



## Impact of dietary supplementation of cinnamon oil on the oxidative stress indices, immune response and intestinal morphology in broiler chickens

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### ABSTRACT

An experiment was conducted to study the effect of different levels of cinnamon essential oil (CEO) on the oxidative stress indices, immune response and intestinal morphology of broiler chickens. Day old broiler chicks (n=180) were randomly allocated to six dietary groups. Control group (T<sub>0</sub>) was fed basal diet without antibiotic growth promoter (AGP), Group 2 basal diet with AGP (T<sub>1</sub>) while basal diet containing graded level of CEO (200, 300, 400 and 500 mg/kg) was fed to Groups T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> respectively. Significantly lower concentration of Malondialdehyde (MDA) was reported in birds fed CEO supplemented diet as compared to control. Significantly increased villi height was observed in histological section of duodenum and jejunum due to increase in concentration of CEO in T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> as compared to control. No significant change in the histo-morphology of liver was observed due to supplementation of CEO at different levels in the diet of broiler chicken. It is concluded that dietary supplementation of cinnamon essential oil @ 400 mg/kg improved the immune response and intestinal morphology of broiler chickens without any adverse effect.

**Keywords:** Broiler, Cinnamon oil, Histology, Immunity, Oxidative stress

Several feed additives including antibiotic growth promoters (AGPs) are usually included in poultry feeds to improve the efficiency of production, product quality and to prevent diseases (Brenes and Roura 2010). In recent years, the use of antibiotics has been restricted due to the growing concerns regarding development of antibiotic-resistant bacteria and transfer of antibiotic residues in meat and eggs, which may cause side-effects in humans (Tehseen *et al.* 2016). The restriction on the use of antibiotics as feed additives has driven nutritionists and feed manufacturers to develop alternatives such as enzymes, probiotics, prebiotics, synbiotics, organic acids and plant extracts (phytobiotics) as feed additives in diets of monogastric animals (Zeng *et al.* 2015). Various natural substitutes of AGP have been found efficacious with different mechanisms of action, including use of dietary plant derived natural bioactive compounds (phytobiotics) like essential oils (EOs). Essential oils are complex compounds, and their chemical composition and concentrations of various bioactive compounds are variable (Torki *et al.* 2015). Studies have revealed that EOs exhibit anti-microbial, antiviral, antifungal, immune-modulatory, hypolipidemic, digestive stimulation effect and growth-promoting properties (Gopi *et al.* 2014). Due to positive

modulation of gastrointestinal microbiota of birds, essential oils are recently being used in poultry feed as growth promoters (Wade *et al.* 2018). Cinnamon (*Cinnamomum zeylanicum*) commonly known as ‘dalchini’ is one of the oldest medicinal plants, widely used as condiment in India. Cinnamon is commonly used in food industry because of its special aroma. Additionally, it has strong antibacterial properties, anti-candidal, antiulcer, analgesic, antioxidant, hypo-cholesterolemic activities and regulates the lipogenesis (Ciftci *et al.* 2010). Cinnamon bark oil contains 505–977 g/kg of cinnamaldehyde (Mohamed *et al.* 2020), an aromatic aldehyde that has greater antibacterial activity than the other oils of phenolic nature such as carvacrol and thymol. Recent studies have shown that cinnamaldehyde alone or in combination with other essential oils had a wide array of beneficial effects in poultry. The knowledge regarding the application of cinnamon essential oil as feed additives is quite rudimentary. Thus the present study was designed to evaluate the effect of different dietary levels of cinnamon essential oil on the oxidative stress indices, immune response and histo-morphology of small intestine and liver of broiler chicken.

### MATERIALS AND METHODS

*Birds, housing and management:* Day-old Ross AP strain broiler chicks (180) from the same hatch were procured and reared under deep litter system for 35 days. The chicks were divided randomly into 6 dietary treatment

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groups (T<sub>0</sub>-T<sub>5</sub>). Each treatment group had three replicates of 10 chicks. Similar management practices were followed in all the groups.

