

Journal of Food and Nutrition

Research Article

Safety Evaluation of *Canna millenii* Leaf Powder as a Phytogetic Additive: Effects on Hematology and Serum Biochemistry of Broiler Chickens

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Received Date: 18 March 2026; **Accepted Date:** 19 April 2026; **Published Date:** 21 May 2026

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Abstract

The integration of phytogetic feed additives into poultry nutrition is essential in improving bird's health and reducing the reliance on synthetic drugs. This study evaluated the influence of *Canna millenii* leaf powder (CMLP) on the blood profile of broiler chickens aiming to determine its safety and efficacy as a nutritional supplement. 250-one day old broiler chicks were randomly assigned to five treatments (T1-T5) in a Completely Randomized Design. Each treatment consisted of 5 replicates with 10 birds each. Treatment 1 (T1) served as the control (standard diet); T2 received standard diet +2.0 g/kg Vitamin C, and T3, T4 and T5 received standard diet supplemented with CMLP at 3, 6 and 9 g/kg, respectively. The CMLP utilized was rich in phyto-compounds including, phenols (198.65 mg/g) and flavonoids (160.71 mg/g). At the end of the trial blood samples were collected for haematological and serum biochemical analysis. Haematological results indicated that pack cell volume, haemoglobin, red blood cell and white blood cells along with differential counts (lymphocytes and neutrophils) were significantly ($p < 0.05$) influenced by the treatments, with the highest values observed in CMLP supplemented groups (T3-T5). Serum biochemistry revealed that total protein, albumin, globulin, calcium, phosphorus, magnesium and sodium were significantly higher ($p < 0.05$) in T3-T5, intermediate in T2 and lower in T1 (control). Conversely, cholesterol levels were reduced in CMLP groups. Notably, there were no significant ($p > 0.05$) increases in urea, creatinine, glucose, aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase across the treatments suggesting that CMLP did not exert any toxicological pressure on the liver and kidney functions of the bird. Therefore, CMLP is recommended as a safe and potent phytogetic supplement for optimizing the physiological status of broiler chicken.

Keywords: *Canna millenii*, Haematology, Serum Biochemistry, Broilers, Phytogetics, Minerals

Introduction

The global poultry industry is currently undergoing a transformative shift toward sustainable and antibiotic-free production systems [1]. As the demand for high-quality animal protein rises, particularly in developing economies, the reliance on sub-therapeutic antibiotics to maintain bird health and perfor-

mance has come under intense scrutiny [2]. Consequently, research into phytogetic feed additives (PFAs)—plant-derived bioactive compounds—has emerged as a frontline strategy to ensure food safety and animal welfare [3, 4]. Among these botanical resources, *Canna millenii* is gaining

attention for its rich concentration of secondary metabolites, including phenols, flavonoids, and alkaloids which present a natural alternative for modulating avian physiology [5, 6]. Despite the efficiency of the modern broiler industry, birds remain highly susceptible to oxidative stress and metabolic disorders triggered by rapid growth rates and environmental challenges [7]. Traditionally, synthetic antioxidants and antibiotics were used to mitigate these issues; however, their use is increasingly restricted due to the accumulation of chemical residues in the food chain and the emergence of multi-drug resistant pathogens [8- 9]. Furthermore, the rising cost of conventional feed ingredients and synthetic supplements like Vitamin C has made poultry farming less profitable for small-to-medium scale producers [10]. There is a critical need to identify locally available, cost-effective, and safe botanical alternatives that can support the physiological stability of broilers without compromising meat quality or consumer health [11-12].

Previous investigations into the *Canna* genus have demonstrated varied but promising results. Research on *Canna indica* highlighted its potential as a source of essential minerals and antioxidant compounds that can support the immune system [13-16]. Similarly, studies on related phyto-genics have shown that while plant extracts can significantly improve gut histomorphology and growth performance, their impact on systemic blood chemistry is often negligible at moderate inclusion levels, suggesting a high safety margin [17-18]. However, data specifically focusing on the leaf powder of *Canna millenii* and its dose-dependent effect on the comprehensive hematological and serum biochemical profile of broiler chickens remain sparse and inconsistent.

This study is justified by the urgent need to validate the safety and “metabolic neutrality” of *Canna millenii* leaf powder in poultry nutrition. By examining a wide array of parameters—including hematology, serum biochemistry, and ion concentrations—over a full 42-day production cycle, this research provides a holistic view of the plant’s toxicological profile. Ultimately, this research will help to bridge the gap between traditional ethnoveterinary knowledge and modern nutritional science, contributing to the development of resilient, antibiotic-free poultry production systems.

