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Effectiveness of Vitamin C-Enriched Tubifex Worms (*Tubifex* sp.) as Live Feed for African Catfish (*Clarias* sp.) Larvae

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ABSTRACT

Background: Live feed is a critical factor in the successful rearing of African catfish (*Clarias* sp.) larvae, particularly during the early developmental. Tubificid worms (*Tubifex* sp.) are commonly used as live feed due to their high protein content; however, their nutritional value can be further improved through enrichment techniques.
Aims: This study evaluated the effects of vitamin C-enriched Tubifex worms on the growth performance and survival of African catfish larvae.
Methods: A completely randomized design (CRD) consisting of three treatments and three replications was employed: unenriched Tubifex worms (control), enrichment with 2.5 g L^{-1} vitamin C (A), and enrichment with 3.5 g L^{-1} vitamin C (B). Data on survival rate (SR), specific growth rate (SGR), and growth rate (GR) were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at a 95% confidence level. Seven-day-old larvae were reared for seven days and fed enriched worms according to the respective treatments. Growth performance was assessed using survival rate (SR), specific growth rate (SGR), and growth rate (GR).
Results: The results showed that vitamin C enrichment significantly affected ($P < 0.05$) the survival rate, specific growth rate, and growth rate of African catfish larvae. The treatment enriched with 2.5 g L^{-1} vitamin C produced the highest survival rate (86.67 ± 12.09%), specific growth rate (1.70 ± 0.66% day^{-1}), and growth rate (0.125 ± 0.055 g day^{-1}). These values were significantly higher than those of the control treatment and numerically higher than those of the 3.5 g L^{-1} treatment.
Conclusion: In conclusion, enrichment of Tubifex worms with vitamin C at 2.5 g L^{-1} is an effective strategy for improving growth performance and survival of African catfish larvae and may be applied as a practical nutritional approach during the early rearing stage.

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1. Introduction

African catfish (*Clarias* sp.) is one of the most important freshwater aquaculture commodities in Indonesia, characterized by its high economic value and continuously increasing market demand. This growing demand has stimulated the expansion of aquaculture activities, particularly in the hatchery sector, which plays a critical role in supplying high-quality fish seed. The success of the hatchery phase largely determines production performance during the grow-out stage, especially during the larval phase, which is characterized by relatively high mortality rates due to nutritional limitations and susceptibility to environmental fluctuations and disease outbreaks. Fish larvae possess an immature immune system and limited disease resistance compared with juvenile and adult fish. During this developmental stage, innate immune mechanisms are not fully established, making larvae highly susceptible to pathogenic infections and environmental stress. Therefore, nutritional intervention through functional feed supplementation is required to enhance physiological performance and support larval survival.

Feed is one of the primary factors influencing the growth and survival of African catfish larvae. Feed costs may account for approximately 60–70% of the total production expenses in catfish farming, particularly when commercial feeds are utilized (Dewi *et al*., 2016; Dewi *et al*., 2017). This condition highlights the need for strategies to improve feed efficiency through the use of natural feeds that meet the physiological requirements of fish larvae. One of the most commonly used live feeds during the larval stage is the tubificid worm (*Tubifex* sp.), owing to its high protein content, suitable size for larval mouth opening, soft texture, and high digestibility.

Although Tubifex worms possess relatively good nutritional value, their nutritional quality can be further improved through enrichment techniques. Live feed enrichment has been widely developed as an approach to increase the concentration of specific nutrients, thereby enhancing fish growth performance and health. According to Okon *et al*., (2025), the incorporation of feed additives can improve nutrient utilization efficiency, promote fish growth, and reduce aquaculture production costs. Tubifex worms are widely used as live feed because of their high protein content, palatability, and digestibility. However, despite their excellent nutritional value, Tubifex worms do not always provide sufficient levels of specific micronutrients required to optimize larval growth and physiological development. Nutritional enrichment is therefore commonly applied to live feeds to improve the availability of essential nutrients, antioxidants, vitamins, and immunostimulants before feeding. Vitamin C enrichment is particularly important because fish are unable to synthesize this vitamin endogenously and must obtain it from dietary sources. Consequently, enrichment of Tubifex worms with vitamin C may serve as an effective strategy to improve nutrient delivery and enhance larval performance.

