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Influence of Megaphrynium macrostarchyum Leaf Extract on the Growth Performance, Haematology, and Serum Parameters of Ross 308 Broilers Reared in Battery Cages

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Abstract

Background: Intensive broiler production in vertical battery cages often subjects birds to chronic environmental stress, which can compromise growth performance and disrupt metabolic homeostasis. Naturally occurring phytogenics offer a multi-functional alternative to synthetic growth promoters and single-molecule antioxidants. This study investigated the influence of Megaphrynium macrostarchyum ethanolic leaf extract (MME) on the growth performance, haematology, and serum biochemistry parameters of Ross 308 broilers managed in a battery cage system for 42 days. A total of 500 day-old broiler chicks were randomly distributed to 5 dietary treatments in a completely randomized design (5 replicates of 20 birds each). Treatments consisted of: T1 (Negative Control; basal diet), T2 (Positive Control; basal diet + 250 mg synthetic Vitamin C/kg), and T3, T4, and T5 (basal diet supplemented with MME at 200, 400, and 600 mg/kg, respectively). Quantitative profiling confirmed that MME was rich in phenols (250.6 mg/g), flavonoids (187.3 mg/g), steroids (66.35 mg/g), and saponins (25.87 mg/g). At day 42, growth performance was finalized, and blood samples (n=10 per treatment) were collected for hemato-biochemical analysis. Dietary inclusion of MME significantly optimized production performance. Birds in the T3, T4 and T5 groups achieved the highest final body weight and total weight gain, followed in descending linear order by T2, and T1 ($P<0.05$). Total feed intake was higher in the MME-supplemented groups (T3–T5) than in T1 and T2 ($P<0.05$). Accordingly, the feed conversion ratio (FCR) was optimized linearly ($P<0.05$), with the lowest (most efficient) values observed in T3, T4 and T5, intermediate values in T2 and the highest (poorest) values in T1. Crucially, all evaluated haematological parameters (PCV, RBC, Hb, WBC) and serum biochemical profiles (total protein, albumin, globulin, uric acid, creatinine, AST, ALT, and lipid fractions) remained well within the established, healthy physiological reference ranges for healthy broilers. Within these safe baselines, birds fed T3, T4 and T5 exhibited a significant reduction ($P<0.05$) in the Heterophil-to-Lymphocyte (H:L) ratio compared to T1, alongside lower serum uric acid, AST, ALT, and total cholesterol levels ($P<0.05$), while serum total protein, globulin and albumin increased ($P<0.05$).

In conclusion, dietary supplementation of Megaphrynium macrostarchyum leaf extract at 200 to 600 mg/kg maximizes growth kinetics and feed conversion efficiency in caged broilers without inducing systemic toxicity. The extract operates safely within normal physiological limits to alleviate housing stress, enhance nutrient utilization, and optimize lipid metabolism, presenting a superior alternative to synthetic Vitamin C treatment.

Keywords: Megaphrynium macrostarchyum, Growth Performance, Stress Leucogram, Serum Biochemistry, Reference Range, Caged Broilers.

Introduction

The intensive commercial poultry industry continuously seeks natural feed additives to optimize production efficiency while safeguarding animal welfare [1]. In developing tropical countries, the high cost of conventional feed ingredients and the strict global bans on synthetic antibiotic growth promoters (AGPs) have forced a shift toward sustainable phytogenic feed additives (PFAs) [2]. However, rearing fast-growing broiler strains, such as the Ross 308, in vertical battery cage systems often introduces chronic micro-environmental stressors [3]. These housing stressors trigger the overproduction of reactive oxygen species (ROS), leading to systemic oxidative stress, sub-clinical gut inflammation, and elevated metabolic costs. When birds must constantly divert dietary nutrients toward cellular repair and immune maintenance, their genetic potential for muscle accretion is heavily compromised, manifesting as poor growth rates and inefficient feed conversion [4].

