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IMPACT OF GARLIC EXTRACT ADMINISTRATION FREQUENCY ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

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ABSTRACT

Phytogenic additives like garlic are increasingly valued in poultry production for their natural growth-promoting, antimicrobial, and health-enhancing properties, making garlic a viable alternative to antibiotics in broiler production. This study investigated the impact of garlic extract administration frequency on the haematological and serum biochemical indices of broiler chickens. A total of 300-day-old COBB -500 chicks were assigned to five treatment groups: T1 (standard check with antibiotics), T2 (garlic extract three days/week), T3 (five days/week), T4 (daily), and T5 (control with water only), each with four replicates of 15 birds over 42 days. Garlic extract was prepared by soaking 50 g of ground garlic in 1 litre of boiled water for 12 hours and administered at a concentration of 7.5 g per litre through drinking water according to treatments. Birds were raised on deep litter with *ad libitum* access to feed and water. Blood samples were collected on days 21 and 42 for analysis. One-way ANOVA was employed for data evaluation using SAS. There were significant variations ($P<0.05$) in eosinophil levels, mean corpuscular volume, and mean corpuscular haemoglobin concentration at the finisher phase. Total protein, globulin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), levels at the starter phase, along with total protein, albumin, globulin, Alkaline phosphatase (ALP) and uric acid at the finisher phase, were also significantly influenced by frequency of garlic extract administration. Garlic extract (7.5 g per litre) administration frequencies positively affected haematological and biochemical parameters in broiler chickens at starter and finisher phases.

Keywords: Phytogenic additives, Growth performance, Antimicrobial effects, Immune response, Alternative to antibiotics

INTRODUCTION

In Nigeria, limited intake of animal protein, particularly in the rural areas, has significant nutritional implications. Poultry meat, as a rich protein source, plays a vital role in addressing this deficiency. However, increasing the profitability of poultry production while managing feed and medication costs remains a primary challenge, leading to widespread efforts to enhance the efficiency and nutritional value of broiler diets through feed supplements and additives. Antibiotics have reportedly been used as growth promoters and infection preventatives in poultry (Ogle, 2013), but its extensive use has contributed to the development of antibiotic-resistant bacteria, prompting global restrictions on antibiotics in animal feeds.

The poultry industry now seeks effective, natural alternatives to antibiotics. Herbs and medicinal plants, known for their diverse health benefits, have emerged as potential replacements (Brenes & Roura, 2010). Garlic (*Allium sativum)* is notable for its antibacterial, antiviral, antifungal, antioxidant, and other health-promoting properties (Hanieh *et al*., 2010). Allicin, the primary bioactive compound in garlic, rapidly decomposes into various organosulfur compounds with well-documented biological activities, making garlic a promising alternative to antibiotics (Shang *et al*., 2019). These compounds support immune and digestive functions in poultry, potentially copying antibiotic benefits without associated risks (Bot *et al*., 2019).

In broiler production, which is an accessible livelihood option in rural communities, fast growth has been traditionally achieved through antibiotic growth promoters (AGPs). Yet, growing concerns over AGPs' role in microbial resistance and human health impacts have shifted focus toward natural growth enhancers like garlic. Garlic contains inulin, a prebiotic that boosts probiotic activity, alongside a nutrient-rich profile including proteins, fats, vitamins, and volatile oils with therapeutic effects (Esmail, 2012).

Blood analysis remains essential for assessing the health and physiological status of animals, with haematological parameters providing insights into the impacts of dietary changes (Evans, 2002). Studies indicate that garlic can positively influence haematological indices, enhance immune response, and improve feed efficiency and growth rates in poultry (Abd El-Ghany, 2024). Garlic's bioactive compounds offer antimicrobial and antioxidative benefits, making it a promising addition to poultry diets (Puvača *et al*., 2015). However, the optimal dosage and frequency of garlic administration in broilers remain under-researched, highlighting a gap for further study.

Considering the pressing need to reduce antibiotic use, garlic's bioactive components, such as diallyl polysulfide, offer an attractive alternative with minimal risk of resistance (Kim *et al*., 2018). This study aimed to determine the impact of administration frequency of garlic extract on the haematological and serum biochemical indices of broiler chickens, potentially contributing to sustainable poultry health and productivity.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The farm is situated within the rainforest vegetation zone of South-Western Nigeria, at latitude 7° 13' 49.46"N, longitude 3° 26' 11.98"E, and an altitude of 76 meters above sea level (Google Earth, 2023). The climate in this region is humid, with a mean annual rainfall of 1037 mm, a mean temperature of 34.7°C and 83% relative humidity.

