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Impact of *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* Leaves Powder on Antioxidant Activity in Broiler Chickens

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Abstract

This study evaluates the effects of dietary supplementation with *Mangifera indica* (mango), *Lawsonia inermis* (henna), and *Carica papaya* (papaya) leaf powders on antioxidant activity, lipid metabolism, immune response, and meat quality in broiler chickens. Fresh leaves were shade-dried, ground into powder, and incorporated into feed at specific concentrations for 600-day-old chicks divided into five groups (n = 120/group). Key parameters—including blood biochemistry, antioxidant enzyme activity (MDA, SOD, GPx), lipid profiles, and immune markers—were assessed using standardized protocols. Results indicate that mixed herbal supplementation (Group E) demonstrated the highest DPPH radical scavenging activity (45.23% ± 1.48%, $p < 0.0001$), significantly exceeding the single-herb groups. Additionally, oxidative stress markers improved, with MDA levels lowest in Group E (1.24 ± 0.31 nmol/mL, $p = 0.0103$), and SOD (92.61 ± 10.08 U/mL) and GPx (90.62 ± 12.08 U/mL) activities highest in this group ($p < 0.05$). Lipid metabolism was optimized, showing moderate cholesterol (86.00 ± 0.78 mg/dL) and the lowest LDL levels (41.33 ± 0.44 mg/dL, $p < 0.0001$). *Carica papaya* supplementation (Group D) effectively reduced cholesterol (56.00 ± 0.43 mg/dL) and triglycerides (108.33 ± 0.66 mg/dL). Furthermore, immune responses improved, with increased IgG (4.30 ± 0.14 mg/dL), IgM (2.85 ± 0.13 mg/dL), and Newcastle disease antibody titers (7.00 ± 0.22, $p < 0.0001$). The combination of herbal additives also enhanced meat quality by improving water-holding capacity and reduc-

ing cooking loss. These findings suggest that dietary inclusion of these herbal leaf powders can enhance broiler health, immune function, and meat quality.

Keywords

Antioxidant Activity, Broiler Chickens, Herbal Supplementation, Immune Response

1. Introduction

In poultry management, it's common to administer antibiotics through drinking water to promote growth and control infectious bacterial diseases [1]. This practice helps maintain bird health, reduce mortality, and enhance nutrient utilization for optimal growth and profit. However, synthetic antibiotics, especially growth promoters, have notable side effects [2]. These concerns have led to bans on antibiotic growth promoters, mainly due to the development of cross and multiple resistance. Consequently, researchers have shifted focus toward organic or natural supplements to improve economic outcomes in poultry production [3].

Medicinal plants, including herbs and their extracts, are being explored as natural sources of substances that can positively impact poultry health and productivity. The use of medicinal plants, either alone or in combination, is under active scientific investigation for potential therapeutic benefits [4]. Certain medicinal plant products are known to enhance the host's natural resistance to infection due to bioactive phytochemicals or phyto-nutrients [5]. For instance, mango leaf extracts possess health-promoting properties such as analgesic, antioxidant, antimicrobial, anti-inflammatory, and antifungal activities, which may marginally improve broiler birds' growth performance and feed conversion ratio [6]. Despite the pharmacological potential of mango leaf phytochemicals, their use in poultry production remains underexplored. Therefore, this study aims to investigate the effects of extracts from three plant leaves on the growth performance and sensory properties of broiler birds. The current study investigates the impact of dietary supplementation with mango, henna, and papaya leaf powders on the antioxidant activity, lipid metabolism, and overall health of broiler chickens. By assessing key biochemical and physiological parameters, this research aims to determine whether these herbal additives can serve as viable alternatives to synthetic antibiotics and growth promoters in poultry production.

2. Materials and Methods

2.1. Collection and Preparation of Plant Leaves

Freshmature leaves of *Mangifera indica* (mango), *Lawsonia inermis* (henna), and *Carica papaya* (papaya) were collected from the Poultry Experimental Station, Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, these leaves were washed thor-

oughly three times with distilled water to remove surface contaminants and then shade-dried in a well-ventilated area at ambient temperature (25˚C - 30˚C) for 5 - 7 days to prevent the degradation of heat-sensitive phytochemicals Once completely dried, the leaves were milled into a fine powder using a laboratory grinder fitted with a 60-mesh sieve. Each plant species was processed separately to prevent cross-contamination. The powders were stored in airtight opaque containers at 4˚C until further use.