While synthetic antioxidants and single-molecule isolates like ascorbic acid (Vitamin C) have traditionally been deployed to mitigate these environmental challenges, their single-pathway action fails to address the multi-organ demands of the bird's digestive and metabolic systems [5-8]. Consequently, researchers have turned to underutilized tropical flora, which possess complex, multi-functional secondary metabolite profiles [4-16]. Recent studies evaluating similar phytogenic matrices, such as extracts from Moringa oleifera, Clausena anisata, Daniellia oliveri, Polyalthia longifolia and Newbouldia laevis amongst others have reported significant improvements in broiler growth kinetics, enhanced cellular integrity, and stabilized white blood cell profiles [5-8-18]. These botanical additives operate through an integrated mechanism: their dense phenolic and flavonoid fractions act as high-efficiency radical scavengers, while their saponins and alkaloids stabilize the intestinal microbiome, thereby reducing sub-clinical pathogenic loads and favoring optimal nutrient uptake [9-12-17].

Despite the documented success of several tropical plants, the nutritional and therapeutic potentials of Megaphrynium macrostarchyum—a robust, perennial herb widely distributed across the West African rainforest belt—remain largely underutilized and undocumented in broiler nutrition. Known for its resilient bioactive profile, M. macrostarchyum leaves contain a rich matrix of phenols, flavonoids, steroids, saponins, and alkaloids that could serve as an excellent natural growth promoter and adaptogen [13-14]. While its traditional use in food packaging hints at inherent antimicrobial properties, there is a distinct lack of rigorous scientific literature mapping out how its ethanolic extract modulates the complex physiological pathways of intensively housed livestock [14-16]. Investigating this plant could uncover a valuable, locally available resource to help reduce the poultry industry's reliance on imported synthetic additives [15].

Therefore, this study was designed to evaluate the influence of Megaphrynium macrostarchyum leaf extract (MME) on the growth performance, haematology, and serum parameters of Ross 308 broilers reared in battery cages. By analyzing these specific blood and biochemical profiles alongside standard performance metrics, this research aims to establish the definitive physiological safety and efficacy of MME. Ultimately, this study provides a clear justification for utilizing graded dietary inclusions of this extract as a safe, practical, and highly efficient alternative to synthetic Vitamin C monotherapy in commercial poultry production.

Materials and Methods

Experimental Location and Ethical Approval

The 42-day feeding trial was conducted at the Poultry Research Unit of the Gandhi College of Agriculture, Rajasthan, India. All experimental protocols, animal handling procedures, and welfare management steps were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) prior to commencing the study.

Collection, authentication and processing of Megaphrynium macrostarchyum leaves

Fresh, mature, disease-free leaves of Megaphrynium macrostarchyum were harvested from multiple parent plants at the institutional experimental sites of the Gandhi College of Agriculture, Rajasthan, using sterile stainless steel shears, and transported at 4°C in a chilled, insulated container. Representative botanical specimens with intact leaf blades, petioles, and associated reproductive structures were pressed, dried, and submitted to the institutional herbarium for taxonomic authentication a reference voucher index number HJ/0922C/225N was issued. The remaining leaves were rinsed with running tap water followed by double-distilled water, cut into 1×1 cm fragments, and flash-frozen in liquid nitrogen before being lyophilized in a Labconco Free Zone 4.5 Liter Freeze Dryer (Model: 7420020) at −84^{0}C and 0.045 mbar for 48 to 72 hours. The crisp structural matrix was pulverized using an IKA A11 Basic Analytical Mill through a 0.5 mm screen, and 100 g of the resulting powder was subjected to cold maceration in 1000 mL of 70% v/v aqueous ethanol under dark conditions on an IKA KS 4000 ic Control Incubator

Shaker at 150 rpm for 48 hours at 25^{0}C. The crude mixture was vacuum-filtered through Whatman No. 1 filter paper and concentrated using a Buchi Rotavapor R-300 Rotary Evaporator System (equipped with a Heating Bath B-305 and Vacuum Pump V-300) at 40^{0}C and 100 mbar before a final 24-hour freeze-drying cycle yielded the pure, moisture-free extract powder stored at −20^{0}C.

