Immunostimulatory Effect of Garlic Powder (*Allium sativum*) on Growth and Haematological Parameters of Clariid Catfish (*Clarias gariepinus*) Juveniles

RESEARCH ARTICLE

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ABSTRACT

This study investigated the immunostimulatory effects of garlic powder (*Allium sativum*) on the growth performance, haematological parameters, serum biochemical indices, innate immune response, and disease resistance of African catfish (*Clarias gariepinus*) juveniles. Fresh garlic bulbs were processed into powder and incorporated into five experimental diets at graded inclusion levels of 2 g/kg, 3 g/kg, 4 g/kg, and 5 g/kg–a fifth diet without garlic powder served as the control. The feeding trial, designed as a completely randomized design (CRD), lasted for 56 days with 150 catfish juveniles stocked in 10 tanks (15 fish per tank), receiving diets at 5% body weight twice daily. Bi-weekly weight measurements and weekly water quality assessments were recorded. Following the feeding trial, fish were intramuscularly injected with 0.1 mL of *Aeromonas hydrophila* to assess disease resistance. Haematological and biochemical indices were analysed using standard procedures. The results revealed that fish fed 2 g/kg garlic inclusion (T1) exhibited the highest growth performance, while the control group recorded the lowest. Haematological parameters, including haemoglobin (Hb), packed cell volume (PCV), and red blood cell count (RBC), were highest in the control group and decreased with increasing garlic levels. However, white blood cell (WBC) counts increased with higher garlic inclusion, peaking at 5 g/kg (T4), indicating enhanced immune response. The challenge test results demonstrated that T2 (3 g/kg) had the lowest mortality rate, suggesting improved disease resistance at moderate garlic inclusion levels. The findings suggest that garlic powder supplementation at 2 g/kg promotes growth, while 3 g/kg enhances disease resistance without compromising blood parameters. Excessive inclusion (5 g/kg) may weaken immune defence mechanisms. This study concludes that moderate garlic supplementation is beneficial for African catfish culture, supporting sustainable aquaculture practices.

Methodology

Completely randomized design with 150 catfish juveniles across 5 treatments over 56 days

Key Variables

Garlic inclusion levels: 0, 2, 3, 4, and 5 g/kg with growth and haematological assessments

Main Finding

2 g/kg garlic promotes growth; 3 g/kg enhances disease resistance with zero mortality

Keywords: *Allium sativum*, *Clarias gariepinus*, immunostimulatory, growth performance, haematology, serum biochemistry.

INTRODUCTION

Aquaculture (fish farming) is a vital component of the global food industry, providing high-quality protein to meet growing seafood demand (Soliman, 2017). The industry has rapidly expanded due to technological advancements, research innovations, and increasing consumer demand for fish protein (Nunes, 2009). In Nigeria, aquaculture has grown significantly over five decades, yet domestic production remains insufficient to meet local demand (Digun-Aweto & Oladele, 2017).

The African catfish, *Clarias gariepinus*, is a fast-growing, omnivorous species widely cultivated in African and Asian aquaculture (Kaur & Ansal, 2020). Its streamlined, elongated body with scaleless, slimy skin protects against pathogens. Hardy and capable of surviving in low-oxygen environments due to its accessory breathing organ (Greenwood, 1976), this species is highly valued for its rapid growth, resilience to harsh conditions, and high market demand (Teugels, 1986). However, despite its hardy nature, bacterial infections, particularly from *Aeromonas hydrophila*, pose a significant challenge, causing substantial economic losses (Saharia *et al.,* 2021).

01

Aquaculture Growth

Global fish farming expansion driven by technological advances and increasing demand for high-quality protein sources.

02

Nigerian Context

Significant aquaculture growth over five decades, but domestic production still insufficient to meet local demand.

03

Disease Challenges

Bacterial infections like *Aeromonas hydrophila* cause substantial economic losses despite the hardy nature of African catfish.

These infections lead to high mortality rates, reduced growth performance, and poor feed conversion efficiency (Campbell & Adams, 2010). Specifically, *Aeromonas hydrophila* infections cause haemorrhagic lesions, fin rot, and high mortality (Campbell & Adams, 2010); trigger oxidative stress and tissue damage (Hamed *et al.,* 2017); and reduce serum complement activity and phagocyte function, weakening the fish's immune defence (Pridgeon & Klesius, 2011).

Garlic (*Allium sativum*) is a perennial bulbous plant rich in organosulphur compounds like allicin, which possess antimicrobial, antioxidant, and immunomodulatory properties (Gong *et al.,* 2022). These compounds inhibit bacterial growth and enhance immune responses in animals (Zaefarian *et al.,* 2017). As a natural additive, garlic has proven antimicrobial, antioxidant, and immunostimulatory benefits (Sangouni *et al.,* 2021). Previous studies show that garlic supplementation in aquatic species improves growth performance, immune response, and survival rates (Ndong & Fall, 2011; Zaefarian *et al.,* 2017). Additionally, garlic is cost-effective, readily available, and safe for long-term aquaculture use.

The aquaculture sector faces challenges related to fish health management, particularly bacterial diseases that reduce productivity and profitability. Synthetic antibiotics, commonly used to manage infections, contribute to antibiotic resistance, environmental contamination, and regulatory concerns (Miranda *et al.,* 2018). Therefore, there is an urgent need to explore natural, eco-friendly alternatives to promote fish health and enhance disease resistance.

STUDY OBJECTIVES

To address these challenges, there is increasing interest in using natural feed additives, such as garlic powder (*Allium sativum*), to enhance the immune response and growth performance of cultured fish (Zaefarian *et al.,* 2017). Garlic has long been recognised for its antimicrobial, immunomodulatory, and antioxidant properties, making it a promising alternative to synthetic antibiotics (Elbaz et al., 2021). However, limited research has been conducted on its effects on *Clarias gariepinus*, particularly concerning its ability to enhance immunity against *Aeromonas hydrophila* infections.

