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Concentration of Vitamin C and Vitamin E Supplementation in *Artemia* Sp. on The Level of Stress Resistance, Survival, and Growth of Banggai Cardinalfish (*Pterapogon Kauderni* Koumans, 1933)

Devi Elvina Sari
Universitas Airlangga, Indonesia
Email: devi.elvina.sari02@gmail.com

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ABSTRACT

The Banggai cardinalfish (*Pterapogon kauderni*) is an endemic marine ornamental fish from Indonesia, facing population decline due to habitat degradation and overexploitation. Controlled cultivation is essential for conservation; however, challenges such as stress, low survival rates, and slow growth remain significant. This study employs a qualitative research approach through literature review and library research to analyze the impact of vitamin C and E supplementation in *Artemia sp.* on stress resistance, survival, and growth performance of *P. kauderni*. Vitamin C and E are known antioxidants that mitigate oxidative stress and enhance immune responses in fish. Previous studies indicate that optimal supplementation levels can improve metabolic efficiency, stress tolerance, and overall survival rates in aquaculture species. Our findings suggest that a balanced combination of vitamin C (230 mg/L) and vitamin E (115 mg/L) in *Artemia sp.* provides the most effective results in reducing stress-induced glucose levels, increasing survival rates, and improving growth performance. These results highlight the potential of dietary antioxidant supplementation as a sustainable strategy for *P. kauderni* aquaculture and conservation. Further research is recommended to explore long-term effects and adaptability in different aquaculture settings.

Corresponding Author:
Devi Elvina Sari
E-mail: devi.elvina.sari02@gmail.com

INTRODUCTION

Banggai cardinalfish (Pterapogon kauderni) is an endemic marine ornamental fish of 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 cultivation is a conservation solution, but it still faces obstacles such as low growth and survival due to environmental stress and unstable water quality (Andayani et al., 2018; Artayasa et al., 2022).

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

Stress causes metabolic disorders, lowers 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 warding off ROS, boosting 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 and Takahashi, 2018). The combination of vitamins C and E has been shown to be effective in increasing fish's 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 (Suarsana and Priatna, 2009). Artemia sp. was chosen as a supplementation medium because it is in accordance with the mouth opening of Banggai cardinalfish, easy to digest, and attracts the attention of fish (Septian et al., 2017). However, research on the supplementation of Artemia sp. with the combination of vitamins C and E in Banggai cardinalfish is still limited. Therefore, this study aims to evaluate the effect of vitamin C and E supplementation on Artemia sp. on the level of stress resistance, survival, and growth of Banggai cardinalfish in a controlled maintenance system. The results of the research are expected to be the basis for innovation in the cultivation of marine ornamental fish to support its preservation and sustainable development.

METHOD

The research was carried out in September-November 2024, which was carried out at the Kambal Beach Fish Seed Center of the Mamboro Installation of the Marine and Fisheries Service, Mamboro Village, Palu City, Central Sulawesi Province. The tools used in the research are aquariums, digital scales, digital calipers, secers, syringes/syringes, glucometer test kits, thermometers, pH meters, DO meters, refractometers, aerators, aeration hoses, aeration stones, cameras and stationery. The materials used in the study were, juvenile Banggai cardinalfish (size 18-25 mm), weight ± 0.5-0.8 g as many as 80 fish, seawater, fresh water, *Artemia* sp., vitamin C, vitamin E, chlorine and sodium thiosulfate.

