Enriched Rotifers (*Brachionus plicatilis*) on the growth and survival rate of Barramundi (*Lates calcarifer*) larvae

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Abstract. The availability of high-quality natural feed is one of the key factors in the successful cultivation of barramundi (*Lates calcarifer*) larvae. Rotifers (*Brachionus plicatilis*) are the main feed during the early larval stage; however, their nutritional content needs to be improved through an enrichment process. Rotifers, as natural feed for fish larvae, not only provide protein and other nutrients, but are also considered living capsules capable of transferring micromolecules. Another advantage of rotifers is their very slow movement, making them easy for fish larvae to catch. Several studies on the enrichment of rotifers using specific types of vitamins have been conducted, but they have not yet been applied to specific fish species. This study aimed to analyze the effect of enriched rotifers on the growth and survival of barramundi larvae. The study was conducted from day 2 to day 17. The treatments tested were: A (*Chlorella* sp.+ yeast), B (*Chlorella* sp. + yeast + Ascorbic acid), C (*Chlorella* sp. + yeast + Cobalamin), and D (*Chlorella* sp. + yeast + Ascorbic acid + Cobalamin). The results showed that treatment D produced the highest absolute length growth of 0.95 cm, while treatment B resulted in the highest survival rate of 41.7%.

1 Introduction

Several research studies on barramundi larvae have reported low survival rates. [8] Reported that a survival rate of barramundi larvae only 8.89%. Also reported that mortality barramundi larvae have rates of up to 100% [9]. Fish mortality can occur due to unsuitable low-quality feed and environmental conditions [2]. Therefore, the availability of high-quality and sufficient natural feed is very important for larval rearing.

One type of zooplankton that is commonly used is *Branchionus plicatilis*, a type of rotifer that has been used as feed for various fish and shellfish larvae. Providing rotifers at a density of 10–20 individuals/ml can increase survival rates by up to 72%. The quality and quantity of rotifers are determined by the type of nutrition they receive [10]. Rotifer nutrition can be enhanced through nutrient enrichment. Common types of feed given to rotifers include algae, yeast, vitamins, and enrichment emulsions [7].

The growth of barramundi larvae requires vitamins that serve as supplements for the juveniles [13]. A combination of ascorbic acid and cobalamin is considered ideal. Cobalamin

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can aid in the secretion of digestive enzymes, thereby enhancing fish growth. Cobalamin deficiency can lead to symptoms such as reduced growth and decreased feed intake (anorexia) [5].

The aim of this study is to determine the effect of rotifer enrichment using various enrichment materials on the growth and survival of barramundi larvae.

2 Materials and methods

The barramundi larvae used in this study were two days old (D2), with an initial length and weight of 0.21 ± 0.04 cm and 0.49 ± 0.06 mg, respectively. A total of 300 larvae were used per container, equivalent to 10 larvae per liter. At this age, the larvae had entered the early exogenous phase, which is the stage where they begin actively searching for and consuming food from their environment, as the yolk sac reserves start to deplete. By D2, the morphological structure of the larvae's digestive tract had developed sufficiently to begin digesting external feed such as rotifers [12]. The containers used had a capacity of 40 liters, totaling 12 units, each filled with 30 liters of seawater. Each container was equipped with moderate aeration to diffuse oxygen and assist in larval movement.

This study used a Completely Randomized Design (CRD) with 4 treatments and 3 replications. The stocking of barramundi larvae into the experimental in the morning, with a stocking density of 10 fish/L for all treatments [14]. Each containers used were 30 liter water. The enrichment materials used were *Chlorella* sp. with a density of 1.5–2.0 × 10^6 cells/mL yeast = 200 µg/mL [11] ascorbic acid = 4 µg/mL [7] and and cobalamin = 1.4 µg/mL.

The treatments that have been tested in this study are:
A: Chlorella + Yeast
B: Chlorella + Yeast + Ascorbic acid
C: Chlorella + Yeast + Cobalamin
D: Chlorella + Yeast + Ascorbic acid + Cobalamin

Chlorella was cultured in 2.5-liter jars. The density of Chlorella was measured using a hemocytometer to determine the number of cells each milliliter. Harvesting was carried out by directly collecting the required amount of Chlorella as needed.

