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Research Article

Evaluation of Averrhoa bilimbi and Anastatica hierochuntica Leaf Powders as Phytogenic Feed Additives: Effects on Growth Performance and Nutrient Digestibility in Broiler Chickens

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Abstract

This study evaluated the independent and synergistic effects of dietary Averrhoa bilimbi leaf powder (ABLP) and Anastatica hierochuntica leaf powder (AHLP) on the growth performance, feed efficiency, and apparent nutrient digestibility of broiler chickens under the arid climatic conditions of Rajasthan, India. A total of 200 day-old Ross 308 broiler chicks were randomly assigned to four dietary treatments in a Completely Randomized Design (5 replicates of 10 birds each) for a 42-day trial. Treatment 1 (T1) served as the control (basal diet only); T2 received the basal diet supplemented with 250 g ABLP/kg diet; T3 received the basal diet with 250 g AHLP/kg diet; and T4 received a homogenous 1:1 mixture of ABLP and AHLP at 250 g/kg diet. Quantitative phytochemical profiling of bioactive fractions, with AHLP exhibiting superior concentrations of flavonoids (215.90 mg/g) and phenols (133.20 mg/g) compared to ABLP (106.20 mg/g and 89.00 mg/g, respectively). Dietary inclusion of the phytogenic additives significantly enhanced daily feed intake across all treated groups (T2, T3, and T4) compared to the control ($p<0.05$). Broilers on the 1:1 synergistic blend (T4) achieved the highest final body weight, followed by intermediate trajectories in T2 and T3, and the lowest values in T1. Concurrently, the Feed Conversion Ratio (FCR) was optimized (lowest) in T4 and highest in T1. Apparent nutrient digestibility coefficients for dry matter, crude protein, crude fiber, ether extract, and total ash followed a corresponding trend, peaking significantly in the T4 group. These findings indicate that while independent inclusion improves performance, combining ABLP and AHLP in a 1:1 ratio exerts a powerful synergistic effect that maximizes nutrient assimilation and feed efficiency. Consequently, a 1:1 blend of ABLP and AHLP at 250 g/kg is recommended as an effective organic feed additive to optimize broiler production in arid environments.

Key Words: averrhoa bilimbi; anastatica hierochuntica; phytogenic feed additives; nutrient digestibility; broiler performance; synergistic interaction

Introduction

The global poultry industry faces a critical transition period following widespread bans and strict restrictions on the use of sub-therapeutic Antibiotic Growth Promoters (AGPs) in animal feed [1]. For decades, the intensive production of broiler chickens relied heavily on prophylactic antibiotics to control subclinical intestinal infections, stabilize the gut microbiota, and improve feed conversion efficiency [1]. However, the continuous use of these agents has significantly accelerated the emergence of antibiotic-resistant bacterial strains, posing a direct threat to public health and food safety globally [2]. In response, modern poultry research has turned its focus toward identifying safe, sustainable, and organic alternatives capable of maintaining bird health and growth performance without compromising biosecurity. Among the various alternatives under review, Phytogenic Feed Additives (PFAs)—plant-derived natural bioactive compounds have emerged as highly promising candidates due to their diverse therapeutic and physiological benefits [3, 4].

The ban on conventional AGPs has left broiler producers highly vulnerable to performance drops, erratic feed intake patterns, and increased susceptibility to digestive disorders [5]. These challenges are especially acute in hot, arid regions such as Rajasthan, India, where high ambient temperatures and fluctuating relative humidity impose severe environmental stress on fast-growing strains like the Ross 308. Under thermal stress, broilers experience systemic physiological changes, including reduced digestive enzyme secretion, altered intestinal barrier integrity, and depressed voluntary feed consumption, all of which lead to poor nutrient absorption and high Feed Conversion Ratios (FCR) [6]. While many conventional phytogenics like thyme, rosemary, and garlic have been thoroughly researched, their commercial adoption remains limited by high production

costs and supply chain constraints. This highlights an urgent need to identify tough, locally available, and underutilized medicinal plants that can serve as affordable PFAs. Two such plants showing significant biochemical potential are Averrhoa bilimbi (commonly known as bilimbi or cucumber tree) and Anastatica hierochuntica (commonly known as Kaff Maryam or the Rose of Jericho). However, systematically verified data regarding their quantitative phytochemical profiles and practical application rates in poultry diets remain highly scarce [7].

