International Journal of Marine Engineering Innovation and Research, Vol. 8(2), Jun. 2023. 330-336
(pISSN: 2541-5972, eISSN: 2548-1479)

Effectiveness of Adding Different Types of Vitamin C in Improving the Quality of Artemia Salina sp.

Ajeng Sukmasari[1], Nuhman[2], Ninis Trisyani[3]
(Received: 26 May 2023 / Revised: 07 June 2023 /Accepted: 07 June 2023)

Abstrak- **Artemia is a natural feed that contains pigments (canthaxanthin), protein, vitamin C and some essential fatty acids to help fish and shrimp grow. Artemia contains vitamin C, but it is still suspected that additional vitamin C is needed for larval development, gonad maturity and gamete quality. The aim of this study was to analyse the effectiveness of vitamin C (ascorbic acid) absorption in laboratory enrichment of Artemia sp. The method used was laboratory experiments. The absorption of vitamin C was analysed using the UV-Vis spectrophotometry method at a wavelength of 450-750 nm, followed by the BNT test. UV-Vis spectrophotometer method to determine the amount of vitamin C absorbed during the immersion process of Artemia. The stocking density of Artemia cysts used was 2 g per 2 liters of water with a dose of Vitamin C 100 mg/liter. The results of the calculation of Hatching Percentage (HP) obtained results of 54.22% and 53.69%. UV-Vis spectrophotometer results showed that the concentration of Vitamin C absorption after the immersion process for 6 hours obtained an average of 0.8640 ppm and 0.8642 ppm. The conclusion obtained is that the addition of different vitamin C does not get any difference, this is indicated by the results of the BNT test which are not significantly different.**

Key Wordi—Artemia, UV-Vis Spectrophotometer, Vitamin C (ascorbic acid)

I. INTRODUCTION

The of artemia in the world of fisheries is very important, especially in shrimp farmers. Artemia body has vitamin C content. According to Gammanpila et al. (2007), vitamin C is required by fish for larval development, gonad maturation and gametes quality, and according to M-land et al. (2000), the vitamin C content of Artemia is 692 ± 89 mg/kg dry weight, Irmasari (2002) the protein content of Artemia is 63% of dry weight, but Artemia has a low vitamin C content of 19.99 µg/g dry weight. So it is necessary to increase the content of vitamin C through enrichment in order to improve the quality of shrimp fry or artemia itself, because especially shrimp in the early stages have a digestive tract that is still very simple so it requires food nutrients microorganisms that have high nutritional value.

Vitamin C functions as an anti-oxidant and increases the immune system of shrimp and fish, but vitamin C cannot be synthesized by the body of shrimp and fish. Therefore, to meet the larvae's need for vitamin C, it is expected that it can be provided through naupli artemia which is used as a natural feed with enrichment techniques using vitamin C. Artemia's advantage as a natural feed is that it contains pigments (canthaxanthin), protein, vitamin C and some fatty acids essential for larval growth and survival (Hafezieh et al., 2009). Previous research according to Hamdani, 2001 describes the content of different salinity levels (35ppt, 75ppt and 150ppt) on the growth rate of Artemia populations. Intrinsic growth rate (r) Artemia at salinity 150‰ about 0.901-1.281, while at salinity 35‰ and 75‰ the value of r is -1.330 to -0.877 thus at salinity 150‰ the survival of Artemia population is maintained, while at salinity 35‰ and 75‰ the survival of Artemia population is stopped.

On the basis of the above description, a study was carried out, namely artemia hatching trials with the addition of different types of vitamin C in improving the quality of Artemia salina sp using well water with a salinity of 32 ppt. This is related to the utilization of abundant water sources around the cultivation site.

II. METHOD

This study used materials such as artemia cysts, vitamin C (IPI), vitamin C (L-ascorbic acid WS FG), iodine 0.01 N (technical), distilled water, 96% ethanol, sodium thiosulfate, 10% acetic acid (technical), 5% H2SO4 (technical), 0.04% oxalic acid (0.2 ppm), vitamin C (0.2 ppm), 5% ammonium molybdate.

The tools used are glass jars with a capacity of 3lt, aerator hose, aerator, aeration stone, aerator faucet, LED lamp, digital pH, digital thermometer, DO meter, binocular microscope, optilap camera, stationery, camera, pipette, analytical balance, erlenmeyer, Whatmann number 1 filter paper, funnel, test tube, measuring cup, UV-Vis spectrophotometry.