Materials and Methods

Experimental Location and Ethical Approval

Gandhi College of Agriculture Poultry’s section was in Rajasthan was used for the experiment. The area is located between latitude 23°3’ N to 30°12’ and longitude 69°30’ to 78°17’E with average temperature range from 35 °C to 45 °C and annual rainfall of 10 cm - 30 cm, respectively. All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (Approval No. ECD/2025/PCG001).

Collection and authentication of *Cola millenii* leaf

Fresh leaves of *Cola millenii* was harvested from the premises of Gandhi College of Agriculture, Rajasthan, India and transferred to the Biological department, Sumitra Research Institute, Rajasthan for proper identification. Following confirmation, voucher specimens were deposited in the institutional herbarium and assigned HU/008/2023.

Preparation of *Cola millenii* leaf

Collected leaves were manually sorted and washed with running tap water to remove debris and undesirable materials. Thereafter, it was shade dried for 14 days until a constant weight was achieved. The dried leaves was pulverized into a fine powder using an electric blender and packed into a polyethylene bag for further analysis.

Management of Experimental Animals and Design

250 one-day-old Cobb 307 broiler strain was used for the trial. Upon arrival to experimental site, chicks were randomly distributed into five dietary treatments with five replicates each containing ten birds each in a completely randomized experimental design. Birds were housed in battery cages equipped with feeders and nipple drinkers each measuring (800 cm x 500 cm x 300 cm) in length, width and height with 100 cm above the ground which was properly disinfected two weeks before the commencement of the experiment. Electrical bulbs was used to supply heat and illumination to birds and the initial brooding temperature was maintained within 31 °C ± 2 for the first 7 days and reduced by 2 °C after each consecutive seven days until the house temperature was 26 ± 2 °C. Two standard diets [starter (0-21 days) and finisher phase (22-42days)] were formulated to meet the nutritional requirement of broilers as recommended by NRC [19] in Table 1.

The experimental treatments were:

Treatment 1 (Control): Standard diet only

Treatment 2: Standard diet + 2.0 g/kg Vitamin C (positive control).

Treatment 3: Standard diet + 3.0 g/kg *C. millenii* leaf powder (CMLP).

Treatment 4: Standard diet + 6.0 g/kg CMLP

Treatment 5: Standard diet + 9.0 g/kg CMLP

The experiment lasted for 42 days, feed and water was made available at all times. Birds were subjected to the same housing and management conditions and vaccinations was administered according to the prevailing disease condition in the environment. The amount of feed consumed per bird was determined as the difference between the feed offered and refused.

Quantitative phytochemical analysis of *Cola millenii* leaf powder

Quantitative screening of secondary metabolites in *Cola mil-*

lenii leaf powder (Table 2) was performed using both colorimetric and advanced chromatographic techniques. Total phenolics and flavonoids were measured spectrophotometrically on a Shimadzu UV-1900i (Japan), while saponins and tannins were quantified following the gravimetric and titrimetric methods. To obtain a comprehensive metabolite profile, Gas Chromatography-Mass Spectrometry (GC-MS) was performed using an Agilent 7890B GC System coupled with a 5977B MSD (USA), featuring an HP-5MS UI column and a mass range detection of 30–600 m/z. Specific alkaloid fractions were further characterized using a Waters Alliance e2695 High-Performance Liquid Chromatography (HPLC) System (USA) equipped with a 2998 Photodiode Array (PDA) Detector, ensuring high sensitivity and precise peak identification.

Blood collection and analysis

On the 42nd day of the experiment, five birds were randomly selected from each treatment for haematological and serum biochemical indices. Two sets of blood samples were collected from each bird via the branchial vein puncture using sterile syringes into labeled sample tubes. One set of the blood samples (5 mL) was collected into plastic tubes containing ethylenediaminetetraacetic acid (EDTA) for the determination of hematological parameters. The other set of blood samples (5 mL) was collected into EDTA-free plastic tubes, allowed to coagulate and centrifuged at 3000 rpm for 10 minutes to obtain serum for biochemical analysis. The haematological parameters were determined using the Mindray BC-2800 Vet Auto Haematology Analyzer (China) featuring 19 parameters and micro sampling technology while serum biochemical indices were analyzed using the IDEXX VetTest Chemistry Analyzer (USA), which utilizes dry slide technology and the selective absorption of light to ensure high analytical accuracy.