Experimental Design and Dietary Treatments A total of 600-day-old broiler chicks (Cobb-600) were obtained from a certified commercial hatchery. After initial body weight recording, birds were randomly assigned to five dietary treatment groups, with 120 chicks per group and six replicates of 20 birds each:

- **Group A:** Control (basal diet without supplementation).
- **Group B:** Basal diet + 0.5% *Mangifera indica* leaf powder (0.5%/100kg feed).
- **Group C:** Basal diet + 0.15% *Lawsonia inermis* leaf powder (0.15%/100kg feed).
- **Group D:** Basal diet + 0.5% *Carica papaya* leaf powder (0.5%/100kg feed).
- **Group E:** Basal diet + 0.58% herbal mixture (half dose of each treatment in Groups B-D).

The inclusion levels of each leaf powder were determined based on previous literature reporting the effective and safe use of these plants in poultry nutrition, preliminary pilot studies conducted in our laboratory, and traditional ethnoveterinary practices. These dosages were selected to balance biological efficacy, animal safety, and practical feed formulation.

All groups were fed for 42 days. Birds were housed in environmentally controlled pens with ad libitum access to feed and water. Standard broiler management practices regarding temperature, ventilation, lighting, and vaccination (ND and IBD) were followed throughout the trial. The proximate composition (crude protein, crude fiber, ash, moisture, and ether extract) of the leaf powders was analyzed following AOAC (2016) methods.

2.2. Sample Collection and Analysis

2.2.1. Blood Biochemistry

At day 42, blood samples were collected from the jugular vein of 64 randomly selected birds (n = 12 - 13 per group) using non-heparinized vacutainers. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20˚C until analysis. The following serum biochemical parameters were measured using a Merck Microlab-300 analyzer with commercial kits (Human Diagnostics, Germany):

Blood glucose (Kit #10260)

Total protein (Biuret method; Kit #157004)

Cholesterol (CHOD-PAP method)

Urea (Kit #10505)

2.2.2. Plasma Macro Minerals

Blood samples from 30 birds (n = 6 per group) were collected in heparinized vacutainers. Plasma was separated and stored at −20˚C. Concentrations of calcium

(Ca), phosphorus (P), sodium (Na), potassium (K), and magnesium (Mg) were determined using atomic absorption spectroscopy (AAS) according to standard protocols.

2.2.3. Intestinal Morphometry

Segments (2 cm) of the small intestine (duodenum, jejunum, ileum) were excised post-mortem, fixed in 10% neutral buffered formalin for 72 hours, dehydrated, embedded in paraffin, and sectioned at 5 µm thickness. Slides were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus CX31). Villus height, width, and crypt depth were measured using ImageJ software, and the villus height to crypt depth (VH: CD) ratio was calculated.

2.2.4. Lipid Peroxidation (MDA)-TBA Assay

Malondialdehyde (MDA) levels in breast meat samples were assessed as a marker of lipid peroxidation using the thiobarbituric acid (TBA) assay). Briefly, 1 mL of tissue homogenate was mixed with TBA reagent and heated in a water bath at 95˚C for 60 minutes. The absorbance of the supernatant was measured at 532 nm, and MDA concentration was calculated using a standard curve.

2.2.5. Glutathione Peroxidase (GPx) Activity

The GPx activity was estimated by measuring the rate of oxidation of glutathione (GSH) in the presence of hydrogen peroxide (H_2O_2), based on the decrease in NADPH absorbance at 340 nm. Absorbance was recorded at 412 nm after 10 minutes of incubation, and enzyme activity was expressed in U/mL.

2.2.6. Superoxide Dismutase (SOD) Activity

SOD activity was determined using a commercial assay kit (Human Diagnostics, Germany) as per the manufacturer's instructions. Absorbance was read at 560 nm using a spectrophotometer, and results were expressed in U/mL.

2.3. Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to evaluate the effects of treatment and replication. Means were compared using Tukey's Honest Significant Difference (HSD) test. Results were considered statistically significant at $p < 0.05$. All data are presented as mean ± standard deviation (SD).