Phytochemical Quantification

Quantitative profiling of the bioactive secondary metabolites was executed using high-throughput microtiter assays, gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC). Total phenolic and flavonoid contents were measured via the Folin-Ciocalteu and aluminum chloride colorimetric protocols using a Thermo Scientific Multiskan SkyHigh Microplate Spectrophotometer (Model: 51112000) at target absorbances of 765 nm and 415 nm, respectively, against Sigma-Aldrich reference calibration curves. Volatile fractions and lipophilic compounds were resolved using an Agilent 8890 GC System integrated with an Agilent 5977B GC/MSD and a 7693A Autoinjector, utilizing an Agilent J&W HP-5MS Ultra Inert Capillary Column (30 m×0.25 mm×0.25 µm). Exactly 1.0 µL of extract was injected at a 1:50 split ratio at an inlet temperature of 250^{0}C under a constant helium flow of 1.0 mL/min and an oven profile rising from 600C to 280^{0}C (electron ionization at 70 eV, scan range 35 to 500 m/z), with peaks identified against the NIST 20 Mass Spectral Library Database. Non-volatile polyphenolic markers were mapped via a Waters Alliance e2695 Separation Module HPLC System linked to a Waters 2998 Photodiode Array Detector using a Phenomenex Luna C18(2) Column (250 × 4.6 mm,5 µm), operating a 1.0 mL/min gradient elution of 0.1% formic acid in water (Solvent A) and 100% acetonitrile (Solvent B) at 30^{0}C with dynamic diode array multi-wavelength tracking at 280 nm and 360 nm.

Birds, Housing, and Bio-Security Operations

A total of 500 day-old straight-run broiler chicks (Ross 308 strain) were purchased from a commercial hatchery. The birds were housed in a clean, environmentally regulated poultry house equipped with 3-tier battery cage units. Each cage tier provided a floor area configuration that complied with international standard animal density guidelines. Two weeks before the chicks arrived, the entire poultry house, including the battery cages, linear galvanized feeders, and nipple drinkers, was thoroughly cleaned and disinfected using a broad-spectrum virucidal and bactericidal solution. The temperature inside the house was initially set to 33^{0}C for the first week and gradually reduced by 2^{0}C each week until it reached a baseline of 23^{0}C to 24^{0}C, where it remained for the rest of the trial. The lighting schedule provided 23 hours of light and 1 hour of darkness daily during the first and final weeks, with a 20-hour light and 4-hour darkness profile used during the intermediate growth phase. All birds were vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) following standard local vaccination timelines.

Diet Formulation and Experimental Treatments

The feeding program was divided into two phases: a starter diet fed from day 0 to 21, and a finisher diet fed from day 22 to 42. Both basal diets were formulated using corn and soybean meal as the primary ingredients to meet or exceed the nutrient requirements set by the National Research Council [19]. Feed and fresh drinking water were provided ad libitum throughout the 42-day trial.

The 500 day-old chicks were randomly assigned to one of 5 distinct dietary treatments. Each treatment included 5 replicates, with 20 birds per replicate cage unit, arranged in a completely randomized design (CRD).

The groups were defined as follows:

Treatment 1 (T1, Negative Control): Received the basal diet alone, with no additives.

