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Research Article:

Dietary Cinnamaldehyde and Eugenol Enhance Growth Performance in Broiler Chickens: A Natural Alternative to Antibiotic Growth Promoters

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Abstract

Increasing restrictions on antibiotic growth promoters (AGPs) have been a challenge for the broiler chicken industry. The study aimed to evaluate the effect of three inclusion levels (3mg, 6mg, 9mg per kg of feed) of cinnamaldehyde and eugenol on the growth performance of broiler chickens as a potential substitute for AGPs. A total of 480 one-day-old Cobb 500 chicks were raised to the age of 35 days in a completely randomized design with eight dietary treatments, each replicated six times with 10 birds per replicate. Treatments were based on added growth promoters (GP) to the 1kg of basal diet: negative control-NC (without GP), positive control-PC (AGP; zinc bacitracin, 50 ppm), cinnamaldehyde at 3 mg/kg (T3), 6 mg/kg (T4), 9 mg/kg (T5) of cinnamon oil or eugenol at 3 mg/kg (T6), 6 mg/kg (T7), 9 mg/kg (T8) of clove oil. Weekly measurements of feed intake and live body weight were obtained from each replicate pen, which was considered the experimental unit. The cumulative body weight gain, feed conversion ratio (FCR), and performance efficiency factor (PEF) were calculated for the total growing period. One-way analysis of variance (ANOVA) was used to compare the performances of the treatments. Broilers receiving the positive control diet exhibited significantly greater ($p < 0.05$) final body weight (2056±22.23 g), weight gain (2009±22.07 g), and feed intake (2887.9±38.4 g) than birds assigned to all other treatments. Except for the group receiving the highest level of cinnamaldehyde (T5), all other groups supplemented with cinnamaldehyde and eugenol showed increased ($p < 0.05$) live weight and weight gain compared with the NC group. The lowest ($p < 0.05$) FCR was recorded in 3 mg/kg cinnamaldehyde-fed birds (T3). The highest PEF was also recorded in birds fed 3 mg/kg cinnamaldehyde, and that was similar ($p > 0.05$) to PC. In conclusion, this study showed that broiler chickens fed diets containing 3 mg/kg and 6 mg/kg cinnamaldehyde and 3 mg/kg, 6 mg/kg, and 9 mg/kg eugenol improved performance compared with birds fed diets without growth promoters.

Keywords: Antibiotic growth promoters, feed conversion ratio, Inclusion levels

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1. Introduction

The demand for chicken meat is growing rapidly worldwide because of its nutritional value and affordability. To meet the increasing demand for poultry meat, specialized broiler lines have been developed through intensive genetic selection and crossbreeding for rapid growth and high feed efficiency (Tallentire et al., 2016; Bennett et al., 2018). These genetic improvements have primarily focused on improving feed conversion ratio (FCR), growth rate, yield and quality of meat (Choi et al., 2023). However, such intensive strategies have negatively affected the broilers' natural resistance to diseases and environmental stress factors. Consequently, modern broiler chickens often require continuous human intervention and proper nutritional management to survive until slaughtering age.

In the broiler chicken industry, various feed additives have been used to prevent diseases, increase growth, balance gut microflora, and regulate external stress factors. For a long time, antibiotics have been utilized for disease prevention, therapeutic purposes, and growth promotion (Dibner & Richards, 2005; Mehdi et al,2018, Ayalew et al., 2022). Antibiotic growth promoters help broilers to achieve their maximum growth by reducing the number of harmful pathogenic bacteria in the gut and developing favourable gut microflora. Although AGPs have positive effects, the overuse and misuse of antibiotics in livestock have led to the development of antibiotic-resistant bacteria. Many countries have instituted restrictions on the use of antibiotics in the broiler industry due to concerns about their residues in meat (Dibner & Richards, 2005, Mehdi et al., 2018, Rahman et al., 2022).

Consequently, considerable research efforts have been directed toward identifying sustainable and effective AGPs in broiler production. Among these, essential oils have gained more attention due to their natural bioactive properties. In ethnomedicine, essential oils have been utilized and have demonstrated antimicrobial, antioxidant, immune-stimulant, and anti-inflammatory properties on living entities. The natural growth-promoting effects of plant-derived oils such as cinnamon, turmeric, garlic, clove, ginger, and rosemary are being researched (Ertas et al., 2005; Tiihonen et al., 2010; El Hack et al., 2022). However, the efficacy of essential oils is influenced by multiple factors, including the plant's physiological characteristics, environmental and soil conditions during growth, the method of essential oil extraction, dosage, and the mode of administration (via feed or drinking water) (Özbilgin et al., 2023).