Research Gap

- Limited information on garlic effects on *Clarias gariepinus* immunity
- Need for optimal inclusion levels in catfish diets
- Assessment against *Aeromonas hydrophila* infections

Study Objectives

- Evaluate effects on growth performance
- Assess haematological and biochemical parameters
- Test disease resistance capabilities

Despite extensive research on other aquatic species, there is limited information on the effects of garlic on the innate immunity and disease resistance of *Clarias gariepinus*. Investigating the optimal inclusion levels of garlic powder in catfish diets will provide valuable data for farmers, researchers, and policymakers to promote sustainable aquaculture practices.

This study, therefore, aims to evaluate the effects of dietary garlic powder on the growth performance, haematological and serum biochemical parameters, and disease resistance of *Clarias gariepinus* juveniles. The findings will provide insights into the potential use of garlic powder as a natural immunostimulant and growth promoter in catfish aquaculture.

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria.

Procurement of Experimental Fish

A total of 150 healthy *Clarias gariepinus* fingerlings (average weight: 9.09 ± 0.01 g) were purchased from a reputable fish farm in Ondo town. The fish were transported to the research facility in a 50-litre container during the early hours to minimise transport stress.

Acclimatisation of Fish

Upon arrival, the fingerlings were stocked in a concrete tank (2 × 2 × 4 ft) and acclimatised for 14 days. They were fed ad libitum with a 3 mm Blue Crown commercial diet twice daily at 08:00 and 17:00 hours.

Garlic Preparation

Fresh garlic bulbs were processed into fine powder through drying and grinding procedures.

Feed Formulation

Garlic powder was incorporated at graded levels: 0, 2, 3, 4, and 5 g/kg into commercial feed.

Experimental Design

Completely randomised design with 150 fingerlings distributed across 10 tanks for 56 days.

Preparation of Garlic Additive

Fresh garlic bulbs were sourced from Oja-Oba market, Akure. The preparation procedure followed Gong et al. (2022). Garlic bulbs were peeled, washed, and sliced into smaller pieces. The slices were sun-dried for 7 days, followed by oven drying at 30°C for 1 hour to remove residual moisture. The dried garlic slices were ground into a fine powder using an electric blender and passed through a fine sieve. The garlic powder was stored in an airtight container to maintain its potency.

Experimental Feed Formulation

The experimental diets were prepared using Blue Crown commercial feed, which was pulverised to a fine powder. The garlic powder was incorporated into the feed at graded levels: 0 g/kg garlic (no additive), 2 g/kg garlic, 3 g/kg garlic, 4 g/kg garlic, 5 g/kg garlic. For each diet batch, 1 kg of the feed was mixed with 400 mL of boiling water and kneaded into a dough-like consistency. The dough was pelleted using a hand-operated pelleting machine with a 2 mm die. The pellets were sun-dried for 48 hours and stored in labelled plastic containers under cool, dry conditions.

Experimental Design and Feeding Trial

The study employed a **Completely Randomized Design (CRD)** with five treatments, each having two replicates. 150 fingerlings were randomly distributed into 10 tanks (15 fish per tank). Fish were fed twice daily (08:00 and 17:00 hours) at 5% body weight for 56 days. Feed quantities were adjusted bi-weekly based on the recorded weight gains. Tanks were siphoned daily to remove faecal matter and uneaten feed. Water was partially replaced every 3-4 days using dechlorinated borehole water.

Data Collection

Growth Performance Measurement

Fish were collectively weighed bi-weekly using an electronic weighing balance. Growth parameters such as weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) were calculated using standard formulas (Heidarieh *et al.,* 2013):

Average Weight Gain (AWG):

AWG = Final Weight (g) - Initial Weight (g)

Specific Growth Rate (SGR, %/day):

SGR = 100 x (ln final weight - ln initial weight) / days

Feed Conversion Ratio (FCR):

Feed Conversion Ratio (FCR) = Feed Input (consumed) / weight gain

Water Quality Parameters

Water quality was monitored weekly using a Hanna Instruments (HI 9813-61) Portable Metre. Temperature was recorded using the temperature probe of the metre. The pH was measured with the pH sensor after calibration. Salinity was assessed using the salinity mode on the metre and recorded in ppm. Conductivity **(µS/cm) was measured via the conductivity sensor.**

Statistical Analysis

Data collected on growth performance, water quality, haematological indices, serum biochemistry, and mortality rates were analysed using descriptive statistics and one-way analysis of variance (ANOVA) in Microsoft Excel 2016. Differences between treatment means were determined using Duncan's Multiple Range Test (DMRT) at a 5% significance level ($p < 0.05$).

Growth Metrics

- Average Weight Gain (AWG)
- Specific Growth Rate (SGR)
- Feed Conversion Ratio (FCR)

Water Quality

Weekly monitoring of temperature, pH, salinity, and conductivity using Hanna Instruments metre

Statistical Method

ANOVA with Duncan's Multiple Range Test at 5% significance level using Microsoft Excel 2016

Bacterial Preparation and Challenge Test

Aeromonas hydrophila was isolated from a sewage sample collected from the FUTA Teaching and Research Farm. The isolation followed the method of Pridgeon & Klesius (2011). 1 mL of the sewage sample was serially diluted in sterile distilled water. The diluted samples were plated on Ampicillin Dextrin Agar (ADA) and incubated at 36°C for 24 hours. Single colonies were sub-cultured in nutrient broth and incubated for 24 hours at 36°C. The broth culture was centrifuged at 20 rpm for 5 minutes to separate bacterial cells. The pellet was washed twice and suspended in sterile water.

Challenge Test Protocol

At the end of the 56-day feeding trial, 10 fish were randomly selected from each treatment group for the challenge test. Fish were intramuscularly injected with 0.1 mL of *Aeromonas hydrophila* suspension (1 × 106 CFU/mL) using sterile syringes (Mosbah *et al.,* 2018). The fish were monitored for 12 days, and mortality rates were recorded.

Sample Collection for Haematological and Biochemical Analysis

Blood Sampling:

Blood samples were collected from the caudal vein using sterile 2 mL syringes. Samples for haematological analysis were placed in EDTA-coated tubes, while samples for serum biochemical analysis were placed in plain Eppendorf tubes and allowed to clot.