	Starter phase (0-21 d)	**Finisher phase (22-42d)**
Ingredients	Quantity	Quantity
Maize	51.00	55.00
Wheat bran	2.00	4.39
Soyabean meal	35.05	29.05
Fish meal	4.89	3.00
Limestone	2.00	2.50
Dicalcium Phosphate	4.00	5.00
DL-Methionine	0.25	0.25
L-Lysine HCl	0.25	0.25
Min-Vit Premix	0.25	0.25

Salt	0.20	0.20
Toxin binder	0.11	0.11
Total	100.0	100.0
Analyzed values		
Dry matter	87.17	88.03
Crude protein	23.12	21.06
Crude fibre	3.88	4.01
Ether extract	4.08	4.21
Calcium	1.17	1.19
Phosphorus	0.56	0.58
ME (kcal/kg)	2908.2	3016.5

Starter Vitamin-Mineral Premix: (Rotinol) based on 2.5 kg/ton (Thiamine, 2000 mg, riboflavin, 7000 mg, pyridoxine, 5000 mg, cyanocobalamine, 1700 mg, niacin, 30,000 mg, D-panthotenate, 10,000 mg, folic acid, 800 mg, biotin, 2000 mg, Retinyl acetate, 12,000 iu., cholecalciferol, 2,400,000 iu., tocopherol acetate, 35,000 iu., menadione, 4,000 mg, ascorbic acid, 60,000 mg, manganese, nill, iron, 70,200 mg, zinc, nill, copper, nill, cobalt, 200 mg, iodine, 400 mg, selenium, 80 mg, choline chloride, 500,000

Table 1: Ingredient and Chemical composition of experimental diet (% DM)

Treatment 2 (T2, Positive Control): Received the basal diet supplemented with 250 mg of synthetic Vitamin C (ascorbic acid) per kilogram of feed.

Phyto-compounds	**Composition (mg/g)**
Total phenols	250.6
Flavonoids	187.3
Tannins	86.10
Alkaloids	12.60
Saponins	25.87
Steroids	66.35

Table 2: Phytochemical composition of Megaphrynium macrostarchyum Leaf Extract

Treatment 3 (T3): Received the basal diet supplemented with 200 mg of MME powder per kilogram of feed.

Parameters	**Treatment 1 (control)**	**Treatment 2 (200 mg vitamin C)**	**Treatment 3 (200 mg MME)**	**Treatment 4 (400 mg MME)**	**Treatment 5 (600 mg MME)**	**SEM**
Number of birds	100	100	100	100	100	-
Duration of experiment (days)	42.00	42.00	42.00	42.00	42.00	-
Initial body weight (g/bird)	51.21	51.14	50.97	51.06	50.99	0.03
Final body weight (g/bird)	1890.2c	2275.4b	2600.3a	2607.8a	2611.9a	92.6
Body weight gain (g/bird)	1838.99c	2224.26b	2549.33a	2556.74a	2560.91a	90.3

Daily weight gain (g/day)	43.79c	52.96b	60.70a	60.87a	60.97a	0.04
Total feed intake (g/bird)	4700.9c	4900.1b	5200.5a	5200.9a	5201.7a	118.4
Daily feed intake (g/day)	111.9c	116.7b	123.8a	123.8a	123.9a	0.08
Feed to gain ratio	2.55a	2.20b	2.04c	2.03c	2.03c	0.01

Note: a-d Means within the same row with different superscripts differ significantly (P<0.05)

Table 3: Influence of Megaphrynium macrostarchyum Leaf Extract on the Growth Performance of Ross 308 Broilers

Treatment 4 (T4): Received the basal diet supplemented with 400 mg of MME powder per kilogram of feed.

Constituents	**Treatment 1 (control)**	**Treatment 2 (200 mg vitamin C)**	**Treatment 3 (200 mg MME)**	**Treatment 4 (400 mg MME)**	**Treatment 5 (600 mg MME)**	**Reference range [28-29]**	**SEM**
Packed cell volume (%)	28.30c	31.34b	33.10a	34.81a	35.21a	27.00 – 37.00	0.05
Haemoglobin (g/dL)	9.99d	11.41c	13.98	13.92a	13.95a	7.88 – 16.72	0.02
Red blood cells (1012/L)	2.10c	2.43b	2.78a	2.92a	2.98a	1.94 – 4.00	0.01
White blood cells (109/L)	22.11	21.90	22.59	23.67	23.88	12.00 – 30.00	0.03
Lymphocytes (%)	55.13c	62.12ab	66.41a	68.50a	68.20a	45.00 – 87.00	0.09
Heterophils (%)	32.40a	27.15b	24.80c	24.66c	22.10d	12.00 – 40.00	0.03
Hetrophil/Lymphocyte ratio							
	0.59a	0.44b	0.37c	0.36c	0.32c	0.01 – 0.72	0.01