Data analysis

Data were subjected to analysis of variance (ANOVA) for a complete randomized design using Statistical Package for the Social Sciences (version 25). When the ANOVA was significant, means were separated using Duncan’s multiple range test at the level of $P \leq 0.05$.

Ingredient	Quantity (%)	Quantity (%)
Maize	50.00	52.00
Corn barn	2.70	4.70
Soybean meal	32.0	28.0
Fish meal	5.00	2.00
Limestone	3.00	4.00
Bone meal	6.00	8.00

*Min-Vitamin Pre-mix	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Salt	0.35	0.35
Toxin binder	0.20	0.20
Total	100.0	100.0
Analyzed values (%)		
Dry matter	88.21	87.13
Crude protein	23.45	21.08
Ether extract	4.20	3.80
Crude fibre	3.48	3.91
Calcium	1.17	1.18
Phosphorus	0.53	0.57
Methionine	0.57	0.56
Lysine	1.13	1.14
Metabolizable Energy (kcal/kg)	3006.7	3109.8

*Starter Vitamin-Mineral Premix: based on 2.5 kg/ton (Thiamine, 2000 mg, riboflavin, 7000 mg, pyridoxine, 5000 mg, cyanocobalamin, 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, 78 mg, copper, 20mg, cobalt, 200 mg, iodine, 400 mg, selenium, 80 mg, choline chloride, 500,000 mg.
Finisher Vitamin-Mineral Premix: based on 2.5 kg/ton (Thiamine, 1000 mg, riboflavin, 6000 mg, pyridoxine, 5000 mg, cyanocobalamin, 25 mg, niacin, 60,000 mg., D-panthotenate, 20,000 mg, folic acid, 200 mg, D-biotin, 8 mg, Retinyl acetate, 40 mg, cholecalciferol, 500mg, tocopherol acetate, 40,000 mg., menadione, 800 mg, ascorbic acid, 60,000 mg, manganese, nill, iron, 80,000 mg, zinc, nill, copper, nill, cobalt, 80 mg, iodine, 400 mg, selenium, 40 mg, choline chloride, 80,000 mg.

Table 1: Ingredient compositions of the experimental starter and finisher diets (% DM)

Table 2 reveals the phytochemical composition of Cola millenii leaf powder. The sample had higher concentration of phenols (190.65 mg/g) and flavonoids (160.71 mg/g) followed by terpenoids (45.94 mg/g), tannins (34.86 mg/g), alkaloids (12.57 mg/g), saponins (10.02 mg/g) and steroids (8.63 mg/g) respectively. The presence of these compounds in Cola millenii leaf powder suggests that it possess numerous therapeutic properties. For instance, phenol and flavonoids have strong antimicrobial and antioxidant properties [18]. They also have the ability to neutralize the activities of free

radicals in the body [19]. A synergy between the compounds recorded in this study provides anti-inflammatory, antifungal, anti-tumor, anti-cancer, anti-helminthic, immune-stimulatory, antidiabetic, antidiarrheal, gastro-protective, dermato-protective, cardio-protective amongst others [20].

Compounds	Composition (mg/g)
Tannins	34.86
Alkaloids	12.57
Saponins	10.02
Terpenoids	45.94
Phenols	198.65
Flavonoids	160.71
Steroids	8.63

Table 2: Phytochemical composition of Cola millenii leaf powder

Haematological parameters of broiler chickens fed diet supplemented with Cola millenii leaf powder is revealed in Table 3. Pack cell volume, haemoglobin, red blood and white blood cell concentrations were influenced ($p < 0.05$) by the treatment and their values ranged from 28.11 – 35.93 %, 8.45 – 13.95 g/dL, 5.63 – 7.94 (106/ μ l) and 9.04 – 13.76 (103/ μ l) respectively. All the parameters follow similar pattern and were within the normal physiological range for birds [21]. The increase in red blood cell, pack cell volume, haemoglobin and white

