

Effectiveness of Octopus (*Octopus* sp.) Ink Extract on the Growth of Mozambique Tilapia (*Oreochromis mossambicus*) Infected with *Aeromonas hydrophila*

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Received:

April 18th, 2026

Accepted:

May 10th, 2026

Published:

May 18th, 2026

Keywords:

Octopus Ink Extract,
Aeromonas hydrophila,
Mozambique Tilapia, Feed Additive, Growth Performance

ABSTRACT

Mozambique tilapia (*Oreochromis mossambicus*) is an important aquaculture commodity in Indonesia; however, its productivity can be constrained by disease outbreaks, including *Aeromonas hydrophila* infection. The use of natural feed additives containing nutritional and bioactive compounds is a promising strategy to improve fish growth and health. This study aimed to evaluate the effectiveness of octopus (*Octopus* sp.) ink extract supplemented in feed on the growth performance of Mozambique tilapia infected with *A. hydrophila*. The study was conducted for 45 days using a completely randomized design with five treatments and three replications. The treatments consisted of a positive control, namely feed without octopus ink extract with *A. hydrophila* infection (K+); a negative control, namely feed without octopus ink extract and without *A. hydrophila* infection (K-); and feed supplemented with octopus ink extract at 50, 100, and 150 ml/kg feed followed by *A. hydrophila* infection (A, B, and C, respectively). The observed growth parameters were absolute weight gain, absolute length gain, specific weight growth rate, and specific length growth rate. The results showed that octopus ink extract supplementation improved all growth parameters compared with the positive control. The best performance was obtained in treatment B (100 ml/kg feed), with an absolute weight gain of 10.77 ± 1.53 g, absolute length gain of 2.65 ± 0.39 cm, specific weight growth rate of $2.59 \pm 0.11\%$ /day, and specific length growth rate of $2.16 \pm 0.05\%$ /day. Therefore, octopus ink extract has potential as a natural feed additive to enhance the growth performance of Mozambique tilapia challenged with *A. hydrophila*.

INTRODUCTION

Mozambique tilapia (*Oreochromis mossambicus*) is one of the fishery commodities widely cultivated in Indonesia. The Ministry of Marine Affairs and Fisheries recorded that Mozambique tilapia production in Indonesia reached 15,109 tons, with a production value of IDR 385.77 billion in 2022. This species has several advantages, including relatively fast growth, high adaptability to various environmental conditions, high economic and nutritional value, and a relatively affordable price (Restika, 2018). In tilapia aquaculture, feed may constitute the largest production cost, reportedly accounting for approximately 60-70% of total production costs; therefore, feed innovation is an important strategy to improve the efficiency of aquaculture enterprises (Firdaus *et al.*, 2020).

One widely developed approach to overcoming constraints related to fish growth and health is the use of feed additives. Feed additives are supplementary ingredients administered in certain amounts to improve feed quality, enhance nutrient utilization, improve immune responses, and support fish growth and survival (Vijayaram *et al.*, 2022). These additives may include natural materials that are considered more environmentally friendly, relatively safe, and suitable as alternatives to synthetic additives or antibiotics in fish farming.

Octopus ink is a fishery by-product with potential for development as a natural feed additive because it contains nutritional and bioactive compounds. Kipalahi *et al.* (2024) reported that the gross chemical composition of octopus ink consists of $8.32 \pm 0.042\%$ protein, $0.08 \pm 0.0018\%$ fat, $6.33 \pm 0.306\%$ carbohydrate, and $2.79 \pm 0.014\%$ ash. The protein and amino acid contents of octopus ink may support fish growth because protein is the main nutrient required for tissue formation, whereas amino acids are required for protein synthesis and growth metabolism. In addition to serving as a nutrient source, octopus ink also has potential as a natural immunostimulant in aquaculture. Octopus ink extract has been reported to exhibit strong antioxidant activity, with an IC₅₀ value of 94.4661 ppm, and to contain alkaloids, saponins, phenols, and steroids (Affandi *et al.*, 2025). These compounds are associated with biological roles as immunostimulants, antibacterials, antivirals, antiparasitics, antifungals, and antioxidants; therefore, octopus ink has the potential to improve fish resistance to disease.

