

Effect of dietary inclusion of phytosome conjugated carvacrol essential oil on growth, nutrient digestibility, carcass traits and gut health in broilers

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Abstract

This study evaluated phytosome-conjugated carvacrol essential oil as an alternative to antibiotic growth promoters in broilers. A total of 240 day-old chicks were assigned to four treatments: a control diet (T0), antibiotic-supplemented diet with standard antibiotic (T1), carvacrol essential oil at 100 mg/kg (T2), and phytosome-conjugated carvacrol at 100 mg/kg (T3). Although T2 and T3 showed numerically higher body weights and weight gains than the control, differences were not statistically significant. Feed intake, FCR, and nutrient digestibility were also similar across treatments, though T2 and T3 showed numerically improved cumulative FCR. Caecal *E. coli* and total viable counts were significantly reduced ($p \leq 0.0001$) in T2 and T3, confirming the antimicrobial activity of carvacrol. Carcass traits, including dressing yield and organ weights, were unaffected. Overall, phytosome-conjugated carvacrol (T3) provided comparable performance and superior gut health benefits relative to the control, indicating its potential as a sustainable alternative to antibiotics in broiler production.

Key words: broiler; carvacrol; phytosome; growth performance; carcass traits; gut health

Introduction

Antibiotics have long been used at sub-therapeutic levels in broiler production to enhance growth, reduce mortality, and prevent disease outbreaks (Abd El-Hack et al., 2020). However, continuous antibiotic use has raised major public health concerns, including the emergence of antibiotic-resistant bacteria and the presence of drug residues in poultry products (Yonatan et al., 2023). These issues prompted regulatory authorities such as the European Union to impose strict bans on antibiotic growth promoters (AGPs) in poultry production (Namdeo et al., 2020). Consequently, the poultry industry is compelled to identify alternative strategies that maintain productivity without compromising bird health or consumer safety (Tsogoeva et al., 2021).

In response, researchers have focused on alternatives capable of supporting broiler growth and gut health. Effective AGP replacements must beneficially modulate gut microbiota, enhance nutrient absorption, and strengthen immune responses. Essential oils (EOs) have emerged as promising candidates, offering multiple bioactive properties relevant to poultry production. Extracted from various plant sources and widely accepted as safe feed additives, EOs possess antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and digestion-enhancing activities (Kansal et al., 2017; Gomathi et al., 2018).

Among these, carvacrol—a monoterpene phenol has received substantial attention for its broad biological activities. Carvacrol has been widely investigated for antibacterial, antioxidant, anti-inflammatory, growth-promoting, and immunomodulatory effects in poultry. Both *in vitro* and *in vivo* studies demonstrate its ability to inhibit pathogenic bacteria, improve feed efficiency, and support gut health in broilers. Additionally, carvacrol modifies rumen microbial fermentation and reduces methane production in ruminants, underscoring its versatility as a functional phytochemical (Hashemipour et al., 2013).

Despite their potential, the application of essential oils in animal feed is hampered by poor bioavailability due to low water solubility. When provided in their natural form, EOs often show reduced efficacy because of incomplete absorption and degradation by digestive enzymes and gastric acids (Saha et al., 2013). These limitations necessitate higher doses, which are costly for large-scale poultry operations. Further, isolated EO components may lose part of their natural bioactivity when removed from the plant matrix, diminishing their effectiveness as feed additives.

To overcome these challenges, recent advances in delivery systems have led to the development of phytosomes—complexes formed between plant polyphenolic compounds (e.g., flavonoids, terpenoids, and phenolics) and phospholipids. These lipid-based carriers enhance the stability, solubility, and bioavailability of plant bioactives, improving their *in vivo* performance (Anitha et al., 2019). Phytosome-conjugated products consistently show enhanced bioactivity compared to non-conjugated forms, making them an appealing strategy for improving the efficacy of essential oils in broiler diets (Doost et al., 2020).

Carvacrol particularly benefits from phytosome incorporation, exhibiting increased absorption, stability, and bioavailability, which allows lower doses to provide similar or superior effects compared with pure carvacrol. Enhanced intestinal uptake improves bioactivity (Saha et al., 2013), while the phospholipid matrix protects carvacrol from environmental and digestive degradation.