Source and preparation of test ingredients

The test ingredient, garlic (*Allium sativum*) bulbs, was purchased from an open market in Abeokuta. A portion was carefully peeled and blended using an electric blender; 50 grams of the blended garlic paste was added to a litre of boiled water at 80°C and kept for 12 hours (overnight). The following morning, the garlic solution was sieved and added to the drinking water of the birds at 15% inclusion level (150 ml of garlic extract per litre of water) giving a concentration of 7.5 g per litre.

Experimental birds and management

Three hundred (300) day-old broiler chicks of the COBB-500 strain were procured from a reputable hatchery for a 6-week study. Before the chicks' arrival, the pen and all equipment were cleaned, disinfected, and allowed to rest for two weeks. The pen surroundings were also cleared. In preparation for brooding, wood shavings were spread on the floor, coal pots were placed strategically as a source of heat, and electric bulbs were installed to provide light and additional heat.

Upon arrival, the chicks were weighed and assigned to five treatment groups. Each treatment was divided into four replicates consisting of 15 broiler chicks per replicate. The treatment groups were as follows:

Treatment 1 (T_1): Positive control, antibiotics (Colistin) was administered through water according to the manufacturer's dosage twice during the experimental period (at a week old and at 4 weeks old). (Standard check).
Treatment 2 (T_2): Garlic extract was administered through drinking water three consecutive days a week (3 days/week).
Treatment 3 (T_3): Garlic extract was administered through drinking water for five consecutive days a week (5 days/week).
Treatment 4 (T_4): Garlic extract was administered through drinking water every day of the week (Everyday).
Treatment 5 (T_5): Negative control, the birds were given only fresh water without any additives (Control).

The birds were fed commercial broiler starter feed (CP: 22%, Metabolizable Energy: 3000 Kcal/Kg, CF: 3.5%, Methionine: 0.6%) ad libitum from 0 to 3 weeks, and commercial broiler finisher feed (CP: 21%, Metabolizable Energy: 2800 Kcal/Kg, CF: 3.66%, Methionine: 0.59%) from 3 to 6 weeks. Water was provided *ad libitum*, and the vaccination and medication schedule were strictly followed throughout the study.

Collection of blood sample

Blood samples were collected twice during the study, at the end of both the starter and finisher phases. Two birds were randomly selected from each replicate across the treatments for sampling.

For haematological analysis, approximately 3 ml of blood was drawn from the jugular vein of each selected bird and transferred into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles. For serum biochemical analysis, about 2 ml of blood was also collected from the jugular vein and placed in plain collecting bottles. These samples were immediately placed in an ice pack and transported to the laboratory for further analysis.

Hematological Analysis

Key haematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) concentration, and packed cell volume (PCV), were measured using established methods. The RBC and WBC counts were performed using a hemocytometer and Natt-Herrick solution, which are essential for evaluating oxygen transport capacity and immune response, respectively (Barde *et al*,.2021). Haemoglobin concentration was measured using the cyanmethaemoglobin method, which involves mixing blood with Drabkin's reagent and measuring absorbance at 540 nm (Jain, 1986). PCV was determined by the microhematocrit technique, where blood samples were centrifuged at high speeds to estimate the proportion of red blood cells. Differential leukocyte counts were also performed on Giemsa-stained blood smears to calculate leukocyte subtypes, such as heterophils and lymphocytes, which are indicative of immune status and stress levels in broilers (Gross and Siegel, 1983).

Serum Biochemical Analysis

Serum biochemical indices were measured to evaluate metabolic and liver function. Total protein concentration was determined using the Biuret method, while albumin levels were quantified using the Bromo Cresol Green method. Globulin levels were calculated by subtracting albumin values from total protein (Arthur *et al*., 2012). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured as indicators of liver health through colorimetric assays involving 2, 4-dinitrophenylhydrazine. Glucose, creatinine, and Uric acid content were quantified using spectrophotometric or ion-selective electrode methods (Arthur *et al*., 2012).

Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) using SAS (2012). Significant difference among means, accepted at $p < 0.05$, was separated using the Duncan multiple range test as contained in the same statistical model.

