

Effect of Vitamin C Bioencapsulation in Natural Feed on Protein, Fat, Energy, and Mortality of Milkfish Larvae (*Chanos chanos*)

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Abstract

Indications of milkfish larvae problems show lack symptoms of vitamin C in the tissues, which will cause defective bone formation and become an inhibitor factor in growth and mortality. This research aims to investigate the influence of vitamin C bioencapsulation on rotifer and artemia in protein retention, fat, energy, and the cause of death in milkfish larvae (*Chanos chanos*). The dose of vitamin C used in this study were 0 (control), 50, 100, 150, and 200 mg L⁻¹. The treatment starts from D-1 to D-30 stadia of milkfish larvae, with 30-day maintenance. The density of milkfish larvae utilized is 20 L⁻¹. The protein and fat content of milkfish larvae bodies were measured using a proximate analysis, and the energy content of the feed was measured using a bomb calorimeter. Mortality was observed under the Ministry of Health of Republic Indonesia standard binocular microscope by identifying larvae that died daily in each container. This research shows that vitamin C bioencapsulation in rotifer and artemia affects the retention rate of protein, fat, energy and the cause of milkfish larvae mortality. The dose of vitamin C of 150 and 200 mg L⁻¹ is effective in improving protein, fat, and energy retention, which can minimize the cause of milkfish larvae mortality. The best results are obtained by bioencapsulating Vitamin C on rotifer and artemia with dose of 150 mg L⁻¹.

Keyword: larvae quality, natural feed, production and technology improvements

INTRODUCTION

Milkfish (*Chanos chanos*) is a valuable source of animal protein with a high nutritional value and a large market share (Kresnasari, 2021). In 2021, Indonesia can produce 784,941.13 tons of milkfish worth IDR 15.56 billion. South Sulawesi's average milkfish production is 85,666.3 tons (Indonesian Data, 2022). In order to increase the profitability and sustainability of the milkfish business, milkfish hatchery activities must be of appropriate quantities, high quality, and ongoing continuity.

Feed issues in milkfish hatcheries cause slow fish growth, low survival, disease attacks, stress levels, abnormal larvae morphology, problems with technology, and a lack of supportive larvae management (Baiduri *et al.*, 2018). Low nutritional intake, including minerals and protein, can cause low metabolism, growth, survival, and stress levels in milkfish larvae. Natural feed consumption is strongly influenced by fish characteristics (Hatta *et al.*, 2019). Milkfish technology still depends on natural feed such as rotifer and artemia. The limitations of rotifer and artemia cultures include lower nutritional value due to a lack of quality and quantity, culture techniques, and culture duration. The amount and quality of natural feed will be affected by nutrient intake (Firmansyah *et al.*, 2013).

One of the vitamins that have an essential role in the growth and stress resistance of milkfish larvae is vitamin C. Vitamin C plays a role in normalizing immune function, reducing stress, and accelerating wound healing in fish. Indications of problems with milkfish larvae show lack symptoms of vitamin C in the tissue, which will lead to imperfect bone growth and act as a growth inhibitor factor when a deficiency occurs (Kursistiyanto *et al.*, 2013). According to Ambarwati *et al.* (2014), a lack of vitamin C in feed can cause structural changes and deformation of the skeleton (scoliosis and lordosis), indicated by loss of appetite, decreased growth, and even death.

Characteristics of rotifer and artemia as biocapsules that given to larvae can be improved nutritional quality with the addition of vitamin C (Alfisha *et al.*, 2020). Vitamin C has several benefits, including increased growth, stress resistance, immunity against disease, collagen production in fish, increased interferon levels and cell immunity activation, lymphocyte and macrophage activity, repair migration and mobility of leukocytes from viral infection (As'ari, 2021). Vitamin C has multiple roles in cell respiration and enzyme activity. The function of vitamin C is to oxidize phenylalanine to tyrosine, reduce ferric ions to ferrous in the digestive system so that iron ions can easily absorbed, convert folic acid to folinic acid (in its active form), and play a role in the production of steroid hormones from cholesterol. It is also an active antioxidant that prevents the disruption of fatty acid chains into various toxic cells compounds such as aldehydes and various hydrocarbons such as ethane and pentane, which can cause severe damage to cell membranes, including the membranes of erythrocytes (Sumitro and Afandi, 2021).

