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# Comparative Effects of *Bacillus subtilis* Probiotic and *Abutilon indicum* Leaf Powder on Growth Performance, Nutrient Digestibility, Antioxidant Status, and Carcass Traits of Broiler Chickens

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## Abstract

The search for viable, non-antibiotic alternatives in poultry nutrition has intensified focus on both microbial and phytogenic feed additives to optimize gut health and production efficiency. This study evaluated the comparative effects of *Bacillus subtilis* probiotic (Gallipro Max) and *Abutilon indicum* Leaf Powder (AILP) on the growth performance, nutrient digestibility, antioxidant status, and carcass traits of Ross 307 broiler chickens. A 42-day feeding trial was conducted using 500 one-day-old broiler chicks randomly assigned to 5 dietary treatments (n=100 birds/treatment), with each treatment subdivided into 5 replicates of 20 birds each under a Completely Randomized Design (CRD). Treatment 1 (T1) was fed a basal control diet alone; T2 received the basal diet supplemented with Gallipro Max at 200 g/kg; while T3, T4, and T5 were fed the basal diet supplemented with AILP at 100, 200, and 300 g/kg, respectively. Final body weight, feed intake, carcass yield, and dressing percentage were lowest in T1, intermediate in T3-T4, and significantly higher in T2 and T5. Feed Conversion Ratio (FCR) was optimized in T2 and T5 and poorest in T1. Mortality was exclusively recorded in T1, whereas all supplemented groups (T2-T5) recorded 100% survival rates. Relative weights of high-value cuts (breast, thigh, leg, shank) and gizzard were significantly increased in T2 and T5, while liver, heart, spleen and kidney weights showed no significant variations across all groups ($P$>0.05). Apparent nutrient digestibility coefficients for dry matter, crude protein, crude fat, crude fiber, and ash were substantially enhanced in T2-T5 compared to the control. Physiologically, dietary supplementation significantly reduced Malondialdehyde (MDA) levels while concurrently boosting serum immunoglobulins (IgG, IgA, IgM) and endogenous antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) in T2-T5 relative to T1. In conclusion both *Bacillus subtilis* probiotic at 200 g/kg and *Abutilon indicum* leaf powder at 300 g/kg serve as potent, safe bio-enhancers. They maximize broiler growth, tissue accretion, and nutrient digestibility while simultaneously reinforcing systemic antioxidant status and humoral immunity.

**Keywords: *Abutilon indicum*; Antioxidant status; *Bacillus subtilis*; Broiler Performance; Carcass traits; Nutrient Ddigestibility**

## Introduction

The global poultry industry faces a critical transition phase driven by mounting regulatory restrictions and consumer pushback against Antibiotic Growth Promoters (AGPs) [1]. For decades, sub-therapeutic doses of antibiotics were routinely incorporated into broiler feeds to suppress pathogenic gut microbiota, improve feed conversion efficiency, and lower mortality rates [2]. However, the indiscriminate use of these agents has accelerated the emergence of multi-drug-resistant bacterial strains and left hazardous chemical residues in poultry meat, posing a severe public health threat worldwide [2]. Consequently, animal scientists and poultry nutritionists are actively exploring safe, natural biosecurity strategies to support gut health and maintain production efficiency without relying on conventional antibiotics [3]. Among these alternatives, feed-grade probiotics and Phytogenic Feed Additives (PFAs) have emerged as highly promising tools [3].

Commercial probiotics, particularly spore-forming bacterial strains like *Bacillus subtilis*, are

widely recognized for their ability to stabilize the gastrointestinal environment [4]. These live microbial supplements survive the harsh conditions of feed manufacturing and avian gastric acidity, actively colonizing the intestinal mucosa [5]. Once established, they work through competitive exclusion - occupying binding sites and exhausting resources to prevent pathogens like *Clostridium perfringens* and *Escherichia coli* from taking hold [5]. Concurrently, phytogenic feed additives derived from aromatic plants, herbs, and spices are gaining traction due to their rich concentrations of secondary metabolites, including flavonoids, alkaloids, saponins, and tannins [6]. These bioactive compounds possess potent antimicrobial, anti-inflammatory, and antioxidant properties [6]. They stimulate endogenous enzyme secretions, optimize intestinal morphology, and protect tissues from oxidative damage, making them an excellent natural alternative for poultry production [7].

Numerous studies demonstrate the production and physiological benefits of supplementing broiler diets with *Bacillus subtilis* - based probiotics [4]. Research shows that *B. subtilis* actively secretes highly efficient extracellular enzymes, including proteases, amylases, and lipases, which break down complex dietary compounds and significantly improve apparent nutrient digestibility coefficients for crude protein and dry matter [8]. This enzymatic support consistently translates into higher final body weights, increased feed intake, and optimized Feed Conversion Ratios (FCR). Furthermore, microbial colonization primes the host's immune system [9]. Previous trials report substantial elevations in serum immunoglobulins (IgG, IgA, and IgM) alongside improved intestinal architecture, characterized by increased villus height and reduced crypt depth. This structural optimization expands the functional absorptive surface area of the gut while reinforcing the mucosal barrier against systemic infections [10-15]. Parallel to these microbial interventions, the evaluation of innovative phytogenic candidates has shown promising results in poultry nutrition. Plants belonging to the Malvaceae family, such as *Abutilon indicum* (commonly known as Indian Mallow), are increasingly recognized for their diverse pharmacological properties [11, 14]. While literature on the direct application of *Abutilon indicum* Leaf Powder (AILP) in commercial broiler diets remains limited, studies on closely related botanical extracts demonstrate that their inclusion improves growth performance, enhances carcass yield, and minimizes mortality under intense production conditions.

