



## Effects of Garlic (*Allium sativum*) Extract on the Growth and Survival of Nile Tilapia (*Oreochromis niloticus*) Fingerlings at High Stocking Density

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**Abstract:** This study aimed to evaluate the effect of garlic extract supplementation as an immunostimulant and feed additive on the survival of Nile tilapia fry reared in an intensive aquaculture system under high stocking density, and to determine the most effective dosage. The study employed an experimental method using a Completely Randomized Design (CRD) consisting of four treatments with three replicates: no extract, 5 g/kg, 10 g/kg, and 15 g/kg of feed administered over a 28-day rearing period. The primary parameter observed was survival rate, supported by growth data. Data were analyzed using ANOVA at a 95% confidence level, followed by the Least Significant Difference (LSD) test. The results showed that garlic extract supplementation improved survival compared with the control, with values of 53.3%, 61.7%, 70.0%, and 65.0%, respectively. The treatment with 10 g/kg feed produced the highest and most optimal survival rate. A higher dose did not result in further improvement, presumably because of reduced feed palatability and the physiological response of the fish. It can be concluded that garlic extract supplementation significantly affected the performance of Nile tilapia fry, with 10 g/kg identified as the most effective dose for enhancing resistance to environmental stress. This study also provides a practical contribution by recommending an optimal dose of natural feed additive for intensive aquaculture systems.

**Keywords:** Nile tilapia fry; garlic extract; immunostimulant; intensive aquaculture; survival

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### INTRODUCTION

Tilapia (*Oreochromis niloticus*) culture is one of the leading commodities in the freshwater aquaculture sector and plays an important role in supporting food security, particularly as an affordable and sustainable source of animal protein (Oktami et al., 2024). This species is characterized by a rapid growth rate, high adaptability to various environmental conditions, and efficient feed conversion, making it widely cultivated across different production scales. In addition, its economic value is relatively stable, with market demand continuing to increase at both local and global levels (FAO, 2022; El-Sayed, 2019). These conditions have encouraged the development of intensified production systems through the application of high stocking densities to maximize yield within limited space (Boyd, 2020).

However, intensification through high stocking density has ecological and physiological consequences, particularly during the early life stages, which are more sensitive to environmental changes. Excessive density may increase competition among individuals, accelerate the accumulation of metabolic waste, and reduce the quality of the rearing medium. These effects may subsequently lead to stress, reduced appetite, and increased susceptibility to disease. Such conditions contribute to low fish survival rates, highlighting the need for management strategies capable of maintaining biological stability throughout the culture period (Ruslaini et al., 2025; Wedemeyer, 1996; Ashley, 2007).

A major challenge in intensive aquaculture systems is the high mortality of juveniles caused by environmental stress. High stocking density increases interactions among individuals in competition for feed and space, thereby disturbing the physiological balance of the organism. Moreover, increased biomass in culture containers accelerates the accumulation of uneaten feed and metabolic waste, which adversely affects water quality, including dissolved oxygen levels and pH stability (Tumembouw et al., 2025; Boyd & Tucker, 2012). Such unstable environmental conditions can trigger prolonged stress, resulting in immune suppression and greater vulnerability to pathogen infection. This condition disrupts metabolism, reduces feed intake, and increases the risk of mortality, especially during the early rearing phase. If not properly managed, these problems will reduce production efficiency and threaten the sustainability of aquaculture operations.

To date, disease control in aquaculture has remained heavily dependent on the use of chemicals and antibiotics. Uncontrolled use may promote microbial resistance and leave residues in fishery products, thereby posing risks to both the environment and human health (Batubara et al., 2023; Cabello, 2006; Romero et al., 2012). Safer, greener, and more sustainable alternatives are therefore needed, such as using natural feed-based immunostimulants.

One natural ingredient with considerable potential is garlic (*Allium sativum*), which contains active compounds such as allicin, flavonoids, and organosulfur compounds that function as antibacterial, antioxidant, and immunostimulatory agents (Corzo-Martínez et al., 2007; Nya & Austin, 2009). Previous studies have shown that garlic supplementation in feed can improve fish growth, feed efficiency, and immune response (Hismah et al., 2022; Talpur & Ikhwanuddin, 2012). Nevertheless, existing findings remain inconsistent, likely due to differences in species, fish size, rearing conditions, and administered dosage.

