



Effects of Sun-Dried and Blanched Avocado Leaf Meal on Growth Performance, Haematological Parameters, and Oxidative Stress Biomarkers in Broiler Chickens

Olafalay, Roseline Feyisayo^{1*} and Alamuoye, Oluwatoyin Folake²

Ekiti State University, Ado-Ekiti, Nigeria.

*Corresponding Author

olafalayeroseline@gmail.com

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Abstract: This study evaluated the effects of dietary inclusion of sun-dried and blanched avocado (*Persea americana*) leaf meal (ALM) on growth performance, haematological indices, and oxidative stress biomarkers in broiler chickens. One hundred and eighty broiler chickens were randomly assigned to nine dietary treatments: control (T1), sun-dried ALM at 0.25% (T2), 0.50% (T3), 0.75% (T4), and 1.00% (T5); and blanched ALM at 0.25% (T6), 0.50% (T7), 0.75% (T8), and 1.00% (T9) in a completely randomized design. Growth performance parameters (final body weight, body weight gain, feed intake, feed conversion ratio) were monitored from days 28-56. Haematological parameters (PCV, RBC, WBC, Hb, platelet counts, differential leukocyte counts, MCH, MCHC) and oxidative stress markers (superoxide dismutase, glutathione peroxidase, catalase, malondialdehyde, total antioxidant capacity) were determined at the end of the experiment. Results showed no significant differences ($p > 0.05$) in growth performance parameters across treatments, although T5 (1% sun-dried) numerically improved body weight gain (466.67 g). Haematological parameters revealed significant treatment effects: PCV ranged from 34.00% (T9) to 54.00% (T6); WBC ranged from $2400.00 \times 10^3/\mu\text{L}$ (T1) to $4500.00 \times 10^3/\mu\text{L}$ (T9); and haemoglobin ranged from 11.30 g/dL (T9) to 17.65 g/dL (T7). The 1% blanched ALM (T9) induced haematological alterations characterized by reduced PCV, RBC, and Hb, and elevated WBC. Catalase activity was significantly higher ($p < 0.05$) in T3 (0.162 U/mg) and T8 (0.162 U/mg). Malondialdehyde concentrations were significantly reduced ($p < 0.05$) in T3 (0.317 V/mg), T8 (0.295 V/mg), and T9 (0.320 V/mg) compared to control (0.526 V/mg). Dietary inclusion of sun-dried ALM at 0.5-1.0% and blanched ALM at 0.5-0.75% supported broiler performance comparable to control and enhanced antioxidant capacity by reducing lipid peroxidation and improving catalase activity. However, 1% blanched ALM induced adverse haematological effects, indicating a threshold-dependent toxicity. Blanched ALM at 0.5-0.75% inclusion is recommended as the optimal processing method and inclusion level, while sun-dried ALM can be effectively utilized at up to 1.0% inclusion without adverse effects.

Keywords: Avocado leaf meal, blanching, broiler chickens, haematology, oxidative stress, growth performance, antioxidant capacity.

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1. Introduction

The global poultry industry continues to evolve in response to increasing consumer demand for high-quality, safe, and environmentally sustainable animal products (Rafiq *et al.*, 2022). Producers face pressure to enhance production efficiency while maintaining or improving meat quality and safety standards. In recent years, there has been a marked shift toward the use of natural feed additives as alternatives to synthetic growth promoters and antibiotics, which have raised concerns due to their potential residues and contribution to antimicrobial resistance (Rafiq *et al.*, 2022; Ali *et al.*, 2021).

Among the various natural additives explored, plant-based compounds have garnered significant attention for their multifunctional properties. These phytogetic feed additives, derived from herbs, spices, and plant by-products, are known to possess antioxidant, antimicrobial, and immunomodulatory effects that can positively influence animal health and performance (Ali *et al.*, 2021). One such promising candidate is avocado (*Persea americana*) leaf meal (ALM), a by-product of avocado cultivation that is often discarded despite its rich phytochemical profile.

Avocado leaves are abundant in bioactive compounds such as flavonoids, tannins, saponins, and phenolic acids, which have been associated with various biological activities including antioxidant, anti-inflammatory, and antimicrobial effects (García-Vargas *et al.*, 2021; Ademoyegun *et al.*, 2024). These properties suggest that avocado leaf meal could serve as a functional feed additive capable of enhancing broiler chicken performance and meat quality. The antioxidant potential of these compounds, in particular, may contribute to improved oxidative stability, thereby reducing oxidative stress and enhancing physiological function (Abdelli *et al.*, 2021).

However, the efficacy of avocado leaf meal as a feed additive is influenced by the method of processing. Raw plant materials often contain anti-nutritional factors such as oxalates, phytates, and tannins, which can interfere with nutrient absorption and metabolism in poultry. Processing techniques such as sun-drying and blanching have been shown to mitigate these effects by altering the chemical composition and reducing the concentration of anti-nutritional compounds (Kurt *et al.*, 2023). Sun-drying, a traditional and cost-effective method, relies on solar energy to dehydrate plant materials, while blanching involves brief heat treatment to inactivate enzymes and improve digestibility (Che-Galicia *et al.*, 2020).

