

Effects of Lemongrass (*Cymbopogon Citratus*) Extract on Blood Profile of ISA Brown Layers

Mercy E. Akpudi¹, Jerome U. Unukevwere², Onyekachukwu S. Eboma³,
Peter Uvwo⁴ and Lawrence Bratte⁵

^{1,2,4,5}Animal Science Department, Delta State University, Abraka, Nigeria.

³Department of Animal Science, University of Pretoria, Hatfield South Africa.

Corresponding Author Email: stmercilov@gmail.com

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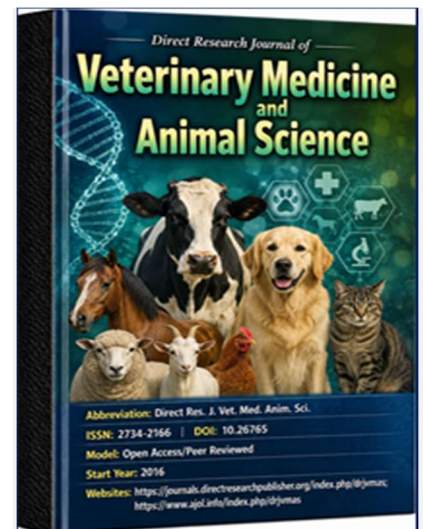
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ABSTRACT

Global restriction on antibiotic use in poultry production, driven largely by concerns over antimicrobial resistance and drug residues, has intensified the search for safe and natural, phytogetic alternatives like lemongrass. This study evaluated the effects of varied levels of aqueous lemongrass (*Cymbopogon citratus*) extract, through drinking water, on the blood profile, of ISA Brown layers. In a completely randomized design (CRD), seventy-five (75) seven-month-old layers, randomly allotted into five Treatment (T) of fifteen (15) birds each over a twelve (12) week period. T1 (control) received plain water, while T 2, 3, 4, and 5 received 50 mL, 100 mL, 150 mL, and 200 mL of lemongrass extract per litre of drinking water, respectively. Blood samples were collected after 12 weeks and analyzed following standard procedure. Data was subjected to one-way Analysis of Variance, significant means were separated using Duncan's Multiple Range Test ($p < 0.05$). Packed cell volume, haemoglobin concentration, mean corpuscular haemoglobin concentration, white blood cell count, total cholesterol, total protein, were not significant ($p > 0.05$) while, Red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, heterophils, lymphocytes, monocytes, and basophils were significantly ($p < 0.05$) influenced by lemongrass extract supplementation across treatments, reflecting an effect of the extract on the immune system of the hens. Serum glucose, albumin, and plasma protein concentrations were also significantly ($p < 0.05$) affected, with glucose increasing progressively to a peak of 77.38 mg/dl in T4, while albumin and plasma protein declined with increasing extract concentration. Results demonstrate that lemongrass extract exerts a dose-dependent effect on the physiological and productive performance of ISA Brown, with moderate inclusion levels consistently producing the most favourable haematological and biochemical outcomes. Overall, lemongrass extract was found to be a safe and beneficial phytogetic that can serve as a viable natural alternative to antibiotic growth promoters in ISA Brown layer.



Keywords: phytogetic, lemongrass, ISA Brown layers, haematology, Serum biochemical indices

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INTRODUCTION

Poultry producers have traditionally used synthetic antibiotics as growth promoters, for disease prevention and treatment. Although, these drugs can improve feed efficiency and productivity, their prolonged and extensive use contributes to antimicrobial resistance and drug residues, creating health risks for both animal and human (Agu and Olabode, 2024; De Mesquita *et al.*, 2022). Resistant bacteria other organisms and drug residues may spread from poultry to people through the food chain, while antibiotic residues in meat and eggs can pose health risks to consumers (Azizi *et al.*, 2024; Mund *et al.*, 2017), and those released in poultry litter and wastewater may also affect the ecosystem (Abd El Hack *et al.*, 2022). Restrictions or ban on antibiotic growth promoters, have therefore increased interest in sustainable phyto-genic alternatives that support poultry health without contributing to resistance or residue concerns (Aminullah *et al.*, 2025). Lemongrass (*Cymbopogon citratus*) is a tropical herb which has essential medicinal uses. Its bioactive constituents include citral, which has demonstrated biological activity. Several Studies of poultry supplementation suggest that lemongrass can support blood and immune status. These effects may be linked to the antioxidant activity of citral, flavonoids, and phenolic compounds, which can protect cells from oxidative damage (Ali and Abdel Zalmi, 2022). Lemongrass supplementation has also been associated with improved growth, carcass quality, and biochemical and immunological indices in poultry (Alagawany *et al.*, 2021). Despite these reported benefits, empirical evidence on lemongrass extract on blood indices, and productivity of ISA Brown layers remains limited. Maintaining bird health and efficient production in this breed requires assessment of both biological outcomes and production performance. This study therefore evaluates the effects of lemongrass extract on ISA Brown layers, focusing on blood parameters. By examining these outcomes, the study aims to contribute evidence on whether lemongrass extract can serve as a more sustainable practical phyto-genic supplement for layer production..