Under high-density conditions, the response of fish to herbal additives such as garlic has not been fully understood, particularly with respect to the balance between growth and survival. In addition, excessive supplementation may reduce feed palatability, resulting in suboptimal feed intake and consequently affecting growth performance. This indicates that the dose–response relationship is an important aspect requiring further investigation in order to determine the optimal level of administration (Nursatia et al., 2017). To date, studies specifically examining the effectiveness of garlic extract under high stocking density conditions remain limited, leaving an important research gap to be addressed.

Based on these considerations, this study aimed to evaluate the effect of dietary garlic (*Allium sativum*) extract supplementation on the survival of Nile tilapia (*Oreochromis niloticus*) juveniles reared under high stocking density and to determine the most effective dosage. The novelty of this study lies in testing garlic extract in a high-density culture system that represents environmentally stressful conditions in intensive aquaculture. In addition, this study simultaneously integrates growth and survival parameters to obtain a more comprehensive understanding of the biological responses involved. The findings are expected to provide both scientific and practical contributions to the development of sustainable aquaculture through the use of natural feed additives that are safe, effective, and applicable.

## METHOD

### Experimental Design

This study employed an experimental design with a quantitative approach to evaluate the effect of garlic extract supplementation as a feed additive on the growth

and survival of Nile tilapia fry under high stocking-density conditions. The experiment was arranged in a Completely Randomized Design (CRD) with one treatment factor, namely the dosage of garlic extract incorporated into the feed.

Four treatment levels were applied: 0 g/kg feed (P0, control), 5 g/kg feed (P1), 10 g/kg feed (P2), and 15 g/kg feed (P3). Each treatment was replicated three times, resulting in a total of 12 experimental units. All units were randomly assigned to minimize the influence of uncontrolled external factors.

The independent variable was the garlic extract dosage, whereas the dependent variables were absolute weight growth, absolute length growth, and survival rate. Other experimental conditions, including stocking density, water volume, feeding rate, water quality management, and rearing duration, were maintained uniformly across all treatments to ensure that the observed differences were attributable to the garlic extract dosage.

### Experimental Fish

The experimental fish were Nile tilapia (*Oreochromis niloticus*) fry with an average length of approximately 3 cm. A total of 240 fry were used in the study. Before the experiment began, the fish were acclimatized for 1 h to adapt to the rearing environment. Each experimental unit contained 20 fry.

### Treatments

The treatments consisted of four levels of garlic extract added to the feed as follows:

**P0:** without garlic extract (control)

**P1:** 5 g garlic extract/kg feed

**P2:** 10 g garlic extract/kg feed

**P3:** 15 g garlic extract/kg feed

All treatments were conducted under the same high stocking-density condition, namely 20 fish per container, so that the only experimental factor tested was the garlic extract dosage.

### Materials and Equipment

The materials used in this study included Nile tilapia fry (*Oreochromis niloticus*), fresh garlic (*Allium sativum*) as the raw material for extract preparation, commercial pellet feed as the basal diet, 96% ethanol as the extraction solvent, distilled water, and clean water as the rearing medium. The commercial feed contained at least 30% protein, 5–8% lipid, and not more than 5% crude fiber.

The equipment used included 30-L plastic buckets as rearing containers, aerators and air stones, a digital balance, a ruler, a blender, knives, a cutting board, beakers, measuring cylinders, spray bottles for feed coating, a thermometer, a pH meter, a dissolved oxygen (DO) meter, and writing and documentation tools.

### Preparation of Garlic Extract

Garlic extract (*Allium sativum*) was prepared by first producing dried garlic powder and then extracting it using the maceration method. Fresh garlic bulbs of good quality were selected, peeled, washed, and sliced thinly to increase the surface area for drying and extraction. The slices were dried indirectly, either by air-drying or in an oven at a low temperature (approximately 40–50°C), until the material reached a dry condition. Low-temperature drying was intended to preserve heat-sensitive bioactive compounds such as allicin (Hismah et al., 2022).

The dried garlic was then ground into powder and sieved to obtain a more uniform particle size, thereby increasing contact between the material and solvent and

improving extraction efficiency (Batubara et al., 2023). A total of 100 g of garlic powder was macerated in 500 mL of 96% ethanol for 3 × 24 h with periodic stirring. The solution was then filtered to separate the filtrate from the residue, and the filtrate was evaporated to obtain a concentrated extract.

