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

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ABSTRACT

Catfish (*Clarias* sp.) is an economically important freshwater aquaculture commodity in Indonesia. However, bacterial infections can compromise fish health and growth performance, highlighting the need for natural feed additives that support sustainable aquaculture. Octopus (*Octopus* sp.) ink, a fishery by-product containing nutrients and bioactive compounds with potential antioxidant and antimicrobial properties, represents a promising alternative. This study aimed to evaluate the effectiveness of octopus ink extract as a natural feed additive in improving the growth performance of catfish challenged with *Staphylococcus aureus*. A 50-day experiment was conducted using a completely randomized design with five treatments and three replicates. The treatments comprised K+ (infected fish without extract), K− (uninfected fish without extract), and three extract supplementation levels: A (25 mL/kg feed), B (50 mL/kg feed), and C (75 mL/kg feed), with all supplemented groups subjected to bacterial infection. Growth performance was assessed through absolute weight gain, absolute length gain, specific weight growth rate, and specific length growth rate. Octopus ink extract supplementation significantly affected growth performance ($P < 0.05$). Treatment C produced the highest absolute weight gain (8.50 ± 0.06 g), absolute length gain (5.18 ± 0.33 cm), and specific length growth rate (1.27 ± 0.07%/day), whereas treatment B achieved the highest specific weight growth rate (2.75 ± 0.14%/day). These findings suggest that octopus ink extract has potential as a natural feed additive for catfish, with 75 mL/kg feed providing the most favorable overall growth performance under the experimental conditions.

INTRODUCTION

Catfish (*Clarias* sp.) is a freshwater fish species widely cultivated in Indonesia. The Ministry of Marine Affairs and Fisheries (KKP) recorded that catfish production in Indonesia reached 1.12 million tons in 2022. Catfish is widely cultured because of its relatively rapid growth compared with other freshwater fish species, ease of maintenance, and suitability for cultivation in limited spaces. Catfish also offers several advantages as an aquaculture

commodity, including the ability to survive in various freshwater environments, relatively high disease resistance, and an omnivorous feeding habit, which makes it relatively easy to culture at low production costs. Although catfish can consume a wide variety of food sources, the use of formulated pellet feed remains essential in intensive aquaculture systems. Feed is one of the largest components of production costs in catfish farming, accounting for approximately 60–70% of total production costs. Therefore, feed innovation is an important strategy for improving aquaculture production efficiency (Aldo, 2019).

One strategy that can be implemented to improve fish growth and health in aquaculture is the incorporation of feed additives. These substances play a role in improving feed quality, optimizing nutrient utilization, and supporting immune responses, growth, and fish survival (Vijayaram *et al*., 2022). In recent years, natural feed additives have received increasing attention as potential alternatives for supporting more sustainable aquaculture practices by reducing dependence on synthetic additives and antibiotics.

Octopus ink is a fishery by-product with potential for use as a natural feed additive because it contains nutritional components and bioactive compounds. Octopus ink has a chemical composition consisting of 8.32 ± 0.042% protein, 0.08 ± 0.0018% fat, 6.33 ± 0.306% carbohydrates, and 2.79 ± 0.014% ash (Kipalahi et al., 2024). Its protein and amino acid content may support fish growth, as protein contributes to body tissue formation, while amino acids are required for protein synthesis and various metabolic processes. In addition to its nutritional value, octopus ink also has potential as a source of natural immunostimulants in aquaculture. (Affandi *et al*., 2025) reported that octopus ink extract exhibits antioxidant activity, with an IC50 value of 94.4661 ppm, and contains several secondary metabolites, including alkaloids, saponins, phenols, and steroids. The presence of these compounds indicates potential biological activities relevant to fish health, including antioxidant and antimicrobial effects. Therefore, the use of octopus ink as a feed additive may support fish growth and improve resistance to health challenges in aquaculture.

Therefore, research on the use of octopus ink as a natural feed additive in catfish feed is important to support increased aquaculture productivity, reduce dependence on synthetic feed additives, and promote the sustainable utilization of fishery by-products.