RESULTS

Impact of the Frequency of Garlic Extract Administration on the Haematological and Serum Biochemical Indices of Broiler Chickens at Starter Phase

The haematological parameters of broiler chickens were not significantly affected by the frequency of garlic administration at the starter phase (Table 1). However, the serum biochemical parameters revealed some significant differences in total protein, globulin, aspartate aminotransferase (AST), and alanine aminotransferase (ALP). Birds administered garlic everyday (T_4) showed higher total protein values compared to those administered garlic for three days (T_2) and five days (T_3) – Table 2. The globulin level was significantly higher with birds treated with standard check (T_1) and with birds treated with garlic treatment everyday (T_4) compared to birds on 3 days/week and 5 days/week. The AST level was significantly higher in birds on standard check than those on 3 days/week. The ALP level was significantly higher in birds on control compared to those on standard check.

Table 1: Frequency of oral administration of garlic on the haematological indices of broiler chickens at starter phase

Parameters	Standard check	3 days/week	5 days/ week	Everyday	Control	SEM	Reference Range
PCV (%)	33.50	35.50	32.75	34.75	36.25	0.73	35.0 - 55.0
Haemoglobin (g/dl)	11.30	11.95	11.15	11.73	12.48	0.29	11.0 - 17.0
RBC (10^{12}/l)	3.97	4.11	3.76	4.07	4.235	0.11	2.5 - 3.5
WBC (10^{12}/l)	13.10	11.03	11.55	13.18	11.53	1.35	1.2 - 3.0
Heterophils (%)	19.25	45.25	23.00	42.25	39.50	5.17	15.0 - 50.0
Lymphocytes (%)	73.25	45.75	68.75	53.25	55.50	5.17	45.0 - 70.0
Basophils (%)	1.25	1.75	1.25	2.25	0.00	0.34	Rare
Monocytes (%)	3.25	2.25	5.75	1.50	2.00	0.68	5.0 - 10.0
Eosinophils (%)	3.00	5.00	1.25	0.75	3.00	0.68	1.5 - 6.0
MCV(FI)	85.38	86.48	87.05	85.33	86.15	0.85	95.0-187
MCH (pg)	28.8	29.1	29.63	28.78	29.45	0.29	25.0-59.0
MCHC (g/dl)	33.75	33.65	34.05	33.70	34.28	0.18	30.2 -36.2

SEM-Standard Error of Mean, PCV - Pack Cell Volume, RBC - Red blood Cell, WBC – White blood cell, MCV – Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCH - Mean corpuscular haemoglobin concentration.
Source of range value: (PharmD, 2024).

Table 2: Effect of the frequency of aqueous garlic administration on serum indices of broiler chicken at the starter phase

Parameters	Standard check	3 days/ week	5 days/ week	Everyday	Control	SEM	Reference Range
Glucose (Mg/dL)	82.23	90.88	86.15	92.33	87.15	4.73	105.9–200.8
Total Protein (Mg/dL)	45.80^{ab}	41.58^{c}	42.83^{bc}	46.93^{a}	45.40^{ab}	0.61	2.64–3.98
Albumin (Mg/dL)	29.65	28.88	29.88	30.35	30.70	0.33	1.09–1.98
Globulin (g/dL)	16.15^{a}	12.70^{b}	12.95^{b}	16.58^{a}	14.70^{ab}	0.53	1.55–2.00
AST (U/L)	108.38^{a}	69.10^{b}	81.15^{ab}	70.55^{b}	57.35^{b}	5.65	10–40
ALT (U/L)	29.65	24.93	27.00	25.23	21.23	1.59	9–43
ALP (U/L)	144.03^{c}	149.38^{bc}	188.08^{ab}	165.08^{bc}	225.98^{a}	8.57	92–294
Uric acid (Mmol/L)	3.81	4.27	3.61	4.78	5.15	0.21	6–30
Creatinine (Mg/Dl)	0.83	0.85	0.95	0.85	0.95	0.03	0.8-1.3

$^{a,b,c:}$ Means within the same row having different superscripts differ significantly ($P<0.05$).
SEM-Standard Error of Mean, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase.
Source of range value: (PharmD, 2024).

Impact of the Frequency of Garlic Extract Administration on the Haematological and Serum Biochemical Indices of Broiler Chickens at Finisher Phase

The haematological parameters generally showed no significant differences across treatments, except for eosinophils, mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) - Table 3.

