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Effect of Octopus (*Octopus* sp.) Ink Extract on Haematological of the Catfish (*Clarias* sp.) Infected with *Aeromonas hydrophila*

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ABSTRACT

The catfish (*Clarias* sp.) is a freshwater fish species with economic value, leading to widespread cultivation by fish farmers. The growing demand for catfish consumption has driven a shift from traditional to intensive farming methods. However, intensive farming often faces issues such as disease outbreaks, which can cause significant mortality among the farmed fish. A common disease affecting catfish is Motile Aeromonas Septicemia (MAS), caused by *Aeromonas hydrophila*, which produces exotoxins that damage the infected fish's surface. One preventive measure that can be applied is the use of immunostimulants. Octopus ink extract is a potential immunostimulant known to enhance the immune system of the catfish. This extract contains bioactive compounds, including alkaloids, saponins, and lysine, which are believed to boost growth. This study investigated the effects of octopus ink extract on the immune system of catfish infected with *Aeromonas hydrophila*. The experimental method involved five treatments with three replications: Treatment 1 (Control), Treatment 2 (40 ml/kg), Treatment 3 (80 ml/kg), Treatment 4 (120 ml/kg), and Treatment 5 (160 ml/kg). The research steps included extracting octopus ink, determining the Lethal Concentration 50 (LC_{50}), preparing equipment and materials, supplementing octopus ink extract in the feed, applying treatments, challenging with *A. hydrophila*, and maintaining the fish. The parameters measured during the research included immune system parameters. The immune system parameters assessed were erythrocytes, leukocytes, leukocytes differential, and hematocrit. The results of the study showed that Treatment 5 (160 ml/kg) yielded the best outcomes for immune system function. Immune system improvements were indicated by the highest values for erythrocytes (2.56 x 10^6 cells/mm^3), leukocytes (3.91 x 10^4 cells/mm^3), and hematocrit (32.9%). The addition of octopus ink extract into the catfish feed significantly improves the immune system (erythrocytes, leukocytes, and hematocrit).

INTRODUCTION

The catfish (*Clarias* sp.) ranks among the most economically valuable freshwater fish species, making it a favorite choice for many fish farmers **(Wardika *et al.*, 2014)**.

This popularity stems from the fact that the catfish farming is relatively straightforward, requiring neither complex infrastructure nor significant water resources. Additionally, farmers can start raising the catfish without needing large amounts of initial capital **(Su'udi & Wathon, 2018)**.

The catfish is classified as one of the aquaculture commodities with an annually increasing production demand. According to production data, in 2015, the catfish production reached 719,619 tons/year, rising to 764,797 tons/year in 2016. By 2017, the national catfish production soared to 1,771,867 tons, reflecting a 131.7% increase from the previous year **(Lutfiyanah & Djunaidah, 2020)**. The increasing public interest in consuming the catfish will indirectly shift the farming system from traditional to intensive methods.

In intensive fish farming, the production output is significantly higher. Intensive aquaculture refers to a farming system that involves high stocking density **(Yunarty *et al.*, 2022)**. However, despite the potential profit gained from high stocking density, intensive farming carries risks, particularly disease outbreaks. The occurrence of diseases can become a major obstacle in aquaculture operations, as it may lead to high mortality rates among the cultured organisms **(Utami *et al.*, 2016)**. Harvest failure is often caused by disease outbreaks affecting the cultivated organisms. Diseases that cause mortality in aquaculture species can be triggered by bacteria, viruses, parasites, or other toxic microorganisms. In the catfish, red spot disease is commonly observed, caused by the bacterium *Aeromonas hydrophila*. *A. hydrophila* is a pathogen responsible for MAS (Motile Aeromonas Septicemia) in the catfish **(Muslikha *et al.*, 2016)**. This bacterium can be found in a wide range of environments, from freshwater fish to, occasionally, marine fish. Disease control can be carried out through preventive measures and treatment.

Prevention can be achieved through the application of antibiotics, chemicals, and natural substances. The application of chemical materials has also caused new problems concerning the increase of environmental pollution **(Rairakhwada *et al.*, 2007)**. Another preventive approach involves the use of immunostimulants **(Marentek *et al.*, 2013)**. Immunostimulants are substances capable of enhancing the natural immune system or innate immune response, thereby helping fish improve their resistance to diseases and reducing mortality rates caused by infections **(Muahiddah & Dwiyanti, 2023)**. When applied, immunostimulants do not leave residues in the fish's body or the environment, nor do they cause any side effects on human health. Immunostimulants can be sourced from herbal plants or animals **(Junaidi *et al.*, 2020)**.

