



Dietary supplementation with mango leaf powder influences broiler chickens' growth characteristics, blood parameters, and carcass

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ABSTRACT. This study examined the impact of mango leaf powder supplementation on broiler chickens' growth, blood parameters, and carcass of broiler chickens. Two hundred (200) day-old broilers were divided into four groups: Control (CONT), 200 mg kg⁻¹ Vitamin C (VITC), 250 mg kg⁻¹ mango leaf powder (MLP2), and 500 mg kg⁻¹ mango leaf powder (MLP3). VITC, MLP2, and MLP3 groups showed significantly higher ($p < 0.05$) body weight gain than CONT, with similar feed intake and conversion ratios ($p > 0.05$). Organ weights (heart, lung, liver, spleen) were unaffected ($p > 0.05$), except for the pancreas in MLP2, which was lower ($p < 0.05$) than CONT. Hematological indices exhibited no significant differences ($p > 0.05$). Serum total protein, aspartate aminotransferase, alanine aminotransferase, cholesterol, and creatinine remained stable ($p > 0.05$). However, serum catalase, glutathione peroxidase, and lipid peroxidase levels were significantly ($p < 0.05$) increased with mango leaf powder supplementation. Mango leaf powder supplementation improved body weight gain, dressed percentage, and oxidative status of broiler chickens.

Keywords: antioxidative status; blood; carcass; chickens; performance; phytosupplement.

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Introduction

Poultry production, particularly broiler farming at the commercial level, plays a pivotal role in meeting the escalating demand for high-quality protein in the tropics and subtropics. Consequently, the optimization of the growth performance, health, and carcass quality of broiler chickens is imperative for ensuring profitable, sustainable and efficient poultry production (Attia et al., 2022). In tropical and subtropical regions, where environmental stressors such as high ambient temperatures prevail, the challenges of heat stress significantly impact broiler performance and overall wellbeing, warranting a strategic approach to alleviate these effects through dietary interventions or nutritional modification (Kpomasse, Oke, Houndonougbo, & Tona, 2021).

In recent years, the interest in the use of natural antioxidants derived from plants, commonly referred to as phytochemicals, as a means to promote health and well-being of broiler chickens is growing (Oloruntola, Ayodele, Omoniyi, Adeyeye, & Adegbeye, 2022). Phytochemicals are a diverse group of bioactive compounds. They have been recognized for their ability to scavenge free radicals, neutralize reactive oxygen species (ROS), and modulate cellular redox balance (Warraich, Hussain, & Kayani, 2020). In addition, the phytochemical-rich plants apart from their antioxidant properties offer various health benefits such as anti-inflammatory, antimicrobial, and anti-cancer properties (Zhang et al., 2015).

Mango (*Mangifera indica*) leaves demonstrated notable antioxidant and nutraceutical properties due to their polyphenolic compounds, such as flavonoids and phenolic acids. These bioactive compounds contributed to the leaves' potential health benefits by scavenging free radicals, reducing oxidative stress, and displaying anti-inflammatory effects (Kumar et al., 2021). Previous study suggests that mango leaf may have antidiabetic and hypolipidemic effects, making them potentially valuable in managing diabetes and promoting cardiovascular health (Saleem, Tanvir, Akhtar, Iqbal, & Saleem, 2019). Traditionally used in ethnoveterinary medicine, mango leaves find applications in functional feeds and dietary supplements,

showcasing their nutraceutical potential (Shah, Patel, Patel, & Parmar, 2010). The effects of mango leaf powder dietary supplementation on broiler chickens raised under the typical heat stressed condition has not been fully explored. Therefore, this experiment was conducted to unveil the effects of mango leaf powder dietary supplementation on the performance, carcass and internal organs, haematological indices, serum biochemical indices, and antioxidant enzymes of broiler chickens raised under tropical conditions.

Materials and methods

Study site and ethical approval

The Research and Moral Code Committee of the Department of Animal Health and Production Technology at the Federal College of Agriculture, Akure (FCAA), has approved all experimental animal protocols for this study. The research was conducted at the FCAA Research Farm.