Rotifers (*Brachionus plicatilis*) used as live feed for the larvae were of SS type size and cultured under controlled conditions. Rotifers are cultured using a 25-liter container with the provision of nutrients according to the treatment being tested. Observations on individual density (ind/ml) are conducted every day for 7 days in each treatment to evaluate their growth. Nutrient enrichment of the rotifers was carried out for 4 hours prior to feeding [11]. Nutrients were administered to the rotifers twice a day, at 06:00 am and 00:00 pm, and were used as feed for the larvae at 11:00 am and 16:00 pm. The rotifers were provided to the barramundi larvae at a density of 10 individuals/ml. Rotifer density was measured before and after enrichment to ensure consistency in the number provided to the larvae.

The nutrient enrichment doses used were Chlorella at 1.5–2.0 × 10^6 cells/mL, yeast at 200 µg/ml, ascorbic acid at 4 µg/ml, and cobalamin at 1.4 µg/mL. The observed water quality parameters included temperature, pH, salinity, and dissolved oxygen (DO). Water quality parameters were measured daily in the morning and afternoon [8].

Growth monitoring was carried out by sampling every 7 days. Ten fish each container were carefully caught using a scoop net and a small container to prevent stress, then measured and recorded.

Monitoring of barramundi larval growth was conducted to assess the effects of administering rotifers enriched with various tested nutrients on both growth and survival of the barramundi larvae. The growth parameter observed was the absolute increase in length [11]. This approach aligns by [13] who stated that fish growth parameters can be assessed through changes in body length. Larval length measurements were taken every 7 days over a

15-day rearing period. These measurements were intended to monitor the growth dynamics of the larvae under each treatment.

3 Result

3.1 *Chlorella* sp. growth

The growth of *Chlorella* sp. during the culture period showed a characteristic dynamic corresponding to the growth phases of microalgae. Observations were made daily over a certain period to determine the growth rate based on cell density (cells/mL) [4]. These observations provide an overview of the adaptation ability, nutrient utilization, and the potential of *Chlorella* sp. as a live feed. The growth data of Chlorella during the culture period can be seen in Figure 1.

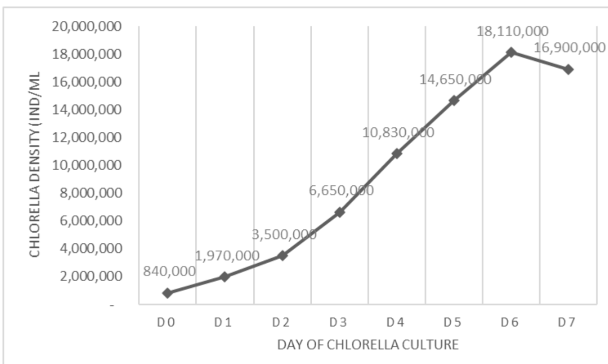

Fig. 1. *Chlorella* sp. growth graph in 7 days culture.

Based on Figure 1, the density of *Chlorella* sp. on the first day was recorded at 840,000 cells/ml. From the first day to the third day, there was a relatively slow increase in density due to the initial lag phase. According to [2], the lag phase occurs from day 0 to day 3, characterized by a slow growth rate because the algae have not yet optimally utilized nutrients for growth. Subsequently, from the fourth to the sixth day, there was a significant and steady increase in cell numbers as the culture entered the logarithmic (exponential) phase. This phase is highly optimal because metabolic processes and cell division occur actively [1] However, starting on the seventh day, the growth rate began to decline, entering the death phase. This is indicated by a decrease in cell density, which aligns with the statement by [2] that the death phase in *Chlorella* sp. occurs on day 7. *Chlorella* sp. was harvested on the sixth day, during the exponential (logarithmic) phase, because at this stage the algal cells experience their most active growth and cell density reaches its optimal peak.

3.2 Rotifers growth

The growth of Rotifers shows a dynamic pattern influenced by the type of nutrients provided. Rotifer growth can be seen in Figure 2. Rotifer density increased in all treatments until day 5, then decreased on days 6 and 7. The highest increase occurred in treatment D (yeast + Chlorella + ascorbic acid + cobalamin), followed by treatments C, B, and A. This indicates that the addition of ascorbic acid and cobalamin in the feed affects the growth rate of the rotifer population.