In addition to nutritional challenges, disease outbreaks remain one of the major constraints in catfish aquaculture, resulting from interactions among the host, pathogens, and the culture environment (Olafsen, 2001). The continuous use of antibiotics and synthetic chemicals for disease control may lead to adverse consequences, including pathogen resistance, environmental degradation, and increased production costs. Therefore, safer and more sustainable alternatives are required, particularly through nutritional strategies aimed at enhancing the fish immune system.

Vitamin C is an essential micronutrient that plays a vital role in metabolism, collagen synthesis, tissue formation, immune responses, and resistance to environmental stress in fish. Supplementation with vitamin C at appropriate levels has been reported to improve fish growth and health from the egg stage through adulthood (Sunarto *et al*., 2008; Anggriyani *et al*., 2023). During the larval stage, vitamin C becomes even more critical because the immune system is not yet fully developed, while tissue growth and differentiation occur at a rapid rate. Therefore, enriching Tubifex worms with vitamin C is expected to improve the nutritional quality of this live feed and subsequently enhance the growth and survival of African catfish larvae. Previous studies have demonstrated beneficial effects of vitamin C supplementation on growth performance, immune response, and survival in juvenile and adult catfish

(Kumari & Sahoo, 2005; Sembiring *et al*., 2025). However, information regarding the use of vitamin C-enriched Tubifex worms as a live feed enrichment strategy for African catfish larvae remains scarce. Therefore, the present study aimed to evaluate the effectiveness of vitamin C-enriched Tubifex worms on larval growth and survival.

However, information regarding the use of vitamin C-enriched Tubifex worms as live feed for African catfish larvae remains limited, particularly concerning its effectiveness in improving growth performance and survival. Therefore, this study was conducted to evaluate the effects of vitamin C enrichment of Tubifex worms (*Tubifex* sp.) on the growth and survival of African catfish larvae (*Clarias* sp.) as an effort to develop a more effective, economical, and sustainable nutritional strategy for catfish hatchery operations.

2. Methods

2.1 Study Period and Location

The study was conducted in May 2025 at the Hatchery Facility of the IPB University Sukabumi Campus, West Java, Indonesia. The larval stage was selected because it represents a critical developmental phase that is highly influenced by feed quality and rearing environmental conditions.

2.2 Equipment and Materials

The equipment used in this study included rearing trays measuring 53 cm × 38 cm × 16 cm, aerators, air stones, aeration hoses, enrichment containers, a digital balance, measuring cylinders, plastic basins, and other supporting equipment. The materials consisted of 7-day-old African catfish (*Clarias* sp.) larvae, tubificid worms (*Tubifex* sp.), vitamin C, and reservoir water.

2.3 Experimental Design

A completely randomized design (CRD) was employed with three treatments and three replicates, resulting in a total of nine experimental units. The treatments were as follows:
K: Tubifex worms without vitamin C enrichment (control)
A: Tubifex worms enriched with vitamin C at 2.5 g L^{-1}
B: Tubifex worms enriched with vitamin C at 3.5 g L^{-1}

2.4 Experimental Procedures

2.4.1 Preparation of Rearing Units

All rearing containers and equipment were thoroughly cleaned and disinfected prior to use. Each rearing tray was filled with 8 L of water and supplied with continuous aeration. The enrichment containers were filled with 1 L of water and provided with aeration throughout the enrichment process.

2.4.2 Enrichment of Tubifex Worms

Vitamin C was dissolved in water according to the designated treatment concentrations. Subsequently, 30 g of Tubifex worms were immersed in the enrichment solution for 15 min under continuous aeration. In the control treatment, the worms were immersed in water without vitamin C. The enrichment process was conducted daily throughout the experimental period.

