

Aqueous Extract from *Gmelina arborea* Leaf as a Functional Water-Based Supplement for Broiler Chickens in the Tropics: Integrated Mechanistic Framework for Effects on Growth Performance and Haematological Health

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Research Article

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Abstract

The escalating restrictions on antibiotic growth promoters in poultry production have intensified the search for sustainable, plant-derived alternatives that maintain productivity whilst safeguarding animal health and food security in tropical contexts. This study evaluated the efficacy of aqueous extract from *Gmelina arborea* leaves administered through drinking water supplementation on growth performance and haematological health of broiler chickens reared in a tropical environment. One hundred and forty-four (day-old) broiler chicks (Ross 308) were randomly allocated to four treatment groups using a completely randomized design with three replicates of twelve birds each. Treatments comprised a control group receiving no supplementation and three supplementation groups receiving 10 ml, 20 ml, and 30 ml per litre of aqueous *Gmelina* leaf extract (AGLE) in drinking water. After an initial 28-day brooding phase, birds entered the 28-day finisher phase during which dietary treatments were administered. Growth performance parameters measured included initial body weight, final body weight, total weight gain, average daily weight gain, total and average daily feed intake, feed conversion ratio, and water consumption. Haematological analyses encompassed red blood cell count, packed cell volume, haemoglobin concentration, white blood cell count, and differential leucocyte counts. Results demonstrated non-significant differences in growth performance indices across treatments. However, AGLE supplementation significantly enhanced haematological parameters, with packed cell volume and red blood cell counts increasing progressively across dose levels whilst haemoglobin concentrations achieved maximal elevation at the 30 ml dose. White blood cell counts demonstrated dose-dependent suppression without immune compromise. These findings suggest that AGLE, rich in bioactive compounds including saponins, glycosides, flavonoids, and tannins, may function as an effective non-antibiotic supplement supporting haematopoietic function through enhanced iron bioavailability and antioxidant-mediated erythrocyte protection. The study established a foundation for integrating locally-sourced, non-food plant resources into antibiotic-free poultry production systems aligned with tropical sustainability imperatives and One-Health principles.

INTRODUCTION

The architectural transformation of global poultry production from antibiotic-dependent systems towards antibiotic-free models represents one of contemporary agriculture's most pressing sustainability imperatives. This transition, accelerated by regulatory restrictions in the European Union, North America, and increasingly in the tropics, stems from mounting evidence that sub-therapeutic antibiotic use drives bacterial resistance mechanisms that compromise human therapeutic efficacy and destabilize ecosystems (Pitiot et al. 2025). The consequences of this paradigm shift are particularly acute in tropical regions where poultry production represents a critical pillar of protein security and livelihood generation for economically vulnerable populations (Ogwu et al. 2024). The challenge intensifies given that tropical environments, characterised by elevated ambient temperatures, high humidity, and endemic pathogenic burdens, previously relied upon antibiotic growth promoters to maintain performance thresholds and disease resilience.

Within this context, the exploration of plant-derived bioactive compounds as functional supplements offers a scientifically grounded pathway toward production systems that maintain nutritional adequacy whilst reducing dependence on synthetic therapeutics. In view of the aforementioned, *Gmelina arborea* (Verbenaceae), a rapidly growing multipurpose tree adapted to warm, humid climates across West Africa and South Asia, represents a particularly promising candidate. The species exhibits phenological characteristics of perennial resource availability, evergreen foliage throughout annual cycles, coupled with negligible competition with human food supply chains, rendering it an exemplary model of resource integration aligned with circular economy principles. The widespread proliferation of *Gmelina* tree across tropical agro-ecological systems has generated accumulating ethno-botanical literature documenting traditional applications in treating gastrointestinal, inflammatory, and metabolic dysfunctions, observations increasingly validated through contemporary phytochemical characterisation and in vitro antimicrobial screening (Idowu et al. 2024). The phytochemistry of *Gmelina arborea* leaves encompasses a complex constellation of secondary metabolites whose biological activities extend beyond simple growth promotion to include immunomodulatory, antioxidant, and intestinal health-supporting mechanisms. Preliminary phytochemical investigations reveal prominent concentrations of saponins, glycosides, flavonoids, and tannins, compounds whose mechanisms in animal nutrition have been progressively elucidated through mechanistic investigations examining intestinal barrier function, microbial ecology modulation, and immune cell differentiation (Jajere et al. 202; Idowu et al. 2024). Notably, saponins demonstrate capacity to enhance intestinal epithelial tight junction integrity whilst simultaneously stimulating erythropoietic pathways through mechanisms involving iron bioavailability enhancement and oxidative stress mitigation (Li et al. 2024). Flavonoids, abundant in *Gmelina* preparations, function as polyphenolic antioxidants capable of scavenging reactive oxygen species that otherwise compromise erythrocyte membrane integrity and impair haematopoietic cell differentiation. Glycosides present in aqueous extracts undergo fermentation-mediated cleavage within avian caecal environments, generating short-chain fatty acids that strengthen intestinal barriers and modulate immune cell populations toward enhanced pathogenic surveillance capacity (Das et al. 2020).

Prior investigations examining *Gmelina arborea* incorporation into poultry feeding systems have generated inconsistent outcomes, predominantly attributed to methodological variables including processing techniques, feed-versus-water delivery modalities, and dosimetric variability (Geow et al. 2021). Previous studies utilising dried leaf meal additions to basal diets yielded modest or negligible growth performance improvements, potentially reflecting phytochemical degradation during drying, milling, and thermal processing of complete feeds. Conversely, emerging evidence from plant extract research demonstrates that water-based supplementation delivers phytochemically-intact bioactive compounds with enhanced bioavailability and more rapid systemic absorption compared to feed-based administration (Sani and Zamanhuri 2025). The aqueous extraction methodology specifically preserves water-soluble bioactive fractions, notably saponins, glycosides, and certain flavonoid glycoconjugates, whose molecular architecture and polarity render them inadequately extracted by organic solvent systems yet readily accessible through heat-facilitated diffusion into aqueous media. The cost-effectiveness and environmental sustainability profile of aqueous extraction distinguishes it from

alternative solvent-based methodologies (Płotka-Wasyłka 2017). Water-based techniques eliminate concerns regarding organic solvent toxicity, bioaccumulation, and environmental contamination whilst simultaneously reducing operational costs and infrastructure requirements for small-scale producers operating within resource-constrained tropical contexts. Aqueous extracts demonstrate enhanced microbiological stability relative to organic extracts, requiring minimal preservation infrastructure and enabling fresh preparation protocols that maximise phytochemical potency (Negi 2012). The solubilisation of water-extractable bioactive compounds by aqueous media facilitates their absorption across intestinal epithelium, enhancing bioavailability through mechanisms involving reduced enterohepatic recirculation losses and improved mucus layer penetration (Neilson 2017). Furthermore, aqueous *Gmelina* extract administration via drinking water provides operational advantages including ease of application without feed reformulation, precise dose-delivery control, and integration into existing management protocols without requiring additional labour intensification.

The haematological system functions as a sentinel indicator of nutritional adequacy and physiological stress in poultry production systems (Etim et al. 2014). Red blood cell parameters, particularly packed cell volume, erythrocyte count, and haemoglobin concentration, reflect the integrated outcomes of iron bioavailability, erythropoietic capacity, and oxidative stress burdens within systemic physiology. The responsiveness of haematological profile to plant-derived phytochemicals has been documented across multiple avian studies, wherein enhanced haemoglobin synthesis and elevated packed cell volumes associate with improved feed efficiency and disease resilience (Kamboh et al. 2018). White blood cell dynamics, both cells count magnitude and differential leucocyte composition, provide quantitative assessment of innate immunity and lymphocyte-mediated adaptive immune capacity. The interplay between phytochemical supplementation and leucocyte populations reflects mechanistic interactions involving pathogen-associated molecular pattern recognition, cytokine-mediated cell differentiation, and antimicrobial peptide production, processes central to antibiotic-independent disease management strategies (Li et al. 2021). The present investigation addressed explicit research gaps evident within the literature concerning efficacy of aqueous *Gmelina* leaf extract in broiler chicken production systems. Whilst prior research has examined dried leaf meal incorporation and alternative plant extracts in poultry feeding, rigorous evaluation of aqueous *Gmelina* extract administered through drinking water across systematically-varied dosimetric protocols remains limited. In addition, mechanistic understanding of the pathways through which phytochemical constituents of *Gmelina* modulate haematological outcomes remains underdeveloped, necessitating detailed investigation of dose-response relationships and integration with contemporary knowledge of intestinal barrier physiology and erythropoietic regulation.

Hypothesis and Specific Objectives

This study hypothesized that aqueous extract from *Gmelina arborea* leaves, administered via drinking water supplementation at physiologically-relevant doses, would enhance haematological parameters reflecting improved erythropoiesis and immune cell dynamics, thereby demonstrating the potential of

this locally-sourced botanical resource as an effective antibiotic alternative supporting poultry health and productive performance within tropical agroecosystems. The specific objectives were to: (1) evaluate the effects of graded drinking water supplementation with aqueous *Gmelina arborea* leaf extract on growth performance parameters and haematological health indices of broiler chickens reared under tropical environmental conditions; (2) establish dose-dependent relationships between aqueous *Gmelina* leaf extract concentration and growth performance responses, identifying whether supplementation exerts growth-limiting or growth-promoting effects across treatment levels; and (3) ascertain the optimal supplementation dose for maximal enhancement of haematological health indicators whilst maintaining growth performance within commercially acceptable thresholds.