Determination of Haematological Parameters

Haematological parameters were determined using the methods described by Svobodová *et al.* (1991). The Packed Cell Volume (PCV) was measured using the Micro-haematocrit reader (microhaematocrit method). A haemoglobinometer was used to determine the haemoglobin count (HB), and a Neubauer haemocytometer was used to count the white blood cells (WBC) and red blood cells (RBC) (Olowolafe *et al.*, 2022). The following red blood cell indices, including mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV), and haematocrit (HCT), were determined using the formulas provided below.

Red Blood Cell Indices:

Mean Cell Haemoglobin Concentration (MCHC)

This measures the average haemoglobin concentration in the red blood cells, which is an indicator of their oxygen-carrying capacity.

$$MCHC = \frac{HB}{PCV} \times 100$$

Mean Cell Haemoglobin (MCH)

This expresses the average amount or concentration of haemoglobin in a single red blood cell.

$$MCH = \frac{HB}{RBC} \times 10$$

Mean Cell Volume (MCV)

This defines the average volume of a red blood cell. It is important in assessing certain blood disorders.

$$MCV = \frac{PCV}{RBC} \times 10$$

Determination of Serum Biochemical Parameters

Serum biochemical indices were determined using standard analytical methods as described by Olowolafe *et al.* (2022). Enzyme activities, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were measured using a colourimetric assay. Antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx), were analysed using spectrophotometric methods, while serum protein and creatinine parameters, such as total protein (TP) and creatinine (CREA), were determined using the biuret and Jaffe methods, respectively.

Proximate Analysis of Experimental Diets

The proximate composition of the five experimental diets was analysed following the procedures of the Association of Official Analytical Chemists (AOAC, 2010) at the Nutrition Laboratory, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure. Moisture content was determined by oven drying at 105°C until a constant weight was obtained. Ash content was measured by incinerating the samples in a muffle furnace at 550°C for 24 hours. Crude protein was analysed using the Kjeldahl method (Nitrogen content × 6.25), crude lipid was determined using Soxhlet extraction with petroleum ether, and crude fibre was assessed by sequential acid and alkali digestion. Nitrogen-Free Extract (NFE) was calculated by difference:

NFE = 100 − (Moisture + Ash + Crude Protein + Crude Lipid + Crude Fibre)

RESULTS

This chapter presents the results of the study, including water quality parameters, growth performance, haematological indices, serum biochemical parameters, and mortality rates observed during the 12-day challenge test. The results are presented using tables, charts, and figures, followed by descriptions and interpretations.

Water Quality Parameters

The water quality parameters monitored weekly during the 56-day feeding trial are presented in Figure 1. The values were within the acceptable range for *Clarias gariepinus* culture as recommended by Teugels *et al.* (1991).

Temperature ranged from 25.01°C to 25.05°C, with the highest value recorded in the control group and the lowest in T2. According to De Graaf & Janssen (1995), *Clarias gariepinus* thrives best at temperatures between 25°C and 30°C.

pH: ranged from 5.80 to 5.94, with T3 recording the lowest and the control the highest. Slightly acidic pH values are consistent with findings by Olowolafe et al. (2022).

Salinity: ranged from 273.18 ppm (T2) to 307.38 ppm (T1).

Conductivity: ranged from 0.38 μS/cm (Control) to 0.42 μS/cm (T1).

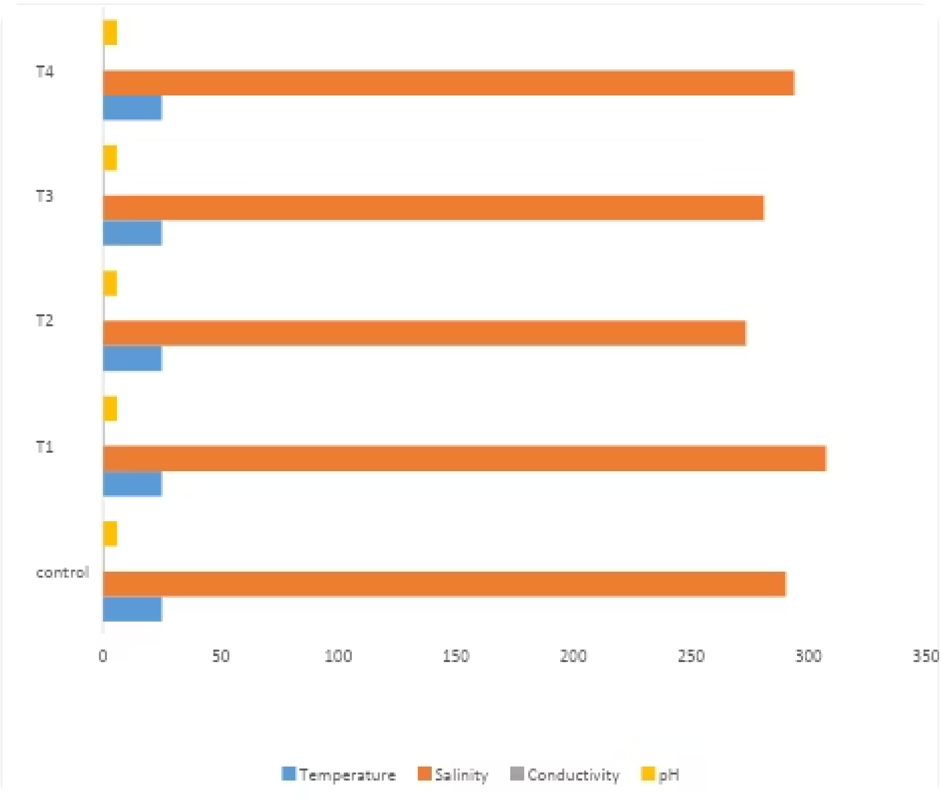

Figure 1: Water Quality Parameters Measured Weekly During the 56-Day Feeding Trial

(Source: Experimental Data, 2023)

Growth Performance of *Clarias gariepinus* Juveniles

The growth performance of fish fed varying levels of garlic powder is shown in Figure 2. From the study, the highest average weight gain (AWG) and SGR were observed in T1 (2 g/kg garlic), followed by T3 (4 g/kg), T2 (3 g/kg), and T4 (5 g/kg). The control recorded the lowest growth performance. This result aligns with findings by Ndong and Fall (2011), who reported improved growth in hybrid tilapia fed garlic-supplemented diets.