This study uses an experimental method by comparing the content of vitamin C and E combinations in the feed supplementation *of Artemia* sp. in the cultivation of Banggai cardinalfish. The experimental design used in the study used a Complete Randomized Design (RAL) with 5 treatments and 4 replicates, so that 20 experimental units were used. The placement of the research container is randomized using Microsoft Excel, resulting in the layout of the research container as shown in Figure 6. Based on the results of previous studies that have been carried out, it has been shown that the dose

International Journal of Social Discussion
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Volume 1 No. 1 2024

of natural feed *of Artemia* sp. enriched with vitamin C of 115 mg/L has an effect on the survival and growth of Banggai cardinalfish (. Based on this, this study was conducted to determine the best dose to support the level of stress resistance, survival and growth of Banggai cardinalfish in a controlled container. The treatment applied is as follows: Sambulaka *et al.* 2023)

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)

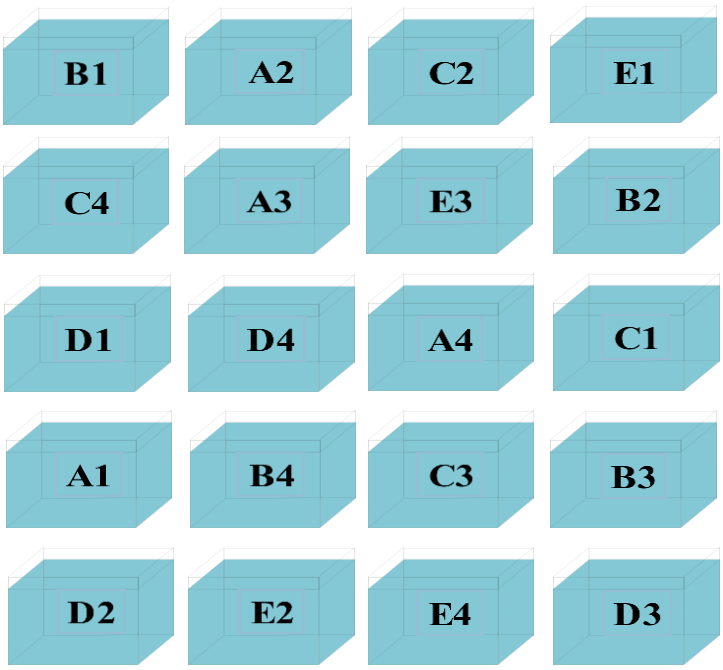

Figure 1. Layout of research containers

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The study used 20 aquariums measuring 40×35×35 cm^3 with aeration and filled with 30 L of seawater. The aquarium is cleaned, dried, and then randomly arranged. Banggai cardinalfish undergo acclimatization 24 hours before being put in the aquarium. The seawater used is aerated and filtered for 24 hours before maintenance to improve its quality.

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

Artemia sp. cyst through hydration in fresh water for 1–2 hours (1 g/L), then filtered and decapsulated using a chlorine solution (1.5 mL/g cyst) for 20–30 minutes until it turns brick red (Adloo et al., 2012). The cysts were washed and neutralized with a solution of sodium thiosulfate, then hatched in 33 ppt salinity seawater with strong aeration for 18–24 hours. After 24 hours, the nauplius is harvested and raised until it reaches the Instar II stage.

Vitamin C is dissolved in water (4000 mg/L), while vitamin E is dissolved using tween 80 as a non-ionic solvent in a ratio of 3:25. Dilution was carried out based on the formula M1×V1=M2×V2 to obtain the concentration according to the treatment. The solution is then mixed and homogenized before being used for Artemia sp. enrichment. (Norouzitallab *et al.*, 2009)

Nauplius Instar II (1.5 g) is placed in a vitamin C and E enrichment solution (1000 mL) for 3 hours with optimal aeration; Artemia sp. that have been supplemented are washed back with seawater before being given as feed for the Banggai cardinalfish. Fish are weighed and measured before stocking (10 fish/30 L) in an aquarium with a salinity of 33 ppt for 60 days (Unisa, 2000). Artemia sp. that has been supplemented is given as feed as much as 3% of the body weight of the fish, twice a day. Every three days a fondue (25% of the water volume is replaced) is carried out to maintain water quality (Adloo *et al.* , 2012) (Ramadan) *et al.* , 2021) (Hamsah *et al.* , 20 18) ..

