

Effect of Vitamin C and E Supplementation on Stress Response Banggai Cardinalfish (*Pterapogon kauderni*)

^{1*}Devi Elvina Sari, ²Ahmad Shofy Mubarak, ³Laksmi Sulmartiwi

¹Master of Fisheries Science Program, Faculty of Fisheries and Marine Sciences, Airlangga University, Surabaya, Indonesia.

^{2,3}Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Airlangga University,

Surabaya, Indonesia.

*Corresponding Author e-mail: devi.elvina.sari02@gmail.com

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Abstract: This study aims to analyze the effect of giving a combination of vitamins C and E as Artemia sp. supplementation on the level of stress resistance of Banggai cardinalfish. The research method used a completely randomized design. The treatments used were a combination of vitamins C and E in supplementation of Artemia sp. as feed: A (vitamin C 57.50 mg/L + vitamin E 28.75 mg/L), B (vitamin C 115 mg/L + vitamin E 57.50 mg/L), C (vitamin C 230 mg/L + vitamin E 115 mg/L), D (vitamin C 460 mg/L + vitamin E 230 mg/L), and E (vitamin C 920 mg/L + vitamin E 460 mg/L), each treatment was repeated 4 times. The main parameters observed were the level of stress resistance (frequency of pectoral fin movement, frequency of operculum movement, frequency of mouth movement) and blood glucose. Data were analyzed using analysis of variance (ANOVA) and further tests were carried out to determine the differences between treatments. The results showed that supplementation of vitamin C and E in Artemia sp. with different dose combinations had a significant effect (P < 0.05) on the frequency of pectoral fin movement frequency, and mouth movement frequency after hypoxia treatment at the 2nd, 4th and 8th hours and blood glucose levels after hypoxia treatment. The best stress resistance level during the study was in treatment C (vitamin C 230 mg/L + vitamin E 115 mg/L), while the lowest was in treatment A (vitamin C 57.50 mg/L + vitamin E 28 mg/L).

Keywords: Banggai cardinalfish; stress response; Vitamin C and E supplementation on Artemia sp.

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INTRODUCTION

Banggai cardinalfish (*Pterapogon kauderni*) is a marine ornamental fish endemic to the Banggai Islands, Central Sulawesi, and has been designated as a national marine ornamental fish (MMAF, 2021). Its population has declined due to habitat degradation and overexploitation, so it is categorized as a limited protected species (MMAF, 2018). Controlled container culture is a conservation solution, but it still faces obstacles such as stress levels (Andayani et al., 2018; Artayasa et al., 2022).

Stress causes metabolic disorders, decreases immunity, and increases the production of Reactive Oxygen Species (ROS) which triggers oxidative stress and cell damage (Komalasari et al., 2017). Antioxidants such as vitamin C and vitamin E play a role in counteracting ROS, improving the immune system, and supporting fish growth (Gasco et al., 2018). Vitamin C helps neutralize free radicals and supports antioxidant enzymes, while vitamin E protects cell membranes from lipid peroxidation (Biller & Takahashi, 2018). The combination of vitamin C and E has been shown to be effective in increasing fish resistance to stress and improving metabolic efficiency (Rahimnejad et al., 2021; Anzabi et al., 2023).

Since fish cannot synthesize vitamins C and E optimally, supplementation through feed is an effective strategy to maintain physiological functions and enhance

stress resistance (Sambulaka et al., 2023). Vitamin C (ascorbic acid) functions as a key antioxidant, aids in collagen synthesis, and supports the immune system, while vitamin E (α -tocopherol) protects cell membranes from oxidative damage caused by reactive oxygen species (NRC, 2011; Hamre et al., 2013). Deficiencies in these vitamins can result in reduced growth rates, poor wound healing, and increased vulnerability to infections, especially in intensive aquaculture systems where stress levels are typically higher (Buentello et al., 2000).

To ensure efficient delivery of these essential micronutrients, Artemia sp. was selected as the medium for supplementation. Artemia, commonly used as a live feed in aquaculture, possesses high palatability, digestibility, and the ability to be enriched or bioencapsulated with water-soluble and fat-soluble compounds (Dhont & Lavens, 1996). This makes it ideal for delivering micronutrients like vitamins C and E in a bioavailable form directly to fish larvae and juveniles. Furthermore, the natural motility of Artemia enhances feeding stimulation and ingestion efficiency, ensuring a more consistent nutrient uptake (Bengtson et al., 1991).

