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Growth performance, haematological, immune and anti-oxidative response of broiler birds administered pawpaw *(Carica papaya)* leaf aqueous extract in place of conventional antibiotics

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Abstract

This study evaluated the effects of pawpaw (Carica papaya) leaf aqueous extract (PLAE) on the growth performance, hematological, immune and oxidative responses of broiler chickens as a natural alternative to conventional antibiotics. A total of 150 day-old Arbor Acre broiler chicks were randomly assigned to five treatments in a completely randomized design: negative control (plain water), positive control (oxy tetracycline, 1 g/L), and 10, 20, and 30 g/L of PLAE. The trial lasted six weeks. Results showed that feed intake showed significant difference (P<0.05) across the treatments (3.63 – 4.50). Other growth parameters showed no significant difference (P>0.05). Results indicated significant (P < 0.05) improvements in packed cell volume (PCV), hemoglobin (HB), and red blood cell (RBC) counts in birds administered 20 and 30 g/L PLAE compared with the controls. White blood cell (WBC) and lymphocyte counts also increased, suggesting enhanced immune competence. The absence of adverse effects on hematological indices confirms the safety of the extract. These findings highlight Carica papaya leaves as a viable, climate-smart substitute for antibiotics in broiler production, promoting sustainable poultry health management, reducing antimicrobial dependence, and supporting environmentally friendly livestock systems adaptable to climate change challenges.

Keywords: *climate-smart agriculture; haematology; pack cell volume; phytogenic.*

Description of the Problem

The swift development of poultry production has exaggerated the search for operative, safe, and sustainable approaches to advance bird health and productivity. For decades, antibiotic growth promoters (AGPs) have been broadly utilized in broiler nutrition to boost performance and avert diseases. Nevertheless, anxieties over the advent of antimicrobial-resistant pathogens and the occurrence of drug residues in poultry products have led to stringent global restraints on antibiotic use in animal feed (1); (2). Therefore, research commitment has moved in the direction of phytogenic feed additives, bioactive plant derived mixtures known for their growth promoting, antioxidant, and immune modulatory effects (3); (4). Among several medicinal plants of importance, *Carica papaya* (pawpaw) has received considerable attention due to its rich phytochemical composition and therapeutic potential. The leaves of *C. papaya* encompass alkaloids, flavonoids, saponins, tannins, papain, and phenolic compounds that parade antibacterial, anti-inflammatory, and antioxidant stuffs (5); (6). These bioactive ingredients are acknowledged to moderate immune reaction, excite hematopoiesis, and safeguard cells against oxidative injury (7).

Studies have established that pawpaw leaf extract advances gut health, improves nutrient absorption, and improvement of disease resistance in poultry, making it a favorable alternative to artificial AGPs (8); (9). Hematological indices are vibrant indicators of the physiological and health position of animals. Parameters such as packed cell volume (PCV), hemoglobin concentration, red blood cell (RBC) and white blood cell (WBC) counts offer important evidence on oxygen transport capacity, immune proficiency, and overall metabolic purpose (10). Disparities in these parameters can mirror the impact of feed additives, stress, or disease situations on the birds' homeostatic equilibrium (11). Likewise, immune response pointers: such as lymphocyte count, heterophil to lymphocyte ratio, and antibody titers, offer valuable insights into the bird's (11) ability to counterattack infection and uphold physiological flexibility under production stress.

Integrating natural phytogenic additives like *Carica papaya* into broiler diets supports the ideologies of climate-smart and sustainable livestock production, as they are renewable, locally accessible, and environmentally benign (3); (2). In contrast to synthetic antibiotics, plant centered extracts diminish chemical deposits in meat and shrink environmental pollution while improving immune function and disease resistance. Therefore, understanding the hematological and immunological responses of broiler chickens to pawpaw leaf extract can aid in establishing its efficiency and safety as a natural feed additive for antibiotic-free poultry production. This study was consequently designed to appraise the effects of aqueous *Carica papaya* leaf extract on the hematological parameters and immune response of broiler chickens. The findings are expected to provide insight into its potential as a natural immune stimulant and contribute to the improvement of sustainable, climate-smart poultry production systems.