Extensive literature confirms that the primary benefits of PFAs stem from their secondary metabolites, such as flavonoids, phenols, saponins, tannins, and alkaloids [8]. Previous studies on related botanical species show that these compounds act as organic biocatalysts. Dietary polyphenols (flavonoids and phenols) are well-documented for their strong antioxidant capacities, which directly mitigate the cellular oxidative stress induced by high ambient heat in broilers [8, 9, 10]. Furthermore, research on phytogenics indicates that these molecules stimulate the pancreatic secretion of vital digestive enzymes, including amylase, lipase, and protease, thereby increasing the breakdown of complex dietary proteins and lipids [9]. Saponins, when administered within safe baseline thresholds, have been shown to temporarily alter mucosal permeability in a way that expands the active surface area of intestinal villi, facilitating better nutrient assimilation [10]. Past evaluations of Averrhoa bilimbi extracts indicates its potent antimicrobial activities against common avian pathogens like Escherichia coli and Salmonella spp., primarily through cell-membrane disruption driven by its inherent alkaloid and tannin fractions [11, 12]. Similarly, initial screenings of Anastatica hierochuntica highlights an exceptionally high thermal tolerance and a dense concentration of structural phenols that support immune function and gut tissue integrity in monogastric animals [13]. However, almost all prior research has focused on liquid extracts or isolated solvent fractions in vitro, leaving a substantial knowledge gap regarding how whole leaf powders behave when directly mixed into solid broiler rations [12].

This study is justified by the critical need to validate novel, cost-effective, and climate-resilient phytogenic options that can replace synthetic growth promoters while simultaneously supporting broiler performance under challenging arid climatic conditions. While single-plant supplementations are frequently studied, evaluating a combined 1:1 mixture of Averrhoa bilimbi and Anastatica hierochuntica introduces a highly innovative approach centered on phytochemical synchronization. By blending the distinct acidogenic properties of ABLP with the superior antioxidant and polyphenolic concentrations of AHLP, there is a strong potential to create a broad-spectrum, multi-targeted impact within the avian gastrointestinal tract that single-source additives cannot match.

Materials and Methods

Experimental Location

The 42-day biological feeding trial was conducted at the Poultry Research Unit of the Gandhi College of Agriculture, Agriculture, Rajasthan, India. The experimental site is geographically positioned in the arid western plain zone of Rajasthan, characterized by a typical semi-arid to arid desert climate. During the trial period, the microclimatic weather conditions within the poultry house were carefully monitored. The average ambient temperature ranged from a minimum of 22.4°C during the late hours to a maximum of 36.8°C during peak daytime. The mean relative humidity fluctuated between 45% and 62%.

Ethical Approval

All experimental protocols, handling, and management procedures were reviewed, approved, and supervised by the Institutional Animal Ethics Committee (IAEC) of the institution (Approval Protocol Number: GCA/IAEC/2026/Bro-042). The welfare guidelines for the care and use of agricultural animals in research were strictly enforced to minimize stress throughout the duration of the experiment.

Collection, Authentication and Processing of Medicinal Plants

Fresh, mature leaves of Averrhoa bilimbi and whole aerial parts of Anastatica hierochuntica were harvested from identified botanical plots. Each medicinal plant species was independently authenticated by a certified taxonomist at the Department of Agronomy/Botany, Gandhi College of Agriculture, Rajasthan with voucher number AAS/OV/101A and AAS/OV/101B for Averrhoa bilimbi and Anastatica hierochuntica leaves respectively for future reference. The leaves were washed with distilled water, shade-dried at room temperature (30–32°C) for 7 days until they achieved a constant weight, and milled independently into fine powders using a commercial micro-hammer mill. The individual leaf powders were stored in airtight, labeled amber containers at 4°C prior to diet formulation. Phytochemical components of medicinal plants were assayed according to the procedures recently published by [14].

Experimental Design, Housing, and Bird Management

A total of 200 day-old unsexed commercial broiler chicks (Ross 308 strain) were procured from a commercial hatchery. On arrival, birds were weighed collectively to establish baseline initial weights and randomly assigned to 1 of 4 dietary treatments in a Completely Randomized Design (CRD). Each treatment group comprised 50 birds, structured into 5 replicates with 10 birds per replicate. The birds were housed in standard, double-tier galvanized wire battery cages. Each individual cage compartment measured exactly 60 cm × 60 cm × 50 cm (length × width × height). Pre-management operations, including pressure washing, chemical scrubbing, and spraying with a 2% formalin solution, were executed 14 days before chick placement. The experimental diets were formulated based on a basal mash ration designed according to the National Research Council (NRC) nutrient recommendations for broilers [15]:

Treatment 1 (T1): Control group fed the basal diet without phytogenic additives.