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Research and Demonstration farm of Delta State University, Abraka, Delta State Nigeria.

Experimental Design

The study adopted a completely randomized design (CRD), in which 75 layers were divided into five groups each having 15 hens, consisting of 3 replicates, for 12 weeks. The lemongrass extract was administered in doses (mL) of “0, 50, 100, 150, and 200” representing

T1 to T5, respectively.

Preparation of the Test Ingredient

Fresh lemongrass leaves were harvested and washed thoroughly and air-dried at room temperature; 25–30°C, to a constant weight. Aqueous extract was prepared by boiling 100g of air-dried lemongrass per Litre of distilled water for 30 minutes (100g/L-30mins). The mixture was allowed to cool, filtered using a muslin cloth, and stored at 4°C. The extract was freshly prepared daily to maintain potency and was administered daily to the hens via drinking water.

Data Collection

Blood Collection

To determine the blood and biochemical characteristics of the birds at the end of 12weeks trial, one bird was selected at random from each replicate (3 birds per treatment) from which 5mL of blood sample was collected into through the wing vein and released into two containers. About 2.5 mL into tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) used as an anti-coagulant. The remaining 2.5 mL was released into a plain sterile tubes for serum biochemical analysis according to the procedure of Baker and Silverton (1998). Haematological analysis of Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Haemoglobin concentration and Packed Cell Volume (PCV) were determined by the method described by Jain (1986). The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated, respectively. Serum biochemical indices: The blood samples in plain sterile containers for serum were allowed to clot before centrifuge at 3000 rpm for 15mins in a micro-centrifuge to obtain serum that is free from cell debris. After this, separated sera were decanted into another sterile tubes which was used determine serum metabolites such as: total serum protein, albumin, glucose and total cholesterol, according to the enzymatic colorimetric method described by Schmidt and Schmidt (1963) using a commercial diagnostic reagent kits (Randox Laboratories Ltd., UK).

Data Analysis

A one-way Analysis of Variance (ANOVA) was performed on each response variable Where the ANOVA yielded a significant F-ratio ($p < 0.05$), Duncan's multiple range test was applied as a post-hoc procedure to separate the treatment means.

RESULTS

Result of haematology shows that there were significant difference ($p < 0.05$) among the means of PCV, RBC, MCV,

Table 1: Effect of Lemongrass Extract on Blood Indices of ISA Brown Layers.

Parameters	T1	T2	T3	T4	T5	F value	P value	SEM
PCV(%)	35.00	31.00	31.00	34.66	32.00	1.00	0.44	0.87
Hb(g/dl)	11.80	10.43	10.55	12.00	11.10	1.33	0.32	0.28
RBC($\times 10^{12}/L$)	3.76 ^a	2.07 ^c	2.27 ^c	3.26 ^b	3.57 ^a	14.01	0.00	0.20
WBC($\times 10^9/L$)	255.67	116.80	355.83	23.07	206.75	0.65	0.68	67.12
MCV(fl)	93.73 ^c	149.92 ^a	142.21 ^a	106.46 ^b	90.70 ^c	7.66	0.00	7.61
MCH (pg)	31.62 ^c	50.43 ^a	48.35 ^b	38.15 ^c	31.42 ^c	7.06	0.0	2.51
MCHC (g/dl)	33.71	33.65	34.13	35.78	34.70	0.55	0.70	0.49
Heterophils (%)	26.00 ^a	23.67 ^b	22.00 ^c	25.00 ^a	27.33 ^a	17.41	0.00	0.52
Lymphocytes (%)	59.00 ^b	61.33 ^a	62.00 ^a	58.00 ^b	56.67 ^c	20.55	0.00	0.57
Monocytes (%)	8.00 ^b	8.00 ^b	8.00 ^b	9.00 ^a	8.33 ^b	8.50	0.03	0.12
Eosinophils (%)	5.00	5.00	6.00	5.00	5.00	-	-	0.11
Basophils (%)	2.00 ^c	2.00 ^c	2.00 ^c	3.00 ^a	2.67 ^b	10.00	0.02	0.13