The choice of processing method not only affects the nutritional profile of avocado leaf meal but also its palatability and safety for poultry consumption. Studies have demonstrated that processed avocado leaves retain significant levels of beneficial phytochemicals while exhibiting reduced toxicity and improved bioavailability (Zaki *et al.*, 2020). These improvements may translate into enhanced feed conversion ratios, growth rates, and physiological status in broiler chickens, making avocado leaf meal a viable additive in commercial poultry diets.

Haematological parameters serve as reliable indicators of the physiological, nutritional, and immunological status of broiler chickens (Kokore *et al.*, 2021; Ogbuagu *et al.*, 2022). Red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration, and white blood cell (WBC) counts provide insights

into oxygen-carrying capacity, immune competence, and overall health status. Similarly, oxidative stress biomarkers including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA) reflect the balance between reactive oxygen species production and endogenous antioxidant defense mechanisms (Tejchman *et al.*, 2021).

Despite the growing interest in phytogetic feed additives, limited information exists on the comparative effects of differently processed avocado leaf meal on broiler performance, haematology, and oxidative status. This study was therefore designed to evaluate the effects of graded levels of sun-dried and blanched avocado leaf meal on growth performance, haematological parameters, and oxidative stress biomarkers in broiler chickens, with the aim of identifying optimal processing methods and inclusion levels for sustainable poultry production.

Materials and Methods

Study Location and Biosecurity Protocols

The experiment was conducted at a dedicated poultry facility located within Ado-Ekiti, Ekiti State, Nigeria (Latitude: 7.623°N, Longitude: 5.221°E; approximate elevation: 455 m above sea level). This site was strategically selected to optimize research conditions, offering advantages in proximity for intensive monitoring, logistical efficiency for resource management, and minimization of environmental variables contributing to experimental error. Rigorous biosecurity protocols were implemented prior to the arrival of experimental chicks, including thorough mechanical cleaning of the brooding house floor, followed by disinfection of all surfaces, feeding troughs, and drinking troughs using 2% Virkon® S solution to establish a sanitized experimental environment.

Experimental Birds, Management, and Dietary Treatments

A total of 200 unsexed day-old broiler chicks (*Gallus gallus domesticus*) were sourced from a certified commercial hatchery, ensuring genetic quality and health status. For the initial four weeks, the chicks were collectively reared under uniform management conditions, receiving a nutritionally complete commercial broiler starter ration devoid of avocado leaf meal. This baseline period established a control condition before the experimental dietary interventions.

At 28 days post-hatch, the chicks were systematically randomized into nine distinct dietary treatment groups: one control group receiving untreated basal diet, four groups receiving diets supplemented with avocado leaf meal (ALM) derived from sun-dried leaves, and four groups receiving diets supplemented with ALM derived from blanched leaves. Each treatment was replicated three times ($n=3$ replicates per treatment), with seven birds assigned to each replicate pen. The birds were housed in 2.6 m × 3 m deep-litter pens bedded with fresh wood shavings, providing a comfortable environment that allowed natural scratching behavior and managed waste. The experimental dietary treatments comprised:

T1: Control (unmodified basal diet without *P. americana* supplementation)

T2: Sun-dried ALM at 0.25% (w/w, as-fed basis)

T3: Sun-dried ALM at 0.50% (w/w, as-fed basis)

T4: Sun-dried ALM at 0.75% (w/w, as-fed basis)

T5: Sun-dried ALM at 1.00% (w/w, as-fed basis)

T6: Blanched ALM at 0.25% (w/w, as-fed basis)

T7: Blanched ALM at 0.50% (w/w, as-fed basis)

T8: Blanched ALM at 0.75% (w/w, as-fed basis)

T9: Blanched ALM at 1.00% (w/w, as-fed basis)

Throughout the trial, the birds had continuous access to their assigned experimental diets and clean drinking water, and received standard prophylactic veterinary care, including scheduled vaccinations and health monitoring to ensure animal welfare and minimize confounding health issues.

Growth Performance Parameters

Quantitative data on broiler growth performance were systematically recorded from

day 28 to day 56 post-hatch. The following parameters were measured:

Body Weight (BW): Recorded weekly using a calibrated electronic balance (Model PGW 4502e, Adam Equipment, UK) after a 12-hour fast to standardize gut fill, following protocols established in Nigerian poultry studies (Fajemilehin *et al.*, 2025).

Feed Intake (FI): Determined gravimetrically by subtracting residual feed from total feed offered per pen over 7-day intervals, with corrections for spillage and mortality (Folorunso *et al.*, 2025).

Mortality: Recorded twice daily (0800 h and 1600 h). Dead birds were weighed to adjust feed intake and performance indices.