This research examined how the bioencapsulation of vitamin C on rotifer and artemia affects the retention of protein, fat, energy and the reasons for mortality of milkfish (*Chanos chanos*) larvae.

MATERIALS DAN METHODS

This research was carried out during September – October 2021 at Milkfish Production Unit, Takalar Brackish Water Aquaculture Fishery Center (BPBAP), Takalar, South Sulawesi. Analysis of retention of protein, fat, energy, causes of mortality and water quality was carried out at the Takalar BPBAP Test Laboratory. This research used milkfish larvae stage D-1 to D-30. The larvae were hatched at the BPBAP Takalar Hatchery Station. The test substance was commercial ascorbic acid (AA) powder as vitamin C. This research used rotifer (*Nannochloropsis* sp.) and artemia (*Artemia nauplii*) as natural feed and NRD microencapsulate (with diameter 30 – 90 μm) as artificial feed. The research containers used were 12 plastic buckets filled with 70 L of marine water with a density of 20 L⁻¹ milkfish larvae, as shown in Figure 1. Marine water has 30 – 33 ppt salinity with a green water system. The water quality parameters measured were temperature using a mercury thermometer with a precision of 0.1 °C, salinity using a hand refractometer with an accuracy of 0.1 ppt, pH using a pH-meter with an accuracy of 0.1, dissolved oxygen using a DO meter and ammonia using a spectrophotometer (American Public Health Association, 2017).

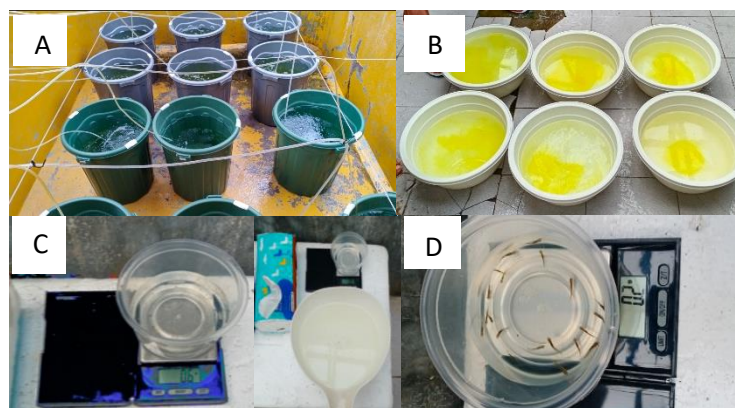


Figure 1. Research materials. (A) Milkfish maintenance containers, (B) Vitamin C solution, (C) Milkfish larvae before treatment, (D) Milkfish larvae after treatment.

Feed Preparation Procedure

Before being given to the larvae, the natural feed was enriched with vitamin C. Rotifer and artemia were enriched separately with vitamin C by immersing them in 1 L conical container filled with 0.5 L of marine water and provided with aeration. The experimental containers were randomly put in a closed room at 29 – 30 °C and 32 – 35 ppt of salinity. The container is filled with rotifer with a density of 500,000 ind./L and artemia with a density of 300,000 ind./L of water media, which is enriched with vitamin C based on the treatment dose for 4 – 6 hours. After enrichment, the rotifer were filtered using a 40 µm plankton net (Zainuddin and Aslamiah, 2012).

Feeding Procedure

The feeding schedule for milkfish larvae is shown in Table 1. From stage D-1 to stage D-5, milkfish larvae were fed with rotifer at a density of 10 – 15 ind./mL of medium water daily. After the milkfish larvae reach stage D-5, rotifer is mixed with artemia at a 5 – 20 ind./mL density of water medium until they reach stage D-10. Milkfish larvae were fed in the morning and evening at 0.5 – 2 ppm/day. Feed density was maintained during the research. Figure 2 shows the rotifer and artemia given to milkfish larvae.