The extract was applied to the feed by the coating method. The extract was dissolved according to the treatment dose, sprayed evenly onto the pellet feed, and then air-dried before use. To maintain the stability of active compounds and reduce the risk of contamination, the coated feed was stored in closed containers and used within a short period, or alternatively prepared shortly before feeding.

### **Fish Rearing Procedure**

The fry were reared for 28 days in 30-L plastic buckets containing 10 L of water. Each bucket was stocked with 20 fish, representing a uniform high stocking density across all treatments. Aeration was provided continuously throughout the rearing period.

The experimental feed was administered three times daily at 08:00, 12:00, and 16:00 WIB at a rate of 5% of total biomass. Feed amount was adjusted every seven days based on the latest biomass measurement. Partial water exchange was performed every two days by replacing 30–40% of the water volume to maintain environmental stability. Fish behavior and general condition were observed routinely throughout the experiment.

### **Observed Parameters**

The parameters observed in this study were absolute weight growth, absolute length growth, survival rate, and supporting water quality variables. Growth parameters were determined based on the difference between the initial and final measurements, whereas survival rate was calculated from the proportion of fish that remained alive until the end of the rearing period.

#### **a. Absolute Weight Growth**

Absolute weight growth was calculated according to Effendie (1997) using the following formula:

$$\mathbf{WG = Wt - W0}$$

Note:

**WG** = absolute weight growth (g)

**Wt** = average final weight of fish (g)

**W0** = average initial weight of fish (g)

#### **b. Absolute Length Growth**

Absolute length growth was calculated according to Effendie (1997) using the following formula:

$$\mathbf{LG = Lt - L0}$$

Note:

**LG** = absolute length growth (cm)

**Lt** = average final length of fish (cm)

**L0** = average initial length of fish (cm)

#### **c. Survival Rate**

Survival rate was calculated at the end of the experiment using the following formula:

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

Note:

**SR** = survival rate (%)

**Nt** = number of fish alive at the end of the rearing period

**N0** = number of fish at the beginning of the rearing period

#### d. Water Quality

Water quality was monitored as a supporting parameter and included temperature (°C), pH, and dissolved oxygen (DO, mg/L). Measurements were conducted every two days during the 28-day rearing period. The recorded values were used to confirm that environmental conditions remained within a suitable range for Nile tilapia fry culture.

**Table 1.** Range of water quality values during the experimental period

Parameter	Unit	P0	P1	P2	P3
Temperature	°C	27.0–28.5	27.5–29.0	28.0–29.5	28.5–29.0
pH	–	6.9–7.5	7.0–7.8	7.2–8.0	6.8–7.4
DO	mg/L	4.0–5.0	4.2–5.5	4.5–6.0	4.0–4.8

#### Data Analysis

The data were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level to determine the effect of garlic extract dosage on absolute weight growth, absolute length growth, and survival rate. When significant differences were detected among treatments, the Least Significant Difference (LSD) test was performed at the 5% significance level to identify differences between treatment means. The results were presented as mean  $\pm$  standard deviation and discussed descriptively.

## RESULTS AND DISCUSSION

The effects of garlic extract supplementation on the growth performance and survival of Nile tilapia fry during the 28-day rearing period are presented and discussed below.

#### Growth Performance and Survival

For an overall comparison of treatment responses, the final values of absolute weight gain, absolute length gain, and survival rate are presented in Table 2.

**Table 2.** Final growth performance and survival of Nile tilapia fry fed diets supplemented with garlic extract

Treatment	Absolute Weight Gain (g)	Absolute Length Gain (cm)	Survival Rate (%)
P0 (Control)	3.43 $\pm$ 0.21 <sup>a</sup>	3.10 $\pm$ 0.15 <sup>a</sup>	53.3 $\pm$ 2.50 <sup>a</sup>
P1 (5 g/kg feed)	4.13 $\pm$ 0.25 <sup>b</sup>	3.75 $\pm$ 0.20 <sup>b</sup>	61.7 $\pm$ 3.10 <sup>b</sup>
P2 (10 g/kg feed)	5.43 $\pm$ 0.18 <sup>c</sup>	4.50 $\pm$ 0.18 <sup>c</sup>	70.0 $\pm$ 2.00 <sup>c</sup>
P3 (15 g/kg feed)	4.80 $\pm$ 0.22 <sup>b</sup>	4.10 $\pm$ 0.17 <sup>b</sup>	65.0 $\pm$ 2.60 <sup>b</sup>

**Note:** P0 = without extract; P1 = 5 g/kg feed; P2 = 10 g/kg feed; P3 = 15 g/kg feed. Different superscript letters within the same column indicate significant differences based on the Least Significant Difference (LSD) test at the 5% significance level ( $p < 0.05$ ).