The primary mode of action for these phytogenics lies in their exceptional antioxidant and cytoprotective capabilities. Broilers fed diets rich in plant polyphenols consistently exhibit a dramatic reduction in serum Malondialdehyde (MDA) levels a key biomarker of lipid peroxidation [8, 13]. At the same time, these diets trigger a up-regulation of vital endogenous antioxidant enzymes, including: Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px) [9]. This dual action minimizes systemic oxidative stress, allowing birds to direct more metabolic energy toward muscle tissue accretion rather than cellular repair [10-12]. Despite the documented benefits of commercial probiotics and the therapeutic potential of *Abutilon indicum* in traditional medicine, there is a clear gap in research comparing them directly as feed additives in modern broiler production. Most existing phytogenic research focuses on oil extracts rather than whole leaf meals, which are often more practical and cost-effective for small-to-medium scale poultry farmers to process. It is also essential to carefully evaluate the toxicological safety profile of high AILP inclusion rates by monitoring vital organ weights, ensuring these botanical compounds can be used safely without causing metabolic or physiological harm.

## Materials and Methods

### Experimental location and weather conditions

The 42-day feeding trial was conducted at the Poultry Research Unit of the Gandhi College of Agriculture, located in Rajasthan, India. Geographically, the research site is situated approximately at a latitude of $26^0 55'$ N and a longitude of $75^0 49'$ E, sitting at an elevation of roughly 431 meters above sea level. The environmental profile of this region is characterized by a typical semi-arid to arid climate.

### Collection, identification, and processing of plant material

Fresh, healthy leaves of *Abutilon indicum* (Linn.) Sweet were collected during the early morning hours from natural habitats across Rajasthan. The botanical identification and authentic confirmation of the plant material were performed at the Department of Botany and Plant Sciences, and a voucher herbarium specimen (Voucher No. AI-M-085) was deposited for future reference. The harvested leaves were thoroughly washed with tap water to eliminate dust and soil contaminants, followed by a secondary rinse with distilled water then spread in a thin layer on clean mats and allowed to air-dry completely under a shaded, well-ventilated pavilion at room temperature (28-32$^0$C) for 14 days to preserve the integrity of heat-sensitive phytochemical compounds. The completely dried leaves were milled into a fine, homogenous powder using a heavy-duty commercial laboratory hammer mill (Model 300-H, Apex Industrial, India) fitted with a 1.0 mm stainless steel mesh screen. The resulting *Abutilon indicum* Leaf Powder (AILP) was packed tightly into airtight, light-shielded polyethylene containers and kept at room temperature until it was mixed into the experimental diets.

### Sanitation, disinfection, and brooding setup

Prior to the arrival of the chicks, the experimental poultry house at the Gandhi College of Agriculture was subjected to a rigorous biosecurity and sanitation protocol. All previous litter material was completely removed, and the concrete floors, walls, mesh wires, and equipment (feeders, drinkers, and brooding cages) were thoroughly washed with pressurized water and a 5% sodium hypochlorite solution. Following structural drying, the entire facility was disinfected using an iodine-based compound and left vacant for a 14-day down period.

Three days before stocking, fresh, dust-free wood shavings were distributed evenly to establish a 5 cm deep litter base in each pen. The heating system, consisting of infrared heat lamps, was activated 24 hours prior to chick placement to ensure the concrete floor and litter reached a stable thermal equilibrium of 33±1$^0$C. On Day 1, immediately upon arrival, the chicks were unboxed, given an oral rehydration solution containing 5% glucose and vitamins to alleviate transportation stress, and allowed to acclimate for two hours before individual initial body weights were recorded and dietary treatments were introduced.

### Ethical approval and experimental design

All animal care, handling, and management procedures were strictly executed in accordance with the standard guidelines for the care and use of laboratory animals. The experimental protocol received official clearance and ethical approval from the Institutional Animal Ethics Committee (IAEC) under approval certificate registration number GCA/POUL-AE-04-2025.

A total of 500-day-old, unsexed Ross 307 strain broiler chicks were acquired from a commercially certified hatchery. Upon arrival, the

chicks were weighed collectively to ensure uniformity and randomly assigned to one of five distinct dietary treatments using a Completely Randomized Design (CRD). Each treatment group consisted of 100 birds, subdivided into 5 replicates containing 20 birds per pen. The pens were identical deep-litter structures (2.0 m×1.5 m floor dimensions) bedded with fresh wood shavings to a uniform depth of 5 cm. Standard commercial brooding, biosecurity, and management practices were rigorously maintained across all groups.

The experimental diets were formulated to be iso-nitrogenous and iso-caloric, meeting or exceeding the nutrient specifications set by the National Research Council [16] for broiler chickens. The treatments were assigned as follows:

T1: (Control): Basal diet alone.

T2: Basal diet+commercial probiotic (Gallipro Max, Chr. Hansen, Denmark) at an inclusion level of 200 g/kg of feed.

T3: Basal diet+*Abutilon indicum* Leaf Powder (AILP) at 100 g/kg of feed.

T4: Basal diet+AILP at 200 g/kg of feed.