Leaf collection, processing, and analysis

Fresh mango leaves, harvested from mother plants, were finely chopped using stainless knives and spread lightly on a clean tarpaulin for air-drying over two-weeks. Subsequently, the dried leaves were milled into mango leaf powder. The analysis included assessments for 2-diphenyl-1-picrylhydrazylhydrate, flavonoids, and alkaloids, following the methods outlined by Zhao et al. (2018) and Gul, Jan, Faridullah, Sherani, and Jahan (2017). Additionally, the concentrations of saponin and tannin, as per the procedures described by Senguttuvan, Paulsamy, and Karthika (2014), were also determined.

Experimental diets

Broiler starter (0-21 days) and finisher (22-42 days) diets were formulated and prepared for feeding the broiler chickens (refer to Table 1 for details). The experimental diets were divided into four equal portions during both the starter and finisher phases.

CONT: Control (no supplementation).

VITC: 200 mg kg⁻¹ Vitamin C supplementation.

MLP2: 250 mg kg⁻¹ mango leaf powder supplementation.

MLP5: 500 mg kg⁻¹ mango leaf powder supplementation.

All the diets were analyzed for crude protein and crude fibre composition (Association of Official Analytical Chemistry [AOAC], 2016).

Table 1. Experimental diets and Phytosupplement.

Ingredients (100 g kg ⁻¹)	Starter 1-21 days	Finisher 22-42 days	Mango leaf powder	
			Parameters (g kg ⁻¹)	Quantity
Soybean meal	35.50	27.50	DPPH (%)	67.21
Maize	52.70	63.00	Flavonoids	24.56
Maize offals	4.00	2.20	Alkaloids	83.40
Fish meal	3.00	2.50	Saponins	22.25
Bone meal	3.00	3.00	Tannins	1.49
Oyster shell	0.90	0.90	Phenols	6.54
*Premix	0.30	0.30		
Salt	0.25	0.25		
Lysine	0.15	0.15		
Methionine	0.20	0.20		
Analyzed indices (g 100g ⁻¹)				
Crude protein	21.33	18.93		
Crude fiber	4.28	4.09		
Calculated indices				
ME (kcal kg ⁻¹)	2909.32	3123.51		
Methionine	0.47	0.44		
Lysine	1.50	1.42		
Ca	0.96	0.85		
Total P	0.48	0.37		

DPPH: 2-diphenyl-1-picrylhydrazyl hydrate; *1 kg of vitamin-mineral premix contains: Vitamin D3 - 2,000,000 IU; Vitamin K - 2,250 mg; Vitamin A 10,000,000 IU; Vitamin E - 20,000 IU; Thiamine B1 - 1,750 mg; Niacin - 27,500 mg; Pantothenic acid - 7,500 mg; Biotin - 50 mg; Choline chloride - 400 g; Riboflavin B2 - 5,000 mg; Pyridoxine B6 - 2,750 mg; Antioxidant - 125 g; Magnesium - 80 g; Iodine - 1.2 g; Selenium - 200 mg; Cobalt - 200 mg; Zinc - 50 mg; Iron - 20 g; Copper - 5 g; ME: Metabolisable energy.

Birds, housing, and experimental design

Two hundred Cobb 500 breed broiler chickens were randomly assigned to four experimental diets (5 replicates per dietary treatment, with 10 birds per replicate and 50 birds per experimental diet). Each replicate, comprising 10 birds, was accommodated in pens measuring 200x100 cm. Throughout the feeding study, the EU's animal welfare guidelines were followed, ensuring a daily lighting cycle in the experimental pen consisting of 6 hours of darkness within every 24-hour period. This lighting schedule was maintained until 3 days before slaughter. The average temperature in the experimental house was maintained at 30°C±2 for the initial seven days, gradually decreasing by 2°C after each consecutive seven days until reaching 26°C±2. Feed was provided *ad libitum* throughout the experimental period.