Therefore, it is crucial to investigate the most efficient dose of different constituents of essential oils individually, in relation to the growth performance of broiler chickens. In this context, the present study was conducted to evaluate three doses of cinnamaldehyde from cinnamon and eugenol from clove essential oil for their effectiveness in promoting the growth performance of broiler chickens.

2. Materials and Methods

All procedures conducted in this experiment were approved and monitored by the Animal Ethics Committee (VRI/ETC/004/5/24) of the Veterinary Research Institute, Gannoruwa, Peradeniya, Sri Lanka.

2.1. Experimental design and Animals

A total of four-hundred- and eighty-day-old chicks (Cobb 500 strain) of similar mean weight were purchased from a reputed local hatchery (Nawgala breeders, Dombawela, Mathale, Sri Lanka). Based on initial body weight, they were randomly assigned to eight dietary treatments in a completely randomized design. Each treatment was replicated six times with ten birds per replicate and raised until 35 days of age in respective pens. Forty-eight deep litter pens were disinfected, thoroughly cleaned and littered with dry paddy husk in an open-sided poultry house located at the Animal Experimental Farm, Veterinary Research Institute, Gannoruwa, Peradeniya, Sri Lanka. Each pen provided

0.09 m² of floor space per bird. Floor brooding continued until 14 days of age in the respective pen for all replicates to support thermoregulation. Initially, the brooding temperature was maintained at 34°C with the aid of artificial lighting fora 24-hour lighting schedule and gradually reduced to environmental temperature (27–28°C). Birds had ad libitum access to feed and clean water. Recommended guidelines provided by the Cobb broiler management guide were followed throughout the experimental period.

2.1.1. Experimental diets

The maize-soybean-based diet was formulated to meet the nutrient needs suggested by the Cobb 500 nutritional requirement guide (Cobb-Vantress Inc. (2022) according to the ingredient composition given in Table 1.

Table 1 Ingredients and nutrient Composition of the basal diet used for the experimental period

Items	**Broiler booster (Day 1- 12)**	**Broiler starter (Day 13 – 28)**	**Broiler finisher (day 29-35)**
Ingredients (%)			
Maize	33.60	28.25	37.57
Broken rice	10.40	17.15	14.65
Rice Polish	9.68	14.80	9.70
Soybean meal	38.60	33.13	30.80
Fish meal 60	0.57	0.20	0.20
Dicalcium phosphate	1.43	0.43	0.33
Calcite	1.07	1.31	1.24
Salt	0.20	0.20	0.20
Vegetable oil	3.30	3.35	4.10
DL-methionine	0.25	0.24	0.25
Lysine	0.02	0.04	0.06
Vitamin/mineral Premix	0.30	0.30	0.30
Phytase enzyme	0.10	0.05	0.10
Vit E	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05
Choline chloride	0.10	0.10	0.10
Calculated Nutrient Composition			
Metabolizable energy (kcal/kg)	2960	3004	3130
Crude protein (%)	22.7	21.0	18.6
Ether extract (%)	4.70	5.20	6.30
Available phosphorus (%)	0.45	0.42	0.38
Calcium (%)	0.96	0.89	0.85
Lysine	1.12	1.05	0.94
Methionine	0.54	0.36	0.30

The calculated chemical composition for the ingredients of the diet according to the National Research Council NRC

The purified bioactive compounds derived from essential oils were incorporated into the basal diet at three different inclusion levels and compared with the non-supplemented and antibiotic-fortified groups of birds. The treatment groups were, negative control group (NC; T1) fed basal diet without any growth promoters (GPs), positive control group (PC; T2)fed basal diet with antibiotic growth promoter, zinc bacitracin(50 ppm), T3, T4 and T5 groups fed basal diet containing graded levels of cinnamaldehyde at 3 mg/kg, 6 mg/kg or 9 mg/kg per kg basal diet, while remaining three groups (T6, T7 and T8) fed graded levels of eugenol at 3 mg/kg, 6 mg/kg or 9 mg/kg per kg basal diet, respectively.