The proper application of immunostimulants requires the correct dosage and frequency of administration. Continuous use of immunostimulants is recommended to enhance the immune system's ability to provide optimal protection **(Febriani *et al.*, 2013)**. One of the potential immunostimulant materials is octopus ink. Octopus ink can serve as an alternative immunostimulant source that may improve the immune system of

the catfish. Tyrosinase, an enzyme found in squid ink, is recognized for its crucial role in defending against microbes **(Takai *et al.,* 1992)**. Typically, octopus ink is underutilized, making it a potential waste product. Therefore, repurposing octopus ink to boost the immune system of the catfish is necessary to maximize its beneficial components and to reduce the risk of waste that could pollute the environment. Research on giving octopus ink extract to the catfish contributes to Sustainable Development Goals (SDGs) 2 (Zero Hunger), 3 (Good Health and Well-being), 12 (Responsible Consumption and Production), and 14 (Life Below Water) by improving fish health, supporting sustainable aquaculture, and reducing the use of synthetic inputs. The aim of this research was to evaluate the effect of octopus ink extract on the immune system of the catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria.

MATERIALS AND METHODS

Time and location

This research was carried out for a duration of 45 days from August- October 2024. The research activities were conducted at the Fish Production and Reproduction Laboratory, as well as the Fish Health Laboratory in the Department of Fisheries and Marine Science at the University of Mataram.

Research procedures

The first activity carried out was the preparation of the rearing media. The media used in this study involved the use of containers. The containers were cleaned using running water and were then dried under direct sunlight.

The next step involved preparing the test animals. The test animals used were catfish measuring 6– 7cm in length. Before applying the treatments, the catfish were acclimatized for 14 days. During the acclimatization period, the test fish were fed at 3% of their body weight **(Wahjuningrum *et al.,* 2010)**.

The process of making octopus ink extract began with extraction. The extraction was carried out by soaking the octopus ink in 96% methanol solvent, stirring it for 1.5 hours, and then storing it at room temperature for one week **(Affandi *et al.,* 2019)**. The octopus ink was then filtered, followed by evaporation to obtain pure octopus ink extract **(Smiline *et al.,* 2012)**.

The bacteria used in this study was *Aeromonas hydrophila*, obtained from BBPBAP Jepara. Before being spread on NA media, the bacteria were diluted to a density of 10^8 **(Maulidya *et al.,* 2017)**.

The LC_{50} (Lethal Concentration 50) test method used in this study was adapted from the procedures outlined by **Rosidah *et al.* (2018)**, **Kalor *et al.* (2019)** and **Kumari *et al.* (2020)**. The LC_{50} test method used was the oral method, in which the feed was mixed with octopus ink extract according to the test doses of 0, 25, 50, 75, and 100ml/ kg. The catfish were placed in the rearing media at a density of 20 fish/container **(Kalor *et al.,* 2019)**.

The feed used in this study was commercial feed, mixed with octopus ink extract according to the predetermined doses. After mixing, the feed was left to dry for 15 minutes. According to **Sukendar *et al.* (2021)**, the extract used was evenly sprayed onto the feed. After that, the feed was measured at 5% of the total body weight of the test fish **(Haetami *et al.*, 2023)**.

The fish were treated for 21 days with 20 fish/container **(Rosidah *et al.*, 2018)**. Feed provided at 5% of the total biomass of the fish, and sampling was conducted every 10 days **(Syahrizal *et al.*, 2016)**. The feeding method used in this study was *ad libitum*, meaning that the feed was given according to the fish's biomass. The feed was administered three times a day at 07:00, 12:00, and 17:00.

The preparation of *A. hydrophila* bacteria at a concentration of 10^8 CFU/ml **(Karina *et al.*, 2015)** for infecting fish was conducted using Nutrient Agar (NA) as the growth medium. The process began by transferring a loopful of A. hydrophila bacterial sample from a slant agar medium onto the NA medium. The sample was then streaked onto the medium and incubated for 24 hours.

