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Physiological and Biochemical Responses in the Blood of Striped Catfish (*Pangasianodon hypophthalmus*) to the Addition of Papaya Seed Extract (*Carica papaya* L.) to Feed

Respons Fisiologis dan Biokimia Darah Ikan Jambal Siam (Pangasianodon hypophthalmus) dengan Penambahan Ekstrak Biji Pepaya (Carica papaya L.) pada Pakan

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Abstract

Striped catfish (*Pangasianodon hypopthalmus*) is a group of catfish that has become one of the leading freshwater commodities. This gives striped catfish (*P. hypopthalmus*) a fairly good market potential. A frequent constraint in fish farming is the risk of disease outbreaks. One factor contributing to the failure of fish farming and resulting in significant mortality is disease. One pathogenic bacterium responsible for disease is *Aeromonas hydrophila*, which is often found in freshwater and causes 80-100% of fish mortality in a short period. The method used is an experimental method employing a Completely Randomized Design (CRD) with one factor, 5 treatment levels, and 3 replications. The treatments used in this study refer to. The study on the addition of papaya seed extract (*Carica papaya* L.) to the feed found that it affects the physiological and biochemical responses of striped catfish (*P. hypophthalmus*). The best feeding dosage with the addition of papaya seed extract of 3.0 mL/kg of feed results in glucose levels of 70.33 mg/dL, calcium 12.53 mg/dL, magnesium 2.73 mg/dL, phosphorus 8.66 mg/dL, lysozyme activity 275 units/mL, absolute weight gain 14.92g, absolute length gains 5.25cm, and survival rate reaching 93.33%. The researcher recommends conducting further studies on the addition of papaya seed extract at a dosage of 3.0 mL/kg of feed to improve growth in different test fish and advises against using papaya seed extract in high doses.

Keywords: *Aeromonas hydrophila*, Physiological response, Blood biochemistry

Abstrak

Ikan jambal siam (*Pangasianodon hypopthalmus*) merupakan kelompok ikan jambal yang telah menjadi salah satu komoditas air tawar terkemuka. Hal ini memberikan potensi pasar yang cukup baik bagi ikan jambal siam (*P. hypopthalmus*). Salah satu kendala umum dalam budidaya ikan adalah wabah penyakit. Salah satu faktor penyebab kegagalan budidaya ikan yang menyebabkan kematian massal adalah infeksi penyakit. Salah satu bakteri patogen yang bertanggung jawab atas penyakit adalah *Aeromonas hydrophila*, yang sering ditemukan di air tawar dan bertanggung jawab atas 80-100% tingkat kematian ikan dalam waktu singkat. Metode yang digunakan adalah metode eksperimental dengan Desain Acak Lengkap (CRD) dengan satu faktor, 5 tingkat perlakuan, dan 3 ulangan. Perlakuan yang digunakan dalam studi ini merujuk pada. Hasil penelitian tentang penambahan ekstrak biji pepaya (*Carica pepaya L.*) dalam pakan mempengaruhi respons fisiologis dan biokimia ikan jambal siam (*P. hypophthalmus*). Dosis pakan terbaik dengan penambahan ekstrak biji pepaya sebesar 3,0 mL/kg pakan menghasilkan kadar glukosa 70,33 mg/dL, kalsium 12,53 mg/dL, magnesium 2,73 mg/dL, fosfor 8,66 mg/dL, aktivitas lisozim 275 unit/mL, penambahan berat absolut 14,92 g, penambahan panjang absolut 5,25 cm, dan tingkat kelangsungan hidup mencapai 93,33%. Peneliti merekomendasikan untuk melakukan penelitian lebih lanjut mengenai penambahan ekstrak biji pepaya dengan dosis 3,0 mL/kg pakan untuk meningkatkan pertumbuhan pada ikan uji yang berbeda dan menyarankan untuk tidak menggunakan ekstrak biji pepaya dalam dosis tinggi karena bersifat toksik bagi ikan.