3. Results

The results are presented in sections based on antioxidant activity, lipid profile, immune status, and enzyme activity in broiler chickens supplemented with *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* leaf powders.

3.1. Antioxidant Enzyme Activity in Breast Muscle

This section reports the levels of Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) in the breast muscle of broilers across

different treatment groups (Table 1).

Table 1. Effect of *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* leaf powder supplementation on antioxidant enzyme levels in breast muscle.

Parameter	GROUPS					p-value
	A	B	C	D	E	
MDA (nmol/mL)	1.97 ± 0.36^{a}	1.90 ± 0.24^{a}	1.65 ± 0.27^{ab}	1.40 ± 0.25^{ab}	1.24 ± 0.31^{b}	0.0103
SOD (U/mL)	70.39 ± 8.61^{ab}	69.48 ± 8.61^{b}	70.92 ± 9.11^{ab}	80.26 ± 11.40^{ab}	92.61 ± 10.08^{a}	0.0236
GPx (U/mL)	72.29 ± 7.58^{ab}	68.38 ± 8.61^{b}	71.82 ± 9.14^{ab}	83.25 ± 11.40^{ab}	90.62 ± 12.08^{a}	0.0226

Key: Values with different superscript letters within the same row differ significantly ($p < 0.05$). Group E = 3% mixed herbs per 100 kg feed.

3.2. Malondialdehyde (MDA)

Malondialdehyde (MDA) MDA levels were significantly ($p = 0.0103$) reduced in all supplemented groups compared to the control. Group E showed the lowest MDA concentration (1.24 ± 0.31 nmol/mL), indicating enhanced antioxidant protection. Group A and B showed the highest MDA levels, with no statistical difference between them. Intermediate values were observed in Groups C and D.

3.2.1. Superoxide Dismutase (SOD)

SOD activity was significantly higher ($p = 0.0236$) in the herbal mix group (E) than in the control and *M. indica* groups. Group E had the highest SOD activity (92.61 ± 10.08 U/mL), followed by Group D. Group B recorded the lowest activity.

3.2.2. Glutathione Peroxidase (GPx)

GPx activity significantly increased across all treated groups compared to the control ($p = 0.0226$). Group E again showed the highest GPx activity (90.62 ± 12.08 U/mL). Group B recorded the lowest enzyme activity among treatments.

3.3. Antioxidant Enzyme Activity in Blood

Blood MDA, SOD, and GPx levels were evaluated to assess systemic antioxidant responses (Table 2).

Table 2. Effect of leaf powder supplementation on blood antioxidant enzymes in broilers.

Parameter	GROUPS					p-value
	A	B	C	D	E	
MDA (nmol/mL)	4.5 ± 0.2^{a}	3.8 ± 0.1^{b}	3.5 ± 0.2^{c}	3.2 ± 0.3^{d}	1.24 ± 0.31^{b}	0.043
SOD (U/mL)	45.2 ± 1.5^{c}	52.3 ± 1.8^{b}	54.8 ± 2.1^{ab}	56.4 ± 1.7^{a}	57.6 ± 2.0^{a}	0.036
GPx (U/mL)	28.5 ± 1.2^{b}	35.7 ± 1.5^{a}	36.2 ± 1.4^{a}	37.5 ± 1.3^{a}	38.0 ± 1.2^{a}	0.044

Key: ^{abc}Mean values in the same row that do not share a common letter differ significantly ($p < 0.05$). Legends: Group-A = Control, Group-B = 0.5% *Mangifera indica*, Group-C = 0.15% *Lawsonia inermis*, Group-D = 0.5% *Carica papya*, Group-E = 0.58% mixture of herbs g/100kg feed.

3.4. Malondialdehyde (MDA)

MDA, a biomarker of lipid peroxidation and oxidative stress, exhibited a significant reduction ($p = 0.0103$) in all supplemented groups compared to the control. The highest MDA levels were observed in the control group (Group A: 4.5 ± 0.2^{a}), while the mixed herbal supplementation group (Group E) demonstrated the lowest MDA concentration (1.24 ± 0.31^{b}), indicating enhanced antioxidant protection. Among individual herbal treatments, *Carica papaya* (Group D) showed markedly reduced MDA levels (3.2 ± 0.3^{d}), outperforming *Mangifera indica* (Group B: 3.8 ± 0.1^{b}) and *Lawsonia inermis* (Group C: 3.5 ± 0.2^{c}).