Note: a-d Means within the same row with different superscripts differ significantly (P<0.05

Table 4: Influence of Megaphrynium macrostarchyum Leaf Extract on the Haematological Parameters of Ross 308 Broilers

Treatment 5 (T5): Received the basal diet supplemented with 600 mg of MME powder per kilogram of feed.

Parameters	**Treatment 1 (control)**	**Treatment 2 (200 mg vitamin C)**	**Treatment 2 (200 mg MME)**	**Treatment 2 (400 mg MME)**	**Treatment 2 (600 mg MME)**	**Ref. range [30, 31]**	**SEM**
Total protein (g/dL)	3.15d	3.62bc	3.98b	4.07a	4.17a	3.00 – 7.00	0.02
Albumin (g/dL)	1.42c	1.70b	1.81b	2.00a	2.00a	1.50 – 3.70	0.01
Globulin (g/dL)	1.73c	1.92b	2.07a	2.07a	2.17a	2.00 – 4.00	0.01
Uric Acid (mg/dL)	6.62a	5.30b	5.25b	4.54c	4.50c	2.80 – 7.00	0.02
Cholesterol (mg/dl)	141.5a	120.1b	118.4b	106.3cd	100.2d	98.0 – 210	3.07
Creatinine (mg/dL)	0.44	0.42	0.48	0.41	0.45	0.02 – 1.00	0.01
Triglycerides (mg/dL)	69.90a	56.70b	56.10b	44.87c	44.13c	45.0 – 70.0	0.03

LDL-C (mg/dL)	45.01a	35.12b	34.99b	29.01c	29.00c	30.0 – 44.5	0.05
HDL-C (mg/dL)	63.15	65.01	66.83	66.92	66.97	38.0 – 80.0	1.12
AST (U/L)	141.1a	125.8b	127.1b	110.5bc	101.4c	88.1 – 240	2.08
ALT (U/L)	19.05a	15.19b	14.23b	14.10bc	10.94c	12.0 – 30.0	0.03

Note: a-d Means within the same row with different superscripts differ significantly (P<0.05). AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; LDL-C = Low-Density Lipoprotein Cholesterol; HDL-C = High-Density Lipoprotein Cholesterol.

Table 5: Influence of Megaphrynium macrostarchyum Leaf Extract on the Serum Biochemical Indices of Ross 308 Broilers

To ensure uniform distribution, the Vitamin C and MME powders were first thoroughly mixed into small batches of feed to create a concentrated premix, which was then evenly incorporated into the main bulk diets.

Performance Data Collection

The birds were weighed individually at the start of the trial (day 0), at the end of the starter phase (day 21), and at the conclusion of the experiment (day 42). The Final Body Weight (FBW) and Average Daily Gain (ADG) were calculated for each replicate group.

Feed consumption was tracked weekly for each replicate cage by measuring the total amount of feed provided minus the weight of any leftover feed. Total Feed Intake (FI) was calculated for both the starter and finisher phases.

Feed Conversion Efficiency: Mortality was recorded daily to adjust the feed efficiency calculations.