METHODS

Time and Place

This research was conducted over 50 days, from September to October 2026. The research was carried out 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 tools used in this research included aerators, autoclave, 45 L containers, corning tubes, digital scale, erlenmeyer, hot plate, inoculation needles, micropipettes, microtubes, nets, petri dishes, rotary evaporator, spoon, spray botle and vortex mixer. The materials used in this research were *Staphylococcus aureus*, aluminum foil, alcohol, distilled water, freshwater, Catfish (measuring 4-6 cm), methanol, NaCl solution, NA media, Octopus ink, TSB media, NA media, soap, pellets, and tissue paper.

Research Design

The method used in this study was an experimental method with a completely randomized design (CRD) consisting of five treatments and three replications, resulting in 15 experimental units. Each experimental unit used 20 Catfish.

K+ (Control +) : Feed without octopus ink extract + bacterial infection
K- (Control -) : Feed without octopus ink extract (without bacterial infection)
A : Feed supplemented with 25 mL/kg octopus ink extract + bacterial infection
B : Feed supplemented with 50 mL/kg octopus ink extract + bacterial infection
C : Feed supplemented with 75 mL/kg octopus ink extract + bacterial infection

Research Procedures

- **Preparation of Rearing Containers and Media**

A total of 15 rearing containers, each with a capacity of 45 L, were used in this study. Before use, the containers were washed with detergent, rinsed thoroughly with clean water, and sun-dried. Once dry, the containers were transferred to the research location and filled with 20 L of water. Each container was equipped with an aeration unit to ensure adequate oxygen supply. Finally, the containers were covered and labeled according to their respective treatment groups.

- **Preparation of Test Animals**

The experimental fish used in this study were catfish measuring 4–6 cm in length, sourced from the Batu Kumbung Fish Seed Center (BBI), located in Lingsar District, West Lombok Regency, West Nusa Tenggara, Indonesia. Prior to stocking, the fish underwent an acclimatization period to allow them to adjust to the new rearing environment. This process is essential for minimizing stress and reducing the risk of mortality during the study (Augusta, 2012)

- **Preparation of Octopus Ink Extract**

Octopus ink was obtained from an octopus processing facility or warehouse located in the Mataram City area. The ink sacs were carefully dissected, and the ink was collected for further extraction. The extraction process was conducted using the maceration method, in which the collected ink was mixed with methanol at a ratio of 1:3, using 1,500 mL of methanol as the solvent. The mixture was placed in an Erlenmeyer flask and stored in a refrigerator at 4°C for 7 days (Sabathini *et al*., 2026). After maceration, the extract was concentrated through evaporation using a rotary evaporator operated at 62 rpm for 5 hours.

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

Commercial feed was used in this study at a rate of 1 kg per treatment. Octopus ink extract was incorporated into the feed by spraying it uniformly over the feed surface to ensure even distribution. The feed was then left to air-dry at room temperature before use.

- **Challenge Test with *Staphylococcus aureus***

The challenge test was carried out to assess the immune response of catfish after receiving octopus ink extract as a natural feed additive. Bacterial infection was induced through the immersion method by introducing a suspension of *Staphylococcus aureus* into the rearing water. The bacterial isolate was obtained from the Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram. The bacterial suspension was prepared at a concentration of 10^8 cells/mL, which has been reported to cause infection symptoms and mortality in fish within 24 hours (Nuryati *et al*., 2024). Following bacterial exposure, the fish were monitored regularly to observe changes in immune responses, survival rates, and clinical signs associated with *S. aureus* infection. All research parameters were evaluated on the 14th day following the challenge test.

- **Absolute Weight**

Absolute weight was calculated to measure the increase in fish weight during the rearing period. The formula for absolute weight according to Nazlia & Zulfiadi (2018) is as follows:

$$W = Wt - W0$$

Where:
W : Absolute weight gain
Wt : Average fish weight at the end of rearing
W0 : Average fish weight at the beginning of rearing

- **Absolute Length**

Absolute length was calculated to measure the increase in fish length during the rearing period. The formula for absolute length according to Mata *et al*. (2022) is as follows:

$$L = Lt - L0$$

Where:
L : Absolute length gain (cm)
Lt : Average final length (cm)
L0 : Average initial length (cm)