3.5. Superoxide Dismutase (SOD)

SOD activity, reflecting enzymatic defense against superoxide radicals, was significantly elevated in supplemented groups compared to the control ($p = 0.0236$). The control group (Group A) displayed the lowest SOD activity (45.2 ± 1.5^{c}), whereas the mixed herbal group (Group E: 57.6 ± 2.0^{a}) and *Carica papaya* group (Group D: 56.4 ± 1.7^{a}) exhibited the highest activity. Intermediate values were recorded for *Mangifera indica* (Group B: 52.3 ± 1.8^{b}) and *Lawsonia inermis* (Group C: 54.8 ± 2.1^{ab}).

3.6. Glutathione Peroxidase (GPx)

GPx activity, critical for detoxifying hydrogen peroxide, increased significantly ($p = 0.0226$) in all supplemented groups relative to the control. The lowest GPx activity was observed in the control group (Group A: 28.5 ± 1.2^{b}), while the mixed herbal group (Group E: 38.0 ± 1.2^{a}) achieved the highest activity. All herbal treatments showed elevated GPx levels: *Mangifera indica* (Group B: 35.7 ± 1.5^{a}), *Lawsonia inermis* (Group C: 36.2 ± 1.4^{a}), and *Carica papaya* (Group D: 37.5 ± 1.3^{a}).

3.7. Antioxidant Activity of Broiler Meat

The antioxidant activity of broiler chicken meat was evaluated using the DPPH radical scavenging method across five treatment groups: one control and four experimental groups supplemented with *Mangifera indica*, *Lawsonia inermis*, *Carica papaya* leaf powders, and their mixture. Significant variation in antioxidant activity was observed among the groups ($p < 0.05$), with herbal supplementation markedly enhancing the meat's antioxidant capacity as shown in Table 3.

Table 3. The effect of *Mangifera indicia*, *Lawsonia inermis* and *Carica papya* leaves powder supplementation on the Antioxidant DPPH Radical Scavenging Activity (%) of meat of broiler chickens.

Treatment Group	DPPH Radical Scavenging Activity (%)	p-value
Control	25.34 ± 1.23^{a}	0.003
Mangifera indica leave powder	35.67 ± 1.45^{b}	0.003

Continued

Lawsonia inermis leave powder	30.12 ± 1.37^{ab}	0.015
Carica papya leave powder	40.89 ± 1.56^{c}	0.001
Mix of herbs leave powder	45.23 ± 1.48^{c}	0.000

Key: Values with different superscript letters (a, b, c) within a column differ significantly ($p < 0.05$). **Legends:** Group-A = Control, Group-B = 0.5% *Mangifera indica*, Group-C = 0.15% *Lawsonia inermis*, Group-D = 0.5% *Carica papya*, Group-E = 0.58% mixture of herbs g/100kg feed.

3.7.1. Group A

The control group exhibited the lowest DPPH radical scavenging activity (25.34% ± 1.23%), significantly differing from all treatment groups ($p = 0.003$). This highlights the limited antioxidant potential of the basal diet without herbal supplementation.

3.7.2. Group B

Supplementation with 0.5% *Mangifera indica* leaf powder significantly improved antioxidant activity, achieving a DPPH value of 35.67% ± 1.45% ($p = 0.003$), indicating moderate antioxidant enhancement. In contrast, 0.15% *Lawsonia inermis* supplementation resulted in intermediate DPPH activity (30.12% ± 1.37%), which, while not significantly different from the control ($p = 0.015$), was lower than *Mangifera indica* and *Carica papaya* groups, suggesting a milder effect.

3.7.3. Group C

The 0.5% *Carica papaya* group demonstrated the highest antioxidant activity among single-herb treatments, with a DPPH value of 40.89% ± 1.56% ($p = 0.001$), underscoring its potent antioxidant properties.

3.7.4. Group D

The mixed herbal supplementation (0.58% combination of *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya*) yielded the highest DPPH activity (45.23% ± 1.48%, $p = 0.000$), significantly outperforming all other groups. This synergistic effect highlights the superior efficacy of combining these herbs in enhancing meat quality through improved antioxidant activity.

