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# Effect of cinnamon oil supplementation into broiler chicken diets on growth, carcass traits, haemato-biochemical parameters, immune function, antioxidant status and caecal microbial count

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**ABSTRACT.** This study aims to investigate the effect of supplementing dietary cinnamon oil (CO) into broiler chicken diets on growth performance, carcass traits, haemato-biochemical parameters, immune function, antioxidant status, and caecal microbial count. Three hundred one-day old broilers were distributed randomly, with 5 replications, into 5 groups (12 birds per replicate). Dietary treatments comprised the basal diet (control group), and the basal diet supplemented by either 10 mg/kg avilamycin or 500, 1000 and 1500 mg/kg of CO. Results indicated that birds from CO-treated groups had higher body weight, weight gain and feed conversion ratio than the control group. Also, birds from CO supplemented groups had lower total cholesterol, triglycerides, low-density lipoproteins than those from the control group; the lowest values were recorded in the group treated with 500 mg/kg CO. Treatment with CO increased the relative weight of spleen, thymus, bursa of Fabricius and plasma content of IgM when compared to the control and the antibiotic-treated groups. In chickens fed CO a significant decrease in caecal total microbial count, total yeast and mold count, *Escherichia coli*, and *Salmonella* was noted in comparison with the control group but was similar to animals from antibiotic-treated group. The count of lactic acid bacteria increased in the caecum of chickens fed CO in comparison with those from the control group; the lowest level was observed in the antibiotic group. So, CO can be used in broiler chicken diets as a natural alternative to antibiotic growth promoters to improve gut health and consequently growth performance.

## Introduction

Subtherapeutic antibiotics have been used extensively as growth promoters in poultry production to enhance growth performance, treating and improving the immunity of sick and sensitive birds. However, the overuse of antibiotics has resulted in the development of antibiotic-resistant bacterium strains that may be transmitted from animals to humans (Anthony et al., 2010). The use of antibiotics as growth promoters was thus completely banned by the European Union in 2006 due to its adverse effects on human health. So, it is important to search for alternative growth promoters to avoid the negative effects and consequences of using such antibiotics. Many studies have been conducted to investigate the use of plants, plant extracts and oils as effective substitutes for antibiotics. In poultry diets, herbs and herbal products are used to replace synthetic and chemical products (Alagawany et al., 2020). Because of their antimi-

crobial effects (Valero and Salmero, 2003), and their stimulating effects on animal digestive systems, aromatic plants and essential oil extracts received more attention. Although the antimicrobial effects of essential oils are proven, the studies concerning their effects on growth performance in poultry are variable and need more investigation (Reda et al., 2020a,b,c).

Cinnamon oil (CO) is mostly used in the animal and poultry feed industry because of its special aroma (Abo Ghanima et al., 2020). The main effects of CO are due to its content of cinnamaldehyde followed by eugenol with the strongest antibacterial, antifungal and antioxidant properties (Abd El-Hack et al. 2020). Some studies have stated that CO could be considered a natural alternative to dietary antibiotics and have positive effects on broiler chicken growth performance (Mehdipour and Afsharmanesh, 2018). However, other studies did not observe any significant improvement in chicken growth (Hernández et al., 2004). From the previous studies, it is hypothesized that the dietary supplementation of CO is expected to exert beneficial impacts on the performance and heatlh of broiler chickens. Therefore, this study was implemented to assess the effect of CO as a natural alternative to antibiotics on growth performance, carcass traits, haemato-biochemical parameters, immune function, antioxidant status, and caecal microbial count in broiler chickens.