**Experimental diet:** The diets were formulated as per ICAR (2013) specification for pre-starter (0-14 days), starter (15-21 days) and finisher (22-35 days) stages using maize, soy DOC, soy oil along with minerals and vitamin premixes. Control group (T<sub>0</sub>) was fed basal diet without antibiotic growth promoter (AGP), Group 2 basal diet with AGP (T<sub>1</sub>) while basal diet containing graded level of CEO (200, 300, 400 and 500 mg/kg) was fed to Groups T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> respectively. The cinnamon bark oil used for the dietary supplementation was purchased from Allin Exporters, Noida. All the diets were made iso-caloric and iso-nitrogenous. At 28 d, blood samples were collected from three birds per replicate in eppendorf tubes containing anti-coagulant acid citrate dextrose for oxidative stress indices. Lipid peroxidation in haemolysate was measured by the method of Placer *et al.* (1966). Reduced Glutathione in RBC suspension was measured by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) method as per Prins and Loos (1969). For immunological studies, the blood was collected from three birds each replicate in non-heparinized clean test tube on 28 and 35 day of experiment. The mean serum HI titre was evaluated against Newcastle disease virus as per method described by Allan and Gough (1974). The *in vivo* cell mediated immune response to PHA-P was evaluated in nine birds per treatment as per method described by Cheng and Lamont (1988). For histo-morphological evaluation, three birds per replicate were slaughtered on 35<sup>th</sup> day and representative sample of duodenum, jejunum and liver were collected. The tissue samples were fixed, processed and stained with Haematoxylin and Eosin as per the procedure of Singh and Sulochana (1997). Measurements of height of the villi and depth of crypt were based on at least 10 well-oriented villi per section under 10× objectives using an inverted microscope (T capture software). The slides of liver were examined to assess the histo-pathological changes, if any. For interpretation of the results, the data were analyzed by one-way Analysis of Variance and mean value were compared using SPSS package (SPSS ver 26.0) and significant difference was expressed as 1 and 5% of probability.

## RESULTS AND DISCUSSION

**Oxidative stress indices:** The dietary supplementation

of CEO accrued significant (P<0.05) effect on MDA irrespective of CEO level. The lowest concentration of MDA was observed in Group T<sub>5</sub> followed by T<sub>1</sub>, T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub> and T<sub>0</sub> (Table 1). Malondialdehyde (MDA) is a degraded product of lipids that can be used as a biomarker of lipids peroxidation and oxidative stress (Yang *et al.* 2019). The reduced glutathione concentration in erythrocyte did not vary significantly among the groups due to dietary supplementation of CEO or AGP. The result corroborates with the findings of previous researchers (Faix *et al.* 2009, Ciftci *et al.* 2010, Yang *et al.* 2019) who reported that the plasma or serum MDA levels of broilers decreased after CEO supplementation in diets.

**Cell mediated immune response:** The results indicated no significant difference in the foot index (FI) in response to PHA-P among the dietary treatment groups, though the thickness was non-significantly higher in all the treatment groups as compared to control group (Table 1).

**Humoral immune response:** The mean serum HI titre against NCDV at 28<sup>th</sup> and 35<sup>th</sup> day indicated significant (P<0.05) improvement in HI titre with the increase in CEO concentration in Groups T<sub>3</sub> and T<sub>4</sub> as compared to control (Table 1). The findings of present study are in line with previous researchers (Sadeghi *et al.* 2012, Sang-oh *et al.* 2013, Chowdhury *et al.* 2018, Parveen *et al.* 2020) who reported increased antibody titer in CEO supplemented birds.

**Histo-morphological study:** There was improvement (P<0.05) in villus height of duodenum due to increase in concentration of CEO in T<sub>4</sub>, T<sub>5</sub> and T<sub>3</sub> Groups as compared to T<sub>0</sub> (Table 2, Figs 1 and 2). No significant effect of AGP or CEO supplementation on crypt depth and villus height to crypt depth ratio was observed in the present study. In jejunum also, the maximum villus height was recorded in Group T<sub>4</sub> which was significantly higher (P<0.05) as compared to T<sub>0</sub>. No significant impact of CEO was observed on CD and VH:CD ratio. The results corroborated with the findings of previous researchers (Chowdhury *et al.* 2018, Abudabos *et al.* 2018, Reis *et al.* 2018, Parveen *et al.* 2020) who reported significantly (P<0.05) increased height of villi of duodenum and jejunum as compared to the control due to essential oil supplementation. The increase in villi height leads to more surface area for absorption of nutrients, which, in turn, results in better digestion and absorption of nutrients (Murugesan *et al.* 2014). In contrast to present finding, Yang *et al.* (2019) concluded that 50 to 400 mg CEO per kg diet did not affect villus height in the