3.8. Blood Lipid Profile and Total Protein Levels

The Level of blood lipid and total protein was evaluated across five groups, which included, one control and four treated groups supplemented with *Mangifera indica*, *Lawsonia inermis*, *Carica papaya* leaf powders, and their mixture.

All the lipid parameters (cholesterol, triglycerides, LDL, HDL, VLDL, and non-HDL) showed statistically significant differences between the groups ($p < 0.0001$). The Least Significant Difference (LSD) values indicate significant distinctions between groups for each lipid parameter shown in Table 4.

Table 4. The effect of *Mangifera indicia*, *Lawsonia inermis* and *Carica papya* leaves powder supplementation on the blood lipid and total protein of broiler chickens.

Group	Blood lipid and Total protein							
	Cholesterol	Lipid (Triglyceride)	LDL	HDL	LDL	Non-HDL	p-value	LSD
A	$99.66^{a} \pm 0.57$	$115.00^{ca} \pm 1.00$	$26.66^{c} \pm 0.57$	$77.66^{c} \pm 0.57$	$19.67^{a} \pm 0.58$	$37.33^{c} \pm 0.55$	0.0000	1.6272
B	$76.00^{c} \pm 1.00$	$126.67^{a} \pm 0.57$	$29.33^{a} \pm 0.50$	$90.33^{b} \pm 0.47$	$15.40^{c} \pm 0.53$	$35.33^{d} \pm 1.15$	0.0000	1.2428
C	$99.00^{a} \pm 1.22$	$118.00^{b} \pm 0.66$	$29.00^{c} \pm 0.33$	$78.33^{b} \pm 0.57$	$19.33^{d} \pm 1.15$	$38.66^{c} \pm 0.57$	0.0000	1.4092
D	$56.00^{d} \pm 0.43$	$108.33^{d} \pm 0.66$	$44.66^{b} \pm 0.33$	$80.00^{a} \pm 0.57$	$11.40^{d} \pm 0.28$	$70.33^{b} \pm 0.65$	0.0000	1.2881
E	$86.00^{b} \pm 0.78$	$117.33^{b} \pm 0.33$	$44.00^{d} \pm 0.57$	$41.33^{a} \pm 0.44$	$17.40^{b} \pm 0.30$	$76.00^{a} \pm 0.57$	0.0000	1.4854

Key: Different superscript letters within rows denote significant differences ($p < 0.05$).

3.8.1. Group A

Exhibited the highest cholesterol levels ($99.66^{a} \pm 0.57$), significantly differing from the other groups. This group also showed moderate HDL and LDL levels but relatively lower VLDL ($19.67^{a} \pm 0.58$). Group A had the second-lowest non-HDL level ($37.33^{c} \pm 0.55$).

3.8.2. Group B

Had the lowest cholesterol ($76.00^{c} \pm 1.00$) among all groups, with the highest lipid triglyceride level ($126.67^{a} \pm 0.57$). The LDL level was the highest ($90.33^{b} \pm 0.47$), but HDL was lower ($15.40^{c} \pm 0.53$) than Groups A and C. Non-HDL levels were moderately low ($35.33^{d} \pm 1.15$).

3.8.3. Group C

Presented cholesterol levels ($99.00^{a} \pm 1.22$) comparable to Group A. The lipid triglyceride levels were lower than Group B but higher than Group D. LDL levels ($78.33^{b} \pm 0.57$) were relatively lower than Group B but higher than Group A. Group C displayed the highest HDL levels ($19.33^{d} \pm 1.15$) among all groups.

3.8.4. Group D

Showed the lowest cholesterol ($56.00^{d} \pm 0.43$) and lipid triglyceride ($108.33^{d} \pm 0.66$) levels. LDL levels ($80.00^{a} \pm 0.57$) were moderately high, while VLDL was the lowest ($11.40^{d} \pm 0.28$). Non-HDL levels ($70.33^{b} \pm 0.65$) were significantly higher compared to Groups A, B, and C.

3.8.5. Group E

Exhibited moderate cholesterol ($86.00^{b} \pm 0.78$) and triglyceride ($117.33^{b} \pm 0.33$) levels. LDL levels ($41.33^{a} \pm 0.44$) were the lowest, but this group had the highest VLDL levels ($17.40^{b} \pm 0.30$) and the most elevated non-HDL levels ($76.00^{a} \pm 0.57$) among all groups.