T5: Basal diet+AILP at 300 g/kg of feed

Feed and clean water were provided ad libitum throughout the 42-day trial

### Phytochemical Analysis of Plant Powder and proximate analysis of experimental diet

Analysis of phyto-components in *Abutilon indicum* leaf powder was carried out using standard laboratory procedures previously published by [17]. Proximate analysis of experimental diet was done using FOSS Near Infra-Red Feed Analyzer (Model NIRS DS3/DS2500 F, Denmark) which is maintained at a wavelength range (400-2500 nm) and data resolution of 0.5 nm to ensure high precision in results. Mineral analysis of basal diet was assayed according to standard procedures of AOAC [18].

### Process for performance parameters

Growth performance traits were measured systematically throughout the 42-day experimental period. The initial body weights of individual chicks were recorded on Day 1 using a high-precision digital platform scale (Model scout-SPX, Ohaus Corporation, Parsippany, NJ, USA; accuracy±0.01 g). Broilers were subsequently weighed individually at weekly intervals to document Body Weight Gain (BWG).

Feed Intake (FI) was calculated weekly per replicate pen by subtracting the weight of the refused or leftover feed from the total quantity of feed provided over that seven-day period. The Feed Conversion Ratio (FCR) for each replicate was calculated at the conclusion of each weekly phase and summarized for the full 42-day period using the formula:

FCR=Total Live Weight Gain (g)/Total Feed Consumed (g)

Mortality was monitored and recorded twice daily (morning and evening). The weight of any dead bird was immediately recorded and factored into the final FCR calculation to adjust for final bird-day feed consumption across all pens.

### Apparent nutrient digestibility evaluation

At the end of the feeding trial, a 7-day digest trial was executed to measure apparent nutrient digestibility coefficients using an indicator-free total fecal collection approach. Two representative birds from each replicate pen (totaling 10 birds per treatment group) were transferred to individual metabolic cages designed for separate, clean fecal recovery. Birds were placed on a two days acclimatization period. Feed consumption was precisely documented, and clean, uncontaminated excreta samples were collected daily after the adjustment period from plastic sheets placed beneath the cages. The daily fecal collections from each replicate were pooled, freed of shed feathers and scale debris, acidified with 10% sulfuric acid to trap nitrogen, and stored at -18$^0$C. At the end of the collection phase, the samples were dried in a forced-air drying oven (Model Memmert UF110, Memmert GmbH, Schwabach, Germany) at 65$^0$C for 72 hours, then ground to pass through a 1.0 mm screen. Proximate analysis of both the feed inputs and the dried excreta was conducted in duplicate according to [18]. Protocols to determine Dry Matter (DM), Crude Protein (CP, *via* the Kjeldahl method), crude fat (*via* ether extraction), Crude Fiber (CF), and total ash content. The apparent nutrient digestibility coefficients were determined using the formula:

Digestibility Coefficient =(Nutrient Intake (g)−Nutrient Excreted (g)/Nutrient Intake (g))×100

### Carcass characteristics and organ weight indices

At the conclusion of the 42-day trial, two representative broilers from each replicate pen whose body weights were within ±5% of the group's mean value were selected for carcass analysis. The chosen birds were fasted for 12 hours (with continuous water access), individually weighed to record final live weight, and humanely sacrificed *via* cervical dislocation followed by rapid exsanguination. Following feathers removal, scalding, and evisceration, the hot carcass weight was immediately taken to calculate the dressing percentage: The carcass was then dissected to isolate primal cuts, including the breast, thigh, leg, and shank. The relative weights of these cuts, along with the internal organs (liver, kidney, heart, spleen, and gizzard), were calculated and expressed as a percentage of the bird's fasted live weight using a precision analytical balance (Model electronic balance Pioneer PX224, Ohaus Corporation, USA; readability 0.1 mg).

### Serum immunoglobulins and oxidative stress (antioxidant) biomarkers analysis

During the final processing on day 42, 5 mL blood samples were harvested from the brachial wing vein of two birds per replicate using sterile, non-heparinized vacutainer tubes. The blood was left at room temperature for 2 hours to clot, then centrifuged at 3500×g for 15 minutes at 4$^0$C using a refrigerated laboratory centrifuge (Model 5425-R, Eppendorf AG, Hamburg, Germany) to isolate clean serum. The serum was transferred into sterile 1.5 mL Eppendorf tubes and kept at -80$^0$C for subsequent biochemical processing.

Serum titres of Immunoglobulin G (IgG), Immunoglobulin A (IgA), and Immunoglobulin M (IgM) were determined using chicken-specific Enzyme-Linked Immunosorbent Assay (ELISA) kits (Cusabio Technology LLC, Houston, TX, USA) according to the manufacturer's strict protocol.

Serum Malondialdehyde (MDA) concentrations, along with the activities of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GSH-Px), were analyzed using specialized commercial colorimetric assay kits (Cayman Chemical Company, Ann Arbor, MI, USA). All ELISA and colorimetric assay absorbances were quantified on an automated Microplate Reader (Model Sunrise-01, Tecan Group Ltd., Männedorf, Switzerland) running

specialized Magellan data processing software to track standard calibration curves and absolute sample calculations.

### Statistical analysis

All data gathered were subjected to a One-way Analysis of Variance (ANOVA) for a Completely Randomized Design (CRD) using SPSS software. Significant differences among treatment means were separated using Duncan's Multiple Range test at a confidence level of $p<0.05$.