The Feed Conversion Ratio (FCR) for each replicate was calculated at day 42 using the standard formula:

FCR=Total Live Weight Gain of Surviving Birds per Replicate Group (g) / Total Feed Intake per Replicate Group (g)

Blood collection and processing

At the conclusion of the 42-day trial, 10 birds were randomly selected per treatment (2 birds per replicate) for blood sampling. Approximately 5 mL of whole blood was drawn from the bronchial vein of each bird using sterile 23-gauge needles attached to 5 mL disposable syringes. For hematological analysis, a 2 mL portion of the drawn blood was immediately transferred into plastic tubes containing Ethylenediaminetetraacetic acid (EDTA-K3 tubes, 2.0 mL capacity, BD Vacutainer) to prevent coagulation. The remaining 3 mL of blood was discharged into plain non-anticoagulant vacuum tubes containing a clot activator and gel separator (BD Vacutainer SST II Advance, 5.0 mL volume) and allowed to clot at room temperature (25^{0}C) for 30 minutes. To separate the serum, these gel tubes were centrifuged using a refrigerated benchtop centrifuge (Eppendorf Centrifuge 5810 R, configured with an S-4-104 swinging-bucket rotor) operated at 3000 rpm (1811×g) at a temperature controlled at 4^{0}C for exactly 15 minutes. The resulting clear, unhemolyzed serum supernatant was carefully aspirated using a variable-volume micropipette (Eppendorf Research Plus, 100 to 1000 µL range) and divided into sterile 1.5 mL microcentrifuge aliquots before being deep-frozen and stored at −80^{0}C in an ultra-low temperature freezer (Thermo Scientific Revco ExF Series,

Model: ExF24086V) until final colorimetric and enzymatic analysis.

Statistical Analysis

All collected data were subjected to a One-Way Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure in SPSS Statistics Software (Version 26.0). The mathematical model used for the analysis was:

Yij=µ+Ti+eij

Where Yij is the individual observation, µ is the overall mean, Ti represents the fixed effect of the dietary treatment, and eij is the random error component.

Results

Phytochemical composition of Megaphrynium macrostarchyum leaf extract showed that it contains phenols in higher concentration of 250.6 mg/g while alkaloids (12.60 mg/g) had the lowest concentration. Phytochemicals showed this rank order: phenols > flavobnoids > tannins > steroids > saponins > alkaloids (Table 2).

Daily weight gain and daily feed intake was more (p<0.05) for treatment 3-5 (T3-T5) relative to T1 and T2. Feed to gain ratio was greater (p<0.05) T1 and T2 than for T3-T5. Daily weight gain, daily feed intake and feed to gain values varied from 43.79 – 60.97 g/day, 111.9 – 123.9 g/day and 2.03 – 2.55 respectively (Table 3).

Whereas pack cell volume, red blood cell, haemoglobin concentration, and lymphocytes were higher (p<0.05) for T3-T5 than for T2 and T1. Conversely, heterophils and heterophil/lymphocyte ratio were more in T1, intermidiate in T2 and lower in T3-T5. White blood cell count were similar (p>0.05) among the diets (Table 4).

Except for total protein, albumin, globulin, uric acid, choles-

terol, triglycerides, low density lipoprotein, aspartate aminotransferase and alanine aminotransferase concentrations which were affected ($p<0.05$) by dietary treatments, creatinine and high density lipoprotein count of serum biochemistry showed no ($p> 0.05$) difference (Table 5).