On the 22nd day, after the test fish had been treated with octopus ink extract for 21 days, bacterial infection was performed on the test fish after the rearing period. The fish were infected with *Aeromonas hydrophila* bacteria at a density of 10^8 CFU/mL **(Maulidya *et al.*, 2017)** using an immersion method. The observation of parameters was conducted on the seventh day after bacterial infection **(Wahjuningrum *et al.*, 2013)**.

The total red blood cell (erythrocyte) count was determined using the following method: A blood sample was drawn from an Effendorph tube and aspirated into an erythrocyte pipette (capillary tube) containing a small red bead, up to the 0.5mL mark. Hayem's solution was then added until the total volume reached the 101 mark. The mixture was homogenized by gently shaking it in a figure-eight motion. To eliminate air bubbles, the first two drops of the solution were discarded. A drop of the remaining mixture was then placed into a haemocytometer that had been covered with a glass cover slip.

Observation was conducted under a microscope at 100× magnification (10× objective lens and 10× ocular lens). Erythrocytes were counted in five fields within the small squares of the haemocytometer grid. The final erythrocyte count was calculated using the formula described by **Yanto *et al.* (2015)**:

$$\text{Total erythrocytes} = n \times 10^4 \text{ cells/mm}^3$$

Where:

n : number of erythrocyte cells in 5 small boxes of counting rooms

10^4 : dilution factor

The total white blood cell (leukocyte) count was carried out as follows: A blood sample was taken from an Effendorph tube and aspirated into a leukocyte pipette (capillary tube) containing a small white bead, up to the 0.5 mL mark. Turk’s solution

was then added until the volume reached the 11 mark. The solution was homogenized by gently shaking it in a figure-eight motion. To remove air bubbles, the first two drops were discarded, and a drop of the remaining solution was placed into a haemocytometer that had been covered with a glass cover slip.

The sample was observed under a microscope at 400× magnification (40× objective lens and 10× ocular lens). White blood cells were counted in five fields of view within the small squares of the haemocytometer grid. The total leukocyte count was then calculated using the formula provided by **Kurniawan *et al*. (2013)**:

$$\text{Total leukocytes} = \frac{\text{N1 x 20}}{0.4}$$

Where:

N1 : number of leukocytes in 5 fields of view

20 : dilution factor

0.4 : total blood volume in 5 fields of view (mm^3)

The differential leukocyte count was performed based on the method described by **Agustiana *et al.* (2020)**. This involved calculating the percentage of each type of leukocyte observed across 10 fields of view under a microscope. The leukocyte types identified and counted included lymphocytes, monocytes, and neutrophils. Each cell type was recorded separately according to its morphological characteristics.The formula used to calculate the number of lymphocyte, monocyte, and neutrophil is as follows:

$$\% \text{ Lymphocyte} = \frac{\text{L}}{100} \text{ x } 100\%$$

$$\% \text{ Monocyte} = \frac{\text{M}}{100} \text{ x } 100\%$$

$$\% \text{ Neutrophil} = \frac{\text{N}}{100} \text{ x } 100\%$$

Hematocrit level calculation was performed using blood samples collected in Eppendorf tubes. The blood was drawn into a hematocrit capillary tube until it reached approximately ¾ of the tube's length. The open end of the tube was then sealed with *kretoseal* (sealing wax). The sealed capillary tubes were placed in a microhematocrit centrifuge and spun for 15 minutes at a speed of 3,500 rpm.

After centrifugation, the hematocrit level was determined by comparing the volume of packed red blood cells to the total volume of blood in the tube, using a hematocrit reader or scale. The calculation followed the formula provided by **Royan *et al*. (2014)**:

$$\frac{\text{Length of volume of red blood cells that settle}}{\text{Total length of blood volume in the tube}} \text{ x } 100\%$$

RESULTS

Erythrocytes

Based on this study, the number of erythrocytes in the catfish during a 45-day rearing period with varying doses of octopus ink extract, following the administration of immunostimulants, ranged from 2.31 - 2.73 x 10^6 cells/mm^3, and after infection, it ranged from 1.61 - 2.56 x 10^6 cells/mm^3.