## Results

Phytochemical components in *Abutilon indicum* leaf powder revealed that it contained phenolic compounds at 302.0 mg/g, flavonoids (209.5 mg/g), terpenoids (176.1 mg/g), tannins (85.12 mg/g), steroids (80.65 mg/g), saponins (34.57 mg/g) and alkaloids (25.71 mg/g). In ranking order: phenols>flavonoids>terpenoids>tannins>steroids>saponins>alkaloids (Table 1 and 2).

Body weight gain, average daily weight gain was more ($p<0.05$) in T2 and T5, intermediate in T3, T4 and lower in T1. Average daily feed intake was higher in T2-T5 relative to T1 ($p<0.05$). Feed conversion ratio and mortality rate was influenced by the treatment (Table 3).

Carcass characteristics of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets are revealed in Table 4. Dressed weight, eviserated weight and dresssing percentage were higher ($p<0.05$) in T2 and T5, intermediate in T3-T4 and lower in T1. Except for weights of gizzard, head, neck, wing, breast and shank which were affected ($p<0.05$) by dietary treatments, other carcass parameters (spleen, liver, kidney, heart) showed no ($p>0.05$) difference (Table 4).

**Table 1:** Ingredient and determined values of basal diet.

| | Starter phase (0-21 d) | Finisher phase (22-42d) |
|---|---|---|
| Ingredients | Quantity | Quantity |
| Maize | 50.00 | 55.00 |
| Wheat bran | 3.00 | 4.39 |
| Soyabean meal | 35.05 | 29.05 |
| Fish meal | 4.89 | 3.00 |
| Limestone | 2.00 | 2.50 |
| Dicalcium Phosphate | 4.00 | 5.00 |
| DL-Methionine | 0.25 | 0.25 |
| L-Lysine HCl | 0.25 | 0.25 |
| Min-Vit Premix | 0.25 | 0.25 |
| Salt | 0.20 | 0.20 |
| Toxin binder | 0.11 | 0.11 |
| Total | 100.0 | 100.0 |
| Analyzed values | | |
| Crude protein | 23.12 | 21.06 |
| Crude fibre | 3.88 | 4.01 |
| Ether extract | 4.08 | 4.21 |
| Calcium | 1.17 | 1.19 |
| Phosphorus | 0.56 | 0.58 |
| ME (kcal/kg) | 2908.2 | 3016.5 |

2.5 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.

**Table 2:** Phytochemical components in *Abutilon indicum* leaf powder.

| Compounds | Concentration (mg/g) |
|---|---|
| Phenols | 302.0 |
| Flavonoids | 209.5 |
| Terpenoids | 176.1 |
| Tannins | 85.12 |
| Steroids | 80.65 |
| Saponins | 34.57 |
| Alkaloids | 25.71 |

Nutrient digestibility of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets (Table 5). Dry matter, crude protein, crude fibre, ether extract and ash digestibility were influenced ($p<0.05$) by the treatment. Values obtained were more in T2-T5 relative to T1.

Malondialdehyde concentration was lower ($p<0.05$) in T2-T5 than that of T1. Conversely, superoxide dismutase, catalase, glutathione peroxidase concentration was higher ($p<0.05$) in T2-T5 relative to T1 as presented in Table 6.

Immunoglobulin response of broiler chickens fed Abutilon indicum leaf powder supplemented diets is presented in Table 7. Immunoglobulin A, G and M values follow similar pattern and were influenced by the treatment. Immunoglobulin A (IgA) values ranged from 2.05-5.08 mg/dL, IgG (4.01-7.95 mg/dL) and IgM (0.91-1.98 mg/dL). Immunoglobulin concentrations were more ($p<0.05$) in T2 and T5, intermediate in T3, T4 and lower in T1.

## Discussion

The variations in final body weight, feed intake, and Feed Conversion Ratio (FCR) highlight the nutritional efficiency of the experimental treatments. Broilers in T2 and T5 achieved the highest final body weights, supported by increased feed intake and the lowest (most efficient) FCR values. The control group (T1) exhibited the lowest growth performance. The superior performance in T2 can be attributed to Gallipro Max (*Bacillus subtilis*), which establishes a highly favorable microbial balance in the gut [19]. These beneficial bacteria secrete exogenous digestive enzymes (amylases, proteases, and lipases) that break down complex dietary treatment, directly translating to enhanced feed utilization and rapid weight gain [20]. Concurrently, the improvement seen across the Abutilon indicum treatments (T3 to T5) indicates that the leaf powder contains rich concentrations of bioactive phytochemicals, such as flavonoids, alkaloids, and saponins. At the highest inclusion level (300 g/kg in T5), these secondary metabolites act as natural growth promoters. They stimulate endogenous digestive enzyme secretions from the pancreas and intestinal mucosa, while optimizing the architecture of the intestinal villi to maximize the surface area available for nutrient absorption [21]. This explains why T5 birds consumed more feed and converted it into skeletal muscle mass far more efficiently than the control birds. The result obtained in this study aligns with the report of [22] who recorded a higher body weight in broilers fed diet supplemented with bitter melon and basil leaves powder. Similarly, outcome observed by [23] showed that feed consumption of broilers fed different levels of alfalfa extract increased across the treatment. Crucially, the fact that mortality was exclusively recorded in the control group (T1), while T2 through T5 recorded zero mortality, emphasizes the protective biosecurity role of both additives. By

