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The Influence of Enrichment with Ascorbic Acid and Fermipan on *Artemia* sp. Exposed to Salinity Shock

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Abstract

Artemia sp. plays a vital role in the natural feed used for cultivation due to its rich nutritional composition, primarily consisting of proteins and amino acids. Despite its nutritional value, the current profile of *Artemia* sp. falls short of being optimal for sustaining its life. To address this, a study focused on enriching *Artemia* sp. with ascorbic acid and fermipan during the salinity shock test. The research utilized a laboratory experimental method with a completely randomized design (CRD). The toxicity test for ascorbic acid on *Artemia* sp. employed the Brine Shrimp Lethality Test (BSLT) method, and the obtained toxicity values were used to determine treatment dosages. *Artemia* sp. enrichment involved a 3-day soaking process with seven distinct treatments, each repeated for accuracy. The salinity test subjected *Artemia* sp. to salinities ranging from 25 ppt to 0 ppt, recording the survival rate every 4 hours until mortality occurred. The toxicity test revealed an LC_{50} value of 200.84, indicating the toxic nature of ascorbic acid against *Artemia* sp. In the salinity shock test, *Artemia* sp. demonstrated varying resistance levels, with the longest survival time observed in the AS 2 treatment (20 hours), followed by FR, C1, and C2 (16 hours), AS 3, C3, and the control (12 hours), and AS 1 (8 hours). The enrichment of ascorbic acid and fermipan significantly influenced the salinity shock test, but the combination of both did not have a significant impact on *Artemia* sp.'s response to salinity shock.

1. Introduction

Fishery cultivation is a crucial subsector that contributes to the mission of enhancing community welfare in the fisheries and marine sector. Fishery cultivation plays a significant role in boosting the national economy, and Indonesia stands as one of the world's largest producers in fishery cultivation. In 2021, Indonesia's shrimp production reached 1.21 million tons, indicating a 9.20% increase compared to the previous year. The growth of vannamei shrimp is influenced by salinity levels. Water quality factors such as salinity in shrimp cultivation lead to an increase in larval mortality. Salinity can impact the osmoregulation process, where organisms in a water environment strive to control the balance of water with their surroundings (Jayanti *et al*., 2022).

Ascorbic acid serves to enhance the immune system of larvae against stress. It also functions as an antioxidant in the body. The absorbed ascorbic acid in the *Artemia* sp. body is expected to improve the growth and survival of shrimp and fish larvae (Mudiarti and Kursitiyanto, 2019). Ascorbic acid is highly effective in the formation of steroid hormones and boosts the body's resilience when exposed to stress (Kaspul, 2011). Fermipan, with its high protein and carbohydrate content, can be utilized in feed. Fermipan can act as an organic substrate, thereby enhancing the growth in fish larvae (Iksan *et al*., 2015). Fermipan contains immunostimulants that can elevate the immune response in shrimp growth.

The administration of ascorbic acid and fermipan tested on *Artemia* sp. is an initial step before being provided to shrimp. The objectives of this research include determining the toxicity level of ascorbic acid in *Artemia* sp. and examining the influence of enriching ascorbic acid, fermipan, and a combination of both. This study is expected to yield positive results, providing valuable references for future endeavors.

2. Material and methods

2.1 Material

This research utilized the test organism *Artemia* sp., Supreme Plus brand, from the Great Salt Lake. The ascorbic acid used in this study is from the HL Vitamin brand. Fermipan, in sachet form, under the brand Fermipan, was employed for enrichment in *Artemia* sp.

2.2 Research Design

This study employed a laboratory experimental approach with a Completely Randomized Design (CRD). There were eight treatments, namely Fermipan 15 ppm, AA (ascorbic acid) 100 ppm, AA 50 ppm, AA 25 ppm, AA 50 ppm + Fermipan 7.5 ppm, AA 25 ppm + Fermipan 7.5 ppm, AA 12.5 ppm + Fermipan 7.5 ppm, and control.

2.3 Preparation of *Artemia* sp.

The hatching of *Artemia* sp. cysts used sterilized seawater. Seawater was filtered and heated to 100 °C for sterilization. *Artemia* sp. cysts weighing 50-100 mg were placed in bottles filled with sterile seawater. After aeration for 24 hours, hatched *Artemia* sp. were ready for use as test animals.