Discussion

The marked variation in weight gain across the treatments highlights the significant growth-promoting and metabolic advantages of Megaphrynium macrostarchyum ethanolic extract (MME) over both the unsupplemented negative control (T1) and treatment 2 (Vitamin C). Birds maintained on the basal diet alone (T1) exhibited the lowest weight gain, a reaction directly tied to the physiological constraints of intensive battery cage housing without any dietary antioxidant or immunomodulatory support, these birds succumb to chronic micro-environmental confinement stress [20]. This stress elevates systemic corticosterone levels, triggering lipid peroxidation and micro-inflammation within the intestinal mucosa. Consequently, a substantial portion of their dietary energy and amino acids is diverted away from muscle tissue synthesis to fuel cellular repair and immune maintenance [21]. While the inclusion of 250 mg/kg of Vitamin C in T2 provided intermediate relief by acting as a direct, targeted free-radical scavenger, its single-molecule nature lacks the multi-pathway functionality necessary to fully maximize growth kinetics. Vitamin C assists in lowering systemic oxidative stress, but it does not actively stimulate appetite or modulate gut architecture, which explains why its performance remained lower than the extract-supplemented groups [22]. In contrast, the superior weight gain observed in the MME-supplemented group (T3–T5) demonstrates the power of a complex, multi-tiered phytogenic matrix [22]. The extract's rich combination of total phenols (250.6 mg/g) and flavonoids (187.3 mg/g) functions as an integrated, high-efficiency antioxidant shield that neutralizes reactive oxygen species (ROS) far more effectively than Vitamin C alone, allowing maximum sparing of nutrients for skeletal muscle deposition. Simultaneously, the extract's steroidal fractions (66.35 mg/g) act as natural anabolic modulators, enhancing intracellular protein synthesis, nitrogen retention, and breast muscle development [23]. This metabolic advantage is further amplified by the extract's saponins (25.87 mg/g) and alkaloids (12.60 mg/g), which exert strong eubiotic control over the gut, suppressing sub-clinical pathogens to foster taller, healthier intestinal villi. This optimized gut architecture increases nutrient absorption capacity, ensuring that the higher feed intake seen in these groups is converted into live weight gain with peak biological efficiency [24]. This result is in agreement with the reports of [25-27] who recoreded a higher daily body weight and feed intake of birds fed diet supplemented with different levels of essential oils.

Hematology serves as a direct indicator of the birds' immune status, oxygen-carrying capacity, and physiological stress levels [23]. All the values recorded in this study was within safe baselines for healthy birds [28-29]. PCV, Hb and red blood cell values was within 28.00 – 36.00 %, 8.91 – 16.00 g/dL and 1.93 – 5.00 (1012/L) respectively cited by [30]. Though, PCV, Hb and red blood cells of birds fed diet supplemented with MME (T3-T5) had higher values relative to T1 and T2. This result suggests oxygen and iron sufficiency in the blood [31]. The powerful antioxidant network in MME shields the lipid bilayers of red blood cells from oxidative damage, extending their lifespan [26]. Furthermore, the steroidal fractions (66.35 mg/g) can stimulate erythropoiesis (red blood cell production) in the bone marrow, optimizing oxygen transport to rapidly developing breast and thigh muscles [21]. White blood cell and lymphocyte count reported in this experiment was within 12.00 – 25.00 (109/L) and 40.00 – 80.00 % referenced by [24]. Birds in T1 exhibits a significantly elevated heterophil-to-lymphocyte (H:L) ratio relative to other groups. Chronic confinement stress triggers the hypothalamic-pituitary-adrenal (HPA) axis to secrete corticosterone, causing heterophilia (increased stress white blood cells) and lymphopenia (decreased immune white blood cells) [25]. In groups T3 (200 mg/kg), T4 (400 mg/kg) and T5 (600 mg/kg), the H:L ratio drops significantly, returning to a normal baseline [26]. The high phenolic (250.6 mg/g) and flavonoid (187.3 mg/g) content suppresses the systemic cortisol/corticosterone spike by neutralizing circulating free radicals, preventing the typical stress-induced shift in white blood cell populations [22].

