



## Impact of Dietary Garlic (*Allium sativum*) Rhizome Powder Supplementation on Haematological and Serum Biochemical Indices of Broiler Starter Chickens

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### Abstract

*This study evaluated the effects of dietary garlic (*Allium sativum*) rhizome powder supplementation on the haematological and serum biochemical profiles of broiler starter chickens. A total of 120 day-old Abor Acre broiler chicks were randomly assigned to four dietary treatment groups, each with three replicates of 10 birds (30 birds per treatment). The experimental diets included: T1 (control – basal diet without garlic), T2 (basal diet + 0.5% garlic powder), T3 (basal diet + 1.0% garlic powder), and T4 (basal diet + 1.5% garlic powder). Chicks were fed a starter diet from week 1 to 4. Blood samples were collected at the end of the starter phase (week 4) for haematological and serum biochemical analysis. Haematological parameters assessed included haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), heterophils, lymphocytes, monocytes, and eosinophils. Serum biochemical indices measured were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, total protein, albumin, globulin, uric acid, and creatinine. Results revealed that dietary inclusion of garlic powder had significant effects ( $P < 0.05$ ) on several haematological and serum biochemical parameters. Notably, serum cholesterol levels decreased significantly with increasing levels of garlic supplementation. However, creatinine levels were not significantly affected ( $P > 0.05$ ) by garlic inclusion. These findings suggest that garlic rhizome powder can positively influence the blood profile of broiler starter chickens, particularly in lowering serum cholesterol, without adversely affecting kidney function*

**Keyword:** Dietary garlic, *Allium sativum*, Rhizome Powder, Haematological, Serum Biochemical Indices

### Introduction

Poultry meat ranks as the second-largest global food commodity, accounting for approximately 33% of world meat production (Manning et al., 2007). Chicken, in particular, comprises nearly 86%–87% of poultry meat production globally (FAO, 2007). The industry's prominence stems from poultry's affordability, lower fat content, convenience, and efficient feed-to-meat conversion (Manning & Baines, 2004; FAO, 2023).

In tropical regions, high feed costs and frequent disease outbreaks limit poultry productivity, driving reliance on inexpensive feed ingredients and antibiotic usage to sustain

production (George, 2020).. Antibiotic growth promoters (AGPs) have traditionally been used to elevate growth rates and enhance feed efficiency in broiler production (El-Ghany, 2024). However, AGP use is linked to disruption of gut microbiota, development of antimicrobial resistance, and consumer health concerns from drug residues in poultry products (Mehdi et al., 2018).

Recognizing these risks, consumers increasingly prefer organic and antibiotic-free poultry, prompting a shift toward natural growth promoters such as phytochemical feed additives, probiotics, and medicinal plants (Rafiq et al., 2022; Wikipedia, Natural Growth Promoter) These natural alternatives support healthy gut flora, improve digestion,



boost immunity, and enhance growth performance without the risks associated with AGPs (Wikipedia, 2023).

Medicinal plants have long been recognised as sources of bioactive compounds used in therapeutic applications and modern drug development (Evans, 2002). Among them, garlic (*Allium sativum*)—a member of the Alliaceae family—is valued for its nutritional and medicinal properties. Native to Central Asia and Northeastern Iran, garlic and related species (onions, leeks, chives) are widely used globally (El-Ghany, 2024). The health-promoting effects of garlic are attributed to its sulfurous bioactives—particularly allicin and ajoene—which exhibit potent antimicrobial, antioxidant, and lipid-regulating actions (Navidshad et al., 2018; El-Ghany, 2024). Additional compounds—such as allyl methyl thiosulfate, allyl propyl disulfide, glycosides, and coenzyme Q10—along with vitamin C, potassium, and phosphorus, are believed to improve feed intake, nutrient utilisation, and blood lipid profiles (Rastad et al., 2020; Navidshad et al., 2018).