Performance indices were calculated using standard formulas:

Body Weight Gain (BWG) = (Final BW – Initial BW) / Number of experimental days
Feed Conversion Ratio (FCR) = Total FI / Total BWG (adjusted for mortality)

These metrics are consistent with National Research Council (NRC, 1994) guidelines and have been validated in Nigerian poultry research (Olawumi and Fajemilehin, 2012).

Blood Sample Collection and Haematological Analysis

At 56 days post-hatch, one bird per replicate was subjected to venipuncture via the jugular vein. Blood intended for haematological analysis was collected into EDTA-coated tubes pre-chilled at 4°C to prevent coagulation.

Packed Cell Volume (PCV) Determination: Packed cell volume was measured using the microhaematocrit technique. Specifically, 75 µL of EDTA-treated blood was transferred into heparinized capillary tubes and centrifuged at 12,000 × g for 5 minutes. Readings were obtained using a calibrated haematocrit reader following the procedures outlined by Campbell and Dein (2007) and Jones (2015).

Erythrocyte and Leukocyte Quantification: Total red blood cell (RBC) and white blood cell (WBC) counts were performed using Natt and Herrick's solution in a Neubauer hemocytometer under light microscopy at 400× magnification (Campbell and Ellis, 2007).

Haemoglobin (Hb) Concentration: Haemoglobin levels were determined using the cyanmethemoglobin method. A 20 µL aliquot

of EDTA-preserved blood was mixed with 5 mL of Drabkin's reagent, incubated at 25°C for 10 minutes, and absorbance read at 540 nm using a UV-visible spectrophotometer (Jones, 2015., 2019; Ogunlade and Egbunike, 2013).

Erythrocyte Indices Calculation: Erythrocyte indices were derived using standard formulae to assess cellular morphology:

$$\text{MCV (fL)} = (\text{PCV} \div \text{RBC}) \times 10$$

$$\text{MCH (pg)} = (\text{Hb} \div \text{RBC}) \times 10$$

$$\text{MCHC (g/dL)} = (\text{Hb} \div \text{PCV}) \times 100$$

These indices provide insights into anaemia classification and erythrocyte function, calculated per methods described by Campbell and Ellis (2007) and Gbore *et al.* (2013).

Differential Leukocyte Counts: Blood smears were prepared, stained with Giemsa stain, and examined under oil immersion (1000×) for differential counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Determination of Oxidative Stress Biomarkers and Antioxidant Capacity

Sample Collection and Preparation: Biological specimens were collected under aseptic conditions to minimize contamination and preserve biochemical integrity. Immediately following collection, samples were stored at -80°C to prevent oxidative degradation and enzymatic activity that could compromise analytical outcomes (Hadwan, 2018). Tissue homogenization was performed in phosphate-buffered saline (PBS, pH 7.4) using a motorized homogenizer. The homogenates were centrifuged at 10,000 × g for 15 minutes at 4°C, and the resulting supernatants were used for subsequent biochemical assays (Senthilkumar *et al.*, 2021).

Determination of Lipid Peroxidation (Malondialdehyde - MDA): Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay. This method relies on the reaction of MDA with thiobarbituric acid (TBA) under acidic and high-temperature conditions to form a pink chromogen (MDA-TBA₂ adduct), which exhibits maximal absorbance at 532 nm (Senthilkumar *et al.*, 2021; Zeb and Ullah, 2016). Samples were treated with 20% trichloroacetic acid (TCA) and 1% TBA in 0.05 M NaOH, heated at 95°C for 60 minutes, cooled, and centrifuged at 3,000 × g for 10 minutes. The absorbance of the supernatant was measured spectrophotometrically, and MDA concentration was calculated using an extinction coefficient of 1.56 × 10⁵ M⁻¹cm⁻¹ (Ohkawa *et al.*, 1979).

Superoxide Dismutase (SOD) Activity Assay: Superoxide dismutase (SOD) activity was assessed based on its ability to inhibit the reduction of WST-1 by superoxide anions generated via xanthine oxidase-mediated oxidation of xanthine. The reaction mixture included WST-1 reagent, xanthine substrate, xanthine oxidase, and assay buffer. Samples were incubated at 37°C for 20 minutes, and absorbance was measured at 450 nm. SOD activity was expressed as units per milligram of protein (Senthilkumar *et al.*, 2020; Zhang *et al.*, 2017).

Catalase (CAT) Activity Assay: Catalase activity was determined by measuring the decomposition of hydrogen peroxide (H₂O₂). Residual H₂O₂ was quantified via its reaction with ammonium molybdate, forming a yellow complex with absorbance at 405 nm

(Kadhun and Hadwan, 2021). Samples were incubated with 30 mM H₂O₂ in 0.05 M phosphate buffer (pH 7.0) for 3 minutes, followed by addition of ammonium molybdate to terminate the reaction. CAT activity was expressed as $\mu\text{mol H}_2\text{O}_2$ decomposed per minute per milligram of protein.