2.4 Toxicity Test of Ascorbic Acid with BSLT

The toxicity test on *Artemia* sp. with ascorbic acid was conducted using the Brine Shrimp Lethality Test (BSLT) method. Testing was performed with concentrations of ascorbic acid at 100 ppm, 200 ppm, 400 ppm, 600 ppm, 1000 ppm, and control. A total of 360 *Artemia* sp. were required, with three repetitions.

2.5 Preparation of Ascorbic Acid Stock Solution

The stock solution of ascorbic acid 1000 ppm was prepared by dissolving 0.08 g of ascorbic acid in 80 mL of seawater with a salinity of 25 ppt.

2.6 Media Preparation

The media was prepared by dissolving ascorbic acid and fermipan in seawater with a salinity of 25 ppt. Treatments included Fermipan 15 ppm, AA 100 ppm, AA 50 ppm, AA 25 ppm, AA 50 ppm + Fermipan 7.5 ppm, AA 25 ppm + Fermipan 7.5 ppm, AA 12.5 ppm + Fermipan 7.5 ppm, and control. Fermipan concentration followed Mahdalena's guidelines (2021), which is 0.15 g/l.

2.7 Toxicity Test with BSLT

The toxicity test using the Brine Shrimp Lethality Test (BSLT) method served as a preliminary test to determine the required dosage for further bioactivity testing.

2.8 *Artemia* sp. Testing

Artemia sp. submerged in the solution was collected using a plankton net, transferred to a measuring glass, and placed in a 50 mL vial. Each vial contained 20 *Artemia* sp. The test was repeated three times using 20 test animals in a 10 mL petri dish. Observations were made at hours 1, 2, 4, 8, 12, 16, 20, and 24.

2.9 Data Analysis

Data analysis was performed using One Way Analysis of Variance (ANOVA) with SPSS 23 software and Microsoft Excel 2013. Normality and homogeneity tests were conducted as prerequisites for the ANOVA. If significant differences were found, further analysis was carried out using the Duncan test to detect significant differences among treatment groups.

3. Results

The toxicity test on *Artemia* sp. with ascorbic acid was conducted using the Brine Shrimp Lethality Test (BSLT) method. The test included concentrations of 100 ppm, 200 ppm, 400 ppm, 600 ppm, and 1000 ppm. The results are presented in Table 1, and the regression analysis graph is illustrated in Figure 1. The probit analysis yielded a coefficient of determination (R2) of R2=0.8364. This indicates that 83.64% of the variability is controllable, attributed to the examined variable, ascorbic acid. The concentration probit values indicate a linear correlation, while 16.36% is uncontrolled due to various factors beyond ascorbic acid addition.

The toxicity test of ascorbic acid on *Artemia* sp. was calculated using MS Excel 2010 with probit analysis of LC_{50}-24 hours, as shown in Table 2. The LC_{50}-24 hours value for ascorbic acid was determined to be 200.84 ppm.

The LC_{50}-24 hours hours value of 200.84 ppm was utilized to determine the dosage of ascorbic acid, setting the highest treatment dosage at 100 ppm. Treatments included Fermipan 15 ppm, AA 100 ppm, AA 50 ppm, AA 25 ppm, AA 50 ppm+Fermipan 7.5 ppm, AA 25 ppm+Fermipan 7.5 ppm, AA 12.5 ppm+Fermipan 7.5 ppm, and control. Data from all treatments, each with 60 *Artemia* sp., were collected for further research.

The toxicity test on *Artemia* sp. with ascorbic acid was conducted in three repetitions, using concentrations of 0 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, and 1000 ppm over a 24-hour period. Table 2 displays the probit analysis results, revealing an LC_{50} value of 200.84 ppm.

Observations on the resistance of *Artemia* sp. were performed in three repetitions with 20 test animals in a 10 mL petri dish, conducted every 4 hours during the salinity stress test. The data is presented in Figure 2, indicating that the AS 50 ppm treatment demonstrated a resilience of 24 hours, while the AS 100 ppm treatment exhibited the lowest resilience, lasting for 8 hours.