Table 1. Effect of cinnamon oil supplementation on oxidative stress indices, cell mediated and humoral immune response

Particular	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	P value
Lipid peroxidation (MDA <sub>1</sub> nM/mg Hb)	4.52 <sup>a</sup> ±0.23	3.42 <sup>b</sup> ±0.23	3.72 <sup>b</sup> ±0.31	3.56 <sup>b</sup> ±0.24	3.55 <sup>b</sup> ±0.37	3.32 <sup>b</sup> ±0.26	0.006
GSH (M /g Hb)	0.27±0.01	0.29±0.03	0.28±0.01	0.27±0.01	0.27±0.02	0.27±0.01	0.688
FI (mm) in response to PHA-P at 24 h	0.44±0.10	0.60±0.10	0.48±0.08	0.55±0.16	0.65±0.07	0.53±0.06	0.756
HI titer (log 2) at 28 d	2.00 <sup>c</sup> ±0.58	3.67 <sup>ab</sup> ±0.33	3.00 <sup>bc</sup> ±0.58	3.67 <sup>ab</sup> ±0.67	4.67 <sup>a</sup> ±0.33	4.67 <sup>a</sup> ±0.33	0.018
HI titer (log 2) at 35 d	2.33 <sup>c</sup> ±0.33	3.67 <sup>abc</sup> ±0.33	3.00 <sup>bc</sup> ±0.58	4.00 <sup>ab</sup> ±0.58	4.33 <sup>a</sup> ±0.33	2.67 <sup>bc</sup> ±0.33	0.040

T<sub>0</sub>, Control; T<sub>1</sub>, C + AGP; T<sub>2</sub>, CEO @ 200 mg/kg; T<sub>3</sub>, CEO @ 300 mg/kg; T<sub>4</sub>, CEO @ 400 mg/kg; T<sub>5</sub>, CEO @ 500 mg/kg. Means in the same row with different superscript (a, b, c) are significantly different (\*P<0.05, \*\*P<0.01).

Table 2. Effect of dietary levels of cinnamon essential oil on intestinal histomorphology of broilers at 35 d of age

Particular		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	P value
Duodenum	VH (µm)	906 <sup>a</sup> ±137.49	1065 <sup>bc</sup> ±46.60	1038 <sup>bc</sup> ±41.65	1137.67 <sup>a</sup> ±18.89	1338 <sup>a</sup> ±86.77	1191 <sup>ab</sup> ±21.93	0.018
	CD (µm)	318.67±42.15	339.33±9.70	224±37.86	337±6.66	343.33±80.25	328.67±60.34	0.309
	VH:CD	2.54±0.27	3.14±0.08	4.94±0.98	3.38±0.03	4.26±0.78	3.92±0.80	0.310
Jejunum	VH (µm)	1167 <sup>c</sup> ±48.66	1403 <sup>ab</sup> ±81.55	1231 <sup>c</sup> ±68.64	1183 <sup>c</sup> ±33.72	1438 <sup>a</sup> ±18.15	1251 <sup>bc</sup> ±27.10	0.011
	CD (µm)	423±17.93	375±42.14	263±31.79	313±17.19	376.67±54.23	333±56.01	0.201
	VH:CD	2.76±0.10	3.89±0.66	4.84±0.74	3.80±0.24	3.97±0.53	4.02±0.77	0.759

T<sub>0</sub>, Control; T<sub>1</sub>, C + AGP; T<sub>2</sub>, CEO @ 200 mg/kg; T<sub>3</sub>, CEO @ 300 mg/kg; T<sub>4</sub>, CEO @ 400 mg/kg; T<sub>5</sub>, CEO @ 500 mg/kg. Means in the same row with different superscript (a, b, c) are significantly different (\*P<0.05, \*\*P<0.01).

