

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

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ABSTRACT

One material that can be used to improve feed quality is octopus ink. Octopus ink can serve as an alternative nutrient source to optimize catfish growth. Ink produced by cephalopods such as squid, octopus, and cuttlefish contains various components, including fat, protein, ash, and carbohydrates. The aim of this research is to determine the effect of administering octopus ink extract (*Octopus sp.*) on the growth of catfish (*Clarias sp.*) infected with *Aeromonas hydrophila* bacteria. The method used in this research was experimental, involving five treatments with three replications: treatment 1 (Control/P1), treatment 2/P2 (40 ml/kg), treatment 3/P3 (80 ml/kg), treatment 4/P4 (120 ml/kg), and treatment 5/P5 (160 ml/kg). The research steps included octopus ink extraction, a Lethal Concentration 50 (LC₅₀) test, preparation of tools and materials, supplementation of octopus ink extract in feed, application of test treatments, challenge tests with *Aeromonas hydrophila*, and maintenance. The results of this research showed that treatment 5 (160 ml/kg) yielded the best outcomes in growth parameters, including an absolute weight of 1.26 grams, a specific growth rate of 0.57%/day, a feed conversion ratio (FCR) of 1.66, and a survival rate of 90%.

INTRODUCTION

Catfish (*Clarias sp.*) is one of the freshwater fish species with significant economic value, making it highly popular among fish farmers (Wardika *et al.*, 2014). This is not without reason; catfish farming is relatively simple as it does not require special facilities or large amounts of water. Furthermore, cultivating catfish does not necessitate substantial initial capital for farmers to begin their operations (Su'udi & Wathon, 2018).

Catfish is classified as one of the aquaculture commodities with an annually increasing production demand. According to production data, in 2015, catfish production reached 719,619 tons per year, rising to 764,797 tons per year in 2016. By 2017, national catfish production soared to 1,771,867 tons, reflecting a 131.7% increase from the previous year (Lutfiyannah & Djunaidah, 2020).

In catfish farming, the primary focus is on the nutritional content of the feed provided. This is because feed plays a crucial role in supporting growth and the survival rate of aquatic organisms. As a key component in fish farming, feed significantly determines the growth of fish. According to (Indra *et al.*, 2021) approximately 50%-80% of feed is required to accelerate the growth rate of fish.

The proper nutritional content is a fundamental factor in achieving optimal fish growth. Nutrients in feed, such as proteins, fats, carbohydrates, vitamins, and minerals, are essential for supporting metabolism, enhancing digestive efficiency, and accelerating fish growth. A balanced nutrient composition not only affects body growth but also impacts survival rates, health, and disease resistance in fish.

One material that can be used to improve feed quality is octopus ink. Octopus ink can serve as an alternative nutrient source to optimize catfish growth. Ink produced by cephalopods such as squid, octopus, and cuttlefish contains various components, including fat, protein, ash, and carbohydrates (Riyad *et al.*, 2020). Generally, octopus ink remains underutilized and often becomes waste. Therefore, reutilizing octopus ink to enhance catfish growth is essential, as it maximizes the beneficial content of the ink while reducing potential waste that could harm the environment. The aim of this research is to determine the effect of administering octopus ink extract (*Octopus sp.*) on the growth of catfish (*Clarias sp.*) infected with *Aeromonas hydrophila* bacteria.

METHODS

Time and Place

This research was conducted over 45 days, from August 2024 to October 2024. The research occurred at the Fish Production and Reproduction Laboratory and the Fish Health Laboratory, Department of Fisheries and Marine Science, University of Mataram.

Tools and Materials

The equipment used in this research included aerators, writing tools, autoclave, petri dishes, centrifuge, erlenmeyer flasks, hemocytometer, hot plate, inoculation needles, microscope slides, cover glasses, camera, filter paper, 45 L containers, microhematocrit tube, micropipettes, microscope, microtubes, rotary evaporator, container racks, nets, siphon hoses, corning tubes, digital scale, and vortex mixer.