Materials and Methods

Experimental location

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Health and Production Technology, the Oke-Ogun Polytechnic, Saki, Oyo State, Nigeria. The area lies within the derived savannah zone of southwestern Nigeria, characterized by a tropical climate with distinct wet (April–October) and dry (November–March) seasons, an annual rainfall of approximately 1,200 mm, and an average ambient temperature ranging from 26°C to 34°C.

Preparation of pawpaw (*Carica papaya*) leaf extract

Fresh, matured leaves of *Carica papaya* were gathered from healthy, disease-free plants within the neighborhood of the institution. The leaves were carefully washed under running tap water to eradicate dirt and debris, then weighed into three portions corresponding to 10 g, 20 g, and 30 g of fresh leaves. Each portion was pulverized using a sterile mortar and pestle to obtain a fine paste. The paste from each treatment was infused in 1 liter of distilled water at room temperature to facilitate extraction of the bioactive compounds. The mixtures were filtered using clean muslin cloth to obtain the aqueous extract.

Experimental birds and design

A total of 150 day-old Arbor Acre broiler chicks were used for the study. The birds were randomly allotted to five treatments, each with three replicates of ten birds, using a completely randomized design (CRD). The treatments were as follows:

T1: Control (plain water, no extract)

T2: Positive control (oxytetracycline, 1 g/L)

T3: Pawpaw leaf extract at 10 g/L

T4: Pawpaw leaf extract at 20 g/L

T5: Pawpaw leaf extract at 30 g/L

The experiment lasted six weeks. Commercial Feed (22.00 and 18.00% CP for starter and finisher phase respectively with 2900Kcal/Kg and 3050Kcal/Kg energy for starter and finisher phase respectively) and water were supplied *ad - libitum*, and all management practices were in accordance with standard broiler production guidelines.

Data collection

Growth performance

Data on body weight, weight gain, feed intake, feed conversion ratio (FCR), and mortality were recorded weekly. Feed intake was determined as the difference between feed offered and feed remnant, while FCR was calculated as feed intake divided by weight gain.

Blood sample collection

At the end of the trial, two birds per replicate (six per treatment) were randomly selected and fasted overnight. Approximately 3 mL of blood was collected from the wing vein using sterile syringes and needles. Blood samples were divided into two sets: one portion was collected into ethyl ene-diamine-tetra acetic acid (EDTA)-treated tubes for hematological analysis, while the second portion was placed in plain tubes to obtain serum for immune response assays.

Hematological and immune response analysis

Hematological parameters, including packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, and white blood cell (WBC) count, were determined using standard procedures described by (4). Differential leukocyte counts were performed using blood smears stained with Giemsa. Serum samples were analyzed for total protein, albumin, and globulin concentrations using spectrophotometric methods. Immune response indicators such as antibody titers against Newcastle disease virus were determined using the hemagglutination inhibition test as described by (5).

Statistical analysis

All data were analyzed using one-way analysis of variance (12) in SAS software (1999). Means were separated using Duncan's Multiple Range Test of the same package and significance was accepted at $p < 0.05$.