Kata kunci: *Aeromonas hydrophila*, Respons fisiologis, Biokimia darah

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is one of the leading freshwater commodities, and its production in Indonesia has experienced rapid growth. Initially, this fish was produced only from wild catches, but it is now widely cultivated in aquaculture systems. This development is evident in the increase in breeding, rearing, processing, and utilization of large aquaculture areas, enabling it to meet domestic consumption needs and providing good market opportunities (Tamba et al., 2021).

However, the main obstacle in fish farming is disease outbreaks, especially those caused by pathogenic bacteria. One of the bacteria that often attacks freshwater fish is *Aeromonas hydrophila*, the cause of *Motile Aeromonas Septicemia* (MAS), which can cause mortality rates of 80–100% within a short period of time (Maryani et al., 2020). Cases of pathogen attacks have been reported in Koto Masjid Village, XIII Koto Kampar, Riau, with the discovery of *Aeromonas* sp, *Edwardsiella* sp, and *Pseudomonas* sp in catfish. *Aeromonas* sp. was identified in all ponds, making it opportunistic and a serious threat to the success of aquaculture (Tamba et al., 2021). Until now, fish farmers have often relied on antibiotics to treat fish diseases. However, long-term use of antibiotics at inappropriate doses can have negative impacts, including water pollution and bacterial resistance (Syawal et al., 2019). Therefore, the use of herbal ingredients is a more environmentally friendly alternative for improving fish growth, health, and immune function. One plant with potential for use is papaya (*Carica papaya* L.), which grows easily in various regions of Indonesia.

Papaya seeds contain active compounds, including alkaloids, flavonoids, saponins, tannins, and triterpenoids, with antimicrobial and antibacterial properties (Yonarta et al., 2022). These compounds can inhibit the growth of pathogenic bacteria and increase the immune system of fish. Thus, papaya seeds have the potential to be used as a natural supplement in feed to prevent disease and reduce dependence on antibiotics in freshwater fish farming. The objective of this study was to determine the optimal dose of papaya seed extract solution added to feed to prevent Striped catfish carp from being infected by *A. hydrophila*, as assessed by the physiological and biochemical responses in fish blood.

2. Material and Method

2.1. Time and Place

This research was conducted from June to December 2024. Observations of physiological responses, including blood glucose levels, growth, and fish survival, were conducted at the Parasite and Fish Disease Laboratory of the Faculty of Fisheries and Marine Sciences at the University of Riau. In contrast, biochemical blood analyses for calcium, magnesium, phosphorus, and lysozyme were performed at the Balivet Laboratory in Bukittinggi.

2.2. Methods

The method used was a completely randomized design (CRD) with one factor, five treatment levels, and three replicates. The treatments applied were as follows:

Kn : Feeding without papaya seed extract and without testing against *A. hydrophila* (negative control)
Kp : Feeding without papaya seed extract and challenged with *A. hydrophila* (positive control)
P1 : Feeding with papaya seed extract at 1.0 g/1 kg feed and challenged with *A. hydrophila*
P2 : Feeding containing papaya seed extract 2.0 g/1 kg feed and tested against *A. hydrophila*
P3 : Feeding containing papaya seed extract 3.0 g/1 kg feed and tested against *A. hydrophila*

2.3. Procedures

2.3.1. Production of Papaya Seed Extract

Ripe papaya fruit is harvested for its seeds, which are dried by sun-drying under a parasol cover for 3–4 days until completely dry, yielding approximately 180 g of dry seeds from 1 kg of wet seeds. The dried seeds are then ground into a powder using a blender, and 500 g of the powder is macerated in a dark glass jar with 1,500 ml of 96% ethanol at room temperature for 3 days, with periodic stirring (Wantah et al., 2018). After the maceration process, the mixture is filtered through cotton and a glass funnel, and the filtrate is then evaporated in a rotary evaporator at 50°C to produce 50 g of papaya seed extract.

2.3.2. Feed Preparation

The test feed used in the study was PF 800 floating feed, a commercial feed with a protein content of 45%. Papaya seed extract was added to the feed by dissolving 200 mL of the extract in distilled water for each treatment dose, then stirring until homogeneous. Next, the dissolved extract was sprayed evenly onto 1 kg of pellet feed and left for ± 10 minutes until the extract water was completely absorbed into the feed, then air-dried (Syawal et al., 2019).