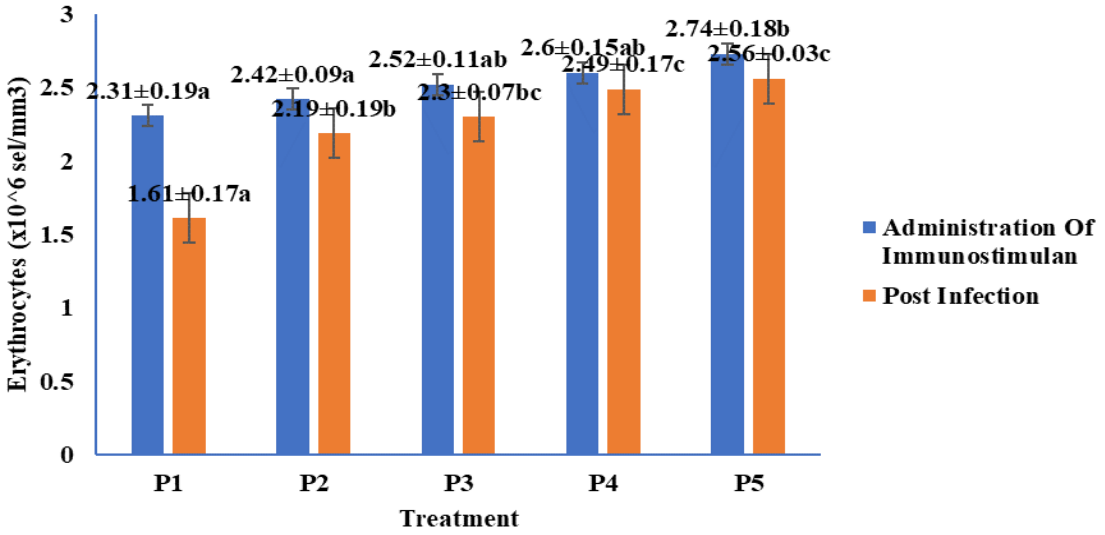

Fig. 1. Erythrocytes of catfish (*Clarias* sp.)

Leukocytes

Based on this research, the leukocyte count in catfish during a 45-day rearing period with different doses of octopus ink extract, following the administration of immunostimulants, ranged from 2.81 - 3.53 x 10^4 cells/mm^3, and after infection, it ranged from 3.07 - 3.91 x 10^4 cells/mm^3.

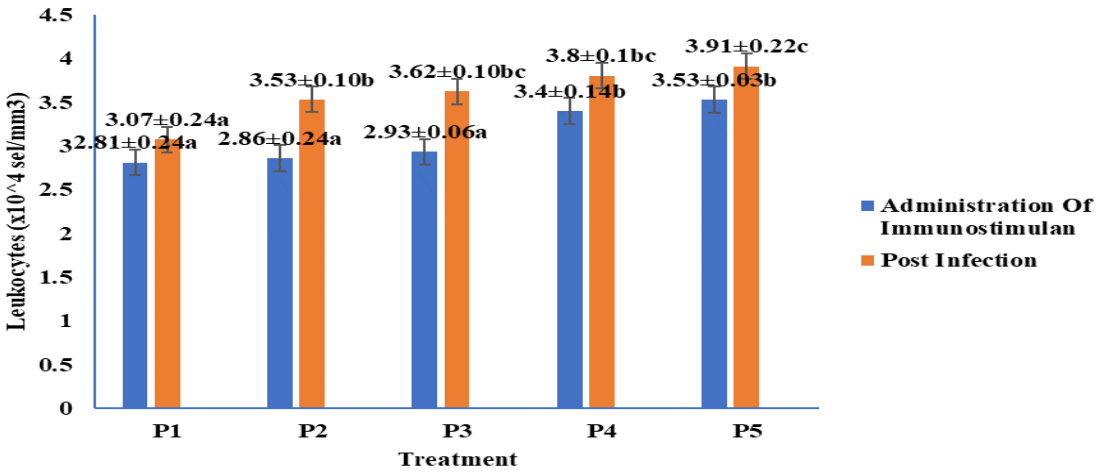

Fig. 2. Leukocytes of catfish (*Clarias* sp.)