# Material and methods

## Description of the experimental treatments

The present study was conducted at a private poultry farm located in San-El-Hagar, Sharkia Governorate, Egypt. In total, 300 unsexed, one-day-old Arbor Acre broiler chicks were randomly allocated in 5 treatment groups, 60 chicks and 5 replicates per treatment (12 birds in each replicate). Chicks of each replicate were placed in separate pens (100 × 120 cm). Birds were fed the same basal diets (Table 1) which were formulated to conform to broiler requirements during starter and finisher phases according to the National Research Council (NRC, 1994). Dietary treatments comprised: the basal diet with no additives (control group; T1), the basal diet supplemented with 10 mg/kg of avilamycin (group T2), 500 (group T3), 1000 (group T4) and 1500 mg/kg of CO (group T5). Cinnamon oil purchased from El Hawag Company for Natural Oils (Cairo, Egypt) was mixed with a soyabean oil as a carrier and then uniformly sprayed on the diet. The concentration of some cinnamon bark oil components is presented in Table 2, these major and

**Table 1.** Basal diet composition, g/kg

| Items, as-fed basis | Starter diet (days 1–21 of experiment) | Finisher diet (days 22–42 of experiment) |
|---|---|---|
| Ingredient, g/kg | | |
| maize (8.5%) | 530.3 | 592.1 |
| soybean meal (44%) | 350.0 | 270.0 |
| maize gluten meal (62%) | 50.0 | 50.0 |
| soybean oil | 29.0 | 48.2 |
| limestone | 14.0 | 13.7 |
| Di-calcium phosphate | 15.0 | 15.5 |
| salt | 3.0 | 3.0 |
| vitamin and mineral premix[1] | 3.0 | 3.0 |
| L-lysine | 1.50 | 1.50 |
| DL-methionine | 1.20 | – |
| choline chloride (50%) | 3.0 | 3.0 |
| Composition[2] | | |
| metabolizable energy, MJ /kg | 12.54 | 13.37 |
| crude protein | 230.1 | 200.1 |
| calcium | 10.2 | 10.0 |
| nonphytate P | 4.50 | 4.50 |
| lysine | 13.2 | 11.0 |
| TSAA | 9.20 | 7.20 |

[1] consisted per 2.5 kg of diet: IU: vit. A 12 000, vit $D_3$ 2 000; g: vit. E 10, vit. K 3.2, vit. $B_2$ 49, vit. $B_6$ 105, pantothenic acid 10, niacin 20, biotin 50, Zn 45, Fe 30, Mn 40, Cu 3; mg: vit. $B_1$ 1 000, vit. $B_{12}$ 10, folic acid 1 000, choline chloride 500, Co 200, Si 100; [2] according to NRC (1994); TSAA – total sulphur amino acids

**Table 2.** Concentration of some cinnamon bark oil components

| Compound | Concentration in cinnamon oil, %[1] |
|---|---|
| Cymene | 1.28 |
| Cinnamyl acetate | 2.15 |
| Caryophyllene oxide | 0.33 |
| Cinnamaldehyde | 77.16 |
| Linalool | 3.80 |
| Limonene | 0.31 |
| Benzaldehyde | 0.29 |
| Benzyl benzoate | 0.28 |
| Benzyl alcohol | 0.15 |
| Benzenepropanal | 0.39 |
| Eugenol | 1.86 |
| Myrcene | 0.36 |
| Delta-3-Carene | 0.29 |
| α-Muurolene | 4.30 |
| α-Pinene | 0.45 |
| α-Humulene | 0.25 |
| β-Phellandrene | 0.24 |
| β-Caryophyllene | 1.98 |
| β-Pinene | 0.13 |
| Phenylethyl alcohol | 0.14 |
| Coumarin | 0.46 |
| Hinesol | 0.35 |
| T-cadinol | 2.44 |

[1] determined by gas chromatography/mass spectrometry (GC/GC-MS)

minor components were determined by GC/GC-MS (gas chromatography/mass spectrometry) instruments (Agilent Technologies 6890 series, Wilmington, DE, USA) based on the methodology described by Adams (2017).

The experiment period lasted 42 days (to slaughter age). All birds were reared in controlled environmental conditions (Fan-Pad Evaporative Cooling Systems, Tabreed, Wadi Group Company, Sheikh Zayed, Giza, Egypt) with 23/1 light/dark cycle. The average indoor temperature for the first three days of the experiment was 35 °C; it was slowly lowered to 24 °C until the end of experiment. Standard procedures for management, vaccination, and husbandry were enforced throughout the experimental period. Over the entire experimental period, feed and water were provided *ad libitum*.

