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Effectiveness of Octopus (*Octopus* sp.) Ink Extract on the Growth of Nile Tilapia (*Oreochromis niloticus*) Infected with *Staphylococcus aureus*

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ABSTRACT

Octopus ink contains bioactive compounds with the potential to serve as natural immunostimulants in aquaculture. This study aimed to evaluate the effect of supplementing feed with octopus (*Octopus* sp.) ink extract on the growth performance of Nile tilapia (*Oreochromis niloticus*) infected with *Staphylococcus aureus*. A completely randomized design was employed, consisting of five treatments with three replicates each: a positive control (K+), a negative control (K−), and three experimental groups receiving octopus ink extract at doses of 50 (A), 100 (B), and 150 mL/kg of feed (C). The fish were reared for 50 days, and growth performance was assessed based on absolute weight gain, absolute length gain, specific growth rate (SGR), and specific length growth rate (SLGR). The results indicated that treatment B yielded the highest numerical values for all growth parameters, with an absolute weight gain of 9.64 ± 0.32 g, an absolute length gain of 4.03 ± 0.32 cm, an SGR of 2.48 ± 0.03%/day, and an SLGR of 2.27 ± 0.03%/day. No significant difference was observed between treatments B and C regarding SLGR. These findings suggest that supplementing feed with octopus ink extract—specifically at a dose of 100 mL/kg—has the potential to enhance the growth performance of Nile tilapia infected with *S. aureus*. Consequently, octopus ink extract may be considered a promising natural feed additive for Nile tilapia aquaculture.

INTRODUCTION

Nile Tilapia (*Oreochromis niloticus*) is one of Indonesia's leading aquaculture commodities with high economic value. This species is widely cultured due to its relatively easy farming practices and its ability to thrive under various environmental conditions. Its high adaptability makes tilapia a popular species for large-scale aquaculture production (Indriati & Hafiludin, 2022). These characteristics contribute to the continuously increasing demand and relatively stable market value of tilapia. Indonesia is the world's second-largest tilapia producer, contributing 25.9% of total global tilapia production (Gustiano *et al*., 2023). This

condition highlights the significant role of tilapia as an important fishery commodity in the Indonesian economy.

The growth of tilapia is influenced by various factors, including both internal and external factors. External factors include stocking density, feeding frequency and quantity, temperature, pH, dissolved oxygen, and water quality. These factors can affect body weight, feed consumption, feed utilization efficiency, fish health, and survival. Therefore, proper water quality management and appropriate feeding practices are essential aspects of supporting optimal tilapia growth (El-Hack *et al*., 2022).

In addition to environmental and dietary factors, fish health also plays an important role in successful aquaculture production. Infections caused by pathogenic microorganisms can pose significant challenges to aquaculture productivity. One of the bacterial pathogens reported in tilapia is *Staphylococcus aureus*. Mohammed *et al*. (2020) reported the presence of *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in tilapia (*O. niloticus*) samples collected in Maiduguri, Nigeria. This finding indicates that the presence of this bacterium should be considered in fish health management.

Maintaining fish health can be achieved by enhancing the body's defense mechanisms. One approach that can be applied is the administration of immunostimulants. Immunostimulants are substances capable of stimulating immune responses, thereby potentially helping fish withstand pathogen exposure. Wang *et al*. (2023) explained that the use of herbal ingredients, probiotics, prebiotics, and various immunostimulant products represents several strategies that can be applied to improve fish health and immune responses in tilapia.

One natural material with potential for development as an immunostimulant candidate is octopus ink (*Octopus* sp.). Octopus ink is a fishery by-product that has considerable potential for utilization in aquaculture. Research conducted by Affandi *et al*. (2025) demonstrated that octopus ink extract contains bioactive compounds, including alkaloids, saponins, phenols, and steroids, and exhibits antioxidant activity with an LC_{50} value of 94.4661 ppm. These findings indicate the potential of octopus ink as an immunostimulant candidate, although further investigations involving aquatic organisms are required to establish its effectiveness.

Based on this potential, further research on the use of octopus ink (*Octopus* sp.) as a natural immunostimulant in tilapia (*O. niloticus*) challenged with *Staphylococcus aureus* is necessary. This study aims to evaluate the effects of octopus ink administration on the disease resistance and growth performance of tilapia. The findings are expected to provide scientific information regarding the potential utilization of octopus ink as a natural ingredient to support fish health and productivity in aquaculture.

