Biosight 2026; 07(01): 7-15

Impact of Papaya Leaves Powder supplementation on Growth and Antioxidant Markers of Broiler.

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Abstract: Current research study was conducted to determine the impact of *Carica papaya* leaves powder supplementation on the growth and antioxidant activity in broiler chicken**.** A total of one hundred twenty (n=120) broiler birds were procured from a hatchery and allocated into two groups (Group-A & B) with three replicates and each group comprised sixty (n=60) birds. Broiler chicks of Group-A (Control) were fed on basal diet only and birds of group-B were supplemented with 1.5% papaya leaves powder along with basal diet. The significant impact of the Papaya (*Carica papaya)* leaves powder supplementation was recorded on the growth and antioxidant activity in the broiler chicken. The live body weight (2868.7±43.60g) and FCR (1.40±0.0230) levels were significantly (P<0.05) improved with lower feed intake (3488.4±29.20g) in the broiler chicken supplemented with *Carica papaya* leaves powder than the live body weight (2240.0±28.50g), FCR (1.70±0.0154) and high feed intake (3716.7±16.66g) control group fed only on basal diet. However, the levels of antioxidant enzymes Glutathione peroxidase (79.19±10.89µmol) and Superoxide dismutase (82.88±9.98U/mg) were markedly increased and malondialdehyde level (1.30±0.19nmol/g) decreased in papaya leaves powder supplemented group compared to the control group (65.30±7.99 µmol, 69.78±7.58 U/mg and 1.89±0.38 nmol/g) of birds resulting in better antioxidant activity contrast to control group fed of basal diet only.

Keywords: Papaya leaves powder, growth performance, antioxidant activity, broiler chicken.

Received: 19-9-2025
Accepted: 10-12-2025
DOI: 10.46568/bios.v7i1.234

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Introduction

Broiler chicken is recognized as a rich source of low-fat white meat with high nutritional value. Increasing consumer demand for broiler meat has led to the rapid growth of broiler farming, making it a profitable sector of the poultry industry. Besides, additives used in the feed have been reported to increase the productivity of poultry and are becoming popular as a viable alternative to antibiotics in animal's feeding. Following the worldwide ban on antibiotics in foods (1), there is an attempt to concentrate on utilizing byproducts and extracts of plants as new natural additives to poultry feed and minimize antibiotics administration in diets of broiler chicken as they can influence the health of consumers. A considerable number of references support the idea that medicinal plants can be considered a good alternative to synthetic additives and impact the immune system, intestinal microbiome, and antioxidant status of broiler chickens (2). In this

regard, performing the potential of the antimicrobial and antioxidant action of medicinal plants must achieve at least a minor degree of regular and organization tests within a large range of production patterns. Nowadays, the feed antibiotics of animal feed are replaced by botanical plants and extracts that are applied as new feed additives (3). Medicinal portions of different plants have been as supplements or additions to livestock production for several purposes regarding performance and health significance (4). Papaya leaves and papaya seeds contain nutritional and antioxidant values. Papaya contains a lot of vitamins A, B, C, papain and Chymopapain. The papaya leaves and seeds possess 2,2-diphenyl-1-picrylhydrazyl (7.8 mg/ml and 1.0 mg/ml), phenol (424.89 mg GAE/100 g dry weight and 30.32 mg GAE/100 g dry weight) and flavonoids (333.14 mg GAE/100 g dry weight and 59.54 mg GAE/100 g dry weight) respectively (5). It has been estimated that some papers have tested the papaya seeds and leaves powder that has been incorporated in the diet of poultry as a supplement of protein and herbal proteolysis enzymes to increase the digestibility of consumed feed in the gastrointestinal tract accelerating growth, increasing the immune response and health of the birds (5,6). Different studies have been documented on the impact of papaya leaves on the growth of birds, and it has been cited by the authors that supplementing the diet with papaya leaf extract significantly improved daily weight gain, feed conversion ratio, protein digestibility, and overall growth performance in broiler chickens (7). It improves the antioxidants activity and strengthens the immune response in broilers, evidenced by increased relative weights of the bursa of Fabricius and spleen and a reduced heterophil/lymphocyte ratio, which acts as a stress indicator. The superoxide dismutase, glutathione peroxidase, and catalase levels which are the indicators of antioxidant activity are also significantly improved with the addition of papaya leaves in diets of birds (8). Keeping in the literature highlighting the therapeutic and growth promoting effects of papaya, the present study was designed to observe the impact of papaya leaves on the growth and antioxidant profile of broiler chicken.