2.3.3. Provision of Aeromonas hydrophila isolates

The bacterium *Aeromonas hydrophila* is one of several bacterial collections belonging to the Parasite and Fish Disease Laboratory of the Faculty of Fisheries and Marine, Universitas Riau. Isolates of *A. hydrophila* were cultured on TSA medium and incubated in an incubator for 18-24 hours at 28-30 °C. After the bacterial colonies grew, they were separated using a Pasteur pipette and cultured on GSP medium, then incubated in an incubator for 18-24 hours at 28-30 °C. If the GSP changed color to yellow and physical and biochemical tests showed that the bacteria were *A. hydrophila*, the bacteria were recultured on TSA medium.

2.3.4. Challenge Test Aeromonas hydrophila

The Striped catfish fighting fish challenge test was conducted on day 32 with *A. hydrophila.* Before the challenge test, the fish were anesthetized with clove oil at a dose of 0.1 mL/L to render them unconscious. The fish were infected with *A. hydrophila* at a density of CFU/mL by intramuscular injection of 0.1 mL into the back. After the injection, the fish were kept for 14 days to observe clinical symptoms.

2.3.5. Fish Blood Collection

Six test fish from each treatment were anesthetized with clove oil at 0.1 mL/L for approximately 5 minutes before blood sampling. After that, blood was collected from the fish using a 1 mL syringe without an anticoagulant. Blood was collected from the caudal vein, then the blood in the syringe was placed in a microtube and centrifuged at 3000 rpm for 10 minutes. After that, the blood serum was taken and transferred to a 10 mL microtube using a micropipette, and 1 mL of AS FS calcium kit, XL FS magnesium kit, and FS REF phosphorus kit were added. Blood sampling from the test fish was performed three times: before treatment, after 30 days of maintenance, and 14 days after the *A. hydrophila* challenge test.

2.4. Measured Parameters

2.4.1. Blood Biochemistry

Blood biochemical tests were conducted at the Bukittinggi Veterinary Laboratory (calcium, magnesium, phosphorus), and lysozyme tests at the Fish Health Laboratory, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. These tests used serum obtained from the test fish. Fish blood was collected using a 1 mL syringe without an anticoagulant, then placed in a microtube and centrifuged at 3000 rpm for 10 minutes. The serum was collected and transferred to a microtube, then stored at -20 °C. The serum packaging process before shipment via courier involved placing the microtubes containing serum into ziplock bags, which were then labeled according to the treatment. The ziplock bags containing serum were placed into Styrofoam boxes filled with dry ice to keep the serum frozen.

2.4.2. Serum Calcium

Fish blood was collected from the caudal vein using a 1 mL syringe, then placed in a microtube and centrifuged at 3000 rpm for 10 minutes. The serum was transferred to a reaction tube using a micropipette (10 µm), and 1 mL of Calcium AS FS kit was added. Serum calcium was measured using a spectrophotometer.

2.4.3. Magnesium Serum

Fish blood was collected from the caudal vein using a 1 mL syringe, then placed in a microtube and centrifuged at 3000 rpm for 10 minutes. After that, the serum was collected and transferred to a microtube using a micropipette (10 µm), and 1 mL of the Magnesium XL FS kit was added. Serum magnesium was measured using a spectrophotometer.

2.4.4. Phosphorus

Fish blood was collected from the caudal vein using a 1 mL syringe, then placed in a microtube and centrifuged at 3000 rpm for 10 minutes. After that, the serum was transferred to a microtube using a micropipette (10 µm), and 1 mL of the Fosfor FS REF kit was added. The phosphorus content was analyzed using a spectrophotometer.

