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Phytosome-conjugated carvacrol: A novel approach for improving growth performance, intestinal morphology and economics of production in Broiler Chicken

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ABSTRACT

Essential oils are plant-derived aromatic volatile oils, and they contain bioactive compounds that have been shown to improve poultry nutrition. However, considering problems associated with the solubility and bioavailability of polyphenolic compounds, the study was planned to find out the effect of the novel feed-grade delivery system, phytosomes for conjugation of plant-derived polyphenolic compound carvacrol on the growth performance of broiler chickens. The experiment was conducted, on 240 broiler chicks for a period of 6 weeks. The chicks were divided into 4 groups having 4 replicates of 15 birds each. The birds in the control group (T_0) offered a standard diet as per BIS (2007) specification. Group T_1 received a standard diet supplemented with Bacitracin Methylene Disalicylate (BMD) antibiotic at standard dose and group T_2 received a standard diet supplemented with carvacrol essential oil @100 mg/kg feed. Group T_3 received a standard diet supplemented with phytosome-conjugated carvacrol essential oil (carvacrol @16.6%) @100 mg/kg feed. The performance of all the treatment groups was assessed with respect to the different performance parameters. The supplementation of phytosome-conjugated carvacrol essential oil (carvacrol @16.6%) @ 100 mg/kg feed was found beneficial in terms of growth performance, feed efficiency, and intestinal morphometry. In terms of economics of broiler production, the results revealed that the addition of phytosome-conjugated carvacrol essential oil and carvacrol essential oil in diets was found beneficial in reducing the cost of broiler production, thereby enhancing the margin of profit in broiler production and fetching higher net profit than the control group.

Keywords: Broiler, Carvacrol, Economics, Growth, Intestinal morphology, Phytosome

Due to the extensive use of antibiotics in poultry production, the emergence of antimicrobial resistance has become a large threat to One World Health in the coming decades. In response to this challenge, many countries have banned or restricted the use of in-feed antibiotics in the hope to reduce the development of bacterial resistance (Magnusson 2020). In the contemporary pursuit of alternatives to antibiotic growth promoters, essential oils have garnered significant attention. The essential oil carvacrol extracted from oregano have become prominent as it possess antimicrobial activity and therefore, substitute antibiotic-based growth promoters, in addition to its antioxidant actions, anti-inflammatory and analgesic properties (Galli *et al.* 2020).

Encapsulation involves structuring systems capable of preserving the chemical, physical, and biological properties of active compounds as well as releasing or delivering them under desired conditions. The process of encapsulation involves coating either a single or a mixture of bioactive materials with another one or a combination of materials (Sonawane *et al.* 2020). Phytosome is made using encapsulating standardized plant extracts that are linked to phospholipids, specifically phosphatidylcholine from soy, to create a lipid-compatible complex (Pal *et al.* 2021). The nomenclature “phyto” pertains to plant, while “some” refers to cell-like structures. Phytosome technology involves the creation of miniature cellular entities (50 nm to 100 µm), wherein plant extracts or their active components find safeguarding against degradation caused by gastric secretions and gut bacteria. These phytosomes play a pivotal role in diminishing the necessary dosage of active agents or drugs, attributed to the heightened assimilation of primary constituents.

Generally, extensive research has delved into the application of encapsulation technology across diverse sectors; however, very limited work has been conducted so far to investigate its application in broiler production. In view of this, current study was designed to explore a pioneering approach, the phytosome-conjugation of carvacrol essential oil utilizing a lower carvacrol

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concentration (16.6%). The underlying hypothesis posits that this innovation could enhance the overall performance of broiler chickens by improving bioavailability, absorption, and cost-effectiveness in production.