The materials used in this research were freshwater, aluminum foil, alcohol, anticoagulants, distilled water, *Aeromonas hydrophila* bacteria, catfish (measuring 6-7 cm), methanol, Giemsa solution, hayem solution, NaCl solution, turk solution, TSB media, NA media, dish soap, pellets, and tissue paper.

Research Design

The method applied in this research was an experimental method using a Completely Randomized Design (CRD) consisting of 5 treatments with 3 replications, resulting in a total of 15 experimental units. Each treatment involved 20 catfish. The tested treatments involved the administration of octopus ink extract through feed, with concentrations determined based on the LC₅₀ test results. The treatments in this researches were as follows:

P1: Control without octopus ink extract + infection with *Aeromonas hydrophila* bacteria.

P2: Feed containing octopus ink extract at a dose of 40 ml/kg + infection with *Aeromonas hydrophila* bacteria.

P3: Feed containing octopus ink extract at a dose of 80 ml/kg + infection with *Aeromonas hydrophila* bacteria.

P4: Feed containing octopus ink extract at a dose of 120 ml/kg + infection with *Aeromonas hydrophila* bacteria.

P5: Feed containing octopus ink extract at a dose of 160 ml/kg + infection with *Aeromonas hydrophila* bacteria.

Research Procedures

• Preparation of Containers and Maintenance Media

The maintenance media used in this research consisted of 15 containers. Before use, the containers were cleaned with detergent, rinsed with water, and left to dry for 24 hours. Afterward, the containers were moved to the research site, filled with 20 liters of water each, and equipped with one aerator per container to supply oxygen to the water. The containers were then covered and labeled according to their respective treatments.

• Preparation of Test Animals

The test animals used in this research were catfish (*Clarias* sp.) measuring 6-7 cm in length. The test animals were obtained from BBI Batu Kumbung, Lingsar District, West Lombok Regency. The preparation of the test fish began with an acclimatization process for 14 days before the fish were transferred to the containers. During this process, the fish were fed artificial feed three times a day. The amount of feed given (feeding rate, FR) was 3% of the total fish biomass (Wahjuningrum *et al.*, 2010). The purpose of the acclimatization process was to help the fish adapt to and adjust to their environment, reducing stress and vulnerability to disease (Prihandini & Umami, 2022). After the 14-day acclimatization period, the catfish were transferred to the containers.

• Preparation of Octopus Ink Extract

The preparation of octopus ink extract was carried out by collecting ink sacs from octopus (*Octopus* sp.). The ink sacs were cut open, and the ink was collected. The extracted ink was then placed in a sterile container and stored in a refrigerator to prevent spoilage. The extraction process began with maceration, which involved soaking the octopus ink with a solvent. The ink was mixed with the solvent in a ratio of 1:3, using 1500 ml of methanol as the solvent in this research. The ink and solvent were measured with a graduated cylinder, transferred into an Erlenmeyer flask, and manually homogenized for 1.5 hours. The mouth of the Erlenmeyer flask was then sealed with aluminum foil and tied securely. Next, the flask was stored in a refrigerator at 4°C for 7 days (Affandi *et al.*, 2019). After the 7-day period, the mixture was subjected to evaporation using a rotary evaporator at a speed of 62 rpm for 4.5 hours (Smiline *et al.*, 2012).

• Preparation of *Aeromonas hydrophila*

The *Aeromonas hydrophila* bacteria used in this research were obtained from BBPBAP Jepara. Before conducting the challenge test, media preparation was necessary for bacterial growth. This was done by preparing 1.5 g of TSB (Tryptic Soy Broth) powder and adding 50 ml of distilled water into an erlenmeyer flask. The mixture was then heated on a hot plate until boiling and sterilized using an autoclave. Before introducing *Aeromonas hydrophila* into the growth media, the bacteria were diluted using a 10⁸ dilution factor (Maulidya *et al.*, 2017).

• LC₅₀ Test

The LC₅₀ (Lethal Concentration 50) test method used in this research was a modification of the methods from Kalor *et al.* (2019), Kumari *et al.* (2020), and Rosidah *et al.* (2018). The LC₅₀ test was conducted to determine the maximum concentration of octopus ink extract that causes 50% mortality in catfish after 24 hours of exposure. The method used in the LC₅₀ test involved oral administration by supplementing feed with octopus ink extract at doses of 0 ml/kg, 25 ml/kg, 50 ml/kg, 75 ml/kg, and 100 ml/kg (Kalor *et al.*, 2019), with three replications.