2.4.5. Lysozyme

The method used for lysozyme testing is as follows: in the first stage, prepare an agar medium containing 15 mL of NaH_2PO_4 and $N_{a2}HPO_4$ as a buffer, add 1% agarose gel, then add 50 µg/mL of *Micrococcus leuteus*. After that, spread the medium evenly on a glass object. Three 6 mm diameter holes are made in the agar medium on the glass slide using a pipette. Fifteen mL of blood plasma is placed in one of the holes, while another hole is filled with 15 µL of chicken egg white lysozyme (Sigma) as a positive control, and another hole is filled with lysozyme buffer (Sigma) as a negative control. The glass object was left at room temperature for 10 minutes, then incubated at 25 °C for 17 hours. Lysozyme activity was observed by measuring the diameter of the inhibition zone formed (Zainun, 2017).

2.5. Data Analysis

Data on clinical symptoms observed in fish were analyzed descriptively. Meanwhile, research data, including blood glucose, serum calcium, serum magnesium, phosphorus, lysozyme, absolute weight and length growth, survival rate, and water quality, were tabulated in tables or graphs. The research data were then analyzed using the SPSS application, including Analysis of Variance (ANOVA) if the treatment showed a significant difference ($P<0.05$), a Newman-Keuls follow-up test was performed to determine differences between treatments (Sutisna, 2020).

3. Result and Discussion

3.1. Clinical Symptoms of Striped Catfish

Clinical symptoms of the striped catfish were observed during 30 days of maintenance and 14 days after the challenge test. The clinical symptoms observed included the fish's behavior during maintenance, such as appetite, movement, body color, belly shape, fins, and eyes. Changes in the clinical symptoms of Striped catfish after the challenge test are shown in Table 1.

Table 1. Clinical Symptoms of Post-challenge Striped Catfish

	Clinical Symptoms				
Treatment	Appetite	Movement	Eye	Body surface	Fin
Kn	Normal	Active	Normal	Bright color, normal mucus	Normal
Kp	Decreasing	Passive	*Exopthalmia*	Pale color, excessive mucus, and ulcers on the skin surface, and an enlarged abdomen	Anal fin irritation and bleeding
P1	Decreasing	Passive	*Exopthalmia*	Pale color, excessive mucus, sores on the skin surface, and an enlarged abdomen	Anal fin irritation and bleeding
P2	Decreasing	Active	*Exopthalmia*	Pale color, normal mucus	Anal fin irritation and bleeding
P3	Normal	Active	Normal	Bright color, normal mucus	Normal

Table 1 shows that the negative control (Kn) did not show any clinical symptoms. This is because the Striped catfish in Kn was not tested with *A. hydrophila*. After testing, the Striped catfish showed clinical symptoms in the positive control treatment (Kp). The clinical symptoms in the Kp treatment group included paler-colored Striped catfish, excessive mucus production, body surface ulcers, exophthalmos (protruding eyes), an enlarged or bloated abdomen, and fin rot on the tail and abdominal fins. The clinical symptoms after the challenge test are shown in Figure 1.

Post-challenge observation results for *Aeromonas hydrophila* in Striped catfish showed clinical symptoms of excessive mucus production, body ulcers, exophthalmia, and fin erosion in treatments Kp, P1, and P2. These symptoms are consistent with the reports by Rosidah et al. (2018); Tamba et al. (2021) that fish infected with *A. hydrophila* generally experience exophthalmia, dropsy, hemorrhage, and fin damage. The severity of symptoms in the P1 treatment was higher than in P2, presumably because the dose of papaya seed extract administered was lower, making it less effective in inhibiting the bacteria. Conversely, in P3, the higher dose suppressed the development of clinical symptoms.

Clinical symptoms in the form of bleeding (hemorrhage) occur due to the activity of the hemolysin enzyme produced by *A. hydrophila*. This enzyme breaks down erythrocyte cells, causing hemoglobin to leak out and produce red spots on the fish's body. Rizvi et al. (2025) stated that hemolysin dissolved in the blood can lyse erythrocytes and release hemoglobin, causing blood to leak through wounds and exacerbating hemorrhage. In addition, ulcers or wounds were found at the site of intramuscular injection. Ulcers were caused by high bacterial density in the infected area, leading to increased toxin production and accelerating tissue damage.