T1 (2 g/kg) had the best FCR, indicating efficient feed utilisation, consistent with the report by Zaefarian *et al.* (2017).

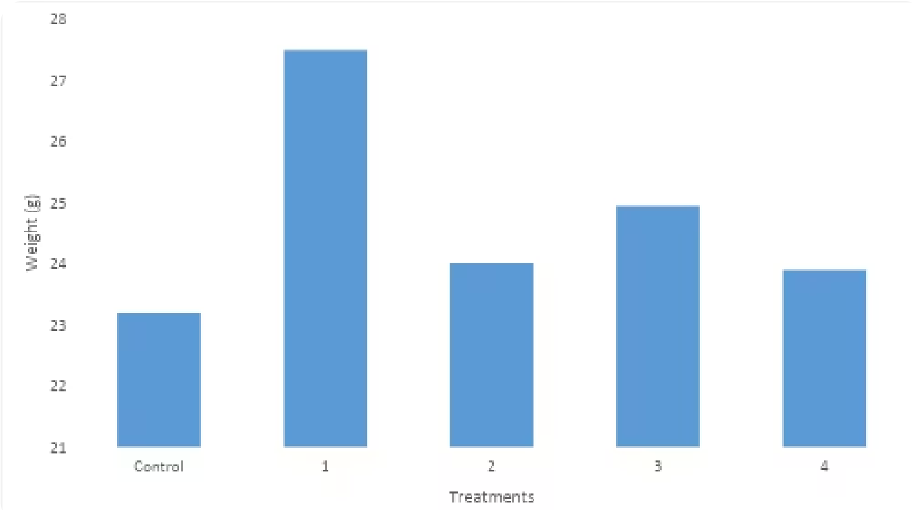

Figure 2: Growth Performance of *Clarias gariepinus* Juveniles Fed Garlic-Supplemented Diets

(Source: Experimental Data, 2023)

Haematological Parameters and Indices

The results of haematological analysis are presented in Figures 4.3 - 4.5. Haemoglobin (Hb), Packed Cell Volume (PCV), and Red Blood Cell (RBC) counts decreased with increasing garlic inclusion, with the highest values recorded in the control group and the lowest in T4 (5 g/kg) (Figure 3). Adeshina *et al.* (2021) observed a similar trend where high herbal inclusions reduced RBC counts in tilapia.

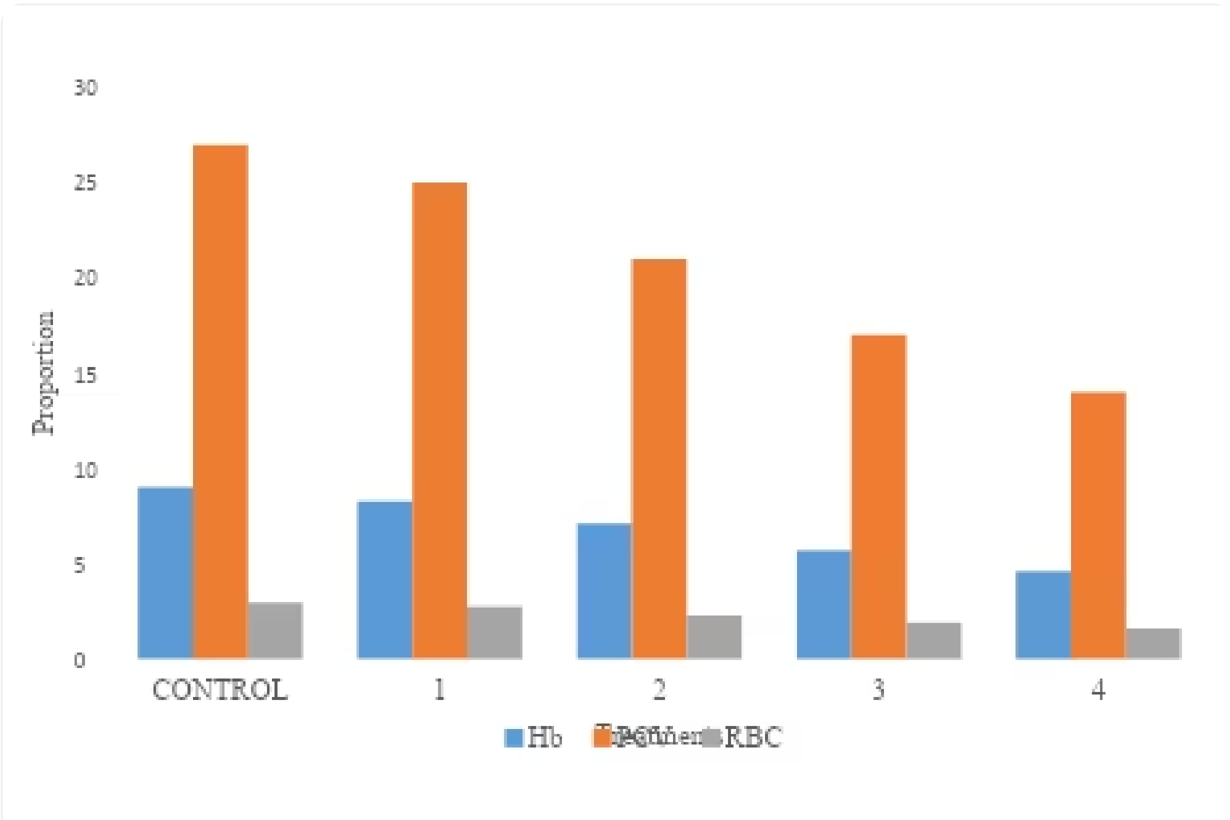

Figure 3: Haematological Parameters (Hb, PCV, RBC) of *Clarias gariepinus* Juveniles fed with varying concentrations of garlic powder and dosed with *Aeromonas hydrophila*.