**Table 3:** Growth performance of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets.

| Parameters | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 | Treatment 5 | SEM |
|---|---|---|---|---|---|---|
| Number of birds | 100 | 100 | 100 | 100 | 100 | - |
| Duration of experiment (days) | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 | - |
| Initial body weight (g/bird) | 45.62 | 45.71 | 45.63 | 46.11 | 45.09 | 0.02 |
| Final body weight (g/bird) | 1900.2$^{c}$ | 2690.8$^{a}$ | 2205.7$^{b}$ | 2213.2$^{b}$ | 2701.6$^{a}$ | 98.55 |
| Body weight gain (g/bird) | 1854.58$^{c}$ | 2645.09$^{a}$ | 2160.07$^{b}$ | 2167.09$^{b}$ | 2656.51$^{a}$ | 90.76 |
| Daily weight gain (g/day) | 44.16$^{c}$ | 62.98$^{a}$ | 51.43$^{b}$ | 51.60$^{b}$ | 63.25$^{a}$ | 4.82 |
| Total feed intake (g/bird) | 4800.9$^{b}$ | 5203.1$^{a}$ | 5200.5$^{a}$ | 5200.9$^{a}$ | 5201.7$^{a}$ | 113.2 |
| Daily feed intake (g/day) | 114.3$^{b}$ | 123.9$^{a}$ | 123.8$^{a}$ | 123.8$^{a}$ | 123.9$^{a}$ | 8.85 |
| Feed to gain ratio | 2.58$^{a}$ | 2.00$^{c}$ | 2.40$^{b}$ | 2.40$^{b}$ | 2.00$^{a}$ | 0.01 |

Note on Superscripts: $^{a, b, c, d}$ Means along the same row with different superscripts are significantly different ($p$<0.05). Rows without superscripts show no significant difference ($p$>0.05); T1: (Control): Basal diet alone; T2: Basal diet+commercial probiotic (Gallipro Max, Chr. Hansen, Denmark) at an inclusion level of 200 g/kg of feed; T3: Basal diet+*Abutilon indicum* Leaf Powder (AILP) at 100 g/kg of feed; T4: Basal diet+AILP at 200 g/kg of feed;T5: Basal diet+AILP at 300 g/kg of feed.

**Table 4:** Carcass characteristics of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets.

| Parameters | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 | Treatment 5 | SEM |
|---|---|---|---|---|---|---|
| Live weight (g) | 1808.2$^{c}$ | 2711.2$^{a}$ | 2219.2$^{b}$ | 2281.5$^{b}$ | 2769.3$^{a}$ | 89.31 |
| Dressed weight (g) | 1508.1$^{c}$ | 2511.2$^{a}$ | 1985.1$^{b}$ | 2045.9$^{b}$ | 2528.6$^{a}$ | 77.54 |
| Eviscerated weight (g) | 1199.9$^{c}$ | 2207.1$^{a}$ | 1662.4$^{b}$ | 1698.2$^{b}$ | 2289.4$^{a}$ | 67.01 |
| Dressing percentage (%) | 66.36$^{c}$ | 81.42$^{a}$ | 74.90$^{b}$ | 74.43$^{b}$ | 82.67$^{a}$ | 0.03 |
| Organ weight (% Live weight) | | | | | | |
| Liver (%) | 2.09 | 2.13 | 2.15 | 2.17 | 2.19 | 0.02 |
| Kidneys (%) | 0.21 | 0.22 | 0.25 | 0.26 | 0.29 | 0.01 |
| Spleen (%) | 0.12 | 0.15 | 0.17 | 0.18 | 0.19 | 0.01 |
| Heart (%) | 0.36 | 0.37 | 0.39 | 0.40 | 0.41 | 0.02 |
| Gizzard (%) | 3.02$^{b}$ | 4.38$^{a}$ | 4.31$^{a}$ | 4.40$^{a}$ | 4.43$^{a}$ | 0.03 |
| Cut parts (% Live weight) | | | | | | |
| Head (%) | 3.09$^{b}$ | 4.02$^{a}$ | 4.18$^{a}$ | 4.23$^{a}$ | 4.28$^{a}$ | 0.03 |
| Neck (%) | 3.35$^{b}$ | 4.59$^{a}$ | 4.62$^{a}$ | 4.76$^{a}$ | 4.78$^{a}$ | 0.02 |
| Wing (%) | 7.11$^{b}$ | 9.57$^{a}$ | 9.88$^{a}$ | 9.92$^{a}$ | 9.97$^{a}$ | 0.04 |
| Breast (%) | 14.67$^{b}$ | 24.33$^{a}$ | 24.04$^{a}$ | 24.01$^{a}$ | 24.81$^{a}$ | 0.09 |
| Thigh (%) | 7.82$^{b}$ | 11.35$^{a}$ | 11.24$^{a}$ | 11.57$^{a}$ | 11.62$^{a}$ | 0.15 |
| Shank (%) | 4.86$^{c}$ | 8.15$^{a}$ | 6.85$^{a}$ | 6.98$^{b}$ | 8.81$^{a}$ | 0.11 |

Note on Superscripts: $^{a, b, c, d}$ Means along the same row with different superscripts are significantly different ($p$<0.05). Rows without superscripts show no significant difference ($p$>0.05); T1: (Control): Basal diet alone; T2: Basal diet+commercial probiotic (Gallipro Max, Chr. Hansen, Denmark) at an inclusion level of 200 g/kg of feed; T3: Basal diet+*Abutilon indicum* Leaf Powder (AILP) at 100 g/kg of feed; T4: Basal diet+AILP at 200 g/kg of feed;T5: Basal diet+AILP at 300 g/kg of feed.