Table 1. Feeding Schedule for Milkfish (*Chanos chanos*) Larvae.

Feed	Larvae Stadia					
	D-1	D-5	D-10	D-15	D-20	D-30
Rotifer	15 ind./mL	15 ind./mL	15 ind./mL			
Artemia		5 ind./mL	15 ind./mL	15 ind./mL	20 ind./mL	
Artificial Feed		NRD 1 ppm	NRD 1 ppm	NRD 1.5 ppm	NRD 2 ppm	NRD 2 ppm



Figure 2. Rotifer and Artemia Given to Milkfish Larvae.

The research design used a completely randomized design (CRD) which consisted of four treatments and three repetitions. The dose of vitamin C used in this study were 0 (control), 100, 150, and 200 mg L⁻¹ (Setiawati, 2013). Protein and fat content of the test fish bodies were measured using proximate analysis at the beginning and the end of the research, while the energy content of the feed was measured using a bomb calorimeter.

This study used an equation according to Salam (2022) to calculate protein retention, fat retention, and energy retention, as follows:

Protein retention

$$RP (\%) = \frac{Pt - Po}{Total\ protein\ consumed} \times 100 \dots \dots \dots (1)$$

Note: RP = Protein retention (%); P_t = Body protein content after treatment (g); P_o = Body protein content before treatment (g).

Fat retention

$$RL (\%) = \frac{L_t - L_o}{Total\ fat\ consumed} \times 100 \dots \dots \dots (2)$$

Note; RL = Fat retention (%); L_t = Body fat content after treatment (g); L_o=Body fat content before treatment (g).

Energy retention

$$RE (\%) = \frac{E_t - E_o}{Total\ energy\ consumed} \times 100 \dots \dots \dots (3)$$

Note: RE = Energy retention (%); E_t = Body energy content after treatment (kcal); E_o = Body energy content before treatment (kcal).

Milkfish larvae mortality was observed using a standard binocular microscope from the Ministry of Health of Republic Indonesia by identifying larvae that died every day in each treatment container. Previous killed larvae were removed from each container as samples and identified based on the cause of death. The causes of milkfish larvae mortality were calculated using an equation according to Shalihin et al. (2017), as follow:

$$Mt (\%) = \frac{L_t}{L_o} \times 100 \dots \dots \dots (4)$$

Note: M_t = Mortality percentage due t; L_t = Larvae that die because t; L_o = Total of dead larvae.

Data Analysis

The research data were analyzed using the mean value, then presented as a frequency distribution table. Regression correlation was used to determine the relationship as a response to treatment (Purwanti et al., 2014). Physical and chemical parameters of water will be analyzed descriptively based on the viability of milkfish larvae. Furthermore, SPSS version 23.0 software was used for statistical tests.

RESULT AND DISCUSSION

Retention of protein, fat, and energy of Milkfish larvae with various doses of vitamin C enrichment in rotifer and artemia at the end of maintenance is presented in Table 2.

Table 2. Results of Measurement of Protein, Fat and Energy Retention of Milkfish Larvae at Various Doses of Vitamin C Enrichment.

Vitamin C Doses (mg L ⁻¹)	Nutrition Retention (%)		
	Protein	Fat	Energy
0	8.56 ± 0.09	4.88 ± 0.09	8.39 ± 0.12
100	9.19 ± 0.16	6.47 ± 0.06	9.37 ± 0.09
150	11.32 ± 0.06	8.02 ± 0.05	11.36 ± 0.09
200	14.42 ± 0.09	9.62 ± 0.40	12.38 ± 0.05

Measurements of protein, fat, and energy (carbohydrate) retention in milkfish larvae obtained results where for protein retention ranged from 8.56 ± 0.09 to 14.42 ± 0.09%. The highest protein retention measurements were found in the 200 mg L⁻¹ treatment and the lowest in the

treatment of 0 mg L⁻¹. Measurement of fat retention in milkfish larvae that had been treated with bio-encapsulation of vitamin C obtained results ranging from 4.88 ± 0.09%. The highest fat retention results were found in the 200 mg L⁻¹ treatment of 9.62%, and the lowest was in the 0 mg L⁻¹ treatment of 4.88%. Measurement of the energy retention of milkfish larvae obtained results in the range of 8.39 ± 0.123%, while the highest energy retention was found in the 200 mg L⁻¹ treatment, which was 12.38 ± 0.05%, and the lowest energy retention was in the 0 mg L⁻¹ treatment, which was 8.39 ± 0.123%.