MATERIALS AND METHODS

Experimental birds and design of experiment: The current experimental trial involved the utilization of two hundred forty-day-old unsexed broiler chicks of the Vencobb 430 breed, sourced from commercial hatchery. The essential oils, namely carvacrol essential oil and phytosome conjugated carvacrol (composed of 16.6% carvacrol and 16.6% tween), were procured from IgY Immunologist India Pvt. Ltd., Hyderabad. These essential oils were homogeneously blended with the respective feed allocated to the broiler subjects.

The initial weights of the broiler birds were recorded on day 1, following which they were randomly allocated into four distinct treatment groups. All experimental groups were further divided into 4 replicates with 15 birds per replicate in a completely randomized block design. The control group (T_0) received a standard diet in adherence to the specifications outlined by the Bureau of Indian Standards (BIS) in 2007. The treatment group T_1 received a standard diet supplemented with BMD antibiotic at a standard dose. Group T_2 was provided with a standard diet enriched with carvacrol essential oil @100 mg/kg of feed. The last treatment group, T_3, was supplied with a standard diet containing phytosome conjugated carvacrol essential oil (carvacrol @16.6%) @100 mg/kg of feed. The comprehensive composition of the experimental diet, including ingredients and nutrient content, is detailed in Table 1. The broiler birds were accommodated in a well-ventilated housing system designed with a deep litter system. Throughout the experimental phases encompassing pre-starter (1 to 7 days), starter (8 to 21 days), and finisher (22 to 42 days), the broilers were provided with a corn-soya-based mash feed formulated according to the BIS (2007) guidelines. All diets across the treatment groups were designed to be iso-caloric and iso-nitrogenous.

Continuous access to both feed and water was offered to the experimental broiler birds throughout the study duration. For the initial two weeks, brooding conditions were established using electric bulbs, and standard vaccination protocols were applied to all birds. Consistent management practices were uniformly employed throughout the 42-day experimental time frame. The performance of all the treatment groups was assessed with respect to the different performance parameters.

The birds from each group were evaluated for growth performance in terms of weekly body weight, weekly feed conversion ratio, average feed intake, and average daily gain. The histomorphological characteristics of the small intestine (duodenum, jejunum, and ileum) to discern the potential impact of essential oils on key parameters, including villus height (VH), crypt depth (CD), and villus height-crypt depth ratio. The economic facets of broiler production were determined through a comprehensive analysis encompassing the summation of production cost, and miscellaneous expenditure. Subsequently, the profit per kg of live body weight was calculated post-sale of birds within the local market, where live body weight served as the basis for pricing.

Table 1. Ingredient and nutritional composition of various experimental diets

Feed ingredient	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
D L-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
Coccidiostat	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.

Statistical analyses were performed on the data collected across various parameters throughout the study period. The complete randomized design was employed, and SPSS-21 software was used for analysis. One-way ANOVA was executed on all parameters, and subsequent separation of treatment means was carried out via Duncan's multiple range test (1995). The experimental units were the replicates for all parameter analyses. Statistical significance was established at $P \leq 0.05$.

RESULTS AN DISCUSSION

Weekly live weight: The average live body weights of broilers (Table 2) at 6th weeks of age revealed numerically higher live body weights in birds from the group supplemented with carvacrol essential oil (T_2) and phytosome conjugated carvacrol essential oil (T_3) than control group (T_0), however, there were no significant differences in body weights between treatments. The per cent improvement in live body weight of broilers in T_1, T_2 and T_3 treatment groups over the control was 2.29, 4.64, and 1.86 %, respectively, at the 6th week of age.

The findings of Madrid *et al.* (2003), who investigated the impact of a plant extract (mix of oregano, cinnamon, and pepper essential oil) on broiler performance, are similar to the findings of the present study. Similar findings were also observed by Amad *et al*. (2013), Hoffman and Wu (2010) and Saadat Shad *et al*. (2016). It was suggested that the antimicrobial activity of essential oil may be masked by diet composition and/or environment, resulting in non-significant effect of essential oil on growth performance when a well-balanced diet was fed and the birds were kept in a clean environment, as was done in this study. However, in contrary to the present findings, the authors, viz. Cross *et al*. (2007), Al-Kassie (2009), Ben-Mahdi *et al*. (2010), and Gole *et al.* (2022) have reported significantly higher body weights in treatment groups.