2.4.3 Larval Rearing

A total of 160 African catfish larvae were stocked into each rearing unit and maintained for 7 days. The larvae were fed enriched Tubifex worms according to the respective treatments on an apparent satiation basis. Uneaten feed and debris were removed daily to maintain water quality.

2.5 Growth Performance Parameters

The observed parameters included survival rate (SR), specific growth rate (SGR), and growth rate (GR).

2.5.1 Survival Rate (SR)

Survival rate was calculated according to the method described by Effendie (2002):

$$SR\ (\%) = \frac{Nt}{N0} \times 100$$

where:
Nt = number of surviving fish at the end of the experiment
N0 = initial number of fish stocked

2.5.2 Specific Growth Rate (SGR)

Specific growth rate was calculated following Muchlisin *et al*., (2016):

$$SGR\ (\%\ day{-}1) = \frac{\ln Wt - \ln W0}{t} \times 100$$

where:
Wt = final average body weight (g)
W0 = initial average body weight (g)
t = culture period (days)

2.5.3 Growth Rate (GR)

Growth rate was calculated according to Mojer & Al-Dubakel (2024):

$$GR\ (g\ day{-}1) = \frac{Wt - W0}{t}$$

where:
Wt = final average body weight (g)
W0 = initial average body weight (g)
T = culture period (days)

2.6 Statistical Analysis

All data were analysed using one-way analysis of variance (ANOVA) at a 95% confidence level. When significant differences among treatments were detected ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was performed as a post hoc analysis to identify differences between treatment means.

3. Results and Discussion

3.1 Survival rate (SR)

The results showed that vitamin C enrichment of *Tubifex* worms significantly influenced the survival rate (SR) of African catfish larvae. The SR value in the control treatment (K) was 63.33 ± 25.16%, whereas Treatment A, enriched with 2.5 g L^{-1} vitamin C, produced the highest SR value of 86.67 ± 12.09%. Treatment B, enriched with 3.5 g L^{-1} vitamin C, resulted in an SR value of 74.33 ± 12.09%. Statistical analysis revealed that Treatment A differed significantly from the control treatment ($P < 0.05$), while no significant difference was observed between Treatments A and B. The survival rates of African catfish larvae during the rearing period are presented in Figure 1.

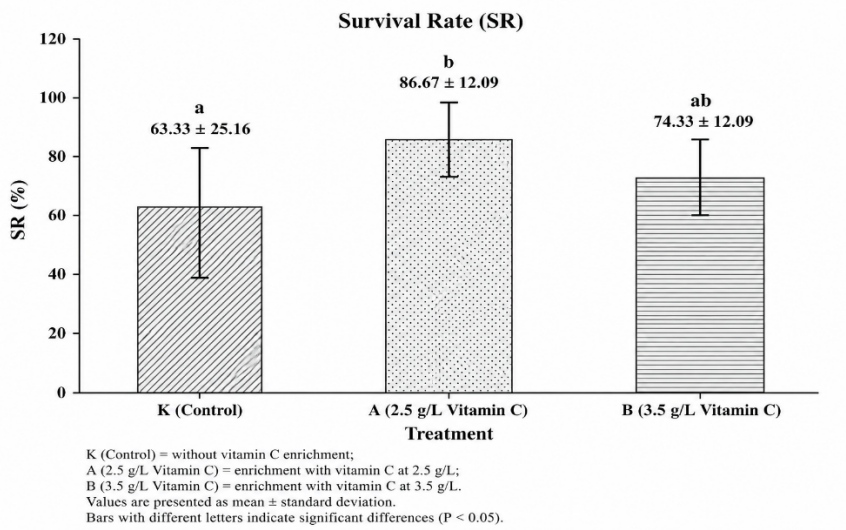

Figure 1. The survival rate (SR) of African catfish larvae during the rearing period