Protein retention illustrates the quantity of protein delivered that can be absorbed and used to construct or repair damaged body cells and utilized for daily metabolism (Kurniawan *et al.*, 2017; Ananda *et al.*, 2022). According to Setiawati *et al.* (2013), Protein retention is the accumulation of proteins derived from diet and retained in the fish's body. There was a link between vitamin C supplementation and increased protein retention in this research. The highest protein retention results were found in the 200 mg L⁻¹ treatment at 14.42% and the lowest in the 0 mg L⁻¹ treatment at 8.56%. According to Siahaya (2020) higher protein and fat retention increase fish growth, then feed usage becomes more efficient. It can be seen in the increased feed efficiency value, which correlates to higher quantities of vitamin C in the feed.

Fat retention describes the ability of fish to store and utilize feed fat, as well as to construct or repair damaged body cells and to be used for daily metabolism. The 0 mg L⁻¹ treatment had the lowest fat retention value of 4.88%, while the 200 mg L⁻¹ treatment had the highest fat retention value of 9.62%. The fat retention value for the 100 mg L⁻¹ treatment was 6.47%. Nevertheless, it was 8.02% with the 150 mg L⁻¹ treatment. These data show that the fat retention acquired in this research is proportionate to protein retention. The higher protein retention, then the higher fat retention. Siahaya (2020) states that differences in vitamin C levels in the feed also cause an increase in fat retention. As a result, the fat content of the fish will increase.

Energy retention describes the ability of fish to store and utilize energy (carbohydrates) in feed to construct or repair damaged body cells and for daily metabolism (Sukmaningrum and Setyaningrum, 2014). In this research, energy retention was assessed following the end of maintenance, with the 200 mg L⁻¹ treatment achieving the highest energy retention of 12.38%. The 0 mg L⁻¹ treatment has the lowest energy retention of 8.39%, the 100 mg L⁻¹ treatment had 9.37%, and the 150 mg L⁻¹ treatment was 11.36%. According to Sukmaningrum and Setyaningrum (2014), energy retention shows a significant contribution of feed energy consumed to increased fish energy. The feed given is a source of energy used for fish maintenance, metabolic activity, and growth. Moreover, Tantri *et al.* (2016) said that the balance between energy and protein levels plays an important role in growth. If energy requirements are lacking, protein will be broken down and used as an energy source. Utilization of protein as an energy source will inhibit the growth of cultivars. Energy retention tends to be low when compared to several other studies related to energy retention. Saputra (2014) states that this occurs because the body expends much of the energy produced for metabolism, reproductive activity, and biosynthesis and is lost in the form of heat. The stored energy is utilized in the synthesis of cell components and used as fuel in the cell's energy production.

The relationship between the dose of bioencapsulated vitamin C in rotifer and artemia with protein retention in milkfish larvae after maintenance is shown in Figure 3. Based on Figure 3, the pattern tends to be linear with regression equation result of milkfish larvae protein retention is $y = 1.714x + 5.9456$, where the value of the determinant coefficient R^2 is 0.9259. This suggests that 93% the effect of bio-encapsulation of vitamin C at various doses will affect milkfish larvae retention. Vitamin C contributes in hydroxylation of amino acid proline and lysine to hydroxyproline and hydroxylysine, which are essential components in collagen synthesis. Collagen is the most predominant protein that necessary for maintaining biomechanical characteristics and other

biological processes in fish tissues (Guo *et al.*, 2022).