The pathogen *A. hydrophila* produces extracellular enzymes, including hemolysin, protease, and elastase, which contribute to tissue damage. These enzymes can trigger inflammation and ulceration on the surface of the fish's body. According to Tamba et al. (2021), ulcers form as a result of bacterial proteolytic activity, in which extracellular products such as proteases and cytokines hydrolyze and lyse host tissue. This explains the relationship between the enzymatic activity of *A. hydrophila* and the physical damage that appears in infected fish.

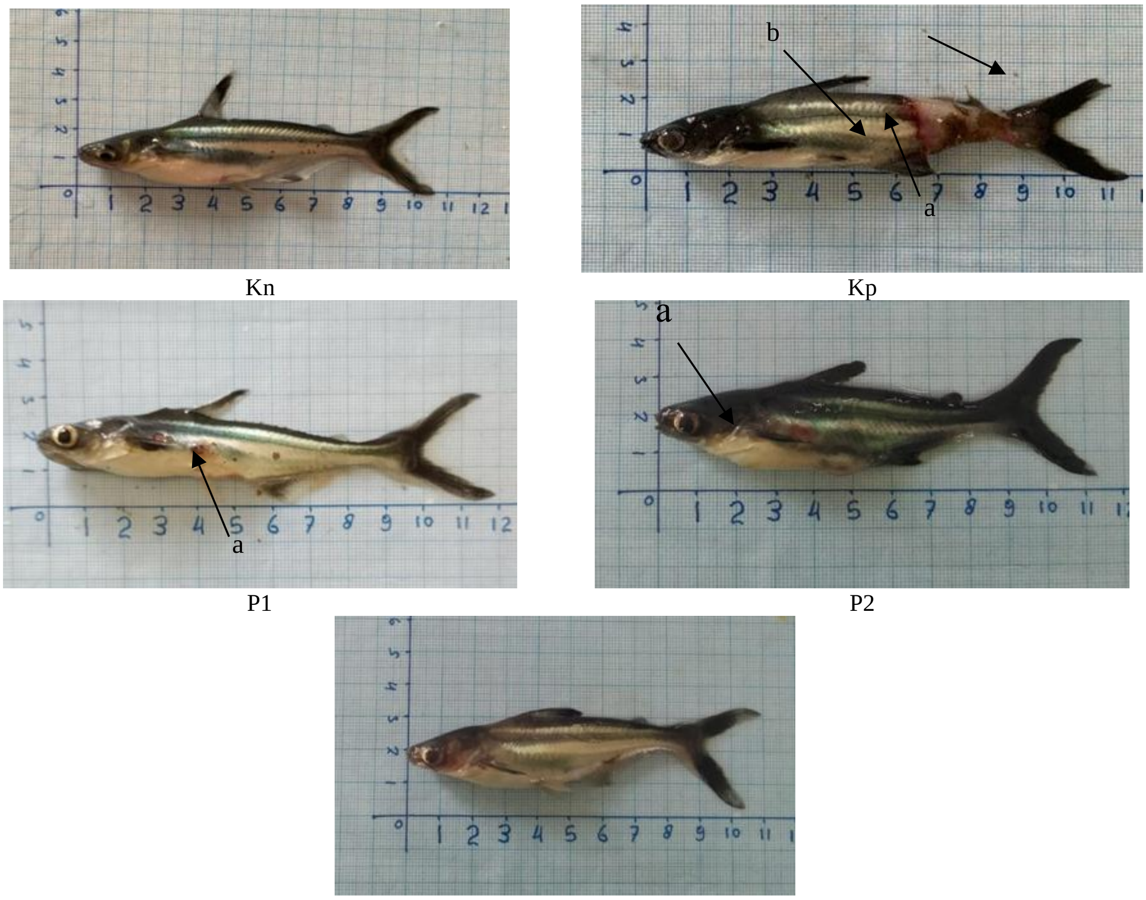

Figure 1. Clinical Symptoms after the Challenge Test

Meanwhile, fish in the P3 treatment showed normal clinical conditions, with maintained appetite, body surface without ulcers, no excessive mucus, and fins without damage. This condition indicates an increase in the fish's immune system, which is thought to be influenced by papaya seed extract administration. The triterpenoid content in papaya seeds is known to exhibit antibacterial activity by damaging bacterial cell membranes (Wulansari et al., 2020). This supports the idea that administering papaya seed extract can suppress the growth of *A. hydrophila* while boosting the immune system of Striped catfish.