Glutathione Peroxidase (GPx) Activity Assay: GPx activity was measured using a coupled enzymatic assay involving glutathione reductase and NADPH. GPx catalyzes the reduction of H₂O₂ using reduced glutathione (GSH), producing oxidized glutathione (GSSG), which is recycled by glutathione reductase with concurrent oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was monitored spectrophotometrically and used to calculate GPx activity, expressed as nmol NADPH oxidized per minute per milligram of protein (Ahmed *et al.*, 2021; Flohé and Günzler, 1984).

Total Antioxidant Capacity (TAC): TAC was evaluated using the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method, which measures the ability of antioxidants to reduce Cu²⁺ to Cu⁺. The reduced Cu⁺ forms a colored complex with neocuproine, detectable at 490 nm. Samples were incubated with Cu²⁺ reagent, chromogenic substrate, and assay buffer at room temperature for 10 minutes. TAC was expressed in Trolox equivalents ($\mu\text{mol TE/mg protein}$) (Hadwan, 2018; Bekdser *et al.*, 2014).

Protein Quantification: Protein concentration in samples was determined using the Bradford assay (Bradford, 1976), providing a basis for normalizing enzyme activities to protein content to ensure accurate inter-sample comparisons.

2.6 Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using a completely randomized design. Significant differences between treatment means were separated using Duncan's Multiple Range Test at a 5% significance level. All statistical analyses were

performed using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA). Results are presented as mean $\pm$ standard error of the mean (SEM).

Results:

Growth Performance Parameters

Table 1 presents the growth performance parameters of broiler chickens under different dietary treatments. Final body weight (FBW) values ranged from 2.40 ± 0.10 kg (T7) to 3.00 ± 0.26 kg (T5), with no significant differences among treatments. Initial body weight (IBW) ranged from 2.11 ± 0.10 kg (T7) to 2.58 ± 0.16 kg (T3), with no significant variation. Body weight gain (BWG) ranged from 233.33 ± 61.10 g (T9) to 466.67 ± 152.75 g (T5). T5 (1% sun-dried ALM) and T6 (0.25% blanched ALM) numerically improved body weight gain compared to other treatments. Feed intake (FI) values ranged from 120.33 ± 20.57 g (T8) to 197.62 ± 14.87 g (T6), with no significant differences. Feed conversion ratio (FCR) ranged from 1.67 ± 0.54 (T9) to 2.58 ± 0.24 (T2), with no significant treatment effects.

Haematological Parameters

Table 2 presents the haematological parameters of broiler chickens subjected to different dietary treatments. Packed cell volume (PCV) values ranged from $34.00 \pm 0.00\%$ (T9) to $54.00 \pm 4.00\%$ (T6), with significant differences among treatments ($p = 0.002$). T9 had significantly lower PCV compared to all other treatments. White blood cell (WBC) counts ranged from $2400.00 \pm 100.00 \times 10^3/\mu\text{L}$ (T1) to $4500.00 \pm 0.00 \times 10^3/\mu\text{L}$ (T9), with highly significant differences. T9 exhibited markedly elevated WBC counts compared to all other treatments. Red blood cell (RBC) counts ranged from $2.90 \pm 0.00 \times 10^6/\mu\text{L}$ (T9) to $5.85 \pm 0.05 \times 10^6/\mu\text{L}$ (T1), with significant differences. T9 had significantly lower RBC counts compared to T1, T3, T6, and T7.

Table 1: Growth Performance of Broilers under Different Dietary Treatment

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	T9	p-value
FBW (kg)	2.63±0.21	2.83±0.29	2.86±0.23	2.50±0.26	3.00±0.26	2.90±0.66	2.40±0.10	2.43±0.06	2.63±0.32	0.224
IBW (kg)	2.20±0.10	2.40±0.44	2.58±0.16	2.28±0.25	2.53±0.38	2.53±0.65	2.11±0.10	2.20±0.10	2.43±0.32	0.598
BWG (g)	433.33±152.75	433.33±152.75	263.33±55.08	263.33±70.95	466.67±152.75	373.33±110.15	290.00±85.44	276.67±25.17	233.33± 61.10	0.088
FI (g)	193.81±44.41	166.67±50.17	133.33±21.82	145.24±17.98	183.33±28.87	197.62±14.87	157.15±51.51	120.33±20.57	142.29±25.47	0.106
FCR	2.29±0.78	2.58±0.24	2.05±0.68	1.85±0.68	2.50±0.51	1.90±0.60	1.86±0.21	2.32±0.23	1.67±0.54	0.427

T1 = control; T2 = sun-dried ALM 0.25%; T3 = sun-dried ALM 0.50%; T4 = sun-dried ALM 0.75%; T5 = sun-dried ALM 1.00%; T6 = blanched ALM 0.25%; T7 = blanched ALM 0.50%; T8 = blanched ALM 0.75%; T9 = blanched ALM 1.00%