The high survival rate observed in Treatment A indicates that enrichment of *Tubifex* worms with vitamin C at 2.5 g L^{-1} effectively improved larval viability during the rearing period. This improvement may be attributed to the role of vitamin C as an antioxidant that enhances immune function, maintains physiological balance, and helps larvae cope with environmental stress during early development. Vitamin C is also involved in tissue formation, collagen synthesis, and the enhancement of non-specific immune responses, thereby reducing mortality during the larval rearing stage (Kumari & Sahoo, 2005; Wan *et al*., 2014). Vitamin C enhances immune function by stimulating leukocyte activity, increasing phagocytic capacity, promoting antioxidant defense systems, and reducing oxidative stress. Furthermore, vitamin C contributes to collagen synthesis and tissue repair, thereby improving the integrity of epithelial barriers that serve as the first line of defense against pathogens. These mechanisms collectively improve disease resistance and survival in fish larvae.

Adequate vitamin C availability enables metabolic processes to proceed more efficiently, allowing nutrients and energy derived from feed to be utilized more effectively for maintaining physiological functions. Consequently, larvae exhibit greater resilience to environmental fluctuations and stress conditions during culture. These findings are consistent with those reported by Sembiring *et al*., (2025), who demonstrated that dietary vitamin C supplementation increased the survival rate of African catfish by 76.6% compared with unsupplemented treatments. Similarly, Ghughuskar (2012) reported that vitamin C supplementation improved physiological performance and stress resistance in fish larvae, which are particularly vulnerable to environmental changes.

Although Treatment B exhibited a higher SR than the control, increasing the vitamin C concentration to 3.5 g L^{-1} did not result in better survival compared with Treatment A. This finding suggests that vitamin C requirements during the larval stage have an optimal threshold, and excessive supplementation may not necessarily provide additional physiological benefits. Excessive vitamin C levels may reduce nutrient utilization efficiency and potentially disrupt metabolic balance, thereby increasing physiological stress in larvae. Previous studies have reported that the effectiveness of vitamin C supplementation largely depends on its suitability to the physiological requirements of the cultured organism (Ai *et al*., 2006; Sunarto *et al*., 2008).

3.2 Specific Growth Rate (SGR)

Vitamin C enrichment of *Tubifex* worms also affected the specific growth rate (SGR) of African catfish larvae. The control treatment produced an SGR value of 1.15 ± 0.86% day^{-1}, while Treatment A resulted in the highest SGR value of 1.70 ± 0.66% day^{-1}. Treatment B yielded an SGR value of 1.49 ±

0.36% day^{-1}. Statistical analysis indicated that Treatment A differed significantly from the control treatment ($P < 0.05$), whereas no significant difference was observed between Treatments A and B. Although Treatment A produced higher values than Treatment B, the statistical analysis indicated no significant difference between both treatments. This result suggests that the vitamin C concentrations of 2.5 and 3.5 g L^{-1} provided comparable physiological benefits. The numerical differences observed between treatments were likely associated with biological variability among replicates rather than a true treatment effect. Therefore, increasing the vitamin C concentration beyond 2.5 g L^{-1} did not provide additional measurable benefits. The SGR values of African catfish larvae are presented in Figure 2.