Birds administered garlic for three days (T_2) had a significantly higher eosinophil count compared to those on ordinary water (T_5), although the count was similar to other groups. The MCV was significantly higher in birds on control compared to those on garlic for five days (T_3), though it was similar to other groups. MCHC was significantly higher at 34.13% in birds on standard check (T_1) compared to those on control.

Regarding serum biochemical parameters, the frequency of garlic administration did not lead to significant differences in most indices, except for total protein, albumin, globulin, alkaline phosphatase (ALP), and uric acid (Table 4). The total protein level was significantly higher in control birds than in those administered garlic for three days (T_2). The globulin level was also significantly higher in birds on control compared to those on 3 days/week. ALP levels were also significantly higher in birds on control compared to those in the standard check group, though similar to those in 3 days/week and 5 days/week. Uric acid levels were significantly higher in birds on control compared to those in the 3 days/week (Table 4).

Table 3: Frequency of oral administration of garlic on the haematological indices of broiler chickens at the finisher phase

Parameters	Standard check	3 days/ week	5 days/ week	Everyday	Control	SEM	Reference Range
PCV(%)	37.50	36.25	37.50	37.50	34.25	0.79	35.0 - 55.0
Haemoglobin(g/dl)	12.80	12.23	12.63	12.55	11.35	0.29	11.0 - 17.0
RBC(10^{12}/l)	4.34	4.22	4.43	4.41	3.85	0.11	2.5 - 3.5
WBC(10^{12}/l)	12.95	15.13	15.35	10.20	16.08	1.15	1.2 - 3.0
Heterophils(%)	30.00	37.50	37.25	42.25	33.50	3.44	15.0 - 50.0
Lymphocytes(%)	62.00	54.75	54.25	50.00	66.00	3.31	45.0 - 70.0
Basophils(%)	1.50	0.50	0.00	1.50	0.50	0.24	Rare
Monocytes(%)	1.50	1.25	1.00	4.00	3.25	0.49	5.0 - 10.0
Eosinophils(%)	5.00^{ab}	6.00^{a}	3.50^{ab}	2.25^{ab}	1.25^{b}	0.66	1.5 - 6.0
MCV(FI)	86.53^{ab}	85.88^{ab}	84.78^{b}	85.43^{ab}	88.95^{a}	0.59	95.0-187
MCH(pg)	29.53	28.98	28.60	28.50	29.50	0.22	25.0-59.0
MCHC(g/dl)	34.13^{a}	33.73^{ab}	33.68^{ab}	33.35^{ab}	33.10^{b}	0.14	30.2 -36.2

$^{a,b:}$ Means within the same row having different superscripts differ significantly (P<0.05).
SEM-Standard Error of Mean, PCV - Pack cell volume, RBC - Red blood cell, WBC - White blood cell, MCV - Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC - Mean corpuscular haemoglobin concentration.
Source of range value: (PharmD, 2024).

Table 4: Effect of the frequency of aqueous garlic administration on serum indices of broiler chicken at the finisher phase

Parameters	Standard check	3 days/ week	5 days/ week	Everyday	Control	SEM	Reference Range
Glucose (Mg/dL)	132.35	142.68	123.83	112.65	121.78	4.94	105.9–200.8
Total Protein (Mg/dL)	48.35^{c}	47.10^{c}	52.95^{b}	50.45^{bc}	58.70^{a}	1.08	2.64–3.98
Albumin (Mg/dL)	28.95^{c}	30.50^{bc}	32.18^{bc}	33.38^{ab}	36.75^{a}	0.76	1.09–1.98
Globulin (g/dL)	19.58^{b}	16.63^{c}	20.78^{ab}	17.08^{c}	21.95^{a}	0.54	1.55–2.00
AST (U/L)	123.40	75.10	91.10	118.15	91.60	7.67	10–40
ALT (U/L)	31.40	17.30	32.13	33.88	30.65	2.83	9–43
ALP (U/L)	145.60^{c}	152.05^{c}	156.38^{c}	188.58^{b}	217.70^{a}	6.94	92–294
Uric acid (Mmol/L)	4.28^{bc}	3.48^{c}	4.48^{b}	4.93^{b}	6.11^{a}	0.23	6–30
Creatinine (Mg/Dl)	0.80	0.95	1.05	1.00	1.18	0.05	0.8-1.3