Materials & Methods

Experimental birds and housing

One hundred twenty (n=120) day-old broiler chicks were procured from a well reputed hatchery. After initial weight, chicks were equally distributed into two experimental groups (A & B); each group consisted of 60 birds and each group had three replicates, each replicate had twenty (n=20). Between both two groups, birds of control group (A) fed on basal diet while other group (B) was supplemented with *Carica papaya* leaves powder @ 1.5% per kg feed. This design was allowed to observe how *Carica papaya* leaves powder affects the growth and antioxidant activity in broiler chicken.

Preparation of supplement

The papaya leaves were collected from the vicinity of the study area, properly washed and dried with convection forced drying method. After drying the dried leaves were grinned into the electrical grinding machine till it became powder. The papaya leaves powder further analyzed for its proximate and/or chemical analysis. The proximate analysis of papaya leaves powder is mentioned in the table below.

Table 1. Proximate/chemical composition of papaya leaves powder

Supplement	Crude protein (%)	Fat (%)	Moisture (%)	Ash (%)	Dry matter (%)
Papaya leaves powder	6.12	12	8.79	20.4	52.69

Housing facilities preparation for birds

One hundred twenty (n=120) day-old broiler chicks were brought from reliable commercial hatchery and housed at well-managed poultry experimental station, Sindh Agriculture University Tandojam. Shades of birds were cleaned, washed, and disinfected before arrival of birds to prevent any bacterial or viral infection and wood shaving was dispersed (2-inch thickness) and used as their bedding material. The brooders, drinkers and feeders were placed and initiated prior to arrival of birds to ensure required temperature of experimental house.

Following isoenergetic and isonitrogenous diets with the addition of papaya leaves powder supplement were formulated for the experimental trial.

Table 2. Feed formulation chart with papaya leaves powder supplementation

Ingredient	Group A (Control)	Group B
Starter ration		
Maize	55%	54.5%
Soybean Meal	30%	30%
Fish Meal	5%	5%
Wheat Bran	5%	5%
Vegetable Oil	2%	2%
Dicalcium Phosphate	1%	1%
Premix (Vitamins, Minerals)	1%	1%
Supplement	-	1.5% papaya leaves powder
Grower ration		
Maize	57%	56.5%
Soybean Meal	28%	28%
Fish Meal	6%	6%
Wheat Bran	4%	4%
Vegetable Oil	2%	2%
Dicalcium Phosphate	1%	1%
Premix (Vitamins, Minerals)	1%	1%
Supplement	-	1.5% papaya leaves powder
Finisher ration		
Maize	59%	58.5%
Soybean Meal	25%	25%
Fish Meal	7%	7%
Wheat Bran	3%	3%
Vegetable Oil	2%	2%
Dicalcium Phosphate	1%	1%
Premix (Vitamins, Minerals)	1%	1%
Supplement	-	1.5% papaya leaves powder

The recommended vaccination schedule for broiler chicken by National Disease control Committee, Pakistan Poultry Association has been followed accordingly.

For observing the impact of papaya leaves powder supplementation on the production and antioxidant markers birds were randomly selected from each replicate of each group, slaughtered and used for sampling to analyze the growth performance and antioxidant activity in the broiler chicken.

Growth performance

Body weight (g)

After the arrival of day-old broiler chicks at the research station, each chick was individually

weighed. Subsequently, body weights for each replicate were recorded on a weekly basis throughout the experimental period. Moreover, at the end of the experiment (6^{th} week) the birds from both groups (A&B) were weighed to record final body weight by using electronic weighing scale.