Leukocytes differential

Based on this study, the leukocyte differential count in catfish during the 45-day rearing period with different concentrations of octopus ink extract showed that lymphocytes levels ranged from 70.33 – 77.0%, monocytes from 12.00 – 19.67%, and neutrophils from 8.0 – 11.0%. After infection, the lymphocytes levels ranged from 77.7 – 80.0%, monocytes from 12.7 – 15.3%, and neutrophils from 6.0 – 8.0%.

Table 1. Leukocytes differential of catfish (*Clarias* sp.)

Leukocytes Differential	Treatment	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Start of maintenance	P1	70.0 ± 1.7	16.3 ± 1.5	13.7 ± 3.2
	P2	71.3 ± 2.3	16.7 ± 1.5	12.0 ± 3.0
	P3	70.3 ± 1.5	18.7 ± 2.5	11.0 ± 3.5
	P4	71.7 ± 1.5	18.3 ± 1.5	10.0 ± 2.6
	P5	71.0 ± 1.0	18.0 ± 1	11.0 ± 1.0
Administration of Immunostimulant	P1	70.33 ± 0.5^{a}	19.67 ± 0.5^{b}	10.0 ± 0^{ab}
	P2	72.67 ± 0.5^{b}	18.33 ± 1.1^{b}	9.0 ± 1^{ab}
	P3	72.33 ± 0.5^{bc}	19.67 ± 1.5^{b}	8.0 ± 1.7^{a}
	P4	74.0 ± 1^{c}	18.0 ± 1^{b}	8.0 ± 1^{a}
	P5	77.0 ± 1^{d}	12.00 ± 1^{a}	11.0 ± 1^{b}
Post Infection	P1	77.7 ± 0.5^{a}	14.3 ± 0.5^{bc}	8.0 ± 0^{c}
	P2	78.7 ± 0.5^{a}	15.3 ± 0.5^{c}	6.0 ± 0^{a}
	P3	78.3 ± 0.5^{ab}	14.7 ± 0.5^{c}	7.0 ± 0^{b}
	P4	79.7 ± 0.5^{bc}	13.3 ± 0.5^{ab}	7.0 ± 0^{b}
	P5	80.0 ± 1^{c}	12.7 ± 0.5^{a}	7.3 ± 0.5^{b}

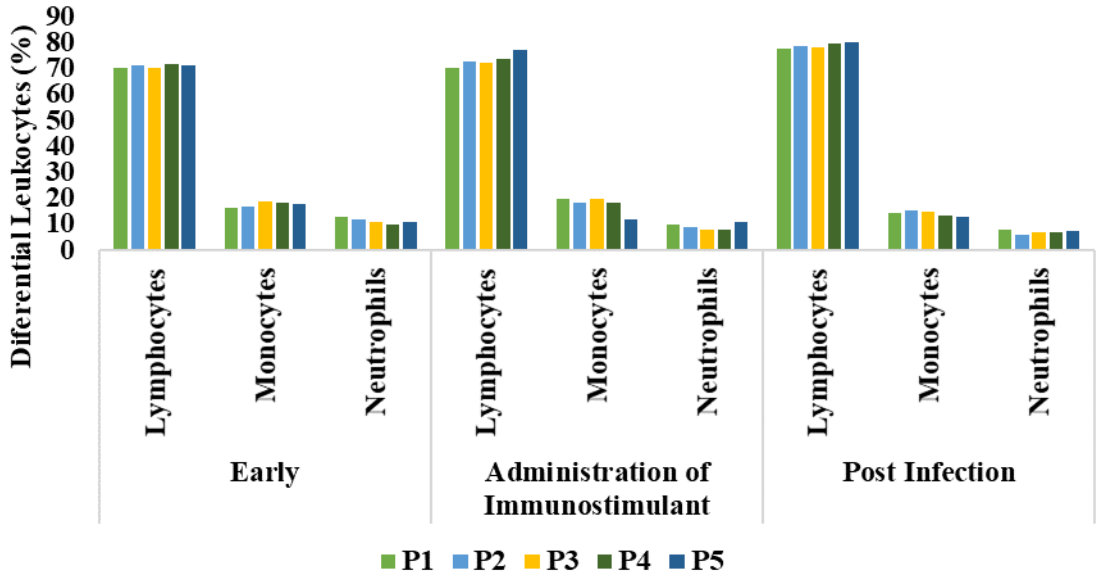

Fig. 3. Leukocytes differential of catfish (*Clarias* sp.)