Research demonstrates that dietary garlic supplementation in broilers leads to significant improvements in growth performance, feed conversion ratio, immune function, gut health, antioxidant capacity, and favourable changes in haematological and serum biochemical profiles, including reduced cholesterol (El-Ghany, 2024; Urban et al., 2025). Moreover, garlic has been shown to increase lymphocyte counts, spleen and thymus weights, and modulate gut microbiota, supporting its potential as an antibiotic alternative (Navidshad et al., 2018; Rastad et al., 2020).

Evaluation of haematological parameters provides vital insights into the physiological, nutritional, and pathological status of animals. These measures are sensitive indicators of systemic health, nutritional balance, stress responses, and the effects of dietary treatments such as garlic supplementation (El-Ghany, 2024; Rafiq et al., 2022).

This study aims to evaluate the effects of dietary garlic supplementation on growth performance, feed efficiency, immune response, haematological and serum biochemical markers, and overall health status of broiler chickens. The objective is to assess garlic's potential as a viable natural alternative to conventional AGPs in poultry production.

## Materials and Methods

### Experimental Site

The experiment will be conducted at the Teaching and Research Poultry Unit, Department of Animal Science and Technology, Faculty of Agriculture, **Nnamdi Azikiwe University, Awka**, Anambra State, Nigeria. This site is geographically situated between latitudes **6.24°N–6.28°N** and longitudes **7.00°E–7.08°E**. The region experiences a tropical wet and dry climate with two distinct seasons: a rainy season (April–July, September–October) and a dry harmattan season (November–March), typical of the **tropical rainforest zone** (Awka lies below 300 m above sea level in a valley on the plains of the Mamu River. Mean daytime temperatures average around **27 °C**, rising to **32–34 °C** in March, with relative humidity reaching ~80% at dawn. Annual rainfall

averages around **1,600 mm** (local meteorological station records since 1978).

### Sourcing and Preparation of Garlic Powder

Fresh garlic bulbs were obtained from **Eke Awka market**, Anambra State. Bulbs were sliced into thin pieces, sun-dried over three weeks, then oven-dried at **50 °C for 15 hours**, and finally milled into fine powder. For optimal dryness and storage stability, the resultant powder was further sun-dried before use.

### Experimental Design and Diets

A completely randomised design (CRD) was employed. Broiler chicks were allocated into four dietary treatment groups (T-1 to T-4):

- **T1:** Basal diet without garlic powder (control)
- **T2:** Basal diet + 0.5% garlic powder
- **T3:** Basal diet + 1.0% garlic powder
- **T4:** Basal diet + 1.5% garlic powder

Starter (0–28 days) diets were formulated (Table 1) to meet recommended nutrient requirements for broiler chickens, maintaining consistent composition across treatments except for garlic inclusion levels (per similar methods in comparable broiler garlic studies).

**Table 1: Cross composition of broiler starter diet with graded levels of dried garlic powder.**

| Feed ingredients (kg)  | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|------------------------|--------|--------|--------|--------|
| Maize                  | 54.00  | 54.00  | 54.00  | 54.00  |
| Wheat offal            | 6.00   | 5.50   | 5.00   | 4.50   |
| Soya bean meal         | 25.00  | 25.00  | 25.00  | 25.00  |
| (PKC) palm kernel cake | 8.00   | 8.00   | 8.00   | 8.00   |
| Fish meal              | 3.00   | 3.00   | 3.00   | 3.00   |
| Bone meal              | 3.00   | 3.00   | 3.00   | 3.00   |
| Methionine             | 0.25   | 0.25   | 0.25   | 0.25   |
| Lysine                 | 0.25   | 0.25   | 0.25   | 0.25   |
| Vitamin premix         | 0.25   | 0.25   | 0.25   | 0.25   |
| Garlic                 | -      | 0.50   | 1.00   | 1.50   |

|               |         |         |         |         |
|---------------|---------|---------|---------|---------|
| Salt          | 0.25    | 0.25    | 0.25    | 0.25    |
| Total         | 100     | 100     | 100     | 100     |
| Crude protein | 23.50   | 23.45   | 23.42   | 23.40   |
| Me (kcal/kg)  | 3005.00 | 3004.95 | 3004.85 | 3004.74 |

### Experimental Birds

One hundred and twenty (120) Arbor Acres broiler chicks of mixed sex were procured from FIDAN Farm, Ibadan North, Oyo State. The day-old chicks were randomly assigned to four treatment groups, each comprising thirty (30) birds, further subdivided into three replicates of ten (10) birds per replicate.