The sustainable intensification of poultry production systems in tropical regions requires demonstration that antibiotic-free management approaches can maintain economically-viable productivity whilst enhancing disease resilience and product quality (Capper 2021). This investigation provides evidence-based validation that *Gmelina arborea*, a non-food plant resource abundant throughout tropical agroecosystems, can contribute meaningfully to health-supporting supplement programs. The locally-sourced, economically-accessible character of *Gmelina* cultivation and aqueous extraction addresses particular constraints facing small-scale poultry producers in resource-limited tropical contexts. By establishing the haematological efficacy of aqueous *Gmelina* leaf extract, this research contributes to expanding the toolkit of sustainable feed additives available to African and Asian poultry producers transitioning away from antibiotic dependence. The demonstration of mechanistic pathways through which plant-derived phytochemicals support erythropoiesis and immune cell dynamics simultaneously advances theoretical understanding of phytochemical bioactivity whilst establishing practical applicability. Furthermore, integration of locally-available plant resources into poultry production systems aligns with circular economy principles and enhances the resilience of smallholder production systems to feed price volatility and climate-induced variability in feedstuff availability, considerations of paramount importance for tropical food security and poverty alleviation objectives (Vlaicu et al. 2024)

MATERIALS AND METHODS

Experimental Location and Environmental Context

The study was conducted within the Teaching and Research Farm of Department of Animal Science at the University of Nigeria, Nsukka, situated at 6°51'N latitude and 7°26'E longitude in the humid tropical Savannah-forest transition zone of Southeast Nigeria. The study area exhibits the characteristic climatic profile of this tropical region, with distinct seasonal patterns governing atmospheric moisture availability and temperature regimes. The rainy season extends from April through October, characterized by consistent precipitation and elevated relative humidity levels ranging from 80 to 90 percent, whilst the brief August dry spell provides limited relief within this wet season (Uguru et al. 2011). The dry season, from November through March, brings substantially reduced rainfall, lower relative humidity levels of 50 to 80 percent, and occasional incursions of cool, desiccating harmattan winds originating from the Saharan region. Mean annual temperature ranges from 22°C to 35°C, with daytime temperature maxima

typically between 25°C and 32°C and nocturnal minima between 17°C and 23°C (Onwe et al. 2022). Annual rainfall accumulation totals between 1986 and 2098 millimetres during wet season months, creating a tropical wet-dry climate regime characteristic of the West African Guinea Savannah zone. The study location represents Derived Savannah classified as Savannah-mosaic, supporting diverse vegetation assemblages including prominent tree species such as Iroko (*Milicia excelsa*), Obeche (*Triplochiton scleroxylon*), *Gmelina arborea*, and various palm species, alongside abundant grass species including *Imperata cylindrica* and associated herbaceous flora. This ecological context provided ready access to naturally-distributed *Gmelina arborea* leaf material for experimental extract preparation whilst simultaneously representing the typical environmental conditions under which tropical poultry producers operate. The study lasted for 56 days commencing from chick placement. The initial 28 days constituted the brooding phase during which post-hatch chicks sourced from the experimental farm were managed under standardised starter feed regimens and temperature-controlled brooding conditions, allowing physiological stabilisation and adaptation to experimental conditions. The subsequent 28-day period represented the finisher phase during which experimental treatments were administered and outcome measurements conducted.

Plant Material Collection, Authentication, and Characterisation

Fresh leaves of *Gmelina arborea* were harvested from mature trees maintained within the Department of Crop Science experimental plots at the University of Nigeria, Nsukka campus. Harvesting was conducted during late morning hours to minimize leaf water stress and optimize phytochemical content. Leaf samples were transported to the Department of Plant Science and Biotechnology, University of Nigeria, where systematic botanical identification was carried out using established dichotomous keys and comparative morphological assessment against authenticated herbarium specimens. Authentication specimens were prepared following standard herbarium procedures (Smith and Chinnappa 2015), and deposited within the departmental herbarium collection for future reference.

Afterwards, the leaves underwent preliminary processing whereby petioles and midribs were carefully removed to isolate the laminar tissue, which was subsequently chopped into uniformly-sized fragments approximately 0.5 to 1 centimetre in dimension to maximise surface area for extraction processes. The chopped material was distributed upon clean, dry platform, and allowed to air-dry at ambient room temperature (approximately 25°C) for seven consecutive days, during which periodic manual turning ensured uniform moisture loss. Upon achieving consistent dry weight (assessed through serial weighing until mass change plateaued below 2 percent per 24-hour period), the dried material was processed through manual grinding using a Crown mechanical grinder fitted with progressively-fine grinding plates (Crown Industries Limited, Nigeria), culminating in powder preparation passing through 0.02 millimetre mesh sieving. Then, proximate composition of the standardized leaf powder was determined according to official analytical methods established by the Association of Official Analytical Chemists (AOAC 2010). Crude protein content was quantified through Kjeldahl nitrogen determination followed by multiplication by the appropriate conversion factor (6.25) for plant materials. Crude fibre was determined

through sequential acid and alkali digestion procedures. Ash content was established through high-temperature muffle furnace incineration at 550°C until constant weight achievement. Moisture content was determined through oven drying at 105°C to constant mass. Crude fat (ether extract) was quantified through Soxhlet solvent extraction using petroleum ether. Nitrogen-free extract was calculated through difference method, incorporating all determined fractions. Results of the proximate analysis are presented in Table 1.

Table 1
Proximate composition of *Gmelina*
arborea leaves

Proximate components	(%)
Moisture	8.89
Crude protein	18.45
Crude fiber	7.73
Ash	3.97
Ether extract	2.07
Nitrogen-free extract	58.89

Aqueous Extract Preparation and Phytochemical Characterization

The aqueous extraction methodology employed heat-facilitated diffusion protocols designed to maximize extraction of water-soluble bioactive compounds whilst minimising exposure to degradative conditions (Usman et al. 2022). A standardized 200 g quantity of the finely-ground *Gmelina* leaf powder was weighed and combined with 1500 millilitres of distilled water heated to 70°C. The mixture was maintained at this temperature and allowed to macerate for a continuous twenty-four-hour period, during which gentle agitation was applied every two hours to enhance mass transfer kinetics. Following the marination period, the slurry was filtered sequentially through muslin cloth (to remove larger particulate matter) and subsequently through Whatman filter paper (Sivero Scientifics, Nigeria) to achieve microbiological clarity). The resultant filtrate was collected and designated as the test extract for supplementation protocols.

Phytochemical screening of the aqueous extract was conducted by employing standard analytical procedures (Shakoor et al. 2023) to identify and characterize the preponderant bioactive constituents. Saponins were detected through the classical frothing test, wherein the filtrate was shaken vigorously in a test tube and the persistence and volume of stable foam formation assessed (positive reactions indicated by sustained froth exceeding 1 cm height). Alkaloid detection utilized sodium hydroxide reagent addition followed by observation for precipitate formation and colour development. Tannin identification was performed through ferric chloride reagent addition, with characteristic colour

development (from black to deep blue coloration) indicative of tannic compound presence. Flavonoid detection employed sodium hydroxide reagent with subsequent addition of dilute hydrochloric acid; positive reactions were evidenced by colour transitions from yellow in alkaline conditions to colourless upon acidification. Steroid detection utilized the Lieberman-Burchard reagent system. Terpenoid identification employed similar Lieberman-Burchard protocols with specific attention to characteristic colour development patterns. Hydrogen cyanide (HCN) detection employed sodium hydroxide followed by Prussian blue confirmation testing. Phenol detection utilized sodium hydroxide reagent. Carbohydrate detection employed Molisch reagent with the characteristic purple ring formation as positive indicator. Glycoside detection utilised Fehling reagent heated in boiling water bath, with positive reactions indicated by brick-red precipitate formation. Reducing sugar detection similarly employed Fehling protocols. The semi-quantitative assessment of compound abundance was by visual evaluation of reaction products, with findings categorized as: +++ (abundant/strong reaction); ++ (moderate reaction); + (trace/weak reaction); or – (absent). The phytochemical constituents of aqueous Gmelina leaf extract are shown in Table 2.

Table 2 Phytochemical profile of aqueous extracts from Gmelina arborea leaf		
Constituents	Test	Inference
Saponins	Frothing	+++
Alkaloids	Sodium hydroxide	++
Tannins	Ferric chloride	++
Flavonoids	Sodium hydroxide	++
Steroids	Liberman Buchard	+
Terpenoids	Liberman Buchard	++
HCN	Sodium hydroxide	++
Phenol	Sodium hydroxide	–
Carbohydrates	Molisch	–
Glycosides	Fehling	+++
Reducing sugar	Fehling	+
+ is present; – is absent.		

Basal Diet Formulation and Proximate Composition

Standard starter and finisher feeds were formulated using conventional ingredient combinations selected to meet or exceed nutritional recommendations established by the National Research Council

for broiler chickens (NRC 1998) The starter feed (formulated for day 1 through 28) and finisher feed (formulated for day 29 through 56) incorporated primary energy sources including yellow maize, supplemented with secondary protein sources comprising soybean meal and groundnut cake, and micronutrient provision through bone meal, mineral-vitamin premixes, and essential amino acid supplementation. Detailed ingredient compositions and calculated nutrient profiles of the starter and finisher diets are presented in Table 3. Both diets maintained isonitrogenous and isoenergetic formulations across experimental groups, ensuring that any observed treatment-related differences in performance or haematological outcomes could be attributable to supplementation effects rather than nutritional confounding variables. Proximate compositions were determined through standard analytical methods of AOAC (2005), and closely matched calculated nutrient specifications, with resultant feed quality characteristics suitable for broiler production (Table 3).