RESULTS AND DISCUSSION

Result

The results of the analysis showed that the blood glucose level of Banggai cardinalfish before treatment was in the range of 55-59 mg/dL, the highest in treatment C. Based on the ANOVA test, there was no real effect between treatments (P>0.05). After being treated and hypoxia tested, the blood glucose levels of Banggai cardinalfish increased significantly (P<0.05) between treatments to a range of 102-177 mg/dL, the highest in treatment A. Increased blood glucose levels indicated a stress response in fish due to hypoxia exposure, shown in Figure 2.

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

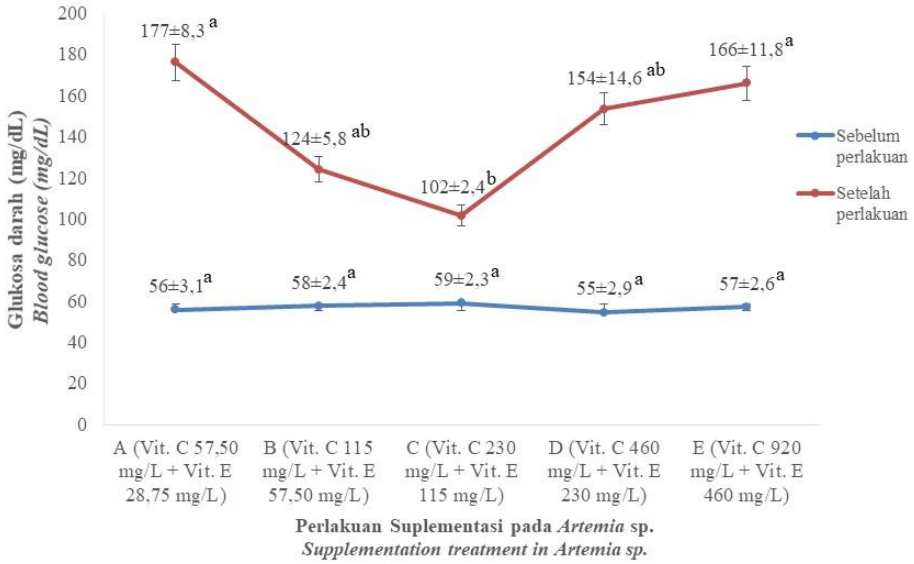

Figure 2. Blood glucose Banggai cardinalfish

Banggai cardinalfish in the study supplemented *with Artemia* sp. with vit. C and vit. E has a survival rate of 65.00% to 87.50%, shown in Figure 3.

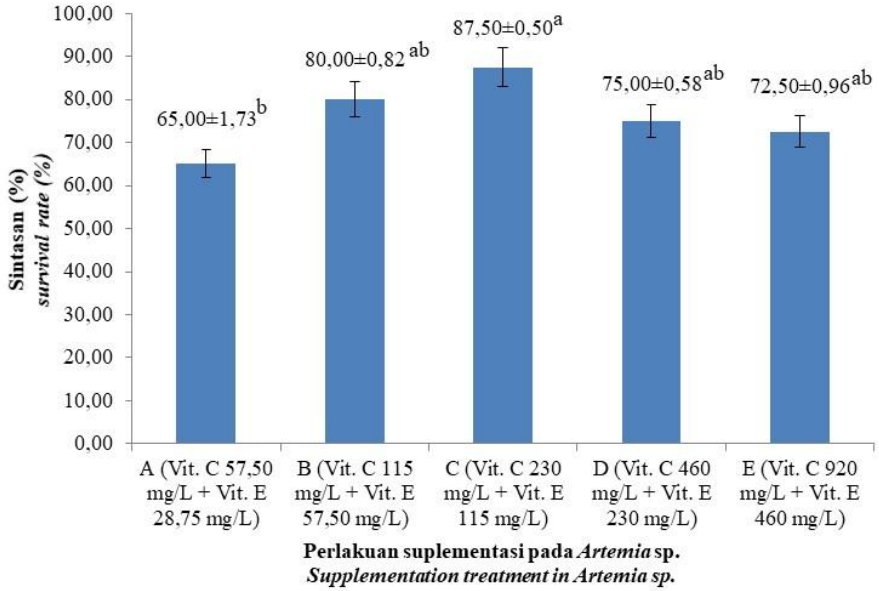

Figure 3. Banggai cardinalfish Survival

The results of the study on the growth of absolute weight of Banggai cardinalfish based on the results of the ANOVA test showed that there was a real difference ($P<0.05$) between the treatments. Based on follow-up tests, treatment C was the highest treatment (1,000 g) compared to other treatments. The growth of the absolute weight of the Banggai cardinalfish is shown in Figure 4.