### Experimental Design and Bird Management

A completely randomised design (CRD) was employed. Initially, the brooding pen, feeders, and drinkers were disinfected before chick arrival. All chicks were re-distributed randomly into treatment groups (T1–T4) with 30 birds per group and 10 birds per replicate.

Birds were housed on a deep litter system, with kerosene stoves and lanterns maintaining brooding temperatures between 33–35 °C during the first few weeks. Feed and water were provided ad libitum, and feeders and drinkers were replenished every morning and evening.

Chicks were weighed at the start of the trial to record initial body weights, then weighed weekly. Daily feed intake per replicate was calculated as feed offered minus orts measured the following morning. The feeding trial lasted four (4) weeks. Routine management practices included scheduled vaccination and litter replacement.

### Blood Collection

At week 4 (midpoint), blood samples were collected from two randomly selected birds per replicate via the wing (brachial) vein using sterile 2 ml syringes.

Approximately 2.5 ml of blood per bird was dispensed into EDTA tubes (for haematological assays) and separate plain tubes (for serum biochemical analysis). Samples were stored in cool conditions and centrifuged to harvest serum, which was then frozen pending biochemical evaluation.

### Haematological Analyses

Standard techniques were applied based on peer-reviewed protocols:

#### Haemoglobin (Hb):

Measured using the Sahli's acid haematin method, where 20 µl of whole blood was mixed with acid haematin, diluted dropwise with distilled water until colour matching was achieved, and read against a haemometer standard.

#### Packed Cell Volume (PCV):

Determined using the microhaematocrit centrifuge method; capillary tubes filled with blood were sealed, centrifuged at 3000 rpm for 20 minutes, and PCV read via a microhaematocrit reader (Coles, 1986).

#### Red Blood Cell (RBC) Count:

Performed using the Neubauer counting chamber following dilution with formal-citrate solution; counts from specified grids were multiplied by 1.5 (as per standard dilution factor) to express values in  $\times 10^6/\mu\text{l}$ .

#### White Blood Cell (WBC) Count:

Carried out using the Turk's solution dilution and Neubauer chamber; counts in the primary grids were multiplied by 50 to yield values in  $\times 10^3/\mu\text{l}$ .

#### Differential Leukocyte Counts:

Performed on stained blood smears to determine percentages of heterophils, lymphocytes, monocytes, eosinophils, and basophils.

#### Erythrocyte Indices (MCV, MCH, MCHC):

$\text{MCV (fL)} = (\text{PCV\%} \times 10) \div \text{RBC count} (\times 10^6/\mu\text{l})$

$\text{MCH (pg)} = (\text{Hb in g/dL} \times 10) \div \text{RBC count}$

$\text{MCHC (\%)} = (\text{Hb} \times 100) \div \text{PCV}$

These protocols align with published broiler haematology standards (e.g. Agboola et al., 2013; Chauhan et al., 2020)

#### Serum Biochemical Analyses

Serum samples were analyzed using commercial assay kits (e.g. Sigma, Randolph kits). Measured parameters included:

- Total serum protein
- Albumin
- Globulin (calculated as total protein – albumin)
- Albumin/globulin ratio
- Serum cholesterol
- Glucose
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Creatinine
- Uric acid

Serum parameters were measured using standard colorimetric or enzymatic assays consistent with previously reported methodology (Olabisi et al., 2021; Chauhan et al., 2020)

#### Statistical Analysis

Data were subject to one-way ANOVA using the PROC GLM procedure in SAS (2000) software. Significant differences between treatment means were separated using Duncan's Multiple Range Test at a probability level of  $P < 0.05$ .

## Results and Discussion

### Haematological Profile of Starter Broiler Chickens (0–4 Weeks)

The haematological parameters of broiler chicks fed graded levels of garlic powder during weeks 0–4 are presented in Table 3.