- **Specific Weight Growth Rate**

Specific length growth rate represents the percentage increase in fish length per day during the rearing period. The calculation of specific length growth rate follows the formula proposed by Wicaksana *et al*. (2015), as presented below:

$$SWGR = \frac{(\ln Wt - \ln Wo)}{t} \times 100\%$$

Where:
SWGR : Specific weight growth rate (%/day)
W0 : Average fish weight at the beginning of rearing (g)
Wt : Average fish weight at the end of rearing (g)
t : Rearing period (days)

- **Specific Length Growth Rate**

Specific length growth rate (SLGR) indicates the daily percentage increase in fish length throughout the rearing period. It is calculated based on the formula described by Sitio *et al*. (2017), as follows:

$$SLGR = \frac{(\ln Lt - \ln Wo)}{t} \times 100\%$$

Where:
SLGR : Specific length growth rate (%/day)
L0 : Fish length at the beginning of rearing (cm)
Lt : Fish length at the end of rearing (cm)
t : Rearing period (days)

- **Data Analysis**

The data obtained were statistically analyzed using SPSS version 16.0. Analysis of variance (ANOVA) was performed at a 95% confidence level ($p < 0.05$). If significant differences were found, further analysis was conducted using Duncan's test.

RESULTS

Absolute Weight

Based on the research results, the absolute weight gain of catfish during the 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 6.15 to 8.05 g.

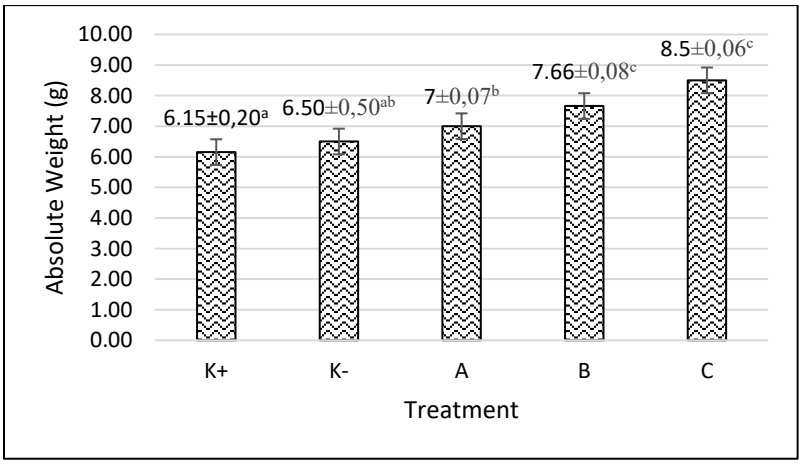


Figure 1. Absolute Weight of Catfish (*Clarias* sp.)

Absolute Length

Based on the research results, the absolute length gain of catfish during the 50-day rearing period, with feed supplemented with octopus ink extract, ranged from 4.08 to 5.18 cm.

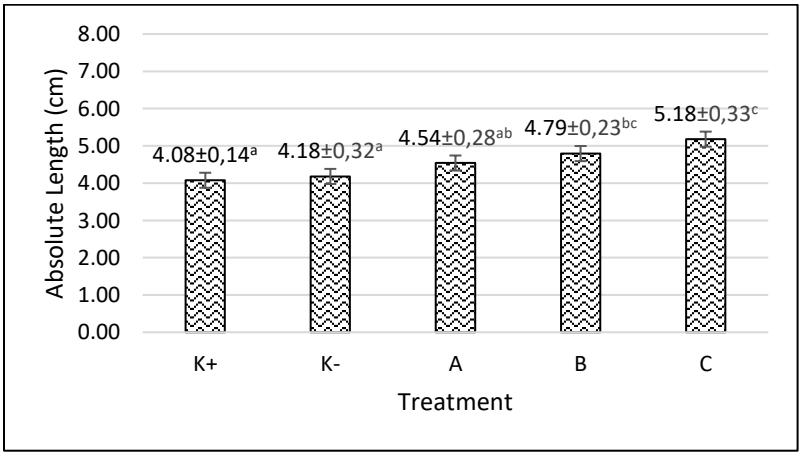


Figure 2. Absolute Length of Catfish (*Clarias* sp.)

Specific Weight Growth Rate

Based on the research results, the specific weight growth rate gain of catfish during the 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 2.29 to 2.75%/day.