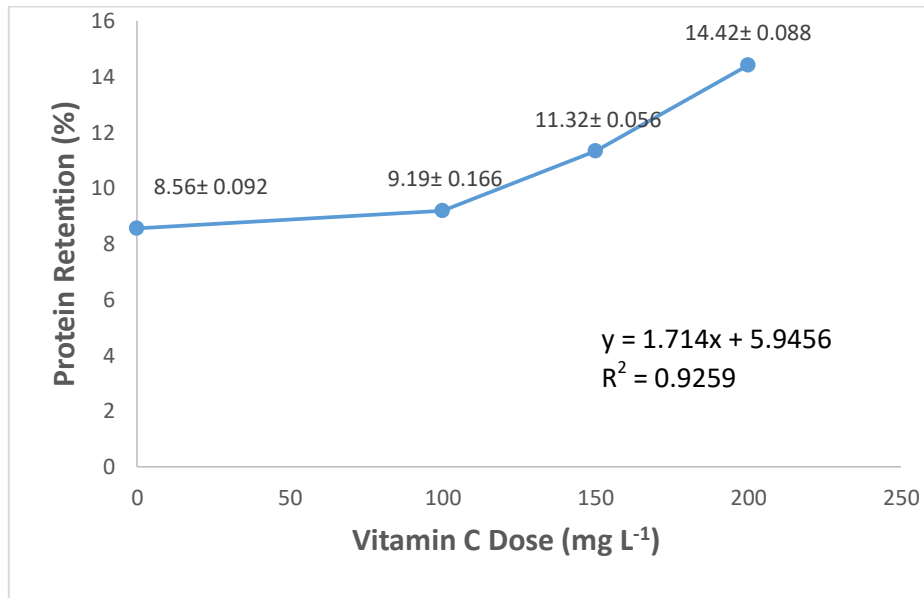


Figure 3. The pattern of protein retention in milkfish larvae (*Chanos chanos*) at various doses of natural feed enrichment with vitamin C.

The results of the fat retention patterns of milkfish larvae (*Chanos chanos*) at various doses of natural feed enriched with vitamin C are shown in Figure 4. The relationship between fat retention and doses of vitamin C enrichment in rotifer and artemia, as shown in Figure 4, shows a positive correlation. Based on Figure 4, the linear pattern formed and the regression equation for milkfish larvae protein retention is $y = 1.5767x + 3.3047$ with a determinant coefficient value of $R^2 = 0.9999$. This value indicates that 99% the effect of bioencapsulation of vitamin C with different doses have an impact on the fat retention of milkfish larvae. As an antioxidant, vitamin C can inhibit the oxidation of unsaturated fatty acids, which are components of lipid in cell membranes. Lipid in cell membranes play an essential role in cell fluidity, contributing to fish metabolism (Sersermudy *et al.*, 2020).