Table 3. Vaccination schedule for the broiler chicken

Days	Vaccines	Route of administration
1	IB H120+ I.B variant IBD	Spray Injection
3	ND killed (ND + H9 killed)	Injection
6	ND Live	Spray
12	IB H120	Spray
17	ND Live	Spray

Feed intake (g)

The weight of given feed, daily feed intake and feed refusal by the birds of each replicate was recorded and following formula was used to calculate the overall feed intake from the recorded data of feed for each bird.

Feed intake = Feed offered-Feed residue in a specified period × No of birds

Feed conversion ratio

On the basis of body weight gain (g) of each bird the Feed conversion ratio was calculated through following formula.

Feed Conversion Ratio (FCR) = Feed intake (g) / Weight gain (g)

Antioxidant enzymes

Malondialdehyde

The Malondialdehyde enzyme was used to estimate the protein peroxidation in serum according to the method reported by Drapper and Hadley (9) by using Malondialdehyde MDA: A003-1 kit manufactured by Nanjing Jiancheng Bioengineering Institute, China. In the serum phase Malondialdehyde was permitted to interact with thiobarbituric acid. The Malondialdehyde-thiobarbituric acid adduct in acidic medium was formed during the reaction, extracted to the organic coating. The absorbance was calculated at 532nm in the spectrophotometer model (721-VIS Spectrophotometer). The combination of supernatant (4ml) and serum homogenate (0.4ml) was used with the combined mixture of thiobarbituric acid (0.8 ml) and acetic acid (1.5ml) at 3.5 pH level added with distilled water, boiled in water bath at 95°C for 1hour. After 1 hour, the mixture was removed and the level of malondialdehyde (MDA) in the supernatant was measured at 532 nm using a spectrophotometer. The final MDA concentration was calculated from a standard curve and expressed as nmol MDA/mg protein.

Glutathione peroxidase (GPx)

Raddam et al. (10) method was used to determine tissue glutathione peroxidase (GPx) level. In the presence of H_2O_2 and $NaNO_3$GPx activity was determined and measured by the decreased glutathione level after incubation. A period of ten minutes was spent incubation for the reagents with the PBS solution. After incubation, the reaction was stopped by adding 2 milliliters of 1.65 percent HPO32- to the mixture, and then the mixture was spun at 1500 revolutions per minute for ten minutes to complete the stop step. After centrifugation, 1 ml of DTNB and 2 ml of Na2HPO4 were added to 2 ml of the supernatant, and then another 1 ml of DTNB was added to the mixture.

Following the mixing, yellow-colored solution and absorbance was determined at a wavelength of 412 nm.

Superoxide dismutase (SOD)

For the determination of SOD level, the method reported by Giannopolitis and Ries (11) was used. Following steps were taken to estimate the SOD through Spectrophotometry. Tissue samples of liver and heart were collected from broilers and immediately placed in ice until processing. Tissue samples were homogenized with phosphate-buffered saline (PBS) to remove erythrocytes that may interfere with the assay. The tissue was homogenized in a suitable ice-cold buffer. The typical ratio is 1:9 (weight to volume). Homogenate was centrifuged at high speed (10,000 g to 14,000 g at 4°C for 15-30 minutes) to remove cellular debris and obtain the supernatant, which contains soluble enzyme. The concentration of protein in supernatant was determined by using a Bradford protein assay. This is essential for normalizing the SOD activity results. The SOD assay relies on the enzyme's ability to inhibit the photochemical reduction of NBT to a blue formazan product. The decrease in color development is measured spectrophotometrically. Following steps were followed to analyze the SOD levels.