Haemoglobin (Hb) concentrations ranged from 11.30 ± 0.00 g/dL (T9) to 17.65 ± 0.35 g/dL (T7), with significant differences ($p = 0.015$). T9 had significantly lower Hb compared to all other treatments except T6. Platelet (PLT) counts ranged from $140.00 \pm 0.00 \times 10^3/\mu\text{L}$ (T9) to $405.00 \pm 130.00 \times 10^3/\mu\text{L}$ (T6), with significant differences ($p = 0.045$). T9 had significantly lower platelet counts compared to all other treatments. Differential leukocyte counts revealed significant differences across treatments. Neutrophil percentages ranged from $33.00 \pm 1.00\%$ (T1) to $68.50 \pm 9.53\%$ (T2), with T2 and T9 showing elevated values. Lymphocyte percentages ranged from $30.00 \pm 0.00\%$ (T9) to $57.00 \pm 1.00\%$ (T1), with T9 showing the lowest value. Monocyte, eosinophil, and basophil percentages also showed significant variations among treatments.

Mean corpuscular haemoglobin (MCH) values ranged from 18.40 ± 0.00 pg (T2) to 28.90 ± 0.00 pg (T9), with significant differences ($p = 0.009$). Mean corpuscular haemoglobin concentration (MCHC) values ranged from 21.50 ± 0.23 g/dL (T2) to 32.30 ± 0.00 g/dL (T9), with significant differences ($p = 0.012$).

Table 2: Haematological Parameters of Broiler Chickens under Different Treatments

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	T9	p-value
PCV (%)	51.00±1.00 ^a	50.50±1.44 ^a	49.00±2.00 ^a	51.00±3.00 ^a	47.50±1.50 ^a	54.00±4.00 ^a	53.00±1.00 ^a	50.00±2.00 ^a	34.00±0.00 ^b	0.002
WBC ($\times 10^3/\mu\text{L}$)	2400.00±100.00 ^b	2560.00±80.83 ^b	2622.5±102.50 ^b	2555.50±155.50 ^b	2690.00±40.00 ^b	2491.00±70.00 ^b	2455.50±44.50 ^b	2605.50±94.50 ^b	4500.00±0.00 ^a	0.000
RBC ($\times 10^6/\mu\text{L}$)	5.85±0.05 ^a	4.50±0.35 ^{abc}	4.80±1.00 ^{ab}	4.50±0.60 ^{abc}	3.90±0.10 ^b ^c	5.80±0.10 ^a	5.51±0.40 ^{ab}	4.85±1.05 ^{ab}	2.90±0.00 ^c	0.044
Hb (g/dL)	16.95±0.35 ^{ab}	16.80±0.46 ^{ab}	16.30±0.70 ^{ab}	17.05±0.95 ^{ab}	15.80±0.50 ^{ab}	14.05±2.45 ^b ^c	17.65±0.35 ^a	16.65±0.55 ^{ab}	11.30±0.00 ^c	0.015
PLT ($\times 10^3/\mu\text{L}$)	327.50±7.50 ^a	277.50±1.44 ^a	328.50±1.50 ^a	274.50±2.50 ^a	328.00±1.00 ^a	405.00±130.00 ^a	298.50±22.50 ^a	299.00±22.00 ^a	140.00±0.00 ^b	0.045
N%	33.00±1.00 ^c	68.50±9.53 ^a	50.50±2.50 ^c	48.50±7.50 ^d	45.50±8.50 ^d	34.00±0.00 ^f	38.00±7.00 ^c	45.00±12.00 ^d	60.00±0.00 ^b	0.043
L%	57.00±1.00 ^a	46.50±4.91 ^c	39.50±2.50 ^c	42.00±8.00 ^d	44.50±8.50 ^{cd}	56.00±0.00 ^a	49.50±1.50 ^b	45.00±12.00 ^c	30.00±0.00 ^c	0.049
MCH (pg)	25.90±3.00 ^b	18.40±0.00 ^c	26.05±0.35 ^b	25.65±1.05 ^b	25.70±2.60 ^b	22.25±0.15 ^c	20.55±0.15 ^c	23.35±2.55 ^{bc}	28.90±0.00 ^a	0.009
MCHC (g/dL)	27.05±1.25 ^b	21.50±0.23 ^c	27.25±1.15 ^b	26.15±0.45 ^b	27.50±1.40 ^b	28.85±0.55 ^{ab}	25.05±1.45 ^{bc}	28.05±3.75 ^{ab}	32.30±0.00 ^a	0.012

a,b,c,d: means within a row with different superscripts differ significantly ($p < 0.05$). PCV = Packed cell volume; WBC = White blood cells; RBC = Red blood cells; Hb = Haemoglobin; PLT = Platelet; N = Neutrophil; L = Lymphocyte; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration

Oxidative Stress Biomarkers and Antioxidant Capacity

Table 3 presents the oxidative stress markers and total antioxidant capacity across the nine experimental treatments. Superoxide dismutase (SOD) activity ranged from 59.95 ± 1.65 U/mg (T7) to 64.65 ± 2.21 U/mg (T6), with no significant differences ($p > 0.05$) among treatments.