3.2. Blood Glucose Levels in Striped catfish (P. hypophthalmus)

The results of blood glucose measurements in Striped catfish during the study are shown in Table 2.

Table 2. Blood Glucose Levels of Striped catfish (*P. hypophthalmus*)

Treatment	Blood Glucose (mg/dL)		
	H-0	H-30	H-46
Kn	44,00	46,00 ± 1,00^{a}	54,66 ± 3,51^{a}
Kp	40,00	47,00 ± 1,00^{a}	111,33 ± 6,02^{e}
P1	44,00	59,66 ± 2,0^{b}	80,00 ± 1,00^{d}
P2	45,00	65,33 ± 2,30^{c}	73,66 ± 0,57^{c}
P3	43,00	70,33 ± 1,52^{e}	66,66 ± 1,5^{b}

The results of glucose level observations in Striped catfish showed that at the beginning of the maintenance period, values ranged from 43.00 to 45.00 mg/dL, which remained within the normal blood glucose range for fish 40–90 mg/dL (Shabrina et al., 2018). However, glucose levels increased from the start of cultivation through post-challenge, indicating a physiological response to the cultivation conditions and the treatment. On the 30th day of maintenance, the fish's blood glucose levels increased to 46.00–70.33 mg/dL. A higher increase occurred in fish fed papaya seed extract, namely P1 (59.66 mg/dL), P2 (65.33 mg/dL), and P3 (70.33 mg/dL). The values for P1 and P2 remained within the normal range, whereas P3 was higher due to the large treatment dose, indicating that the fish were not yet fully able to adapt to the papaya seed extract.

Fourteen days after infection, blood glucose levels increased sharply, ranging from 54.66 to 111.33 mg/dL. A significant increase in the control fish (Kp) was observed, with a level of 111.33 mg/dL, despite receiving no special treatment and being infected only with *A. hydrophila*. This condition was triggered by stress caused by a bacterial infection that produced a hemolysin toxin, which damaged erythrocytes, disrupted oxygen diffusion, decreased appetite, and increased stress in fish (Rizvi et al., 2025).

Meanwhile, in treatments P1 and P2, glucose levels remained within normal limits. This is thought to be because papaya seeds contain active compounds such as alkaloids, flavonoids, saponins, tannins, and phenolic acids that can lower glucose levels and act as antioxidants, anti-stress agents, and antibacterials (Puspadewi & Aisyah, 2019). In P3, glucose levels decreased to 66.66 mg/dL, presumably due to the optimal dose of extract, allowing flavonoids to act as antimicrobials that damage bacterial cell membranes, prevent inflammation, and boost the fish's immune system. This decrease in glucose levels also indicates that fish utilize energy to adapt to stress (Djauhari et al., 2020). In general, increases or decreases in blood glucose levels indicate stress in fish, which is physiologically reflected in metabolic changes aimed at maintaining homeostasis (Hertika et al., 2021).

3.3. Blood Biochemistry of Striped catfish (P. hypophthalmus)

The results of the biochemical observations of Striped catfish blood during the study are shown in Table 3.

Table 3. Blood Biochemistry of Striped catfish (*P. hypophthalmus*)