1. Reaction mixtures were fed in 96-well plate (for microplate readers).
2. All the tubes were controlled that lack the enzyme sample but contain all other reagents to determine the maximal NBT reduction rate (0% inhibition).
3. Appropriate amount of the chicken tissue extract (sample) was added to the reaction mixture to start the reaction.
4. The plates were exposed to a light source (e.g. 15W fluorescent lamp in an aluminum foil-lined box) for a specific period (typically 10-15 minutes). The light triggers the reaction that generates superoxide radicals.
5. Reaction was stopped, often by adding a stopping agent like glacial acetic acid or allopurinol (an XOD inhibitor if using the xanthine/xanthine oxidase method).
6. The absorbance of the reaction mixture was measured by using a spectrophotometer at the appropriate wavelength, typically 560 nm for the NBT method.

For calculation determine the percentage inhibition of NBT reduction caused by the sample compared to the control:

$$\text{Inhibition (\%)} = \frac{\text{Control rate} - \text{Sample rate}}{\text{Control rate}} \times 100$$

Statistical analysis

For the analysis of data, the average values from each replicate were used to compare the means of groups. The mean and standard error descriptive statistical tools were used to analyze the data, and the differences among the groups were also evaluated through the analysis of variance (P-value) at 5% level of probability by using the SAS statistical software.

Results

Growth parameters

Beneficial effect of papaya (*Carica papaya)* leaves powder supplementation was observed on the growth performance of broiler chicken and results are presented in Table-1. Significant impact of papaya (*Carica papaya)* leaves powder supplementation was observed on the growth performance, and it was noted that the live body weight and feed conversion ratio was significantly ($P<0.05$) improved in the papaya (*Carica papaya)* leaves powder supplementation group with low feed intake, while higher feed intake was noted in the control group only fed on basal diet.

Table 4. Effect of papaya *(Carica papaya)* leaves powder supplementation on growth performance of broiler chicken

Growth Parameters	Treatment groups		P-value (0.05)
	Group-A (Control; only Basal diet)	Group-B (1.5% *Carica papaya* + Basal diet)	
Live body weight (g)	2240±26.204	2668±31.520	0.0004
Feed conversion ratio	1.70±0.0428	1.47±0.0306	0.0021
Feed intake(g)	3710±48.414	3490±16.403	0.0038

*The data analyzed are the average values of the three replicates of each group.

Antioxidant enzymes

The effect of papaya (*Carica papaya)* leaves powder supplementation was noted on antioxidant activity in broiler chicken and results are presented in Table-2. Significant impact of papaya (*Carica papaya)* supplementation was observed on the Glutathione peroxidase and Superoxide dismutase and Malondialdehyde, and it was noted that the levels of Glutathione peroxidase and Superoxide dismutase enzymes were significantly ($P<0.05$) improved and Malondialdehyde content was reduced, compared to the control group of broiler chicken.

Table 5. Effect of papaya *(Carica papaya)* leaves powder supplementation on Antioxidant enzymes of broiler chicken

Antioxidant markers	Treatment groups		P-value (0.05)
	Group-A (Control; only Basal diet)	Group-B (1.5% *Carica papaya* + Basal diet)	
Malondialdehyde (nmol/g protein)	1.89±0.0306	1.30±0.0365	0.0001
Glutathione peroxidase (µmol protein)	65.30±0.5774	79.19±0.3170	0.0015
Superoxide dismutase (U/mg protein)	69.78±0.5774	82.88±0.5774	0.0012

*The data analyzed are the average values of the three replicates of each group.

Discussion

Growth performance: The current results provide some useful knowledge on the influence of these natural food additives on chicken output. Group of broilers supplemented with Papaya leaves powder showed a very superior growth performance, live body weight and feed conversion ratio with low feed intake. In a related study by authors (5) demonstrating that papaya leaves improve nutrient use and promote development because they are bioactive, bearing numerous flavonoids and antioxidants. Although the control group showed relatively low weight gain, supplementation with mixed herbs improved feed conversion efficiency, suggesting a synergistic effect among the herbal components. These findings support previous reports indicating that combinations of phytonutrients can enhance production efficiency. The papaya-supplemented group exhibited the highest feed conversion ratio, indicating the potential influence of papaya leaf