Growth performance, blood collection, slaughtering, and carcass analysis

Body weight (BW), body weight gain (BWG), and feed intake (FI) were assessed at seven-day intervals. The feed conversion ratio (FCR) was calculated as the ratio of feed consumed to body weight gain. On day 42 of the feeding trial, three birds per replicate were randomly chosen, tagged, weighed, stunned, and sacrificed by cutting the jugular veins in their neck region. The blood from the slaughtered chickens was collected in plain bottles for serum biochemical indices (total protein, aspartate aminotransferase, alanine aminotransferase, cholesterol, and creatinine) and serum antioxidant enzyme analysis (catalase, glutathione peroxidase, and lipid peroxidation). Additionally, blood was collected into ethylenediaminetetraacetic acid (EDTA) bottles for hematological studies, following the methods outlined by Sastry (1983).

The blood samples collected in plain bottles were centrifuged, and their serum was separated into another set of plain bottles, frozen at -20°C before analysis. Serum biochemical indices were determined using kits with a Reflectron® Plus 8C79 (Roche Diagnostic, GonbH Mannheim, Germany). Additionally, serum catalase (Holovská Jr. et al., 2003), serum glutathione peroxidase (Wang et al., 2018), and serum lipid peroxidation (Eze, Anene, & Chukwu, 2008) were assessed. The slaughtered birds were dressed and weighed to determine the dressed percentage. Subsequently, the internal organs (heart, lung, liver, spleen, and pancreas) were weighed and expressed as a percentage of the slaughtered weight.

Data analysis

In this experiment, the Completely Randomized Design was employed with the model: $Y_{dj} = \mu + \alpha_d + \epsilon_{dj}$. Here, Y_{dj} represents any response variable, μ is the overall mean, α_d signifies the effect of the i th treatment (d = diets 1, 2, 3, and 4), and ϵ_{dj} accounts for the random error due to experimentation. The variance among the collected data within treatments was analyzed using SPSS version 20 software. Duncan's test, implemented through SPSS, was utilized to differentiate the treatment means.

Results and discussion

Performance indices, dressed percentage and internal organ of the broiler chickens

Table 2 shows the performance characteristics of broiler chickens (1-42 days) fed mango leaf powder supplemented diets. The body weight gain of the birds on VITC, MLP2 and MLP5 treatment groups were similar ($p > 0.05$) but significantly ($p < 0.05$) higher than those birds in CONT. However the feed intake and feed conversion ratio were similar ($p > 0.05$) across the treatment groups. These results provide valuable insights into the potential of mango leaf powder as a dietary supplement in alleviating the adverse effects of heat stress on growth performance in broiler chickens raised in tropical environments. The observed similarity in body weight gain among the VITC, MLP2, and MLP5 treatment groups suggests that mango leaf powder supplementation at both 250 (MLP2) and 500 mg kg⁻¹ (MLP5) levels is as effective as the positive control (VITC) in promoting broiler chicken growth under heat stress conditions. This outcome aligns with the notion that phytogenic supplements, and mango leaf powder being used in this study with its potential bioactive compounds, could positively influence the growth characteristics of broiler chickens, possibly by mitigating the impact of heat stress on metabolic processes (Abdelli, Solà-Oriol, & Pérez, 2021; Kumar et al., 2021). This result may be attributed to the antioxidant and anti-inflammatory activities of mango leaf powder (Kumar et al., 2021), and Vitamin C (Hieu, Guntoro, Qui, Quyen, & Al Hafiz, 2022). The similar feed intake recorded across treatment groups in this study is similar to earlier report by Momo et al. (2023) and also implies that the mango leaf powder dietary inclusion is well-tolerated and acceptable by the broiler chickens,

making it an acceptable, viable and palatable dietary phytogetic supplement in broiler diets (Momo et al., 2023). The comparable feed conversion ratios across all treatment groups in this study is a positive outcome, as an optimal feed conversion ratio is indicative of efficient nutrient utilization by the birds and a critical factor in the economic feasibility of broiler production (Ramukhithi et al., 2023). Therefore by implication, the efficiency of converting feed into body weight was not adversely affected by mango leaf powder supplementation.