2.2. Data Collection and Calculations

Growth performance

A weighed quantity of feed was given to all the treatments, and the residual feed was collected and weighed separately at the end of each week. The average weekly feed consumption was calculated using the following equation.

$$Feed\ Intake = Given\ feed\ (g) - Remain\ feed\ (g)$$

Equation 1

Initial average body weights of the broiler chicks were recorded at the beginning of the experiment. Thereafter, the body weight of the birds from each treatment was taken at weekly intervals. The average weekly weight gain was calculated by subtracting the previous week's average weight from the present weekly average weight of the birds separately for each treatment.

The feed conversion ratio (FCR) was calculated.

$$Feed\ conversion\ ratio = \frac{Feed\ Intake\ (kg)}{Live\ Weight\ Gain\ (kg)}$$

Equation 2

The performance efficiency factor was calculated for each treatment.

$$PEF = \frac{Live\ weight\ (kg) \times Liveability\ (\%)}{Day\ of\ slaughter \times FCR} \times 100$$

Equation 3

PEF - Performance efficiency factor

2.3. Data analysis

The experiment unit for ANOVA was the replicate pen (6 replicate per treatment). The data were analyzed using the one-way analysis of variance (ANOVA) procedure in R Studio software (Version 2023.03.1). Means were compared by Tukey's HSD test, and statistical significance was declared at $p<0.05$.

3. Results and discussion

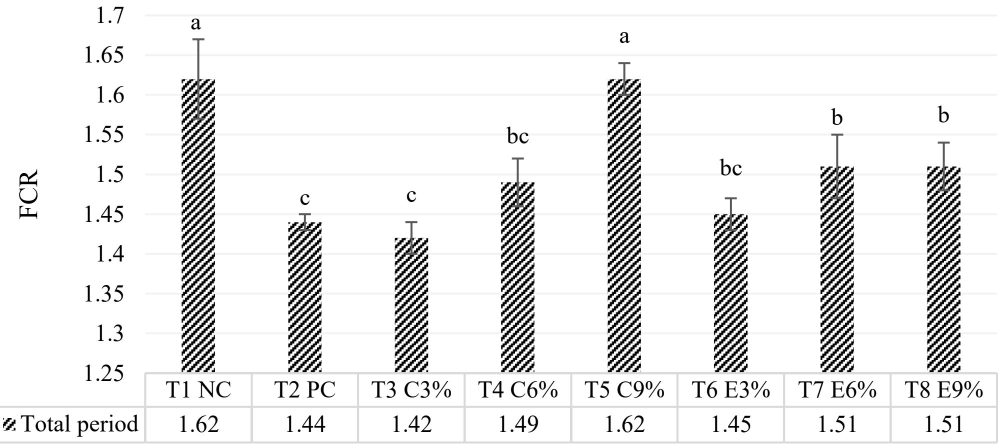
The effects of cinnamaldehyde and eugenol on the cumulative weight gain, feed intake, feed conversion ratio and performance efficiency factors of broilers over the 35-day study period are presented in Table 2. The highest final live weight (2056.2 ± 22.2 g) and total weight gain (2009 ± 22.0 g) were recorded in birds fed diets supplemented with antibiotic growth promoters. Many years of research have shown that antibiotic growth promoters effectively regulate gut microflora, facilitating healthy growth in broiler chickens. Alongside their beneficial effect on growth, antibiotic growth promoters have accelerated the emergence of antibiotic-resistant bacteria. As a result, Phytochemicals are being highlighted as a sustainable alternative for AGPs (Demir et al*.*, 2005; Abd El-Hack et al*.*, 2020).

Notably, except for the group fed cinnamaldehyde at the 9 mg/kg inclusion level, all other essential oil constituent-supplemented treatments showed higher ($p<0.05$) final live weight and total weight gain compared with birds fed the basal diet without any growth promoters. Among the cinnamaldehyde-supplemented groups, birds fed the 3 mg/kg inclusion level recorded the highest ($p<0.05$) final live weight, emphasizing a dose-dependent response. A similar trend was observed in the eugenol-supplemented groups, where birds fed 3 mg/kg eugenol achieved higher ($p<0.05$) final live weight than those fed 6 mg/kg or 9 mg/kg. As illustrated in Figure 1, the lowest FCR was recorded in birds on diets containing 3 mg/kg

Table 2: Live body weight, total feed intake, total body weight gain, feed conversion ratio and performance efficiency factors of broiler chickens fed different treatment diets at 35 days of age.