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

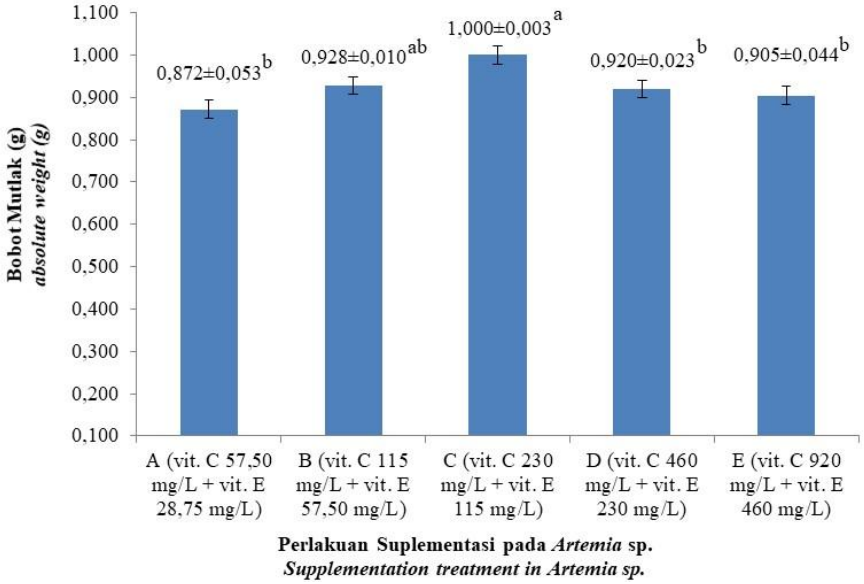

Figure 4. Absolute weight growth Banggai cardinalfish

Absolute length

The results of the study on the absolute length of Banggai cardinalfish based on the results of the ANOVA test showed that there was a real difference (P<0.05) between treatments. Based on the follow-up test, treatment C was the highest treatment (14.19 mm) and the lowest treatment was treatment A (11.79 mm). The absolute length growth of the Banggai cardinalfish is shown in Figure 5.

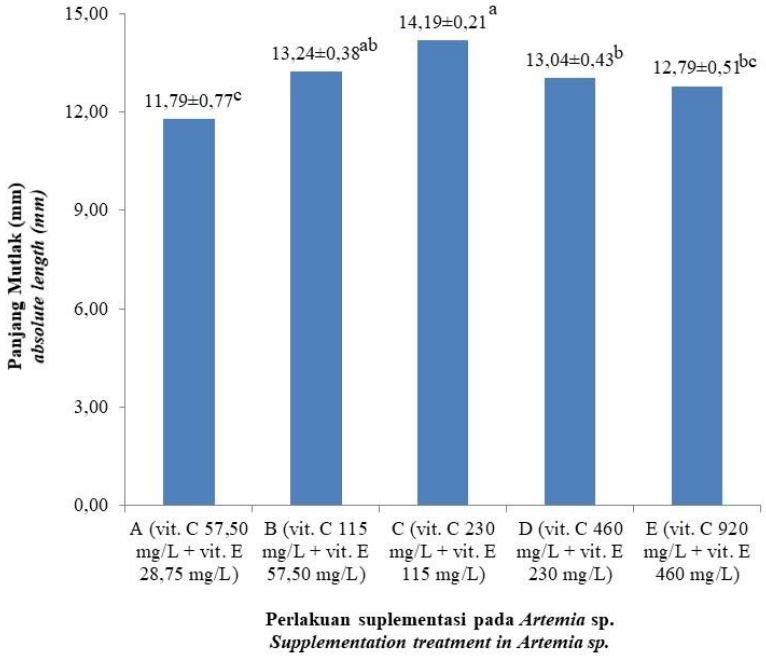

Figure 5. Absolute long growth of Banggai cardinalfish

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

Specific growth rate (SGR)