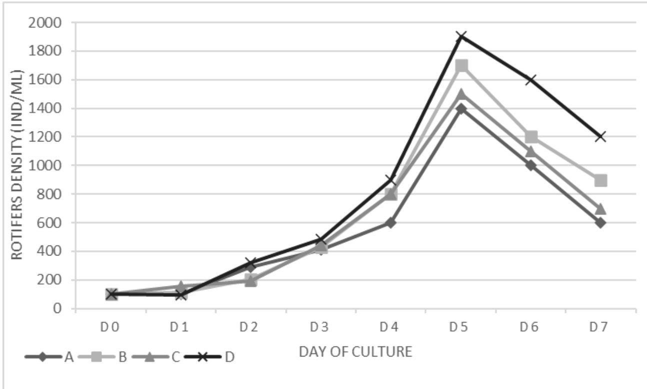

Fig. 2. Growth graph of rotifers with different nutritional treatments during 7 days of culture.

Ascorbic acid is known to act as an antioxidant that supports the immune system and energy metabolism, thus accelerating the reproduction rate of rotifers. Research by [12] shows that feeding yeast enriched with ascorbic acid significantly increases Rotifers density.

Cobalamin functions in DNA synthesis and cellular metabolism, contributing to growth of rotifer cells. Research by [6] shows that the use of yeast, ascorbic acid and cobalamin as feed enrichers has a very significant effect on the increase in rotifer populations, and the best treatment being a combination of all.

Treatment A (yeast + *Chlorella* sp) resulted in the lowest density compared to other treatments of rotifers. Although *Chlorella* sp. contains important nutrients such as proteins, lipids, and essential fatty acids, without additional vitamin enrichment, rotifers' reproductive capacity remains limited. [12] who reported that Chlorella supports only weak rotifers growth unless enriched with cobalamin.

The observed pattern of rapid growth up to day 5, followed by a decline, is likely due to diminished media quality from accumulating metabolic waste and uneaten feed. Once peak density is reached, increased competition for space and oxygen causes the rotifer population to decrease. Therefore, effective management of water quality and nutritional balance to maintaining optimal population growth.

Treatment B (yeast + *Chlorella* sp + Ascorbic acid) produced a higher rotifer density compared to treatments A and C. This indicates that ascorbic acid exerts a stronger influence on increasing the rotifer population density depend on only used cobalamin. Ascorbic acid functions as an antioxidant that protects cells from oxidative damage and enhances the efficiency of energy metabolism. According to [11] adding ascorbic acid to rotifer enrichment media significantly accelerates the reproductive rate by supporting the rotifers' resilience and physiological activity. However, treatment C, which contained only cobalamin (without ascorbic acid), the results were lower than treatments D and C but not lower than treatment A. Although is important in DNA synthesis and protein metabolism, its solitary use is not optimally effective under intensive culture conditions, which may induce oxidative stress in rotifers. Without ascorbic acid as an antioxidant protector, the effectiveness of cobalamin may diminish, so the reproductive rate does not increase maximally [14]. This aligns [4] who stated that combinations of vitamins are more effective than single vitamins, as they provide both metabolic support and cellular protection simultaneously.

Barramundi larvae reared until day 17 showed different average rotifer consumption levels across each treatment. Treatment A, the larvae consumed rotifers at a density of 1–2 ind/mL. Treatment B consumption level of 3–6 ind/mL. Treatment C ranged between 2–6 ind/mL. Treatment D highest with consumption reaching 6–8 ind/mL. Although all treatments used the same number of larvae and rotifers, the differences in consumption indicate that feeding efficiency is influenced by the feed composition provided in each treatment. This finding supports the statement by [9], that the efficiency of live feed consumption in larvae is strongly determined by physiological conditions, feed quality, and rearing management.

3.3 Absolute length growth larvae

Figure 3 presents a graph of the absolute length growth of barramundi larvae throughout the study. The values displayed represent the increase in larval length over a 15-day rearing period of rotifers enriched with different combinations of substances. The largest length increment was observed in treatment D (0.95 cm), next by treatment B (0.89), treatment C (0.83 cm), and the lowest in treatment A (0.75 cm). The highest growth occurred in larvae fed rotifers enriched with yeast + *Chlorella* sp + Ascorbic acid + Cobalamin, while the lowest growth corresponded to rotifers enriched with yeast + *Chlorella* sp. Subsequently, statistical tests were conducted to compare growth differences among treatments.

