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Effect of Feeding Neem and Papaya Leaf Meal as Feed Additive on Performance, Immune response, Antioxidant status and Serum biochemical parameters in Broilers

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ABSTRACT

The present study was conducted to evaluate the impact on performance, immune response, antioxidant level, and serum biochemical components of broiler production by supplementing Neem Leaf Meal and Papaya Leaf Meal, individually as well as combined. 220 day-old VenCobb-400 commercial broiler chicks were divided randomly into 11 treatment groups with four replicates per treatment, keeping five chicks per replicate. In treatment groups, besides basal diet, neem leaf meal, as well as Papaya leaf meal, at two different levels, individually (0.5 and 1.0 % diet) and three different combinations (1:1, 1:2, and 2:1 part NLM:PLM @ 1.0 and 2.0 % diet), were supplemented. Body weight, weight gain, feed consumption, and feed conversion ratios (FCR), as well as performance index (PI), were recorded weekly up to 42 days. Humoral immunity against Newcastle disease virus was evaluated on 30th and 42nd day post-feeding trial through hemagglutination inhibition (HI) test. At the end of trial, on 42nd day, two birds per replicate were sacrificed to estimate antioxidant property in liver and breast muscles. Biochemical estimations were done on serum samples at completion stage. The results revealed that 1:1 ratio of neem, as well as Papaya leaf meal (at 1.0 %), and 1:2 ratio (at 2.0 % diet level) of NLM:PLM supplement, individually, possessed a significant (P<.05) impact on their performance. Their humoral immunity, as well as antioxidant levels, were found to be significantly (P<.05) high. Biochemically, serum levels of total triglycerides, total, LDL-cholesterol, and HDL-cholesterol were found to reduce and increase, significantly (P<.05), due to supplementation.

KEYWORDS: Antioxidant status, Broilers, Immune response, Neem Leaf Meal, Papaya Leaf Meal, Serum biochemical.

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INTRODUCTION

The ever-growing rate of chicken production has traditionally utilized antibiotic growth promoters. In this manner, the overuse of antibiotics has been associated with antimicrobial resistance, antibiotics within chicken products and a rising level of public health concerns. This has led to restrictions on antibiotics by the government, necessitating to find a suitable alternative that is safe and reliable (Soren et al., 2025). In this respect, phytogenic feed supplements are identified as capable alternative to antibiotics in poultry nutrition. They are obtained from natural herbs and leaves; contain bioactive compounds such as phenolic compounds, flavonoids, saponins and tannins, which exhibit antimicrobial, antioxidant and immunomodulatory activities in poultry diets (Obianwuna et al., 2024). Some recent findings have revealed that phytogenic supplements significantly enhance growth performance by modulating the intestinal microbial population, stimulating the activities of enzymes and alleviating oxidative stress in the body, consequently increasing nutritional utilization and immune abilities without selective antimicrobial resistance (Singh et al., 2019 and Mounika et al., 2025). Likewise, poly-herbal feed additives have shown a great potential as natural alternations to synthetic growth promoters in broilers (Rao, 2021).

Neem leaves (*Azadirachta indica*) contain biological active compounds like azadirachtin, nimbin, and salannin, which possess anti-bacterial, anti-viral, anti-fungal, hepatoprotective, and immunostimulatory actions that ultimately improve the health of the intestinal tracts and immune and efficacy of feeds in poultry (Islam et al., 2025). Papaya leaves (*Carica papaya*) are endowed with proteolytic enzymes like papain and chymopapain, which increase the digestion of proteins and make amino acids more available, while the phenolic constituents of papaya leaves possess powerful antioxidant actions that protect tissues against oxidative injury (Okonkwo et al., 2021). The combined supplementation of neem and papaya leaf meal will, therefore, have a synergistic effect. Therefore, the current study aims to explore their effect on the immune, antioxidant value and the serum biochemical constituents of the broiler chickens.