The results of the specific growth rate (SGR) of Banggai cardinalfish based on the results of the ANOVA test showed no real difference between treatments (P>0.05). The specific growth rate was highest in treatment C (2.198%) and lowest in treatment A (2.038%). The specific growth rate of Banggai cardinalfish is shown in Figure 6.

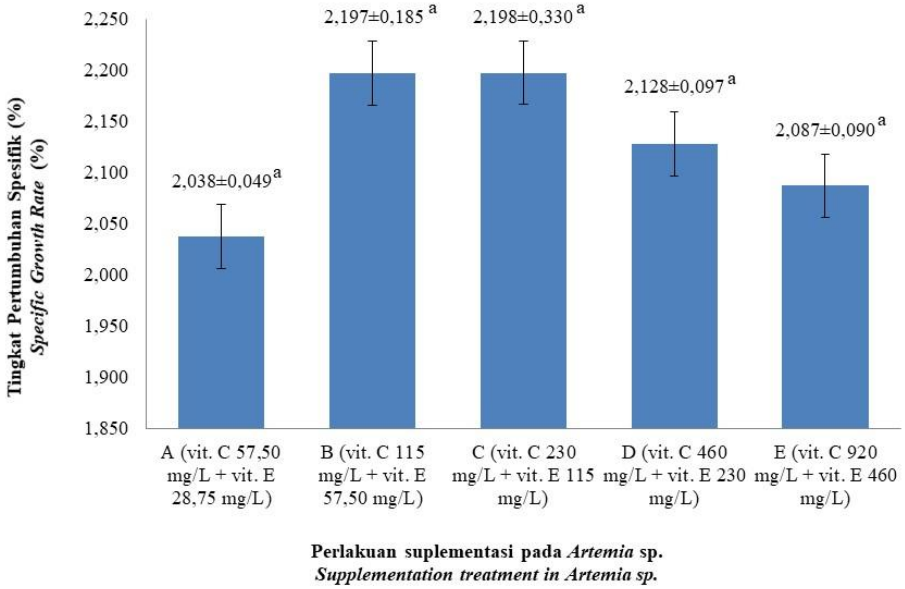

Figure 6. Specific growth rate of Banggai cardinalfish

Feed Utilization Efficiency (EPP)

The results of the research on the efficiency of Banggai cardinalfish feed utilization based on the results of the ANOVA test showed a real difference (P<0.05) on the 30th day and on the 60th day, the highest in treatment C. While on day 0 there was no real difference (P>0.05), the highest in treatment D. The efficiency of Banggai cardinalfish feed utilization is listed in Table 1.

Table 1. Feed utilization efficiency of Banggai cardinalfish

Treatment	Dosage (mg/L)	EPP Day 0 ± Elementary School	EPP 30th day ± elementary school	EPP 60th day ± elementary school
A	Duck. C 57.50 + Vit. E 28,75	8,546 ± 0,872A	22,647 ± 1,274b	34,205 ± 2,097b
B	Duck. C 115 + Screws. E 57,50	8.235 ± 0.318a	25,784 ± 2,316ab	36,403 ± 0,407ab
C	Duck. C 230 + Screws. E 115	7,547 ± 0,804a	28,614 ± 0,731a	39,225 ± 0,122a
D	Duck. C 460 +	8,664 ± 1,319a	28,508 ± 1,574a	36,082 ± 1,742b

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

E	screws. E 230 Duck. C 920 + screws. E 460	7,021 ± 1,022a	23,022 ± 1,125b	35,481 ± 0.914b

Food Convertion Ratio **(FCR)**

The results of the Banggai *cardinalfish food conversion ratio* study based on the results of the ANOVA test showed a real difference (P<0.05) on the 30th day and on the 60th day, the lowest in treatment C. While on day 0 there was no real difference (P>0.05), the lowest in treatment D. *The food conversion ratio* of Banggai cardinalfish is listed in Table 2.