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

METHODS

Time and Place

This research was conducted over 45 days, from December 2025 to January 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 bottle and vortex mixer.

The materials used in this research were *Aeromonas hydrophila*, aluminum foil, alcohol, distilled water, freshwater, Mozambique tilapia (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 Mozambique tilapia. The doses of octopus ink extract administered through feed were determined based on previous research (Saputra *et al.*, 2025).

K+ (Positive Control) : Feed without octopus ink extract (infected with *Aeromonas hydrophila*)

K- (Negative Control) : Feed without octopus ink extract (without *Aeromonas hydrophila* infection)

A : 50 ml/kg feed + *Aeromonas hydrophila* infection

B : 100 ml/kg feed + *Aeromonas hydrophila* infection

C : 150 ml/kg feed + *Aeromonas hydrophila* infection

Research Procedures

• Preparation of Rearing Containers and Media

The rearing containers used in this study were 15 containers with a capacity of 45 L. The containers were cleaned with detergent, rinsed with water, and then dried under sunlight. After drying, the containers were moved to the research site, filled with 20 L of water, and equipped with one aeration unit. The containers were then covered and labeled according to the respective treatments.

• Preparation of Test Animals

The test animals used in this study were Mozambique tilapia measuring 4-6 cm, obtained from the Batu Kumbung Fish Seed Center (BBI), Lingsar District, West Lombok Regency, West Nusa Tenggara Province. Before being placed in the rearing containers, the fish were acclimatized. Acclimatization is important to reduce stress and prevent mortality (Wardani *et al.*, 2024).

• Preparation of Octopus Ink Extract

Octopus ink extract was prepared by collecting ink sacs from octopus (*Octopus sp.*) obtained from an octopus processing factory or warehouse around Mataram City. The ink sacs were cut, and the ink was collected. Octopus ink extraction was carried out using the maceration method, in which the ink was mixed with solvent at a ratio of 1:3. The solvent used in this study was 1500 ml of methanol. The Erlenmeyer flask was then stored in a refrigerator at 4°C for 7 days (Affandi *et al.*, 2025). Subsequently, evaporation was performed using a rotary evaporator at 62 rpm for 5 hours.

• Supplementation of Octopus Ink Extract into Feed

In this study, the feed used was commercial feed at a quantity of 1 kg per treatment. The mixing method referred to 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 or air-dried.

• Fish Rearing Process

Acclimatized Mozambique tilapia were stocked at 20 fish per prepared rearing medium and reared for 45 days. Feed was administered based on 5% biomass, with feeding frequency three times daily at 07:00, 12:00, and 17:00. Water quality maintenance was also important. Water replacement of 10-50% was carried out every two days to maintain stable culture water quality, using siphoning to reduce residual feed and waste deposited at the bottom of the rearing tanks so that ammonia and nitrite levels could be controlled (Panjaitan *et al.*, 2024).

- **Challenge Test with *Aeromonas hydrophila***

The challenge test was conducted to evaluate the immune response of Mozambique tilapia after administration of the natural feed additive from octopus ink extract. The challenge was performed by immersion in *Aeromonas hydrophila* by adding the bacterial suspension to the rearing water. The *A. hydrophila* used in this study was obtained from the Fish Health Laboratory, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram. The bacterial suspension had a density of 10^8 cells/mL, a density that can cause infection symptoms and mortality in fish within 24 hours (Nuryati *et al.*, 2024). After immersion, fish were observed periodically to evaluate immune responses, survival rate, and clinical symptoms caused by exposure to *Aeromonas hydrophila*. The test parameters were observed on day 14 after immersion.