In the present study, essential oil-supplemented groups exhibited satisfactory results in the substitution of the growth promoters, and no negative effect was observed on growth performance when compared to the birds receiving diets containing antibiotic growth promoters during the entire trial phase. The explanation for this equivalence of growth performance between the birds from all treatment groups could be attributed to increased secretions of digestive enzymes resulting in better nutrient utilization and rate of feed passage in gut and to the equilibrium of the intestinal microbiota of the birds supplemented with carvacrol essential oil, phytosome conjugated carvacrol, and antibiotic, as found by Hernandez *et al.* (2004) and Bosetti *et al*. (2020), leading to better intestinal mucosa development, stimulation of the enzymatic secretion, better nutrient digestion and absorption.

Cumulative weekly body weight gain: The numerically highest average cumulative weekly body weight gain (Table 2) was observed in group T_2 (carvacrol essential oil supplementation) followed by T_1, T_3, and T_0, however, the statistical analysis revealed a non-significant difference in all the groups over the entire period of trial. The results revealed that at the end of 6th week, the supplementation of phytosome-conjugated carvacrol essential oil (T_3) and carvacrol essential oil (T_2) resulted in increased live body weight by 1.86 % and 4.63%, respectively. The numerically higher body weight gain in groups supplemented with essential oil may be associated with its antimicrobial action and stimulation of various digestive enzymes resulting in improved nutrient utilization. It is pertinent to note that the phytosome conjugated carvacrol essential oil group (T_3) also revealed cumulative weight gain at par with the carvacrol essential oil supplemented group (T_2) during all the weeks under the present study even at less concentration of carvacrol (16.6%).

The present findings for the average cumulative weekly body weight gain corroborate with the findings of Hoffman and Wu (2010), Amad *et al*. (2013) and Saadat Shad *et al*.

Table 2. Mean weekly live body weight and cumulative weekly body weight gain of broilers in the experimental groups

Week	Mean weekly live body weight (g)				CD	Cumulative weekly body weightgain(g)				CD
	T_0	T_1	T_2	T_3		T_0	T_1	T_2	T_3	
Day old	44.45±0.11	43.70±0.35	43.36±0.18	43.43±0.70	NS					
1st	44.45±0.11	43.70 ±0.35	43.36 ±0.18	43.43 ±0.70	NS	127.83±3.37	134.42±2.00	137.42±6.41	125.65±5.83	NS
2nd	172.28 ±3.36	178.11±1.67	180.78±6.56	169.08±6.13	NS	425.55±6.67	442.30±6.13	451.30±13.24	465.24±30.70	NS
3rd	470.00±6.59	486.00±6.31	494.66±13.39	508.66±30.53	NS	930.22±12.30	943.97±6.95	957.13±22.9	938.57±14.41	NS
4th	974.66±12.20	987.66±7.15	1000.50±23.04	982.00±13.93	NS	1598.62±13.03	1614.63±24.25	1628.66±45.39	1613.90±27.53	NS
5th	1643.07±12.94	1658.33±24.27	1672.02±45.44	1657.68±27.12	NS	2315.38±15.64	2350.13±56.82	2368.87±48.10	2321.57±74.39	NS
6th	2359.83±15.54	2393.83±56.68	2412.23±48.20	2365.00±74.45	NS	2973.55±29.01	3043.30±78.17	3114.63±62.57	3030.82±64.84	NS

*$P<0.05$. NS, non-significant.