As shown in Table 2, dietary supplementation with garlic extract improved the growth performance and survival of Nile tilapia fry compared with the control treatment. The highest values of absolute weight gain, absolute length gain, and survival rate were consistently recorded in treatment P2, while P1 and P3 showed intermediate values and P0 produced the lowest response. Overall, these results indicate that garlic

extract supplementation generated a positive biological response up to a certain dose, after which the response declined slightly at the highest inclusion level tested.

This pattern is biologically plausible because garlic contains allicin, a bioactive compound known for its antibacterial and immunostimulatory properties. These properties may help suppress pathogenic microorganisms, improve physiological stability, and enhance the capacity of fish to cope with environmental stress under high stocking-density conditions (Hismah et al., 2022). In addition, improved immune condition and digestive balance may enhance nutrient utilization efficiency, thereby contributing to better growth and survival performance (Nursatia et al., 2017; Batubara et al., 2023).

### Absolute Weight Gain

To determine whether the dietary treatments significantly affected absolute weight gain, the ANOVA results are presented in Table 3.

**Table 3.** Analysis of variance (ANOVA) for absolute weight gain

Source of Variation	SS	df	MS	F-value	F-table	
					5%	1%
Treatment	2.06	3	0.68	136**	4.07	7.59
Error	0.04	8	0.005			
Total	2.10	11				

**Note:** \*\* highly significant

As presented in Table 3, garlic extract supplementation had a highly significant effect on absolute weight gain. The calculated F-value (136) was much higher than the critical F-values at both the 5% and 1% significance levels, indicating that the treatments produced statistically different responses. Referring to Table 2, treatment P2 resulted in the highest absolute weight gain ( $5.43 \pm 0.18$  g), followed by P3, P1, and P0.

The superior weight gain observed in P2 suggests that supplementation at 10 g/kg feed provided the most favorable condition for growth. This effect may be associated with the antibacterial activity of allicin, which can reduce the proliferation of harmful microorganisms in the digestive tract and thereby support more stable physiological conditions (Hismah et al., 2022). Under such conditions, nutrient digestion and absorption may proceed more efficiently, resulting in improved biomass accumulation.

In addition, the immunostimulatory effect of garlic extract may have contributed to improved resistance to environmental stress, allowing energy to be allocated more effectively toward growth rather than toward physiological defense (Nursatia et al., 2017). Garlic extract may also help maintain intestinal microflora balance, which is important for digestive efficiency and nutrient assimilation (Batubara et al., 2023). These mechanisms are consistent with the higher weight gain recorded in P2.

Nevertheless, the response did not continue to increase at the highest dose. Although P3 still showed better performance than the control, its value was lower than that of P2. This result suggests that excessive garlic extract inclusion may reduce feed palatability and consequently lower feed intake. Therefore, while garlic extract provides beneficial biological effects, its efficacy remains dependent on the proper dosage, and the present results indicate that 10 g/kg feed was the most effective level within the tested range.

### Absolute Length Gain

To assess whether the treatments also significantly influenced linear growth, the ANOVA results for absolute length gain are presented in Table 4.

**Table 4.** Analysis of variance (ANOVA) for absolute length gain

Source of Variation	SS	df	MS	F-value	F-table	
					5%	1%
Treatment	0.67	3	0.22	44.61**	4.07	7.59
Error	0.04	8	0.005			
Total	0.71	11				

**Note:** \*\* highly significant

Table 4 shows that garlic extract supplementation also had a highly significant effect on absolute length gain. The calculated F-value (44.61) exceeded the critical F-values at both significance levels, confirming that dietary treatment significantly affected linear growth. As shown in Table 2, the highest absolute length gain was recorded in P2 ( $4.50 \pm 0.18$  cm), followed by P3, P1, and P0.

The similarity between the weight and length responses suggests that garlic extract promoted balanced somatic growth rather than merely increasing body mass. Linear growth reflects effective nutrient assimilation and proper tissue development over time, and thus supports the interpretation that garlic extract improved overall growth performance. The antibacterial action of allicin may have stabilized the internal environment of the fish by suppressing harmful microorganisms in the digestive tract (Hismah et al., 2022), while its immunostimulatory activity may have enhanced resistance to environmental stress and improved nutrient allocation for tissue formation (Nursatia et al., 2017).