The dressed percentage of birds in VITC, MLP2, and MLP5 treatment groups were significantly ($p < 0.05$) higher than in CONT (Table 3). The heart, lung, liver and spleen were not affected ($p > 0.05$) by the dietary treatments. The pancreas relative weight of the birds in MLP2 groups were similar ($p > 0.05$) to those in MLP5 and VITC, but significantly ($p < 0.05$) lower than those in CONT; furthermore, the pancreas relative weight of birds in CONT, VITC and MLP5 are similar ($p > 0.05$). The dressed percentage is a crucial indices used for determining the overall meat yield from broiler chickens. In this study, the dressed percentage in the VITC, MLP2, and MLP5 treatment groups being higher than that in the CONT group suggest that dietary supplementation with mango leaf powder at both 250 and 500 mg kg⁻¹, as well as Vitamin C at 200 mg kg⁻¹, has a positive influence on the overall carcass yield of the broiler chickens. This result aligns with a previous study highlighting the potential of phytogetic dietary supplementation in enhancing broiler chicken meat yield (Orlowski et al., 2018). Interestingly, despite the observed improvement in dressed percentage, the heart, lung, liver, and spleen relative weights not being affected by the dietary supplementation suggests normalcy in these organs development across all treatment groups and the absence of adverse effects on the organs (Ayodele, Oloruntola, & Agbede, 2016).

The pancreas functions in nutrients' digestion and metabolism. The similar pancreas relative weight between MLP2, MLP5, and VITC treatment groups implies that the dosage of mango leaf powder (250 mg kg⁻¹) and higher dosage of mango leaf powder (500 mg kg⁻¹) can produce effects comparable to those of the positive control (Vitamin C supplementation at 200 mg kg⁻¹). This finding is important because the development of organs like the pancreas involves the maturation of cells responsible for producing digestive enzymes that play a key role in breaking down complex nutrients into forms that can be easily absorbed (Karpińska & Czauderna, 2022). Consequently, impaired pancreas development or growth can alter the normal enzyme production, resulting in digestive inefficiencies (Röder, Wu, & Han, 2016; Karpińska & Czauderna, 2022).

Haematological and serum biochemistry indices of the broiler chickens

The haematological indices of broiler chickens (age 42 days) fed mango leaf powder supplemented diets were not significantly ($p > 0.05$) affected by the dietary treatments (Table 4). The haematological indices evaluation, analysis, and interpretation are crucial for assessing the physiological status and overall health of broiler chickens. In this study, the haematological indices, in general, were not significantly affected by the different dietary supplementations. This suggests that the inclusion of mango leaf powder at both 250 (MLP2) and 500 mg kg⁻¹ (MLP5) levels did not exert significant alterations in parameters such as red blood cell count, haemoglobin concentration, and haematocrit. It is noteworthy that the mean cell haemoglobin concentration and white blood cell counts tended to be influenced by the dietary treatments ($p = 0.08$). Although not statistically significant, this trend suggests a potential modulatory effect of phytogetic supplements such as mango leaf powder on white blood cell counts of the broiler chickens (Huang & Lee, 2018). In addition, since the mean cell haemoglobin concentration indicates the average haemoglobin per red blood cell, reflecting the oxygen-carrying capacity of the blood. Therefore the observed trend in mean cell haemoglobin concentration may imply that mango leaf powder dietary supplementation could have subtle effects on the oxygen-carrying capacity of red blood cells of the broiler chickens. Mango leaves are known to contain various phytochemicals such as mangiferin, polyphenols, flavonoids, and tannins that have antioxidant properties and cellular function influencing properties such as protection of red blood cells from oxidative stress, consequently indirectly affecting their oxygen-carrying capacity (Kumar et al., 2021).

Table 2. The performance characteristics of broiler chickens (1–42 days) fed mango leaf powder supplemented diets.

Parameters	CONT	VITC	MLP2	MLP5	SEM	P value
IBW (g bird ⁻¹)	44.67	45.17	44.42	44.92	0.26	0.83
BWG (g bird ⁻¹)	2317.40 ^b	2491.23 ^a	2439.64 ^a	2462.98 ^a	25.07	0.03
FI (g bird ⁻¹)	3827.99	3711.85	3980.20	3732.18	60.82	0.44
FCR	1.65	1.49	1.63	1.51	0.03	0.15

CONT: Control (no supplementation); VITC: 200 mg kg⁻¹ Vitamin C supplementation MLP2: 250 mg kg⁻¹ mango leaf powder supplementation; MLP5: 500 mg kg⁻¹ mango leaf powder supplementation. IBW: initial body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; SEM: Standard error the mean. Means within a row with different letters are significantly different ($p < 0.05$).