(Source: Experimental Data, 2023)

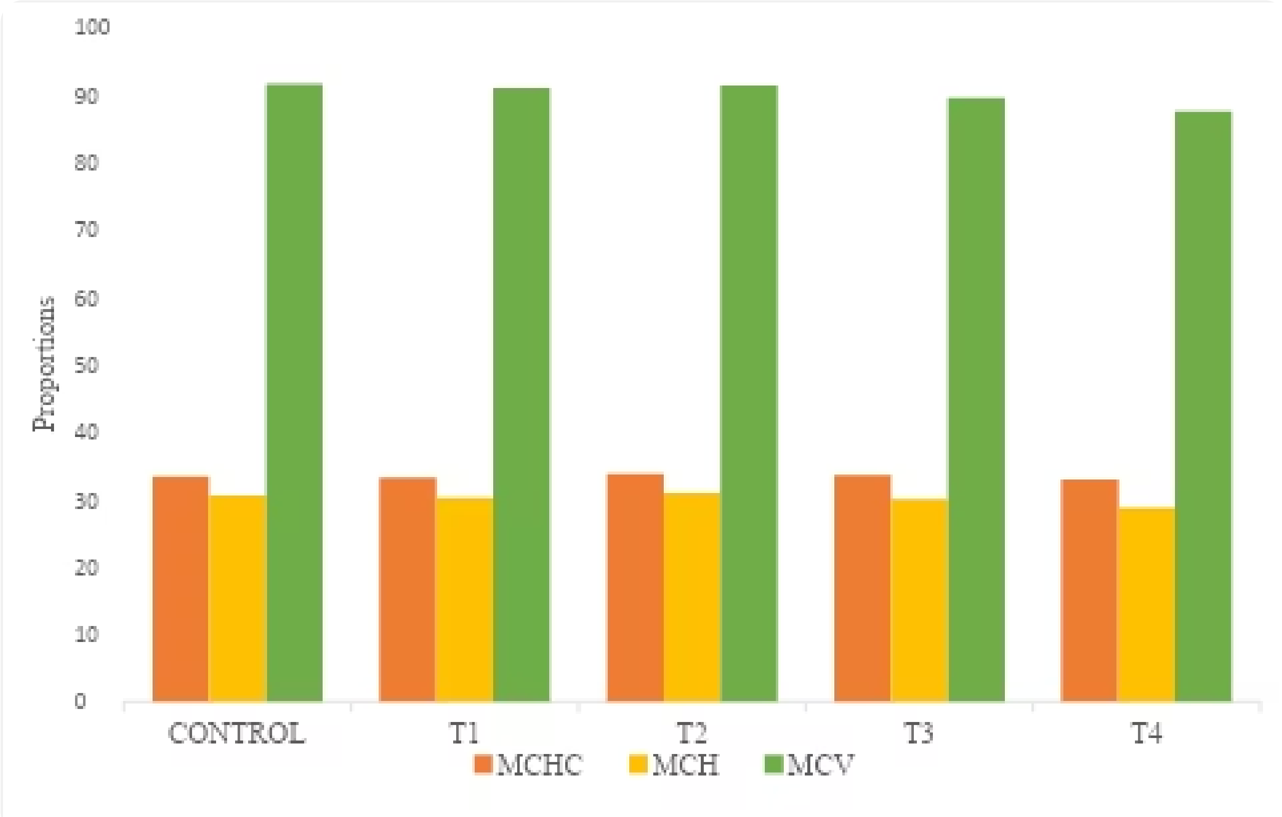

Figure 4: Result of the haematological indices (MCHC, MCH, and MCV) determined from the blood of *Clarias gariepinus* fed with varying concentrations of garlic powder and dosed with *Aeromonas hydrophila*.

(Source: Experimental Data, 2023)

The WBC count increased with higher garlic levels, with T4 recording the highest value (8600) (Figure 4). Douglas and Jane (2011) emphasized that higher WBC counts indicate enhanced immune response.

2g/kg

Optimal Growth

Best average weight gain and specific growth rate observed at this inclusion level