The present results are also consistent with the assumption that a balanced intestinal microflora contributes to more efficient nutrient absorption, which in turn supports structural growth (Batubara et al., 2023). However, as with weight gain, the response declined slightly in P3 relative to P2. This finding again suggests that although garlic extract is beneficial, excessive supplementation may reduce feed acceptance and limit the expected growth response. Thus, the dose-response pattern observed in this study indicates the existence of an optimum supplementation level.

### Survival Rate

To facilitate a clearer evaluation of treatment effects on viability, the survival data are summarized separately in Table 5.

**Table 5.** Survival rate of Nile tilapia fry fed diets supplemented with garlic extract

Treatment	Survival Rate (%)
P0 (Control)	$53.3 \pm 2.50^a$
P1 (5 g/kg feed)	$61.7 \pm 3.10^b$
P2 (10 g/kg feed)	$70.0 \pm 2.00^c$
P3 (15 g/kg feed)	$65.0 \pm 2.60^b$

**Note:** Different superscript letters indicate significant differences based on the LSD test at the 5% significance level ( $p < 0.05$ ).

Based on Table 5, the highest survival rate was observed in P2 ( $70.0 \pm 2.00\%$ ), followed by P3 ( $65.0 \pm 2.60\%$ ), P1 ( $61.7 \pm 3.10\%$ ), and P0 ( $53.3 \pm 2.50\%$ ). According to the statistical analysis, P2 differed significantly from all other treatments, whereas

P1 and P3 were not significantly different from each other but both were significantly higher than the control. These results indicate that the beneficial effect of garlic extract on survival was most evident at the 10 g/kg feed level.

The higher survival observed in P2 was likely associated with the dual role of allicin as an antibacterial and immunostimulatory compound. By suppressing pathogenic microorganisms and strengthening the immune response, garlic extract may have improved the ability of fish to withstand environmental pressure, particularly under high stocking-density conditions (Hismah et al., 2022). Improved immune status may also allow metabolic energy to be used more efficiently to maintain physiological stability rather than being diverted to defense processes (Nursatia et al., 2017). In addition, improved intestinal microbial balance may help support overall health and resilience (Batubara et al., 2023).

The lower survival in P3 compared with P2 suggests that higher extract inclusion did not provide additional benefit and may instead have reduced treatment effectiveness. One possible explanation is that excessive garlic extract affected palatability and reduced feed consumption, resulting in less efficient nutrient intake and weaker physiological performance. Under high-density rearing conditions, such a reduction in nutritional adequacy may compromise the capacity of fish to adapt to environmental stress.

Overall, the results demonstrate that garlic extract supplementation improved both the growth performance and survival of Nile tilapia fry, with the best overall response recorded in P2. This finding indicates that 10 g/kg feed was the most effective dose within the tested range, providing the best balance between growth and survival. Although higher supplementation still produced better results than the control, excessive inclusion did not enhance performance further and instead tended to reduce the response. Therefore, 10 g/kg feed may be recommended as an effective supplementation level for improving the performance of Nile tilapia fry under intensive rearing conditions.

## CONCLUSION

The supplementation of garlic extract in the diet significantly affected the growth performance and survival of Nile tilapia fry reared under high stocking-density conditions. Among the tested treatments, the dose of 10 g/kg feed (P2) produced the best overall response, resulting in the highest absolute weight gain ( $5.43 \pm 0.18$  g), absolute length gain ( $4.50 \pm 0.18$  cm), and survival rate ( $70.0 \pm 2.00\%$ ). These findings indicate that garlic extract can function effectively as a natural feed additive to enhance fry performance, likely through its antibacterial and immunostimulatory properties. However, increasing the dose to 15 g/kg feed did not further improve the response and tended to reduce performance relative to the optimum treatment. Therefore, 10 g/kg feed can be recommended as the most effective dosage of garlic extract for improving the growth and survival of Nile tilapia fry in intensive culture systems.

## RECOMMENDATION

Based on the findings of this study, garlic extract at a dose of 10 g/kg feed is recommended as the optimal level for improving the growth and survival of Nile tilapia (*Oreochromis niloticus*) fry. Future studies are recommended to examine physiological parameters such as feed conversion ratio (FCR) and immune response, as well as to conduct longer rearing trials to evaluate its long-term effectiveness.

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