Hematocrit

Based on this research, the hematocrit levels of catfish during the 45-day rearing period with different concentrations of octopus ink extract ranged from 32.0 - 35.1% after immunostimulant administration, while after infection, the hematocrit levels ranged from 23.6 - 32.9%.

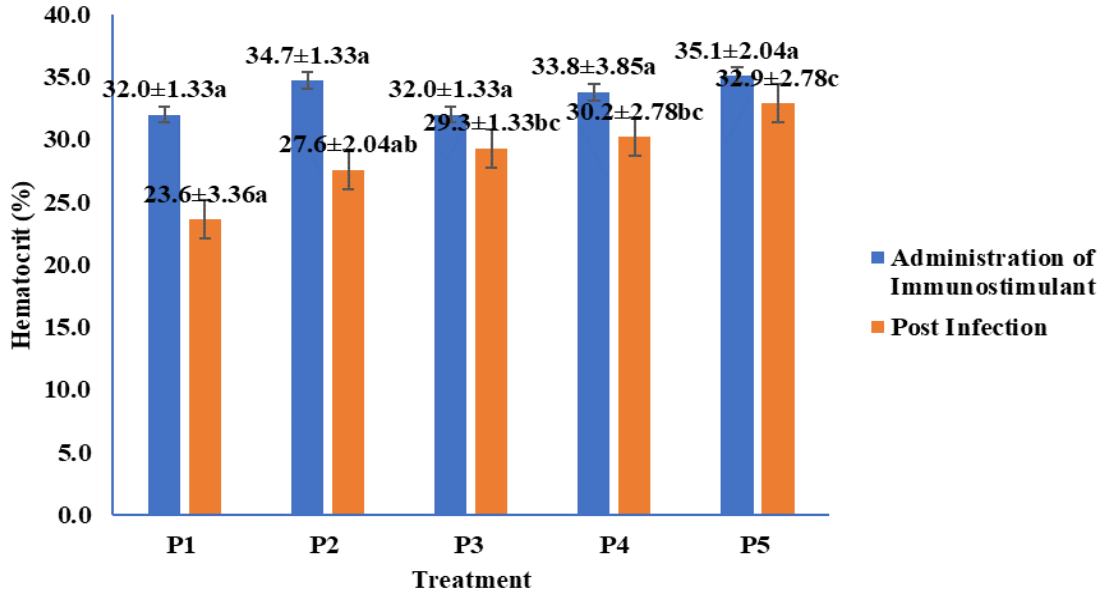

Fig. 4. Hematocrit of catfish (*Clarias* sp.)

DISCUSSION

Erythrocytes

Red blood cells (erythrocytes) play a crucial role in distributing oxygen and nutrients throughout the body. According to the graph in Fig. (1), the average erythrocyte count recorded during the study ranged from 2.28–2.29 x 10^6 cells/mm^3 at the start of the study (day 21), 2.31–2.74 x 10^6 cells/mm^3 after immunostimulant administration (day 35), and 1.61–2.56 x 10^6 cells/mm^3 seven days post-infection with *A. hydrophila* (day 43). The graph shows that the total erythrocyte count on days 21 and 35 remained within the normal range, however a significant decline was observed on day 43. According to **Hastuti and Subandiyono (2015)**, the optimal erythrocyte range in catfish blood is 2–3 x 10^6 cells/mm^3.

The erythrocyte count of catfish post-infection (day 43) showed a notable decrease, presumably due to the bacterial infection caused by *A. hydrophila*. This finding aligns with **Sarjito *et al.* (2019)**, who stated that *Aeromonas hydrophila* can produce hemolysin, which lyses erythrocytes, leading to a reduction in the total erythrocyte count.

In treatments 2, 3, 4, and 5, the total erythrocyte count in the catfish post-infection remained within the optimal range. This may be attributed to bioactive compounds present in octopus ink, which can enhance the immune system of the catfish. This result is consistent with that of **La Basy *et al.* (2023)**, who explained that the ink produced by cephalopods such as squid, octopus, and cuttlefish contains melanin, which acts as an antioxidant and antibacterial agent. The ink glands in cephalopods have also been shown to contain various melanogenic enzymes such as tyrosinase, dopachrome tautomerase, and peroxidase **(Prota, 2000)**. Furthermore, it is supported by **Dangeubun and Metungun (2017)**, who noted that a normal erythrocyte count in infected fish indicates that the immune system can stimulate erythrocyte production to replace those lysed by bacterial infection.