Although phytosome technology has been well studied in pharmaceutical, medical, and food applications (HariPriya and Kumaran, 2023), research on its use in broiler production is limited. Nonetheless, advantages such as increased bioavailability, reduced dosage requirements, and improved cost-effectiveness (Anitha et al., 2019) have generated interest in phytosome-conjugated essential oils as potential antibiotic alternatives.

Therefore, the present study aimed to evaluate the effects of dietary phytosome-conjugated carvacrol essential oil on broiler growth performance, feed efficiency, nutrient digestibility, carcass traits, and gut health. The objective was to determine an optimal inclusion level capable of matching or surpassing the effects of pure carvacrol or antibiotic supplementation. By exploring its growth-promoting and antimicrobial attributes at lower dosages, the study seeks to establish phytosome-conjugated carvacrol as a sustainable and economically viable alternative to antibiotic growth promoters in modern broiler production.

Materials and Methods

Experimental Site and Birds

A total of 240, straight-run, day-old broiler chicks (Vencobb 430) were procured from a commercial hatchery. The birds were housed in a well-ventilated deep litter system, following constant conditions of temperature, relative humidity, ventilation, light cycle and floor space throughout the experimental period of 42 days.

Procurement and Preparation of Essential Oils

The essential oils utilized in this study included carvacrol essential oil and phytosome-conjugated carvacrol (which contained 16.6% carvacrol and 16.6% Tween). These oils were procured from IgY Immunologix India Pvt. Ltd., Hyderabad, India and were thoroughly mixed with the feed to ensure uniform distribution and intake among the respective broiler groups.

Experimental Design, Dietary Treatments, and Management

The experimental design consisted of four treatment groups, each with four replicates containing 15 broiler chicks per replicate. The birds were weighed on day 1 and randomly assigned to the following dietary treatments:

Control Group (T0): Standard diet as per Indian feeding standards; Antibiotic-Supplemented Group (T1): Standard diet (T0) supplemented with Bacitracin Methylene Disalicylate (BMD) antibiotic at @ 250 mg/ kg feed; Carvacrol Essential Oil Group (T2): Standard diet (T0) supplemented with carvacrol essential oil at 100 mg/kg feed; Phytosome-Conjugated Carvacrol Essential Oil Group (T3): Standard diet (T0) supplemented with phytosome-conjugated carvacrol essential oil at 100 mg/kg feed.

The corn–soy–based diets were formulated to be isocaloric and isonitrogenous across treatments and to meet nutritional requirements as per Indian feeding standards. The nutrient composition of the experimental diets is shown in Table 1. Birds had *ad libitum* access to feed and water. Standard vaccination schedules and uniform management practices were followed for all treatment groups. The study adhered to the guidelines of the Committee for the Control and Supervision of Experimentation on Animals (CCSEA), Government of India.

Growth Performance Evaluation

One-day-old birds were weighed to determine initial group body weight. Mortality, house temperature, and humidity were recorded daily. Individual body weights were recorded weekly, and feed intake was measured per replicate. Clinical signs, feed and water intake, and any abnormal symptoms or mortality were monitored daily. All dead birds were dissected, and a detailed post-mortem was conducted. Feed conversion ratio (FCR) was calculated as feed intake divided by body weight gain for each replicate.

Nutrient Digestibility

A metabolic trial was conducted at the end of six weeks. Two randomly selected birds per replicate were housed in metabolic cages for five days (two days adaptation, three days fecal collection). Daily feed intake and fecal output were recorded. Representative samples of feed and feces were analyzed for proximate composition following AOAC (2016) methods. Nutrient digestibility was calculated from dry matter intake and fecal output.

Table 1: The percent ingredient composition and chemical composition of various experimental diets.