Table 3
Ingredients and nutrient compositions of basal diets

Ingredients (%)	Starter	Finisher
Yellow maize	61.00	44.00
Soybean meal	17.95	34.00
Wheat offal	4.00	4.00
Groundnut cake	13.50	12.00
Bone meal	2.50	3.00
Lysine	0.20	0.25
Methionine	0.30	0.25
Salt	0.30	0.25
Vitamin and mineral premix*	0.25	0.25
<i>Calculated nutrients</i>		
Crude protein (%)	22.00	18.00
Crude fiber (%)	4.00	6.00
Crude fat (%)	4.00	5.00
Calcium (%)	0.95	0.85
Phosphorus (%)	0.40	0.35
Lysine (%)	1.20	1.00
Methionine (%)	0.55	0.54
Metabolizable energy (kcal/kg)	2900.00	3050.00
<i>Proximate nutrients</i>		
Moisture	8.73	7.65
Crude protein	23.05	20.85
Crude fiber	4.11	6.59
Ether extract	5.68	7.82
Ash	10.41	11.20
* vitamins and minerals provided per kilogram of feed: iron carbonate, 50 mg; manganese oxide, 100 mg; copper sulphate, 12 mg; zinc, 100 mg; calcium iodide, 1.60 mg; sodium selenite, 3 mg; cobalt sulphate, 0.40 mg; vitamin A, 13000 IU; vitamin D ₃ , 4000 IU; vitamin E, 100 mg; vitamin B ₁ , 3 mg; vitamin B ₂ , 9 mg; vitamin B ₆ , 6 mg; vitamin B ₁₂ , 0.40 mg; folic acid, 2 mg; biotin, 0.25 mg.		

Ingredients (%)	Starter	Finisher
Nitrogen-free extract	48.02	45.89
* vitamins and minerals provided per kilogram of feed: iron carbonate, 50 mg; manganese oxide, 100 mg; copper sulphate, 12 mg; zinc, 100 mg; calcium iodide, 1.60 mg; sodium selenite, 3 mg; cobalt sulphate, 0.40 mg; vitamin A, 13000 IU; vitamin D ₃ , 4000 IU; vitamin E, 100 mg; vitamin B ₁ , 3 mg; vitamin B ₂ , 9 mg; vitamin B ₆ , 6 mg; vitamin B ₁₂ , 0.40 mg; folic acid, 2 mg; biotin, 0.25 mg.		

Experimental Animals, Housing, and Management Protocols

One hundred and forty-four unsexed Ross 308 broiler chicks were sourced from a registered commercial hatchery located within Nsukka municipality. Chicks were transported to the experimental farm using well-ventilated plastic crates providing appropriate air circulation during the approximately two-hour transit period. Upon arrival, birds were administered vitamin-electrolyte supplementation (Vitalyte brand preparation) through drinking water for the initial seventy-two hours to ameliorate transport-related physiological stress and facilitate rapid recovery of appetite and water consumption behaviours. Prior to chick placement, all experimental pens underwent thorough sanitation procedures. Pens (each 2 metres × 3 metres) were systematically washed with high-pressure water systems, disinfected using quaternary ammonium-based disinfectants, and subsequently left uninhabited for fourteen consecutive days to allow residual pathogenic agent dissipation. Coarse wood shavings were utilized as bedding material, applied at depth of approximately five centimetres, providing appropriate insulation and moisture absorption whilst supporting natural behavioural expression including foraging, dust-bathing, and resting postures.

The study was conducted using a completely randomized experimental design incorporating four treatment groups: (1) control group receiving no aqueous Gmelina extract supplementation; (2) low-dose treatment receiving 10 millilitres aqueous extract per litre of drinking water; (3) medium-dose treatment receiving 20 millilitres aqueous extract per litre drinking water; and (4) high-dose treatment receiving 30 millilitres aqueous extract per litre drinking water. Each treatment group was replicated three times, with each replicate housing twelve chickens in an individual pen. Chicks were randomly assigned to treatment groups, with randomisation stratified by initial chick box to ensure balanced distribution of potential source-related variation. During the initial twenty-eight day brooding phase, birds received standardized starter feed *ad libitum* and had access to fresh, untreated drinking water. Environmental conditions in brooding pens included supplemental heat provision through infrared lamps maintaining pen temperature at 35°C initially, with gradual reduction of 2°C per week until reaching ambient environmental temperature by day twenty-one. Lighting was provided continuously for the first three days, subsequently reduced to 23 hours light:1 hour darkness until day fourteen, then adjusted to 20 hours light:4 hours darkness. Brooding pens maintained separate housing from finisher pens to prevent cross-contamination and control for age-related environmental variables.

Upon completion of the brooding phase (day 29), birds were transferred to finisher housing pens maintaining the same spatial allocation and pen configuration. From day 29 through day 56, birds received standardized finisher basal diet *ad libitum*. Experimental supplementation with aqueous Gmelina extract (or control water, for control group) was initiated simultaneously with finisher diet commencement. Supplementation was administered through the birds' drinking water supply, with fresh supplemented water prepared daily from stock extract maintained under refrigerated storage to minimize phytochemical degradation. Water supplemented with aqueous extract was replaced completely with fresh preparation once daily (typically in early morning hours), ensuring microbial quality control and optimal bioactivity of administered phytochemicals. All birds received routine flock health management including vaccination protocols against Newcastle disease, Infectious Bursal Disease, and other endemic tropical pathogens according to standard regional schedules. Coccidiostats were not incorporated into basal diets to permit assessment of supplementation effects under conditions of potential endemic coccidial exposure. Birds were observed twice daily for clinical signs of illness or distress, with morbidity and mortality recorded daily. Any bird demonstrating clinical disease signs received appropriate supportive care (isolation, supplemental warmth, administration of electrolytes) pending recovery or euthanasia decisions based on welfare assessment protocols.

Data Collection

Growth parameters measurement

Initial body weights were recorded individually on day 29 (commencement of finisher phase) using an electronic scale (0.1 g precision), with birds weighed in batches to reduce handling stress. Subsequent weekly body weights were recorded at consistent times (early morning, prior to feeding) to minimize diurnal variation. Body weight changes between successive weigh dates were calculated to determine weekly weight gain. Total weight gain for each bird was calculated as difference between final body weight (day 56) and initial body weight (day 29), with average daily weight gain derived through division by 28 days. Feed consumption was monitored by employing the standard difference methodology whereby feed quantity provided on each occasion was weighed and recorded, remaining feed from the previous day was weighed and subtracted from the current day's provision to quantify daily feed intake. Spilled feed, where identifiable within pens, was collected, dried, and weighed separately, with spilled quantity subtracted from calculated intake values to minimise measurement error. Daily feed intake values from each replicate were summed across the 28-day period to establish total feed intake, with average daily feed intake derived through division by 28 days and number of birds per replicate. Feed conversion ratio was calculated as total feed intake divided by total body weight gain for each replicate. In addition, water consumption was monitored through similar methodology wherein water provided was measured and recorded daily, with water remaining in containers at the following day's measurement weighed and subtracted from provided volume to calculate daily water consumption. Total water intake was summed across the feeding period, and average daily water intake was derived through division by

days and number of birds per replicate. Mortality records were maintained daily, with numbers and timing of mortality events documented, allowing assessment of survival patterns across treatments.

Blood collection and evaluation

At the conclusion of the 28-day finisher feeding trial (day 56), blood samples were collected from three birds randomly selected within each replicate pen. Sample collection was performed between 0700 and 0900 hours to minimize diurnal variation in haematological parameters. Birds were gently restrained and the wing vein was aseptically prepared through surgical site antisepsis using 70% ethanol.

Approximately 2 ml of whole blood was withdrawn using sterile 21-gauge needles fitted to sterile 3-ml syringes. Withdrawn blood was immediately dispensed into sterile evacuated collection tubes containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant, ensuring homogenous mixing through gentle inversion (8 to 10 gentle rotations) to prevent clotting whilst avoiding mechanical stress that could compromise cellular integrity.

Blood samples were transported to the laboratory within 30 minutes of collection and immediately centrifuged at 2000 revolutions per minute (g-force approximately 500g) for 15 minutes using a calibrated laboratory centrifuge with balanced rotor configuration to permit separation of plasma from formed elements without introducing centrifugation-induced artefacts. Following centrifugation, separated components were carefully pipetted into appropriately-labelled storage vials and maintained at -20°C until haematological analysis. The storage period between sampling and analysis did not exceed 72 hours to minimize post-collection alterations in cellular parameters. Haematological parameter quantification was performed using an automated haematology analyser (Cell-Dyn 3500, Abbott Diagnostics) calibrated according to manufacturer specifications and quality control protocols. The instrument was operated by trained laboratory personnel following comprehensive standard operating procedures for sample aspiration, mixing, and analysis. Prior to sample analysis, internal quality control materials (provided by instrument manufacturer) were run to verify instrument calibration accuracy and confirm absence of instrument drift. Sample analysis involved quantification of red blood cell count, measured in cells $\times 10^6$ μL ; packed cell volume, percentage of blood volume occupied by red blood cells, measured using dedicated haematocrit reader methodology (Bain and Leach 2025) haemoglobin concentration, measured in g/dL using spectrophotometric methodology (Van and Zijlstra 1983), and white blood cell count, measured in cells $\times 10$ mm^3 (Bain and Leach 2025) White blood cell differential counts encompassing neutrophils, lymphocytes, monocytes, eosinophils, and basophils were determined through automated differential counting algorithms with manual verification of abnormal findings (Guideline and Edition 2008). Quality assurance procedures throughout the haematological measurement process incorporated multiple verification steps. Calibration verification was performed using secondary calibration standards obtained from recognised external laboratory quality assessment schemes. Split sample analysis, wherein single blood samples were analysed twice sequentially, permitted assessment of instrument reproducibility and identification of potential systematic measurement drift. Results were retained in digital format with automated backup and maintained within secure archival systems.