**Table 3. Haematological Values of Broiler Starter Chickens Fed Graded Levels of Garlic Powder (0–4 Weeks)**

| Parameter (units)          | T1 (0%)                 | T2 (0.5%)               | T3 (1.0%)               | T4 (1.5%)               |
|----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Hemoglobin (g/dL)          | 9.38±0.02 <sup>d</sup>  | 10.27±0.02 <sup>c</sup> | 9.77±0.06 <sup>b</sup>  | 11.05±0.05 <sup>a</sup> |
| Packed Cell Volume (%)     | 27.83±0.10 <sup>c</sup> | 28.83±0.10 <sup>b</sup> | 28.00±1.0 <sup>bc</sup> | 31.33±0.10 <sup>a</sup> |
| RBC (×10 <sup>12</sup> /L) | 1.46±0.10 <sup>b</sup>  | 1.57±0.10 <sup>b</sup>  | 1.52±0.04 <sup>b</sup>  | 1.82±0.10 <sup>a</sup>  |
| WBC (×10 <sup>12</sup> /L) | 3.10±0.10 <sup>d</sup>  | 4.87±0.10 <sup>b</sup>  | 5.48±0.10 <sup>a</sup>  | 4.23±0.10 <sup>c</sup>  |
| MCV (fL)                   | 19.19±0.02 <sup>b</sup> | 19.31±0.02 <sup>a</sup> | 18.66±0.06 <sup>d</sup> | 18.84±0.05 <sup>c</sup> |
| MCH (pg)                   | 64.16±0.02 <sup>b</sup> | 65.28±0.02 <sup>a</sup> | 63.46±0.05 <sup>c</sup> | 60.88±0.06 <sup>d</sup> |
| MCHC (g/dL)                | 33.37±0.02 <sup>c</sup> | 33.80±0.02 <sup>b</sup> | 34.14±0.05 <sup>a</sup> | 32.37±0.05 <sup>d</sup> |
| Heterophils (%)            | 66.17±0.02 <sup>a</sup> | 62.64±0.02 <sup>c</sup> | 63.50±0.10 <sup>b</sup> | 60.17±0.05 <sup>b</sup> |
| Lymphocytes (%)            | 30.83±0.01 <sup>d</sup> | 33.62±0.20 <sup>b</sup> | 32.50±0.05 <sup>c</sup> | 35.50±0.05 <sup>a</sup> |
| Eosinophils (%)            | 2.83±0.03 <sup>d</sup>  | 3.07±0.04 <sup>c</sup>  | 3.17±0.04 <sup>b</sup>  | 3.33±0.03 <sup>a</sup>  |
| Monocytes (%)              | 0.17±0.01 <sup>d</sup>  | 0.66±0.01 <sup>c</sup>  | 0.83±0.03 <sup>b</sup>  | 1.00±0.01 <sup>a</sup>  |

Values within a row bearing different superscripts differ significantly ( $P < 0.05$ ).

Treatments:

- **T1:** 0% garlic (control)
- **T2:** 0.5% garlic
- **T3:** 1.0% garlic
- **T4:** 1.5% garlic

## Interpretation of Haematological Parameters

### Effect of Garlic Powder on Erythropoiesis and Oxygen Transport

Starter-phase broiler chickens fed garlic powder showed significant improvements ( $P < 0.05$ ) in haemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) counts, particularly at the 1.5% inclusion level (T4: Hb = 11.05 g/dL, PCV = 31.33%, RBC =  $1.82 \times 10^{12}/L$ ). These enhancements suggest that garlic promoted erythropoiesis and enhanced oxygen-carrying capacity. Similar observations have been reported by Ismail et al. (2020), where dietary garlic powder improved Hb, PCV, and RBC in broilers ( $P < 0.01$ ). The stimulatory effect of garlic on erythrocyte production may involve garlic-derived bioactives promoting erythropoietin secretion or enhancing spleen RBC turnover. These findings align with other studies demonstrating increased PCV, Hb, and RBC in poultry fed garlic powder (e.g. Kairalla et al., 2022).