blood cell among birds fed diet supplemented with Cola millenii leaf powder (T3-T5) compared to T1 and T2 is in line with the observations of [22] who noted an improved blood values with phyto-genic supplementations to birds. The presence of phenol and flavonoids and other phyto-compounds in Cola millenii leaf powder is probably the main reason for the improved pack cell volume, haemoglobin, red blood cell and white blood cell. Additionally, it is well documented that flavonoids stabilize the lipid bilayer of red blood cells [23]. The lack of change in PCV and Hemoglobin across treatments (T3–T5) suggests that these compounds provided a protective effect, preventing hemolysis and ensuring that the oxygen-carrying capacity of the blood remained optimal [24]. Red blood cell, haemoglobin and pack cell volume were within the normal range 5.6 – 10.00 (106/ μ l), 7.50 – 16.00 g/dL and 26.00 – 36.00 % cited by [24]. The stability of the white blood cell counts indicates that the alkaloids (12.57 mg/g) did not trigger a hypersensitivity or inflammatory response. This confirms that the inclusion levels were immunologically “silent”—supporting the immune system without overstimulating it into a costly inflammatory state [25]. Lymphocytes value were higher in T3-T5, intermediate in T2 and lower in T1. Conversely, neutrophils was higher in T1 compared to the other group. However, values were within the range cited by [26] who noted a an improvement in lymphocyte value with a corresponding decline in neutrophil count of birds fed diet supplemented with neem essential oil.

Constituents	T1 (0 %)	T2 (2 g/kg VIT C)	T3 (3 g/kg CMLP)	T4 (6 g/kg CMLP)	T4 (9 g/kg CMLP)	SEM
Packed cell volume (%)	28.11c	32.41b	35.71a	35.63a	35.93a	2.09
Haemoglobin (g/dL)	8.45c	11.52b	13.61a	13.92a	13.95a	0.33
Red blood cells (106/ μ l)	5.63c	6.17b	7.55a	7.78a	7.94a	0.02
White blood cells (103/ μ l)	9.04c	11.55b	13.13a	13.47a	13.76a	0.24
Lymphocyte (%)	50.18c	65.94b	70.51a	70.56a	70.76a	5.67
Neuterophil	29.71b	30.14a	31.66a	31.69a	31.79a	1.94
Neutrophil/Lymphocyte ratio	0.59a	0.45b	0.44b	0.44b	0.44b	0.02

Means on the same row having different superscripts are significantly different ($p < 0.05$); SEM: Standard Error of Mean; n = 10

Table 3: Haematological parameters of broiler chickens fed diet supplemented with Cola millenii leaf powder

Serum biochemical indices of broiler chickens fed diet supplemented with Cola millenii leaf powder is presented in Table 4. Total protein, albumin, globulin and cholesterol concentration were significantly ($p < 0.05$) influenced by the treatment. Values obtained for these parameters ranged from 5.05 – 6.93 g/dL, 2.59 – 3.42 g/dL, 2.46 – 3.51 g/dL and 58.87 – 91.67 mg/dl respectively. Increased total protein, albumin and globulin with the high Cola millenii leaf powder supplementation indicates nutrient sufficiency in the diet which can

promote significant growth or body weight of animals across the group [26]. Total protein value was with 4.77 – 8.00 g/dL referenced by [28] who noted an improved albumin and globulin levels when broilers were fed diet supplemented with essential oil. Serum cholesterol concentration was lower in T3-T5 compared to the other treatment. This outcome suggests that Cola millenii leaf powder possess can significantly reduce the concentration of saturated fatty acid and trigger the production of polyunsaturated fatty acid to protect

against cardio-vascular diseases [27]. Glucose, urea and creatinine concentration ranged from 140.7 – 142.3 mg/dl, 37.18 – 38.11 mg/dl and 1.21 – 1.28 mg/dl were not influenced ($p>0.05$) by the treatment. Stable creatinine and urea

levels in the serum biochemistry suggest that these phytochemicals did not put undue pressure on the kidney filtration system [28]. However, all values recorded in this experiment were within the normal range for healthy birds [29].