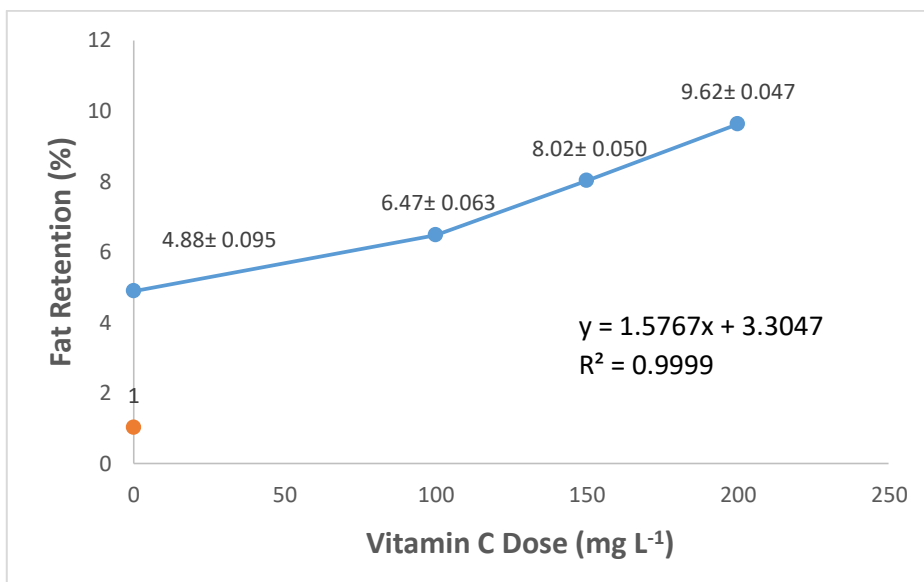


Figure 4. The Fat retention pattern of milkfish (*Chanos chanos*) larvae at various doses of natural feed enrichment with vitamin C.

The results of the energy retention patterns of milkfish larvae (*Chano chanos*) at various doses of natural feed enriched with vitamin C can be seen in Figure 5. The result shows a correlation between doses of bio-encapsulated vitamin C on rotifer and artemia on energy retention of milkfish larvae. In Figure 5, a linear pattern is formed, and the energy retention regression equation for milkfish larvae is $y = 1.395x + 6.8882$ with a determinant coefficient value of $R^2 = 0.9802$. This shows that bioencapsulation at different doses of vitamin C will affect the fat retention of milkfish larvae by 98%. Vitamin C can act as a coenzyme in the metabolic activities of fish. A good metabolism is essential in absorbing feed energy and providing high energy retention (Yanto, 2016).

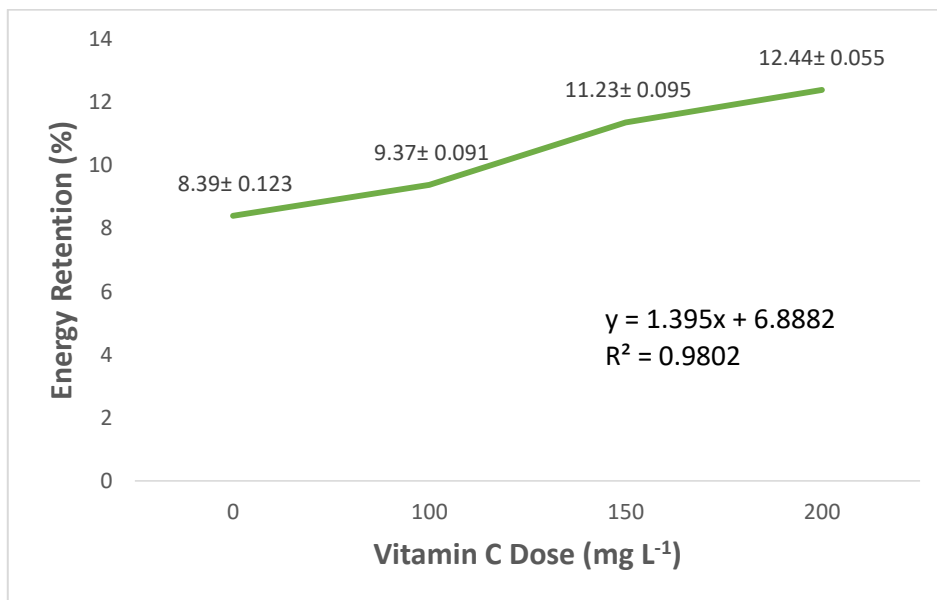


Figure 5. Pattern of energy retention of milkfish larvae (*Chanos chanos*) at various doses of natural feed enrichment with vitamin C.

Causes of Mortality

Causes of milkfish larvae mortality with various doses of vitamin C enrichment in natural feed at the end of maintenance are shown in Table 3.

Table 3. The Calculation results of the causes of mortality of milkfish larvae with various doses of vitamin C enrichment.