Results and Discussion

The effects of pawpaw leaf infusion (PLAE) on the growth performance of broiler chickens are presented in Table 1. Feed intake was influenced by the dietary treatments, whereas live weight, weight gain, feed conversion ratio (FCR) and mortality were not significantly affected ($P > 0.05$). Broilers administered 20 g and 30 g PLAE recorded numerically higher live weights (2.60%) and weight gains (2.56%) compared with the negative (2.46%) and positive (2.20%) controls. Feed intake increased progressively with increasing levels of pawpaw leaf infusion, with birds receiving 20 g and 30 g PLAE showing significantly higher values (4.52 % and 4.50 %, respectively) than the positive control group (4.03 %) ($P < 0.05$). The FCR ranged from 0.56 to 0.70, indicating that inclusion of pawpaw leaf infusion did not compromise feed efficiency. Mortality remained zero across most treatments, except for minimal losses (2.76 %) in the 20 g and 30 g PLAE groups. This might result from physiological stress or metabolic load of the higher doses. The improved live weight and feed intake observed in birds administered pawpaw leaf infusion may be attributed to the presence of phytochemicals and proteolytic enzymes (papain and chymopapain) in *Carica* papaya leaves, which enhance digestive enzyme secretion and nutrient utilization (2). Similar findings were reported by (6), who observed significant increases in body weight and feed consumption among broilers receiving aqueous pawpaw leaf extract via drinking water. The better weight gain recorded at moderate PLAE levels also aligns with the work of (10), who noted that pawpaw leaf meal improved appetite and nutrient absorption due to its stimulatory effect on gut morphology. The non-significant difference in FCR across treatments suggests that the improved intake was efficiently converted into body tissue, reflecting metabolic adaptation to the plant extract. This finding is consistent with the report of (1), who found that inclusion of *Carica papaya* leaf extract up to 2% did not impair feed efficiency or survival in broilers. The low mortality rate and absence of deleterious effects further confirm the safety of the aqueous extract at the inclusion levels tested. Collectively, these results demonstrate that aqueous pawpaw leaf infusion can support growth performance comparable to or better than conventional antibiotic supplementation, highlighting its potential as a natural growth promoter in broiler production.

Table 1: Growth performances of broilers placed on pawpaw leaf infusion (PLAE)

Parameters	Negative control	Positive control	10g PLAE	20g PLAE	30g PLAE	SEM
Live weight (kg)	2.46	2.20	2.50	2.60	2.60	0.12
Weight gain (kg)	2.42	2.16	2.45	2.56	2.56	0.12
Feed intake (Kg)	3.63^{b}	4.03^{ab}	4.38^{a}	4.52^{a}	4.50^{a}	0.14
Feed conversion ratio	0.70	0.56	0.56	0.56	0.66	0.05
Mortality (%)	0.00	0.00	0.00	2.76	2.76	1.74

^{a,b,c} means with different superscript within a row are significantly different (P<0.05)

Hematological parameters

The hematological responses of broiler chickens administered varying levels of pawpaw leaf aqueous extract (PLAE) are presented in Table 2. The results revealed that packed cell volume (PCV), hemoglobin concentration (Hb), and red blood cell count (RBC) were significantly (P < 0.05) influenced by the treatments. Birds given 20 g/L PLAE had the highest PCV (32.00%) and Hb (10.36 g/dL), followed by those on 30 g/L PLAE (30.33% and 9.80 g/dL, respectively), while the 10 g/L group had the lowest values. The positive control (antibiotic-treated) and the negative control groups showed intermediate values. The improvement in PCV, Hb, and RBC observed in birds supplemented with 20–30 g/L PLAE suggests enhanced erythropoiesis and oxygen transport efficiency, possibly due to the bioactive compounds such as alkaloids, flavonoids, and saponins in *Carica papaya* leaves (7); (8). These phytochemicals have been reported to stimulate hematopoietic activity and reduce oxidative stress in broilers (6). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) did not differ significantly (P> 0.05) among treatments, indicating that erythrocyte indices remained within normal physiological ranges (12). This suggests that the inclusion of PLAE did not induce anemia or cellular abnormality. The findings align with those of (11), who reported that herbal extracts enhanced blood quality and immune competence without adverse hematological alterations. Overall, the improved hematological indices in birds given 20 g/L PLAE indicate that this inclusion level may support optimal physiological function and enhance resistance to stress (3).