MATERIAL AND METHODS

Birds, Housing and Experimental Design

The present experiment was conducted in the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.) in year 2020. Total of 220, day old VenCobb-400 broiler chicks were randomly selected for the experiment and were allotted eleven dietary treatments. Each treatment consisted of four replicates with five chicks in each replicate. Experimental diets were formulated in mash form as per VenCobb-400 broiler chickens (Venkys, 2017) and supplemental ingredient i.e. neem and papaya leaf meal was added. The ingredients were procured in one lot for whole experiment. They were grinded and screened properly to get uniform particle size. A phase feeding regimen was followed, comprising three phases: pre-starter (0-14 days), starter (15-28 days), and finisher (29-42 days), consisted of eleven isonitrogenous and isocaloric dietary treatments, by adjusting basal ingredients; therefore, the observed effects were attributed to the bioactive phytochemicals of neem and papaya leaf meal rather than differences in nutrient composition.

Preparation of Leaf meal

Neem and Papaya leaf were airdried; powdered separately and meal was stored in air-tight bags. No phytochemical analysis was conducted in this study, however the presence of phytochemicals was referred from existing literature. The proximate composition of neem leaf meal (NLM) and papaya leaf meal (PLM) on dry matter (DM %) basis is presented in Table 1. Composition of experimental broiler diets used in the study is given in Table 2 and Table 3.

Table 1. Proximate Composition of NLM and PLM (on % DM Basis)

Nutrient Composition (%)	NLM	PLM
Moisture[+]	8.27	11.73
Crude Protein[+]	19.25	24.79
Crude Fibre[+]	15.78	7.64
Ether Extract[+]	5.02	1.70
Ash[+]	6.50	8.94

[+]Calculated values

Treatment-wise dietary supplementation of neem and papaya leaf meal was applied as follows:

T0: Control (basal diet) as per commercial chick feed specifications

T1: Basal diet + Neem leaf meal (NLM) at 0.5% of diet

T2: Basal diet + Neem leaf meal (NLM) at 1.0% of diet

T3: Basal diet + Papaya leaf meal (PLM) at 0.5% of diet

T_4: Basal diet + Papaya leaf meal (PLM) at 1.0% of diet

T5: Basal diet + 1:1 combination of NLM and PLM at 1.0% of diet

T6: Basal diet + 1:1 combination of NLM and PLM at 2.0% of diet

T7: Basal diet + 1:2 combination of NLM and PLM at 1.0% of diet

T8: Basal diet + 1:2 combination of NLM and PLM at 2.0% of diet

T9: Basal diet + 2:1 combination of NLM and PLM at 1.0% of diet

T10: Basal diet + 2:1 combination of NLM and PLM at 2.0% of diet

Table 2. Ingredient composition of Broiler experimental diets

Feed Ingredients	Experimental Diets (%)		
	Pre-starter (0 - 14 days)	Starter (15 - 28 days)	Finisher (29 - 42 days)
Maize	55.21	57.33	59.27
Soybean meal	38.83	35.10	31.52
Calcite/LSP	0.62	0.66	0.69
DCP	1.65	1.64	1.63
Vegetable oil	2.28	3.91	5.60
Methionine	0.22	0.22	0.21
Lysine	0.28	0.28	0.22
Soda Bicarb	0.10	0.10	0.10
Salt	0.24	0.22	0.20
Trace Mineral Premix[1]	0.06	0.05	0.05
Vitamin Premix[2]	0.06	0.05	0.05
Threonine	0.075	0.05	0.02
Maduramicin	0.00	0.00	0.05
Robimidine	0.033	0.033	0.00
Choline Chloride 60 %	0.10	0.12	0.15
Liver tonic	0.05	0.05	0.05
Emulsifier	0.05	0.05	0.05
Toxin binder	0.10	0.10	0.10
Supplemental ingredient	+	+	+
TOTAL	99.99	99.99	99.99

[1]Trace mineral provided per kg diet: Manganese, 120mg; Zinc, 80mg; Iron, 25mg; Copper, 10mg; Iodine, 1mg; and Selenium, 0.1mg.

[2]Vitamin premix provided per kg diet: Vitamin A, 20000IU; Vitamin D3, 3000IU; Vitamin E,10mg; Vitamin K,2mg; Riboflavin, 25mg; VitaminB1,1mg; Vitamin B6, 2mg; Vitamin B12,40mcg and Niacin, 15 mg.

[+] Varying level of NLM and PLM supplement.