The analysis of the survival rate of *Artemia* sp. after feeding with ascorbic acid and fermipan is depicted in Figure 3. Throughout the salinity shock test, the formulation of ascorbic acid enrichment at 50 ppm exhibited a high level of resilience, while the formulation at 100 ppm showed the lowest resilience. One-way ANOVA analysis revealed a significant impact ($p<0.05$) of ascorbic acid and fermipan enrichment in feed on the salinity shock resistance of *Artemia* sp. The observed data indicates a significant difference among treatments, with the AS 50 ppm treatment displaying the highest resilience.

Table 1. Percentage of Mortality of *Artemia* sp. in Ascorbic Acid Toxicity Test with 24 Hours Exposure Time

Concentration (ppm)	Percentage of Mortality (%)
0	3,3
100	5
200	15
400	100
600	100
1000	100

Table 2. Probit Analysis of LC_{50}-24 Hours

Concentration (ppm)	Number of Dead Animals	Total Tested Animals	LC_{50} 24 hours (ppm)
100	3	60	
200	9	60	
400	20	60	200,84
600	20	60	
1000	20	60	

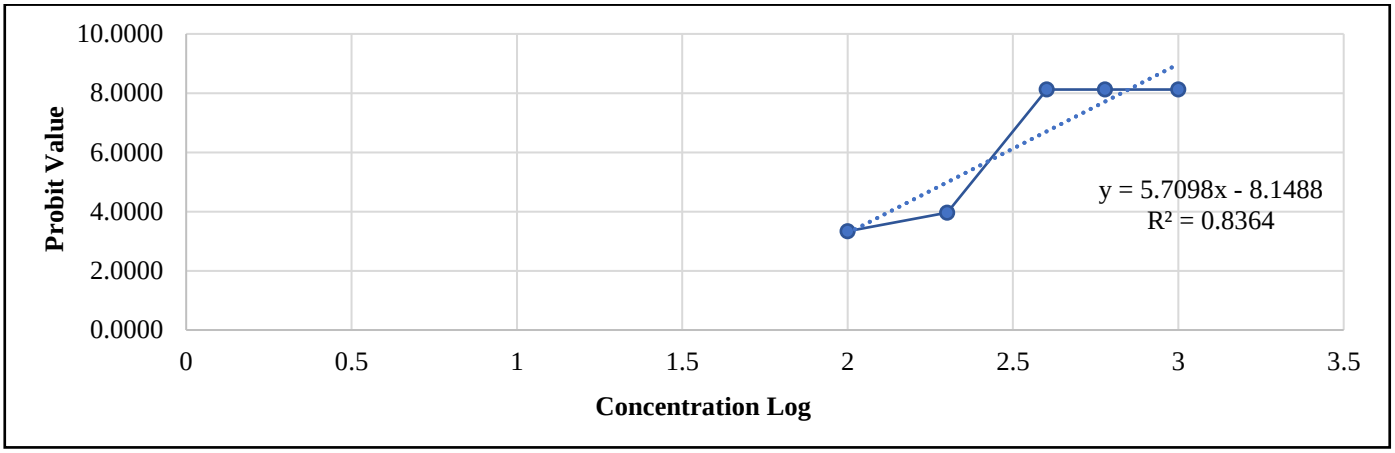
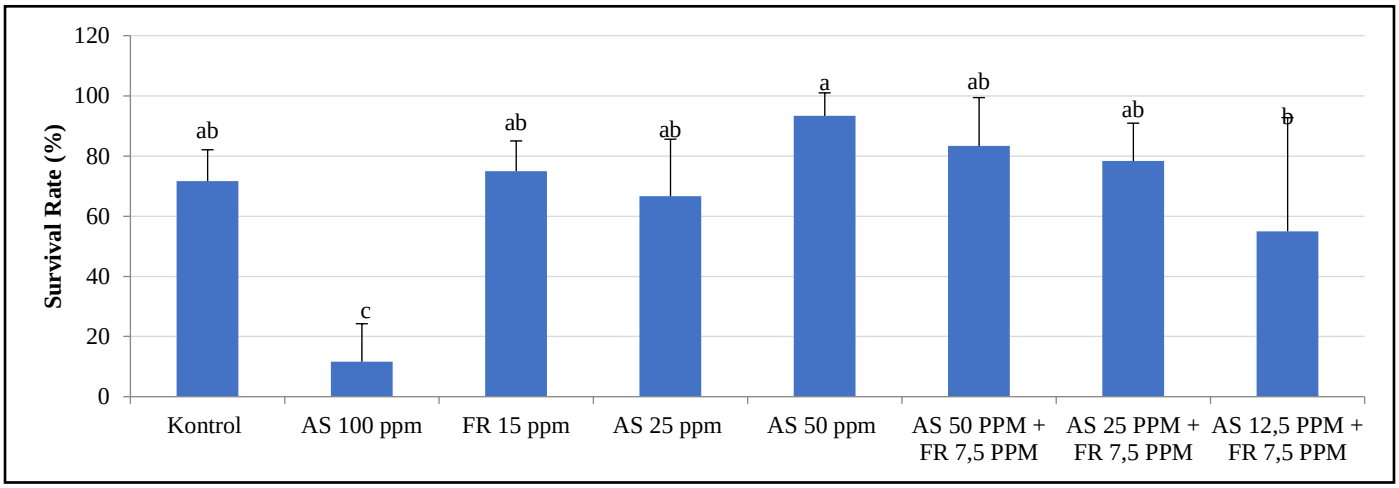
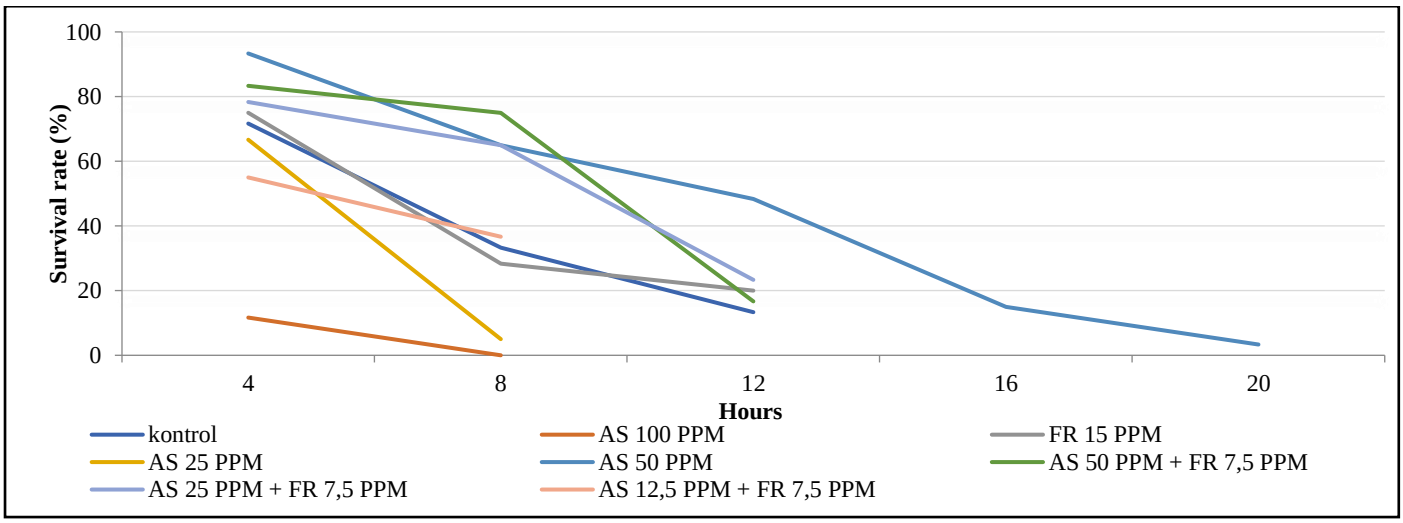


Figure 1. Analysis of Log Concentration Regression with Probit % Mortality of Ascorbic Acid.



Note : AS = Ascorbic Acid
FR = Fermipan

Figure 2. The average survival rate of *Artemia* sp. against salinity shock tests at different time intervals.