Table 2: Hematological result of broilers placed on varying level of PLAE

Parameters	Positive control	Negative control	10g/L PLAE	20G/L PLAE	30G/L PLAE	SEM	Normal range value
PCV (%)	28.33^{ab}	28.66^{ab}	26.33^{b}	32.00^{a}	30.33^{ab}	1.24	22.00-35.00
HB (g/dl)	9.06^{a}	8.86^{b}	8.76^{b}	10.36^{a}	9.80^{ab}	0.38	7.00- -13.00
RBC ($x10^{6/}$ul)	2.82^{ab}	2.89^{ab}	2.65^{b}	3.21^{a}	2.99^{ab}	0.41	2.00– 3.50
MCV (fl)	100.55	99.32	101.90	99.74	98.64	1.56	90.00– 140.00
MCHC (g/dl)	32.03	30.93	32.53	32.55	32.33	0.54	26 - 35
MCH (pg)	32.21	30.70	33.38	32.33	32.78	0.9	33.00– 47.00

$^{a, b, c}$ means with different superscripts within a row are significantly different (P<0.05). PCV: packed cell volume, HB: hemoglobin, RBC: red blood cell, MCV: corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin.

The immune response of broilers administered PLAE is presented in Table 3. White blood cell (WBC) counts increased numerically with PLAE inclusion, with the highest values (18,250 $\times10^3/\mu L$) observed at 30 g/L PLAE, suggesting stimulation of the immune system. Lymphocyte (LYM) coumt was slightly elevated in the extract-treated groups compared to controls, while heterophil (HET), eosinophil (EOC), and basophil (BA) counts remained within normal ranges. The observed increase in total WBC and lymphocyte counts in PLAE-supplemented groups reflects enhanced immunological competence, potentially due to the immune modulatory and antioxidant properties of *C. papaya* leaf constituents such as papain, phenolics, and flavonoids (7); (13). Similar reports by (15) and (9) demonstrated that phytogenic additives boost leukocyte production and improve antibody titers in poultry. The enhanced immune parameters at higher inclusion levels (20–30 g/L PLAE) corroborate findings that herbal extracts can function as alternatives to synthetic antibiotics, promoting disease resistance and maintaining homeostasis in broiler chickens (12); (6). This supports the principle of climate-smart and sustainable poultry production, where natural phytogenic additives improve health and performance while minimizing environmental risks associated with antibiotic residues (8). In summary, dietary administration of PLAE improved hematological indices and immune response in broilers without adverse effects, indicating its potential as a viable natural alternative to antibiotic growth promoters.

Table 3: Immune response of broilers placed on varying levels of PLAE

Parameters	Positive control	Negative control	10g/L PLAE	20g/L PLAE	30g/L PLAE	SEM	Normal range
WBC ($x10^{3}$ul)	14483^{ab}	10067^{b}	14867^{ab}	16967^{ab}	18250^{a}	22.56	
LYM (%)	60.33	58.66	57.66	66.00	60.66	2.49	20-70
HET (%)	34.33	34.33	34.00	27.33	34.00	2.25	20-60
MON (%)	1.66^{bc}	2.33^{ab}	3.33^{ab}	4.00^{a}	1.00^{c}	0.68	0-10
EOS (%)	3.33	4.33	4.66	3.00	4.00	0.73	0-7
BA (%)	0.33	2.33	0.33	0.00	0.33	0.86	0-3

$^{a, b, c}$ means with different superscripts within a row are significantly (P<0.05) different. WBC: white blood cell, LYM: lymphocytes, HET: hetrophiles, MON: monocytes, EOS: eosinophils, BA: basophiles.

