

Effect of *Aloe barbadensis* gel on hematology, mortality rate and behavioral attributes of broiler chickens

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Abstract

The study evaluated the effects of dietary supplementation of *Aloe barbadensis* M. (also known as *Aloe vera*) gel on the hematological, biochemical, and behavioral responses of broiler chickens fed at four dietary levels (T1-T4). Among the hematological indices assessed, Mean Corpuscular Haemoglobin (MCH) was the only parameter significantly affected by treatment, with T4 recording the highest value (35.3 mm/hr) and T3 the lowest (30.3 mm/hr); Duncan's Multiple Range Test (DMRT) separated the means in the order T4 > T1 > T2 > T3, with T3 significantly lower than T4. All other hematological indices, such as PCV, Hb, WBC, RBC, MCV, MCHC, and the neutrophil, lymphocyte, and monocyte differential counts did not significantly differ among the treatments. Whereas serum conjugated bilirubin was the only highly significant parameter in the serum biochemical assessment, with T1 recording the highest value (1.100 µmol/L), significantly greater than T2, T3, and T4, which did not differ from one another. Electrolytes, kidney function markers (urea, creatinine), liver enzymes (ASAT, ALAT, ALP), total bilirubin, total protein, and albumin were unaffected by treatment, and all kidney and liver markers remained within normal physiological ranges across treatments. None of the eight behavioral variables assessed differed significantly among treatments, although Feeding and Drinking behaviors showed numerical trends approaching significance. These findings suggest that *Aloe barbadensis* gel supplementation had a largely neutral effect on the overall hematological, biochemical, and behavioral status of broiler chickens, with isolated effects on MCH and conjugated bilirubin that do not indicate hepatotoxic or nephrotoxic outcomes.

Keywords: *Aloe barbadensis*, *Aloe vera*, hematological indices, serum biochemistry, liver enzymes, behavioral responses

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INTRODUCTION

Broiler meat is a good source of nutrients due to its high protein content, affordability, availability, and low expenses in production, making it a popular choice for consumers (Hassan *et al.*, 2020). Broiler birds make up over 90% of the global poultry population (Biswas *et al.*, 2011), with feed costs accounting for 65-70% of production costs (Shambhvi *et al.*, 2020). Management practices that tends to reduce feed cost of the total production might probably be the poultry farmer's interest in the poultry sub-sector. The intensify search for safer alternatives to antibiotic growth promoters has been deepened in poultry production systems. This has lead interest in phytogenic additives plants such as *Aloe barbadensis* M. especially, the gel (*Aloe vera* gel) because of its broad spectrum of bioactive constituents and perceived safety profile (Oreoluwa *et al.*, 2026).

The biochemical properties contained in *Aloe vera* gel could provide a systematic basis for expecting effects on blood profile and their differentials for physiological status (hematology and serum biochemical parameters) in broiler birds (Khan *et al.* 2023). More so, many studies on broiler birds revealed that *Aloe vera* gel improved blood profile positively (Khan *et al.*, 2023). In addition, previous studies in broilers chickens have concentrated to a great extent on growth performance without evaluating detailed blood profile responses (Oreoluwa *et al.*, 2026).

The benefits of *Aloe vera* gel's ability to modulate reduce pathogen loads, gut microbiota and promote nutrient absorption, leading to superior final body weights and carcass yields (Amber *et al.*, 2021).

Phytobiotic are alternatives to growth promoters, and have been to a great extent accepted and gained attention in many feed industry (Islam *et al.*, 2019). Medicinal and cosmetic plants extracted from these plants have stimulating effects on poultry digestive tracts, antioxidant and antimicrobial properties (Wenk, 2003).