Table 3. Nutrient composition (%) of basal diet during different phases (on % DM Basis)

Nutrient Composition (%)	PHASE		
	Pre-starter (0 - 14 days)	Starter (15 - 28 days)	Finisher (29 - 42 days)
Moisture[++]	11.00	10.70	10.64
Crude Protein[++]	23.57	21.81	20.11
Crude Fibre[++]	4.72	6.33	6.47
Ether Extract[++]	2.18	3.75	4.65
Ash[++]	5.61	6.04	5.23
Metabolisable energy (kcal/kg)[+]	3000	3100	3200
Calcium[++]	1.00	0.90	0.85
Available phosphorus[++]	0.50	0.45	0.40
Lysine[+]	1.35	1.20	1.05
Methionine[+]	0.55	0.50	0.45
Methionine + cysteine (%)[+]	0.88	0.80	0.74
Threonine (%)[+]	0.77	0.70	0.65
Sodium (%)[+]	0.16	0.16	0.16
Chloride (%)[+]	0.18	0.18	0.18

[+]Calculated values [++]Analysed values

Broiler trial

Experimental chicks were reared under controlled conditions in a battery brooder system. Experiment was conducted under tropical climatic conditions (temperatures ranging from 28°C to 34°C and relative humidity between 65-80%), while brooding temperature was maintained at 33-35°C during the first week period under standard management practices.

Birds were weighed individually at weekly intervals up to six weeks of age to know the body weight gain of broilers and weekly feed intake was recorded on a replicate basis by measuring the feed offered and residues at the end of each week. The FCR was calculated using the formula: FCR = Feed consumption (g) / Body weight gain (g). The performance index (PI) was calculated according to Bird (1995) as: PI (%) = Body weight gain (g) × Feed efficiency ratio.

Broilers were vaccinated on 7th day of age with F_1 strain, 14th day with infectious bursal disease and 21st of age with Lasota strain of Newcastle disease. Humoral immunity towards Newcastle disease was measured on day 30th and 42nd by the Haemagglutination inhibition test according to the method of O.I.E. (2005). At the end of trial (42nd day), birds were sacrificed, parts from breast muscle and liver tissue samples (two from each replicate) was taken and analyzed for degree of lipid oxidation. After storage at -20°C for 30 days lipid oxidation was determined as thiobarbituric acid reactive substances (TBARS) according to the extraction method described by Witte et al. (1970). TBARS was expressed as mg of malondialdehyde (MDA)/kg of tissue. Serum biochemical parameters was determined: total protein (g/L), total cholesterol (mg/dL), high density lipoprotein (HDL) cholesterol (mg/dL), low- density lipoprotein (LDL) cholesterol (mg/dL) and triglycerides (mg/dL) by spectrophotometrically using commercial diagnostic kits.

Ethical approval

The experiment on animals including all procedures of this study was approved by Institutional Animal Ethics Committee, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.).

Statistical analysis

The experimental data were analysed using analysis of variance (ANOVA) under a completely randomized design (CRD) following the procedure described by Snedecor and Cochran (1994). Differences among the dietary treatments were tested for significance by using Duncan's Multiple Range Test (1955) to determine statistical significance.

RESULTS AND DISCUSSION

Average Body Weight Gain, Feed Intake, Feed Conversion Ratio and Performance Index

Addition of neem and papaya leaf meal had a significant effect on growth performance of broiler chickens (Table 4). Weight gain was significantly ($P < 0.05$) higher in broiler chickens fed neem and papaya leaf meal than the control during the pre-starter (0-14 days), starter (15-28 days), and finisher (29-42 days) phases. Among the supplemented diets, weight gain was highest in broiler chickens fed 1.0% neem and papaya leaf meal in a 1:1 ratio (T5), followed by T7 (1:2 ratio @ 1.0% concentration) and T8 (1:2 ratio @ 2.0% concentration), and these diets were significantly ($P < 0.05$) better than the control. The improved growth performance may be due to its antimicrobial and growth-stimulating properties (Paul et al., 2020) of neem leaf meal and due to proteolytic enzymes papain and chymopapain in papaya leaf meal (Rahman et al., 2023). Thus, it supports protein digestion and enhance the release of free amino acids required to increase growth of broilers results in growth promoting effect. The feed intake was significantly ($P < 0.05$) different between the treatments, with the highest feed intake recorded for the treatment where the ratio of neem to papaya leaf meal was 1:2 ratio of 1.0% (T7) (Table 04). Higher feed intake in the supplemented groups may be attributed to neem leaf meal's appetite-stimulating, antibacterial and hepatoprotective properties, along with its carminative action, which together improve digestive efficiency (Deka et al., 2019; Ampode et al., 2021). Higher feed intake has also been reported with papaya leaf supplementation in poultry, likely due to improved palatability of the diet (Jimenez et al., 2024). The feed conversion ratio was also improved significantly ($P < 0.05$) in the supplemented groups, with the best FCR being recorded in T5, indicating better feed utilization. The improved feed efficiency can be attributed to the better utilization of nutrients, reduction of pathogenic gut flora and increased digestive activity. The performance index also demonstrated a similar trend, with T5 recording the highest performance index values ($P<0.05$), attributing to the combined effect of increased body weight gain and better feed conversion efficiency.