The effects of Pawpaw Leaf Aqueous Extract (PLAE) on the anti-oxidative response of broiler birds are presented in Table 4 Total protein levels varied across treatments, with the highest value observed in the positive control group (94.89 mg/dL), while the negative control recorded 72.45 mg/dL. Birds administered 10 g/L and 20 g/L PLAE showed slightly reduced total protein levels of 65.79 mg/dL and 66.67 mg/dL, respectively. However, birds treated with 30 g/L PLAE exhibited an improved total protein value (80.00 mg/dL), indicating a dose-dependent trend. Malondialdehyde (MDA), an indicator of lipid peroxidation, remained at 0.00 across all treatment groups, suggesting minimal oxidative stress and effective antioxidant protection irrespective of treatment. Superoxide dismutase (SOD) activity, an important antioxidant enzyme, ranged from 0.20 to 0.40 mg/dL across treatments. The positive control recorded 0.23 mg/dL, while the negative control had 0.32 mg/dL. The 20 g/L PLAE group showed the highest SOD activity (0.40 mg/dL), indicating enhanced antioxidant defense, while the 30 g/L group had a lower SOD value (0.20 mg/dL). The total protein results indicate that inclusion of PLAE influenced the metabolic and physiological status of broiler birds. The slight reduction in total protein at 10 g/L and 20 g/L could be due to increased utilization of available proteins for detoxification and metabolic adaptation to the phytochemical constituents in the extract. The improvement observed at 30 g/L PLAE suggests that higher concentrations may enhance protein synthesis or reduce protein degradation, possibly due to increased antioxidant protection. Similar findings have been reported where plant extracts rich in bioactive compounds improved serum protein profiles in broilers (16); (17). The uniform MDA values (0.00) across treatments demonstrate that birds were not subjected to significant oxidative stress during the experimental period. The absence of lipid peroxidation indicates that both the control and PLAE–supplemented birds maintained optimal oxidative balance. This aligns with earlier studies showing that pawpaw leaf extract contains strong antioxidant compounds such as flavonoids and phenolic acids, which help stabilize cell membranes and prevent lipid oxidation (18); (19). The SOD activity showed variations that reveal how the birds responded to oxidative challenges. The higher SOD activity at 20 g/L PLAE suggests enhancement of endogenous antioxidant enzyme systems, consistent with reports that moderate doses of plant extracts stimulate antioxidant enzyme production (20). The reduced SOD activity in the 30 g/L group compared to 20 g/L may indicate that excessive phytochemical concentration can suppress or overwhelm antioxidant enzyme pathways, as also noted by (21). Nonetheless, SOD levels across all groups fell within normal physiological range for broilers, indicating that PLAE did not induce oxidative stress at any inclusion level. Overall, the results demonstrate that PLAE has beneficial antioxidant properties, especially at moderate inclusion levels, supporting better physiological stability and metabolic function in broiler birds. The extract promoted oxidative balance by enhancing antioxidant enzyme activity while preventing lipid peroxidation.

Table 4: Anti-oxidative response of broiler birds placed on varying levels of PLAE

Parameters	Positive control	Negative control	10g/L PLAE	20g/L PLAE	30g/L PLAE	SEM	Normal value
Total Protein(mg/dl)	94.89	72.45	65.79	66.67	80.00	12.09	
MDA(nmol/mL)	2.15	2.00	1.40	1.30	1.60	0.00	1.00–3.50
SOD(U/mL)	160.00	132.00	161.00	165.00	89.00	0.10	80-200

[a, b, c] means with different superscripts within a row are significantly ($P<0.05$) different. MDA: Malondialdehyde, SOD: Superoxide dismutase