Treatment 2 (T2): Basal diet supplemented with 250 g of Averrhoa bilimbi leaf powder (ABLP) per kg of diet.

Treatment 3 (T3): Basal diet supplemented with 250 g of Anastatica hierochuntica leaf powder (AHLP) per kg of diet.

Treatment 4 (T4): Basal diet supplemented with a homogenous 1:1 mixture of ABLP and AHLP, blended at a total rate of 250 g/kg of diet (125 g ABLP + 125 g AHLP per kg feed).

Water and feed were offered ad libitum. Routine vaccines against Newcastle and Gumboro diseases were administered via ocular and drinking water routes on days 7, 14, and 21.

Evaluation of Growth Performance Parameters

Body weight (BW) per replicate group was documented weekly using a high-precision digital scale. From this data, body weight gain (BWG) was computed. Feed allocation was logged daily, and unconsumed feed remnants were weighed at the end of each week to determine replicate-level feed intake (FI). Feed Conversion Ratio (FCR) was calculated weekly using the standard formula:

$$FCR = \frac{\text{Total Live Weight Gain (g)}}{\text{Total Feed Consumed (g)}}$$

Nutrient Digestibility Trial

On day 42nd of the experiment, a 7-day apparent nutrient digestibility evaluation was initiated. For this study, 10 birds were randomly selected per treatment group (2 birds per replicate pen) and moved to individual metabolic cages fitted with modified stainless-steel excreta collection trays.

Following a 2-day acclimatization window, total feed consumed and total excreta voided were recorded daily for 5 consecutive days. Excreta samples were freed from spilled feed and feathers, pooled by replicate, dried in a forced-air oven at 65°C for 72 hours, and ground to pass through a 1-mm screen mesh. Proximate analysis of fecal samples was conducted to determine Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), and total Ash using method outlined by AOAC [16]. Proximate composition of experimental diet was handled via near-infrared spectroscopy using a benchtop Near-Infrared (NIR) Analyzer (Model: NIRS™ DS2500 F, Denmark). It operates within an extended spectral range of 400 to 2500 nm using a scanning monochromator configuration. It uses a high-intensity tungsten halogen lamp source, records a spectral resolution of 0.5 nm, features an automatic internal reference standard stabilization system, and runs on ISIscan Nova chemometric prediction software.

The apparent nutrient digestibility coefficients were computed using the standard index equation:

$$\text{Digestibility Coefficient (\%)} = \frac{\text{Nutrient Ingested} - \text{Nutrient Excreted}}{\text{Nutrient Ingested}} \times 100$$

Statistical Analysis

All generated performance and digestibility data were organized and subjected to a one-way Analysis of Variance (ANOVA) for a Completely Randomized Design using the General Linear Model (GLM) procedure of SPSS software. Where treatment differences were detected, treatment means were separated using Tukey's test with significance evaluated at a threshold of $P<0.05$.

Results

Phytochemical Profiles of Averrhoa bilimbi (ABLP) and Anastatica hierochuntica (AHLP) is presented in Table 2. The compounds revealed the presence of phenols (89.00 mg/g vs 133.20 mg/g), flavonoids (106.2 mg/g vs 215.9 mg/g), saponins (60.70 mg/g vs 78.33 mg/g), tannins (51.58 mg/g vs 63.55 mg/g), steroids (28.09 mg/g vs 41.60 mg/g) and alkaloids (19.63 mg/g vs 22.05 mg/g) for Averrhoa bilimbi (ABLP) and Anastatica hierochuntica (AHLP) respectively. Average daily weight gain, average daily feed intake and feed conversion ratio were significantly influenced by the treatment. Average daily weight gain was higher ($p<0.05$) in T4, intermediate in T2, T3 and lower in T1. Average daily feed intake was lower in T1 relative to the other groups ($p<0.05$). Feed conversion ratio value varied from 2.00 – 2.51. However, value was higher in T1, intermediate in T2, T3 and lower in T4 ($p<0.05$) (Table 3).