Aloe barbadensis M. has been used over centuries for therapeutic purposes. This plant that looks like a cactus and grows in arid zones of Africa (Boudreau and Beland, 2006). The plant's leaf constitutes of latex and gel. The gel consists 98.5% - 99.5% water (Femenia *et al.*, 1999), and contains more than 75 biologically active compounds, which are useful in treating disease and skin problems (Boudreau and Beland, 2006). *Aloe barbadensis* M. gel contains compounds with antioxidant, antiviral, antifungal, antibacterial, anti-diabetic, immunomodulatory, anti-inflammatory, and wound healing abilities (Christaki and Florou-Paneri, 2010). *Aloe vera* gel also contains acemannan, which affect the humoral immune reaction and cellular immunity, activating macrophages to release inflammatory cytokines such as interleukin-1 and tumour necrosis factor-α.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Unit of Professor Lawal Abdu Saulawa Livestock Teaching and Research Farm, Department of Animal Science, Federal University, Dutsin-Ma, Katsina State, Nigeria. Dutsin-Ma is a Local Government Area in Katsina State, North-western Nigeria. It lies on Latitude 12.27 °18'N, Longitude 07.29°29'E, with onset of rainfall starting from May and ending by October. The average annual rainfall is about 553 mm with annual temperature ranges from 29°C - 45°C (Nimet, 2024).

Experimental Animals and their Management

A total number of 120-day-old Arbor Acres broiler chicks were procured from a reputable commercial hatchery company and the chicks were reared for 2 weeks indoors (brooding). Upon arrival, the brooding pen was prepared with all brooding materials. At the end of the brooding phase (2 weeks), the chicks were placed in a completely randomized design (CRD).

The chicks were randomly allocated to 4 dietary treatment groups, with three replications, each containing 10 birds per replicates. The pens were of equal size, equipped with feeders and drinkers, and located in a well-ventilated poultry house. The birds were raised on a deep litter system, and brooding was conducted under standard conditions for the first 14 days.

Experimental Designs

The study utilized a Completely Randomized Design (CRD). The birds were assigned to four (4) dietary treatment groups, with three replicate pens per treatment. The treatments (T1 – T4) were described as T1 (control) 0.0 mL ABG/L of water, T2 5.0 mL ABG/L of water, T3 7.5 mL ABG/L of water and T4 10 mL ABG/L of water.

Preparation of the *Aloe barbadensis* M.

Fresh *Aloe barbadensis* M was collected from the Dutsin-Ma Wednesday Market. The leaves were cleaned with water and the ABG was extracted from the leaf by peeling off the leaves following Shokraneh *et al.*, (2016) procedure. The latex of the leaves was removed, and the gel was collected and blend using an electric blender (Binatone BLG-595; 400 W, 1.5 L glass jar; Binatone Industries Ltd., Lagos, Nigeria), stocked in a clean container and store in a refrigerator, in line with the stabilization guideline of Ramachandra and Srinivasa Rao (2008). *Aloe vera* gel was added to plain water following the levels specific in each treatment from the commencement of experiment to 42-day, birds in the control group were provided with commercial feed. Water consumption was recorded (Pesti *et al.*, 1985).

Hematological indices determination

Hematological determination of Haemoglobin estimation (Hb), white blood cell (WBC), packed cell volume (PVC), red blood cell, monocytes (M), Heterophil (H), Lymphocytes (L) and Eosinophil (E) were determined as described by Jain (1986). Three (3 mL) of blood was col-

lected from the sub-clavicular vein of each birds using a scalp vein needle set. The blood was placed into sterilized glass tubes/bottles containing Ethylene Diaminetetra-acetic Acid (EDTA) for Hematological studies.

MCH: Mean Corpuscular Haemoglobin, measures the average amount of haemoglobin in one red blood cell.

$$MCH \text{ (pg)} = \frac{Hb(g/dL \times 10)}{RBC(\frac{10^6}{L})}$$

MCV: Mean Corpuscular Volume, it measures the average size of one red blood cell

$$MCV \text{ (fL)} = \frac{PCV(\%) \times 10}{RBC(\frac{10^6}{L})}$$

MCHC: Mean Corpuscular Haemoglobin Concentration; this measures the average concentration of haemoglobin inside one red blood cell. It tells the quality of haemoglobin in RBCs.

$$MCHC \text{ (g/dL)} = \frac{MCH \text{ (pg)} \times 10}{MCV \text{ (fL)}}$$

Mortality

Mortality was recorded as it occurred and percentage mortality was determined as reported by (AGW, 2023).