**Table 5:** Nutrient digestibility of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets.

| Parameters | T1 | T2 | T3 | T4 | T5 | SEM |
|---|---|---|---|---|---|---|
| Dry matter | 70.91$^{c}$ | 84.12$^{a}$ | 80.05$^{b}$ | 80.34$^{a}$ | 85.04$^{a}$ | 0.07 |
| Crude protein | 66.84$^{b}$ | 79.48$^{a}$ | 70.93$^{a}$ | 71.66$^{a}$ | 78.65$^{a}$ | 0.05 |
| Crude fibre | 34.58$^{b}$ | 42.45$^{a}$ | 43.07$^{a}$ | 43.58$^{a}$ | 42.13$^{a}$ | 0.03 |
| Ether extract | 50.45$^{b}$ | 68.13$^{a}$ | 62.19$^{a}$ | 61.96$^{a}$ | 69.83$^{a}$ | 0.05 |
| Ash | 36.14$^{b}$ | 48.23$^{a}$ | 47.13$^{a}$ | 46.48$^{a}$ | 49.05$^{a}$ | 0.03 |

Note on Superscripts: $^{a, b, c, d}$ Means along the same row with different superscripts are significantly different ($p$<0.05). Rows without superscripts show no significant difference ($p$>0.05). T1: (Control): Basal diet alone; T2: Basal diet+commercial probiotic (Gallipro Max, Chr. Hansen, Denmark) at an inclusion level of 200 g/kg of feed; T3: Basal diet+*Abutilon indicum* Leaf Powder (AILP) at 100 g/kg of feed; T4: Basal diet+AILP at 200 g/kg of feed; T5: Basal diet+AILP at 300 g/kg of feed.

suppressing opportunistic pathogens and strengthening intestinal tight junctions, both the probiotic and the phytogenic diets eliminated the subclinical necrotic enteritis and systemic infections that typically drive economic losses and mortality in commercial deep-litter production [24]. This result is in consonance with the result obtained by [25] when phytogenic was included in the diet of broiler chicken.

Carcass yield and dressing percentages followed an identical trend, peaking in T2 and T5, remaining intermediate in T3-T4, and dropping to their lowest levels in T1. This mirrors the overall live weight patterns and underscores efficient dietary protein-to-muscle synthesis. The higher relative weights of primal cuts specifically the breast, thigh, wing, neck and shank in the T2 and T5 groups

**Table 6:** Oxidative stress indices of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets.

| Parameters | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 | Treatment 5 | SEM |
|---|---|---|---|---|---|---|
| Malondialdehyde (nmol/mL) | 9.78[a] | 6.06[b] | 5.94[b] | 5.92[b] | 5.88[b] | 0.02 |
| Superoxide dismutase (U/mL) | 25.71[b] | 33.09[a] | 32.86[a] | 32.19[a] | 32.04[a] | 0.08 |
| Catalase (U/mL) | 10.02[b] | 15.72[a] | 16.88[a] | 16.96[a] | 17.01[a] | 1.21 |
| Glutathione peroxidase (*μ*mol/L) | 19.95[b] | 25.08[a] | 25.17[a] | 26.04[a] | 26.19[a] | 1.87 |

Note on Superscripts: [a, b, c, d] Means along the same row with different superscripts are significantly different ($p<0.05$). Rows without superscripts show no significant difference ($p>0.05$). T1: (Control): Basal diet alone; T2: Basal diet+commercial probiotic (Gallipro Max, Chr. Hansen, Denmark) at an inclusion level of 200 g/kg of feed; T3: Basal diet+*Abutilon indicum* Leaf Powder (AILP) at 100 g/kg of feed; T4: Basal diet+AILP at 200 g/kg of feed; T5: Basal diet+AILP at 300 g/kg of feed.

**Table 7:** Immunoglobulin response of broiler chickens fed *Abutilon indicum* leaf powder supplemented diets.

| Components (mg/dL) | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 | Treatment 5 | SEM |
|---|---|---|---|---|---|---|
| Immunoglobulin A | 2.05[b] | 4.42[a] | 4.94[a] | 5.02[a] | 5.08[a] | 0.02 |
| Immunoglobulin G | 4.01[b] | 7.41[a] | 7.72[a] | 7.91[a] | 7.95[a] | 0.03 |
| Immunoglobulin M | 0.91[b] | 1.69[a] | 1.81[a] | 1.95[a] | 1.98[a] | 0.01 |

Note on Superscripts: [a, b, c, d] Means along the same row with different superscripts are significantly different ($p<0.05$). Rows without superscripts show no significant difference ($p>0.05$). T1: (Control): Basal diet alone; T2: Basal diet+commercial probiotic (Gallipro Max, Chr. Hansen, Denmark) at an inclusion level of 200 g/kg of feed; T3: Basal diet+*Abutilon indicum* Leaf Powder (AILP) at 100 g/kg of feed; T4: Basal diet+AILP at 200 g/kg of feed; T5: Basal diet+AILP at 300 g/kg of feed.

demonstrate that the nutrients absorbed from these diets were preferentially partitioned toward skeletal muscle accretion [26]. The corresponding increase in gizzard weight indicates enhanced mechanical grinding activity, which is a positive adaptation typically driven by the structural fiber components of the leaf powder in T5. This structural stimulation slows digesta transit time, allowing for more thorough enzymatic digestion [27]. The static relative weights observed for metabolic and excretory organs, such as the liver and kidneys, across all treatments provide vital toxicological reassurance. It indicates that despite the high inclusion rates of Abutilon indicum (up to 300 g/kg), the phytogenic compounds did not exert any hepatotoxic or nephrotoxic stress on the birds, establishing a high safety profile for the leaf powder [28]. The result obtained is in agreement with the reports of [28] who supplemented *Asystasia gangetica* leaf meal in the diet of broilers.