The method used was laboratory experiment, with 2 treatments and 10 replications. Then the Vitamin C absorption was analyzed using UV-Vis Spectrophotometry method at 450-750 nm wavelength followed by t test.

Activities in this study began with hatching artemia

Ajeng Sukmasari. The Student of Postgraduate Program on Marine Coastal Engineering, The Faculty of Engineering & Marine Science, Hang Tuah University, Surabaya, Indonesia., Hang Tuah University, Surabaya, 60111, Indonesia. E-mail: ajeng.sukmasari@hangtuah.ac.id.
Nuhman. Departement of Fisheries, Hang Tuah University, Surabaya, 60111, Indonesia. E-mail: nuhman@hangtuah.ac.id.
Ninis Trisyani. Departement of of Fisheries, Hang Tuah University, Surabaya, 60111, Indonesia. E-mail: ninis.trisyani@hangtuah.ac.id.

salina. The artemia used is the Crystal brand artemia type. Artemia was hatched in a 3 liter jar with a volume of 20 pieces and then filled with well water with a salinity of 32 ppt as much as 2 liters and strongly aerated. The hatching density of artemia eggs is 2000 mg / 2 liters, then hatching artemia cysts for 24 hours with a temperature of 26-29°C.

The next step is the calculation of hatching percentage which is calculated using the formula:

$$HP = \frac{N}{C} \times 100\% \quad (1)$$

Description :
HP = Hatching Presentase
N = Number of Artemia nauplius hatched
C = Number of artemia cysts stocked

The third step is the immersion of artemia using vitamin C. Vitamin C which is used as an enrichment material is given to artemia larvae that have hatched. The length of the artemia immersion process against Vitamin C is 6 hours. The concentration of vitamin C to be given is 100 mg/l (Setiawati, 2013). After going through the soaking stage, then proceed with the harvesting process, namely filtering artemia larvae using planktonnet.

The fourth step is artemia harvesting which is done after artemia hatching by taking separate cyst shells and floating on the surface of the hatching media water and also taking unhatched and empty cysts. There are several ways to harvest nauplius. The old way of harvesting Artemia is to turn off the erator and then siphonize.

After the aerator is turned off, the unhatched cysts and empty cysts will float on the surface. The unhatched cysts will settle to the bottom. Nauplius swimming under the unhatched cysts can be siphoned out. The second way, utilizing the nature of Artemia nauplius that moves towards the light (positive phototaxis).

The next step is to analyze the vitamin C content in artemia using the UV-Vis spectrophotometric method. Analysis using spectrophotometry begins with the preparation of a standard solution and the creation of a standard curve.

III. Results and Discussion

A. Artemia Hatching Results

1) Artemia Cyst Count

Researchers calculated the number of artemia cysts in 10 sampling times in 0.0001 gram tafar and summed them up. After that the summation results are averaged or divided by 10 sampling times, in order to reach the number of cysts in 1 gram, the result of the number of cysts that are averaged is multiplied by 10,000.

From the results of these calculations, the researchers obtained the results of sampling Artemia in 1 gram which is as many as 493,000 grains. Because this study uses 2 grams in each container, 493,000 multiplied by 2 results in 986,000. So, it can be concluded that in every 2 grams of Artemia in one jar contains as many as 986,000 grains.

Based on the above statement, it is reinforced by the results of 0.0001 gram scales using the volumetric method (Tombinawa, 2016) in which the calculation found 968,000 / 2 grams of artemia cysts.

2) Calculation of Hatching Percentage (HP) of Artemia

The results of the hatching percentage calculation show the results of the hatching percentage of artemia cysts in each treatment can be seen in Figure 1.