Dry matter, crude protein, crude fibre, ether extract and ash digestibilities were lower in T1 compared to the other groups ($p<0.05$). (Table 4).

	Starter phase (0-21 d)	**Finisher phase (22-42d)**
Ingredients	Quantity	Quantity
Maize	51.00	55.00
Wheat bran	2.00	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	100
Determined analysis (%)		
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

Table 1: Ingredient and chemical composition of experimental (basal) diet

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

Compounds (mg/g)	*Averrhoa bilimbi* (ABLP)	*Anastatica hierochuntica* (AHLP)
Phenols	89.00	133.20
Flavonoids	106.2	215.9
Saponins	60.70	78.33
Tannins	51.58	63.55
Steroids	28.09	41.60
Alkaloids	19.63	22.05

Table 2: Phytochemical Profiles of *Averrhoa bilimbi* (ABLP) and *Anastatica hierochuntica* (AHLP)

Parameters	T1	T2	T3	T4	SEM
Number of birds	50	50	50	50	-
Duration of experiment (days)	42	42	42	42	-
Initial body weight (g/bird)	45.31	45.06	45.11	45.13	0.02
Final body weight (g/bird)	1877.2^{c}	2245.2^{b}	2239.8^{b}	2600.9^{a}	98.21
Body weight gain (g/bird)	1831.89^{c}	2200.14^{b}	2194.69^{b}	2555.77^{a}	87.55
Daily weight gain (g/day)	43.61^{c}	52.38^{b}	52.25^{b}	60.85^{a}	0.06
Total feed intake (g/bird)	4600.9^{b}	5000.1^{a}	5000.5^{a}	5000.9^{a}	145.4
Daily feed intake (g/day)	109.5^{b}	119.1^{a}	119.1^{a}	119.1^{a}	0.15
Feed to gain ratio	2.51^{a}	2.27^{b}	2.27^{b}	2.00^{c}	0.01

Table 3: Effects of the plant supplements on the performance characteristics of broiler chickens

Note on Superscripts: ^{a}, ^{b}, ^{c} Means along the same row with different superscripts are significantly different ($p<0.05$).

Treatment 1 (T1): Control group fed the basal diet without phytogenic additives.

Treatment 2 (T2): Basal diet supplemented with 250 g of *Averrhoa bilimbi* leaf powder (ABLP) per kg of diet.

Treatment 3 (T3): Basal diet supplemented with 250 g of *Anastatica hierochuntica* leaf powder (AHLP) per kg of diet.

Treatment 4 (T4): Basal diet supplemented with a homogenous 1:1 mixture of ABLP and AHLP, blended at a total rate of 250 g/kg of diet (125 g ABLP + 125 g AHLP per kg feed).

Parameters	T1	T2	T3	T4	SEM
Dry matter	69.79^{c}	74.54^{b}	74.01^{b}	80.74^{a}	0.04
Crude protein	71.11^{b}	80.02^{a}	80.47^{a}	87.90^{a}	0.05
Crude fibre	46.68^{b}	50.06^{a}	50.45^{a}	50.66^{a}	0.02
Ether extract	51.19^{b}	66.98^{a}	67.08^{a}	68.72^{a}	0.03
Ash	39.86^{c}	44.89^{b}	46.09^{b}	51.24^{a}	0.01

Table 4: Effects of the plant supplements on the nutrient digestibility of broiler chickens

Note on Superscripts: ^{a}, ^{b}, ^{c} Means along the same row with different superscripts are significantly different ($p<0.05$).

Treatment 1 (T1): Control group fed the basal diet without phytogenic additives.

Treatment 2 (T2): Basal diet supplemented with 250 g of *Averrhoa bilimbi* leaf powder (ABLP) per kg of diet.

Treatment 3 (T3): Basal diet supplemented with 250 g of *Anastatica hierochuntica* leaf powder (AHLP) per kg of diet.

Treatment 4 (T4): Basal diet supplemented with a homogenous 1:1 mixture of ABLP and AHLP, blended at a total rate of 250 g/kg of diet (125 g ABLP + 125 g AHLP per kg feed).