The supplementation of *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* leaf powders significantly influenced the lipid profiles and total protein levels in broiler chickens. Groups varied in cholesterol, HDL, LDL, VLDL, and non-

HDL levels, with statistical significance indicating notable differences in lipid metabolism influenced by the supplementation.

3.9. Blood Enzymes Activity

The activities of blood enzymes, including LDH, AST, ALT, CK, and ALP, varied significantly among the treatment groups ($p < 0.05$). Herbal supplementation influenced these parameters compared to the control, indicating potential hepatoprotective and metabolic effects (as shown in Table 5).

Table 5. The effect of *Mangifera indicia*, *Lawsonia inermis* and *Carica papya* leaves powder supplementation on the activity of blood enzymes of broiler chickens.

Group	Activity of blood enzymes				
	LDH (U/L)	AST (U/L)	ALT (U/L)	CK (U/L)	ALP (U/L)
A	1175.3 ± 10.5	234.07 ± 8.2	22.59 ± 0.9	5561 ± 112	2421 ± 45
B	1150.2 ± 9.8	220.45 ± 7.6	20.75 ± 0.8	5400 ± 105	2300 ± 43
C	1130.5 ± 11.0	210.30 ± 8.0	18.90 ± 0.7	5300 ± 110	2250 ± 40
D	1180.0 ± 12.3	235.50 ± 8.5	23.10 ± 1.0	5580 ± 115	2400 ± 47
E	1165.8 ± 10.7	225.90 ± 7.9	21.50 ± 0.8	5450 ± 108	2350 ± 42
p-value	0.0000	0.0003	0.0000	0.0000	0.0001

Key: Enzymes are expressed in Units per Liter (U/L).

3.9.1. Group A

This group recorded the highest LDH (1175.3 ± 10.5 U/L), AST (234.07 ± 8.2 U/L), and ALT (22.59 ± 0.9 U/L) activities. CK (5561 ± 112 U/L) and ALP (2421 ± 45 U/L) levels were also elevated, representing baseline enzyme activity without supplementation.

3.9.2. Group B (0.5% *Mangifera indica*)

Compared to the control, LDH (1150.2 ± 9.8 U/L), AST (220.45 ± 7.6 U/L), and ALT (20.75 ± 0.8 U/L) were reduced. CK (5400 ± 105 U/L) and ALP (2300 ± 43 U/L) were moderately lower, suggesting improved liver function.

3.9.3. Group C (0.15% *Lawsonia inermis*)

This group showed the lowest enzyme activities among all treatments, with LDH (1130.5 ± 11.0 U/L), AST (210.30 ± 8.0 U/L), ALT (18.90 ± 0.7 U/L), CK (5300 ± 110 U/L), and ALP (2250 ± 40 U/L). These reductions indicate enhanced hepatic health and reduced tissue damage.

3.9.4. Group D (0.5% *Carica papaya*)

LDH (1180.0 ± 12.3 U/L) and AST (235.50 ± 8.5 U/L) were slightly higher than the control, while ALT (23.10 ± 1.0 U/L) was comparable. CK (5580 ± 115 U/L) was marginally higher, and ALP (2400 ± 47 U/L) remained close to control values, suggesting a moderate effect on enzyme activity.

3.9.5. Group E (0.58% Mixture of Herbs)

This group exhibited intermediate enzyme activities with LDH (1165.8 ± 10.7 U/L), AST (225.90 ± 7.9 U/L), ALT (21.50 ± 0.8 U/L), CK (5450 ± 108 U/L), and ALP (2350 ± 42 U/L). These results indicate a balanced modulation of enzyme activity without excessive elevation.

3.10. Immune Response Parameter

The immune response of broiler chickens was evaluated through RBC count, IgM, IgG, Newcastle disease HI titer, and H:L ratio. Significant differences ($p < 0.05$) were observed among groups (as shown in Table 6), indicating that herbal supplementation enhanced immune function compared to the control.

Table 6. Effect of herbal supplementation on immune indicators in broiler chickens.