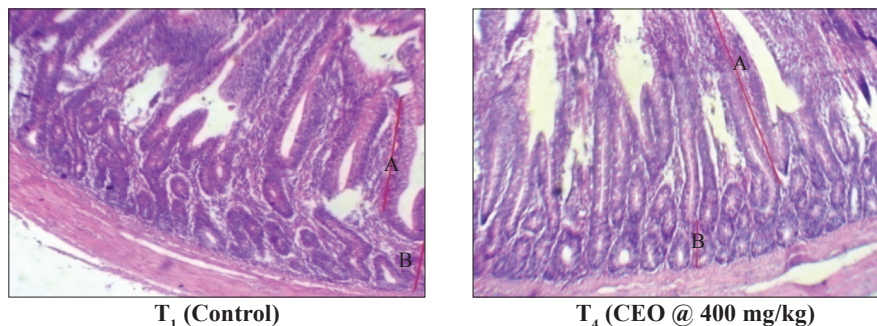


Fig. 1. Micrograph of duodenum showing height of villi (A) and crypt depth (B). (H & E, 10×)

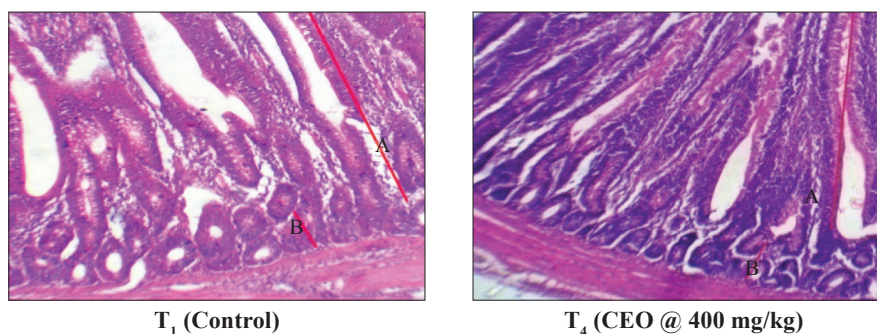


Fig. 2. Micrograph of jejunum showing height of villi (A) and crypt depth (B). (H & E, 10×)

duodenum, jejunum and ileum as compared to the control. The supplementation of CEO at different levels (200, 300, 400, 500 mg/kg) in the diet did not reveal any significant change in the histo-morphology of liver. No pathological changes in liver samples was observed due to increased concentration of CEO in Groups T<sub>4</sub> and T<sub>5</sub>. The present finding is in line with Toghiani *et al.* (2011) who reported that supplementation of cinnamon, caused no significant difference, pathological lesion and damages in heart, gizzard, liver, pancreas, caecum and small intestine.

The results of the current study indicated that dietary supplementation of cinnamon essential oil @ 400 mg/kg improved the immune response and intestinal histo-morphology of broiler chickens without any adverse effect. It is clear that cinnamon can be used as a potential alternative to antibiotics for more safety in the health, environmental and economic aspects of poultry industries.

#### REFERENCES

- Abudabos A M, Alyemni A H, Dafalla Y M and Khan R U. 2018. The effect of phytochemicals on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to *Clostridium perfringens* challenge. *Journal of Applied Animal Research* **46**(1): 691–95.
- Allan W H and Gough R E. 1974. A standard HI test for NCDV I. Comparison of macro and micro method. *Veterinary Record* **95**: 120–25.
- Brenes A and Roura E. 2010. Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology* **158**(1–2): 1–14.
- Cheng S and Lamont S J. 1988. Breeding and genetics; genetic analysis of immunocompetence in White Leghorn chicken line. *Poultry Science* **67**: 989–95.
- Chowdhury S, Mandal G P, Patra A K, Kumar P, Samanta I, Pradhan S and Samanta A K. 2018. Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. *Animal Feed Science and Technology* **236**: 39–47.
- Ciftci M, Simsek U G, Yuce A, Yilmaz O and Dalkilic B. 2010. Effects of dietary antibiotic and cinnamon oil supplementation on antioxidant enzyme activities, cholesterol levels and fatty acid compositions of serum and meat in broiler chickens. *Acta Veterinaria Brno* **79**: 33–40.
- Faix S, Faixová Z, Plachá I and Koppe J. 2009. Effect of *Cinnamomum zeylanicum* essential oil on antioxidative status in broiler chickens. *Acta Veterinaria Brno* **78**: 411–17.