Glutathione peroxidase (GPx) activity ranged from 51.51 ± 1.16 U/mg (T7) to 57.02 ± 0.34 U/mg (T1), with no significant ($p > 0.05$) treatment effects.

Catalase (CAT) activity showed significant differences ($p < 0.05$) among treatments. The highest CAT activities were recorded in T3 (0.162 ± 0.049 U/mg) and T8 (0.162 ± 0.026 U/mg), which were significantly higher than T1 (0.074 ± 0.003 U/mg), T4 (0.070 ± 0.003 U/mg), and T5 (0.074 ± 0.004 U/mg). Intermediate values were observed in T2, T6, T7, and T9.

Malondialdehyde (MDA) concentrations differed significantly ($p < 0.05$) across treatments. The lowest MDA levels were recorded in T8 (0.295 ± 0.040 V/mg), T3 (0.317 ± 0.027 V/mg), and T9 (0.320 ± 0.048 V/mg), which were significantly lower ($p < 0.05$) than the control (T1: 0.526 ± 0.133 V/mg). T2, T4, T5, T6, and T7 showed intermediate MDA values. Total antioxidant capacity (TAC) ranged from 79.3 ± 5.5 μ mol (T4) to 92.6 ± 2.1 μ mol (T5), with no significant differences ($p > 0.05$) among treatments.

Table 3: Oxidative Stress Markers and Antioxidant Capacity of Broiler Chickens Fed the Dietary Treatments

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	T9	p-value
SOD (U/mg)	62.65 ± 2.94	60.63 ± 1.47	60.97 ± 1.21	63.64 ± 0.66	63.97 ± 2.03	64.65 ± 2.21	59.95 ± 1.65	60.63 ± 0.89	61.30 ± 2.54	0.583
GPx (U/mg)	57.02 ± 0.34	54.38 ± 2.16	54.58 ± 3.15	56.25 ± 1.22	52.34 ± 1.65	56.28 ± 0.92	51.51 ± 1.16	52.31 ± 2.85	53.26 ± 1.72	0.401
CAT (U/mg)	0.074 ± 0.003^b	0.101 ± 0.018^{ab}	0.162 ± 0.049^a	0.070 ± 0.003^b	0.074 ± 0.004^b	0.098 ± 0.031^{ab}	0.088 ± 0.022^{ab}	0.162 ± 0.026^a	0.112 ± 0.026^{ab}	0.038
MDA (V/mg)	0.526 ± 0.133^a	0.349 ± 0.020^{ab}	0.317 ± 0.027^b	0.360 ± 0.028^{ab}	0.360 ± 0.036^{ab}	0.390 ± 0.020^{ab}	0.358 ± 0.023^{ab}	0.295 ± 0.040^b	0.320 ± 0.048^b	0.020
TAC (μ mol)	84.2 ± 9.2	85.2 ± 11.3	89.2 ± 3.9	79.3 ± 5.5	92.6 ± 2.1	87.1 ± 2.0	90.3 ± 3.9	80.3 ± 7.2	92.2 ± 2.7	0.747

a,b: means within a row with different superscripts differ significantly ($p < 0.05$). SOD = Superoxide dismutase; GPx = Glutathione peroxidase; CAT = Catalase; MDA = Malondialdehyde; TAC = Total antioxidant capacity

Discussion

The absence of significant differences in growth performance parameters across dietary treatments indicated that inclusion of avocado leaf meal (ALM), regardless of processing method or inclusion level up to 1.0%, did not adversely affect broiler growth performance. This finding is consistent with previous studies reporting that moderate inclusion of phytogetic feed additives could maintain growth performance comparable to control diets (Fajemilehin *et al.*, 2025; Adegbenro *et al.*, 2023).

The numerically higher body weight gain observed in T5 (1.0% sun-dried ALM) and T6 (0.25% blanched ALM) suggested potential growth-promoting effects at specific inclusion levels. This might be attributed to the bioactive compounds present in ALM, particularly flavonoids and phenolic acids, which had been shown to enhance nutrient utilization and metabolic efficiency (Lv *et al.*, 2021; Mutha *et al.*, 2021). However, the lack of statistical significance indicated that these effects were not sufficiently pronounced to achieve detectable differences within the experimental conditions.

The comparable feed conversion ratios across treatments suggested that ALM inclusion did not compromise feed efficiency. This is an important finding from a practical standpoint, as feed efficiency is a primary determinant of production profitability. The numerical improvement in FCR observed in T9 (1.67) compared to control (2.29), despite not reaching statistical significance, warrants further investigation with larger sample sizes to determine whether this represents a biologically meaningful trend.