The effects of Pawpaw Leaf Aqueous Extract (PLAE) on the anti-oxidative response of broiler birds are presented in Table 4 Total protein levels varied across treatments, with the highest value observed in the positive control group (94.89 mg/dL), while the negative control recorded 72.45 mg/dL. Birds administered 10 g/L and 20 g/L PLAE showed slightly reduced total protein levels of 65.79 mg/dL and 66.67 mg/dL, respectively. However, birds treated with 30 g/L PLAE exhibited an improved total protein value (80.00 mg/dL), indicating a dose-dependent trend. Malondialdehyde (MDA), an indicator of lipid peroxidation, ranged (1.30 – 2.15) across all treatment groups, suggesting minimal oxidative stress and effective antioxidant protection across the treatments. Superoxide dismutase (SOD) activity, an important antioxidant enzyme, ranged from 89 to 165 U/mL across treatments. The positive control recorded 160 U/mL, while the negative control had 132 U/mL. The 20 g/L PLAE group showed the highest SOD activity (165 U/mL), indicating enhanced antioxidant defense, while the 30 g/L group had a lower SOD value (89 U/mL). The total protein results indicate that inclusion of PLAE influenced the metabolic and physiological status of broiler birds. The slight reduction in total protein at 10 g/L and 20 g/L could be due to increased utilization of available proteins for detoxification and metabolic adaptation to the phytochemical constituents in the extract. The improvement observed at 30 g/L PLAE suggests that higher concentrations may enhance protein synthesis or reduce protein degradation, possibly due to increased antioxidant protection. Similar findings have been reported where plant extracts rich in bioactive compounds improved serum protein profiles in broilers (16); (17). The MDA values (1.30 – 2.15) across treatments demonstrate that birds were not subjected to significant oxidative stress during the experimental period. The absence of lipid peroxidation indicates that both the control and PLAE–supplemented birds maintained optimal oxidative balance. This aligns with earlier studies showing that pawpaw leaf extract contains strong antioxidant compounds such as flavonoids and phenolic acids, which help stabilize cell membranes and prevent lipid oxidation (18); (19). The SOD activity showed variations that reveal how the birds responded to oxidative challenges. The higher SOD activity at 20 g/L PLAE suggests enhancement of endogenous antioxidant enzyme systems, consistent with reports that moderate doses of plant extracts stimulate antioxidant enzyme production (20). The reduced SOD activity in the 30 g/L group compared to 20 g/L may indicate that excessive phytochemical concentration can suppress or overwhelm antioxidant enzyme pathways, as also noted by (21). Nonetheless, SOD levels across all groups fell within normal physiological range for broilers, indicating that PLAE did not induce oxidative stress at any inclusion level. Overall, the results demonstrate that PLAE has beneficial antioxidant properties, especially at moderate inclusion levels, supporting better physiological stability and metabolic function in broiler birds. The extract promoted oxidative balance by enhancing antioxidant enzyme activity while preventing lipid peroxidation.

Conclusions and Applications

1. This study demonstrates that aqueous pawpaw (*Carica papaya*) leaf infusion (PLAE) can serve as a natural alternative to conventional antibiotics in broiler production.
2. The administration of *Carica papaya* leaf aqueous extract (PLAE) positively influenced the hematological and immune responses of broiler chickens, particularly at inclusion levels of 20–30 g/L. The enhanced blood indices and leukocyte profiles suggest improved physiological and

immunological resilience, which are critical for maintaining productivity under environmental and disease stress.

3. These findings demonstrate that PLAE can serve as a natural, eco-friendly alternative to synthetic antibiotics, aligning with the principles of climate-smart agriculture by promoting animal health, reducing dependency on chemical additives, and minimizing environmental contamination from antibiotic residues. Incorporating locally available plant-based resources such as pawpaw leaves into poultry diets supports sustainable production, enhances food safety, and contributes to resilient livestock systems that adapt to the challenges of climate change.
4. The anti-oxidative response of broiler birds administered pawpaw leaf aqueous extract (PLAE) indicates that the extract supports the maintenance of oxidative balance and enhances antioxidant capacity. Birds given moderate levels of PLAE, particularly at 20 g/L, showed improved superoxide dismutase (SOD) activity, reflecting strengthened endogenous antioxidant defense. The absence of lipid peroxidation across treatments further suggests that PLAE effectively prevented oxidative damage. Although total protein levels varied, higher inclusion levels demonstrated improved metabolic stability.
5. Overall, PLAE shows potential as a natural antioxidant supplement capable of promoting physiological well-being and reducing oxidative stress in broiler production.

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