The test fish were randomly assigned to each aquarium, with 20 fish per aquarium. Before conducting the LC₅₀ test, the catfish were acclimatized. Mortality observations were recorded at intervals of 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, 24 hours, 36 hours, 48 hours, 72 hours, and 96 hours.

- **Supplementation of Octopus Ink Extract into Feed**

In this research, the feed used was commercial feed with a quantity of 1 kg per treatment. The commercial feed was mixed with the predetermined treatment doses in a tray. The mixing method followed the approach of Sukendar *et al.* (2021), where the liquid extract was sprayed onto 1 kg of feed using a spraying technique until evenly distributed. The feed was then dried for 15 minutes at room temperature or air-dried. After the drying process, the feed was weighed to provide 5% of the total biomass or fish weight per treatment or contain (Haetami *et al.*, 2023). Once weighed, the feed was stored in zipper plastic bags according to the specified treatment doses.

- **Administration of Octopus Ink Extract to Fish**

A total of 15 containers were prepared, each filled with 20 liters of water and equipped with aeration. Prior to use, all test fish underwent a 7-day acclimatization period to ensure their health, indicated by a good response to stimuli and feed, no deformities, and no damage to any part of their bodies. After acclimatization, the test fish were placed into aquariums at a density of 20 fish per container (Rosidah *et al.*, 2018). Following acclimatization, the fish were treated by being fed pellets supplemented with octopus ink extract for 21 days. The pellet feed was provided at 5% of the fish's body biomass, which was sampled every 10 days (Syahrizal *et al.*, 2016). Feeding was done *ad libitum*, meaning the feed was given according to the needs of the catfish juveniles based on their weight or biomass. The feed was administered during the maintenance period three times daily at 07:00 WITA, 12:00 WITA, and 17:00 WITA.

- **Preparation of *Aeromonas hydrophila* Bacterial Isolate**

The preparation of *A. hydrophila* bacteria at a density of 10⁸ CFU/ml (Karina *et al.*, 2015) for fish infection was carried out using Nutrient Agar (NA) as the bacterial growth medium. The culturing process began by taking a loopful of *A. hydrophila* bacterial sample from a slant agar medium. The sample was streaked onto an NA medium and incubated for 24 hours.

- **Infection with *Aeromonas hydrophila***

Test fish that had been treated with octopus ink extract for 21 days were subsequently infected with *A. hydrophila* bacteria at a density of 10⁸ CFU/mL (Maulidya *et al.*, 2017) on the 22nd day using an immersion method. Observations of test parameters were conducted from the first to the seventh day following the immersion process (Wahjuningrum *et al.*, 2013).

Research Parameters

- **Absolute Weight Growth**

Absolute weight growth is defined as the rate of weight increase in fish from the beginning to the end of the research. According to Ridwantara *et al.* (2019), absolute weight growth (W) can be calculated using the following formula:

$$W = W_t - W_0$$

Where:

W = Absolute weight growth (g)

W_t = Average fish weight at the end of the research (g)

W₀ = Average fish weight at the start of the research (g)

- **Absolute Length Growth**

Absolute length growth is calculated using the formula from Febri *et al.* (2020):

$$L = L2 - L1$$

Where:

L = Absolute length growth (cm)

L2 = Final length (cm)

L1 = Initial length (cm)

- **Specific Weight Growth Rate**

The weight specific growth rate (SWGR) is calculated using the formula referenced from Wicaksana *et al.* (2015):

$$SWGR = \frac{\ln W_t - \ln W_0}{T} \times 100\%$$

Where:

SWGR = Specific growth rate (% body weight per day)

W0 = Average fish weight at the start of the research (g)

Wt = Average fish weight at the end of the research (g)

T = Duration of the research (days)

- **Specific Length Growth Rate**

The specific length growth rate is calculated using the formula from Nurhidayat (2021):

$$SLGR = \frac{\ln L_t - \ln L_0}{T} \times 100\%$$

Where:

SLGR = Specific length growth rate (% length per day)

L0 = Average fish length at the start of the research (cm)

Lt = Average fish length at the end of the research (cm)

T = Duration of the research (days)

- **Feed Conversion Ratio**

The feed conversion ratio (FCR) is calculated using the formula referenced from Triwinarso *et al.* (2014):

$$FCR = \frac{F}{(W_{t+d}) - W_0} \times 100\%$$

Where:

FCR = Feed conversion ratio

W0 = Biomass weight of fish at the start of the research (g)

Wt = Biomass weight of fish at the end of the research (g)

F = Total feed consumed by the test fish (g)

d = Total weight of the test animals during the research (g)

- **Water Quality**

Water quality is one of the crucial factors in fish farming. During the maintenance period, water quality measurements were conducted to support the research data. The observed parameters included temperature, pH, and dissolved oxygen. Water quality measurements were carried out every 10 days, with a 10% water exchange performed to reduce impurities in the water (Sinaga *et al.*, 2021).

Data Analysis

The observational data were analyzed using Analysis of Variance (ANOVA) with SPSS software at a 5% significance level to determine the effect of treatments in the research. If the data showed significant effects, further analysis was conducted using Duncan's multiple range test with a 95% confidence level.

RESULTS

Absolute Weight Growth

Absolute weight is the increase in fish weight throughout the research, calculated as the difference between the fish's weight at the end and the beginning of the research. The results showed that the weight of catfish after immunostimulant administration ranged from 2.59-2.8 g. The absolute weight of catfish post-infection ranged from 0.61-1.26 g.

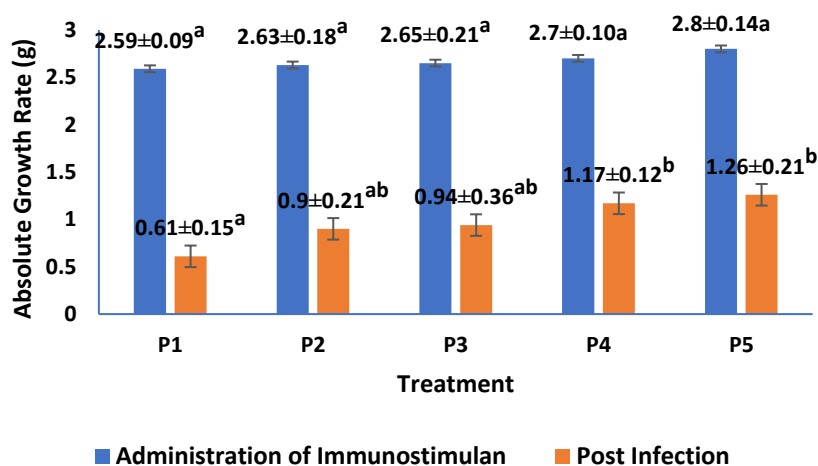


Figure 1. Absolute Growth Rate of Catfish (*Clarias* sp.)

Absolute Length Growth

Absolute length is the measurement of fish growth in length during the research, calculated as the difference between the fish's length at the end and the beginning of the research. The results of this research showed that the absolute length growth of catfish over 45 days of maintenance with different doses of octopus ink extract ranged from 2.73-5.03 cm. The absolute length of catfish post-infection ranged from 0.67-1.5 cm.