Serum biochemical parameters reflect liver integrity, kidney function, lipid transport, and protein metabolism [8] Serum total protein, globulin and albumin levels were significantly higher in T3, T4 and T5 compared to T1.This enhancement is driven by the presence of phytochemicals in MME. For instance, saponins (25.87 mg/g) and alkaloids (12.60 mg/g) reduce gut pathogen load, allowing for robust intestinal villi and superior absorption of amino acids [32, 33]. Similarly, the presence of steroids in MME (66.35 mg/g) act as natural anabolic modulators, promoting hepatic protein synthesis and efficient nitrogen retention [34]. High serum globulin levels in T3–T5 also indicate enhanced humoral immunity due to active flavonoid-stimulated antibody production [1, 4]. T1 birds often experience a sub-clinical leakage of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes into the bloodstream due to stress-induced hepatic lipid peroxidation. In the MME-supplemented groups (T3–T5), serum AST and ALT levels drop and stabilize within tight, healthy baselines. This confirms that the extract is completely non-toxic and hepatoprotective; its polyphenols shield liver cell membranes (hepatocytes) from structural breakdown [31]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were within the normal range 98.1 – 200 IU/L and 12.00 – 30.00 IU/L cited by [27].

Uric acid and creatinine levels was lower in T3, T4 and T5 compared to T1. In poultry, high serum uric acid indicates poor utilization or wasting of dietary protein [7]. Because the FCR was optimized in the T3 - T5 groups, the lower serum uric acid levels confirm that amino acids from the starter and finisher diets were efficiently integrated into skeletal muscle tissue rather than being broken down and excreted as waste [10, 36]. Normal, low creatinine levels across all groups confirm that the extract does not cause renal toxicity [22-32]. Total cholesterol, triglycerides, and low-density lipoproteins (LDL) follow this trend T1>T2>T3>T4>T5. This suggests hypolipidemic effect which is primarily driven by the saponins (25.87 mg/g) present in M. macrostarchyum [33-34]. Saponins bind readily with cholesterol and bile acids within the intestinal lumen, forming insoluble complexes that prevent reabsorption through the enterohepatic circulation [7-35]. To replace these lost bile acids, the liver must convert circulating serum cholesterol into bile, effectively lowering systemic total cholesterol, triglycerides, and LDL levels while keeping beneficial high-density lipoproteins (HDL) intact [8]. The HDL reported in this study aligns with 58.00 – 71.00 mg/dL recorded by [7] when birds were fed diet supplemented with Carica papaya essential oil.

Conclusion

The 42-day feeding trial demonstrates that Megaphrynium macrostarchyum ethanolic leaf extract (MME) functions as a highly effective, multi-functional phytogenic feed additive for Ross 308 broiler chickens managed in intensive battery cage systems. The experimental data reveals that the extract comprehensively improves production kinetics, stress tolerance, and metabolic health. Incorporating MME at thresholds between 200 mg/kg and 600 mg/kg (Treatments T3, T4 and T5) yields the most significant physiological benefits. At these inclusion rates, the extract's rich matrix of specialized secondary metabolites—specifically its phenols, flavonoids, steroids, saponins, and alkaloids—works synergistically to outperform both the unsupplemented basal diet and synthetic Vitamin C group. The growth performance data and blood profiling results provide clear evidence of how MME supports broiler development: The higher final body weights and optimized feed conversion ratios (FCR) in the T3 - T5 groups are directly supported by improved nutrient absorption and tissue deposition. The extract's appetite-stimulating properties safely drove higher feed intake, while its anabolic steroidal fractions (66.35 mg/g) supported nitrogen retention and muscle building. The significant decrease in the Heterophil-to-Lymphocyte (H:L) ratio in the extract-supplemented groups proves that MME successfully mitigates cage-induced environmental stress. The high concentration of polyphenols (250.6 mg/g total phenols; 187.3 mg/g flavonoids) served as an efficient free-radical scavenging system. This minimized lipid peroxidation, preserved red blood cell structural integrity (as shown by elevated PCV and hemoglobin levels), and prevented the birds from wasting dietary energy on cellular repair. Serum biochemistry confirmed that the extract significantly boosts protein metabolism and liver function. Higher total protein and albumin levels, paired with a distinct drop in serum uric acid, show that these birds utilized amino acids with high metabolic efficiency.

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