### White Blood Cell Response and Immunomodulation

White blood cell (WBC) counts increased significantly with garlic supplementation, peaking in the 1.0% inclusion group (T3). The elevated leukocyte counts—particularly lymphocytes, monocytes, and eosinophils—indicate an immunostimulatory effect of garlic. This aligns with the

review by Abd El-Ghany (2024), which highlights garlic's ability to modulate immune responses by increasing WBC and lymphoid cell numbers in poultry. Kairalla et al. (2022) similarly reported significant increases in lymphocyte and monocyte counts with 0.2–0.3% garlic powder supplementation.

### Erythrocyte Index Dynamics (MCV, MCH, MCHC)

Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were highest in T2 (0.5%), while mean corpuscular haemoglobin concentration (MCHC) peaked in T3 (1.0%). These shifts may reflect adaptive haematological changes in response to garlic's bioactive compounds, possibly via mild hemolytic activation or altered erythropoiesis pathways.

### Differential Leukocyte Counts and Stress Response

Heterophils were highest in the control group (T1), while levels of lymphocytes, eosinophils, and monocytes increased with higher garlic inclusion. A lower heterophil count coupled with elevated lymphocytes and monocytes suggests a reduction in physiological stress and improved immune status. The lymphocyte/heterophil ratio, a stress indicator, thus reflects better adaptation in garlic-fed birds.

### Implications for Starter Broiler Health and Performance

Improved Hb, RBC, and PCV values at the starter stage translate to enhanced oxygen delivery and nutrient transport—critical for early growth and metabolic activity. The leukocyte enhancements support better innate immunity against early-life pathogens. Together, these haematological improvements may contribute to the improved growth performance and feed efficiency often observed in garlic-supplemented broilers

### Serum Biochemical Parameters (Starter Phase: 0–4 Weeks)



**Table 4** presents the mean serum biochemical values of broiler starter chickens fed graded levels of garlic powder during the starter phase.

**Table 4. Serum Biochemistry of Broilers Fed Graded Garlic Powder (0–4 Weeks)**

| Parameter           | T1 (0%)                  | T2 (0.5%)                | T3 (1.0%)                | T4 (1.5%)                |
|---------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| ALT (U/L)           | 34.35±0.18 <sup>a</sup>  | 29.66±0.21 <sup>b</sup>  | 27.53±0.31 <sup>d</sup>  | 29.51±0.43 <sup>c</sup>  |
| ALP (U/L)           | 48.47±0.27 <sup>a</sup>  | 45.17±0.03 <sup>c</sup>  | 48.24±0.07 <sup>a</sup>  | 47.53±0.26 <sup>b</sup>  |
| AST (U/L)           | 124.52±0.18 <sup>a</sup> | 122.66±0.21 <sup>b</sup> | 115.51±0.29 <sup>d</sup> | 116.31±0.13 <sup>c</sup> |
| Cholesterol (mg/dL) | 96.28±0.34 <sup>a</sup>  | 92.76±0.68 <sup>b</sup>  | 90.49±0.38 <sup>c</sup>  | 90.43±0.38 <sup>c</sup>  |
| Glucose (mg/dL)     | 137.83±0.03 <sup>c</sup> | 135.37±0.03 <sup>d</sup> | 139.17±0.08 <sup>b</sup> | 145.54±0.12 <sup>a</sup> |
| Total Protein (g/L) | 68.28±0.10 <sup>a</sup>  | 63.16±0.04 <sup>b</sup>  | 61.33±0.03 <sup>c</sup>  | 60.67±0.03 <sup>d</sup>  |
| Albumin (g/L)       | 42.33±0.10 <sup>a</sup>  | 38.52±0.03 <sup>b</sup>  | 37.45±0.03 <sup>c</sup>  | 37.30±0.03 <sup>d</sup>  |
| Globulin (g/L)      | 25.95±0.10 <sup>a</sup>  | 24.67±0.04 <sup>b</sup>  | 23.88±0.03 <sup>c</sup>  | 23.37±0.03 <sup>d</sup>  |
| Uric Acid (mg/dL)   | 40.67±0.03 <sup>b</sup>  | 39.05±0.05 <sup>c</sup>  | 36.83±0.03 <sup>d</sup>  | 41.05±0.05 <sup>a</sup>  |
| Creatinine (mg/dL)  | 0.67±0.02                | 0.74±0.02                | 0.71±0.02                | 0.71±0.04                |

Different superscripts within rows indicate significant differences at  $P < 0.05$ .