Table 3. Cumulative feed consumption and FCR of broilers from different dietary treatments

Week	Cumulative feed intake (g)				CD	Cumulative feed conversion ratio				CD
	T_0	T_1	T_2	T_3		T_0	T_1	T_2	T_3	
1st	144.35±3.91	149.05±1.37	145.75±3.46	141.12±2.26	NS	0.84±0.01	0.84±0.01	0.81±0.01	0.84±0.04	NS
2nd	495.77±10.7	507.44±3.93	515.27±10.17	500.49±9.38	NS	1.06±0.01	1.05±0.01	1.04±0.01	0.99±0.04	NS
3rd	1182.17±15.5	1201.17±3.50	1208.37±26.03	1190.10±13.33	NS	1.21±0.01	1.22±0.01	1.21±0.03	1.21±0.01	NS
4th	2106.85±15.12	2114.72±20.99	2151.86±25.28	2123.18±27.45	NS	1.28±0.01	1.28±0.01	1.29±0.03	1.28±0.01	NS
5th	3234.02±22.85	3246.62±55.41	3280.21±38.83	3261.75±45.96	NS	1.37±0.01	1.36±0.01	1.36±0.01	1.38±0.03	NS
6th	4561.10±69.34	4476.57±86.55	4605.21±53.78	4520.23±72.16	NS	1.51±0.03	1.45±0.01	1.46±0.02	1.47±0.01	NS

*P<0.05. NS, non-significant.

(2016). However, findings made by Cross *et al*. (2007), Al-Kassie (2009), and Gole *et al.* (2022) are not in tune with observations made in the present study.

Cumulative weekly feed consumption: The mean cumulative weekly feed consumption (g/b) for different treatment groups (Table 3) revealed no significant difference among the treatments. Numerically highest feed consumption was observed in group T_2 compared to all other groups. The cumulative weekly feed consumption did not differ significantly among the different treatment groups. The findings on feed consumption of birds supplemented with carvacrol essential oil in the present study are in line with studies reported by Hernandez *et al.* (2004), Saadat Shad *et al.* (2016) and Gole *et al*. (2022). On the contrary, findings reported by Al-Kassie (2010), Feizi *et al.* (2013) and Hashemipour *et al*. (2013) regarding the effect of supplementation with essential oils on feed intake does not corroborate with the present study.

Cumulative weekly feed conversion ratio: The mean cumulative weekly feed conversion ratio in terms of feed intake per unit gain in weight for different dietary treatment groups is presented in Table 3.

The groups T_2 (carvacrol essential oil) and T_3 (phytosome-conjugated carvacrol essential oil) revealed better cumulative feed conversion ratio (FCR) at the 6th week than the control group. The per cent improvement in FCR at the 6th week in broilers in treatment groups T_2 and T_3 over the control was 3.31 and 2.65%, respectively. This may be attributed to the fact that the birds fed with essential oils utilized the nutrients more efficiently than the birds in the control group.

The observations for the average weekly FCR made in the present study synchronize with the findings of Hoffman and Wu (2010), Saadat Shad *et al*. (2016) and Gole *et al*. (2022). However, findings made by Al-Kassie (2010), Ben-Mahdi *et al*. (2010) and Feizi *et al.* (2013) does not corroborate with the present study.

Intestinal tract morphometry: The intestinal morphometry of experimental broiler birds (Table 4) revealed that, villi height was numerically improved at duodenum in T_2 and T_3 groups than other groups (T_0 and T_1). The crypt depth was more in T_2 group followed by T_1, T_3 and control groups, respectively. In jejunum, the higher villus height and crypt depth was observed in group T_1 followed by T_0, T_3, and T_2 group, respectively in decreasing order. The villus height at the ileum was more in group T_3 followed by T_0, T_1, and T_2 groups, respectively in decreasing order, whereas, the increased crypt depth was observed in group T_3 followed by T_1, T_0, and T_2 groups, respectively. However, the statistical analysis of data pertaining to villi morphology of the intestine (duodenum, jejunum, and ileum) in broilers revealed non-significant differences among all the groups. Nonetheless, there were no enteric challenges of infectious agents in the treatment groups, which may be related to the inclusion of the essential oil.