METHODS

Time and Place

This research was conducted over a period of 50 days, from September to October 2026, at the Fish Production and Reproduction Laboratory and Fish Health Laboratory, Department of Fisheries and Marine Science, University of Mataram.

Tools and Materials

The tools used in this research included aerator, autoclave, container racks, digital scale, Erlenmeyer, filter paper, fish nets, haemocytometer, hot plate, inoculating loop, microhematocrit tubes, microscope, microscope slides and cover glasses, microtubes, micropipettes, Petri dishes, rotary evaporator, siphon hoses, and syringe.

The materials used in this research were commercial pellets, distilled water, freshwater, methanol, Nile tilapia (measuring 5-6 cm), NaCl solution, Nutrient Agar (NA) media, octopus ink, soap, *Staphylococcus aureus* bacteria, tissue paper, and Tryptic Soy Broth (TSB) media.

Research Design

The method applied in this study is an experimental method using a Completely Randomized Design (CRD), consisting of five treatments with three replications, resulting in a total of 15 experimental units. Each treatment unit used 20 Nile tilapia. The treatments involve administering octopus ink extract through the feed

K+ (Positive Control) : Feed without octopus ink extract + infected with *S. aureus*
K- (Negative Control) : Feed without octopus ink extract (without bakterial infection)
A : Treatment dose 50 mL/kg feed + *Staphylococcus aureus* infection
B : Treatment dose 100 mL/kg feed + *Staphylococcus aureus* infection
C : Treatment dose 150 mL/kg feed + *Staphylococcus aureus* infection

Research Procedures

- **Preparation of Rearing Containers and Media**

The rearing used in this study were of 15 containers with a capacity of 45 L. Before use, the containers were cleaned with detergent, rinsed with water, and left to dry for 24 hours. After drying, the containers were transferred to the research site and filled with 20 L of water each. Each container was equipped with one aerator to provide an adequate oxygen supply to the water. Subsequently, the containers were covered and labeled according to their respective treatments.

- **Preparation of Test Animal**

The experimental animals used in this study were Nile tilapia (*O. niloticus*), measuring 5-6 cm in length and weighing approximately 1–2 g. The fish were obtained from the Fish Seed Center (BBI) Lingsar, Lingsar District, West Lombok Regency. The preparation of the experimental fish began with a 14-day acclimatization period before the fish were stocked in the rearing containers

- **Preparation of Octopus Ink Estract**

The preparation of octopus ink extract began with the collection of ink sacs from Octopus (*Octopus* sp.) obtained from octopus processing factories or warehouses around Mataram City. The ink sacs were carefully removed, cut open, and the ink was collected. The collected ink was then transferred into sterile containers.

The extraction process began with maceration, which involves soaking the octopus ink in a solvent. The octopus ink was mixed with the solvent at a ratio of 1:3. The solvent used in this study was 1,500 mL of methanol. The Erlenmeyer flask was then stored in a refrigerator at 4°C for 7 days (Affandi *et al*., 2019). After seven days, the extract was evaporated using a rotary evaporator at a speed of 62 rpm for 5 hours.

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

The feed used in this study was commercial pellet feed, with a total amount of 1 kg for each treatment. The commercial feed was subsequently mixed with the predetermined treatment doses in a tray. The mixing method referred to the study by Sukendar *et al*. (2021), in which the liquid extract was sprayed evenly onto 1 kg of feed using a spraying technique. The feed was then dried for 15 minutes at room temperature by air-drying.

After the drying process, the feed was weighed at a rate of 5% of the total biomass or body weight of the fish in each treatment or rearing container (Hendriana *et al*., 2023). After weighing, the feed was stored in zipper plastic bags according to the predetermined treatment doses.

- **Fish Rearing Process**

After the acclimatization period was completed, the experimental fish were given treatment feed supplemented with octopus ink extract for 49 days. The pellet feed was administered at a rate of 5% of the fish's body biomass, with the fish weight measured once every seven days (Pereira *et al*., 2026). Feeding was carried out ad libitum, meaning that the amount of feed was adjusted to the nutritional requirements of the tilapia based on their body weight or biomass. The feed was provided three times a day at 07:00, 12:00, and 17:00 Central Indonesia Time (WITA) throughout the rearing period.

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

The challenge test was conducted to evaluate the response of tilapia following the administration of natural feed additives derived from octopus ink extract. The challenge test was performed using the immersion method, in which a bacterial suspension of *S. aureus* was added directly to the rearing water. The *S. aureus* bacteria used in this study were obtained from the Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram. The bacterial suspension had a density of 10^8 cells/mL. The experimental fish were fed diets supplemented with octopus ink extract for 35 days. On the 36th day, the fish were challenged with *S. aureus* at a bacterial density of 10^8 CFU/mL, following the method described by Maulidya *et al*. (2017), using the immersion method. The experimental parameters were observed on the 14th day after bacterial immersion.