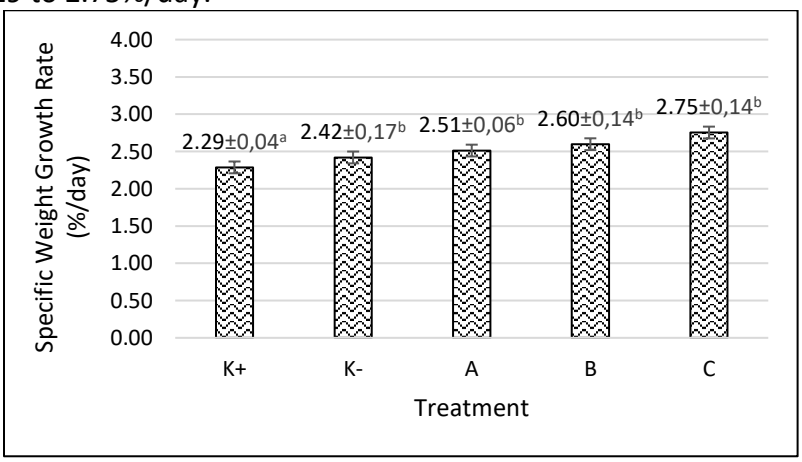


Figure 3. Specific Weight Growth Rate of Catfish (*Clarias* sp.)

Specific Length Growth Rate

Based on the research results, the specific length growth rate gain of catfish during the 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 1.07 to 1.27%/day.

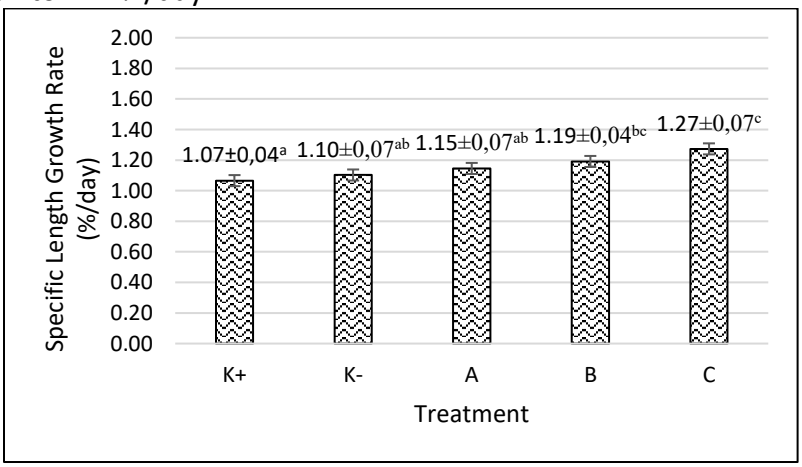


Figure 4. Specific Length Growth Rate of Catfish (*Clarias* sp.)

DISCUSSION

Absolute Weight

Based on the research findings, the highest absolute length of catfish (*Clarias* sp.) was obtained in treatment C, with the addition of octopus ink extract at a dose of 75 mL/kg of feed, reaching 5.18 ± 0.33 cm. The high absolute length observed in treatment C was presumably associated with the potential nutritional content and bioactive compounds present in octopus ink. Anh *et al*. (2021) reported that ink from several cephalopod species, including octopus (*Amphioctopus aegina*), contains protein. Furthermore, Jeyasanta & Patterson (2019) explained that squid ink extract (*Loligo duvauceli*) contains protein and various bioactive compounds, including amino acids. Protein is an essential nutrient that provides amino acids required for protein synthesis and body tissue formation. In fish growth, these processes play an important role in muscle tissue development and increased body size, including length increment.

In addition to its nutritional content, the high absolute length observed in treatment C was also presumed to be associated with the potential antioxidant activity of bioactive compounds in octopus ink. Khandelwal *et al*. (2023) reported that squid ink extract (*Sepioteuthis lessoniana*) exhibits antioxidant activity through its free radical scavenging capacity. This activity may help reduce the effects of oxidative stress, which can interfere with cellular functions and metabolic processes. Maintaining physiological homeostasis may allow metabolic processes and nutrient utilization to function effectively, thereby enabling nutrients from feed to support the formation and development of fish body tissues.