Artemia sp. was chosen as a supplementation medium because it fits the mouth opening of the Banggai cardinal fish, is easy to digest, and attracts the attention of fish (Septian et al., 2017). However, research on supplementation of Artemia sp. with a combination of vitamins C and E in Banggai shad fish is still limited. Therefore, this study aims to analyze the effect of vitamin C and E supplementation in Artemia sp. on the level of stress resistance of Banggai cardinal fish in a controlled maintenance system. The results of this study are expected to be the basis for innovation in marine ornamental fish cultivation to support conservation and sustainable development.

METHOD

The research was conducted in September-November 2024, at the Fish Seed Center of Kampal Beach, Mamboro Installation, Marine and Fisheries Service, Mamboro Village, Palu City, Central Sulawesi Province. The tools used in this study were aquarium, syringe/spuit ($27G \times 1/2$ "), glucometer test kit (EasyTouch GCU), aerator, aeration hose, aeration stone, camera, and stationery. The materials used in this study were 200 juvenile Banggai cardinalfish measuring 18-25 mm and weighing \pm 0.5-0.8 g, obtained from capture in nature, sea water, fresh water, *Artemia* sp. (supreme golden plus), vitamin C (Ascorbic Acid Powder), vitamin E (all-rac- α -Tocopheryl Acetate Powder), chlorine (0,5%), and sodium thiosulfate (Na2S2O3).

This study uses an experimental method by comparing the content of a combination of vitamins C and E in Artemia sp. feed supplementation in Banggai cardinalfish aquaculture. The experimental design used in this study used a completely randomized design (CRD) with 5 treatments and 4 replications, so that 20 experimental units were used. Based on the results of previous studies that have been conducted, it shows that the dose of natural feed Artemia sp. enriched with vitamin C of 115 mg/L affects the survival and growth of Banggai cardinalfish (Sambulaka et al., 2023) and vitamin E of 460 mg/L (Biller & Takahashi, 2018). Based on this, this study was conducted to determine the best dose to support the level of stress resistance of Banggai cardinalfish in a controlled container. The treatments applied were as follows: Treatment A : *Artemia* with the addition of vitamin C (57.50 mg/L) + vitamin E (28.75)

mg/L)

Treatment B : Artemia with the addition of vitamin C (115 mg/L) + vitamin E (57.50 mg/L)

Treatment C : Artemia with the addition of vitamin C (230 mg/L) + vitamin E (115 mg/L)

Treatment D : Artemia with the addition of vitamin C (460 mg/L) + vitamin E (230 mg/L)

Treatment E : Artemia with the addition of vitamin C (920 mg/L) + vitamin E (460 mg/L)

The effect of the combination concentration of vitamin C and E supplemented on *Artemia* sp. obtained data in the form of movement frequency (pectoral fins, operculum, and mouth), and blood glucose, analyzed using analysis of variance (ANOVA). After the analysis of variance and the tested data had an effect, a further Honestly Significant Difference (HSD)/Tukey test was carried out to determine the differences between treatments (Mountogomery and Mastrangelo, 1991).

RESULT AND DISCUSSION

The frequency of pectoral fin movements of Banggai cardinalfish fed with *Artemia* sp. supplemented with a combination of vitamins C and E with different doses and then treated with hypoxia, which was 7-73 times, is shown in Figure 1. The results of ANOVA test showed that, the combination of vitamin C and E supplementation with different doses of *Artemia* sp. as food had a significant effect (P<0.05) on the frequency of pectoral fin movements of Banggai cardinalfish after hypoxia treatment at hour 2, hour 4 and hour 8, but had no significant effect after hypoxia treatment at hour 16.





The frequency of operculum movement of Banggai cardinalfish fed with Artemia sp. supplemented with a combination of vitamins C and E with different doses and then treated with hypoxia, which was 19-188 times, is shown in Figure 2. The results of ANOVA test showed that the combination of vitamin C and E supplementation with different doses of *Artemia* sp. as food had a significant effect (P<0.05) on the frequency of operculum movement of Banggai cardinalfish after hypoxia treatment at hour 2, hour 4 and hour 8, but had no significant effect after hypoxia treatment at hour 16.





The frequency of mouth movements of Banggai cardinalfish fed with *Artemia* sp. with a combination of vitamin C and E supplementation with different doses and then treated with hypoxia, which was 15-179 times, is shown in Figure 3. The results of ANOVA test showed that the combination of vitamin C and E supplementation with different doses of *Artemia* sp. as food had a significant effect (P<0.05) on the frequency of mouth movements of Banggai cardinalfish after hypoxia treatment at hour 2, hour 4 and hour 8, but had no significant effect after hypoxia treatment at hour 16.