Research Parameters

- **Absolute Weight**

Absolute weight was calculated to measure the increase in fish weight during the rearing period. The formula for absolute weight according to Abdani *et al.* (2023) is as follows:

$$W = W_t - W_0$$

Where:

W : Absolute weight gain

W_t : Average fish weight at the end of rearing

W_0 : 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 Mustofa & Djutalov (2023) is as follows:

$$L = L_t - L_0$$

Where:

L : Absolute length gain (cm)

L_t : Average final length (cm)

L_0 : Average initial length (cm)

- **Specific Weight Growth Rate**

Specific weight growth rate is a calculation of fish growth expressed as a daily percentage. The formula for fish specific growth rate according to Wicaksana *et al.* (2015) is as follows:

$$SWGR = \frac{(\ln W_t - \ln W_0)}{t} \times 100\%$$

Where:

SWGR : Specific weight growth rate (%/day)

W_0 : Average fish weight at the beginning of rearing (g)

W_t : Average fish weight at the end of rearing (g)

t : Rearing period (days)

- **Specific Length Growth Rate**

Specific length growth rate is a calculation of fish growth expressed as a daily percentage. The formula for fish specific length growth rate according to Fadhillah & Nasution (2022) is as follows:

$$SLGR = \frac{(\ln L_t - \ln L_0)}{t} \times 100\%$$

Where:

SLGR : Specific length growth rate (%/day)

L_0 : Fish length at the beginning of rearing (cm)

L_t : 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 results, the absolute weight of Mozambique tilapia during 45 days of rearing with different doses of octopus ink extract ranged from 6.87 to 10.77 g.

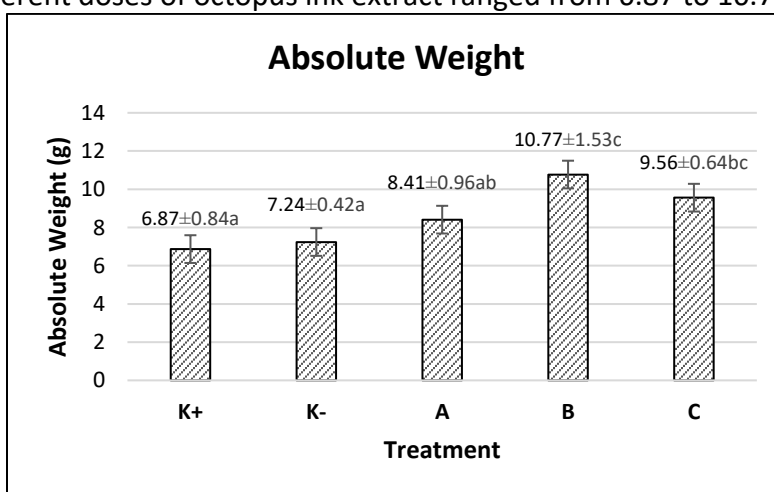


Figure 1. Absolute Weight of Mozambique Tilapia (*Oreochromis mossambicus*)

Absolute Length

Based on the results, the absolute length of Mozambique tilapia during 45 days of rearing with feed supplemented with octopus ink extract ranged from 1.49 to 2.65 cm.

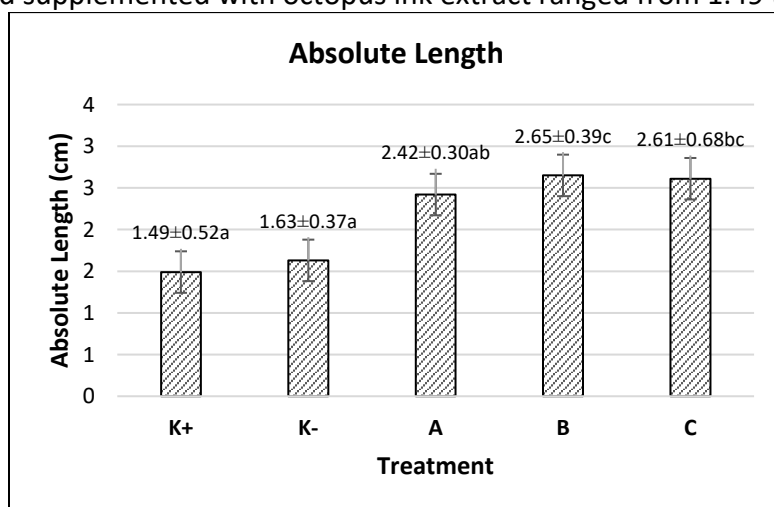


Figure 2. Absolute Length of Mozambique Tilapia (*Oreochromis mossambicus*)

Specific Weight Growth Rate

Based on the results, the specific weight growth rate of Mozambique tilapia during 45 days of rearing with feed supplemented with octopus ink extract ranged from 2.27 to 2.59%/day.