The reduction in final body weight for treatments T4 (sun-dried ALM at 0.75%), T7 (blanched ALM at 0.50%), and T8 (blanched ALM at 0.75%) suggested potential growth-impairing effects at specific inclusion levels. This might be attributed to the presence of persin, a documented toxin in avocado leaves that could cause reduced feeding and metabolic disturbances in animals (Oelrichs *et al.*, 1995; Butt *et al.*, 2006). The inconsistent effect across similar treatment levels implied that toxicity is not solely dose-dependent but is also influenced by processing methods and individual animal sensitivity.

The significant haematological alterations observed, particularly in T9 (1.0% blanched ALM), highlighted the potent bioactivity of phytochemicals present in *P. americana* leaf meal and their processing-dependent effects on broiler physiology. The haematological profile of T9 characterized by suppressed erythroid series (PCV, RBC, Hb, PLT) alongside elevated leukocyte count was highly indicative of a compound-induced bone marrow response.

The depressed RBC count, PCV, and Hb collectively pointed toward impaired erythropoiesis. This could be mediated by bioactive constituents like persin, which has demonstrated cytotoxic properties in other species (Oelrichs *et al.*, 1995). At high inclusion levels, such compounds might inflict low-level oxidative damage on sensitive progenitor cells within the bone marrow, disrupting the normal differentiation and proliferation of erythrocytes and megakaryocytes. The simultaneous elevation in total WBC count, particularly the noted neutrophilia, represented a well-documented reaction to physiological stress or the presence of foreign antigens (Tizard, 2013). It is plausible that the high dose of

blanched ALM acted as a chronic, low-grade stressor or immunogen, provoking an inflammatory-like state that stimulated leukopoiesis even as erythropoiesis was compromised.

The erythrocyte indices provided further diagnostic clarity. The elevated MCHC in treatment with 1% blanched ALM indicated that the few erythrocytes produced were normochromic to hyperchromic, containing high haemoglobin density. This profile was inconsistent with simple nutritional anaemia but characteristic of a regenerative or haemolytic process, where the bone marrow released stress reticulocytes in an attempt to compensate for peripheral cell loss or destruction (Stockham and Scott, 2008). This stands in contrast to the lower MCH and MCHC observed in T2 (0.25% sun-dried ALM), which might reflect a very mild, subclinical interference with haem synthesis an effect not apparent at higher doses of sun-dried ALM.

The general homogeneity of control through 0.75% blanched ALM aligned with the established concept of hormesis, where low doses of phytochemicals could be benign or even beneficial, while high doses became toxic (Calabrese and Blain, 2009). The stark outlier status of T9 (blanched ALM at 1.00%) underscored the critical importance of processing. Blanching, often used to deactivate anti-nutritional factors, might have paradoxically increased the bioavailability or altered the chemical structure of certain thermostable compounds, thereby enhancing their biological potency at the highest inclusion rate. This dose-processing interaction is a key finding, as previous research on phytogetic feed additives has largely emphasized their safety and growth-promoting effects at moderate levels (Windisch *et al.*, 2008), without always highlighting the potential for toxicity at upper limits with specific processing methods.

The non-significant alterations in SOD, GPx, and TAC across treatment groups were scientifically noteworthy. This suggested that the basal diet provided sufficient antioxidant baseline, and supplemented ALM did not provoke substantial upregulation of these particular defense enzymes or the overall non-enzymatic antioxidant pool. This finding aligned with the concept of antioxidant homeostasis, where exogenous antioxidant supplementation might not necessarily boost endogenous enzyme activity unless the system is under significant oxidative challenge or the supplement acted through specific inductive pathways (Birben *et al.*, 2012).

The significant elevation of catalase activity specifically in groups T3 (0.50% sun-dried ALM) and T8 (0.75% blanched ALM) was a pivotal finding. Catalase is uniquely dedicated to the dismutation of high concentrations of hydrogen peroxide (H₂O₂). This selective induction suggested that ALM in these specific treatments might have generated a mild, specific pro-oxidant signal from H₂O₂, sufficient to trigger CAT gene expression via redox-sensitive transcription factors, without overloading the system or significantly affecting SOD which produced H₂O₂ (Ighodaro and Akinloye, 2018). The differential effect of processing is critical here. Blanching, by inactivating polyphenol oxidases, likely preserved a higher level of specific phenolic compounds, such as certain hydroxycinnamic acids, which might be more bioavailable or potent in modulating CAT activity at the 0.75% inclusion level. This enzyme-specific response has been documented; for instance, Zhang *et al.* (2023) found that certain phytochemicals could preferentially enhance catalase activity over other antioxidant

enzymes in broilers, depending on their chemical structure and the metabolic pathways they influence.