Note : AS = Ascorbic Acid
FR = Fermipan

Figure 3. The survival rate with the treatment of the test feed (Different notations at the same maintenance time indicate a significant difference with a confidence interval of 95%).

4. Discussion

Various factors influence the survival of *Artemia* sp., including salinity, temperature, pH, dissolved oxygen, and aeration (Bahari *et al*., 2014). An advantage of *Artemia* sp. lies in its ability to adapt to a wide range of salinities. Testing using ascorbic acid revealed changes in *Artemia* sp., indicating a level of toxicity. Enriching *Artemia* sp. with ascorbic acid provides antioxidants that protect lipids from oxidation (Sunarto *et al*., 2008). Fermipan enrichment contributes immunostimulants that enhance growth immunity in *Artemia* sp. (Manoppo *et al*., 2016).

The smallest size occurred in the control group due to the absence of incoming nutrients. Nutrition is a crucial component for growth. Enriching *Artemia* sp. with Highly Unsaturated Fatty Acids (HUFA) aims to increase DHA and EPA content, enhancing feed quality (Fadila *et al*., 2022). Good feed quality improves growth and stress resistance.

One-way ANOVA analysis revealed a significant impact ($p<0.05$) of ascorbic acid and fermipan enrichment in feed on *Artemia* sp.'s salinity shock resistance. The observed data indicates significant differences among treatments, with the AS 50 ppm treatment displaying the highest resilience. Ascorbic acid at a concentration of 50 ppm was the most effective, demonstrating prolonged resilience compared to other treatments. Excessive ascorbic acid negatively affects larval growth (Faidar *et al*., 2020). A deficiency in ascorbic acid decreases *Artemia* sp.'s immune response. Ascorbic acid plays a role in enhancing growth and immunity, influencing the body's defense and stress resistance (Setiawati *et al*., 2013). High concentrations of ascorbic acid can inhibit the reaction between ascorbic acid and oxygen molecules. The content of ascorbic acid in *Artemia* sp. depends on the soaking process, dosage, and temperature.

The addition of fermipan to feed enhances *Artemia* sp.'s immune response. Fermipan added to feed is expected to produce several energy substrates in the intestinal cells, thereby improving digestion (Dani *et al*., 2015). Adding fermipan to feed enrichment can increase seedling growth in fish. Protein content in feed determines the feed efficiency, with higher protein resulting in higher feed consumption. The addition of fermipan to *Artemia* sp. feed improves feed efficiency, enhancing growth (Jullianty *et al*., 2020). Fermipan can affect the growth rate by containing nucleotides that increase *Artemia* sp.'s appetite, leading to increased feed intake and growth. Nucleotides are semi-essential nutrients crucial for growth and can optimize immune cells (Sumardiyani *et al*., 2020).

Enriching *Artemia* sp. with a combination of fermipan and ascorbic acid did not significantly affect *Artemia* sp.'s resilience. The AS 50 ppm treatment exhibited prolonged resilience to salinity shock. Fermipan, rich in carbohydrates and proteins, can enhance immune resistance. The combination of ascorbic acid and fermipan in enriching *Artemia* sp. did not extend resilience under salinity, as the compound's metabolic properties did not mutually reinforce each other.

5. Conclusions

Ascorbic acid is toxic to Artemia sp., with an LC_{50}-24 hours value of 200.84 ppm (<1000 ppm). Resistance to salinity shock in Artemia sp. can be heightened by administering 50 ppm of ascorbic acid. However, fermipan administration, either alone or combined with ascorbic acid, doesn't significantly enhance the resistance of Artemia sp. The survival rates for fermipan 15 ppm are comparable to the control. Similarly, combinations of fermipan with ascorbic acid at 50 ppm and 25 ppm don't differ significantly from ascorbic acid at 50 ppm and 25 ppm, respectively.

Ethics approval

No need permit to *Artemia* sp.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Credit authorship contribution statement

Zulfa Istiqomah: writing original draft preparation, visualization, project administration and data collecting. Subagiyo and Ervia Yudiati: Conceptualization, methodology, validation, investigation, resources, formal analysis, funding acquisition writing original draft preparation, writing review and editing, supervision, visualization. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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