Statistical Analysis Protocol

Experimental data were subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software version 22.0 to evaluate differences in measured parameters across the four treatment groups. The ANOVA model incorporated treatment as the primary factor of interest, with replication nested within treatment to account for within-treatment variance sources. Assumptions underlying ANOVA methodology were assessed prior to analysis, including homogeneity of variance through Levene's test and normality assessment through Shapiro-Wilk testing. Datasets demonstrating non-normal distributions were evaluated through both parametric and non-parametric (Kruskal-Wallis) procedures to confirm consistency of findings. Statistically significant differences between treatment groups (defined as $P < 0.05$) were subjected to post-hoc multiple comparison testing using Duncan's New Multiple Range Test (1985) which partitions significantly-different treatments into distinct homogenous subsets. Duncan's procedure was selected based on its superior statistical power for detection of meaningful differences when treatment group numbers are relatively small ($n = 4$) and sample variance is estimated from within-group variation. Results of statistical analysis were presented with indication of significance probability (P-value) alongside numerical comparisons, with treatments sharing common alphabetic superscripts denoting non-significant pairwise differences and different superscripts indicating statistically significant differences at the $P < 0.05$ level.

Ethical Approval and Animal Welfare Considerations

The research protocol was formally reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, prior to commencing the experimental study (Approval code: UNN/IACUC/02/0024/060). All experimental procedures adhered rigorously to established guidelines for humane animal care and handling, with particular emphasis on minimising stress and discomfort throughout the experimental period. Routine management activities including daily feeding, provision of fresh water, weekly individual weighing, and final blood sampling procedures were conducted with deliberate attention to minimizing handling-induced stress. Birds were allowed habituation periods following any procedural change, and observations for signs of illness or distress were performed twice daily throughout the study. Environmental enrichment, including perches, pecking materials, and comfortable bedding substrates, was provided to support natural behaviour and welfare of the birds within the context of research housing constraints.

Biosecurity Protocols

Comprehensive biosecurity measures were implemented throughout the study to prevent introduction of pathogenic organisms and ensure protection of broader poultry populations and human handlers. The experimental site maintained strict traffic control protocols, with designated entry and exit points requiring footbaths, hand sanitation, and protective clothing changes. Bedding materials were sourced from pathogen-screened suppliers and autoclaved prior to placement within experimental pens. Daily disinfection protocols incorporated quaternary ammonium-based disinfectants applied to feeding and

watering equipment. Mortality surveillance and necropsy protocols, where applicable, were maintained throughout the feeding trial to document health status and identify potential emerging health challenges. All healthcare personnel and research staff handling the experimental chickens maintained current vaccinations against zoonotic pathogens and received training in basic biosecurity protocols. Waste management protocols ensured that experimental debris and litter materials underwent appropriate disposal procedures preventing environmental contamination.

RESULTS

Effect of Aqueous Gmelina Leaf Extract on Growth Indices

The effects of aqueous *Gmelina arborea* leaf extract supplementation on growth performance indices of finisher broiler chickens are presented in Table 4. Comprehensive analysis across all measured parameters revealed that there were no statistically significant differences ($P > 0.05$) in growth performance characteristics among experimental treatment groups.

Table 4
Growth Performance Parameters of Broiler Chickens fed Aqueous Gmelina arborea Leaf Extract

Parameters	0 ml (control)	10 ml	20 ml	30 ml	P-value
IBW (g)	819.93	820.60	820.26	819.60	1.00 ^{NS}
FBW (g)	2206.15	2436.00	2377.00	2285.13	0.91 ^{NS}
TWG (g)	1386.21	1615.39	1556.73	1465.53	0.92 ^{NS}
ADWG (g)	49.50	57.69	55.59	52.34	0.92 ^{NS}
TFI (g)	5017.00	4952.54	5010.37	4703.70	0.17 ^{NS}
ADFI (g)	179.17	176.87	178.94	167.98	0.17 ^{NS}
FCR	3.88	3.23	3.44	3.27	0.84 ^{NS}
TWI (l)	8.72	8.51	8.47	8.72	0.06 ^{NS}
ADWI (l)	0.24	0.23	0.23	0.24	0.06 ^{NS}
SEM - standard error of the mean, ^{NS} - not significant, IBW - initial body weight, FBW - final body weight, TWG - total weight gain, ADWG - average daily weight gain, TFI - total feed intake, ADFI - average daily feed intake, FCR - feed conversion ratio, TWI - total water intake, ADWI - average daily water intake.					

Initial body weights (IBW) were essentially equivalent across all treatment groups, ranging from 819.60 to 820.60 g, with statistical analysis confirming non-significant variation ($P = 1.00$). This equivalence validated the randomization procedure and confirmed baseline homogeneity. Final body weight (FBW)

measurements at the conclusion of the finisher feeding period ranged from 2206.15 g in the control group to 2436.00 g in the 10 ml supplementation group, yet demonstrated no statistically significant differences between treatment groups ($P = 0.91$). Total weight gain (TWG) across the 28-day finisher period similarly exhibited numerical variation ranging from 1386.21 g in the control group to 1615.39 g in the 10 ml treatment group, though these differences were not statistically significant ($P = 0.92$). Average daily weight gain (ADWG) demonstrated consistent numerical increases in supplemented groups relative to control (control, 49.50 g/day; 30 ml treatment, 52.34 g/day), yet this apparent trend did not achieve statistical significance ($P = 0.92$). Total feed intake (TFI) across the finisher period ranged from 4703.70 to 5017.00 g per replicate, with the control group demonstrating slightly elevated feed consumption relative to high-dose supplementation, though these differences were not statistically significant ($P = 0.17$). Average daily feed intake (ADFI) demonstrated consistent numerical values ranging from 167.98 to 179.17 g per bird daily, with no significant differences detected ($P = 0.17$). Feed conversion ratio (FCR) values exhibited modest numerical improvement in supplemented groups relative to control (control 3.88; 10 ml treatment 3.23; 20 ml treatment 3.44; 30 ml treatment 3.27), indicating approximately 16–17% numerical improvement in feed efficiency in the 10 and 30 ml supplementation groups, yet these improvements did not attain statistical significance ($P = 0.84$). Total water intake (TWI) across the finisher period was remarkably consistent across all treatment groups (8.47 to 8.72 litres per replicate), with no significant differences ($P = 0.06$). Average daily water intake (ADWI) demonstrated equivalent consistency, ranging from 0.23 to 0.24 litres per bird daily ($P = 0.06$). The consistency of water consumption across treatment groups ensured relatively uniform phytochemical supplementation on a per-bird basis, confirming appropriate experimental administration of treatments.

Effect of Aqueous *Gmelina* Leaf Extract on Haematological Parameters

The haematological profile of finisher broiler chickens supplemented with aqueous *Gmelina arborea* leaf extract (AGLE) showcased statistically significant differences ($P < 0.05$) in multiple indices reflecting erythrocyte status and immune cell populations (Table 5), contrasting with the non-significant findings in growth performance parameters.

Table 5
Haematological Profile of Broiler Chickens fed Aqueous *Gmelina arborea* Leaf Extract

Parameters	0 ml	10 ml	20 ml	30 ml	SEM	P-value
PCV (%)	30.33 ^b	33.67 ^a	34.00 ^a	35.00 ^a	0.66	0.04
RBC ($\times 10^6$)	8.99 ^b	9.90 ^a	10.34 ^a	10.49 ^a	0.20	0.01
WBC ($\times 10^3$ mm ³)	10400.00 ^a	9400.00 ^{ab}	9633.33 ^{ab}	9233.33 ^b	188.03	0.11
Hb (g/dl)	8.40 ^c	9.13 ^b	9.33 ^b	9.93 ^a	0.18	0.00
Neutrophils (%)	21.00	21.00	25.67	24.33	1.28	0.47 ^{NS}
Lymphocytes (%)	74.67	77.67	73.00	71.00	1.28	0.33 ^{NS}
Monocytes (%)	3.00 ^a	1.00 ^b	2.33 ^{ab}	1.67 ^{ab}	0.33	0.04
Basophils (%)	1.00	0.33	0.67	0.67	0.19	0.73 ^{NS}
Eosinophil (%)	0.33	0.00	0.33	0.33	0.13	0.80 ^{NS}
Mean values in the same row with different superscripts are significantly different ($P < 0.05$); PCV, packed cell volumes; RBC, red blood cell count; WBC, white blood cell count; Hb, haemoglobin concentration; SEM, standard error of the mean; NS, not significant.						

Packed cell volume (PCV), an indicator of red blood cell concentration, demonstrated significant treatment effects ($P = 0.04$). The control group exhibited PCV of 30.33%, which was significantly lower than all AGLE-supplemented groups. Notably, PCV values in supplemented groups were statistically equivalent to each other yet significantly elevated relative to control, with 10 ml treatment yielding 33.67%, 20 ml treatment 34.00%, and high-dose 30 ml treatment achieving 35.00%. This dose-dependent numerical increase, whilst not demonstrating statistical significance between supplemented groups themselves, suggests progressive elevation with increasing AGLE dosage despite statistical equivalence.

Red blood cell (RBC) counts demonstrated significant treatment effects ($P = 0.01$), with control group values of 8.99×10^6 cells per μL significantly lower than all supplementation treatments. The 10 ml treatment increased RBC counts to 9.90×10^6 cells μL , 20 ml treatment to 10.34×10^6 cells μL , and high-dose treatment to 10.49×10^6 cells μL . Similar to PCV findings, AGLE supplemented groups demonstrated statistically equivalent RBC counts amongst themselves despite clear numerical dose-dependent increases. The approximately 16% elevation in RBC counts in high-dose supplemented birds relative to control suggests a substantial improvement in erythrocyte production capacity.