powder on nutrient digestibility and metabolic utilization. These findings coincide with Nishimwe (12), who observed improved feed conversion in the presence of natural growth promoters in supplemented group compared to control. Sugiharto et al. (13) observed similar trends and credited improved meat yields to better nutrient bioavailability and lower oxidative stress in herbal-supplemented broilers. Good gut health is necessary for nutrient absorption and thus bird performance. Authors further reported that the phytogenic and nutritive potentials of the papaya leaf could be utilized as an additive in feed to promote growth in broiler chickens (14). Likewise, Rahman et al. (15) reported that supplementing with papaya leaf extract slightly increased body weight, weight gain, and the broiler production efficiency factor compared to the control group, suggesting a positive effect on growth. Furthermore, researchers (16) found that inclusion of papaya leaf meals with higher concentration significantly affected feed consumption ($P<0.05$) but had no significant effect ($P>0.05$) on body weight gain and feed conversion ratio in broiler chickens.

Antioxidant activity: The results of this study show how dietary supplementation with *Carica papaya* leaf powder affects broiler chickens' enzyme activity and antioxidant capacity. Significantly increase activity of antioxidant enzymes reflected in lower malondialdehyde levels and higher superoxide dismutase and glutathione peroxidase activity. Similar results from Wallace et al. (17) implied that the papaya leaves are loaded with antioxidants. Researchers reported significantly higher serum levels of superoxide dismutase, glutathione peroxidase, and catalase in birds fed diets supplemented with leaf meals, including pawpaw, compared with the control diet. These improvements in oxidative status and overall performance were attributed to the antimicrobial, anti-stress, antioxidant, and immunomodulatory effects of papaya leaf and seed composites containing phytochemicals such as tannins and terpenoids (8). Other studies using plant-derived bioactive chemicals, such as Kong et al. (18) found comparable trends: The inclusion of *Carica papaya* leaves and other plants powders, separately and/or in a mixture in broiler diets greatly improved the levels of antioxidant enzymes of broiler meat. The bioactive qualities of these plant additives, which have been thoroughly researched in earlier studies, explain this increase in antioxidant capacity. With the lowest antioxidant activity, the control group showed that a basic diet without supplements would not improve meat's antioxidant characteristics. On the other hand, the herbal mix group showed the highest antioxidant radical scavenging capability, pointing to a synergistic effect of mixing these herbs. This is consistent with a study by Tacias-Pascacio et al. (19), which discovered that using many phytogenic feed additives simultaneously results in complementary or synergistic effects, therefore increasing broiler meat's antioxidant capacity. *Carica papaya* leaves powder had the greatest level of antioxidant activity among the herb extracts, followed by *Mangifera indica* and then *Lawsonia inermis*. The more effectiveness of *Carica papaya* may be due to the abundant presence of phenolics, flavonoids, and carotenoids, which are known to have strong free radical scavenging capabilities (20). Moreover, Rahman et al. (2022) documented the antioxidant properties of papaya seed and leaf contributing in part to enhanced growth performance and improved immunity in poultry. Similarly, Sari et al. (16) suggested that papaya leaves are rich in vitamins (C, E, B1), minerals, and enzymes (chymopapain, papain, and lipase) which may increase nutrient digestibility, utilization and antioxidant activity of birds.

Conclusions

The supplementation of papaya (*Carica papaya)* leaves powder positively influenced the growth performance of broiler; the feed efficiency and feed conversion ratio is also improved with low feed intake. Moreover, the antioxidant activity in broiler chicken is triggered with the application of the papaya leaves powder supplementation.

Ethics Approval and Consent To Participate
Not applicable.

Human and Animal Rights
All experimental protocols were performed on the basis of guidelines provided by the national institute of Care and Use.

Consent for Publication
Not applicable.

Availability of Data and Materials
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Funding
None.

Conflict of Interest
There is no conflict of interest shown by the research manuscript authors

Acknowledgements
None

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