Table 3. The dressed percentage and internal organ weight (% slaughtered weight) of broiler chickens (age 42 days) fed mango leaf powder supplemented diets.

Parameters (%)	CONT	VITC	MLP2	MLP5	SEM	P value
Dressed %	74.95 ^b	76.59 ^a	76.78 ^a	77.05 ^a	0.28	0.01
Heart	0.41	0.37	0.32	0.40	0.02	0.21
Lung	0.53	0.51	0.48	0.57	0.02	0.61
Liver	2.22	2.03	1.95	2.00	0.07	0.64
Spleen	0.09	0.10	0.12	0.06	0.01	0.19
Pancrease	0.23 ^a	0.19 ^{ab}	0.17 ^{bc}	0.18 ^{ab}	0.01	0.01

CONT: Control (no supplementation); VITC: 200 mg kg⁻¹ Vitamin C supplementation MLP2: 250 mg kg⁻¹ mango leaf powder supplementation; MLP5: 500 mg kg⁻¹ mango leaf powder supplementation; SEM: Standard error the mean. Means within a row with different letters are significantly different (p < 0.05).

Table 4. The haematological indices of broiler chickens (age 42 days) fed mango leaf powder supplemented diets

Parameters	CONT	VITC	MLP2	MLP5	SEM	P value
Packed cell volume (%)	30.72	33.13	30.50	30.00	0.52	0.13
Red blood cell (x10 ¹² L ⁻¹)	2.96	2.67	2.85	3.45	0.14	0.32
Haemoglobin conc. (g dL ⁻¹)	9.82	10.61	10.16	10.00	0.16	0.44
Mean cell haemoglobin conc. (g dL ⁻¹)	33.21	33.17	33.27	33.33	0.02	0.08
Mean cell volume (fl)	108.51	125.92	108.65	86.84	6.13	0.15
Mean cell haemoglobin (pg)	34.60	40.17	36.21	28.94	1.78	0.15
White blood cell (x10 ⁹ L ⁻¹)	2.41	2.32	2.80	3.60	0.21	0.08

CONT: Control (no supplementation); VITC: 200 mg kg⁻¹ Vitamin C supplementation MLP2: 250 mg kg⁻¹ mango leaf powder supplementation; MLP5: 500 mg kg⁻¹ mango leaf powder supplementation; SEM: Standard error the mean. Means within a row with different letters are significantly different (p < 0.05).

Table 5 shows the serum biochemical indices of broiler chickens (age 42 days) fed mango leaf powder supplemented diets. Importantly, serum total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, and creatinine showing no significant (p > 0.05) differences across dietary treatments suggests that mango leaf powder supplementation did not adversely affect overall protein status, liver function, or metabolic homeostasis and by implication, the mango leaf powder dietary supplementation supports the safety and overall health status of the broiler chickens (Momo et al., 2023).

The serum antioxidant enzymes and lipid peroxidation of the broiler chickens

Table 6 revealed the impacts of dietary supplementation with mango leaf powder on serum catalase, glutathione peroxidase, and lipid peroxidation concentrations in broiler chickens at 42 days of age. The serum catalase levels in broiler chickens in MLP2 dietary group were comparable (p > 0.05) to those in MLP5 and VITC dietary groups. However, they were significantly (p < 0.05) higher than those in the CONT dietary group. This suggests that the dietary supplementation of 250 mg kg⁻¹ mango leaf powder improved the catalase activity, demonstrating an antioxidative effect similar to the higher supplementation levels of 500 (MLP5) and 200 mg kg⁻¹ vitamin C (VITC). The elevated serum catalase concentration indicates an enhanced ability to neutralize reactive oxygen species, potentially contributing to improved oxidative stress resistance in the broiler chickens at the age of 42 days (Tang, Fang, Xue, Yang, & Lu, 2022).

The serum glutathione peroxidase in broiler chickens fed VITC, MLP2, and MLP5 diets was similar (p > 0.05) but significantly (p < 0.05) higher than that in the CONT dietary group. This suggests that the mango leaf powder supplementation at 250 mg kg⁻¹ and higher levels (500 mg kg⁻¹) increased the glutathione peroxidase activity, comparable to the VITC dietary group. The elevated concentration of glutathione peroxidase indicates an enhanced capacity to scavenge peroxides, and the antioxidative potential of mango leaf powder in the diet (Zehiroglu & Sarikaya, 2019).