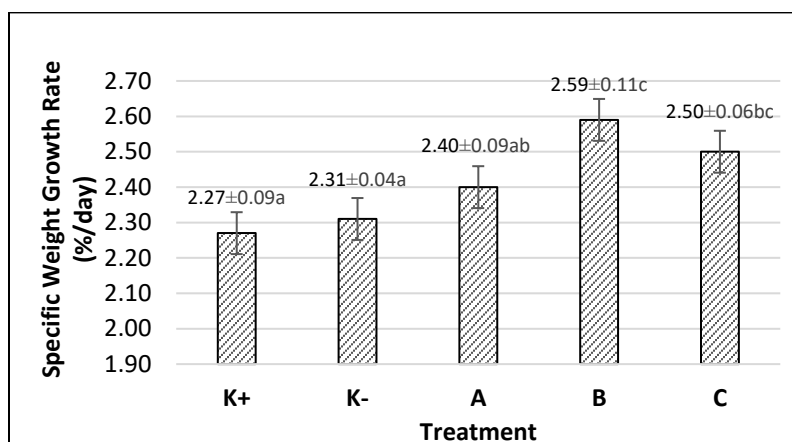


Figure 3. Specific Weight Growth Rate of Mozambique Tilapia (*Oreochromis mossambicus*)

Specific Length Growth Rate

The results showed that the specific length growth rate of Mozambique tilapia during 45 days of rearing with feed supplemented with octopus ink extract ranged from 1.88 to 2.16%/day.

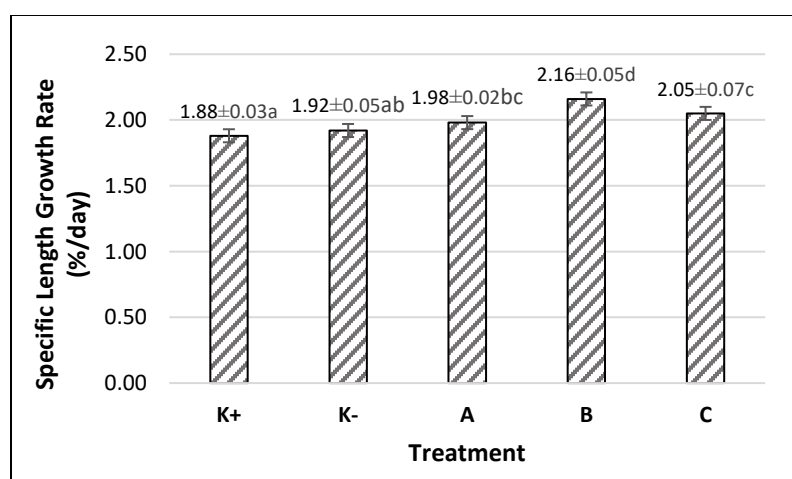


Figure 4. Specific Length Growth Rate of Mozambique Tilapia (*Oreochromis mossambicus*)

DISCUSSION

Absolute Weight

Absolute weight refers to the total increase in fish body weight during the rearing period. Measurement of absolute weight gain provides an overview of fish growth in terms of body weight and indicates the increase in body mass in response to the treatments administered. Based on the graph in Figure 1, administration of the immunostimulant octopus ink extract produced the highest absolute weight gain in treatment B, at 10.77 g, whereas the lowest value was observed in treatment K+, at 6.87 g. The increase in weight of Mozambique tilapia was presumably due to additional protein supplied through feed supplemented with octopus ink extract. According to Agusandi *et al.* (2013), natural melanin in cephalopod ink is a melanoprotein containing at least 10-15% protein, making it a good supplementary protein

source for fish growth. This finding is supported by Riyad *et al.* (2020), who stated that cephalopod ink contains several components, including fat, protein, ash, and carbohydrates. Protein in feed can function as a primary energy source for fish growth processes. Another factor that may influence growth is the age and weight of fish seed. Previous studies have reported that fish seed weighing 2-3 g are already capable of absorbing immunostimulants, thereby improving growth and immune responses compared with smaller fish (Dou *et al.*, 2023).