Leukocytes

Leukocytes, or white blood cells, serve multiple functions, one of which is protecting the body from foreign substances. Leukocytes are essential in the fish's defense mechanism against pathogenic infections **(Anderson *et al.*, 1995)**. According to the graph in Fig. 2, the average leukocyte count recorded during the study ranged from 2.27–2.99 x 10^4 cells/mm^3 at the start of the study (day 21), 2.81–3.53 x 10^4 cells/mm^3 after immunostimulant administration (day 35), and 3.07–3.91 x 10^4 cells/mm^3 seven days post-infection with *A. hydrophila* (day 43). The graph shows that the total leukocyte count on days 21 and 35 remained within the normal range, but a significant increase was observed on day 43. According to **Riauwaty *et al.* (2019)**, in healthy fish, the total leukocyte count ranges from 20,000–150,000 cells/mm^3.

The increase in leukocyte count on day 43 in the catfish treated with octopus ink extract may be attributed to the active compounds in the ink, such as phenols, saponins, and alkaloids, which act as immunostimulants, enhancing the immune system by

promoting leukocyte production **(Delviani, 2024)**. Alkaloids exhibit antimicrobial, anti-tumor, and analgesic properties **(Zhao *et al.*, 2015)**. Based on research by **Affandi *et al.* (2025)**, octopus ink extract that has been tested for phytochemical is known to contain several bioactive compounds such as alkaloids, saponins, phenols and steroids.

The leukocyte count in catfish post-infection (day 43) increased again, likely due to the bacterial infection caused by *A. hydrophila*. This result is consistent with **Rosidah *et al.* (2018)**, who noted that leukocyte production rises during infection, with these cells being transported through the bloodstream to the infection site to suppress and combat invading antigens. At this stage, leukocytes are mobilized to obstruct foreign substances entering the body by traveling through blood vessels to the affected area **(Harikrishnan *et al.*, 2010)**. Additionally, leukocytes play a crucial role in the fish's non-specific immune system by inhibiting pathogenic bacterial infections through the process of phagocytosis.

Leukocytes differential

Leukocyte differential refers to the percentage distribution of various types of white blood cells in fish. Leukocyte measurement is an essential factor in evaluating the function of leukocytes. The common types of leukocytes in fish include lymphocytes, monocytes, and neutrophils. Lymphocytes are white blood cells responsible for the immune system's response. Based on the research, the percentage of lymphocytes in the catfish was recorded on days 21, 35, and 43. According to Table (1), the lymphocyte count at the start of the research (day 21) ranged from 70 - 71%, which falls within the normal range of 71.12–82.88%, as stated by **Ginting *et al.* (2021)**. After feeding the fish with octopus ink extract, the lymphocyte percentage increased to 70–74% on day 35. This increase is likely due to the alkaloid content in octopus ink, which can enhance the immune system. This finding aligns with **Lestari *et al.* (2017)**, who reported that alkaloids serve as antioxidants and have bioactive properties as immunostimulants. On day 43, post-infection, the lymphocyte count further increased to 77–80%, presumably in response to the bacterial infection caused by *Aeromonas hydrophila*. According to **Nainggolan *et al.* (2021)**, an increase in lymphocytes indicates the immune system's successful cellular (non-specific) response. Additionally, the elevated lymphocyte count indicated a significant disruption in the fish's defense system **(Gül *et al.*, 2012).**

Monocytes are another type of white blood cell that helps fight infections. Table (1) shows that the monocyte count at the beginning of the study (day 21) ranged from 16.3-18.7%. After administering the immunostimulant (day 35), the monocyte percentage increased to 12.00–19.67%. **Ginting *et al.* (2021)** noted that the normal monocyte percentage in teleost fish is typically around 0.1% of the total leukocyte count. However, the monocyte percentage decreased post-infection (day 43) to 12.7–15.3%. This decrease is likely due to the bacterial infection caused by *A. hydrophila* during the challenge test. **Kurniawan *et al.* (2013)** explained that a reduction in monocytes may result from

increased lymphocyte production, which generates antibodies essential for defending the body against bacterial infections.