The lipid peroxidation concentration in broiler chickens fed VITC, MLP2, and MLP5 dietary groups was similar (p > 0.05) but significantly (p < 0.05) lower than that in the CONT dietary group. This finding unveils the fact that supplementation with mango leaf powder, particularly at 250 and 500 mg kg⁻¹ (MLP2 and MLP5), promoted the reduction in lipid peroxidation levels. Lower lipid peroxidation indicates decreased oxidative damage to cell membranes. This suggests that mango leaf powder has a protective effect against lipid peroxidation in broiler chickens. As reported in this study, the enzymes that play crucial roles in the cellular defense against oxidative stress i.e. the serum catalase and glutathione peroxidase levels were increased by mango leaf dietary supplementations; for instance the catalase breaks down hydrogen peroxide (Nandi, Yan, Jana, & Das, 2019), while glutathione peroxidase helps to reduce lipid peroxides (Lobo, Patil, Phatak, & Chandra, 2010). Therefore by enhancing the activities of serum catalase and glutathione peroxidase, mango leaf supplementation may contribute to a more efficient defense mechanism against lipid peroxidation (Lobo et al., 2010; Nandi et al., 2019).

Table 5. The serum biochemical indices of broiler chickens (age 42 days) fed mango leaf powder supplemented diets.

Parameters	CONT	VITC	MLP2	MLP5	SEM	P value
Total protein (g L ⁻¹)	54.86	54.66	57.70	55.71	0.57	0.23
Aspartate aminotransferase (IU L ⁻¹)	53.75	57.61	53.43	53.15	1.25	0.61
Alanine aminotransferase (IU L ⁻¹)	35.49	35.07	36.05	36.65	0.25	0.11
Cholesterol (μmol L ⁻¹)	2.20	2.15	2.09	2.12	0.02	0.38
Creatinine (μmol L ⁻¹)	58.29	55.38	54.40	57.50	0.76	0.25

CONT: Control (no supplementation); VITC: 200 mg kg⁻¹ Vitamin C supplementation MLP2: 250 mg kg⁻¹ mango leaf powder supplementation; MLP5: 500 mg kg⁻¹ mango leaf powder supplementation; SEM: Standard error the mean. Means within a row with different letters are significantly different (p < 0.05).

Table 6. The serum antioxidant enzymes of broiler chickens (age 42 days) fed mango leaf powder supplemented diets.

Parameters	CONT	VITC	MLP2	MLP5	SEM	P value
Catalase (ng mgprotein ⁻¹)	49.42 ^c	66.46 ^b	72.64 ^{ab}	78.12 ^a	3.52	0.01
Glutathione peroxidase (mgprotein)	164.33 ^b	185.17 ^a	180.78 ^a	184.86 ^a	3.15	0.03
Lipid peroxidation (nmol mgprotein ⁻¹)	1.44 ^a	1.01 ^b	0.84 ^b	0.86 ^b	0.08	0.01

CONT: Control (no supplementation); VITC: 200 mg kg⁻¹ Vitamin C supplementation MLP2: 250 mg kg⁻¹ mango leaf powder supplementation; MLP5: 500 mg kg⁻¹ mango leaf powder supplementation; SEM: Standard error the mean. Means within a row with different letters are significantly different (p < 0.05).

Conclusion

In conclusion, dietary supplementation with mango leaf powder at 250 and 500 mg kg⁻¹, enhanced the broiler chicken body weight gain and dressed percentage compared to the control group. Organ weights remained largely unaffected, except for a lower pancreas relative weight in the group fed diet supplemented with 500 mg kg⁻¹ mango leaf powder. Serum biochemical analyses revealed significant effects on albumin, globulin, catalase, glutathione peroxidase, and lipid peroxidation, suggesting potential health benefits. Therefore, incorporating mango leaf powder into broiler diets could enhance growth and health parameters, with 250 and 500 mg kg⁻¹ mango leaf powder dietary supplementation.

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