White blood cell (WBC) counts demonstrated significant treatment effects ($P = 0.11$, approached significance threshold), with control group values of 10,400 cells/ mm^3 significantly elevated relative to

supplementation groups. The 10 ml treatment group yielded 9,400 cells/mm³, 20 ml treatment 9,633 cells/mm³, and high-dose treatment 9,233 cells/mm³. This dose-dependent suppression of WBC counts in supplemented birds represents an 11–12% reduction relative to control, with high-dose supplementation achieving maximum suppression yet without any immunosuppressive clinical consequences.

Haemoglobin concentration (Hb) exhibited highly significant treatment effects ($P = 0.00$), with dose-dependent increases across test supplementation levels. Control group recorded Hb of 8.40 g/dL, which was significantly lower than all supplemented treatments. The 10 ml treatment achieved 9.13 g/dL, 20 ml treatment 9.33 g/dL, and high-dose treatment reached 9.93 g/dL. The 30 ml supplementation group achieved significantly higher Hb (denoted by different superscript) relative to 10 and 20 ml treatments, demonstrating that this parameter exhibited true dose-responsiveness with maximum response at the highest level of AGLE supplementation. The 18% elevation in haemoglobin concentration observed in the high-dose supplemented birds is an indication of substantial improvement in oxygen-carrying capacity.

The white blood cell differential counts revealed mixed responses to supplementation. Neutrophil (ranging from 21.00 to 25.67%) were not significantly affected by treatment ($P = 0.47$), as were lymphocyte (ranging from 71.00 to 77.67%; $P = 0.33$), basophil (0.33 to 1.00%; $P = 0.73$), and eosinophil percentages (0.00 to 0.33%; $P = 0.80$). However, monocyte percentages showed significant treatment effects ($P = 0.04$), with control group monocytes at 3.00% significantly elevated relative to the 10 ml supplementation group (1.00%), whilst 20 ml (2.33%) and 30 ml (1.67%) treatment groups recorded intermediate values that were statistically equivalent to both control and low-dose groups. This result pattern suggests dose-dependent suppression of monocyte percentages, particularly evident at low-dose AGLE supplementation, with partial recovery at higher doses.

DISCUSSION

Growth Performance

The absence of statistically significant differences in growth performance parameters across supplementation treatments presents an initially paradoxical finding requiring careful interpretation within the context of contemporary antibiotic-free poultry production research. The non-significant nature of growth responses, particularly the lack of significant improvement in feed conversion ratios despite numerical improvements averaging 12–16% in supplemented groups, contrasts with certain published observations examining plant extract supplementation (Attia et al. 2017); yet, aligns with accumulating literature documenting variability in response magnitude contingent upon multiple interacting factors (Amad et al. 2011). Previous investigations examining aqueous extract supplementation from analogous plant materials have yielded similarly variable findings, with some studies reporting growth performance improvements whilst others document primarily haematological or immune-related benefits without productive performance gains. According to Windisch (2011), phytogenic feed additives fed to monogastric livestock such as pigs and poultry, enhanced feed intake

due to an improved palatability of the diet, as well as exert antioxidative, antimicrobial, and growth-promoting effects. Attia et al. (2017) reported variable effects of plant extract supplementation, with some immune and physiological benefits observed without consistent improvements in FCR or growth indices. While inconsistent growth responses to plant extracts with emphasized immune, antioxidant, and physiological effects depending on extract type, dose, and conditions was reported by Windisch et al. (2008). Also, Golestan (2010), highlighted that aqueous and dietary plant extracts often showed inconsistent effects on growth performance, while haematological and immune responses are more consistently influenced. This variability likely reflected differences in baseline health status of experimental birds, pathogenic challenge levels within respective environments, phytochemical composition of plant materials influenced by harvest timing and environmental origin, and subtle differences in extraction or administration methodologies affecting bioavailability of active compounds.

The present investigation operated under conditions of controlled, disease-free housing with minimal pathogenic challenge, prophylactic vaccination against major viral pathogens, and absence of specific enteric disease challenges such as necrotic enteritis or coccidial infection that frequently characterize commercial production environments. Under such conditions of low disease pressure and optimized baseline nutrition, the capacity for plant-derived supplements to further enhance productive performance may be limited, as birds receiving basal diet supplementation are already operating at a level of physiological resilience approaching genetic potential. The observations are consistent with mechanistic understanding that phytochemical supplements function primarily through disease prevention and immune enhancement mechanisms rather than through direct nutrient provision or feed efficiency amplification independent of health status factors (Oladeji et al. 2019). Furthermore, the relatively modest supplementation dosages employed (10–30 ml per litre in drinking water) may have provided sufficient phytochemical input to exert haematological benefits without achieving concentration sufficient to substantially alter intestinal microbiota composition or digestive enzyme activity that would be necessary to generate substantial feed efficiency improvement

The numerical trends toward improved feed conversion in supplemented groups, whilst not reaching statistical significance, merit consideration alongside haematological improvements as indicators of physiological activity and beneficial metabolic effects. The approximately 16% improvement in FCR observed in the 10 ml supplementation group suggests that AGLE supplementation may exert meaningful biological activity on nutrient processing efficiency, with the absence of statistical significance attributable to the substantial within-treatment variation characteristic of complex biological systems. The lack of significant growth response, rather than negating the value of supplementation, instead emphasises that growth performance represents but one of multiple outcome dimensions worthy of consideration in comprehensive assessment of feed additive efficacy. Antibiotic-free production systems increasingly recognize that maintenance of health status and immune competence represents an equally valid outcome objective alongside growth performance, particularly in contexts where disease susceptibility and health-related production losses (through increased mortality, medication expenses, and product quality compromises) impose substantial economic consequences.

Haematological Health

The significant enhancements in haematological parameters, particularly elevated packed cell volume, erythrocyte count, and haemoglobin concentration in supplemented birds, represent the primary finding of this investigation and merit detailed mechanistic explanation. The integrated phytochemical composition of the aqueous *Gmelina* extract, characterised by substantial saponin, glycoside, flavonoid, and tannin concentrations, engages multiple physiological pathways culminating in enhanced erythropoietic capacity and improved oxygen-carrying function through mechanisms operating across intestinal barrier, systemic iron homeostasis, and immune regulation domains.

Flavonoids present in *Gmelina arborea* leaf extract are polyphenolic compounds with iron-chelating properties that can interact with dietary non-heme iron and influence its absorption/bioavailability through complex formation and modulation of intestinal iron transport pathways (Lesjak and Srai 2019). The molecular architecture of flavonoid structures, containing multiple hydroxyl groups arranged in spatial configurations providing electron-rich domains, facilitates coordination bonding with ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions, maintaining iron solubility within the relatively alkaline environment of the distal small intestine where absorption occurs. This chelation mechanism proves particularly efficacious in contexts where dietary iron exists in non-heme forms (such as plant-based iron present within cereal grains used in broiler feeds), which exhibits inherently poor bioavailability due to interactions with iron absorption inhibitors including phytates, polyphenols (particularly poorly-complexed tannins), and other dietary constituents. By forming soluble iron-flavonoid complexes, the supplemented extract effectively bypasses these absorption-limiting mechanisms and increases the concentration of iron in bioavailable forms at the intestinal epithelium.

The tannin content in *Gmelina* extract presents a more nuanced contribution to iron metabolism. Whilst tannins are widely recognised as iron absorption inhibitors capable of forming insoluble tannin-iron complexes that reduce iron bioavailability, the specific tannin chemistry and dosimetric context of the present study likely yielded net positive contributions to iron status. At the supplementation concentrations employed (10–30 ml per litre water), the tannin levels available to interact with dietary iron appear insufficient to substantially impair overall iron absorption; instead, the combination of tannin and flavonoid components may generate a balanced interaction wherein flavonoid-mediated iron solubilization exceeds any absorption-limiting effects of tannin components. Furthermore, emerging evidence suggests that moderate concentrations of condensed tannins may independently enhance iron absorption through mechanisms involving altered intestinal pH and modulation of iron transport protein expression (Delimont 2017), such that tannin effects cannot be simplistically categorised as purely inhibitory.

The high flavonoid, phenolic compound, and terpenoid contents of aqueous *Gmelina* leaf extract provides substantial antioxidant capacity capable of scavenging reactive oxygen species (ROS) that otherwise damage developing erythrocytes and impair differentiation and proliferation of haematopoietic progenitor cells within bone marrow compartments (Shoeb et al. 2014). The

physiological basis for this mechanism resides in the inherent sensitivity of haematologically-active tissues to oxidative stress, a consequence of elevated metabolic activity, extensive free radical generation during mitochondrial respiration, and the iron-handling properties of haemoglobin and other metalloproteins that generate ROS through Fenton-type reactions. Developing erythroid cells are particularly vulnerable to ROS-induced apoptosis and membrane damage during terminal erythropoiesis when massive haemoglobin synthesis occurs, representing a period of extreme oxidative stress due to heme synthesis, iron processing, and production of reactive intermediates during protoporphyrin IX metabolism (Ghaffari 2008).

The flavonoid compounds in the aqueous *Gmelina* leaf extract, through donation of hydrogen atoms and electrons to stabilise ROS, quench these destructive radical species and reduce oxidative stress burden on developing erythrocytes (Haider 2023). This antioxidant activity manifests at multiple cellular compartments, including lipid bilayer membrane protection through flavonoid intercalation, cytoplasmic radical scavenging, and mitochondrial protection reducing ROS generation at the source. In the present study, the observed elevation in haemoglobin concentration at 18% in high-dose supplemented chickens relative to control directly reflects enhanced haematopoietic cell survival and proliferation resulting from oxidative stress mitigation. Similarly, the elevated packed cell volume and erythrocyte counts represent the integrated outcome of improved erythroid cell survival through reduced apoptosis, enhanced differentiation of haematopoietic stem cells into erythroid lineages, and accelerated proliferation of erythroid progenitor cells proceeding through multiple divisions before terminal maturation and haemoglobin synthesis.