| Feed Ingredients | Pre-Starter (0-1 week) | Starter (2-3 week) | Finisher (4-6week) |
|-----------------------|---------------------------|-----------------------|-----------------------|
| Maize | 57.50 | 59.00 | 61.00 |
| Soybean meal | 36.40 | 33.45 | 30.00 |
| Vegetable oil | 1.75 | 3.20 | 4.50 |
| Monocalcium phosphate | 1.50 | 1.40 | 1.50 |
| Limestone powder | 1.40 | 1.50 | 1.65 |
| Salt | 0.30 | 0.30 | 0.30 |
| Trace Min.premix | 0.05 | 0.05 | 0.05 |
| Vitamin Premix | 0.05 | 0.05 | 0.05 |
| DL-Methionine | 0.30 | 0.35 | 0.30 |
| L-Lysine | 0.30 | 0.25 | 0.25 |
| L-Threonine | 0.15 | 0.15 | 0.10 |
| Choline Chloride | 0.10 | 0.10 | 0.10 |
| Toxin binder | 0.15 | 0.15 | 0.15 |
| Cocciostat | 0.05 | 0.05 | 0.05 |
| Total | 100 | 100 | 100 |
| Dry Matter | 91.00 | 90.86 | 91.12 |
| Crude Protein | 22.84 | 21.73 | 20.05 |
| Ether Extract | 3.32 | 3.96 | 5.44 |
| Crude Fibre | 3.86 | 3.74 | 3.52 |
| Nitrogen Free Extract | 64.92 | 65.79 | 66.17 |
| Total Ash | 5.06 | 4.78 | 4.82 |
| Calcium | 1.12 | 1.16 | 1.16 |
| Phosphorus | 0.86 | 0.78 | 0.86 |
| Methionine* | 0.65 | 0.61 | 0.56 |
| Lysine* | 1.45 | 1.37 | 1.29 |
| ME* (Kcal/kg) | 2965.00 | 3067.00 | 3163.00 |

* Calculated

Carcass Traits

At day 42, two birds (one male and one female) per replicate were randomly selected for carcass evaluation. Birds were fasted for 12 hours but provided water. After weighing, they were slaughtered using standard procedures. Dressed weight was recorded after bleeding, defeathering, and evisceration. Dressing percentage and giblet weights (liver, heart, gizzard) were expressed as a percentage of live body weight.

Microbial Evaluation and Gut Health

Gut health assessment included total viable count (TVC), *E. coli* enumeration, and villus morphology. One bird per replicate was sacrificed on day 42, and 2 g of cecal content was collected in sterile vials. Samples were diluted in 9 ml saline and serially diluted. For TVC, 10 µl of the final dilution was spread on nutrient agar, while EMB agar was used for *E. coli*. Plates were incubated at 37°C for 24 h, and colony-forming units (CFU) were multiplied by dilution factors to determine counts per gram of cecal content.

Statistical Analysis

Data were analyzed using SPSS-21 under a Completely Randomized Design. One-way ANOVA was applied to all parameters, and differences among treatment means were compared using Duncan's multiple range test (1995). Replicates served as the experimental unit. Statistical significance was accepted at $p \leq 0.05$.

Results

Growth Performance

The growth performance of broilers (Table 2) supplemented with carvacrol essential oil, phytosome-conjugated carvacrol, and antibiotics were evaluated across three phases (D1–21, D22–42, and D1–42). Across all periods, birds in the carvacrol group (T2) showed the highest body weight gain (BWG), followed by the antibiotic group (T1) and the phytosome-conjugated group (T3). However, these BWG differences were not statistically significant ($P > 0.05$).

Feed intake (AFI) was also highest in the carvacrol group (T2), with 1208.37 g recorded during the starter phase and 4605.21 g overall, while the control group (T0) had the lowest AFI. By the end of the trial, the live body weights of birds in the carvacrol and phytosome-conjugated groups were 4.63% and 1.86% higher than the control, respectively. Feed conversion ratio (FCR) remained similar across treatments, with the antibiotic group (T1) showing the best efficiency (1.47) and the control the poorest (1.53), although differences were again non-significant ($P > 0.05$).

Table 2: The effect of phytosome conjugated carvacrol essential oil on various performance parameters of broiler chicken (Mean + SE)