		Dietary treatments								*p*- value
Period	**Parameter**	**T1 (NC)**	**T2 (PC)**	**T3 (Cin 3 mg/kg)**	**T4 (Cin 6 mg/kg)**	**T5 (Cin 9 mg/kg)**	**T6 (Eug 3 mg/kg)**	**T7 (Eug 6 mg/kg)**	**T8 (Eug 9 mg/kg)**	
Total Period (1-35 days)	Final Live Weight (g)	1768.2 ±50.7^{c}	2056.2 ±22.2^{a}	1984.1 ±43.6^{ab}	1960.4 ±35.9^{b}	1727.1 ±25.8^{c}	1969.8 ±24.7^{ab}	1922.8 ±41.5^{b}	1907.1 ±31.4^{b}	<0.004
	Total Weight Gain (g)	1720.8 ±50.7^{c}	2009.4 ±22.0^{a}	1937.2 ±43.3^{ab}	1913.4 ±36.1^{b}	1679.9 ±25.9^{c}	1922.7 ±24.5^{ab}	1875.0 ±41.5^{b}	1859.4 ±31.3^{b}	<0.001
	Total Feed intake (g)	2781.6 ±32.2^{bc}	2887.9 ±38.4^{a}	2743.6 ±46.2^{bc}	2837.2 ±40.3^{ab}	2725.2 ±35.1^{c}	2796.1 ±50.1^{abc}	2821.9 ±18.0^{ab}	2807.6 ±39.1^{abc}	<0.012
	Feed conversion ratio	1.62 ±0.05^{a}	1.44 ±0.01^{bc}	1.42 ±0.02^{c}	1.49 ±0.03^{bc}	1.62 ±0.02^{a}	1.45 ±0.02^{bc}	1.51 ±0.04^{b}	1.51 ±0.03^{b}	<0.013
	Performance efficiency factor	314.0 ±18.7^{c}	408.8 ±5.04^{a}	400.7 ±13.5^{a}	378.6 ±13.8^{ab}	304.3 ±6.85^{c}	387.2 ±6.07^{ab}	366.0 ±16.7^{b}	361.5 ±11.83^{b}	<0.030
	Liveability (%)	98.33±1.91	100±0.00	100±0.00	100±0.00	96.67±2.11	100±0.00	98.33±1.67	96.67±3.33	>0.04

Data presented as mean ± SEM (Standard Error of the Mean). a,b,c means within the same row with different superscripts are significantly different (p<0.05). *The treatments were, T1= without growth promoters, T2= with antibiotic growth promoters (Zinc-Bacitracin), T3= Cinnamaldehyde 3 mg/kg, T4= Cinnamaldehyde 6 mg/kg, T5= Cinnamaldehyde 9 mg/kg, T6= Eugenol 3 mg/kg, T7= Eugenol 6 mg/kg, T8= Eugenol 9 mg/kg (1 – 35 days). Experiment units were the replicate pens



	T1 NC	T2 PC	T3 C3%	T4 C6%	T5 C9%	T6 E3%	T7 E6%	T8 E9%
Total period	1.62	1.44	1.42	1.49	1.62	1.45	1.51	1.51

Figure 1. Treatments effect on Feed Conversion Ratio of Broiler chickens (The treatments were, T1= without growth promoters, T2= with antibiotic growth promoters (Zinc-Bacitracin), T3= Cinnamaldehyde 3 mg/kg, T4= Cinnamaldehyde 6 mg/kg, T5= Cinnamaldehyde 9 mg/kg, T6= Eugenol 3 mg/kg, T7= Eugenol 6 mg/kg, T8= Eugenol 9 mg/kg

Cinnamaldehyde (1.42±0.02), followed by those fed on the diet with antibiotic (T2). The highest ($p<0.05$) Performance Efficiency Factor (PEF) was recorded in birds fed a diet containing 3 mg/kg cinnamaldehyde, and that was similar ($p>0.05$) to the birds fed the positive control diet (T2). The results suggest that increasing the inclusion level of essential oil constituents in the diet appeared to reduce their positive effect on the growth performance of broiler chickens. As the dose of Cinnamaldehyde and Eugenol rises, the study shows a reduction in the stimulatory effect on growth. The dose-dependent decline in efficacy is due to the strong pungent taste, which diminishes the feed's palatability. Conversely, the potent antimicrobial properties may be related to gut dysbiosis, disrupting optimal digestion and nutrient absorption (Ali et al., 2021).