Maintenance	Treatment	Calcium (mg/dL)	Magnesium (mg/dL)	Phosphorus (mg/dL)	Lysozyme Activity (Units/mL)
Beginning	Kn	10,5	2,2	6,05	214
	Kp	10,8	2,3	6,36	216
	P1	11,1	2,2	6,49	217
	P2	10,9	2,4	6,08	218
	P3	11,0	2,4	6,50	218
30 Days Maintenance	Kn	11,10 ± 0,10^{a}	2,36 ± 0,57^{a}	7,13 ± 0,15^{a}	222,33 ± 2,51^{b}
	Kp	11,16 ± 0,15^{a}	2,36 ± 0,57^{a}	7,10 ± 0,10^{a}	228,00 ± 2,64^{b}
	P1	12,16 ± 0,15^{b}	2,53 ± 0,57^{b}	8,03 ± 0,15^{b}	250,66 ± 3,05^{b}
	P2	11,80 ± 0,26^{c}	2,53 ± 0,57^{b}	8,10 ± 0,10^{c}	263,33 ± 2,08^{c}
	P3	12,53 ± 0,15^{d}	2,73 ± 0,57^{a}	8,66 ± 0,15^{a}	275,00 ± 4,35^{a}
14 days after-challenge	Kn	11,60 ± 0,20^{b}	2,56 ± 0,05^{ab}	7,63 ± 0,15^{a}	230,33 ± 1,52^{a}
	Kp	11,73 ± 0,15^{a}	1,90 ± 0,10^{a}	5,73 ± 0,15^{a}	484,33 ± 4,50^{e}
	P1	12,40 ± 0,10^{c}	2,76 ± 0,05^{b}	8,55 ± 0,13^{b}	261,00 ± 1,00^{c}
	P2	12,26 ± 0,15^{d}	2,90 ± 0,10^{c}	8,50 ± 0,10^{c}	304,66 ± 4,50^{b}
	P3	12,90 ± 0,10^{e}	3,13 ± 0,15^{a}	9,00 ± 0,10^{a}	280,33 ± 1,52^{d}

Based on the results of the study, the calcium levels of Striped catfish at the start of cultivation ranged from 10.5 to 11.1 mg/dL. After 30 days of cultivation, the levels increased to 11.10–12.53 mg/dL, and 14 days after challenge, the levels ranged from 11.60 to 12.90 mg/dL. According to Syawal et al. (2023), normal blood calcium levels in fish range from 8.6 to 10.30 mg/dL, so the values obtained in this study are higher than the normal range. Calcium plays an important role in various physiological and biochemical functions of the body, including blood clotting, nerve signal transmission, muscle contraction, cellular interactions, enzyme activation, and hormone production.

Calcium levels in control fish (Kn and Kp) during 30 days of maintenance were still within the normal range, indicating good adaptation and normal appetite in the fish. However, in treatments P1, P2, and P3, calcium levels increased significantly beyond normal limits. The addition of papaya seed extract to the feed influences this. The nutritional content of papaya seeds, including minerals such as calcium, phosphorus, and magnesium, has the potential to support fish mineral metabolism. In contrast, active compounds such as alkaloids, flavonoids, saponins, vitamins, and enzymes also have positive effects (Bria et al., 2022).

After 14 days post-challenge, calcium levels in all treatments (Kn, Kp, P1, P2, and P3) were above the normal range. This condition was triggered by an *A. hydrophila* infection, which compromised the fish's immune system and destabilized its physiology. In the P3 treatment, the higher increase in calcium levels was suspected to be due to the excessive dose of papaya seed extract administered, which caused toxic effects. An imbalance in calcium levels can disrupt biochemical functions in the body; although excess calcium can usually be stored in the bones or excreted through urine and feces (Syawal et al., 2023).

Based on Table 5, the magnesium level in the blood of Striped catfish fighting fish at the beginning of the study ranged from 2.20 to 2.40 mg/dL. After 30 days of maintenance, it increased to 2.36–2.73 mg/dL, and 14 days after the challenge, it ranged from 1.90 to 3.13 mg/dL. According to Bojarski et al. (2021), the normal range of magnesium in fish blood is 2.51–3.45 mg/dL. Magnesium is an essential mineral that plays an important role in various physiological functions, including regulating ion balance in blood and body fluids and supporting energy metabolism from carbohydrates, fats, and proteins (Syawal et al., 2023).In addition, magnesium also functions in the respiratory adaptation process in freshwater fish, which is obtained from both feed and the aquatic environment.

The results showed that magnesium levels after treatment in treatments P1, P2, and P3 were still within the normal range, with P3 having the highest level. This increase is thought to be due to the administration of papaya seed extract containing magnesium and essential oils, with active compounds that enhance fish immune systems and suppress bacterial infections. This is in line with Insani (2022), who reported that papaya seeds contain phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, saponins, and steroids, with antibacterial potential. In addition, papaya seeds are rich in essential nutrients, such as calcium, magnesium, and phosphorus, that support the metabolic and immune health of fish.