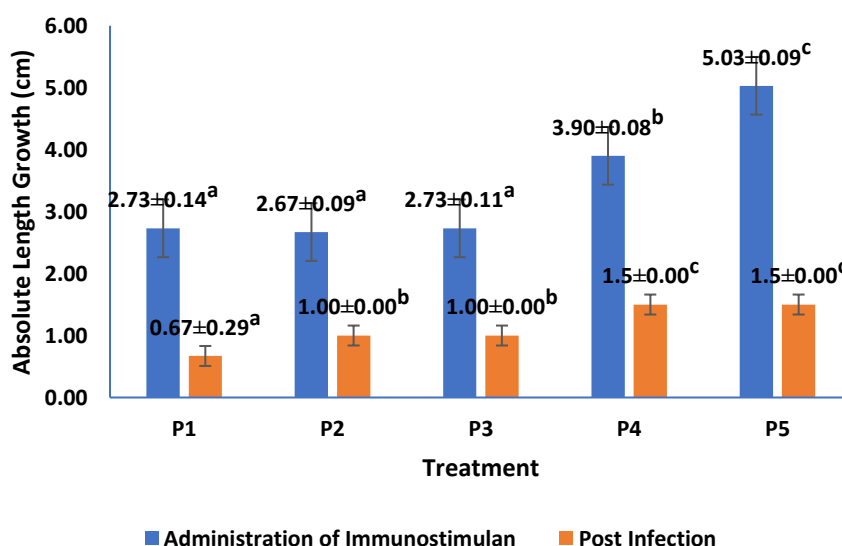


Figure 2. Absolute Length Rate of Catfish (*Clarias* sp.)

Specific Weight Growth Rate

The results of this research showed that the specific weight growth rate of catfish after immunostimulant administration ranged from 2.21-2.34%/day. The specific weight of catfish post-infection ranged from 0.13-0.57%/day.

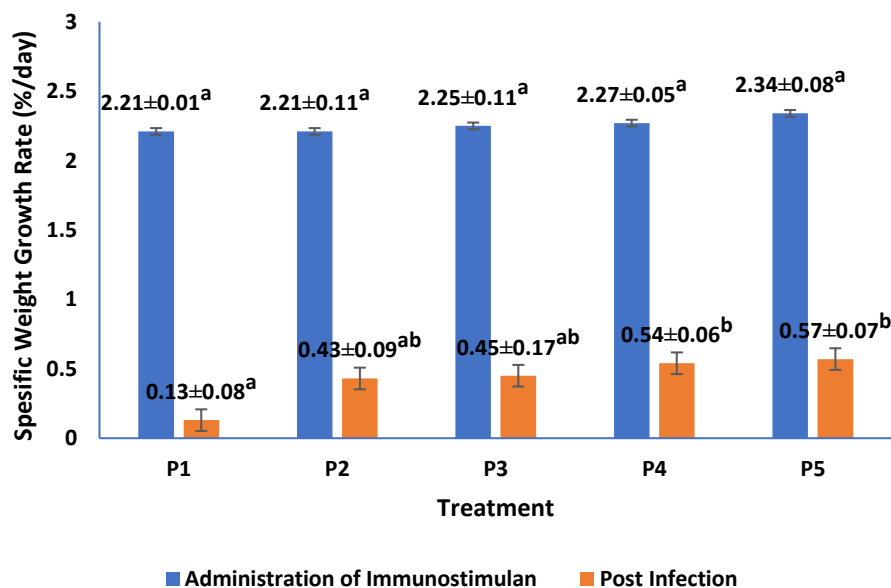


Figure 3. Specific Weight Growth Rate of Catfish (*Clarias* sp.)

Specific Length Growth Rate

The results of this research showed that the specific length growth rate of catfish after immunostimulant administration ranged from 0.88-1.39 %/day. The specific length growth rate of catfish post-infection ranged from 0.17-0.28 %/day.