Mortality (%) = Number of dead chickens x 100/Initial number of chickens at the start of the experiment

Behavioral observations

Behavioral observations were recorded at 22 days old and extended up to 42 days old (Table 1). The behaviors of the chicks were recorded by direct observation using a modified focal sampling technique after modification. For all behavioral patterns, five birds from each group were marked with blue dye. The behaviors were observed during the grower and finishing period of rearing; from day 22 of week 4 to day 40 of week 6. During the days of observations, the behavioral patterns were recorded two times daily as follow; at early morning (7:00-7:30 am), and at evening (6:00-6:30 pm). Data was summed to give the frequency of each behavior performed per chicken per 30-min period as reported by Ferrante *et al.* (2019).

Table 1: Measurement of behaviors and definitions of terms

Behaviors	Description
Standing	Standing, feet or legs, but not belly, on the floor
Sitting	Sitting with breast and belly on the floor
Drinking	Standing or sitting with head in the drinker
Resting	Lying down, not performing any other behavior. May or may not be sleeping
Feeding	Standing or sitting with head in the feeder
Defecation	Defecating of the animals during the test
Escape	Endeavouring to leap out of the test stag
Preening	Manipulating own feathers with the beak while standing

Data Analysis

Data from three experimental datasets (hematological indices, serum biochemical parameters, and behavioral indices were recorded) were recorded and analyzed as a Completely Randomized Design (CRD) using one-way Analysis of Variance (ANOVA) with four dietary treatments supplementation and three replicates per treatment (N = 12 per dataset). Statistical significance were declared at $P < 0.05$. Where the F-test was significant, treatment means were separated using Duncan's Multiple Range Test (DMRT). Significance is further denoted as: ** = highly significant ($P < 0.01$), * = significant ($P < 0.05$), NS = not significant ($P \geq 0.05$). One-way ANOVA was performed on each variables, using SAS and where the F-test indicated significant treatment effects ($P < 0.05$).

RESULTS

The effect of *Aloe barbadensis* M. gel on hematological indices of broiler chickens

The results obtained from the present study depicts that Mean Corpuscular Haemoglobin (MCH) was the only indices with high significant parameter, with T4 recording the highest value (35.3 mm/hr) and T3 the lowest (30.3 mm/hr) (Table 2). Duncan's DMRT revealed $T4 > T1 > T2 > T3$, with T3 significantly lower

than T4. This indicates that dietary supplementation of *Aloe barbadensis* M. gel in the treatment influenced the average amount of haemoglobin per red blood cell, particularly depressing it in T3 broiler birds. All other hematological indices, such as PCV, Hb, WBC, RBC, MCV, MCHC, and differential white cell counts (neutrophils, lymphocytes, and monocytes) were not significantly affected by the inclusion of *Aloe barbadensis* M. gel in the treatment, and this suggesting that the overall hematological and immune status of the birds was similar across treatments.

The effect of *Aloe barbadensis* M. gel on behavioral indices of broiler chickens

None of the eight behavioral variables differed significantly among the four treatments, meaning that, statistically, DMRT letter separation is not warranted for any variable (all treatment means within a behavior belong to the same Duncan grouping, i.e., superscript 'a' throughout). Two variables approached the 5% threshold and are worth noting as trends: Feeding, where T4 (3.87) and T2 (3.67) numerically exceeded Control (2.73) and T3 (2.33); and Drinking, where T3 (3.13) was numerically highest and T4 (2.07) lowest (Table 3). These trends did not reach conventional significance and should be interpreted with caution given the modest replication ($n = 3$ per treatment).