Absolute Length

Absolute length is the difference between fish length at the end of the study and the initial length before treatment. Based on the graph in Figure 2, the highest absolute length gain of Mozambique tilapia after administration of the immunostimulant was found in treatment B, at 2.65 cm, while the lowest value was found in treatment K+, at 1.49 cm. The low absolute length gain in K+ may have been caused by the absence of additional bioactive compounds from octopus ink extract in the feed, meaning that the fish relied only on the protein content of the basal feed. This is in accordance with Silvina *et al.* (2023), who reported that absolute length growth in fish is strongly influenced by dietary protein intake because protein serves as the main building block for body tissues. Feed with an optimal protein level increases growth rate, whereas protein deficiency results in suboptimal growth.

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 during the rearing period was observed in treatment B, with an average value of 2.59%/day, whereas the lowest value was observed in treatment K+, at 2.27%/day. The high specific weight growth rate was presumably due to the administration of octopus ink extract as an immunostimulant, because octopus ink contains components that can improve not only fish immunity but also growth. Gunawan & Rosdiana (2023) explained that ink produced by squid and cuttlefish contains melanin, protein, lipids, and essential amino acids such as lysine, leucine, arginine, and phenylalanine. Lysine functions in activating digestive enzymes, such as protease, which can optimize the ability of fish to digest protein and support growth.

Furthermore, the alkaloid content in octopus ink may increase fish appetite. The bitter taste produced by alkaloids can contribute additional taste and aroma to fish feed, which may ultimately support fish growth and development. In addition, alkaloids can facilitate the digestive system and metabolism of fish, ensuring that feed is digested more efficiently and nutrients are utilized properly (Muahiddah & Diniariwisan, 2024).

Specific Length Growth Rate

Specific length growth rate is a measure of fish growth expressed as the percentage increase in fish length per day during the rearing period. Based on the graph in Figure 4, the highest specific length growth rate of Mozambique tilapia after rearing with octopus ink supplementation was found in treatment B, at 2.16%/day, whereas the lowest value was found in treatment K+, at only 1.88%/day. The high value obtained in treatment B was presumably because the fish received additional protein intake from octopus ink, supported by bioactive compounds at a dose suitable for the needs of the fish. Adawyah *et al.* (2021) stated that natural melanin in cephalopod ink is a melanoprotein containing at least 10-15% protein, making it a sufficient supplementary protein source for fish growth. This is also consistent with the study by Saputra *et al.* (2025), which reported that dietary administration of octopus ink extract to catfish infected with *Aeromonas hydrophila* improved growth, with specific length growth reaching $2.34 \pm 0.08\%$ /day.

In addition to nutritional factors, bioactive compounds in octopus ink may help suppress physiological stress and improve fish immunity. Affandi *et al.* (2025) stated that octopus ink extract has potential as an immunostimulant because it contains compounds that function as antibacterial, antiviral, antiparasitic, antifungal, and antioxidant agents. Better fish health enables more energy from feed to be allocated to growth rather than being used only to defend against stress or infection. Thus, the high specific weight growth rate in treatment B was likely caused by a combination of additional nutrients, particularly protein, and the activity of bioactive compounds in octopus ink that support the health and metabolic efficiency of Mozambique tilapia

CONCLUSION

Based on the results of the study, the addition of octopus ink extract to Mozambique tilapia feed significantly affected growth performance. This was shown by increases in absolute weight gain, absolute length gain, specific weight growth rate, and specific length growth rate. The best result was obtained in treatment B, with a dose of 100 ml/kg feed. Therefore, octopus ink extract has potential as a natural feed supplement to improve the growth of Mozambique tilapia.

ACKNOWLEDGEMENT

I sincerely thank my supervisor Rangga Idris Affandi, S.Pi., M.P. and Awan Dermawan, S.Pi., M.Si. for their invaluable guidance and support throughout this research. I also extend my gratitude to the faculty members, laboratory staff, and all of my friends for their assistance and collaboration. Lastly, I deeply appreciate the encouragement and support from my family, which were essential in completing this research.

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