| Parameters | T ₀ | T ₁ | T ₂ | T ₃ | CD |
|---|-------------------------|-------------------------|-------------------------|--------------------------|--------|
| Growth Performance | | | | | |
| (D1 to 21) | | | | | |
| BWG (g/bird) | 930.22 ±12.30 | 943.97 ±6.95 | 957.13 ±22.9 | 938.57 ±14.41 | NS |
| AFI (g/bird) | 1182.17 ±15.5 | 1201.17 ±3.50 | 1208.37 ±26.03 | 1190.10 ±13.33 | NS |
| FCR (g/g) | 1.27 ± 0.01 | 1.27 ± 0.01 | 1.26 ± 0.03 | 1.27 ± 0.01 | NS |
| D22 to 42 | | | | | |
| BWG (g/bird) | 2043.33 ± 18.69 | 2099.33 ± 72.43 | 2157.50 ± 39.89 | 2092.25 ± 55.79 | NS |
| AFI (g/bird) | 3378.93 ± 59.78 | 3275.40 ± 85.23 | 3396.84 ± 55.87 | 3330.13 ± 63.81 | NS |
| FCR (g/g) | 1.65 ± 0.03 | 1.56 ± 0.02 | 1.57 ± 0.02 | 1.59 ± 0.02 | NS |
| D1 to 42 | | | | | |
| BWG (g/bird) | 2973.55 ±29.01 | 3043.30 ±78.17 | 3114.63 ±62.57 | 3030.82 ±64.84 | NS |
| AFI (g/bird) | 4561.10 ±69.34 | 4476.57 ±86.55 | 4605.21 ±53.78 | 4520.23 ±72.16 | NS |
| FCR (g/g) | 1.53 ± 0.03 | 1.47 ± 0.01 | 1.48 ± 0.02 | 1.49 ± 0.01 | NS |
| Nutrient Digestibility (%) | | | | | |
| DM (%) | 72.85 ±2.57 | 71.27 ±1.15 | 74.82 ±1.47 | 73.82 ±0.42 | NS |
| CP (%) | 62.48 ±4.33 | 61.78 ±2.07 | 67.97 ±2.17 | 62.53 ±1.46 | NS |
| EE (%) | 79.50 ±3.41 | 76.37 ±1.92 | 79.87 ±3.12 | 76.54 ±2.51 | NS |
| CF (%) | 20.53 ±3.06 | 17.91 ±3.47 | 24.54 ±4.23 | 17.70 ±3.60 | NS |
| NFE (%) | 82.66 ±1.57 | 80.13 ±2.06 | 83.22 ±1.05 | 84.23 ±0.50 | NS |
| Carcass traits | | | | | |
| Dressing (%) | 69.66 ±1.20 | 70.57 ±0.70 | 72.34 ±1.85 | 75.32 ±2.23 | NS |
| Heart (%) | 0.46 ±0.03 | 0.50 ±0.04 | 0.53 ±0.03 | 0.48 ±0.03 | NS |
| Liver (%) | 1.71 ±0.07 | 1.72 ±0.14 | 1.61 ±0.14 | 1.71 ±0.10 | NS |
| Gizzard (%) | 1.89 ±0.11 | 1.86 ±0.08 | 1.86 ±0.07 | 1.71 ±0.20 | NS |
| Abdominal Fat (%) | 1.54 ±0.18 | 1.12 ±0.16 | 1.38 ±0.17 | 1.21 ±0.15 | NS |
| Microbial evaluation of intestinal contents | | | | | |
| E Coli Count of caecal contents (10 ⁶ cfu/g)** | 6.50 ^a ±1.70 | 0.61 ^b ±0.07 | 0.24 ^d ±0.05 | 0.42 ^c ±0.06 | 0.0001 |
| Total Viable Count of caecal contents (10 ⁹ cfu/g)** | 6.75 ^a ±2.01 | 2.85 ^b ±0.63 | 0.77 ^c ±0.12 | 1.51 ^{bc} ±0.63 | 0.0001 |

NS= Non-significant, **Means bearing superscripts a, b, c within columns differ significantly ($p \leq 0.0001$) **. BWG: Body weight gain; AFI: Average Feed Intake; FCR: Feed Conversion Ratio, DM: Dry matter; CP: Crude protein; EE: Ether extract; CF: Crude fibre; TA: Total ash; NFE: Nitrogen free extracts

The lack of statistical significance may be related to the relatively low level of phytosome-conjugated carvacrol used, as higher doses might yield more pronounced improvements in growth and feed efficiency. Nonetheless, the numerical trends suggest that both carvacrol essential oil and its phytosome-conjugated form have potential as growth-promoting additives, but further studies with optimized dosages are needed to better evaluate their potential as alternatives to antibiotic growth promoters.