Table 2: The effect of *Aloe barbadensis* M. gel on hematological indices of broiler chickens

Parameter	T1	T2	T3	T4	SEM	F-value	P-value	Remark
PCV %	23.333	23.333	20.667	25.000	2.3094	0.600	0.632	NS
HB g/dl	8.033	7.967	7.333	8.567	0.6489	0.610	0.630	NS
WBC $\times 10^9$ /L	4.100	4.933	5.600	5.067	0.4327	2.060	0.184	NS
RBC mil/uL	2.933	2.800	2.300	2.667	0.3484	0.610	0.626	NS
MCH mm/hr	33.333 ^{ab}	32.667 ^{bc}	30.333 ^{cd}	35.333 ^a	0.7265	8.050	0.008	**
MCV fL	129.667	130.000	127.667	133.000	1.3437	2.680	0.118	NS
MCHC g/dL	31.333	30.000	29.667	32.000	1.0672	1.070	0.416	NS
Neut %	26.667	41.333	22.000	29.333	8.3865	0.970	0.455	NS
Lymp %	71.667	57.667	77.333	70.333	8.3832	0.980	0.449	NS
Mono %	1.667	1.000	0.667	0.333	0.4082	1.940	0.201	NS
MORT%	10.000	3.333	10.000	0.000	4.410	1.290	0.343	NS

a-d means within rows bearing different superscripts differs significantly at $p > 0.05$; SEM: Standard error of means, PCV: packed cell volume, Hb: Haemoglobin, WBC: white blood cell, RBC: red blood cell, MCH: means corpuscular haemoglobin, MCV: mean corpuscular volume, Neut: neutrophils, Lymp: lymphocyte, Mono: monocyte, and MORT: mortality; , NS= not significant ($P > 0.05$); * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$). SEM = Standard Error of the Mean (pooled, from ANOVA error term).

Table 3: Effect of *Aloe barbadensis* M. gel on behavioral indices of broiler chickens

Treatment	T1	T2	T3	T4	SEM	F-value	P-value	Remark
Sitting	3.467	3.400	3.333	3.267	0.395	0.050	0.985	NS
Standing	4.233	4.967	3.967	4.200	0.734	0.350	0.791	NS
Feeding	2.733	3.667	2.333	3.867	0.420	3.070	0.091	NS
Drinking	2.200	2.800	3.867	2.067	0.284	3.140	0.087	NS
Aggression	0.133	0.067	0.067	0.033	0.060	0.490	0.701	NS
Preening	0.300	0.467	3.367	0.333	0.173	0.170	0.912	NS
Packing	0.133	0.100	0.067	0.033	0.029	2.220	0.163	NS
W. diff.	1.067	1.067	1.133	0.733	0.237	0.580	0.644	NS

a-d means within rows bearing different superscripts differs significantly at $p > 0.05$; SEM: Standard error of means, W. diff.: walking difficulties, NS = not significant ($P > 0.05$); * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$). SEM = Standard Error of the Mean (pooled, from ANOVA error term).

DISCUSSION

The result of Nuhu *et al.* (2021) on the effect of *Aloe vera* gel on MCH of Noiler Cocks was in accordance with the present study, in the present study, MCH was highly significant and this could be as a result of either nutritional imbalance, dehydration/hemoconcentration or toxins from the ABG used in the study. In addition, Jonathan *et al.* (2021) also observed linear increases in MCH. Furthermore, Schaer *et al.* (2013) reported that progressive effects were identified for MCH as the level of ABG increased, which is a measure of the average amount of hemoglobin present in the RBC. When the inclusion level of ABG was increased, the MCH levels initially showed decreased before increasing numerically at T4. This corroborated with the finding of Kgaogelo *et al.* (2024). On the contrary, Kgaogelo *et al.* (2024) reported that the use of ABG as nutraceuticals to ameliorate stocking density-induced stress in commercial male broilers has no significant effect on MCH of male broiler birds.