Figure 3. Mouth movements of Banggai cardinalfish fed *Artemia* sp. with different vitamin C and E supplements after hypoxia treatment

The results of the study showed that in stress conditions induced by hypoxia tests, fish showed an increase in the frequency of pectoral fin, operculum, and mouth movements, especially in the 4th hour after air exposure. Fish that received a lower dose of vitamin treatment experienced a longer increase in movement frequency, indicating that the antioxidants given were not sufficient to suppress ROS production and were unable to accelerate the physiological recovery of the fish (Ihsan et al., 2021). Meanwhile, fish that received a higher dose of vitamin treatment actually showed a high frequency of movement for a long time, indicating metabolic disorders due to excessive antioxidant loads (Khara et al., 2016). Disruption of metabolic balance due to the accumulation of vitamin E can trigger physiological stress in fish, which is indicated by an increase in blood glucose levels as a metabolic response to these conditions (Nasichah et al., 2016).

Normal blood glucose levels in fish range from 40-90 mg/dL (Rahardjo et al., 2011). Hartanti et al. 2013, stated that fish in normal conditions have blood glucose levels in the range of 41-150 mg/dL. The same statement was also stated by Widiastuti et al. 2022, that normal blood glucose levels in fish are 40-90 mg/dL, almost the same as human blood glucose, which is 70-110 mg/dL. Stress conditions indicated by increased blood glucose levels above the normal range can disrupt the physiological balance of fish, resulting in decreased survival and inhibited growth (Rusiani et al., 2019). One of the factors that has the potential to trigger this stress is the accumulation of vitamin E in the body due to excessive doses, where vitamin E is fat-soluble and difficult to excrete and can interfere with the normal metabolic function of fish (Fahrudin et al., 2023).

Vitamin E is the main lipophilic antioxidant that functions to capture hydroxyl free radicals in the fish body. In the metabolic framework, vitamin E plays a role in maintaining the integrity of cell membranes by protecting polyunsaturated fatty acids (PUFA) from lipid peroxidation (Biller & Takahashi, 2018). Under normal conditions, the need for vitamin E in fish varies between 90-130 mg/L in the *Artemia* sp. supplementation system, depending on the species and environmental conditions (Hamre et al., 2013). However, when the dose of vitamin E exceeds the physiological needs of the fish, there is an accumulation of vitamin E in body tissues. Vitamin E, as a lipophilic molecule, requires a storage medium in the form of body lipids.

In excess, vitamin E will encourage the accumulation of fatty acids in body tissues, causing an uncontrolled increase in fat storage (adipogenesis) (Huang et al., 2018). This condition burdens the work of bile, because vitamin E must be transported and absorbed through the fat emulsification mechanism. If bile production or function is disrupted due to a high lipid load, digestion and absorption of nutrients become inefficient (Hamre et al., 2013). Vitamin C plays a role in supporting the antioxidant cycle by regenerating oxidized vitamin E. Vitamin C reduces the radical form of vitamin E back to its active form, thereby extending the effectiveness of vitamin E antioxidants (Komalasari et al. 2017). Water-soluble vitamin C is easily excreted through urine when excessive, while fat-soluble vitamin E tends to accumulate in fat tissue and liver, which can disrupt the body's metabolic balance (Gasco et al., 2018; Lung & Destiani, 2017). If there is too much vitamin E in the body, the role of vitamin C in regenerating this becomes ineffective, and unneutralized oxidants can accumulate. Giving higher doses can increase inefficient body fat reserves, as well as worsen oxidative stress leading to high mortality (Hamre et al., 2013; Huang et al., 2018).

CONCLUSION

Based on the research that has been conducted, it can be concluded that supplementation of *Artemia* sp. with a combination of vitamins C and E affects the level of stress resistance (frequency of pectoral fin movement, frequency of operculum movement, frequency of mouth movement, and blood glucose) of Banggai cardinalfish (*Pterapogon kauderni*).

RECOMMENDATION

Recommendations that can be made based on the research results, namely using vitamin C and E supplementation in *Artemia* sp. as a strategy to increase stress resistance using the best dose from the research results, namely (vitamin C 230 mg/L and vitamin E 115 mg/L), can be used as a standard recommendation in fish farming.

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