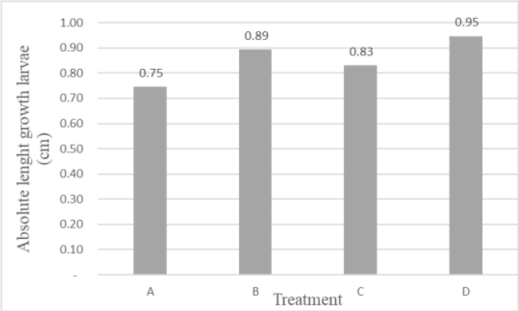

Fig. 3. Absolute length growth barramundi larvae graph.

In treatment A, rotifers enriched with yeast and *Chlorella* sp provided a relatively adequate supply of nutrients to support the growth of barramundi larvae. Yeast is known to contain protein and lipids essential for tissue synthesis and basic metabolic functions. *Chlorella* sp, is rich in unsaturated fatty acids, vitamins, and bioactive pigments, which support energy metabolism and the overall health of the larvae [6]. However, specific micronutrients such as ascorbic acid and Cobalamin crucial roles in stimulating cell division, forming new tissues, and protecting against oxidative stress, and these may not be present in sufficient quantities in yeast and *Chlorella* sp. [2].

The addition of ascorbic acid in treatment B were a significant role in increasing the absolute length growth of the larvae. Ascorbic acid functions as an antioxidant that protects cells from oxidative damage and supports the repair of damaged tissues. By enhancing resistance to stress, larvae are able to allocate more energy toward growth rather than recovery from metabolic disturbances [3]. This aligns with the view of [9], who stated that ascorbic acid is essential for larval development in fish, as most species are unable to synthesize this vitamin. ascorbic acid is involved in several biological processes, including acting as an immunostimulant and supporting stress response. Further noted that a deficiency of ascorbic acid in tissues can result in improper bone growth and may even become a limiting factor for growth. As a result, larvae fed with rotifers enriched with ascorbic acid showed better length growth compared to treatments A and C.

In treatment C, the addition of cobalamin during nutrient enrichment of rotifers contributed positively to the absolute length growth of barramundi larvae, although it was not as effective as treatments B or D. Cobalamin serves as a vital cofactor in DNA synthesis and amino acid metabolism, which are directly involved in cell division and tissue formation especially during the early larval growth phase [9]. Furthermore, cobalamin also affects the nervous system and the production of red blood cells, thereby supporting the overall health and vitality of the larvae [11]. However, without the support of antioxidants such as ascorbic acid, the larvae's ability to cope with environmental stress is more limited, resulting in suboptimal growth.

In treatment D, the combination of ascorbic acid, cobalamin and macronutrients derived from yeast and Chlorella, yielded the highest absolute length growth among the larvae. This combination created a synergistic effect that enhanced multiple metabolic and physiological pathways in the larvae [6]. Ascorbic acid functioned as an antioxidant, reducing oxidative stress and facilitating tissue repair, while vitamin B_{12} supported cell growth and energy utilization [11]. Chlorella contains of highly unsaturated fatty acids (HUFAs), carotenoids, and chlorophyll, which further support immune function and metabolism in the larvae [1]. This nutrient-rich combination enabled the barramundi larvae to maximize nutrient uptake and allocate more energy to growth rather than stress recovery. Therefore, the highest result in absolute length growth was observed in treatment D. Consequently, treatment D produced the greatest gains in absolute length growth. This combination of micronutrients provides the nutritional support critically required during the larval growth phase by accelerating tissue formation and enhancing metabolic efficiency.