Research Parameters

- **Absolute Weight**

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

$$W = Wt - W0$$

Where:
W = Absolute weight growth (g)
Wt = Average fish weight at the end of the rearing period (g)
W0 = Average fish weight at the beginning of the rearing period (g)

- **Absolute Length**

Absolute length growth was calculated using the formula proposed by Effendie (2002), as cited in (Wiranda *et al*., 2025), as follows:

$$L = Lt - L0$$

Where:
L = Absolute length growth (cm)
Lt = Final fish length (cm)
L0 = Initial fish length (cm)

- **Specific Weight Growth Rate**

The specific growth rate was calculated using the formula adopted from (Monier *et al*., 2025), as follows:

$$SWGR = \frac{\text{Ln Wt} - \text{Ln Wo}}{T} \text{x } 100\%$$

Where:
SGR = Specific growth rate (% body weight per day)
W0 = Average fish weight at the beginning of the rearing period (g)
Wt = Average fish weight at the end of the rearing period (g)
T = Rearing period (days)

- **Specific Length Growth Rate**

The specific length growth rate was calculated using the formula adopted from (Rezki *et al*., 2026), as follows:

$$\text{SLGR} = \frac{\text{InLt} - \text{InLo}}{\text{t}} \text{x } 100\%$$

Where:

SLGR = Specific length growth rate (% body length per day)
L0 = Average fish length at the beginning of the rearing period (cm)
Lt = Average fish length at the end of the rearing period (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 result, the absolute weight gain of nile tilapia over a 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 6.91 to 9.64 g.

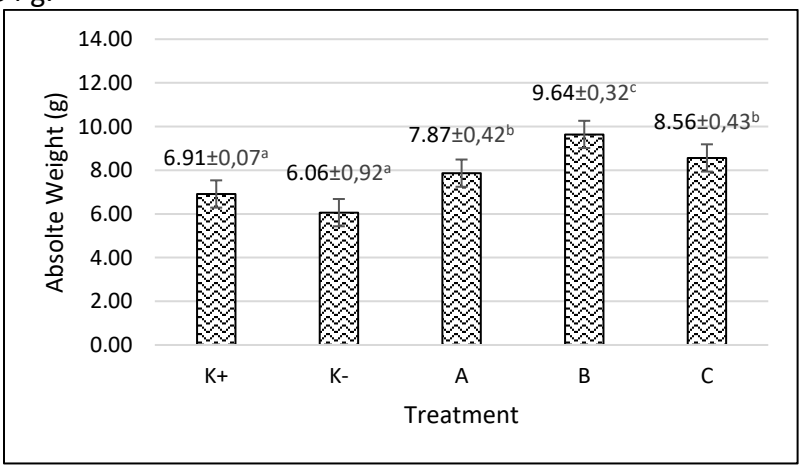


Figure 1. Absolute Weight of Nile Tilapia (*O. niloticus*)

Absolute Length

Based on the research result, the absolute length gain of nile tilapia over a 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 2.27 to 4.03 cm.

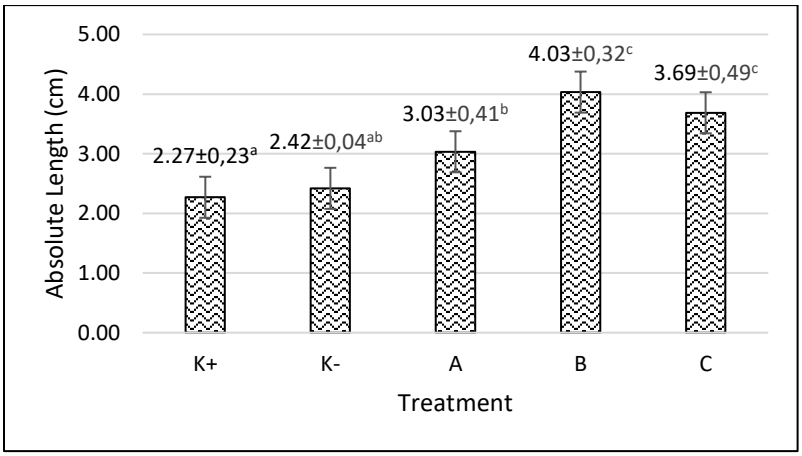


Figure 2. Absolute Length of Nile Tilapia (*O. niloticus*)

Specific Weight Growth Rate

Based on the research result, the specific weight growth rate gain of nile tilapia over a 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 2.21 to 2.48%/day.