Table 4. Growth performance of broilers during pre-starter, starter and finisher phases under different dietary treatments

Treat.	Body weight gain (g/bird)			Feed intake (g/bird)			FCR			Performance Index		
	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher
T0	393.70^{c}	1211.95^{e}	2277.70^{f}	524.60	1897.60^{ab}	4111.10^{bcd}	1.34^{a}	1.68^{a}	2.08^{a}	295.48^{c}	488.03^{e}	513.35^{e}
T1	399.90^{c}	1252.90^{de}	2342.95^{ef}	525.20	1904.40^{ab}	4121.45^{ab}	1.32^{a}	1.62^{ab}	2.03^{ab}	304.99^{c}	527.65^{de}	536.21^{de}
T2	397.10^{c}	1272.80^{de}	2358.45^{ef}	515.30	1882.00^{b}	4073.23^{e}	1.30^{a}	1.57^{b}	2.02^{ab}	306.35^{c}	564.26^{d}	537.98^{de}
T3	400.50^{c}	1286.15^{cd}	2409.90^{de}	519.53	1900.90^{ab}	4116.93^{abc}	1.30^{a}	1.56^{bc}	1.97^{bc}	309.54^{c}	568.65^{d}	570.04^{bcd}
T4	406.30^{bc}	1297.15^{cd}	2445.05^{d}	519.65	1893.93^{ab}	4108.70^{bcde}	1.28^{ab}	1.54^{bc}	1.93^{c}	318.01^{bc}	577.87^{cd}	595.04^{b}
T5	444.45^{a}	1456.70^{a}	2668.20^{a}	512.60	1900.53^{ab}	4142.03^{ab}	1.16^{c}	1.38^{d}	1.85^{d}	388.73^{a}	739.58^{a}	655.24^{a}
T6	404.70^{bc}	1342.50^{bc}	2485.70^{cd}	512.10	1887.85^{ab}	4132.35^{ab}	1.27^{ab}	1.47^{cd}	1.97^{bc}	320.02^{bc}	639.44^{bc}	582.50^{bc}
T7	436.60^{a}	1400.70^{ab}	2570.70^{b}	523.15	1909.00^{a}	4151.50^{a}	1.20^{bc}	1.44^{d}	1.92^{cd}	364.45^{ab}	670.72^{b}	610.52^{b}
T8	430.90^{ab}	1373.90^{b}	2538.25^{bc}	515.05	1897.98^{ab}	4113.43^{bcd}	1.20^{bc}	1.47^{cd}	1.91^{cd}	360.95^{ab}	643.49^{bc}	612.12^{b}
T9	396.40^{c}	1244.00^{de}	2325.55^{ef}	522.55	1889.55^{ab}	4077.33^{de}	1.32^{a}	1.61^{ab}	2.03^{ab}	300.86^{c}	525.89^{de}	535.28^{de}
T1	0395.05^{c}	1249.93^{de}	2344.43^{ef}	521.60	1888.65^{ab}	4082.25^{cde}	1.32^{a}	1.60^{ab}	2.01^{ab}	299.41^{c}	534.81^{de}	546.39^{cde}
SEM	3.66	12.29	19.01	1.49	2.12	4.80	0.01	0.02	0.01	6.23	12.56	7.21
P-value	0.017	0.009	0.006	0.081	0.042	0.010	0.021	0.016	0.009	0.014	0.008	0.011