Cinnamaldehyde and Eugenol are the principal bioactive compounds of cinnamon bark oil and clove bud oil, respectively. Previous studies have reported that the beneficial effects of cinnamon and clove oil/powder at optimal inclusion levels on the growth performance of broiler chickens were attributed to the presence of natural bioactive constituents, such as cinnamaldehyde and eugenol (Ertas et al., 2005, Abd El-Hack et al., 2020, Su et al., 2021, Yaqoob et al., 2022, Dey et al., 2023). Saeed et al. (2018) reported an improvement in body weight gain and FCR in broiler chickens fed a basal diet supplemented with cinnamaldehyde, as compared to the group fed without any growth promoter. Similarly, Toghyani et al. (2011) observed that dietary supplementation of cinnamon significantly improved broiler chicken body weight gain and FCR. In addition, Ertas et al. (2005)highlighted that clove has special characteristics in improving appetite and stimulating the effect of digestion. The pleasant aroma of these compounds may help to increase voluntary feed ingestion of broiler chickens by increasing the palatability of feed and stimulating appetite(Demir et al., 2005). Cinnamaldehyde and eugenol stimulate the activity of pancreatic/intestinal digestive enzymes by stimulating salivary gland secretions (Kim et al.,2010). Especially, these compounds can enhance protein digestion by increasing the secretion of gastric juice(containing HCl and pepsin) (Su et al., 2021).

Furthermore, several *in vitro* studies have revealed that cinnamaldehyde and eugenol exhibit strong antimicrobial activity against enteric pathogenic bacteria, which disturb the balance of beneficial gut microflora (Yaqoob et al*.,* 2022). The enteric pathogenic bacteria can cause depression of appetite, enteric toxin production and diarrhoea. They establish competition with the host bird for nutrients, and the bird is required to spend extra energy to maintain its immune stability. However, the natural antimicrobial properties of cinnamaldehyde and eugenol may suppress the pathogenic effects of microbes and establish a favourable gut environment for the growth of beneficial bacteria, which provide disease-free conditions during the rapid growth period of broilers. Several studies have stated that cinnamaldehyde can improve bile production and utilization of minor nutrients (such as vitamins) by stimulating better hepatic functions via protecting the liver from reactive oxygen species (Saeed et al*.,* 2018). Collectively, these properties would have supported the body weight gain and feed conversion of birds fed diets with cinnamaldehyde and eugenol in the current study.

In conclusion, this study has demonstrated that cinnamaldehyde (at 3 mg/kg and 6 mg/kg mg/kg per kg of feed) and eugenol (at3 mg/kg, 6 mg/kg, and 9 mg/kg per kg of feed) have a positive impacton live weight, weight gain, feed intake and FCR of broiler chickens. For future studies, these selected inclusion levels of cinnamaldehyde and eugenol can be mixed to investigate the synergistic effect of those compounds on growth performance of broiler chickens.

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Declaration of Conflict of Interest

The authors have no conflict of interest to declare.

Use of Artificial Intelligence (AI)

AI tools were utilized in the manuscript preparation solely for language editing.

Author Contributions

Conceptualization; M.W.C.D. Palliyeguru, K.G.N.S. Perera; methodology, M.W.C.D. Palliyeguru, J.K. Vidanarachchi K.G.N.S. Perera; resources, E.W.D.M. Ellawidana, T.R. Wanigasinghe, S.M. Wanigasinghe; writing—original draft preparation, K.G.N.S. Perera; writing— review and editing, M.W.C.D. Palliyeguru, J.K. Vidanarachchi, E.W.D.M. Ellawidana; visualization, K.G.N.S. Perera; supervision, M.W.C.D. Palliyeguru, J.K. Vidanarachchi, E.W.D.M. Ellawidana;

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