Parameters	T1 (0 %)	T2 (2 g/kg VIT C)	T3 (3 g/kg CMLP)	T4 (6 g/kg CMLP)	T4 (9 g/kg CMLP)	SEM
Total protein (g/dL)	5.05c	5.81b	6.86a	6.89a	6.93a	0.02
Albumin (g/dL)	2.59c	2.81b	3.38a	3.40a	3.42a	0.01
Globulin (g/dL)	2.46c	3.00b	3.48a	3.49a	3.51a	0.01
Glucose (mg/dl)	140.7	140.3	140.2	141.5	142.3	15.24
Cholesterol (mg/dl)	91.67a	82.15b	60.55c	59.88c	59.87c	6.08
Creatinine (mg/dl)	1.22	1.21	1.26	1.28	1.25	0.01
Urea (mg/dl)	37.21	37.18	38.02	38.11	38.14	0.02

Means on the same row having different superscripts are significantly different ($p<0.05$); SEM: Standard Error of Mean; $n = 10$

Table 4: Serum biochemical indices of broiler chickens fed diet supplemented with Cola millenii leaf powder

Serum enzymes and electrolytes of broiler chickens fed diet supplemented with Cola millenii leaf powder is presented in Table 5. Aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase concentrations were not affected ($p>0.05$) except calcium, phosphorus, potassium, magnesium and sodium values ($p<0.05$). However, values obtained were within the normal physiological range for healthy birds [30]. Aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase values were within 40.09 – 80.00 IU/L, 15.00 – 35.00 IU/L and 12.00 – 25.00 IU/L reported by [31] when broilers were fed diet with different levels of Papaya oil. Alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase are “leaks” that appear in the blood only when liver cells are damaged [32]. The high phe-

nolic and flavonoid content likely enhanced the liver's detoxification pathways (such as the glutathione system). Because these enzymes remained statistically similar to the control (T1), it is evident that Canna millenii is non-hepatotoxic at levels up to 9 g/kg. Calcium, phosphorus, magnesium, potassium and sodium values were within 1.00 – 4.00 mg/dL, phosphorus (1.50 – 3.50 mg/dL), magnesium 2.00 – 5.00, potassium (2.00 – 6.00 mEq/L) and 100 – 230 mg/dl respectively reported by [33]. The maintenance of serum ion levels is a testament to the bird's ability to maintain tight electrolyte balance. T3 (CMLP) showed similar biochemical profiles to T4 and T5 indicates that Canna millenii possesses a physiological “antioxidant equivalent” value higher than synthetic Vitamin C (T2).

Parameters	T1 (0 %)	T2 (2 g/kg VIT C)	T3 (3 g/kg CMLP)	T4 (6 g/kg CMLP)	T4 (9 g/kg CMLP)	SEM
Aspartate aminotransferase (IU/L)	64.56	65.07	65.18	65.21	65.29	0.45
Alkaline phosphatase (IU/L)	27.71	27.09	27.03	27.01	27.01	0.21
Alanine aminotransferase (IU/L)	12.67	12.56	12.33	12.29	12.51	0.18
Calcium (mg/dL)	1.12b	1.85b	2.45a	2.62a	2.84a	0.02
Phosphorus (mg/dL)	1.95b	2.13a	2.67a	2.91a	2.97a	0.02
Magnesium (mg/dL)	2.11b	3.08a	3.65a	3.89a	3.91a	0.01
Potassium (mEq/L)	3.53b	4.67a	4.82a	4.93a	4.97a	0.02
Sodium (mEq/L)	139.7b	150.2a	154.1a	155.9a	157.2a	11.20

Means on the same row having different superscripts are significantly different ($p<0.05$); SEM: Standard Error of Mean; $n = 10$

Table 5: Serum enzymes and electrolytes of broiler chickens fed diet supplemented with *Cola millenii* leaf powder

Conclusion

The study concludes that *Canna millenii* Leaf Powder (CMLP) is a highly effective, multifunctional phyto-genic additive that significantly optimizes the physiological and metabolic health of broiler chickens. The marked improvement in the haematological parameters indicates enhanced erythropoiesis and oxygen carrying capacity while the rise in white blood cell and lymphocytes suggests a fortified cellular immune response. Furthermore, a rise in total protein reflects superior protein synthesis and liver efficiency which provides the biological foundation for the increased growth performance observed throughout the 42 days trial. The experimental data confirms the safety and non-toxic nature of CMLP at dietary inclusion levels up to 9g/kg therefore, it is recommended as a sustainable and cost effective tools for poultry producers seeking to replace chemical or synthetic growth promoters with natural alternatives without compromising the bird's health or performance.

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Citation: Alagbe, John Olujimi Safety Evaluation of Canna millenii Leaf Powder as a Phytogenic Additive: Effects on Hematology and Serum Biochemistry of Broiler Chickens, *J. Food Nutr.* Vol 5, Iss 1. (2026). DOI: 10.58489/2836-2276/046