Means of column with different superscript differ significantly (P<0.05)

Humoral immune response of broilers (30th and 42nd day)

Results indicate that humoral immune response was significantly improved (P<0.05) by dietary supplementation of neem and papaya leaf meal individually and in combination, when compared with the control. However, maximum and significantly (P<0.05) higher antibody titre (log10 value of HI titre) was observed at 30th and 42nd day of experiment i.e. in T5 and T8 group, respectively (Table 5). Neem and papaya leaves could play a major role in stimulating the humoral immune response because of its significant role in killing or slowing the growth of many pathogenic microorganisms like bacteria, virus, fungi etc. Our results are in agreement with findings that neem leaf supplementation enhances humoral immune response against Newcastle disease virus, likely due to its immunomodulatory effects on B lymphocytes (Ikpendu et al., 2023). This could be attributed to the immunomodulatory effects of neem on B- lymphocyte which differentiate into memory cells and plasma cells. The plasma cells secrete antibodies against antigen and are capable of producing about 2,000 molecules of antibodies per second while the memory cells have the ability to rapidly differentiate into antibody- producing plasma cells when they encounter the same antigen in another infection (Richard and Tracey, 2001). Papaya leaf supplementation has been shown to increase relative weights of lymphoid organs such as the bursa of Fabricius and spleen, indicating enhanced immune function in broiler chickens (Nabhan et al., 2025). Papaya extract also has antibacterial and antifungal properties that may enhance immune activity (Alhodieb et al., 2025). Also factors like saponin present in papaya leaf meal may produce an immunomodulatory effect in the birds (Ojiako et al., 2019).

Table 5. Antibody titre (Log10 value of HI titre) against Newcastle disease virus in broiler under different treatment groups

Treat.	30th Day	42nd Day
T0	2.15e	2.43f
T1	2.34d	2.55de
T2	2.46c	2.74bc
T3	2.18e	2.45ef
T4	2.37cd	2.55de
T5	2.77a	2.96a
T6	2.44cd	2.63cd
T7	2.43cd	2.72bc
T8	2.72a	2.94a
T9	2.56b	2.93a
T10	2.60b	2.80b
SEM	0.03	0.04
P-Value	0.012	0.007

Means of column with different superscript differ significantly (P<0.05)

Antioxidant status

Significantly (P<0.05) better antioxidant status in terms of TBARS values (mg Malondialdehyde/ kg) at 30 days of storage in breast and liver tissue were observed in broilers fed on basal diets supplemented with neem and papaya leaf meal in 1:1 combination at 1.0% of diet (T5) as compared to control (Table 6). Recent research has also shown that supplementation with papaya leaf components enhances antioxidant capacity in quails, marked by increased total antioxidant capacity and enzyme activities alongside reduced oxidative stress markers (El-Rayes et al., 2024). Papaya leaves are a rich source of antioxidant nutrients, including carotenoids, vitamin C, B-complex vitamins (such as pantothenic acid) and essential minerals, which can contribute to improved antioxidant status (Koul et al., 2022) and metabolic health in broiler chickens. Likewise, recent studies have reported that ethanolic extracts of neem contain potent antioxidant compounds such as terpenoids, limonoids, quercetin and phytosterols, which shows strong antioxidant activity and protection against lipid oxidation–induced stress (Zahid et al., 2025).

Table 6. Thiobarbituric acid reactive substances (mg Malondialdehyde (MDA) kg^{1}) in liver tissue and breast muscle of broilers in different treatment groups

Treatment	Fresh		After 30 days storage	
	Liver tissue	Breast muscle	Liver tissue	Breast muscle
T0	0.98a	0.40a	1.66a	1.29a
T1	0.92b	0.39a	1.67a	1.12b
T2	0.86cd	0.37ab	1.46c	0.95c
T3	0.85cd	0.39a	1.56b	0.93cd
T4	0.86cd	0.39ab	1.56b	0.94c
T5	0.77e	0.32c	1.23f	0.72f
T6	0.88bc	0.39a	1.47c	0.93cd
T7	0.85cd	0.37ab	1.41cd	0.86de
T8	0.78e	0.35b	1.31e	0.79ef
T9	0.82de	0.37ab	1.37de	0.84e
T10	0.82de	0.37ab	1.34de	0.83e
SEM	0.01	0.01	0.02	0.02
P-value	0.009	0.015	0.011	0.008