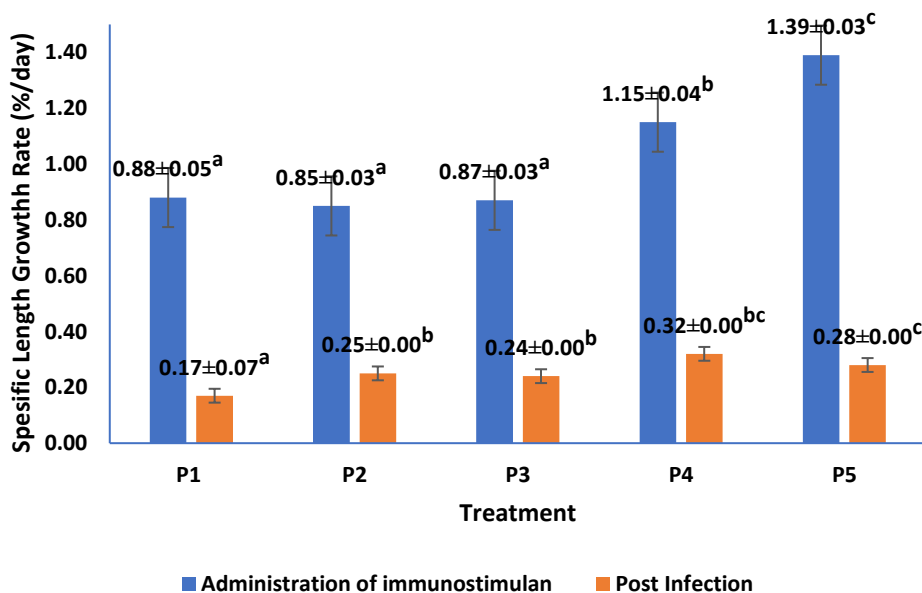


Figure 4. Specific Length Growth Rate of Catfish (*Clarias* sp.)

Feed Conversion Ratio

Based on this research, the feed conversion ratio (FCR) of catfish during the 45-day rearing period with the addition of different doses of octopus ink extract ranged from 1.66-1.97.

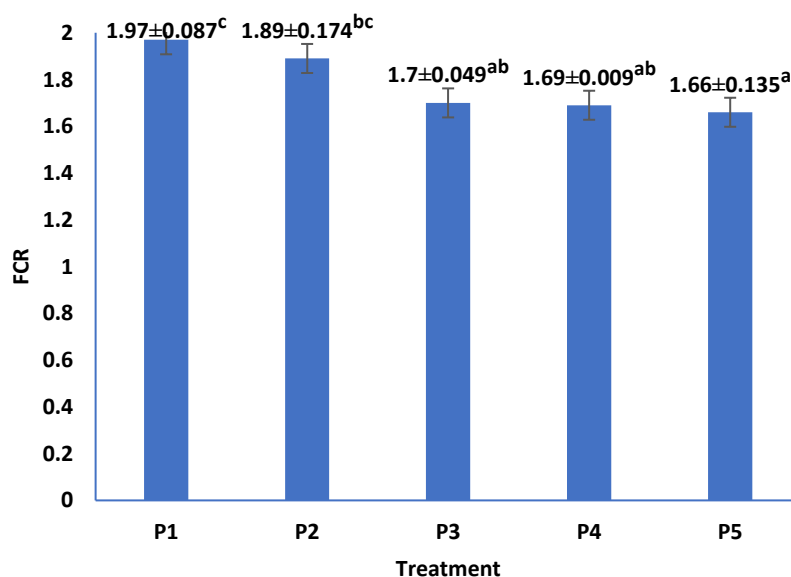


Figure 5. Feed Conversion Ratio of Catfish (*Clarias* sp.)

Water Quality

The results of water quality measurements are presented in Table 1. The measured water quality values during the research were used to determine the optimal range of water conditions suitable for the survival of catfish. The measured parameters included temperature, pH, and dissolved oxygen (DO).

Table 1. Water Quality

Treatment	Temperature (°C)	DO (mg/l)	pH
P1	28.3 – 28.8	6.8 – 7.0	7.4 – 7.6
P2	29 – 29.6	6.6 – 7.4	7.3 – 7.5
P3	30 – 30.3	6.7 – 7.1	7.5 – 7.6
P4	30.3 – 30.4	6.9 – 7.0	7.2 – 7.4
P5	29.3 – 29.7	6.9 – 7.0	7.6 – 7.7
References	28-32 (Patriono <i>et al.</i> , 2022)	>3 (Sugianti & Hafiludin, 2022)	6-8 (Nurhidayat, 2021)