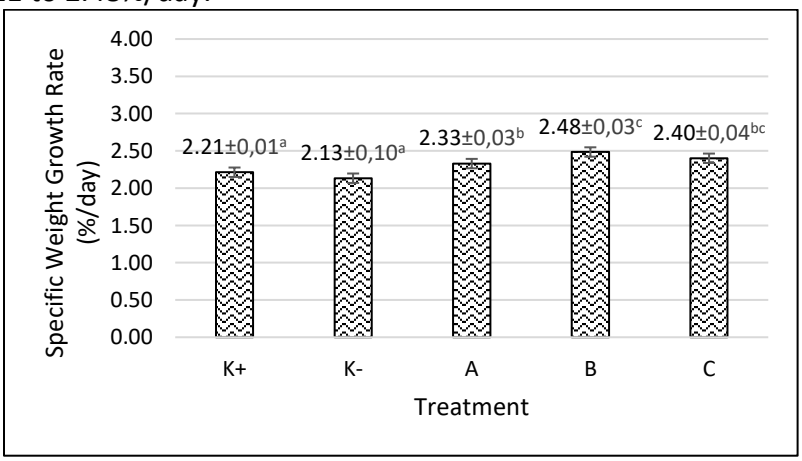


Figure 3. Specific Weight Growth Rate of Nile Tilapia (*O. niloticus*)

Specific Length Growth Rate

Based on the research result, the specific weight growth rate gain of nile tilapia over a 50-day rearing period, following supplementation with different doses of octopus ink extract, ranged from 2.06 to 2.27%/day.

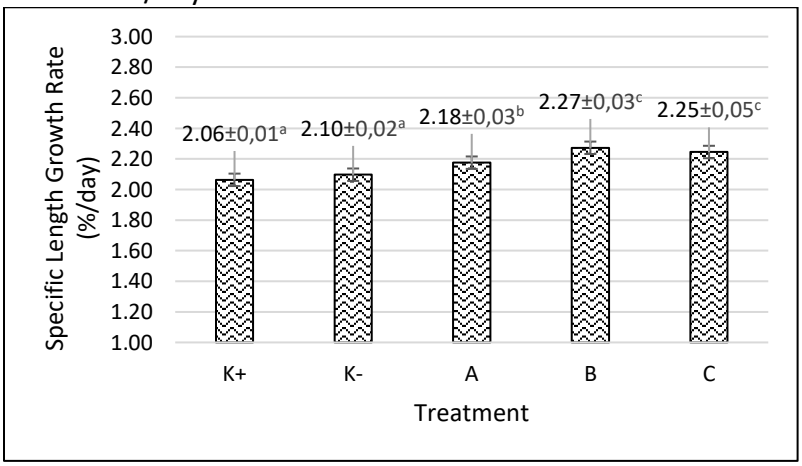


Figure 4. Specific Length Growth of Nile Tilapia (*O. niloticus*)

DISCUSSION

Absolute Weight

Based on the research findings, the highest absolute weight gain in nile tilapia (*O. niloticus*) was recorded in treatment B, which received octopus ink extract at a concentration of 100 mL/kg of feed, reaching 9.64 ± 0.32 g. The higher weight gain in this treatment was presumably influenced by the bioactive compounds contained in octopus ink. These compounds may help maintain fish health and physiological stability. Maintaining a stable physiological condition may enable fish to utilize dietary nutrients more efficiently, allowing the nutrients obtained from feed to support growth processes and increase body weight. Furthermore, the administration of octopus ink extract at an appropriate concentration is presumed to contribute to a positive growth response in fish.

This explanation is consistent with the findings of Affandi *et al*. (2025), who reported that octopus ink extract contains several bioactive compounds with potential applications as immunostimulants in aquaculture. These compounds are also associated with various biological activities, including antioxidant and antimicrobial properties. Further support is provided by Sabathini *et al*. (2026), who reported that dietary supplementation with octopus ink extract at a dose of 100 mL/kg of feed resulted in the best growth performance in Mozambique tilapia (*Oreochromis mossambicus*) infected with *Aeromonas hydrophila*. These findings indicate the potential use of octopus ink extract to support fish growth, although the previous study used a different fish species from the Nile tilapia (*O. niloticus*) examined in the present study.