The significant reduction in malondialdehyde (MDA) levels in T3, T8, and T9 is perhaps the most functionally important result. MDA is a terminal marker of lipid peroxidation, indicating oxidative damage to cell membranes. The lower MDA in these groups provided direct evidence of enhanced protection of cellular lipids from oxidative degradation. The implications of high MDA are severe, encompassing reduced membrane fluidity, increased cellular permeability, and damage to membrane-bound proteins and receptors (Ayala *et al.*, 2014). The reduction observed in T3, T8, and T9, which overlapped with the groups showing high CAT activity (T3, T8), strongly suggested that the combined action of direct free radical scavenging by ALM polyphenols and the enhanced breakdown of H₂O₂ by catalase effectively suppressed the chain reaction of lipid peroxidation.

The efficacy of the 1.00% blanched ALM (T9) in reducing MDA without a concurrent spike in CAT activity indicated that at this higher dosage, the direct antioxidant capacity of the blanched leaf's phytochemicals might be sufficient to quench radicals before they initiated lipid damage, highlighting a potential dose-dependent mechanism for blanched ALM. This aligned with findings from Ibrahim *et al.* (2021), who reported that plant extracts rich in polyphenols significantly reduced MDA levels in broiler meat, correlating with improved lipid stability.

The contrasting responses between 1% blanched ALM inclusion haematological toxicity and its beneficial antioxidant effects (reduced MDA) presented an intriguing paradox. This suggested that at the 1.0% blanched ALM inclusion level, the direct radical-scavenging activity of abundant polyphenols might have protected against lipid peroxidation in peripheral tissues, while the bone marrow, with its high proliferative rate and metabolic activity, remained vulnerable to cytotoxic compounds like persin. This tissue-specific vulnerability underscored the complexity of assessing feed additive safety and efficacy, highlighting the need for comprehensive evaluation across multiple physiological systems rather than reliance on single parameters.

The distinct physiological responses to sun-dried versus blanched ALM could be attributed to their differing phytochemical profiles resulting from processing. Sun-drying, as a slower, less disruptive dehydration process, primarily removed water without causing significant leaching of soluble compounds. This preserved the original proportional composition of solids, resulting in retention of both beneficial phytochemicals and anti-nutritional factors (Benjamin *et al.*, 2022). In contrast, blanching involves brief heat treatment that causes leaching of water-soluble components into the blanch water, effectively reducing concentrations of soluble anti-nutrients such as oxalates and phytates while potentially increasing the relative concentration or bioavailability of thermostable compounds (Rickman *et al.*, 2007; Boda *et al.*, 2025).

The haematological toxicity observed only with 1.0% blanched ALM, not with the equivalent sun-dried ALM level, suggested that blanching might have increased the bioavailability of persin, a lipid-soluble toxin not removed by aqueous processing. This phenomenon has been previously documented during processing of other plant materials, where thermal treatment could disrupt cell wall structures, releasing bound compounds and enhancing their extractability and potential bioaccessibility (Shi *et al.*, 2019).

The antioxidant benefits, particularly enhanced catalase activity and reduced MDA, were observed with both processing methods but at different optimal inclusion levels: 0.50% sun-dried (T3) and 0.75% blanched (T8). This suggested that sun-dried ALM, with its higher retention of water-soluble phenolics, might achieve antioxidant effects at lower inclusion levels, while blanched ALM, with its reduced anti-nutritional load, might be safely included at moderately higher levels before encountering toxicity thresholds.

The findings of this study have several important implications for the practical application of avocado leaf meal in broiler nutrition. First, both sun-dried and blanched ALM could be included in broiler diets up to certain thresholds without compromising growth performance, supporting their potential as sustainable feed additives that valorize agricultural by-products.

Second, the haematological data revealed that the physiological response to ALM was not linear but threshold-dependent, heavily influenced by both inclusion level and processing technique. The results for the 1.0% blanched ALM group signalled a potential risk, suggesting that this specific formulation might induce a state of bone marrow suppression coupled with immunological stimulation, necessitating caution in its application within poultry feed formulations.

Third, the antioxidant benefits, particularly the significant reduction in lipid peroxidation (MDA), demonstrated that ALM supplementation could enhance oxidative stability, which might have downstream effects on meat quality, shelf life, and consumer acceptability. This aligned with the growing interest in natural antioxidants as alternatives to synthetic preservatives in animal production systems.

Fourth, the differential responses to sun-dried versus blanched ALM highlight the critical importance of processing method optimization. Sun-dried ALM appeared safer at higher inclusion levels (up to 1.0%) but might have lower bioavailability of certain beneficial compounds. Blanched ALM offered enhanced phytochemical bioavailability but with a narrower safety margin, requiring more precise inclusion level control.

Avocado leaf meal (*Persea americana*) could be safely included in broiler diets at moderate levels, with optimal benefits observed at 0.5–0.75% (blanched) and up to 1.0% (sun-dried). These levels supported growth and enhanced antioxidant status without compromising haematological health, whereas higher inclusion (1.0% blanched) induced toxicity and should be avoided.

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