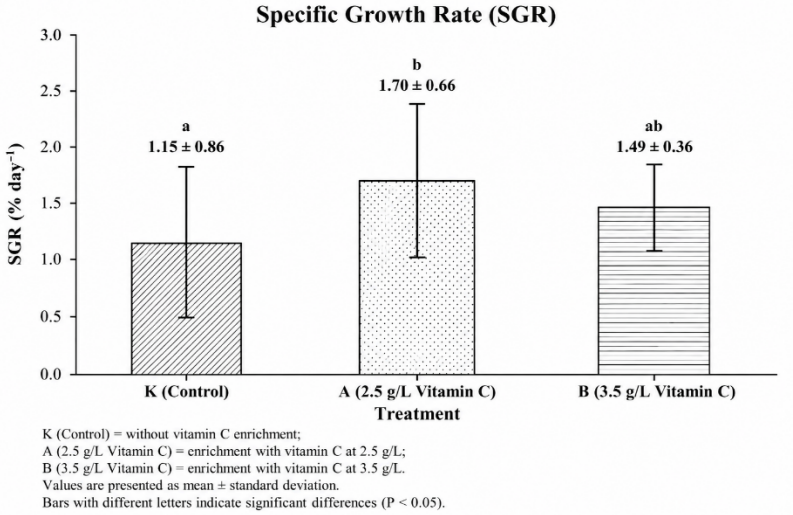

Figure 2. The specific growth rate (SGR) of African catfish larvae during the rearing period

Fish are unable to synthesize vitamin C endogenously because they lack the enzyme L-gulonolactone oxidase, which is responsible for ascorbic acid biosynthesis. Consequently, vitamin C must be supplied through feed to support physiological processes, metabolism, immune function, and growth (Maldgunkar *et al*., 2019). Vitamin C deficiency during the larval stage may impair tissue development, reduce metabolic efficiency, weaken immune responses, and ultimately hinder growth performance. Furthermore, vitamin C plays a crucial role in maintaining antioxidant capacity and enhancing resistance to oxidative stress under intensive culture conditions (Rahman *et al*., 2023).

The present study demonstrated that vitamin C enrichment of *Tubifex* worms improved the SGR of African catfish larvae. These results indicate that vitamin C supplementation at an optimal concentration can significantly enhance larval growth performance. The increased SGR observed in the enriched treatments is likely associated with the role of vitamin C in supporting metabolism, collagen synthesis, cell differentiation, and nutrient utilization. Vitamin C contributes to connective tissue formation and cellular regeneration, thereby facilitating more rapid and efficient growth. In addition, its antioxidant properties protect cells from oxidative damage, which is particularly important during the sensitive larval stage. Omoniyi & Ovie (2018) reported that vitamin C is an essential nutrient required to support physiological and metabolic functions in fish. Adequate vitamin C availability promotes collagen formation and improves growth efficiency. Recent studies have further demonstrated that vitamin C supplementation enhances antioxidant activity, feed utilization efficiency, and growth-related gene expression in cultured fish (Ibrahim *et al*., 2020).

The superior SGR obtained in Treatment A suggests that 2.5 g L^{-1} was the most effective enrichment concentration. Increasing the concentration to 3.5 g L^{-1} did not result in additional growth improvement, indicating that vitamin C requirements during the larval stage are subject to physiological limitations. Optimal growth can only be achieved when vitamin C supplementation is provided according to the nutritional requirements and absorption capacity of the fish (Ai *et al*., 2006; Sunarto *et al*., 2008). Similar

findings were reported by Zehra & Khan (2021), who observed that vitamin C supplementation at optimal levels enhanced growth performance, antioxidant capacity, and immune responses, whereas excessive supplementation did not yield further improvements.

In addition to supplementation level, the nutritional quality of enriched live feed may also contribute to the observed growth enhancement. Vitamin C-enriched *Tubifex* worms likely possessed improved nutritional quality and biological value, thereby supporting better feed utilization and larval development. Tubifex worms are recognized as one of the most nutritious live feeds used in freshwater aquaculture. Previous studies reported that Tubifex worms contain approximately 50–65% crude protein, 10–20% lipid, and essential amino acids required for larval growth and development. Their soft body structure and high digestibility make them particularly suitable for fish larvae and early juveniles (Rihi, 2019). Since the larval stage represents a critical period in freshwater aquaculture, improved growth during this phase can substantially influence subsequent production performance. Khairiman *et al.* (2022) similarly reported that vitamin C-enriched live feed significantly improved the growth and survival of milkfish larvae compared with control treatments.