Absolute Length

The measurement of absolute length growth in Nile tilapia (*O. niloticus*) showed that treatment B, supplemented with octopus ink extract at 100 mL/kg of feed, achieved the highest value of 4.03 ± 0.32 cm, while the lowest value was recorded in treatment K+ at 2.27 ± 0.23 cm. The higher growth observed in treatment B is presumed to be associated with bioactive compounds in octopus ink that may help maintain fish health and physiological condition. Imran *et al*. (2023) reported that Octopus vulgaris ink contains proteins and peptides associated with immune defense and protection against oxidative stress. Furthermore, Imran *et al*. (2023) identified antioxidant and antibacterial activities in octopus ink through in vitro assays.

The highest value in treatment B suggests that the dose of 100 mL/kg of feed produced the best numerical growth response. This is consistent with Mohammady *et al*. (2022), who reported that dietary phytogenic supplementation in Nile tilapia can support growth, feed utilization, and immune and antioxidant responses. However, in treatment C, which received 150 mL/kg of feed, absolute length growth decreased to 3.69 ± 0.49 cm. This suggests that increasing the extract dosage does not necessarily lead to greater growth. Although treatment B had the highest mean value, it was not significantly different from treatment C because both shared the same superscript letter. Therefore, the 100 mL/kg dose produced the highest numerical absolute length growth in this study.

Specific Weight Growth Rate

The results showed that the highest specific growth rate (SGR) was recorded in treatment B, which received octopus ink extract at a dose of 100 mL/kg of feed, reaching 2.48 ± 0.03%/day. This was followed by treatments C, A, K+, and K−, with SGR values of 2.40 ± 0.04, 2.33 ± 0.03, 2.21 ± 0.01, and 2.13 ± 0.10%/day, respectively. The higher SGR observed in treatment B was presumably influenced by the nutritional components and bioactive

compounds present in octopus ink. According to Saputra *et al*. (2025), octopus ink contains several nutritional components, including protein, fat, ash, and carbohydrates, as well as various amino acids, such as lysine, leucine, arginine, and phenylalanine. Protein plays an important role in supporting fish growth, while lysine contributes to the activity of digestive enzymes, such as protease, which helps improve protein digestion and utilization. Furthermore, octopus ink contains alkaloid compounds that may exhibit antimicrobial activity and contribute to supporting the non-specific defense mechanisms of fish. The presence of these various components is presumed to help maintain the physiological condition of fish, allowing nutrients obtained from feed to be utilized more effectively to support growth.

Specific Length Growth Rate

Based on the research results, treatment B with an octopus ink extract dose of 100 mL/kg of feed showed the highest specific length growth rate, reaching 2.27 ± 0.03%/day. This value was higher than that of treatment C (150 mL/kg) at 2.25 ± 0.05%/day, treatment A (50 mL/kg) at 2.18 ± 0.03%/day, K− at 2.10 ± 0.02%/day, and K+ at 2.06 ± 0.01%/day. This result was presumed to be related to the nutritional content of octopus ink. Kipalahi *et al*. (2024) reported that octopus ink contains protein and several amino acids, such as serine, aspartic acid, isoleucine, leucine, and lysine. These components may support the formation of body tissues required for fish growth.

Furthermore, octopus ink also contains various bioactive compounds. Affandi *et al*. (2025) reported that octopus ink extract contains alkaloids, saponins, phenols, and steroids, and exhibits antioxidant activity with an LC_{50} value of 94.4661 ppm. This antioxidant activity may help protect fish cells from damage caused by free radicals. Therefore, the nutritional content and bioactive compounds in octopus ink are presumed to help maintain fish health and support the utilization of feed nutrients for growth

In treatment C, the specific length growth rate was lower than that in treatment B. This indicates that adding a higher dose of octopus ink extract does not necessarily result in better growth. The dose of 100 mL/kg was presumed to be more suitable for supporting fish growth. However, statistical analysis showed that treatments B and C shared the same superscript letter, c, indicating that there was no significant difference between the two treatments.

CONCLUSION

The research results indicate that the addition of octopus ink extract to the feed has a significant effect on the growth performance of Nile tilapia (*O. niloticus*). This is evidenced by improvements in absolute weight gain, absolute length gain, specific growth rate based on weight, and specific growth rate based on length. Treatment B, which utilized an octopus ink extract dosage of 100 mL/kg of feed, yielded the highest growth performance in numerical terms. Therefore, octopus ink extract has the potential to be used as a natural feed additive to support the growth of Nile tilapia (*O. niloticus*).

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