Group	Immune system				
	RBC (Log2)	IgM (mg/dL)	IgG (mg/dL)	Newcastle (HI Titer)	H. ratio
A	6.17 ± 0.12	2.34 ± 0.10	3.83 ± 0.14	6.00 ± 0.18	0.786 ± 0.025
B	6.50 ± 0.15	2.60 ± 0.12	4.10 ± 0.13	6.50 ± 0.20	0.740 ± 0.022
C	6.70 ± 0.13	2.75 ± 0.11	4.25 ± 0.15	6.80 ± 0.19	0.730 ± 0.024
D	6.40 ± 0.14	2.50 ± 0.10	3.95 ± 0.12	6.30 ± 0.21	0.760 ± 0.026
E	6.80 ± 0.16	2.85 ± 0.13	4.30 ± 0.14	7.00 ± 0.22	0.720 ± 0.023
p-value	0.0000	0.0003	0.0000	0.0000	0.0001

Key: SRBC, Sheep red blood cell; H:L, heterophil:lymphocyte. Legends: Group-A = Control, Group-B = 0.5% Mangifer aindica, Group-C = 0.15% Lawsonia inermis, Group-D = 0.5% Carica papya, Group-E = 0.58% mixture of herbs g/100kg feed.

3.10.1. Group A

The control group exhibited the lowest immune indicators, with RBC (6.17 ± 0.12), IgM (2.34 ± 0.10 mg/dL), IgG (3.83 ± 0.14 mg/dL), HI titer (6.00 ± 0.18), and H:L ratio (0.786 ± 0.025).

3.10.2. Group B (0.5% *Mangifera indica*)

This group showed improved values compared to the control, with RBC (6.50 ± 0.15), IgM (2.60 ± 0.12 mg/dL), IgG (4.10 ± 0.13 mg/dL), HI titer (6.50 ± 0.20), and H:L ratio (0.740 ± 0.022).

3.10.3. Group C (0.15% *Lawsonia inermis*)

RBC (6.70 ± 0.13), IgM (2.75 ± 0.11 mg/dL), IgG (4.25 ± 0.15 mg/dL), HI titer (6.80 ± 0.19), and H:L ratio (0.730 ± 0.024) were further improved, showing a strong immune-enhancing effect.

3.10.4. Group D (0.5% *Carica papaya*)

This group also showed enhanced immune responses with RBC (6.40 ± 0.14), IgM (2.50 ± 0.10 mg/dL), IgG (3.95 ± 0.12 mg/dL), HI titer (6.30 ± 0.21), and H:L ratio (0.760 ± 0.026).

3.10.5. Group E (0.58% Mixture of Herbs)

The highest immune responses were recorded in this group, with RBC (6.80 ± 0.16), IgM (2.85 ± 0.13 mg/dL), IgG (4.30 ± 0.14 mg/dL), HI titer (7.00 ± 0.22), and H:L ratio (0.720 ± 0.023), indicating the synergistic effect of combined herbal supplementation.

4. Discussion

The results of this study highlight the effects of dietary supplementation with *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* leaf powders on antioxidant activity, lipid metabolism, enzyme activity, and immune response in broiler chickens. Supplementation significantly enhanced antioxidant enzyme activity, as evidenced by reduced malondialdehyde (MDA) levels and increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Group E (herb mixture) showed the strongest antioxidant activities, with the lowest MDA levels at 1.24 ± 0.31 nmol/mL, and the highest SOD levels at 92.61 ± 10.08 U/mL, and GPx at 90.62 ± 12.08 U/mL. Similar results were found by [7], indicating that mango leaves are rich in antioxidants. Similar trends were reported in other studies involving plant-derived bioactive compounds, such as [8].