Nutrient Digestibility

The metabolic trial conducted at the end of week six showed numerically higher digestibility of dry matter (DM), crude protein (CP), ether extract (EE), and crude fiber (CF) in the essential oil-supplemented groups. Nitrogen-free extract (NFE) digestibility was highest in the phytosome-conjugated carvacrol group. However, none of these differences were statistically significant ($P > 0.05$).

Carcass Traits

Carcass characteristics—including dressing percentage and the relative weights of the liver, gizzard, heart, and abdominal fat—did not differ significantly among treatments ($P > 0.05$). This indicates that supplementation with carvacrol essential oil or phytosome-conjugated carvacrol at 100 mg/kg had no measurable impact on carcass traits.

Microbial Evaluation of Intestinal Contents

Cecal microbial analysis revealed significantly lower ($P \leq 0.05$) *Escherichia coli* (*E. coli*) counts and total viable counts (TVC) in the carvacrol and phytosome-conjugated carvacrol groups compared with the control and antibiotic groups (Table 2). The carvacrol group showed the lowest *E. coli* levels, followed by the phytosome-conjugated group, the antibiotic group, and the control. These findings demonstrate the antimicrobial potential of carvacrol essential oil in reducing harmful gut bacteria.

Discussion

This study found that supplementation with carvacrol essential oil and phytosome-conjugated carvacrol led to numerically higher body weights, weight gains, and improved feed efficiency; however, these differences were not statistically significant at 100 mg/kg inclusion. These findings differ from earlier reports (Yang et al., 2021), possibly due to variations in rearing conditions, as broiler performance can be influenced by housing environment (Mesa et al., 2017). The results are consistent with Saadat Shad et al. (2016) and Amad et al. (2013), who observed no significant effects of carvacrol on performance. The lack of differences may be attributed to the high-quality diet and controlled environment used in this study, which could have masked potential growth-promoting effects. Conversely, several studies (Gole et al., 2022) have reported significant increases in broiler body weight with essential oil supplementation, highlighting variability across experimental conditions.

In terms of nutrient digestibility—an important indicator of gut health—DM, CP, EE, CF, and NFE digestibility values were numerically higher in the essential oil groups, though not significantly different. Previous studies (Bento et al., 2013) reported variable effects of essential oils on nutrient utilization, attributed to their ability to stimulate digestive enzyme secretion. The lack of significant improvement here may relate to the low concentration of carvacrol (16.6%) in the phytosome formulation, potentially limiting its bioavailability.

Organ development is critical for poultry health (Richardson et al., 2025). In this study, no significant changes were observed in heart and liver weights, indicating that carvacrol supplementation did not induce inflammation, as organ enlargement is often associated with inflammatory responses (Cazaban et al., 2015). Carcass traits were unaffected, consistent with Khempaka et al. (2013), who found no significant effects of essential oils on carcass yield. Lack of health challenges during the trial may explain the absence of treatment effects, as carvacrol benefits are often more pronounced under stress or disease conditions. Variability across studies may reflect differences in the form of essential oils used, environmental conditions, dietary composition, dosage, and the physiological status of the birds.

The most noteworthy result was the significant reduction in *E. coli* counts and total viable counts (TVC) in birds receiving carvacrol and phytosome-conjugated carvacrol. This aligns with known antimicrobial properties of carvacrol, which disrupts bacterial membranes and inhibits pathogenic growth (Hashemipour et al., 2013). The reduced microbial load likely contributed to improved gut integrity and nutrient absorption, consistent with the numerical improvements in growth and digestibility observed.

In summary, while growth performance and digestibility responses were not statistically significant, carvacrol—especially in phytosome form—demonstrated clear antimicrobial effects and potential benefits for gut health.

Conclusion

This study demonstrates that both phytosome-conjugated carvacrol essential oil and standard carvacrol essential oil, even at lower dietary inclusion levels, provided improvements in growth performance, nutrient digestibility, and carcass traits comparable to the control group. These findings highlight their potential as sustainable alternatives to antibiotic growth promoters in broiler production. Notably, supplementation with phytosome-conjugated carvacrol at 100 mg/kg enhanced feed conversion ratio and significantly reduced caecal

E. coli and total viable counts, reflecting improved gut health. Overall, the results indicate that carvacrol—particularly in its phytosome-conjugated form—can beneficially modulate gut health and support immune function, making it a promising candidate for antibiotic-free broiler nutrition.

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Conflict of interest

The authors declare no conflicts of interest.

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