- Gopi M, Karthik K, Manjunathachar H V, Tamilmahan, Kesavan M, Dashprakash M, Balaraju B L and Purushothaman M R. 2014. Essential oils as a feed additive in poultry nutrition. *Advance in Animal and Veterinary Science* **2**(1): 1–7.
- ICAR. 2013. *Nutrients Requirements of Poultry*. Indian Council of Agricultural Research, New Delhi. pp 15.
- Mohamed E H, Mahmoud A, Abdel-Moneim E, Abdel M, Noureldeen G, Mohammed A F K, May B, Sarah I, Othman A A A and Shaaban S E. 2020. Cinnamon (*Cinnamomum zeylanicum*) oil as a potential alternative to antibiotics in poultry. *Antibiotics* **9**: 210.
- Murugesan G R, Gabler N K and Persia M E. 2014. Effects of direct-fed microbial supplementation on broiler performance, intestinal nutrient transport and integrity under experimental conditions with increased microbial challenge. *British Poultry Science* **55**: 89–97.
- Parveen S, Mandal G P, Samanta I, Patra A K, Soren S and Roy B. 2020. Effect of essential oil blend on intestinal morphology, gut microbiota and immune response of broiler chickens. *Indian Journal of Animal Health* **59**(2): 228–36.
- Placer Z A, Cushmann L L and Johnson B. 1966. Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical system. *Analytical Biochemistry* **16**: 359–64.
- Prins H K and Loos J A. 1969. *Biochemical Methods in Red Cell Genetics*. Academic Press, London, pp. 127–29.
- Reis J H, Gebert R R, Barreta M, Baldissera M D, Dos Santos I D, Wagner R, Campigotto G, Jaguezeski A M, Gris A, de Lima J L, Mendes R E, Fracasso M, Boiago M M, Stefani L M, Dos Santos D S, Robazza W S and Da Silva A S. 2018. Effects of phytogetic feed additive based on thymol, carvacrol and cinnamic aldehyde on body weight, blood parameters and environmental bacteria in broilers chickens. *Microbial Pathogenesis* **125**: 168–76.
- Sadeghi G H, Karimi A, Padidar J S H, Azizi T and Daneshmand A. 2012. Effects of cinnamon, thyme and turmeric infusions on the performance and immune response in 1- to 21-day-old male broilers. *Brazilian Journal of Poultry Science* 15–20.
- Sang-Oh P, Chae-Min R, Byung-Sung P and Jong H. 2013. The meat quality and growth performance in broiler chickens fed diet with cinnamon powder. *Journal of Environmental Biology* **34**: 127–33.
- Singh U B and Sulochana S. 1997. *A Practical Manual of Histopathological and Histochemical Techniques*. Kothari Publication, Bombay. Pp 1–41.
- Tehseen M, Tahir M, Khan R U, Jabbar A, Ahmad B, Ahsan T, Khan S and Abudabos A M. 2016. Additive effect of *Nigella sativa* and *Zingiber officinale* herbal mixture on performance and cholesterol profile in broilers. *Philippine Agricultural Scientists* **99**: 408–13.
- Toghyani M, Toghyani M, Gheisari A, Ghalamkari G and Eghbalsaied S. 2011. Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. *Livestock Science* **138**: 167–73.
- Torki M, Akbari M and Kaviani K. 2015. Single and combined effects of zinc and cinnamon essential oil in diet on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition. *International Journal of Biometeorology* **59**: 1169–77.
- Wade M R, Manwar S J, Kuralkar S V, Waghmare S P, Ingle V C and Hajare S W. 2018. Effect of thyme essential oil on performance of broiler chicken. *Journal of Entomology and Zoology Studies* **6**: 25–28.
- Yang Y F, Lu-lu Z, Yu-xin S, Xiu-dong L, Li-yang Z and Xu-gang L. 2019. Effects of dietary graded levels of cinnamon essential oil and its combination with bamboo leaf flavonoid on immune function, antioxidative ability and intestinal microbiota of broilers. *Journal of Integrative Agriculture* **18**(9): 2123–32.
- Zeng Z, Zhang S, Wang H and Paio X. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. *Journal of Animal Science and Biotechnology* **6**: 7–15.