Vitamin C Dose (mg L ⁻¹)	Causes of Mortality (%)			
	Unknown	Stress	Malnutrition	Pathogen
0	84	12	0	4
100	85	12	0	3
150	87	11	0	3
200	90	9	0	2

The calculation of milkfish larvae mortality with doses of bioencapsulation of vitamin C was

carried out by looking at pathogenic factors, stress, and malnutrition. As for mortality that the cause cannot be known, it will be included in the cause of unknown mortality. Mortality due to pathogens was obtained by the percentage of mortality ranging from 2 - 4%, with the highest mortality due to pathogens occurring in the 0 mg L⁻¹ treatment of 4%, then downward successively in 150 mg L⁻¹ treatment of 3%. At the same time, the lowest percentage of mortality was in the 200 mg L⁻¹ treatment at 2%. The highest percentage of causes of mortality due to stress occurred in the 0 mg L⁻¹ treatment at 12%. This value is the same as the 100 mg L⁻¹ treatment condition. For the 150 mg L⁻¹ treatment, a mortality percentage of 11% was obtained. In comparison, the smallest percentage for the cause of mortality due to stress is found in the 200 mg L⁻¹ treatment at 9%. There was no mortality due to malnutrition in this research. The highest cause of unknown mortality occurred in the 200 mg L⁻¹ treatment at 90%, followed by the 150 mg L⁻¹ treatment at 87%, the 100 mg L⁻¹ treatment at 85%, then the smallest percentage in the 0 mg L⁻¹ treatment at 84 %.

The use of vitamin C as a natural feed enrichment source for rotifer and artemia plays a role in increasing the body's immunity to milkfish larvae. There are four causes of mortality: stress, malnutrition, pathogens, and unknown. In this research, results were obtained where mortality caused by pathogens was 2 – 4%. The highest results for mortality caused by pathogens or disease occurred at 0 mg L⁻¹ of vitamin C treatment and the lowest at 100 mg L⁻¹ of vitamin C treatment. The results of the 0 mg L⁻¹ of vitamin C treatment were in line with Farida *et al.* (2014) that a vitamin C deficiency in the feed will cause various diseases, such as swimming without direction, pale body color, and bleeding on the body surface. Vitamin C plays a role in the process of maintaining mucous membranes, which can affect immune function and increase resistance to infection (Setiawati *et al.*, 2013).

In this research, the results were obtained where mortality caused by stress was in the range of 9 – 12% mortality. The highest results for death caused by stress or pressure from environmental factors occurred in the 0 mg L⁻¹ treatment and the lowest in the 200 mg L⁻¹ treatment. The need for vitamin C will increase as the level of stress increases (Thabri, 2017). Under stress conditions, vitamin C can stimulate the pituitary-adrenal axis to secrete adrenaline and vitamin C into the blood simultaneously. Cortisol and catecholamine hormone levels in the body of stressed fish will increase. This hormone plays a role in stimulating the production of blood glucose to be used as energy to deal with stress. If the vitamin C levels are optimal, the catecholamine synthesis process can continue well even though environmental conditions are not good. Therefore, the fish can survive the physiological changes in their bodies, or stress does not occur. According to Narra *et al.* (2015), the treatment of 200 mg L⁻¹ vitamin C to catfish feed can help the detoxification process or prevent cell peroxidation so that fish can control stress due to the environment. The treatment of vitamin C in white pomfret feed (*Pampus argenteus*) for 14 days of maintenance affected cortisol metabolism, where cortisol regulation was better during transportation stress than in fish without vitamin C treatment (Dwinanti and Sasanti, 2019).

The undetected or unknown mortality factor was 84 – 90%, where more than 50% of the unknown mortality occurred in the first five days of maintenance. In the 5-day phase at the beginning of maintenance, the size of the larvae was still very small, and it was difficult to find the carcasses of milkfish larvae for observation. This causes the deaths in the first five days of maintenance to be included in the mortality category with unknown causes.

Water Quality

Water quality is one factor that plays an important role in supporting the life and development of milkfish larvae. The physical and chemical parameters measured in this research were salinity, temperature, pH, dissolved oxygen, and ammonia. The results of water measurements during maintenance and their feasibility values are based on standard references, as presented in

Table 4.

Table 4. The Results of Water Quality Measurements During Maintenance Milkfish Larvae (*Chanos chanos*) at various doses of vitamin C enrichment.