3g/kg

Disease Resistance

Lowest mortality rate with zero deaths during challenge test

8600

WBC Peak

Highest white blood cell count indicating enhanced immune response

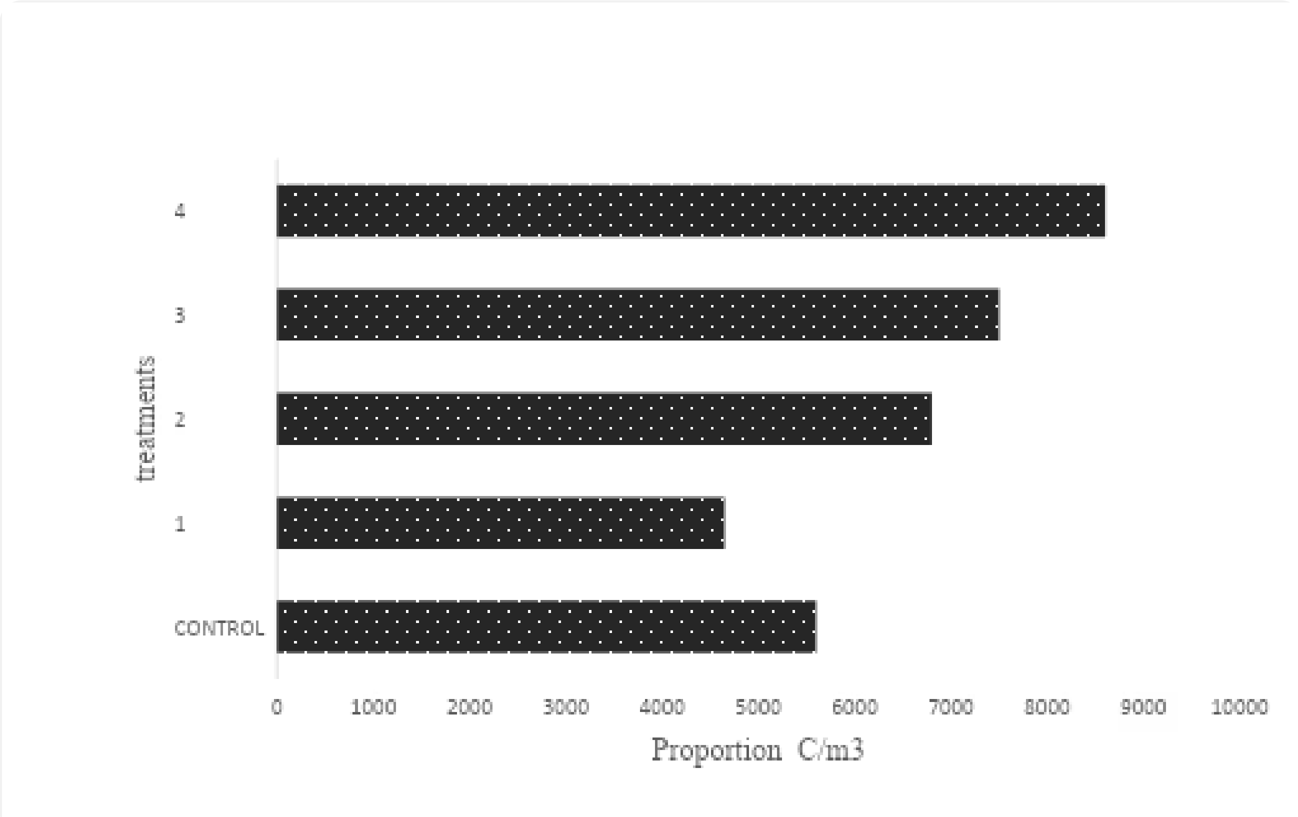

Figure 5: White Blood Cell (WBC) Count of *Clarias gariepinus* Juveniles fed with varying concentrations of garlic powder and dosed with Aeromonas hydrophila.

(Source: Experimental Data, 2023)

Mortality Rate during Challenge Test

The mortality rates recorded during the 12-day *Aeromonas hydrophila* challenge test are shown in Figure 6. From the study, T2 (3 g/kg) recorded zero mortality, indicating the highest resistance to *A. hydrophila*. Similar findings were reported by Pridgeon and Klesius (2011) on garlic-fed tilapia. T4 (5 g/kg) recorded the highest mortality (4 out of 10 fish), suggesting that high garlic levels may impair immunity, as also observed by Adeshina et al. (2018).

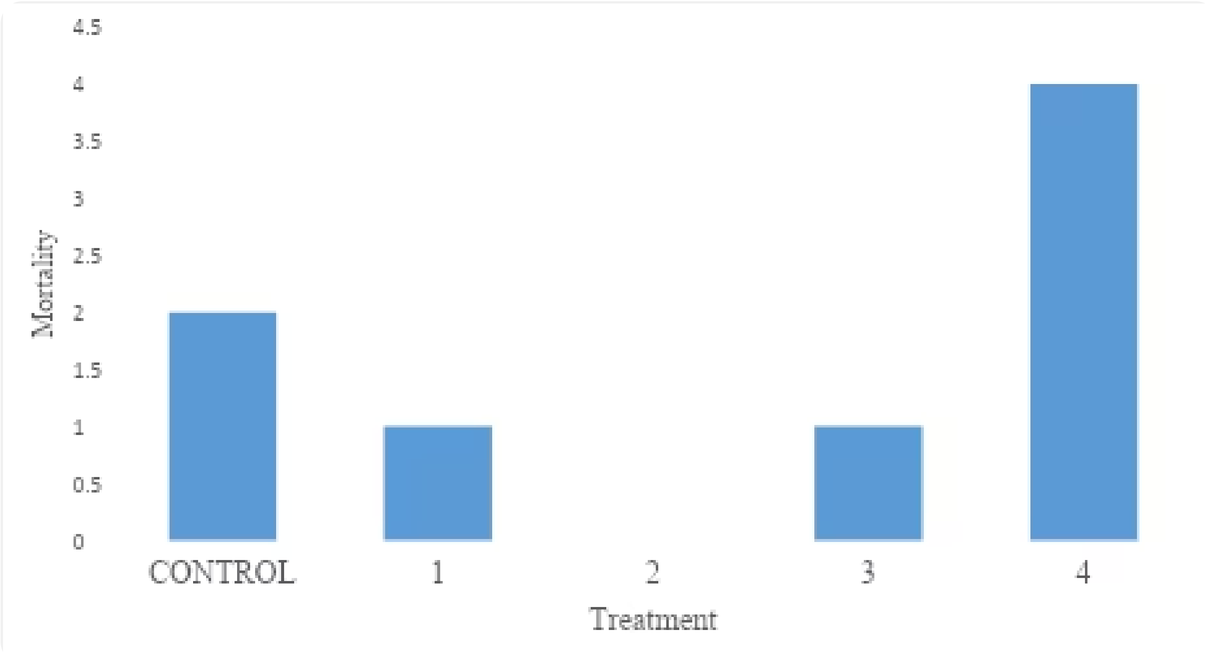

Figure 6: Mortality Rate during 12-Day Challenge Test with *Aeromonas hydrophila*

(Source: Experimental Data, 2023)

DISCUSSION

This study assesses the effect of garlic powder, *Allium sativum* on the growth rate and haematological parameters of the African catfish juveniles. The effects of garlic powder were observed on the growth performance, haematological indices, serum biochemical parameters, and mortality of *Clarias gariepinus* juveniles during the challenge test with *Aeromonas hydrophila*.

The seemingly contradictory results, where 2 g/kg garlic powder optimised growth performance while 3 g/kg provided superior disease resistance, reflect a complex dose-dependent relationship characteristic of bioactive phytochemicals. This biphasic response pattern, detailed in the dosage-response analysis, demonstrates that different physiological pathways are optimised at different garlic concentrations, with growth-promoting effects peaking at lower doses and immunostimulatory effects maximised at moderate doses before declining at higher concentrations.