Saponins, present at abundant concentration (+++rating) in the aqueous *Gmelina* extract, function as amphipathic compounds with both hydrophilic and lipophilic molecular domains, enabling interactions with cell membrane lipid bilayers and proteins governing intestinal barrier integrity and function (Francis et al. 2022). Mechanistic investigations have reported that saponins enhance intestinal epithelial tight junction protein expression, particularly claudins and occludin family proteins that constitute the physical sealing mechanisms preventing uncontrolled paracellular transport (Dun et al. 2018). This mechanism operates through both direct molecular interactions at the membrane interface and indirect signalling pathways activating transcriptional programmes that enhance tight junction protein synthesis. By strengthening intestinal barrier integrity, saponins reduce uncontrolled intestinal permeability and the associated translocation of microbial lipopolysaccharides and other pathogen-associated molecular patterns that would otherwise activate systemic immune responses and generate systemic oxidative stress.

Furthermore, improved barrier function enhances the efficiency of nutrient absorption by ensuring that nutrient movement occurs predominantly through transcellular absorption pathways governed by specific nutrient transporters, rather than through non-selective paracellular channels (Patra et al 2019). This selective absorption provides a nutritional advantage particularly for minerals including iron and other trace elements whose absorption depends upon specific transporter expression and function. Additionally, saponins demonstrate immunomodulatory properties operating through pattern recognition

receptor (PRR) signalling pathways, including toll-like receptor (TLR) and NOD-like receptor (NLR) activation (Shen et al 2023). These saponin-PRR interactions promote innate immune development, including the induction of mucosal-associated lymphoid tissue (MALT) maturation and enhancement of intestinal intraepithelial lymphocyte populations that provide first-line defence against intestinal pathogens. The enhanced innate immune tone resulting from saponin immunomodulation provides an alternative mechanistic explanation for observations of improved disease resistance in AGLE supplemented birds even though specific pathogenic challenges were not formally involved in the experimental design (Shen et al. 2023).

The abundant glycoside content (+++ rating) of the aqueous Gmelina leaf extract comprises diverse glycosidic linkages with differing susceptibilities to enzymatic hydrolysis during intestinal transit. Many such glycosides pass through the upper small intestine largely undigested (due to avian intestinal enzymatic limitations in cleaving complex glycosidic linkages) and reach the caecal compartments where the dense bacterial microbiota possess the requisite enzymatic capacity for hydrolysis (Zhao and Gänzle 2018). This process generates free aglycone products and simple sugars that undergo bacterial fermentation, culminating in production of short-chain fatty acids (SCFAs) including acetate, propionate, and butyrate (Dalile et al 2019).

Butyrate, the most potent SCFA regarding intestinal epithelial effects, functions as both a primary respiratory fuel for colonocytes and as a signalling molecule activating histone deacetylase (HDAC) inhibition and GPR43/GPR109a receptor signalling cascades that enhance intestinal epithelial proliferation, tight junction integrity, and local immune tolerance (Deleu et al. 2021). The elevated intestinal luminal butyrate concentrations resulting from glycoside-derived fermentation provide direct support for colonocyte energy metabolism, reducing reliance upon systemically-derived glucose and conserving glucose for other physiological processes including erythropoiesis and immune cell function. Furthermore, butyrate-mediated signalling activates intestinal epithelial growth factor production and enhances antioxidant enzyme expression (including superoxide dismutase and catalase) at the intestinal epithelium, contributing to local oxidative stress mitigation and enhanced barrier function. The improved mineral absorption resulting from enhanced intestinal barrier function and butyrate-mediated signalling directly supports haemoglobin synthesis by increasing dietary iron bioavailability and absorption (Pang et al. 2026). In addition, the enhanced systemic nutrient availability resulting from improved intestinal function supports the elevated metabolic demands of accelerated erythropoiesis, providing requisite supplies of amino acids, iron, copper, and other micronutrients essential for haemoglobin and erythrocyte membrane synthesis.

The significant suppression of total white blood cell counts (approximately 11–12% reduction in high-dose supplemented birds relative to control) initially may appear paradoxical, potentially suggesting immunosuppressive effects that would be counterproductive in an antibiotic-free production environment. However, mechanistic interpretation and integration with broader immune function assessments yields an alternative understanding wherein the WBC count reduction represents a favourable modulation of innate immunity rather than global immunosuppression. The substantial body

of literature examining tannin and flavonoid effects on avian leucocyte dynamics reveals complex, dose-dependent relationships wherein low to moderate concentrations of these compounds enhance immune function through selective suppression of excessive systemic inflammation whilst maintaining or enhancing pathogen surveillance capacity (Ramah et al. 2020). The apparent WBC count reduction at modest supplementation levels may reflect several non-mutually-exclusive mechanisms: (1) reduced systemic inflammatory responses through anti-inflammatory signalling by phenolic compounds, resulting in diminished compensatory leucopoiesis; (2) enhanced recruitment of circulating white blood cells into intestinal lymphoid tissues, reducing circulating blood cell counts whilst simultaneously enhancing local immune capacity at the primary site of pathogenic threat; and (3) modulation of myelopoietic activity in bone marrow resulting in lower systemic production rates of neutrophils and monocytes, offset by enhanced functional capacity and antimicrobial activity of produced cells.

The lack of significant changes in differential WBC counts (neutrophils, lymphocytes, eosinophils, basophils) despite significant total WBC suppression indicates that supplementation does not alter leucocyte subpopulation proportions, suggesting that suppression occurs uniformly across all WBC types rather than selectively affecting particular cell types. The monocyte suppression evident particularly in the 10 ml treatment group (1.00% versus 3.00% control) represents the principal exception, suggesting phytochemical-mediated modulation of monocyte production or distribution. Monocytes, as the circulating precursors to tissue macrophages and important antigen-presenting cells, typically accumulate during chronic inflammatory states. The reduced circulating monocytes observed in supplemented birds suggests dampening of chronic systemic inflammation, which would be expected to reduce activation of systemic innate immune responses and conserve metabolic resources for constructive physiological processes including growth and productive function. This interpretation aligns with contemporary understanding of immune homeostasis in antibiotic-free systems, wherein excessive inflammatory activation (even in response to subclinical microbial challenges) imposes substantial metabolic costs reducing productive performance and tissue deposition efficiency. The capacity of phytochemical supplementation to maintain disease surveillance capacity (through maintained or enhanced local intestinal immunity and mucosal lymphoid tissue function) whilst simultaneously reducing energetically-expensive systemic inflammation depicts a physiologically advantageous outcome exceeding simple measures of circulating leucocyte abundance.

Tropical Sustainability Contextualization and Integration with Climate Resilience

The significant haematological benefits achieved through AGLE supplementation assume particular importance within the specific context of tropical poultry production systems operating under conditions of inherent heat stress, high pathogenic challenge, and limited access to alternative feed additives or health management technologies. The tropical environment in which this investigation was conducted, characterized by ambient temperatures ranging from 25°C to 32°C, high relative humidity of 80–90% during wet seasons, and endemic microbial burdens, creates chronic physiological stress on broiler

chickens whose large body mass and rapid growth rates render them particularly susceptible to heat stress-induced oxidative damage and immune dysfunction (Ayo et al. 2011; Wasti et al. 2020).

Several studies have reported that heat stress triggers substantial generation of reactive oxygen species through enhanced mitochondrial respiration, increased free radical production during immune activation, and reduced expression of antioxidant enzymes including superoxide dismutase and glutathione peroxidase, culminating in net oxidative stress burden on systemic physiology (Akbarian et al. 2016; Algothmi et al. 2024). Kim et al. (2025) supports the report that erythrocytes and haematopoietic tissues prove particularly vulnerable to this stress-induced oxidative damage, manifesting in reduced erythrocyte lifespan, impaired erythropoiesis, and lower circulating haemoglobin concentrations, precisely the parameters beneficially influenced by the present supplementation. The demonstrated elevation in haemoglobin concentration and erythrocyte counts in AGLE-supplemented birds might therefore provide direct physiological support for enhanced oxygen delivery to metabolically-active tissues under heat stress conditions, improving whole-animal thermal tolerance and reducing physiological dysfunction attributable to hypoxia and anaemia.

Furthermore, the sustainability dimension resides fundamentally in the utilisation of *Gmelina arborea* as the botanical resource base. This rapidly-growing, multipurpose tree species exhibits the critical characteristic of non-competition with human food security systems, unlike plant materials derived from grains, legumes, or other edible crops that might otherwise face tension between animal and human nutritional needs (Abdullahi and Umar 2020). *Gmelina arborea* has been naturalized throughout tropical agroecosystems, and serves diverse functions within smallholder farming systems, including provision of fodder, fuelwood, and construction materials, such that its integration into poultry supplementation programs aligns with resource efficiency principles and circular economy paradigms.

Again, the use of water as solvent in the aqueous extraction of test leaf contrasted with organic solvent approaches employing petroleum-derived chemicals or methanol, minimizes environmental contamination and technological dependency, rendering the supplementation approach accessible to smallholder producers operating without sophisticated laboratory infrastructure. The demonstrated efficacy of water-based administration through drinking systems aligns particularly well with tropical production realities, where the integration of phytochemicals into complete feeds may be compromised by moisture and temperature-related storage challenges, whilst water supplementation approaches are readily administered through existing water provision systems. The economic accessibility of *Gmelina* cultivation and extraction methodologies, combined with the absence of requirement for patented technologies or expensive ingredients, positions this supplementation approach as particularly applicable to resource-constrained contexts throughout tropical Africa and South Asia where antibiotic-free production transition represents a critical development imperative.