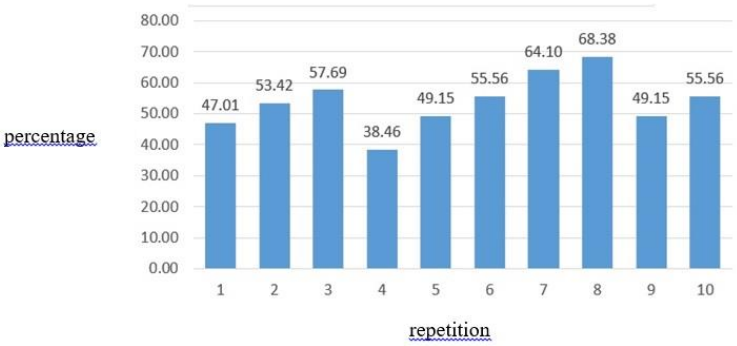

Figure 1. Hatching Percentage on Vitamin IPI.

In the hatching results with 32 ppt salinity control media for Vitamin C IPI immersion treatment, the average hatching data was 53.85%. The condition of the hatched artemia larvae also looks healthy, this is shown by the hatching artemia responding to light and moving actively during the harvesting process. The color of the artemia body tends to be fresh orange.

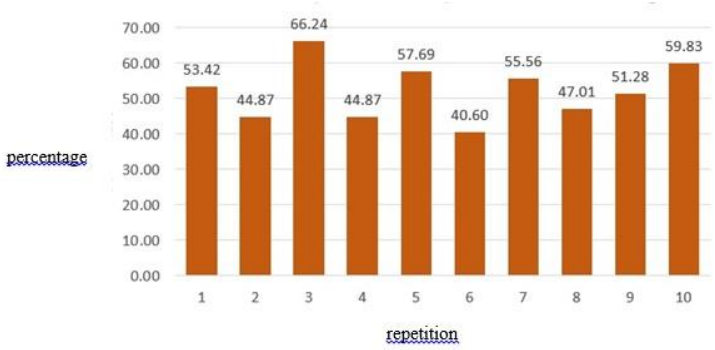

Figure 2. Hatching percentage in ws-fg vitamins.

In the hatching results with 32 ppt salinity control media for the Vitamin C ws-fg immersion treatment, the average hatching data was 52.14%. The condition of the hatched artemia larvae also looks healthy, this is shown by the artemia that hatches in response to light and moves actively during the harvesting process. The color of the artemia body tends to be fresh orange. After knowing the results of artemia hatching based on Wardhana (2020), the hatchability of artemia cysts observed for 24 after incubation at 32 ppt salinity is 65%, the statement above has results that are not much different in the assumption that artemia hatches more than 50% of the stocking amount. The best hatchability of artemia cysts was produced at 32 ppt salinity, namely 65% and the lowest at 26 ppt, namely 51.75%. Therefore, it is appropriate if in this study the hatching medium has a salinity of 32 ppt, temperature 28-29oC, pH 8 and dissolved oxygen 11-12 mg/lt and an average Hatching Percentage (HP) of 53.95%.

B. Test of Vitamin C Content in Artemia

1) Preparation of Standard Solution

Sample analysis using UV-Vis spectrophotometer is done by measuring the maximum wavelength. Measurement of the maximum wavelength can be known through the graph shown on the monitor screen as well as the highest absorbance value.

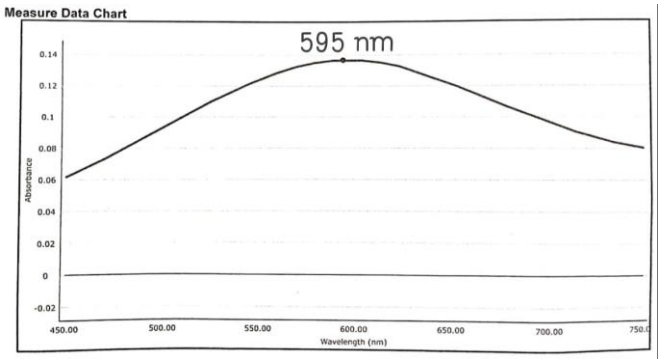

Figure 3. Maximum wavelength curve

The measurement results from the UV-Vis spectrophotometer show that the maximum wavelength of vitamin C is 595 λ with an absorbance value of 0.1368. The purpose of measuring the calibration curve between concentration and absorbance is to obtain a line equation or linear regression.

1) Standard curve generation (displaying results and data interpretation)

The calibration curve is a line that states the relationship between a concentration and absorbance absorbed after linear regression analysis. Calibration curve generation is done by measuring the amount of absorbance with a concentration variation of 0.2 ppm obtained concentration series 0; 0.1; 0.2; 0.4; and 0.5 ml. Data obtained from the measurement results can be seen in Table 1.