The physiological mechanisms driving these structural improvements are clearly illuminated by the birds' antioxidant profiles, immune biomarkers, and nutrient digestibility coefficients. Malondialdehyde (MDA) levels a definitive biomarker of lipid peroxidation and cellular oxidative stress were markedly higher in the T1 control birds. This indicates that un-supplemented broilers were highly susceptible to systemic oxidative damage, which diverts metabolic energy away from growth toward cellular repair [29]. In contrast, T2 through T5 exhibited drastically reduced MDA concentrations alongside significantly elevated levels of endogenous antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase. This radical shift proves that the antioxidant compounds within Abutilon indicum (such as phenols and flavonoids) and the metabolic byproducts of Gallipro Max actively scavenge free radicals, shielding the intestinal epithelium and liver tissues from oxidative degradation or stress [30]. Simultaneously, humoral immunity was profoundly boosted in the treated groups (T2-T5), as evidenced by elevated serum Immunoglobulin G (IgG), IgA, and IgM levels. This robust antibody response confirms that both additives enhanced systemic defense lines (IgG and IgM) and fortified mucosal immunity (IgA) along the gastrointestinal tract, creating a biological barrier against pathogenic invasion [30-32]. These immune and antioxidant fortifications directly supported the digestive tract, as validated by the vastly superior apparent nutrient digestibility coefficients for dry matter, crude protein, crude fat, crude fiber, and ash in T2-T5 compared to T1. By neutralizing oxidative stress, suppressing pathogenic load, and physically optimizing the mucosal lining, the probiotic and Abutilon indicum leaf powder allowed the broilers to break down and assimilate nutrients and essential minerals with maximum biological efficiency [31, 33, 34]. This comprehensive physiological process explains the superior growth, enhanced carcass yields, higher survival rates observed in the treated birds. The result recorded in this study is in line with the report of [35] when essential oil was included in the diet of broilers at 300 mg/kg.

## Conclusion

This 42-day experimental trial establishes that dietary supplementation with either the commercial probiotic Gallipro Max (200 g/kg) or *Abutilon indicum* leaf powder (AILP), particularly at the highest inclusion level of 300 g/kg, significantly optimizes the performance and physiological architecture of Ross 307 broiler chicks. Both additives effectively shift metabolic priorities away from immune maintenance and cellular repair toward efficient tissue accretion. They accomplish this by: elevating apparent nutrient digestibility coefficients, minimizing lipid peroxidation (lowered Malondialdehyde), significantly boosting endogenous antioxidant enzymes (Superoxide dismutase, Catalase, Glutathione peroxidase) and systemic immunoglobulins (IgG, IgA, IgM). The identical performance peaks, improved carcass yields, and zero mortality rates shared between the probiotic group (T2) and the high-AILP group (T5) demonstrate that Abutilon indicum possesses potent phytogenic capabilities. Because it achieved these benefits without inducing any hepatotoxic stress - as evidenced by the unchanged liver and kidney weights - Abutilon indicum leaf powder stands out as a safe, highly effective, and accessible natural growth promoter. It represents a viable commercial alternative to traditional antibiotic feed additives for optimizing broiler welfare, biosecurity, and overall flock productivity.

## References

1. Abdelli N, Solà-Oriol D, Pérez JF. Phytogenic feed additives in poultry: achievements, prospective and challenges. Animals. 2021; 11(12): 3471.
2. Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, et al. Use of antibiotics in broiler production: Global impacts and alternatives. Anim Nutr. 2018; 4(2): 170-178.
3. Ma F, Xu S, Tang Z, Li Z, Zhang L. Use of antimicrobials in food animals

and impact of transmission of antimicrobial resistance on humans. Biosafety and Health. 2021; 3(1): 32-38.