The inclusion of herbal supplements such as *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* leaf powders, both individually and in combination, in broiler diets significantly affected the antioxidant activity of meat, as measured by DPPH radical scavenging activity (%). This improvement in antioxidant capacity reflects the presence of phytochemicals such as mangiferin, flavonoids, phenolic acids, tannins, and carotenoids, which are known to neutralize reactive oxygen species and enhance endogenous antioxidant defenses [9] [10] [11]. The control group had the lowest antioxidant activity (25.34% ± 1.23%), indicating that a basal diet without supplementation cannot enhance meat's antioxidant properties. In contrast, the herbal mixture group exhibited the highest DPPH radical scavenging activity (45.23% ± 1.48%), suggesting a synergistic effect of combining these herbs. This aligns with [9], who found that combining different phytogenic feed additives results in complementary or synergistic effects, improving antioxidant capacity in broiler meat. *Carica papaya* leaf powder recorded the highest antioxidant activity among herb extracts (40.89% ± 1.56%), followed by *Mangifera indica* (35.67% ± 1.45%) and *Lawsonia inermis* (30.12% ± 1.37%). The higher efficacy of *Carica papaya* can be attributed to its abundant carotenoids and flavonoids, which possess strong free radical scavenging properties [10]. The relatively lower activity observed in the *Lawsonia inermis* group may be due to the lower inclusion rate of 0.15%; a higher dose might be required for comparable results. However, studies by [12] show that even low levels of *Lawsonia inermis* can enhance oxidative stability in poultry products, though other dietary factors may influence this effect.

The combination of *Mangifera indica*, *Lawsonia inermis*, and *Carica papaya* (0.58%) in the herbal mixture showed higher antioxidant activity. This synergistic

effect aligns with the hypothesis that diverse phytochemicals with different antioxidant pathways can work in combination to stabilize free radicals more effectively. *Mangifera indica* is rich in mangiferin, a xanthonoid with antioxidant and anti-inflammatory activity; *Carica papaya* contains carotenoids and flavonoids**;** and *Lawsonia inermis* provides phenolic acids and tannins**.** Together, these compounds act on multiple antioxidant pathways [11] [13]. For example, [13] reported a significant reduction in lipid peroxidation of broiler meat with the dietary incorporation of polyphenol-rich plant extracts. According to Alem *et al.* many herbal extracts have antioxidant effects in animals. Similarly, [14] established that a blend of herbal powders has more favorable effects on improving oxidative stability than single additives in broilers.

The enhanced oxidative stability observed in broiler meat has direct practical implications, including extended shelf life and improved health value for consumers. Reduced lipid peroxidation minimizes the risk of rancidity and contributes to meat safety and quality [15]. These results support the use of herbal mixtures as natural alternatives to synthetic antioxidants, aligning with sustainable and residue-free poultry production.

Dietary supplementation also affected lipid metabolism, as evident from changes in cholesterol, LDL, HDL, and triglyceride levels. Group B (*Mangifera indica*) had the lowest cholesterol (76.00 ± 1.00 mg/dL) but higher triglycerides (126.67 ± 0.57 mg/dL). Conversely, Group D (*Carica papaya*, 5%) showed the lowest LDL and cholesterol levels, highlighting its lipid-lowering potential. These effects can be attributed to phytosterols, polyphenols, and flavonoids that inhibit cholesterol absorption and promote hepatic lipid clearance [16] [17]. Previous studies corroborate these lipid-modulating effects of phytogenic feed additives, supporting their role in improving cardiovascular health in poultry.

The immune response also improved, especially in Group E, which showed the highest levels of IgG (4.30 ± 0.14 mg/dL) and IgM (2.85 ± 0.13 mg/dL), along with a higher Newcastle disease antibody titer (7.00 ± 0.22) and lower H:L ratio (0.720 ± 0.023), indicating reduced stress and enhanced immunity. These improvements may result from bioactive compounds that stimulate macrophage activity, cytokine release, and antibody production [18]. Polyphenols and flavonoids are known to act as immunomodulators in poultry.

The activity of blood enzymes (LDH, AST, ALT, CK, and ALP) differed among the treatment groups. The supplemented groups showed a significant decrease in AST and ALT levels, indicating a hepatoprotective effect likely mediated by the antioxidant action of plant-based compounds**.** These findings are consistent with [19], who reported reduced liver enzyme levels in poultry fed phytogenic additives. Intestinal morphology also improved, with increased villus height and VH/CD ratio, especially in Group E. Improved intestinal morphology supports better nutrient absorption and gut integrity, which are essential for growth performance [20]. These outcomes align with previous reports on the benefits of medicinal plants in poultry production [21].

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Author's Contributions

All authors contributed to the study and approved the final manuscript. Abdul Kabir was responsible for writing, review, and editing, as well as designing the figures and tables.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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