The highest growth performance, measured by average weight gain (AWG), specific growth rate (SGR), and feed conversion ratio (FCR), was observed in fish fed 2 g/kg garlic powder (T1). This result aligns with the findings of Ndong and Fall (2011), who reported improved growth rates in tilapia-fed garlic-supplemented diets. The enhanced growth at 2 g/kg inclusion may be attributed to garlic's ability to stimulate appetite and improve nutrient absorption (Zaefarian et al., 2017).

However, growth performance declined as garlic inclusion increased beyond 3 g/kg. This trend is consistent with Tazikeh *et al.* (2020), who found that excessive garlic levels in shrimp diets reduced feed palatability and growth performance. The reduced growth at 5 g/kg (T4) may be due to the anti-nutritional effects of allicin at high concentrations, which can impair digestive efficiency (Elbaz *et al.*, 2021).

Growth Performance

2 g/kg garlic inclusion showed highest growth rates, aligning with previous tilapia studies on appetite stimulation and nutrient absorption.

Haematological Response

Increased WBC counts with higher garlic levels indicate enhanced immune response, while excessive levels may damage erythrocytes.

Disease Resistance

3 g/kg inclusion provided strongest resistance with zero mortality, attributed to immunostimulatory effects of allicin.

Observation of the haematological indices of the challenged catfish juvenile with garlic powder showed that the control group recorded the highest Hb, PCV, and RBC values, with a progressive decline observed as garlic inclusion increased. This finding contrasts the work of Adeshina *et al.* (2021), who reported higher RBC counts in fish fed moderate herbal supplements. Zaefarian *et al.* (2017) observed increased white blood cell counts in brown trout fed garlic-supplemented diets. The reduction in these haematological parameters at higher garlic levels suggests possible oxidative damage to erythrocytes, as noted by Hamed *et al.* (2017).

WBC counts increased with higher garlic inclusion, with T4 (5 g/kg) recording the highest value. This finding is consistent with Douglas and Jane (2011), who highlighted the role of WBCs in enhancing immunity against infections. The increase in WBC at higher garlic levels suggests that garlic stimulated the immune response, consistent with findings by Ndong and Fall (2011) in hybrid tilapia.

The challenge test with *Aeromonas hydrophila* revealed that fish fed 3 g/kg garlic powder (T2) had the lowest mortality rate (0%), indicating strong disease resistance. This result agrees with Pridgeon and Klesius (2011), who reported reduced mortality in garlic-fed tilapia challenged with bacterial infections. The enhanced resistance at moderate garlic inclusion levels is attributed to the immunostimulatory effects of allicin, which enhances phagocytic activity and lysozyme production (Zaefarian *et al.*, 2017).

Conversely, the highest mortality rate was recorded in T4 (5 g/kg), suggesting that excessive garlic inclusion may compromise immune function. Adeshina *et al.* (2018) reported similar findings in carp fed high levels of Moringa leaf meal, attributing the increased mortality to immune suppression and oxidative stress.

The findings from this study recognise the ability of garlic to boost immunity and protect fish from bacterial infections. The study indicates that *Clarias gariepinus* juveniles attained optimal growth at a 2g/kg inclusion level. Higher levels of garlic inclusion in the diet of *Clarias gariepinus* juveniles increased the WBC count, hence enhancing the immunity of the fish. Conversely, higher inclusion levels of garlic led to reduced antioxidant enzyme activity and elevated LPO. The disease-resistant property of garlic powder is best at 3g/kg inclusion level, evidenced by zero mortality during the *A. hydrophila* challenge test.

DOSAGE-RESPONSE ANALYSIS AND MECHANISTIC INTERPRETATION

The findings from this study highlight a complex, dose-dependent effect of garlic powder (*Allium sativum*) on African catfish juveniles, where different concentrations optimise distinct physiological parameters. This often observed pattern in bioactive compounds, known as a biphasic dose-response, necessitates a detailed mechanistic interpretation.

Optimal Growth at 2g/kg

The highest growth performance, measured by average weight gain (AWG), specific growth rate (SGR), and feed conversion ratio (FCR), was observed in fish fed 2 g/kg garlic powder (T1). This result aligns with the findings of Ndong and Fall (2011), who reported improved growth rates in tilapia fed garlic-supplemented diets. The enhanced growth at 2 g/kg inclusion may be attributed to garlic's ability to stimulate appetite and improve nutrient absorption (Zaefarian et al., 2017).

Superior Disease Resistance at 3g/kg

The challenge test with *Aeromonas hydrophila* revealed that fish fed 3 g/kg garlic powder (T2) had the lowest mortality rate (0%), indicating strong disease resistance. This result agrees with Pridgeon and Klesius (2011), who reported reduced mortality in garlic-fed tilapia challenged with bacterial infections. The enhanced resistance at moderate garlic inclusion levels is attributed to the immunostimulatory effects of allicin, which enhances phagocytic activity and lysozyme production (Zaefarian et al., 2017).

Biphasic Dose-Response and Hormesis

The observed pattern, where a low dose (2g/kg) enhances growth and a moderate dose (3g/kg) boosts immunity, with higher doses leading to detrimental effects, is characteristic of a biphasic dose-response relationship. This phenomenon, often referred to as hormesis, describes a beneficial effect at low doses and an inhibitory or toxic effect at high doses of a given substance. In the context of phytochemical supplementation, hormesis suggests that certain compounds, like those in garlic, can trigger adaptive responses that improve physiological functions at sub-toxic concentrations.

Negative Effects and Toxicity Threshold at 5g/kg

Conversely, the highest mortality rate was recorded in T4 (5 g/kg), suggesting that excessive garlic inclusion may compromise immune function. Adeshina et al. (2018) reported similar findings in carp fed high levels of Moringa leaf meal, attributing the increased mortality to immune suppression and oxidative stress. Furthermore, growth performance declined as garlic inclusion increased beyond 3 g/kg, consistent with Tazikeh *et al.* (2020), who found that excessive garlic levels in shrimp diets reduced feed palatability and growth performance. The reduced growth at 5 g/kg (T4) may be due to the anti-nutritional effects of allicin at high concentrations, which can impair digestive efficiency (Elbaz *et al*., 2021). The reduction in haematological parameters at higher garlic levels further suggests possible oxidative damage to erythrocytes, as noted by Hamed *et al.* (2017), indicating that 5g/kg likely represents a toxicity threshold.