DISCUSSION

Absolute Weight Growth

Absolute weight refers to the total weight gain of the fish during the research. Measuring absolute weight provides an indication of the fish's growth in terms of body weight, reflecting the increase in weight as a response to the treatments given. Based on the graph in figure 1 of this research, the administration of immunostimulants, specifically octopus ink extract, resulted in the highest absolute weight in P5 at 2.8 ± 0.14 g, and the lowest in P1 at 2.59 ± 0.09 g. The weight gain in catfish is suspected to be due to the additional protein content in the feed supplemented with octopus ink extract. According to Riyad *et al.* (2020), the ink

produced by cephalopods contains several components, such as fats, proteins, ash, and carbohydrates. The protein content in the feed can act as a primary energy source for the fish's growth process. This is supported by Awaludin *et al.* (2023), who explained that the protein content in the feed can enhance fish growth. The absolute weight of catfish post-infection was highest in P5 at 1.26 ± 0.21 g, while the lowest weight was found in P1 at 0.61 ± 0.15 g. The absolute weight of post-infection catfish with octopus ink extract was higher than in the control group. This is likely due to the presence of saponin compounds in octopus ink. After infection, fish require more energy to repair damaged tissues caused by pathogen attacks, thus reducing the energy available for weight growth. This aligns with the statement by Linayati *et al.* (2024), who explained that saponin acts as an antibacterial agent, helping maintain fish health and potentially stimulating growth.

Absolute Length Growth

Absolute length refers to the difference in the fish's length at the end of the research compared to the initial length before treatment. Based on the graph in figure 2 of this research, the highest absolute length growth in catfish after the administration of immunostimulants was found in P5 at 5.03 ± 0.09 cm, while the lowest was observed in P1 at 2.73 ± 0.14 cm. The highest absolute length of catfish post-infection was found in P5 at 1.5 ± 0.00 cm, and the lowest was in P1 at 0.67 ± 0.29 cm. The lower absolute length in P1 is attributed to the absence of bioactive compounds from octopus ink extract in the feed, meaning the fish relied solely on the protein content in the feed. This is consistent with Setiawati *et al.* (2013) who stated that the utilization of protein in the feed to support fish growth is influenced by factors such as fish size, protein quality, feed energy content, nutritional balance, feed intake rate, and the availability of limiting amino acids.

Specific Weight Growth Rate

Specific growth rate is a measure of fish growth expressed as the percentage increase in body weight per day. Based on the graph in figure 3, the highest specific weight growth rate after the administration of immunostimulants was found in P5 at 2.34 ± 0.08 %/day, while the lowest was in P1 at 2.21 ± 0.01 %/day. This is suspected to be due to the octopus ink extract content administered in each treatment. Gunawan & Rosdiana (2023) explained that ink produced by squids and cuttlefish contains melanin, proteins, fats, and essential amino acids (lysine, leucine, arginine, and phenylalanine). The lysine content in octopus ink, supplemented in the feed, can improve growth rate in catfish. Lysine functions in activating digestive enzymes, such as protease, which optimizes the fish's ability to digest protein and support growth. According to Sari *et al.* (2012), increased growth in fish indicates a healthy immune system.

The specific weight of post-infection catfish was lowest in P1 at 0.13 ± 0.08 %/day. The low value is suspected to be due to decreased appetite in catfish caused by the bacterial infection of *Aeromonas hydrophila*. Fish growth is highly influenced by the amount and quality of feed, as well as the rearing environment and the fish's condition. According to Jumina *et al.* (2024), the decrease in fish weight is likely caused by stress resulting from *A. hydrophila* infection. This infection negatively impacts the fish's immune system due to post-infection stress.

Treatment P5 had the highest specific weight value at 0.57 ± 0.07 %/day. The difference in specific weight values is due to the inclusion of octopus ink in the feed, which relates to the role of octopus ink extract as an immunostimulant, which is believed to enhance growth and immune function in catfish. Octopus ink contains alkaloid compounds. According to Syakirin *et al.* (2022), alkaloids function to disrupt bacterial cell membranes, thereby supporting

increased non-specific immune system activity in fish and inhibiting bacterial growth. Additionally, alkaloids have various benefits, including antimicrobial properties (Wulandari, 2018).

Specific Length Growth Rate

Specific length growth rate is a measure of fish growth expressed as the percentage increase in the length of catfish each day during the research. Based on the graph in Figure 4, the highest specific length growth rate after the administration of immunostimulants was found in P5 at 1.39 ± 0.03 %/day. The highest specific length growth rate of catfish post-infection was still in P5 at 0.28 ± 0.00 %/day. The high value in P5 is suspected to be due to the fact that, in addition to the feed, the fish also received protein from the octopus ink extract provided. This is supported by Gunawan & Rosdiana (2023), who explained that the ink produced by cephalopods contains melanin granules, with melanin being a melanoprotein that contains 10-15% protein, making it a good alternative source of protein.