Absolute Length

Based on the research findings, the highest absolute length of catfish (*Clarias* sp.) was obtained in treatment C, with the addition of octopus ink extract at a dose of 75 ml/kg feed, reaching 5.18 ± 0.33 cm. This value was higher than those obtained in treatments K+, K−, A, and B. The higher absolute length observed in treatment C was presumably associated with the potential nutritional content and bioactive compounds present in octopus ink. Saputra *et al*. (2025) explained that octopus ink has the potential to be utilized as an additional nutritional source because it contains several components, including protein, fat, ash, and carbohydrates.

These nutritional components may contribute to improving the quality of feed consumed by fish. Protein, in particular, is an essential nutrient that provides amino acids for protein synthesis, muscle tissue formation, and body tissue growth. This is consistent with the findings of Saputra *et al*. (2025), who reported that the addition of octopus ink extract to catfish feed resulted in the best growth performance at a dose of 160 ml/kg feed, based on absolute weight gain and specific growth rate parameters.

In addition to its nutritional content, the higher absolute length observed in treatment C was also presumably associated with the presence of bioactive compounds in octopus ink, particularly melanin. Derby (2014) explained that melanin is one of the main components responsible for the black coloration of cephalopod ink. Liu *et al*. (2023) also reported that melanin derived from squid ink possesses free radical-scavenging properties. This antioxidant activity may help reduce the effects of oxidative stress, which can interfere with cellular functions and physiological metabolic processes. Maintaining physiological conditions may allow metabolic processes to function properly, enabling nutrients from feed to be utilized to support fish growth.

Specific Weight Growth Rate

Specific weight 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 during the rearing period was observed in treatment B, with an average value of 2.75%/day. Meanwhile, the lowest value was recorded in treatment K+, at 2.29%/day. The high value was presumably associated with the potential bioactive compounds present in octopus ink, which may support the physiological condition of fish. Sabry *et al*. (2023) explained that cephalopod ink contains various bioactive compounds, including melanin, oligopeptides, polyphenols, flavonoids, alkaloids, polysaccharides, and fatty acids, which exhibit various biological activities. The presence of these compounds indicates that octopus ink has the potential to be utilized as a source of bioactive substances that may support the physiological functions of aquatic organisms.

Furthermore, the high specific weight growth rate observed in treatment C was presumed to be associated with the potential antioxidant activity of melanin found in octopus ink. According to Guo *et al*. (2014), squid ink melanin exhibits superoxide and hydroxyl radical scavenging activities. These antioxidant activities may help reduce the effects of oxidative stress, which can interfere with cellular functions and metabolic processes. Maintaining physiological homeostasis may allow metabolic processes to function properly, thereby enabling nutrients from feed to be utilized more efficiently to support fish growth.

Specific Length Growth Rate

Based on the research findings, the highest specific length growth rate of catfish (*Clarias* sp.) was obtained in treatment C, with the addition of octopus ink extract at a concentration of 75 mL/kg of feed, reaching 1.27 ± 0.07%/day. This value indicates an increasing trend compared with treatments K+, K−, A, and B. The high specific length growth rate observed in treatment C was presumably associated with the contribution of additional nutrients and bioactive compounds contained in octopus ink. Langi *et al*. (2024)explained that the growth of African catfish (*Clarias gariepinus*) is influenced by the nutritional adequacy of feed, particularly protein, lipids, carbohydrates, vitamins, minerals, and amino acids. The availability of nutrients that meet fish requirements plays an important role in supporting body growth and development.

Furthermore, the high specific length growth rate in treatment C was presumed to be associated with the antioxidant activity of melanin compounds found in cephalopod ink. Le *et*

al. (2024) reported that melanin extracted from cuttlefish ink (*Sepia pharaonis*) was capable of reducing intracellular reactive oxygen species (ROS) levels and increasing the activity of antioxidant enzymes.

CONCLUSION

Based on the research findings, the addition of octopus ink extract to catfish (*Clarias* sp.) feed had a significant effect on growth performance. This was demonstrated by increases in absolute weight gain, absolute length gain, specific weight growth rate, and specific length growth rate. The best growth performance was obtained in treatment C, with an octopus ink extract dose of 75 mL/kg of feed. Therefore, octopus ink extract has the potential to be utilized as a natural feed supplement to improve the growth performance of catfish (*Clarias* sp.).

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