Table 2: Effect of Lemongrass Extract on Biochemical Indices of ISA Brown Layers.

Parameters	T1	T2	T3	T4	T5	F-Value	P-value	SEM
Glucose (mg/dl)	57.72 ^c	65.55 ^b	68.09 ^b	77.38 ^a	70.25 ^b	10.43	0.01	1.90
Total Cholesterol (mg/dl)	85.69	73.30	90.23	86.38	54.67	0.93	0.45	6.67
Total Protein (mg/dl)	4.01	4.04	3.80	3.76	4.78	2.79	0.09	0.13
Albumin (mg/dl)	2.00 ^a	1.98 ^a	1.740 ^b	1.96 ^a	1.74 ^a	6.35	0.01	0.04
Plasma Protein (mg/dl)	11.07 ^a	10.67 ^a	9.77 ^b	8.40 ^c	9.47 ^b	8.08	0.00	0.29

a,b,c,d: Means with different superscripts within the same row are significantly different. SEM:Standard Error of Mean

MCH, heterophils, Lymphocytes, Monocytes, and Basophils. However, no significant ($p > 0.05$) differences effects were observed among the means of different levels of lemon grass extract supplementation on PCV, Hb, WBC, and MCHC (Table 1). Red blood cell (RBC) count Ranged from 2.07 (T2) to 3.76 (T1). RBC of hens T1 ($3.76 \times 10^{12}/L$) and T5 ($3.57 \times 10^{12}/L$) had the highest mean values over those of T2 ($2.07 \times 10^{12}/L$), T3 ($2.21 \times 10^{12}/L$) and T4 ($3.26 \times 10^{12}/L$). Mean Corpuscular Volume (MCV) ranged between 90.70 fl (T5) to 149.93fl (T2), with MCV values in T2 (149.93 fl) and T3 (142.21 fl) having significantly ($p < 0.05$) higher values over those of the other treatments. MCH of hens ranged between 31.42 (T5) to 50.43 (T2), with that of T2 (50.43) having the highest value and was significantly different from those of the treatments (Table 1).

Heterophil percentage ranged from 22.0% (T3) to 27.33% (T5). hens in T1 (26.00), T4 (25.00) and T5 (27.33%) were significantly ($p < 0.05$) higher than those of T2(23.67) and T3 (22.00). Lymphocytes values ranged from 50.07 (T5) to 62.00 (T3). Similarly, values observed for Lymphocytes, the highest significant ($p < 0.05$) values were observed in T2 (61.33) and T3 (62.00) and they varied from those of T1 (59.00), T4 (58.00) and T5 (56.67), with T5 (56.67) having the least value across the treatments. Monocyte: significant ($p < 0.05$) difference was observed in the monocyte values of the laying hens, that the means in T1, T2, T3, and T5 were statistically similar and low while T4 was higher and statistically different from other means in the homogenous group, suggesting a greater impact of T4 on the monocytes of the hens. Basophils recorded a significant ($p < 0.05$) difference with those on T4 (2.67) and T5 (3.00) having the highest T1 (2.00) across the treatments.

Table 2 presents the results of biochemical indices of ISA brown layers supplemented with lemongrass extract. Significant effect of lemongrass extract on glucose, albumin and plasma protein ($p < 0.05$). Serum glucose concentration was significantly ($p < 0.05$) affected by lemongrass extract increasing from 57.72 mg/dl in the control to 77.38 mg/dl in T4, with T4 (77.38) having the highest significant ($p < 0.05$) value over the other treatments. However, there was no significant effect of different levels of lemongrass extract on Total cholesterol, and Total protein ($p > 0.05$).