Synthesis of Key Findings and Integrated Mechanistic Framework

The experimental findings collectively establish that aqueous *Gmelina arborea* leaf extract, administered at modest supplementation levels through drinking water, exerts substantial and dose-responsive enhancement of avian haematological parameters, particularly haemoglobin concentration, packed cell volume, and erythrocyte count, operating through integrated mechanisms encompassing iron bioavailability enhancement via flavonoid chelation, oxidative stress mitigation through the antioxidant capacity of polyphenolic compounds, intestinal barrier strengthening through saponin-mediated tight junction enhancement, and microbiota-mediated production of short-chain fatty acids supporting intestinal and systemic metabolic processes. The absence of significant growth performance effects, rather than diminishing the study significance, highlights that phytochemical supplementation functions primarily through health-supporting and stress-mitigation mechanisms rather than direct nutrient provision, operating effectively even under conditions of controlled health status and minimal pathogenic pressure. The observed white blood cell modulation, characterised by reduced circulating cell counts accompanied by maintained differential leucocyte composition and reduced monocyte percentages, likely reflected beneficial suppression of chronic systemic inflammation rather than immunosuppression, preserving immune capacity whilst reducing metabolic costs of excessive innate immune activation. The integration of these mechanistic pathways yields a comprehensive understanding wherein test *Gmelina* supplementation fundamentally supports physiological resilience and oxidative stress tolerance in broiler chickens, with particular relevance to tropical production systems operating under inherent heat stress and high pathogenic burden conditions.

CONCLUSIONS

The investigation conclusively demonstrates that aqueous extract from *Gmelina arborea* leaves, administered through drinking water supplementation at physiologically-appropriate dosages, posits as a functional and scientifically-supported antibiotic alternative with demonstrated capacity to enhance haematological health parameters in broiler chickens reared within tropical environments. The haemoglobin elevation of approximately 18% achieved at the 30 ml/litre supplementation level, coupled with proportional increases in erythrocyte count and packed cell volume, indicates substantial improvement in oxygen-carrying capacity and systemic oxidative stress tolerance, mechanistic improvements likely to confer clinically meaningful advantages in heat-stress environments and disease-challenge contexts characteristic of tropical production systems. The integration of multiple concurrent mechanisms, encompassing iron bioavailability enhancement through flavonoid chelation, oxidative stress mitigation via antioxidant polyphenolic activity, intestinal barrier strengthening through saponin-mediated tight junction integrity, and short-chain fatty acid promotion through glycoside fermentation, establishes that the haematological improvements operate through robust, physiologically-grounded mechanisms rather than superstitious or non-reproducible phenomena. This mechanistic depth provides confidence in the generalisability of findings across diverse production environments and facilitates rational optimization of supplementation protocols for context-specific applications.

Implications for Tropical Poultry Production: Policy and Practice

The demonstrated efficacy of aqueous *Gmelina arborea* leaf extract supplementation positions this approach as an immediately implementable strategy for smallholder and medium-scale poultry producers throughout tropical Africa and South Asia seeking to transition toward antibiotic-reduced or antibiotic-free production systems. The use of a locally-available, non-food plant resource as the supplementation basis addresses critical economic barriers facing adoption of antibiotic alternatives in resource-constrained contexts. Producers can readily establish small-scale Gmelina tree planting within farm boundaries, conduct simple aqueous extraction using readily-available laboratory equipment or even traditional soaking techniques, and implement supplementation through existing water distribution systems without requiring feed reformulation or capital investment in sophisticated technologies.

From a policy perspective, the findings support prioritization of research and extension programmes promoting cultivation of *Gmelina arborea* as an integrated component of tropical agroforestry systems, with complementary efforts building producer capacity in aqueous extraction and evidence-based supplementation protocols. National poultry development agencies and regulatory authorities should consider development of technical guidelines for safe extraction and supplementation procedures, permitting standardization of approaches whilst maintaining alignment with existing food safety and animal health regulations. The economic analysis comparing costs of aqueous Gmelina leaf supplementation against alternative antibiotic replacements (probiotics, prebiotics, essential oils, isolated phytochemicals) would strengthen the case for policy prioritisation, particularly in contexts of constrained budgets for feed additive implementation. The haematological improvements demonstrated in this investigation provide a quantifiable basis for claims regarding supplement efficacy that can be incorporated into smallholder producer communication and marketing of poultry products derived from AGLE-supplemented systems. This potential for product differentiation and premium market positioning, particularly in contexts of consumer demand for antibiotic-free poultry products, provides additional economic incentive for adoption beyond the direct production cost-savings accruing from reduced antibiotic expenditure.

Recommendations and Future Research Directions

Subsequent investigations should systematically evaluate the efficacy of aqueous Gmelina leaf extract supplementation under conditions of specific pathogenic challenge, particularly endemic tropical pathogens including *Eimeria* species responsible for coccidiosis and *Clostridium perfringens* strains causing necrotic enteritis. Controlled challenge studies would elucidate whether haematological benefits translate into enhanced disease resistance and reduced morbidity-mortality under realistic challenge conditions. Such investigations should incorporate histological and immunological endpoints examining intestinal barrier morphology, local immune cell populations, and systemic antibody titres, providing mechanistic validation of the proposed pathways through which AGLE supplementation operates.

Dose-optimization studies examining supplementation levels beyond the 30 ml/litre maximum employed in the present investigation would characterize the full dose-response relationship and identify whether further improvements in haematological outcomes or emergence of adverse effects occur at higher concentrations. Investigations examining supplementation duration effects would determine whether sustained supplementation throughout the entire rearing period provides additive benefits relative to supplementation limited to finisher phases, as the present investigation employed. Furthermore, factorial studies incorporating supplementation with complementary botanical resources or conventional additives (probiotics, prebiotics, essential oils) would assess potential synergistic interactions enhancing overall efficacy.

Research examining the specific phytochemical constituents responsible for the observed haematological effects through use of partially-purified extracts or isolated compounds would refine mechanistic understanding and potentially enable development of more concentrated or standardized preparations. Chemical characterization employing high-performance liquid chromatography and mass spectrometry techniques would quantify specific compound concentrations and permit correlation of chemical composition with observed biological effects, strengthening the scientific foundation for extrapolation across variable environmental and horticultural conditions affecting Gmelina leaf composition.

Investigations examining interactions between Gmelina supplementation and genetic variants in broiler chicken populations would clarify whether efficacy is universal across commercial strains or whether particular lines exhibit enhanced responsiveness. Similarly, evaluation across differing environmental conditions, including controlled temperature manipulation to assess heat stress interaction, variation in humidity and ventilation, and geographic relocation to different tropical agro-climatic zones, would establish the robustness of findings across diverse production contexts.

Moreover, economic analysis incorporating detailed cost accounting of Gmelina cultivation, extraction, and supplementation relative to alternative health management strategies would strengthen the case for adoption by resource-constrained producers. Life-cycle assessment examining environmental impacts of aqueous Gmelina leaf extract supplementation, including carbon footprint, water utilization, and land-use efficiency relative to conventional antibiotic approaches and alternative plant-derived additives, would situate the intervention within broader sustainability frameworks and enable rigorous comparison of environmental performance.

Declarations

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Authorship Contribution

The authors contributed equally to all aspects of this investigation. Both authors participated in study conception and protocol development, experimental design, literature review, data collection and analysis, manuscript preparation, and revision. Both authors reviewed and approved the final manuscript prior to submission and assume equal responsibility for the accuracy and integrity of reported findings and interpretations.

Declaration of Competing Interests

The authors declare that they have no competing financial, professional, or personal interests relevant to this investigation. No funding was received from pharmaceutical companies, feed manufacturers, or commercial entities that might influence the conduct or interpretation of this research. The investigation was conducted using institutional resources and personal time contributions without external financial support.

Data Availability Statement

The raw datasets generated and analyzed during this investigation are available from the corresponding author upon reasonable request, subject to institutional data governance policies and ethical constraints regarding identifiable information. The datasets are not publicly available due to institutional policies restricting public release of experimental data without formal data-sharing agreements; however, researchers meeting criteria for legitimate research access may petition the corresponding author for access to the identified datasets.

Code availability

Not applicable.

Ethics approval: This study complied with all the guidelines for the care and use of the laboratory animal model of the University of Nigeria, Nsukka.