3.4 Survival rate

The research results showed that rotifers given different nutrients exhibited significant effects on the survival rate of barramundi larvae (Fig. 4). Treatment A (yeast + *Chlorella* sp.) achieved a survival rate of 22.7%. This indicates that the nutrient combination was already able to support larval survival.The addition of ascorbic acid in Treatment B increased survival to 41.7%. This aligns with the statement by [9] which noted that ascorbic acid plays an important role in enhancing stress resistance and larval fish survival. Treatment C, which contained cobalamin, also showed an increase in survival to 35.3%, supporting the findings of [11] who reported that cobalamin contributes to larval metabolism and growth. In Treatment D, the addition of both ascorbic acid and cobalamin resulted in a survival rate of 39%. This indicates a potential synergistic effect between the two vitamins on the survival of barramundi larvae. If ascorbic acid and cobalamin work through different but complementary biological pathways, producing a synergistic effect that enhances larval protection and metabolism [12].

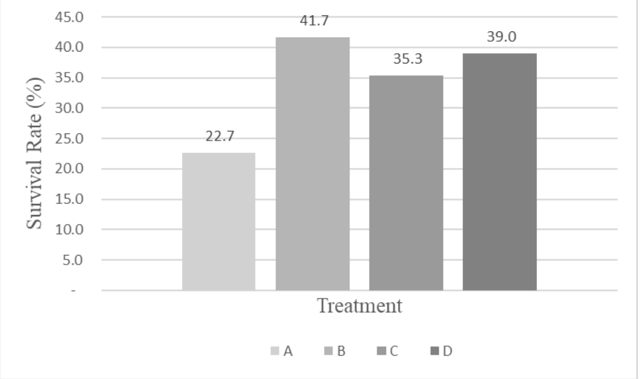

Fig. 4. Survival rate barramundi larvae graph.

According to [2,3], the minimum survival rate standard for barramundi larvae is 20%.Thus, all treatments in this study met and even exceeded the survival rate standard. This indicates that larval survival success also heavily depends on the synchronization between the larvae's nutritional needs and the availability of efficient, high-quality live feed. Therefore, vitamin supplementation particularly the combination of ascorbic acid and cobalamin with yeast and *Chlorella* sp.be recommended as a strategy to improve the survival of barramundi larvae in intensive aquaculture systems.

Physiologically, the relationship between the dosage of additional nutrients and larval survival is very close, as larvae have specific metabolic needs during the early stages of life. ascorbic acid as a powerful antioxidant, protects larval cells from oxidative stress caused by high stocking density and environmental fluctuations, while cobalamin functions as a cofactor in cellular metabolism and DNA synthesis.

The proper dosage of these two vitamins can strengthen the larvae's physiological systems and improve immune resistance, ultimately leading to increased survival rates. In addition, the combination of both vitamins in Treatment D demonstrates a potential synergistic effect that supports the stability of larval survival.

Table 1. Water quality measurement results.

Parameters	Treatment				Optimal range	Source
	A	B	C	D		
Themperature (°C)	28-32.3	28.4-32	28.3-32	28.1-32	28-32	[12]
pH	8.1-8.2	8-8.2	8.1-8.2	8.1-8.2	7.0-8.5	[12]
Salinity(g/L)	30-32	30-32	30-32	30-32	28-32	[12]
DO (mg/L)	5.3-5.6	5.3-5.6	5.3-5.6	5.3-5.6	min 4	[12]

Table 1 shows that the water quality during the rearing of barramundi larvae across all treatments remained within the optimal range to support their survival and growth. The rearing temperature ranged between 28–32°C. This range is considered appropriate and safe for rearing white snapper larvae [4]. The pH values ranged from 7.0 to 8.5, which can optimally support the growth and survival of the larvae. The salinity range of 30–32 g/L is still within the optimal range for rearing barramundi larvae (*Lates calcarifer*). [2] stated that at a salinity of 30 g/L, barramundi larvae exhibited higher growth and survival rates.

Conclusion

The use of *Chlorella* sp., yeast, ascorbic acid and cobalamin as nutrient enrichment for rotifers had an impact on the growth and survival of barramundi (*Lates calcarifer*) larvae. Treatment D resulted in the highest absolute length increment (0.95 cm), while Treatment B achieved the highest survival rate (41.7%).

Recommendation

For barramundi larval rearing, it is recommended to use rotifers enriched with *Chlorella* sp., yeast, and ascorbic acid as the initial feed. Further research is needed using lower dosage variations of ascorbic acid and cobalamin to determine the most effective optimal concentrations for larval growth and survival parameters.

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