Parameter	Vitamin C Doses (mg L ⁻¹)				Feasibility Value
	0	100	150	200	
Temperature (°C)	29 – 32	29 – 32	29 – 32	29 – 32	28 – 32*
Salinity (ppt)	30 – 31	30 – 31	30 – 31	30 – 31	30 – 35 *
pH	8.0 – 8.5	8.1 – 8.4	8.1 – 8.5	8.1 – 8.4	7.0 – 8.5*
DO (mg/L)	5.02 – 5.98	5.04 – 6.02	5.02 – 5.96	5.04 – 6.00	Min 5 *
Ammonia (mg/L)	0.005 – 0.018	0.005 – 0.017	0.005 – 0.016	0.005 – 0.018	< 0.1 *

Note: * National Standardization Agency of Indonesia (BSN) (2011)

DO: Dissolved Oxygen

The results of temperature measurements during maintenance in each treatment were in the range of 29 – 32 °C. The temperature has an important role on larvae metabolism and the activity of microorganisms in water (Boyd and McNevin, 2015). Optimal temperature conditions will stimulate the growth of milkfish larvae to run well, but it can also inhibit the growth of milkfish larvae and cause stress and even death. The temperature in the natural environment for milkfish populations in the waters generally ranges from 25 – 32 °C. The temperature measurements during milkfish larvae maintenance show in the range of 29 – 32 °C. These results are conditions considered optimal in supporting the life of milkfish larvae.

The results of salinity measurements in each treatment were in the range of 30 – 31 ppt. Salinity is one of the parameters of water quality, which is very influential on the survival of larvae. This quantity is still optimal in supporting larvae life (National Standards Agency of Indonesia, 2011). According to Fariedah *et al.* (2021), exposure to salinity above the salinity tolerance limit causes larvae to struggle to sustain their life due to the significant difference in osmotic pressure between them and their environment.

The results of pH measurements for each treatment during maintenance were in the range of 8.0 – 8.5. In maintenance media with relatively low pH values, it will slow the growth of fish, as well as conditions in the range of relatively high pH values. The pH value will affect water quality variables and organism activities (Supono, 2018). The result of pH measurement of this research is supported by Suryani *et al.* (2022) that the optimum pH value for milkfish cultivation is 7.5 – 8.5.

The results of DO or oxygen levels measurements during maintenance in each treatment were in the range of 5.02 - 6.0 mg L⁻¹. Dissolved oxygen levels in water medium significantly impact metabolic activity, feeding, and growth. The dissolved oxygen content in fish farming containers is recommended to be at least 5 ppm (Yuniaanto and Noerbaeti, 2019; Suryani *et al.*, 2022).

The results of ammonia measurements for each treatment were in the range of 0.005 -0.018 mg/L. Ammonia can be created by milkfish larvae excretory residues or feces, usually in the form of gas. An increase the level of ammonia in the water can be caused by poor feed quality and also its management (Saputra and Fotedar, 2021). Moreover, ammonia can be produced by feed which not consumed (decayed) by milkfish larvae, causing ammonia to dissolve in water. According to the National Standards Agency of Indonesia (BSN) (2011), the ammonia range for larvae viability is <0.2 ppm. The quantity of ammonia obtained from all treatments in this research varied from 0.005 - 0.02 mg L⁻¹. This quantity is still appropriate for the survival of milkfish larvae.

CONCLUSION

Bioencapsulation of vitamin C in rotifera and artemia with different doses increased the retention of protein, fat and energy in milkfish larvae. The results showed that the highest protein retention was in the 200 mg L⁻¹ treatment of 14.42 ± 0.09 %, the highest fat retention was in the 200 mg L⁻¹ treatment of 9.62 ± 0.40 %, and the highest energy retention was found in the 200 mg L⁻¹ treatment, which was 12.38 ± 0.05 %. The causes of mortality of milkfish larvae that were given bioencapsulated vitamin C on rotifer and artemia showed a difference with the treatment without bioencapsulation of vitamin C, where treatment with a dose of 150 mg L⁻¹ was the best dose.

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