Based on Table 3, the phosphorus level in the blood of Striped catfish at the beginning of cultivation ranged from 6.05 to 6.50 mg/dL, increased to 7.10–8.66 mg/dL on day 30, and was in the range of 5.73–9.00 mg/dL after challenge. Phosphorus (P) is an essential macro mineral that plays a role in the development and maintenance of the skeletal system, and also participates in various physiological processes in fish. According to Syawal et al. (2023), the normal phosphorus concentration in fish blood serum is in the range of 2.5–4.5 mg/dL. Thus, the phosphorus levels in all treatments in this study were above the normal range. Excessive phosphorus (hyperphosphatemia) can trigger physiological and metabolic disorders that are detrimental to fish health. One effect is the inhibition of calcium absorption, which can lead to hypocalcemia (low calcium levels). An excessively high phosphorus ratio can disrupt mineral balance, as high phosphorus levels limit calcium absorption, and, conversely, calcium imbalance can also affect phosphorus absorption (Syawal et al., 2023). This shows that the balance between phosphorus and calcium is very important for maintaining fish metabolic health.

Based on Table 3, the average lysozyme activity of Striped catfish at the beginning of cultivation ranged from 214 to 218 units/mL, increased to 222.33 to 275.00 units/mL at 30 days of cultivation, and reached 230.33 to 484.33 units/mL after challenge. Lysozyme is an important antimicrobial enzyme in the innate immune system of fish that functions as an initial defense against bacterial and pathogen attacks. This enzyme hydrolyzes the β-(1,4)-glycosidic bonds in the cell walls of bacteria, especially Gram-positive bacteria, thereby causing cell lysis (Syawal et al., 2023). Lysozyme activity can also be used as an indicator of stress levels, where stressed fish have higher lysozyme activity (900 units/mL) compared to normal fish (140 units/mL) (Syawal et al., 2019).

The highest lysozyme activity after challenge was observed in the positive control (Kp), at 484.33 units/mL. This condition indicates activation of the innate immune system following bacterial exposure, as lysozyme plays a major role in the bactericidal activity of monocytes and neutrophils. Increased lysozyme activity is closely correlated with increased phagocytic activity, with higher lysozyme levels resulting in greater phagocyte ability to fight infection (Asmi et al., 2017). This shows that the fish's immune response to bacterial attacks is active, as evidenced by increased production of defense enzymes.

Conversely, the lowest lysozyme activity post-challenge was observed in treatment P1, at 261.00 units/mL. This low lysozyme activity is thought to be influenced by the low dose of papaya seed extract administered, so that the immunostimulants entering the fish's body were insufficient to stimulate optimal lysozyme enzyme production. In fact, immunostimulants play an important role in stimulating phagocytosis and increasing lysozyme secretion. Thus, a higher dose of papaya seed extract tends to be more effective in boosting the innate immune system through increased lysozyme activity in Striped catfish. Blood biochemistry in the positive control fish shows a high mortality rate. This is because the fish did not receive special treatment, such as the administration of immunostimulants in their feed. When *A. hydrophila* infects fish, the bacteria easily penetrate the fish's immune system, leading to a decline in immune function, as evidenced by decreased blood biochemistry levels. According to Syawal et al. (2019), immunostimulants are compounds that can enhance specific or nonspecific defense mechanisms in the body.

4. Conclusions

Based on the study's results, it can be concluded that the addition of papaya seed extract to feed affects the physiological and biochemical responses in the blood of Striped catfish. The optimal feed dosage with the addition of papaya seed extract is 3.0 mL/kg of feed, as seen from the glucose level of 70.33 mg/dL, calcium 12.53 mg/dL, magnesium 2.73 mg/dL, phosphorus 8.66 mg/dL, lysozyme activity 275 units/mL, absolute weight growth 14.92 g, absolute length growth 5.25 cm, and survival rate reaching 93.33%.

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