Among the treatments, the highest monocyte count post-infection was observed in P2. This might be due to the reduced phagocytic activity of monocytes, influenced by the alkaloids in octopus ink, which act as antioxidants. Based on research by **Affandi *et al*. (2025)**, octopus ink extract that has been tested for phytochemical is known to contain several bioactive compounds such as alkaloids, saponins, phenols and steroids. This explanation is consistent with that of **Nainggolan *et al.* (2021)**, who argued that antioxidants are chemical compounds that donate electrons or act as reductants, capable of inhibiting, preventing, or slowing oxidation processes. Additionally, the decrease in monocytes may have resulted from the increased lymphocyte production during the bacterial infection, as supported by **Kurniawan *et al.* (2013)**, who explained that reduced monocyte counts could result from increased lymphocyte activity producing antibodies to neutralize toxins, bind to antigens, and destroy their biological structure.

Neutrophils are critical white blood cells in the immune system. The size of neutrophils varies between 9.6- 10.8µm in diameter **(Levengood *et al.*, 2000)**. According to the study results presented in Table (1), the neutrophil percentage at the beginning of the research (day 21) ranged from 11.00- 13.7%. After immunostimulant administration (day 35), it decreased to 8.0– 11.0%. **Ginting *et al*. (2021)** noted that the normal neutrophil range in fish is typically 6– 8%. Post-infection with *A. hydrophila* (day 43), the neutrophil percentage further decreased to 6.– 8.0%. Neutrophils function by migrating to the site of inflammation or infection, infiltrating the affected area, and subsequently engulfing infectious agents. Proteolytic enzymes and lysozymes then break down the bacteria **(Hrubec *et al.*, 2004)**. The treatment involving octopus ink extract resulted in a lower neutrophil count, which is likely due to the antimicrobial properties of the alkaloids in the ink that suppressed the infection. This finding supports **Wulandari (2018)**, who explained that alkaloids have multiple benefits, including antimicrobial activity. As stated by **Manning and Nakanishi (1996)**, neutrophils are the first cells to arrive at the infection site during a pathogenic bacterial invasion. Furthermore, **Treves-Brown (2000)** observed that neutrophil levels in the blood elevate in response to inflammation triggered by the invasion of pathogens or foreign particles.

Hematocrit

Hematocrit represents the percentage of total red blood cells in the blood volume. According to Fig. (4), the initial hematocrit levels in the catfish ranged between 28- 35%. The average hematocrit levels throughout the research showed that post-infection hematocrit in P1 was 23.6%, while in P2, P3, P4, and P5, the levels ranged from 27- 32% after the challenge test with *Aeromonas hydrophila*. The highest hematocrit level after immunostimulant administration was observed in P5, reaching 35%. The increase in hematocrit after immunostimulant administration is believed to be due to the inclusion of octopus ink in the feed, which can enhance hematocrit levels in the catfish. **Gunawan**

and Rosdiana (2023) stated that the ink produced by cephalopods, such as octopus, contains alkaloids that act as antimicrobials. According to **Cushnie *et al.* (2014)** alkaloids are a primary source of improving performance and immune function.

An increase in hematocrit is typically associated with a rise in total erythrocytes. The catfish fed with octopus ink-enriched feed showed improved hematocrit levels, which remained within the normal range. This finding aligns with **Cerlina *et al.* (2021)**, who stated that normal hematocrit levels in catfish range between 30.8 & 45.5%.

According to Fig. (4), hematocrit levels in catfish decreased post-infection. This decline is likely due to the stress caused by *A. hydrophila* infection, which reduces erythrocyte concentration in the blood, resulting in lower hematocrit levels. The post-challenge infection induced stress in the fish, which negatively impacted their appetite, further contributing to the lower hematocrit values. **Fajriyani *et al.* (2017)** explained that hematocrit levels below 30% indicate erythrocyte deficiency.

CONCLUSION

Based on the current outcomes, it can be deduced that the addition of octopus ink extract to the catfish feed significantly enhanced the immune system, as indicated by increased erythrocyte, leukocyte, and hematocrit levels. Moreover, the optimal treatment was achieved at the P5 dose (160 mL/kg), resulting in an erythrocyte count of 2.56×10^6 cells/mm³, a leukocyte count of 3.91×10^4 cells/mm³, and a hematocrit level of 32.9%.

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