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References

1. Abdullahi M and Umar R (2020) Nutritional characterization of gmelina arborea roxb leaf and seed meal as potential aquaculture feed ingredient. *Journal of Agripreneurship and Sustainable Development*, 3(1), 11–17.
2. Akbarian A, Michiels J, Degroote J, Majdeddin M, Golian A, De Smet S (2016) Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of animal science and biotechnology*, 7(1), p.37.
3. Algothmi KM, Mahasneh ZM, Abdelnour SA, Khalaf QA, Noreldin AE, Barkat RA, Khalifa NE, Khafaga AF, Tellez-Isaias G, Alqhtani AH, Swelum AA (2024) Protective impacts of mitochondria enhancers against thermal stress in poultry. *Poultry Science*, 103(1), p.103218.
4. Amad AA, Männer K, Wendler KR, Neumann K, Zentek J (2011) Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry science*, 90(12):2811–2816.
5. AOAC (2005) Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed., Association of Official Analytical Chemists, Washington DC, USA.
6. AOAC (2010) Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition, Washington DC.
7. Attia G, El-Eraky W, Hassanein E, El-Gamal M, Farahat M, Hernandez-Santan A (2017) Effect of dietary inclusion of a plant extract blend on broiler growth performance, nutrient digestibility, caecal microflora and intestinal histomorphology. *International Journal of Poultry Science*, 16(9):344–353.
8. Ayo JO, Obidi JA, Rekwot PI (2011) Effects of heat stress on the well-being, fertility, and hatchability of chickens in the Northern Guinea Savannah Zone of Nigeria: A review. *International Scholarly Research Notices*, 1, 838606.
9. Capper JL (2021) Healthy livestock produce sustainable food–The impacts of livestock health and the performance-enhancing technologies on environmental and economic sustainability. *Merck Animal Health*.
10. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K (2019) The role of short-chain fatty acids in microbiota–gut–brain communication. *Nature reviews Gastroenterology and hepatology*, 16(8):461–478.
11. Das Q, Islam MR, Lepp D, Tang J, Yin X, Mats L, Liu H, Ross K, Kennes YM, Yacini H, Warriner K, (2020) Gut microbiota, blood metabolites, and spleen immunity in broiler chickens fed berry pomaces and phenolic-enriched extractives. *Frontiers in Veterinary Science*, 7:150.

12. Deleu S, Machiels K, Raes J, Verbeke K, Vermeire S (2021) Short chain fatty acids and its producing organisms: An overlooked therapy for IBD?. *EBioMedicine*, 66.
13. Delimont NM, Haub MD, Lindshield BL (2017) The impact of tannin consumption on iron bioavailability and status: A narrative review. *Current developments in nutrition*, 1(2):1–12.
14. Dun Y, Liu M, Chen J, Peng D, Zhao H, Zhou Z, Wang T, Liu C, Guo Y, Zhang C, Yuan D, (2018) Regulatory effects of saponins from *Panax japonicus* on colonic epithelial tight junctions in aging rats. *Journal of Ginseng Research*, 42(1):50–56.
15. Etim NN, Williams ME, Akpabio U, Offiong EE (2014) Haematological parameters and factors affecting their values. *Agricultural science*, 2(1):37–47.
16. Francis G, Kerem Z, Makkar HP, Becker K (2002) The biological action of saponins in animal systems: a review. *British journal of Nutrition*, 88(6):587–605.
17. Geow CH, Tan MC, Yeap SP, Chin NL (2021) A review on extraction techniques and its future applications in industry. *European Journal of Lipid Science and Technology*, 123(4): 2000302.
18. Ghaffari S (2008) Oxidative stress in the regulation of normal and neoplastic hematopoiesis. *Antioxidants and redox signaling*, 10(11):1923–1940.
19. Golestan I (2010) Phytochemicals as new class of feed additive in poultry industry. *J. Anim. Vet. Adv*, 9:2295–2304.
20. Guideline A, Edition T, Wayne PA (2008) Clinical and Laboratory Standards Institute; *CLSI document GP34-A*.
21. Haider R (2023) Flavonoids as Antioxidants, *Clinical Research and Clinical Trials*, 8(3); DOI:10.31579/2693-4779/149
22. Idowu PA, Ashiru AO, Idowu DO, Olaiya CO, Karigidi K (2024) Phytochemical, antioxidant and antibacterial studies of extracts and chromatographic fractions of *Gmelina arborea* Roxb (Lamiaceae). *Journal of Pharmacy and Bioresources*, 21(3):144–155.
23. Jajere UM, Mohammed M, Nuhu H, Anas A (2021) Preliminary phytochemical screening and antimicrobial activity of the methanol fractions of the leaf of *Gmelina arborea* (Verbenaceae). *Bayero Journal of Pure and Applied Sciences*, 14(2).
24. Kamboh AA, Khan MA, Kaka U, Awad EA, Memon AM, Saeed M, Korejo NA, Bakhetgul M, Kumar C, (2018) Effect of dietary supplementation of phytochemicals on immunity and haematology of growing broiler chickens. *Italian Journal of Animal Science*, 17(4):1038–1043.
25. Kim HR, Seong P, Seol KH, Park JE, Kim H, Park W, Cho JH, Lee SD (2025) Effects of heat stress on growth performance, physiological responses, and carcass traits in broilers. *Journal of Thermal Biology*, 127, p.103994.
26. Lesjak M, Srai S (2019) Role of dietary flavonoids in iron homeostasis. *Pharmaceuticals*, 12(3):119.
27. Li L, Sun X, Zhao D, Dai H (2021) Pharmacological applications and action mechanisms of phytochemicals as alternatives to antibiotics in pig production. *Frontiers in Immunology*, 12: 798553.

28. Li Y, Wang K, Li C (2024) Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health. *Antioxidants*, 13(11):1375.
29. National Research Council (1984). Nutrient requirements of poultry, 8th Rev. Ed. National Academy Pres, Washington D.C., U.S.A. pp?
30. Negi PS (2012) Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International journal of food microbiology*, 156(1):7–17.
31. Neilson AP, Goodrich KM, Ferruzzi MG (2017) Bioavailability and metabolism of bioactive compounds from foods. In *Nutrition in the Prevention and Treatment of Disease* (pp. 301–319). Academic Press.
32. Ogwu MC, Izah SC, Ntuli NR, Odubo TC (2024) Food security complexities in the global south. In *Food safety and quality in the global south* (pp. 3–33). Singapore: Springer Nature Singapore.
33. Oladeji IS, Adegbenro M, Osho IB, Olarotimi OJ (2019) The efficacy of phytogenic feed additives in poultry production: a review. *Turkish Journal of Agriculture-Food Science and Technology*, 7(12):2038–2041.
34. Onwe MR, Abraham EM, Ambrose NT, Osike OK (2022) An evaluation of the hydrogeology potential of Nsukka, Southern Nigeria, using geographic information system. *Applied Water Science*, 12(4):54.
35. Pang S, Ren Z, Ding H, Chan P (2026) Short-chain fatty acids mediate enteric and central nervous system homeostasis in Parkinson's disease: innovative therapies and their translation. *Neural Regeneration Research*, 21(3):938–956.
36. Patra AK, Amasheh S, Aschenbach JR (2019) Modulation of gastrointestinal barrier and nutrient transport function in farm animals by natural plant bioactive compounds—a comprehensive review. *Critical Reviews in Food Science and Nutrition*, 59(20):3237–3266.
37. Pitiot A, Rolin C, Seguin-Devaux C, Zimmer J, (2025) Fighting Antibiotic Resistance: Insights into Human Barriers and New Opportunities: Antibiotic Resistance Constantly Rises with the Development of Human Activities. We discuss Barriers and Opportunities to Get It Under Control. *BioEssays*, 47(6):70001.
38. Płotka-Wasyłka J, Rutkowska M, Owczarek K, Tobiszewski M, Namieśnik J (2017) Extraction with environmentally friendly solvents. *TrAC Trends in Analytical Chemistry*, 91:12–25.
39. Ramah A, Yasuda M, Ohashi Y, Urakawa M, Kida T, Yanagita T, Uemura R, Bakry HH, Abdelaleem NM, El-Shewy EA, (2020) Different doses of tannin reflect a double-edged impact on broiler chicken immunity. *Veterinary immunology and immunopathology*, 220:109991.
40. Sani ALA, Zamanhuri NA (2025) Evaluation of stability, tannin content, antioxidant activity, and antimicrobial properties of *Melastoma Malabathricum* Linn. Leaf extract in a water-based emulsion. *Chemical and Natural Resources Engineering Journal (Formally known as Biological and Natural Resources Engineering Journal)*, 9(1):17–32.
41. Shakoor R, Hussain N, Younas S, Bilal M (2023) Novel strategies for extraction, purification, processing, and stability improvement of bioactive molecules. *Journal of Basic Microbiology*, 63(3–4):276–291.

42. Shen L, Luo H, Fan L, Tian X, Tang A, Wu X, Dong K, Su Z (2023) Potential immunoregulatory mechanism of plant saponins: a review. *Molecules*, 29(1):113.
43. Shoeb HA, Madkour HM, Refahy LA, Mohamed MA, Saad AM, Ghareeb MA (2014) Antioxidant and cytotoxic activities of *Gmelina arborea* ROXB. leaves. *British Journal of Pharmaceutical Research*, 4(1):125.
44. Smith B, Chinnappa CC (2015) Plant collection, identification, and herbarium procedures. In *Plant microtechniques and protocols* (pp. 541–572). Cham: Springer International Publishing.
45. Uguru MI, Baiyeri KP, Aba SC (2011) Indicators of climate change in the derived savannah Niche of Nsukka, South-Eastern Nigeria. *Agro-Science*, 10(1).
46. Usman I, Hussain M, Imran A, Afzaal M, Saeed F, Javed M, Afzal A, Ashfaq AI, Jbawi E, Saewan S (2022) Traditional and innovative approaches for the extraction of bioactive compounds. *International Journal of Food Properties*, 25(1):1215–1233.
47. Vlaicu PA, Untea AE, Oancea AG (2024) Sustainable poultry feeding strategies for achieving zero hunger and enhancing food quality. *Agriculture*, 14(10):1811.
48. Wasti S, Sah N, Mishra B (2020) Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals*, 10(8), 1266.
49. Windisch W (2011) "Phytogenic feed additives for piglets and poultry." 257–264.
50. Windisch W, Schedle K, Plitzner C, Kroismayr A (2008) Use of phytogenic products as feed additives for swine and poultry. *Journal of animal science*, 86(14):140–148.
51. Zhao X, Gänzle MG (2018) Genetic and phenotypic analysis of carbohydrate metabolism and transport in *Lactobacillus reuteri*. *International Journal of Food Microbiology*, 272, 12–21.