4. Alagbe JO. Investigating the effect of dietary supplementation of Ficus exasperata oil on the growth performance, apparent digestibility and nitrogen utilization of weaner rabbits. International Journal of Applied and Advanced Multidisciplinary Research. 2025; 3(9): 661-668.
5. Hernandez M, Alagbe JO. Influence of Odontonema strictum oil on the growth performance and Ruminal fermentation of Barbari bucks. Res Agric Vet Sci. 2025; 9(2): 1-10.
6. Hernandez M, Alagbe JO. Influence of Abrus procatorious crude oil supplementation on the growth performance, Nutrient digestibility, Ruminal fermentation and Microbial population of Malabari Bucks. International Journal of Global Sustainable Research. 2025; 3(7): 527-538.
7. Alagbe JO. Performance, hematology and serum biochemical parameters of weaner rabbits fed different levels of fermented Lagenaria brevifora whole fruit extract. Advances in Research and Reviews. 2020.
8. Alagbe JO, Shittu MD, Ojediran TK, Omokore EA, Oluwayinka EO, Anorue DN, et al. Dietary supplementation of Ocotea aciphylla essential oil: effects on growth performance and nutrient digestibility of weaned pigs. World Journal of Agriculture and Forestry Sciences. 2024; 2(2): 43-49.
9. Omokore EO, Alagbe JO. Efficacy of dried Phyllantus amarus leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. International Journal of Academic Research and Development. 2019; 4(3): 97-104.
10. Tang M, Fang R, Xue J, Yang K, Lu Y. Effects of catalase on growth performance, antioxidant capacity, intestinal morphology, and microbial composition in yellow broilers. Front Vet Sci. 2022; 9: 802051.
11. Adeyemi KD, Olanrewaju SM, Adewunmi PS, Abdulqadir Z. Mango leaf and synthetic additives in broiler diet: impact on growth, caecal microbiota, immunity, blood chemistry, and meat quality. Journal of the Saudi Society of Agricultural Sciences. 2021; 20(7): 482-489.
12. Musa B, Alagbe JO, Adegbite BM, Omokore EA. Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of Balanites aegyptiaca and Alchornea cordifolia stem bark mixture. United Journal for Research and Technology. 2020.
13. Muritala SD, Alagbe JO, Ojebiyi OO, Ojediran TK, Rafiu TA. Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of Polyalthia longifolia leaf extract in place of conventional antibiotics. Anim Sci Genet. 2022; 18(2): 57-71.
14. Alagbe JO, Shittu MD, Ramalan SN, Tanimomo KB Adekunle DA. Growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed Rubia cordifolia root extracts. International Journal of Biological Engineering and Agriculture. 2022; 1(1): 1-13.
15. AlagbeJO, Daniel AN, Muritala SD, Emiola A, Akande T, Emmanuel AA. Impact of dietary supplementation of Carica papaya essential oil on the blood chemistry of broiler chickens. Science Letters. 2023; 11(3): 105-110.
16. NRC Nutrient Requirements of Poultry (19th rev. ed.). The National Academies Press. 1994.
17. Singh S, Alagbe JO, Liu X, Sharma R, Kumar A. Comparative analysis of ethanolic Juniperus thurifera leaf, stem bark and root extract using gas chromatography and mass spectroemetry. International Journal of Agriculture and Animal Production. 2022; 2(6): 18-27.
18. Association of Official Analytical Chemistry. Official methods of analysis of AOAC International (20th ed.). AOAC International. 2016.
19. Daniel AN, Friday U, Alagbe JO. Investigating the effects of pawpaw (Carica papaya) essential oil dietary supplementation on the growth performance and carcass characteristics of broilers. Res Agric Vet Sci. 2023; 7(3): 164-174.
20. Shittu MD, Alagbe JO, Alaba O, Okanlawon EO, Adelakun FA, Emmanuel EO et al. Effect of ginger, garlic and Negro pepper on the gut microbes, gut histomorphometry and pathological assessment of selected organs of broiler chickens. Association of Deans of Agriculture in Nigerian Universities. 2024; 5: 105-121.
21. Olugbenga DO, Simeon AO, Samuel AA, Deborah OA, Ojurereoluwa AA, Olufemi EA, et al. Carcass Characteristics, Meat Antioxidative State, and Gut Microbiota of Broilers Fed with a Mixture of Bitter Melon and Basil Leaves Powder. Livestock Studies. 2024; 64(1): 8-16.
22. Dong XF, Gao WW, Tong JM, Jia HQ, Sa RN, Zhang Q. Effect of polysavone (alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. Poult Sci. 2007; 86(9): 1955-1959.
23. Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, et al. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front Microbiol. 2019; 10: 57.
24. Puvača N, Brkić I, Jahić M, Nikolić SR, Radović G, Ivanišević D, et al. The Effect of Using Natural or Biotic Dietary Supplements in Poultry Nutrition on the Effectiveness of Meat Production. Sustainability. 2020; 2(11): 4373.
25. Murugesan GR, Syed B, Haldar S, Pender C. Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Front Vet Sci. 2015; 2: 21.
26. Oloruntola OD, Agbede JO, Ayodele SO, Oloruntola DA. Neem, pawpaw, and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. J Food Biochem. 2019; 43(2): e12723.
27. Santhiravel S, Bekhit AEA, Mendis E, Jacobs JL, Dunshea FR, Rajapakse N, et al. The Impact of Plant Phytochemicals on the Gut Microbiota of Humans for a Balanced Life. Int J Mol Sci. 2022; 23(15): 8124.
28. Sobayo RA, Adeyemi OA, Sodipe OG, Oso AO, Fafiolu AO, Ogunade IM, et al. Growth response of broiler birds fed Asystasia gangetica leaf meal in hot humid environment. J Agric Sci Envir. 2012; 12(1): 53-59.
29. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Analysis of Kigellia africana fruit's powder antioxidant and phytochemical properties. Brazilian Journal of Science. 2024; 3(7): 38-49.
30. Adewale AO, Alagbe JO, Adekemi GA. Dietary supplementation of Rauvolfia vomitoria root extract as a phytogenic feed additive in growing rabbit's diets: Haematology and Serum biochemical indices. International Journal of Orange Technologies. 2021; 3(3): 31-42.
31. Cichoski AJ, Rotta RB, Scheuermann G, Junior AC, Barin JS. Investigation of glutathione peroxidase activity in chicken meat under different experimental conditions. Food Sci Technol. 2012; 32(4): 661-667.
32. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol. 2024; 98: 1323-1367.
33. Dibaji SM, Seidavi A, Asadpour L, da Silva FM. Effect of a symbiotic on the intestinal microflora of chickens. J App Poult Res. 2014; 23: 1-6.
34. Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia Almazan A, Dominguez-Diaz D, Gonzalez-Rios H. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J Anim Sci Technol. 2017; 59: 8.
35. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol. 2024; 98: 1323-1367.