catfish liver fat as seen from the decrease in fatty degeneration in the catfish liver compared to the control. The higher the dose of cinnamon leaf extract added to the feed, the less fatty degeneration was found in the liver organ. The total erythrocytes, hemoglobin levels, and hematocrit levels of treated fish were lower and significantly different ($P < 0.05$) compared to the control, but the total leukocytes were not significantly different between the treatment and control. This is thought to be due to the high tannin and saponin content in cinnamon leaf extract. The higher the dose of cinnamon leaf extract added to the feed, the more toxic it will be to fish.

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