## Interpretation of Biochemical Findings

### Hepatic Enzymes: ALT, AST & ALP

ALT and AST levels decreased significantly ( $P < 0.05$ ) in garlic-fed groups, with the lowest values at 1.0% inclusion (T3), suggesting potential hepatoprotective effects of garlic. This aligns with findings by Kairalla et al. (2022), where garlic powder supplementation reduced ALT and AST in broiler chickens.

ALP decreased significantly at 0.5% inclusion (T2) but re-elevated at higher garlic levels. Such moderate ALP fluctuations may reflect adaptive hepatic activity changes, as observed in similar garlic-based trials

### Lipid Profile:

#### Cholesterol Reduction

Broilers fed higher levels of garlic exhibited significantly lower serum cholesterol ( $P < 0.05$ ), with untreated birds (T1) showing the highest value (~96.3 mg/dL). Cholesterol dropped modestly from T2 to T4 (~92.8 → ~90.5 mg/dL). This dose-dependent cholesterolemic decrease aligns with Mansoub (2011), who reported decreased cholesterol with 1 g/kg garlic inclusion, and with Stanacev et al. (2011), who demonstrated garlic's suppression of key enzymes in lipid synthesis (e.g. HMG-CoA reductase)

#### Liver Enzyme Profiles (ALT, AST, ALP)

Serum ALT and AST levels decreased significantly with garlic supplementation, particularly at 1.0% (T3), indicating improved hepatocyte integrity. This trend echoes findings by Ibrahim et al. (2024) and El-Katcha et al. (2016), which reported reduced transaminase levels in garlic-fed broilers, suggesting hepatoprotective effects without toxicity.

ALP decreased significantly at 0.5% inclusion (T2), but rose slightly at higher levels (T3-, T4), possibly indicating adaptive metabolic enzyme regulation at moderate doses.

### Protein, Albumin, and Globulin Concentrations

Total protein, albumin, and globulin levels declined progressively as garlic inclusion increased. Birds on the control diet had the highest protein values (~68.3 g/L total protein; ~42.3 g/L albumin). Similar reductions have been previously reported (e.g. El-Katcha et al., 2016; Onunkwo et al., 2019) and may result from improved hydration status, shifts in protein turnover, or downregulated hepatic biosynthesis in response to garlic bioactives.

### Glucose and Uric Acid Levels

Serum glucose rose with garlic inclusion, reaching ~145.5 mg/dL in T4. These elevated levels suggest enhanced gluconeogenesis or altered carbohydrate metabolism due to garlic's bioactive sulfur compounds (e.g. allicin), though this varies across studies. Uric acid decreased at 1.0% inclusion (T3) but returned to control levels at 1.5% (T4), suggesting non-linear modulation of nitrogen metabolism. The lack of significant change in creatinine across treatments confirms that garlic did not impair renal function, which is consistent with prior findings in broiler trials.

## Conclusion

Cholesterol reduction confirms garlic's hypolipidemic effects, supported by the inhibition of key cholesterol biosynthesis pathways (e.g. HMG-CoA reductase). Lower ALT and AST levels at moderate inclusion (1.0%) reflect safer and healthier liver function in starter broilers. Decreased protein fractions across treatments necessitate further study into the impact of garlic on protein metabolism and immune globulin synthesis. Increased glucose at higher inclusion levels suggests augmented metabolic activity, warranting further investigation

on energy balance and feed efficiency. Stable creatinine and variable uric acid indicate retained renal function, supporting garlic's safety at starter-phase inclusion levels.

In summary, graded dietary garlic powder in starter broiler diets significantly:

- Lowers serum cholesterol levels,
- Reduces liver enzymes (ALT, AST),
- Modulates protein fractions and glucose levels,
- Maintains renal function (creatinine).

These results support garlic's role as a functional feed additive that enhances liver health and metabolic profiles in the early developmental stage of broilers.

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