Table 2. *Food convertion ratio* Banggai cardinalfish

Treatment	Dosage (mg/L)	FCR day 0 ± SD	FCR 30th day ± SD	FCR 60th day ± SD
A	Duck. C 57.50 + Vit. E 28,75	11,795 ± 1,231a	4,426 ± 0,252a	2,932 ± 0,178a
B	Duck. C 115 + Screws. E 57,50	12,157 ± 0,465A	3,901 ± 0,330bc	2,747 ± 0.031ab
C	Duck. C 230 + Screws. E 115	13,365 ± 1,444a	3,496 ± 0.091c	2,549 ± 0.008b
D	Duck. C 460 + screws. E 230	11,721 ± 1,568a	3,516 ± 0,192c	2,776 ± 0,134ab
E	Duck. C 920 + screws. E 460	14,454 ± 1,948a	4,352 ± 0,216ab	2,820 ± 0.073a

Water Quality

The results of the water quality study of Banggai cardinalfish during rearing supplemented *with Artemia* sp. with vit. C and vit. E has a temperature range of 26.3-27.5ºC, pH 7.3-8.4, salinity 32.5-33.2 ppt, DO 3.5-4.3 mg/L. Water quality of Banggai cardinalfish is listed in Table 3.

Table 3. Water quality of Banggai cardinalfish

Treatment	Parameters			
	Temperature (ºC)	Ph	Salinity (ppt)	DO (mg/L)

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

A (Vit. C 57.50 mg/L + Vet. E 28.75 mg/L)	26,3 - 27,2	7,3 - 8,3	32,9	3,6 - 4,0
B (Duck. C 115 mg/L + Vit. E 57.50 mg/L)	26,7 - 27,0	7,3 - 8,3	32,9 - 33,2	3,5 - 4,3
C (Duck. C 230 mg/L + Vit. E 115 mg/L)	26,6 - 27,2	7,3 - 8,3	32,8 - 33,0	3,5 - 4,1
D (Duck. C 460 mg/L + Vet. E 230 mg/L)	26,3 - 27,2	7,3 - 8,3	32,5	3,6 - 4,0
E (Vit. C 920 mg/L + Vit. E 460 mg/L)	26,3 - 27,5	7,3 - 8,4	32,5 - 33,1	3,5 - 4,0

Discussion

Blood glucose is one of the important parameters to assess the physiological response of fish to stress. Under stressful conditions, such as exposure to hypoxia, blood glucose levels usually increase due to the activity of the endocrine system, especially the release of stress hormones such as cortisol and catecholamines . This hormone stimulates the process of glycogenolysis and gluconeogenesis in the liver, which leads to an increase in blood glucose levels to provide energy for vital organs, such as muscles and gills in the face of stress. The accumulation of oxidative stress will increase the release of stress hormones, which ultimately trigger high blood glucose levels as the body's response to compensate for the stress. Stress in fish can be indicated by blood glucose levels, if abnormal it can interfere with the survival of fish and even cause death/mortality. In contrast, low blood glucose levels indicate that the fish's metabolism is in a stable state. , stated that normal blood glucose levels in fish ranged from 40-90 mg/dL. The same statement is also stated by , 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. (Nasichah *et al* ., 2016) (Sulmartiwi *et al* ., 2013) (Schreck and Tort, 2016) (Nasichah *et al* ., 2016) Rahardjo *et al* . 2011 Widiastuti *et al*. 2022