Treatment P1 resulted in the lowest specific length growth rate after the administration of immunostimulants, at 0.88 ± 0.05 %/day. The lowest specific length growth rate of catfish post-infection was also found in P1 at 0.17 ± 0.07 %/day. The low specific length growth rate in P1 is suspected to be due to the absence of octopus ink extract. This resulted in a difference in the growth rate of catfish. According to Karimah *et al.* (2018), fish growth can be influenced by several factors, such as protein quality, energy content in the feed, and the nutritional balance of the feed provided.

Feed Conversion Ratio

Feed Conversion Ratio (FCR) is the amount of feed provided to achieve 1 kilogram of body weight in cultured fish. The lower the FCR value, the more efficient the feed is, meaning less feed is needed to optimize fish growth. Based on the graph in figure 5, the highest FCR value was found in P1 with an FCR of 1.97 ± 0.087 , while the lowest was in P5 with an FCR of 1.66 ± 0.135 . The FCR value continued to decrease with the increase in octopus ink extract dosage in each treatment. This suggests that the feed used for catfish growth is relatively efficient, with a high feed intake, enabling the fish's growth needs to be well-met. According to Tangguda *et al.* (2022), a low feed conversion ratio indicates that the applied feed quality is good. Ratulangi *et al.* (2022) added that in catfish farming, the optimal feed conversion ratio ranges between 1-2, and values close to this range indicate that the fish are utilizing the feed efficiently.

Although the FCR value obtained was optimal, the treatments with octopus ink extract resulted in lower FCR values compared to P1. This is suspected to be because the feed provided in these treatments contains lysine. Lysine in the feed aids in muscle growth, which contributes to increasing the fish's body weight. This is supported by Rachmawati *et al.* (2023) who explained that feed containing lysine promotes active hyperplasia and optimizes skeletal muscle growth. Treatment P4 had an FCR value that was not significantly different from P5, indicating that the dosage of octopus ink extract in P4 was optimal for increasing catfish weight, although there was only a slight decrease in FCR in P5. According to Putra *et al.* (2019) a low FCR value signifies that the feed is being utilized very efficiently as energy for growth in fish.

Water Quality

Water quality management is a critical factor in the success of aquaculture activities. Measuring water quality is essential to determine the optimal conditions for the growth and survival of cultured organisms. In this research, the measured water quality parameters included temperature, pH, and dissolved oxygen (DO). Based on table 1, the temperature

ranged from 28.3-30.4°C. The temperature values observed during the research were within the normal range. This is consistent with Patriono *et al.* (2022), who stated that the optimal temperature for supporting the growth and survival of catfish is between 28-32°C.

Another measured water quality parameter was dissolved oxygen (DO), which is essential for respiration and metabolism in fish, as well as for their survival. The dissolved oxygen levels obtained during the research ranged from 6.8-7.4 mg/L. The dissolved oxygen levels were within the optimal range for catfish growth. This is supported by Sugianti & Hafiludin (2022), who explained that dissolved oxygen levels greater than 3 mg/L are ideal for catfish growth.

Additionally, pH was measured as a water quality parameter. pH, or acidity level, refers to the amount of salts present in the water. Low pH values can negatively affect the organisms living in the water. During the research, pH values ranged from 7.2-7.7, which are still within the optimal range. According to Nurhidayat (2021), the ideal pH range for catfish growth is between 6.5-9.0.

CONCLUSION

The conclusions drawn from the research are as follows:

1. The addition of octopus ink extract in the feed of catfish significantly improved the growth parameters (absolute weight, absolute length, specific growth rate, and feed conversion ratio).
2. The best treatment was achieved at the dose of P5 (160 ml/kg), which resulted in absolute weight of 1.26 g, absolute length of 1.5 cm, feed conversion rate (FCR) 1.66 and specific growth rate of 0.57 %/day.

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