The present findings regarding the effect of essential oil on intestinal morphology were found in agreement with

Table 4. Villi morphology of duodenum, jejunum and ileum in broilers from different dietary treatments

Particular	Treatment				CD
	T_0 (Control)	T_1	T_2	T_3	
Duodenum					
Villi height (µm)	1470.02±137.65	1570.40±63.56	1689.91±162.07	1613.51±178.13	NS
Crypt depth (µm)	222.16±45.53	242.64±35.08	387.79±144.11	222.36±30.53	NS
Villi: Crypt	7.92±2.3	6.87±0.93	5.75±1.41	7.37±0.33	NS
Jejunum					
Villi height (µm)	1214.3±123.7	1318.17±44.87	1160.68±109.22	1199.72±101.57	NS
Crypt depth (µm)	199.18±15.10	307.87±107.65	171.27±31.02	182.26±23.07	NS
Villi : Crypt	6.15±0.67	5.51±1.19	7.58±1.47	6.81±0.75	NS
Ileum					
Villi height (µm)	1057.56±177.04	1046.63±153.49	1022.23±105.75	1407.42±467.53	NS
Crypt depth (µm)	163.45±26.32	212.14±20.42	145.45±21.50	224.24±69.90	NS
Villi : crypt	6.53±0.82	4.97±0.61	7.79±1.9	6.21±0.41	NS

*P<0.05. NS, non-significant.

Xue *et al.* (2020), who found that a diet supplemented with a 100 mg/kg EO mixture containing eucalyptus oil, carvacrol, cinnamaldehyde, and capsaicin had no significant effect on the growth performance, carcass traits and intestinal morphology of broilers. Additionally, the type of essential oils, the coating technology, the composition of the ingredients, the dosage administered, the variety and age of the experimental animals are just a few of the variables that could be responsible for the significant discrepancies between the aforementioned studies. The present finding corroborated with Çabuk *et al.* (2006), Agostini *et al*. (2012), and Bosetti *et al*. (2020), who reported no significant improvement in intestinal morphology whereas, Hashemipour *et al*. (2013), Mohammadi *et al*. (2014) and Stamilla *et al*. (2020) observed improvement in villi morphology.

Economics of broiler production: The economics of broiler production of all the treatment groups was worked out by considering the prices of different inputs required for broiler production. The average feed cost per kg was calculated from broiler pre-starter, starter, and finisher feed rates. The broilers were sold at the prevailing rate of ₹75/- kg on a live body weight basis. Other expenses like labour, electricity, and miscellaneous expenses were also considered as uniform input for all the treatment groups. The net profit per kg body weight gain for T_0 to T_3 was ₹12.60, 20.40, 19.78 and 17.31, respectively. The results revealed that the birds from the groups supplemented with phytosome-conjugated carvacrol essential oil (T_3) and carvacrol essential oil (T_2) fetched a higher net profit than the control group, however, the highest net profit of ₹20.40 per kg live body weight was evident in T_1 group, which received antibiotic supplementation. The addition of phytosome-conjugated carvacrol essential oil and carvacrol essential oil in diets was found beneficial in reducing the cost of broiler production, thereby enhancing the margin of profit in broiler production.

The overall results revealed that dietary supplementation of phytosome-conjugated carvacrol essential oil and carvacrol essential oil resulted in increase in live body weights of broilers by 1.86% and 4.63%, respectively over the control group. The gain in body weights and FCR were also better in broilers fed diet supplemented with phytosome-conjugated carvacrol essential oil and carvacrol essential oil, which was reflected in higher economic returns in terms of profit per kg live body weight in these groups than control group. Hence, it is concluded that phytosome-conjugated carvacrol essential oil @100 mg/kg ration could be exploited as dietary additive alternative to the antibiotic growth promoters in the rations of broiler birds, without any detrimental effect on their health and productivity.

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