3.3 Growth Rate (GR)

The results demonstrated that vitamin C enrichment significantly influenced the growth rate (GR) of African catfish larvae. The GR value in the control treatment was 0.11 ± 0.01 g day^{-1}, whereas Treatment A produced the highest GR value of 0.125 ± 0.055 g day^{-1}. Treatment B resulted in a GR value of 0.11 ± 0.023 g day^{-1}. Statistical analysis indicated that Treatment A differed significantly from the control treatment ($P < 0.05$), while no significant difference was observed between Treatments A and B. The GR values of African catfish larvae are presented in Figure 3.

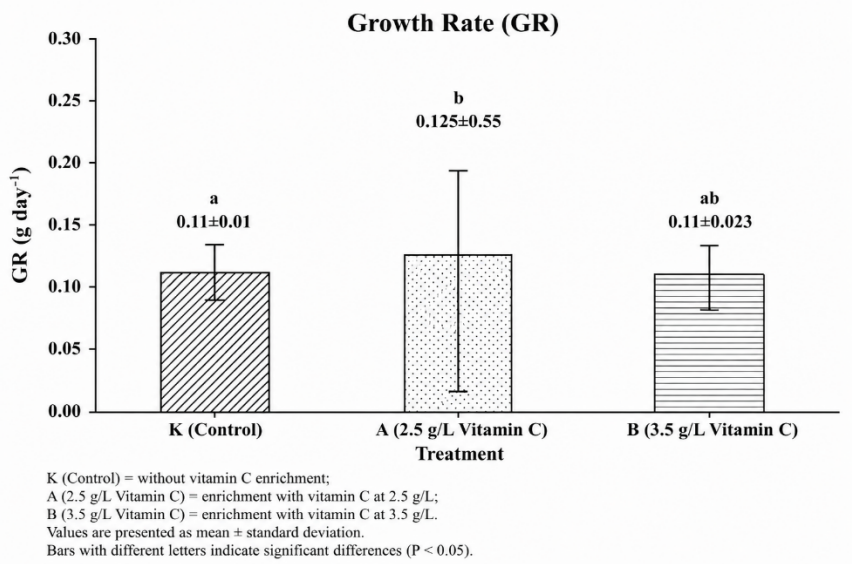

Figure 3. The growth rate (GR) of African catfish larvae during the rearing period

The high GR observed in Treatment A indicates that vitamin C enrichment of *Tubifex* worms effectively enhanced the daily growth performance of African catfish larvae. Vitamin C is known to improve metabolic efficiency and support tissue development, allowing nutrients and energy derived from feed to be utilized more effectively for growth. Abadi *et al.*, (2022) reported that dietary vitamin C supplementation enhances feeding activity and physiological performance, thereby contributing to improved growth.

The improved GR observed in Treatment A suggests that vitamin C enrichment enhanced the nutritional quality of the live feed and increased the efficiency of energy utilization for growth. These findings indicate that vitamin C supplementation at an optimal concentration can effectively promote daily growth in African catfish larvae. The increase in GR may be associated with enhanced nutrient

utilization and metabolic activity within the larvae. Vitamin C plays an important role in supporting physiological processes, stimulating feeding responses, and promoting tissue formation, all of which contribute to improved growth performance. Furthermore, its antioxidant function helps maintain cellular stability and minimize oxidative stress during larval development. Yanto (2016) reported that vitamin C supplementation improves protein utilization efficiency and nutrient retention, resulting in enhanced growth performance in cultured fish.

The highest GR value recorded in Treatment A confirms that 2.5 g L^{-1} was the most effective enrichment concentration for promoting daily growth. At this level, vitamin C likely optimized metabolic processes and energy utilization, enabling available nutrients to be directed efficiently toward tissue development. Rahman *et al*., (2023) reported that vitamin C enhances antioxidant capacity, supports energy metabolism, and improves growth performance under intensive aquaculture conditions.