TABEL 1.
CALIBRATION CURVE DATA

D	Blanko	0,1 ml	0,2 ml	0,3 ml	0,4 ml	0,5 ml
Conc	0.0000	0.1000	0.1800	0.3800	0.5300	0.6600
A595	0.0000	0.0249	0.0440	0.0584	0.1197	0.1820

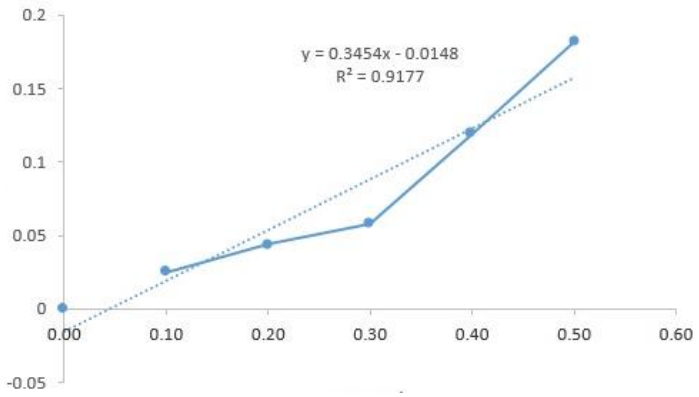

Figure 4. Calibration Curve

The calibration curve of vitamin C standard solution can be seen in Figure 4.

Based on the curve above, it can be seen that the calibration curve formed has the equation y = 0.3454x - 0.0148 with a value of R^2 = 0.9177. This regression value shows the correlation between concentration and absorbance. The correlation is declared perfect if the R value is close to +1, while the zero value states that there is no correlation between the two observed variables, namely concentration and absorbance (Dinararum and Sugiarso, 2013).

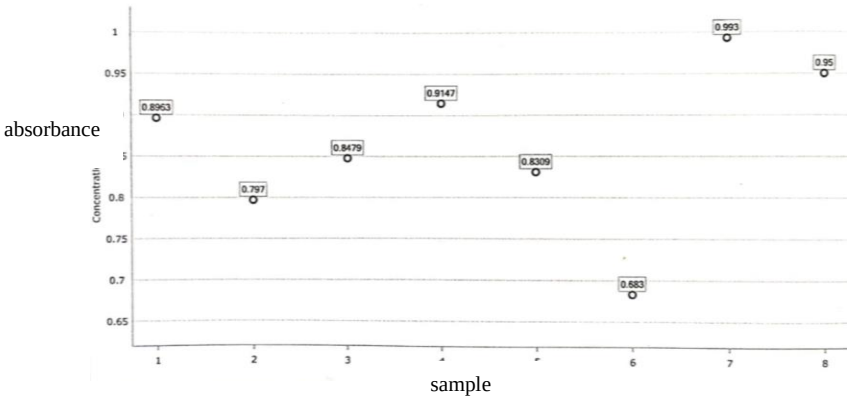

Figure 5. Sample Vitamin C Uptake Data (concentration)

The results of the analysis using the spectrophotometric method obtained the concentration of vitamin C in Artemia with the treatment of the addition of vitamin C IPI and vitamin C ws-fg can be seen in Figure 5.

In general, vitamin C is popular among the public as an antioxidant, anticancer, antibacterial, and so on. According to Subekti et al (2012) in glucogenesis metabolism in animals, vitamin C has a role as an energy provider when animals experience environmental stress. Based on the research of Dwinanti et al (2019) states that vitamin C can increase the growth rate of fish, play a role in non-specific immunity, increase survival, minimize bacterial attacks, and as a potential antioxidant.

Vitamin C given to Artemia is one of the natural feeds known to contain unsaturated fatty acids. As a natural feed, artemia can provide good growth rates in aquatic organisms. Darvishpour et al (2012) state that vitamin C has a role in the process of synthesis of noradrenaline and serotinine, which can reduce stress levels in fish or shrimp. The conversion of dopamine to norepinephrine requires vitamin C. Vitamin C plays a role in converting tryptophan to 5-hydroxytryptophan and serotonin. The hydroxylation of various steroids in adrenal tissue also involves vitamin C. The concentration of vitamin C in the adrenal glands decreases when adrenal hormone activity increases.