This study's findings resonate with similar dose-response patterns observed with other herbal feed additives, where optimal benefits are achieved within a narrow therapeutic window. The general trend suggests that that while lower to moderate concentrations of bioactive compounds can stimulate beneficial physiological pathways, exceeding this optimal range can lead to adverse effects, including anti-nutritional impacts, immune suppression, and oxidative stress. Therefore, precise dosage determination is critical for maximising the benefits of such supplements in aquaculture.

PRACTICAL APPLICATIONS AND ECONOMIC IMPLICATIONS

Building upon the detailed dose-response analysis, this section bridges the gap between scientific findings and real-world aquaculture implementation. It explores the tangible benefits and strategic considerations for integrating garlic powder (*Allium sativum*) as a feed supplement in commercial African catfish (*Clarias gariepinus*) operations.

Cost-benefit analysis of garlic powder supplementation at optimal doses

Implementing garlic powder at optimal doses (e.g., 2g/kg for growth, 3g/kg for disease resistance) can lead to improved feed conversion ratios and reduced mortality, directly impacting profitability. A thorough analyse would weigh the cost of garlic supplementation against the gains in fish biomass and health, demonstrating a favourable return on investment compared to alternative solutions.

Implementation guidelines for commercial aquaculture operations

Clear, evidence-based guidelines are essential for successful adoption. These would include recommendations for dosage specific to developmental stages, mixing protocols to ensure homogeneous distribution in feed, and monitoring strategies to assess effectiveness and detect any adverse effects at higher concentrations. Training for farm staff on precise application is also crucial.

Economic comparison with synthetic alternatives (antibiotics, growth promoters)

Garlic powder offers a natural, sustainable alternative to synthetic antibiotics and chemical growth promoters. Beyond direct cost, this comparison should factor in the reduced risk of antibiotic resistance, lower regulatory hurdles, and enhanced consumer preference for naturally-raised produce, potentially leading to premium market prices.

Scalability considerations for different farm sizes

The methodology for garlic supplementation must be adaptable across various scales, from small-holder farms to large industrial operations. Considerations include sourcing garlic powder in bulk, appropriate mixing equipment for different feed volumes, and logistical challenges associated with storage and distribution, all while maintaining cost-effectiveness.

Integration with existing feeding protocols

Garlic powder should seamlessly integrate into current feed manufacturing and delivery systems. This involves evaluating compatibility with existing feed formulations, ensuring palatability, and confirming that the supplemented feed can be processed and stored without degradation of the active compounds or significant changes to nutritional value.

Market advantages of naturally-raised fish

Fish raised with natural supplements like garlic powder can command a competitive edge in markets increasingly prioritising sustainable and antibiotic-free food sources. Labelling and certification can highlight these practices, appealing to health-conscious consumers and opening new market segments with higher profit margins.

Regulatory compliance benefits

Utilising natural feed additives can simplify compliance with stringent food safety regulations and international trade standards, particularly concerning antibiotic residues and environmental impact. This proactive approach reduces the risk of costly penalties and enhances the industry's reputation for responsible aquaculture practices.

Risk mitigation strategies for disease outbreaks

The improved disease resistance observed at optimal garlic inclusion levels serves as a crucial preventative measure against common bacterial infections like *Aeromonas hydrophila*. Integrating garlic into feed protocols can significantly reduce the incidence and severity of outbreaks, minimising economic losses from mortality and treatment costs.

These practical applications and economic considerations underscore the potential of garlic powder as a valuable and sustainable tool for enhancing productivity and profitability in the aquaculture sector, aligning with global demands for healthier and more environmentally friendly food production systems.

CONCLUSION

The use of natural feed additives to improve fish health and performance has gained attention in aquaculture. Among these additives, garlic (*Allium sativum*) stands out due to its antimicrobial, antioxidant, and immunomodulatory properties (Zaefarian et al., 2017). This study demonstrated that dietary inclusion of garlic powder (*Allium sativum*) positively influences growth performance, haematological indices, serum biochemical parameters, and disease resistance in *Clarias gariepinus* juveniles. The results affirm that moderate inclusion of garlic powder enhances growth and disease resistance, while excessive levels may compromise health due to oxidative stress.

Based on these findings, aquaculture practitioners should consider implementing garlic powder supplementation at 2-3 g/kg feed inclusion rates to optimise both growth performance and disease resistance. The economic benefits, including reduced mortality, decreased antibiotic dependency, and premium market positioning for naturally-raised fish, justify the modest feed cost increase. This research provides a scientifically validated pathway for sustainable aquaculture intensification while meeting growing consumer demand for antibiotic-free fish products.

RECOMMENDATION

Based on the findings, it is recommended that dietary inclusion of **2-3 g/kg garlic powder** is used for enhancing growth and immunity in *Clarias gariepinus*. Garlic levels above **4 g/kg** should be avoided, as they may cause oxidative stress and compromise health. Additional studies should be conducted to examine the long-term effects of garlic supplementation on reproductive performance and survival rates. Secondly, the molecular mechanisms underlying garlic's immunostimulatory properties should be investigated. Fish farmers should consider incorporating garlic powder into commercial feed formulations to reduce dependency on synthetic antibiotics.

01

Optimal Inclusion Level

Use 2-3 g/kg garlic powder for enhancing growth and immunity in *Clarias gariepinus* juveniles.

02

Avoid Excessive Levels

Garlic levels above 4 g/kg should be avoided due to potential oxidative stress and health compromise.

03

Further Research Needed

Investigate long-term effects on reproductive performance and molecular mechanisms of immunostimulatory properties.

04

Commercial Application

Fish farmers should incorporate garlic powder into feed formulations to reduce synthetic antibiotic dependency.

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CONFLICTS OF INTEREST

The author declares no conflicts of interest

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