Water-soluble vitamin C functions in warding off ROS in extracellular fluids, while fat-soluble vitamin E protects cell membranes from oxidative damage. The combination of the two with the right dosage will work synergistically to reduce oxidative stress, thereby lowering the release of stress hormones and stabilizing blood glucose levels. Treatment C is an effective combination of antioxidants to reduce oxidative stress by neutralizing (Komalasari *et al* ., 2017) (Gasco *et al* ., 2018) *the reactive oxygen species* (ROS), thereby reducing the release of stress hormones that trigger an increase in blood glucose, as well as providing an optimal balance between antioxidant effectiveness and physiological tolerance of fish. Supplementation doses higher or lower than the dose in treatment C may

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

be less effective. At high doses, the fish's metabolism can be disrupted due to excessive vitamin intake, while at low doses, supplementation may not be enough to fight ROS optimally. Therefore, moderate dose C treatment proved to be the most effective dose in lowering blood glucose levels and maintaining the metabolic balance of fish by keeping blood glucose levels stable, and also helping to prevent metabolic fatigue that can affect the physiological performance of fish. (Lung and Destiani , 2017) (Renitasari *et al*., 2021)

Survival is the percentage of fish alive at the end of rearing compared to the number of fish at the beginning of rearing. Survival of Banggai cardinalfish with a concentration of vit supplementation. C and vit. E in (Muchlisin *et al* ., 2016) *Artemia* sp. at the end of maintenance shows that treatment C has better survival when compared to other treatments. This is suspected because in treatment C is a dose that is able to increase immunity and suppress the stress level of Banggai cardinalfish so that it can reduce the mortality rate. (Zahedi *et al* ., 2020)

Duck. C and vit. E acts as an antioxidant that helps reduce the effects of oxidative stress. The combination of these two vitamins provides comprehensive protection against cell and tissue damage, thus increasing the fish's ability to survive. The right concentration of supplementation in the right dosage is able to provide optimal antioxidant protection, support physiological function, and help fish better adapt to stress. The higher survival rate reflects the effectiveness of the vit combination. C and vit. E in increasing the fish's immunity and minimizing the risk of death during the study period. (Russian *et al* ., 2019) (Zahedi *et al* ., 2020)

Weight growth is the addition or change of body weight in an organism in a certain time. Vit supplementation. C and vit. E in (Andayani *et al* ., 2018) *Artemia* sp. showed a significant influence on the growth of the absolute weight of the Banggai cardinalfish. The combination of these two vitamins helps improve the metabolic efficiency of fish by reducing oxidative stress that can inhibit growth. Vitamin C, which plays a role in collagen synthesis and protein metabolism, supports the growth of body tissues, while vitamin E protects cell membranes from damage due to lipid peroxidation, thereby increasing the utilization of energy for growth. Treatment C with moderate supplementation doses produced the highest absolute weight compared to other treatments, indicating an optimal balance between antioxidant protection and metabolic efficiency. (Rahimnejad *et al* ., 2021) (Sudarmono *et al*., 2013)

The growth length is the result of the difference between the final length and the initial length of the fish during rearing. The absolute length growth of the Banggai cardinalfish is also affected by vit supplementation. C and vit. E on (Yaningsih , 2018) *Artemia* sp.. The combination of these two vitamins supports the extension of body tissues through protection against oxidative stress, which is often a major inhibitor of fish growth in aquaculture conditions. In treatment C, moderate doses of

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

vitamins were shown to be able to increase fish metabolism without burdening physiological function, resulting in better long growth compared to other treatments. While high doses tend to overload the metabolism of fish, thus reducing growth efficiency. (Khara *et al* ., 2016) (Velez-Alavez *et al* ., 2014)

The specific growth rate (SGR) is an important parameter to assess the efficiency of feed utilization and fish metabolism. Vit supplementation. C and vit. E provides protection against oxidative damage that can inhibit growth and reduce the growth rate of k-specific. In treatment C, the fish showed the highest value indicating more efficient nutrient utilization and optimal metabolism. Moderate doses of the vitamin in this treatment are able to neutralize ROS without causing metabolic disorders, thus supporting consistent growth. While high doses can lead to the accumulation of metabolites that the body does not need, thus overloading the excretory mechanism of fish. This results in a decrease in the performance of the specific growth rate of the fish. (Anzabi *et al* ., 2023) (Velez-Alavez *et al* ., 2014) (Khara *et al*., 2016)