C. Statistical Test

1) Normality test of artemia hatchability

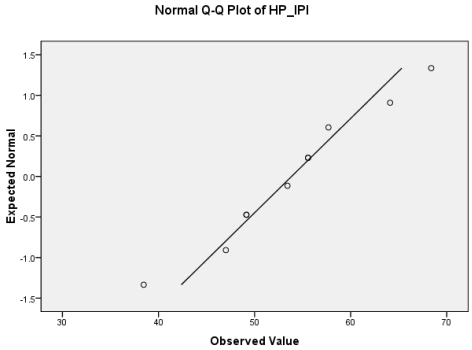

Figure 6. Q-Q plot test result

The Q-Q plot test above is used to test normality. It can be seen that the data is spread around / near the straight line from left to right above, so it can be said that the hatchability of artemia distribution is normal.

2) Static Table Data and Artemia Hatching Power T-Test

TABEL 2.
T-TEST RESULT

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	HP_IPI	53,8480	10	8,59444	2,71780
	HP_WS FG	52,1370	10	7,94437	2,51223

The table above shows the summary statistics of the two variables. The average hatchability of vitamin C IPI is 53.85% and the average hatchability of vitamin C ws-fg is 52.14%

TABEL 3.
T-TEST

		N	Correlation	Sig.
Pair 1	HP_IPI & WS FG	10	0,150	0,679

In the second table above, it can be seen that the correlation between the two variables is 0.150 with a probability value above 0.05 (sig. value in the table 0.679). This states that the correlation between the two variables is weak and not real.

3) T-Test Result of Hatchability of Artemia

TABEL 4.
T-TEST

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Dev	Std. Error	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	vitc_IPI vitc_ws fg	1,71100	10,79206	3,412575	-6,00918	9,43118	0,501	9	0,628

Based on the table above the probability value> 0.05, then H_0 is accepted.

4) Normality Test of Vitamin C Absorbance Value Data

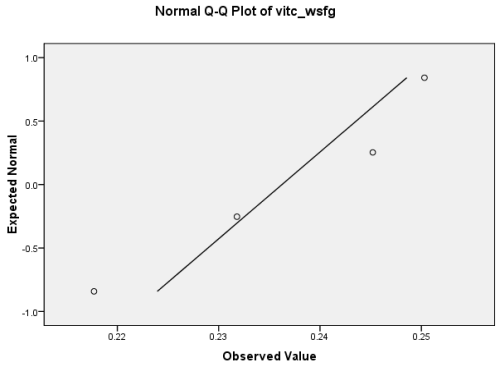

Figure 7. Q-Q plot test result

The Q-Q plot test above is used to test for normality. It can be seen that the data is spread around / near a straight line from left to right above, so it can be said that the distribution is normal.

5) Static Table Data and T Test of Vitamin C Absorbance

TABEL 5.
T-TEST RESULT

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	vitc_IPI	0,236325	4	0,384951	0,0192475
	vitc_ws fg	0,236250	4	0,0146220	0,073110

The table above shows the summary statistics of the two variables. The average absorbance value of vitamin C IPI is 0.236325 and the average absorbance value of vitamin C ws-fg is 0.236250 The amount of data for each variable is 4 times the test.

TABEL 6.
T-TEST

		N	Correlation	Sig.
Pair 1	vitc_IPI & vitc_ws fg	4	0,607	0,393

In the second table above, it can be seen that the correlation between the two variables is 0.607 with a probability value above 0.05 (sig. value in the table 0.393). This states that the correlation between the two variables is strong and not real.

6) T-Test Results of Vitamin C Absorbance Value Data

TABEL 7.
T-TEST

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Dev	Std. Error	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	vitc_IPI-vitc_ws fg	0,0000750	0,0318236	0,0159118	-0,0505635	0,0507135	0,005	3	0,997

Based on the table above the probability value> 0.05, then H_0 is accepted.

IV. CONCLUSION

On the basis of the results that have been obtained in this study, the conclusions that can be drawn are that:

1. The addition of Vitamin C content of different trademarks does not have a significant difference trend / not significantly different.
2. The vitamin C content in Artemia larvae after vitamin C immersion was obtained with an average concentration value of 0.8641.

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