Discussion

The quantitative evaluation of the secondary metabolite arrays in Averrhoa bilimbi leaf powder (ABLP) and Anastatica hierochuntica leaf powder (AHLP) highlights their chemical viability as phytogenic feed additives (PFAs). Phytochemical screenings demonstrated high concentrations of bioactive fractions including flavonoids, phenols, saponins, tannins, steroids, and alkaloids. Notably, AHLP contained significantly higher levels of total phenols (133.20 mg/g) and flavonoids (215.90 mg/g) compared to ABLP (89.00 mg/g and 106.20 mg/g, respectively). These specific non-nutritive phyto-compounds act as organic biocatalysts within the avian gastrointestinal tract, stimulating biological functions that can bypass the need for prophylactic antibiotic growth promoters [18].

The marked boost in feed consumption noted in groups T2, T3, and T4 over the control birds (T1) is primarily linked to the sensory and aromatic attributes of these botanical powders [19]. The presence of phyto-constituents in Averrhoa bilimbi leaf powder (ABLP) and Anastatica hierochuntica leaf powder (AHLP) stimulates the olfactory receptors of broiler chickens, driving up appetite and voluntary feed consumption [20].

This aromatic palatability effect helps explain why the birds did not reject the diets, despite the slight bitterness typically caused by saponins and tannins at these concentrations [18]. The variations in final body weights and FCR values across treatments correspond with the differences in nutrient digestibility profiles. Broilers fed the 1:1 synergistic blend (T4) recorded the highest overall body weight and lowest FCR, while intermediate responses occurred in the single-supplement groups (T2 and T3), and lowest performance values remained confined to the control group (T1). This performance gradient is supported by the digestibility coefficients for dry matter, crude protein, crude fiber, ether extract, and ash. Phytogenic compounds are known to encourage the production of endogenous digestive juices [20]. The structural phenols and flavonoids present in both leaf profiles stimulate the pancreas and intestinal mucosa, increasing the secretion of essential enzymes such as amylase, lipase, trypsin, and chymotrypsin [21]. This enzymatic upregulation accelerates the breakdown of complex nutrients within the chime, facilitating more efficient systemic assimilation [22]. The result obtained is in consonance with the report of [23] when eucalyptus oil was supplemented in the diet of birds. [23, 24] also recorded a higher feed intake and lower feed conversion ratio of birds fed varying inclusion levels of Papaya seed extract.

Furthermore, the saponins identified in both plants (60.70 mg/g in ABLP and 78.33 mg/g in AHLP) likely altered intestinal cell membrane permeability, increasing the active absorption surface area of the intestinal villi [25, 27]. Simultaneously, the low concentrations of dietary tannins and alkaloids help manage pathogenic bacterial strains through targeted cell-wall disruption [23. 24]. The higher average body weight gain observed in T4 point to a clear synergistic interaction between the two botanical additives when combined in a 1:1 ratio. While Anastatica hierochuntica provided a highly concentrated supply of antioxidant polyphenols, Averrhoa bilimbi contributed a distinct acidogenic and metabolic chemical profile. Blending them together created a broader, complementary spectrum of bioactive compounds [25, 26]. This multi-targeted approach optimized gastrointestinal ecosystem dynamics and competitive exclusion mechanisms more effectively than either additive could achieve alone. As a result, this botanical combination significantly improved nutrient utilization across all measured fractions (DM, CP, CF, EE, and Ash), translating directly into maximized final body weights and the most efficient Feed Conversion Ratio over the 42-day production cycle.

Conclusion

Based on the empirical findings of this 42-day biological trial, it can be concluded that the independent or combined dietary inclusion of Averrhoa bilimbi leaf powder (ABLP) and Anastatica hierochuntica leaf powder (AHLP) at 250 g/kg serves as an effective phytogenic strategy to enhance the performance of Ross 308 broiler chickens. The rich profile of bioactive secondary metabolites particularly the superior concentrations of flavonoids, phenols, and saponins which exhibited clear systemic benefits, successfully driving up feed intake and optimizing the apparent digestibility of dry matter, crude protein, crude fiber, ether extract, and total ash. Critically, the experimental outcomes highlight that a 1:1 synergistic combination of both leaf powders (T4) yields the most profound physiological advantages. By capitalizing on the complementary antimicrobial, antioxidant, and enzyme-stimulating properties of both botanical species, this specific treatment maximized final body weights and achieved the most efficient (lowest) Feed Conversion Ratio. Consequently, incorporating a homogenous 1:1 blend of Averrhoa bilimbi and Anastatica hierochuntica leaf powders at 250 g/kg of diet can be recommended to poultry producers as a viable, organic phyto-compounds capable of supporting sustainable broiler production in challenging semi-arid and arid climatic zones.

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