Albumin concentration differed significantly ($p < 0.05$) among the groups with the control (2.00 mg/dl) recording the highest value and T3 and T5 (1.74 mg/dl) had the lowest value. DMRT placed T1 (2.00^b mg/dl), T2 (1.98^b mg/dl) and T4 (1.96^b mg/dl) as high and statistically similar. Plasma protein concentration also differed significantly ($p < 0.05$), decreasing from 11.07 mg/dl in T1 to 8.40 mg/dl in T4. T1 (11.07 mg/dl) as the highest mean value and was not significantly different from that of T2 (10.67 mg/dl). T3 (9.77 mg/dl), and T5 (9.47 mg/dl), were statistically similar, but had lower mean values while T4 (8.40 mg/dl) recorded the lowest mean value. This suggests that T1 had more effect on the plasma protein of the hens.

DISCUSSION

Haematology

RBC of hens ranged from 2.07 (T2) to 3.76 (T1). This aligns with the findings of Onunkwo *et al.* (2021) who documented a significant effect of medicinal plant leaves on RBC. The significantly reduced RBC at low lemongrass doses (50–100 mL/6L) may suggest a mild suppressive

effect on erythropoiesis. MCV values ranged from 90.70 fl (T5) to 149.93 fl (T2) and between 31.42 (T5) to 50.43 (T2) for MCH. Significant differences were recorded in both MCV and MCH ($p < 0.05$) in this study, which is in dissonance with the findings of Udofia, *et al.* (2025) who reported no significant ($P > 0.05$) effect of *Justicia carnea* aqueous leaf extract on MCHC and MCH on broiler chickens, and Alagbe (2019), who documented no significant difference of *Luffa aegyptiaca* leaf extract (LUF) on hematological parameters of Ross broiler chicks ($p > 0.05$). Elevated MCV and MCH in T2 and T3 in this study suggest macrocytic erythrocyte characteristics, which may indicate impaired DNA synthesis in red blood cell precursors at low-dose supplementation levels.

Packed Cell Volume (PCV) and Haemoglobin (Hb) concentration across all treatments showed no significant difference ($p > 0.05$). PCV values ranged from 31.00% to 35.00%, while Hb ranged from 10.43 to 12.01 g/dl across the groups. These results are within the normal physiological range for laying hens, and they are consistent with Alagawany *et al.* (2021), who reported that supplementation with lemongrass additives at moderate levels did not produce significant changes in haemoglobin concentration and packed cell volume in poultry. The result of PCV and Hb also suggests that lemongrass extract at the tested concentrations did not adversely affect erythropoiesis or the oxygen-carrying capacity of the blood in the hens. White Blood Cell (WBC) count showed no significant ($p > 0.05$) difference among the treatments. This result is consistent with findings from Onunkwo *et al.* (2021) and Iheanacho *et al.* (2024), who reported no significant effects of herbal extracts or low levels of ginger and garlic extracts on WBC in broiler chickens. The non-significant effect of the herbal extract suggests that phytogenic additives exert their immunomodulatory effects through specific leucocyte sub-populations rather than total white cell counts.

MCHC did not differ significantly among treatments ($p > 0.05$), with values ranging from 33.65 to 35.78 g/dl. This aligns with Ajibade *et al.*, (2023) who found no effect of *Centella asiatica* as a feed additive on ISA Brown layers. The results of this study fall within the normal range for laying hens, indicating that regardless of lemongrass supplementation level, the haemoglobin concentration within each red blood cell was maintained at physiologically adequate levels.

Significant ($p < 0.05$) differences were observed in both Heterophils and Lymphocytes. Heterophil percentage ranged from 22.0% (T3) to 27.33% (T5) and Lymphocytes values ranged from 50.07 (T5) to 62.00 (T3). These findings are consistent with Krauze *et al.* (2021), who reported that phytobiotic preparations improved immune indices in chickens, including increased lymphocyte activity. Similarly, Alagawany *et al.* (2021) documented that lemongrass supplementation promoted immunological blood markers in broilers, attributing this to the antimicrobial and immunomodulatory properties of