In corroboration with the present study, Oreoluwa *et al.* (2026) reported in their study that the effect of dosage on hematological indices of Noiler birds indicated that packed cell volume, haemoglobin, red blood cell, white blood cell, heterophils, eosinophils, basophils, and monocytes were not significantly affected by varying dosage levels of ABG. On the contrary, Islam *et al.* (2019) reported in their studies that highest significant values were recorded in PCV and Hb. Similarly, Oreoluwa *et al.* (2026) discovered that lymphocyte percentage in their study was significantly affected, with Noiler birds receiving 30 ml/L recording a significantly higher value compared with the control. Indices recorded in the present study were within the normal ranges reported for chickens raised under tropical condition (Oreoluwa *et al.*, 2026). This finding indicated that the inclusion levels of ABG supported hematological constancy. In consistent with the present study, Egena *et al.* (2024) and Khan *et al.* (2023) have similarly reported that moderate inclusion levels of phytogenic supplements do not significantly alter core hematological indices in slow growing or dual-purpose chickens. The significant increase in lymphocyte percentage at higher dosage levels may reflect enhanced immunological effect as dosage increases.

The result of the present study is in accordance with the findings of Adedeji *et al.* (2022) and Idowu *et al.* (2021), who observed that dietary bioactive compounds could modify lymphocyte production in poultry. Also, it was opined that plant-derived bioactive compounds could modulate lymphopoiesis and possibly improve immune performance in poultry without inducing pathological stress Abd El-Hack *et al.* (2022) and Oloruntola *et al.* (2023).

The absence of statistical differences across sitting, standing, feeding, drinking, aggression, preening, pecking and walking difficulties suggests that *Aloe vera* gel did not adversely affect comfort and locomotor ability, or general activity patterns. Which is in consistent with Tudorache *et al.*, (2022) who reported that in poultry management, prolonged sitting is often linked to discomfort, lameness,

or poor litter quality, while excessive standing or pecking may reflect environmental stress. Tudorache *et al.*, (2022) further stressed that feeding and drinking behaviors are direct indicators of appetite, palatability, and metabolic status. Similar findings have been reported in studies evaluating phytogenic additives and herbal extracts, where behavioral changes were minimal despite improvements in growth performance or immunity (Alagawany *et al.*, 2021).

Drinking behavior likewise showed no significant differences among treatments. This indicates that *Aloe vera* gel inclusion did not negatively affect water palatability. Since alterations in water taste or osmotic balance can influence intake patterns, the stability observed here suggests appropriate formulation and dosage. Moreover, the maintenance of consistent drinking behavior aligns with previous reports that *Aloe vera* supplementation in poultry does not impair hydration or electrolyte balance when administered at moderate levels (Deng *et al.*, 2023). Preening behavior, which is typically associated with comfort and feather maintenance, did not differ significantly among treatments. Stable preening frequency may indicate that integumentary condition and comfort levels were comparable across groups. Since *Aloe vera* possesses anti-inflammatory and antimicrobial properties, one might hypothesize potential improvements in skin or feather condition; however, behavioral metrics did not reveal measurable changes. This could reflect either adequate baseline health in all groups or insufficient experimental duration to detect subtle integument-related behavioral modifications (Alagawany *et al.*, 2021).

CONCLUSION

Dietary supplementation of *Aloe barbadensis* M. gel did not produce widespread adverse effects on the hematological or behavioral profiles of broiler chickens under the conditions of this study. With the exception of MCH, which was depressed in T3 relative to T4, all other hematological and behavioral parameters remained statistically comparable across treatments and within normal physiological ranges, indicating that the birds' immune and kidney functions were not compromised by any of the dietary inclusion levels tested. Similarly, none of the behavioral variables assessed were significantly influenced by treatment, suggesting that the general activity budget and welfare status of the birds were unaffected by *Aloe barbadensis* M. gel inclusion. Overall, these results indicate that *Aloe barbadensis* M. gel can be incorporated into broiler diets at 5.0 mL ABG/L of water, 7.5 mL ABG/L of water and 10 mL ABG/L of water without compromising bird health or behavior, though the isolated changes observed in MCH warrant further investigation.

Ethical statement: This study was carried out at the Federal University, Dutsin-Ma, Katsina State, Nigeria. Experiment was conducted in accordance with the Katsina State Health Research Ethical Clearance Committee on Animal Care and Use in Research Committee (MOH/ADM/SUB/1152/1/1173).

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