^{abc} Means with different superscripts along the row are differently significant ($p<0.05$)
SEM-Standard Error of Mean AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline Phosphatase
Source of range value: (PharmD, 2024)

DISCUSSION

The haematological parameters of broiler chickens serve as vital indicators of their health status and the efficient use of consumed feed (Bot *et al.,* 2019). During the starter phase, the frequency of garlic extract administration did not significantly influence the haematological parameters, aligning with the findings of Jimoh *et al.,* (2012), who reported that garlic supplementation in broiler diets did not significantly affect haematological parameters. However, at the finisher phase, garlic usage significantly influenced eosinophils, MCV, and MCHC, which is consistent with the study by Bot *et al.,* (2019) that reported significant differences in haematological parameters.

In this study, the White Blood Cell (WBC) counts varied significantly across treatments and were consistently higher than the reference range, suggesting an immune-modulating effect of garlic. Higher lymphocyte counts observed in birds on standard check at the starter phase and control at the finisher phase align with findings that garlic enhances immunity by promoting lymphocyte proliferation (Amal *et al.*, 2020).

Heterophil and eosinophil percentages fluctuated with administration frequency, potentially indicating responses to stress or allergic reactions. These shifts may reflect garlic's immunomodulatory properties, which can vary depending on dosage and frequency (Kumar and Berwal, 2018). The stability of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) values across treatments demonstrates that garlic does not negatively impact red cell morphology. The MCV in this study was lower than the normal range for broiler chickens.

MCHC was significantly higher in birds given broad-spectrum antibiotics (T_1), indicating a higher concentration of haemoglobin in red blood cells compared to those in ordinary water. Lower MCHC in birds given ordinary water suggests a reduction in haemoglobin concentration, potentially signaling anaemia, iron deficiency, vitamin deficiency, or bone marrow suppression. This finding agrees with the results of Alagbe and Oluwafemi, (2019), who reported that garlic extract improved haematological parameters in broiler chicks.

The serum biochemical indices reflect the metabolic and functional impacts of garlic extract administration frequency. At the starter phase, birds on garlic extract for 5 days/week exhibited the highest levels of total protein and albumin, indicative of improved protein synthesis and liver function (Ademola *et al*., 2009). Similarly, globulin levels, which peaked in birds on 5 days/ week, suggest enhanced immune function, as globulins are critical components of antibody production (Hassan and El-Agmy, 2010). Similar findings were reported by Amouzmehr *et al.,* (2012) who noted that dietary supplementation of garlic essential oils increased total protein and albumin levels while reducing liver enzyme activities. This suggests that garlic may have a hepatoprotective effect, possibly due to its phenolic components and antioxidant properties. The glucose levels across all treatments remained lower than the reference range at the starter phase, potentially reflecting garlic's hypoglycemic effects as reported by Ali *et al.* (2000). This effect, attributed to the inhibition of hepatic glucose production by garlic bioactives, may improve energy utilization efficiency in broilers.

Garlic's effects on liver enzymes such as AST and ALT further support its hepatoprotective role. The AST levels were higher in the control group, with lower values observed in the garlic-treated groups. This aligns with studies by Issa and Omar (2012) and Amouzmehr *et al.,* (2012), who found that garlic supplementation reduced AST and ALT levels in broilers, likely due to the antioxidant properties of allicin and other bioactive substances in garlic. The consistent yet elevated ALP levels in control birds could indicate increased bone development or mild liver stress due to higher metabolic demands induced by frequent garlic administration (Kumar and Berwal, 2018). Studies by Ahmed *et al.* (2015) and Al-Kassie *et al.* (2013) have demonstrated that garlic supplementation lowered serum uric acid levels, potentially improving renal function. The nephroprotective properties of garlic were further supported by findings from Pourali *et al.* (2010), who observed reduced serum creatinine levels in garlic-fed broilers, indicating improved kidney function.

CONCLUSION

The study concluded that Garlic extract administration significantly influenced haematological and serum biochemical parameters, with effects varying across growth phases and administration frequencies. Higher frequencies (5 days/week) generally improved immune and metabolic functions, but potential stress markers such as elevated ALP and uric acid levels warrant further investigation. These results support the inclusion of garlic extract in broiler diets but emphasize the importance of high administration frequency for maximum benefits.

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