Vit supplementation. C and vit. E on *Artemia* sp. has a significant influence on the feed utilization efficiency of Banggai cardinalfish, making it a very effective strategy in increasing the productivity of this ornamental fish farming. The combination of these two vitamins acts as antioxidants that protect fish from the negative effects of oxidative stress, so that the metabolism of nutrients from feed can take place more efficiently. The results showed that treatment C, with a concentration of vit. C and vit. E which is moderate, resulting in the highest EPP value compared to other treatments. This shows that this dose is able to provide optimal antioxidant protection without overloading the fish's metabolism, the fish is able to utilize the nutrients from (Anzabi *et al* ., 2023) *Artemia* sp. to the maximum to support growth, maintain body health, and increase feed conversion efficiency. The right dosage of supplementation on treatment C can improve feed utilization efficiency, providing an ideal balance between antioxidant protection and metabolic efficiency, making it the best solution to improve the cultivation performance of Banggai cardinalfish. (Fahrudin *et al* ., 2023) (Mufidah *et al* ., 2017)

Vit supplementation. C and vit. E in *Artemia* sp. showed a significant influence on the FCR value of Banggai cardinalfish, which indicated the efficiency of the fish in converting feed into growth. Combination of vit. C and vit. E acts as an antioxidant that protects fish from oxidative stress that can interfere with energy metabolism and nutrient utilization. The combination of these two vitamins allows fish to utilize feed nutrients more efficiently, thereby lowering the FCR value. The low FCR value indicates that the fish are able to convert feed very efficiently to support growth, so that the available energy can be fully utilized for the anabolism process. The low FCR value not only indicates high feed efficiency, but also supports the sustainability of fish farming by reducing feed costs, which is one of the largest components in aquaculture production. (Anzabi *et al* ., 2023) (Fahrudin *et al* ., 2023)

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

(Lestari *et al*., 2021)

Water quality is one of the important factors that support the growth and survival of Banggai cardinalfish. The water quality during maintenance is good for the growth and survival of the Banggai cardinalfish, such as the temperature value ranges from 26.3ºC to 27.5ºC. The Banggai cardinalfish in its habitat lives in the temperature range of 27-34ºC. , also stated that Banggai cardinalfish in the cultivation environment can survive and grow in the range of 24-28ºC. The pH range value during the study was 7.3 to 8.4. The pH range of 8.31-8.34 can still support the growth of Banggai cardinalfish, while according to , the pH range of 6.8-8.16 is still the tolerance limit for the growth and survival of Banggai cardinalfish. (Madinawati *et al* ., 2009) (Ndobe *et al* ., 2017) Madinawati *et al* . 2009 (Gunawan *et al* ., 2010) Madinawati *et al.* 2009

Salinity during the study ranged from 32.5 ppt to 33.2 ppt. This range is still in optimal condition for the maintenance of the Banggai cardinalfish, according to , the salinity range in the native habitat of the Banggai cardinalfish is between 33-34 ppt. The DO range ranged from 3.5 mg/L to 4.3 mg/L during the study under conditions that were still feasible for the growth and survival of Banggai cardinalfish, as it is in accordance with the statement, that dissolved oxygen (DO) is still feasible for rearing Banggai cardinalfish in the range of 4.3-5.8 mg/L Carlos *et al* . 2014 Sugama 2018

CONCLUSION

Based on the research that has been carried out, it can be concluded that it is a supplement to Artemia sp. with the combination of vitamins C and E affecting the level of stress resistance, survival, and growth of Banggai cardinalfish (Pterapogon kauderni). The optimal dose of vitamin C and E combination in Artemia sp. which affects the level of stress resistance, survival, and growth of Banggai cardinalfish (Pterapogon kauderni), which is found in treatment C with a dose of vitamin C 230 mg/L + vitamin E 115 mg/L.

International Journal of Social Discussion
ISSN 3063-3036
Volume 1 No. 1 2024

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