Means of column with different superscript differ significantly (P<0.05)

Serum biochemical parameters

Significantly lowered ($P<0.05$) serum concentration of total triglycerides (mg/dL), total cholesterol (mg/dL) and LDL cholesterol (mg/dL) and significantly higher ($P<0.05$) HDL cholesterol (mg/dL) were observed in broilers fed on T5 (neem and papaya leaf meal in 1:1 combination at 1.0% of diet) compared to those assigned control diet (T0). However, the concentration of total protein in serum of broilers was significantly ($P>0.05$) not affected by the supplementation and was statistically similar ($P>0.05$) in all the experimental groups (Table 7).

Findings of the current study are in agreement with Almamury (2024) neem leaf powder supplementation (2.5-3.5 g/kg) in broiler diets led to significantly ($P<0.05$) higher HDL and lower LDL cholesterol levels compared to control birds, suggesting improved lipid metabolism with neem inclusion. Possible reason could be that neem leaf meal exerts inhibitory effects on cholesterol biosynthesis via modulation of HMG-CoA reductase activity, thereby reducing serum cholesterol levels (Asghar et al., 2023). Also, papaya leaf supplementation has been shown to exert hypocholesterolemic effects, significantly reducing serum cholesterol in broiler chickens fed papaya leaf powder (Oloruntola et al., 2024). Some phytochemicals such as saponin and phenolic compounds present in papaya leaf meal have been reported to exert a hypocholesterolemic effect and reduces gut cholesterol uptake through intralumenal physicochemical interaction. This may be responsible for the decrease in cholesterol and LDL level in papaya leaf meal inclusive diets. This equally infers that neem and papaya leaf meal should be used to produce poultry products with desirable reduction in cholesterol level.

Table 7. Serum biochemical parameters of broilers in different treatment groups.

Treatment	Total Protein (g/L)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	LDL(mg/dL)	HDL (mg/dL)
T0	2.50	68.74^{a}	141.60^{a}	71.64^{a}	26.36^{d}
T1	2.29	55.72^{bcd}	136.01^{abc}	58.68^{abc}	28.88^{bc}
T2	2.40	55.13^{cd}	124.95^{e}	59.04^{abc}	27.21^{cd}
T3	2.38	42.12^{f}	129.23^{cde}	50.24^{bc}	29.09^{bc}
T4	2.65	54.40^{d}	129.56^{cde}	53.46^{bc}	29.24^{bc}
T5	2.48	44.94^{e}	124.05^{e}	46.58^{c}	32.02^{a}
T6	2.70	41.70^{f}	133.30^{bcd}	51.22^{bc}	30.22^{ab}
T7	2.33	56.07^{bc}	129.57^{cde}	66.74^{a}	28.16^{bcd}
T8	2.50	54.98^{cd}	126.31^{de}	62.66^{ab}	27.75^{cd}
T9	2.22	56.71^{b}	127.68^{de}	62.00^{ab}	26.59^{d}
T10	2.51	54.67^{cd}	138.66^{ab}	63.30^{ab}	28.17^{bcd}
SEM	0.03	1.61	1.28	1.79	0.37
P-value	0.063	0.012	0.018	0.021	0.009

Means of column with different superscript differ significantly ($P<0.05$)

CONCLUSION

The present study shows that supplementation of broiler diet with a 1:1 blend of neem and papaya leaf meals at 1.0% of the total diet has the potential to enhance several key performance indicators significantly. The birds improved significantly in terms of general performance, indicated by increased growth rate and feed conversion ratio. In addition, the immune response was positively modulated, implying the supplementation enhances the birds' resistance to infection and health maintenance capabilities. The antioxidant status was also significantly improved, implying an intensified defense against oxidative stress during development. Lastly, serum biochemical parameters were within optimal ranges, affirming that the dietary intervention does not compromise metabolic health. These results together validate the promise of neem and papaya leaf meal as an effective and natural alternative to antibiotics for enhancing strong growth and better health in broiler production.

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