citral and other bioactive compounds. The inverse relationship between heterophil and lymphocyte proportions is a well-established finding in avian immunology, where these two cell types constitute the dominant leucocyte subsets (Anthony *et al.* 2024). The heterophil-to-lymphocyte (H:L) ratio is widely used as a stress marker in poultry lower ratios indicate better immune competence and less physiological stress (Bilal *et al.*, 2021). T3 (100 mL/6L) demonstrated the lowest heterophil percentage and one of the highest lymphocyte percentages, suggesting enhanced cell-mediated immune responses at this concentration. Monocyte percentage was significantly different among groups ($p < 0.05$). This is consistent with the findings of Ajibade *et al.* (2023) who reported a significant ($p < 0.05$) difference in monocytes of ISA Brown layers given *Centella asiatica* leaf meal. Monocytes are phagocytic cells that play a key role in innate immunity, and their elevation in T4 may suggest increased phagocytic activity stimulated by 150 mL lemongrass extract, and this is consistent with the immunostimulatory properties of citral (Kazeem *et al.*, 2025). This mild monocytosis at 150 mL may reflect increased response against potential pathogens, which could be a beneficial immune response rather than an indicator of disease. Basophil percentages were significantly different ($p < 0.05$). This contradicts the findings of Udofia *et al.* (2025) who reported no significant effect of *Justicia carnea* on basophils of broiler chickens. Basophils are involved in hypersensitivity and inflammatory responses. Their slight elevation at higher lemongrass concentration may reflect a mild allergic-type response to higher bioactive compounds at 150–200 mL/6L of lemongrass extract. However, the observed values remain within normal physiological ranges.

Serum Biochemical Indices

Serum glucose concentration was significantly ($p < 0.05$) affected by lemongrass extract supplementation ($p < 0.05$). This corroborates the findings of Ajibade *et al.* (2023) who found a significant difference in the levels of glucose of laying hens given varying levels of *Centella asiatica* extract. In this study, glucose mean values increased progressively from (T1) to peak at 77.38 mg/dl in T4, before slightly declining to 70.24 mg/dl in T5. This progressive, dose-dependent elevation in serum glucose up to 150 mL is noteworthy in the context of metabolic health. Glucose in poultry serves as the primary metabolic fuel for egg production, muscular activity, and thermoregulation (Owen *et al.*, 2022). Citral and terpenoids in lemongrass essential oil have been reported to modulate enzyme activity in metabolic pathways, potentially enhancing glucose mobilization and availability (Alagawany *et al.*, 2021). Albumin concentration was significantly ($p < 0.05$) affected by lemongrass extract supplementation, and this aligned with the findings of Owen *et al.* (2022), who documented significant increase

in albumin concentration in laying hens given bio-herbal supplement. Owen *et al.* (2022) noted that albumin, total protein, and liver enzymes frequently accompany blood assessments as indicators of nutritional and physiological well-being in poultry. Plasma protein concentration also differed significantly ($p < 0.05$) across treatment groups, decreasing progressively from 11.07 mg/dl in T1 to the lowest of 8.40 mg/dl in T4. In this study, plasma protein declined with increasing lemongrass extract concentration suggesting a dose-dependent effect on plasma protein dynamics. The reduction in plasma protein at higher doses could reflect increased protein catabolism, redistribution of protein resources for egg production as phyto-genic additives can enhance nutrient partitioning in laying hens (Alagawany *et al.*, 2021). There was no significant ($p > 0.05$) difference recorded in Total cholesterol and Total protein. This is in agreement with the findings of Tella *et al.* (2025) who reported no significant differences in the total protein and cholesterol of broiler chickens fed with bitter leaf extracts. Total protein is a broad indicator of protein metabolism, nutritional adequacy, and hepatic function in poultry. The consistency of total protein and total cholesterol across groups suggests that lemongrass extract supplementation did not impair protein metabolism, liver function or alter lipid metabolism in poultry (Ajibade *et al.*, 2023; Tella *et al.*, 2025).

Conclusion

It can be concluded that aqueous lemongrass (*Cymbopogon citratus*) extract, when incorporated into the drinking water of ISA Brown layers at moderate concentrations of 50 mL and 150 mL per litre of water, enhanced immune-related blood indices, without exerting any harmful effect on the general blood and biochemical health status of birds. The pattern of response observed across the graded concentrations is consistent with the principle of hormesis, where low to moderate concentrations of bioactive phytochemicals confer beneficial physiological and productive effects. The study therefore, established that lemongrass extract is a safe and accessible phyto-genic supplement capable of serving as a viable natural alternative to antibiotic growth promoters in ISA Brown layer.

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