Although Treatment B exhibited a slightly higher GR than the control treatment, increasing the vitamin C concentration to 3.5 g L^{-1} did not produce superior growth performance compared with Treatment A. This finding further supports the existence of an optimal vitamin C requirement for African catfish larvae and suggests that excessive supplementation may reduce nutrient utilization efficiency. Fish growth is most effectively enhanced when vitamin C is supplied at levels that match the physiological requirements of the organism (Ai *et al*., 2006; Sunarto *et al*., 2008).

In addition to supplementation level, the enrichment process itself may have influenced the effectiveness of vitamin C delivery to the larvae. Enriched *Tubifex* worms likely provided improved nutritional quality and biological value, supplying more complete nutrients required for early larval development. Since the larval stage is a critical phase in freshwater aquaculture, successful growth during this period is essential for ensuring optimal performance during subsequent culture stages.

3.4 Water quality

The water quality parameters recorded during the rearing period of African catfish larvae are presented in Table 1. Based on the results of the study, all measured water quality parameters remained within the optimal range for supporting the growth and survival of African catfish larvae.

Water temperature ranged from 26.5–26.6°C, while pH values ranged from 7.9–8.0 throughout the experimental period. These values were within the recommended ranges for African catfish culture, with optimal temperatures of 25–30°C and pH values of 6.5–8.5. Stable temperature and pH conditions are essential for maintaining metabolic processes, enzymatic activity, and efficient nutrient utilization in fish.

Table 1. The water quality of African catfish larvae during the rearing period

Parameters	K	A	B	Optimum range
Temperature (°C)	26,5±0,1	26,6±0,1	26,6±0,1	25–30
pH	8,0±0,1	8,0±0,1	7,9±0,1	6,5–8,5
Nitrite (mg L^{-1})	0,10±0,00	0,01±0,00	0,05±0,00	<1
Nitrate (mg L^{-1})	10,0±0,0	2,5±0,0	5,0±0,0	<50
Ammonia (mg L^{-1})	0,03±0,03	0,03±0,03	0,03±0,03	<0,1

Notes:
K = control without vitamin C
A = vitamin C 2,5 g/L
B = vitamin C 3,5 g/L

Nitrite concentrations ranged from 0.01 to 0.10 mg L^{-1}, whereas nitrate concentrations ranged from 2.5 to 10 mg L^{-1} across all treatments. These concentrations remained below the tolerance thresholds for

cultured fish and therefore were not considered toxic to African catfish larvae. The relatively low nitrite and nitrate levels indicate that organic matter decomposition was well controlled during the rearing period and that continuous aeration effectively maintained stable water quality conditions. Excessive accumulation of nitrite and nitrate can impair physiological processes and reduce growth performance in cultured fish (Hapsari *et al*., 2021).

Ammonia concentrations were consistently recorded at 0.03 mg L^{-1} in all treatments, remaining below the safe limit for freshwater fish culture. The low ammonia concentration was likely associated with proper culture management practices, including continuous aeration and the routine removal of uneaten feed throughout the experiment. Elevated ammonia concentrations can be toxic to fish and may cause respiratory impairment, physiological stress, and reduced survival rates (Marlina & Rakhmawati, 2016).

Overall, water quality conditions throughout the experimental period were suitable for supporting the growth and survival of African catfish larvae. These findings indicate that the observed differences in growth performance and survival rate among treatments were primarily attributable to the vitamin C enrichment of *Tubifex* worms rather than to variations in environmental conditions.

4. Conclusion

Vitamin C enrichment significantly improved the survival and growth performance of African catfish larvae. The enrichment concentration of 2.5 g L^{-1} produced the highest survival rate, specific growth rate, and growth rate. Therefore, vitamin C-enriched Tubifex worms can be recommended as an effective live-feed enrichment strategy for African catfish larval culture.

5. Authors Note

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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