## Data and studied traits

**Growth traits.** The body weight (BW) of birds per replicate was recorded (g) on days 1, 14, 28 and 42 of age, and body weight gain (BWG; g/day) was calculated as the difference between two consecutive weights. Feed intake (FI; g/bird/day) was measured as the difference between the residual and offered feed. Feed conversion ratio (FCR; g feed/g weight gain) was determined as the ratio of FI (g) and BWG (g).

**Carcass traits.** On day 42, five birds from each treatment were chosen at random (after being fasted overnight for 12 h), to represent all treatment replicates, weighed and slaughtered according to the Islamic method (slaughtering birds manually by a sharp knife and allowed to bleed freely while they are conscious without stunting or anaesthesia). After complete bleeding, the feathers were plucked, the carcasses eviscerated, and hot carcasses were weighed. Gizzard, liver, heart and lymphoid organs (thymus, bursa of Fabricius and spleen) were individually weighed (g) for each chicken, and total giblet weight (liver + heart + gizzard) and dressing weight (hot carcass weight + total giblets weight) were determined. All the retios of carcass traits and giblets are related to the live body weight at slughter.

## Haematological parameters

Blood sampling was carried out during slaughtering from the five chickens randomly selected from each treatment. Whole blood samples obtained were used immediately to determine haematological parameters in the whole blood. Red blood cells (RBCs; $10^6/mm^3$) and white blood cells (WBCs; $10^3/mm^3$) were counted manually by a haemocytometer (Martand Medical Services, New Delhi, India) based on the method of Campbell et al. (1995). The haemoglobin levels (Hb; g/dl) were determined using the haematin-acid test of Sahli. The volume of the packed cells (PCV; %) was calculated as described by Schalm (1961). To get the serum, blood samples were collected directly into plain serum bottles, kept at room temperature for 10 min to be coagulated, and then centrifuged at 4000 rpm for 10 min; the resulting supernatant was collected. However, to obtain blood plasma, blood samples were collected into heparinized (anticoagulant) tubes (Shanghai IVEN Pharmatech Engineering Co, New Jinqiao Rd, Pudong, Shanghai, China), centrifuged for 10 min at 4000 rpm, and plasma samples were detached, moved to Eppendorf tubes. Until further analyses, serum and plasma samples were preserved in a deep freezer at −20 °C.

## Blood biochemical parameters

Non-enzymatic colorimetric methods have been used to estimate the total protein, albumin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Nevertheless, triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol, urea and uric acid were estimated by enzymatic colorimetric methods. Serum globulin has been measured by the subtraction of serum albumin from total serum protein. The ratio of albumin/globulin (A/G) was determined by dividing serum albumin by serum globulin. Very low-density lipoprotein (VLDL) of the serum cholesterol was measured by dividing triglycerides (TG) by 5. Low-density lipoprotein (LDL) of the serum cholesterol was assessed by subtracting serum HDL plus VLDL cholesterol from total cholesterol (Friedewald et al., 1972). Plasma content of Immunoglobulin G (IgG), Immunoglobulin M (IgM) and Immunoglobulin A (IgA) was estimated using ELISA sandwich kits (Biodiagnostic Company, 29 El-Tahrir St. Dokki, Giza, Egypt) according to a modified version of the method defined by Bianchi et al. (1995). The plasma levels of superoxide dismutase (SOD) were measured by the xanthine oxidase method; which monitors the inhibition of reduction of nitro blue tetrazolium by the sample (Nishikimi et al. (1972), catalase (CAT) determined according to Aebi (1984) by monitoring the initial rate of disappearance of hydrogen peroxide (initial concentration 10 mmol) at 240 nm in a spectrophotometer, the results were reported as rate constant per s per mg tissue (nmol/g tissue), reduced glutathione (GSH) according to Beutler (1963), total antioxidant ability (TAC) according to Ohkawa et al. (1979) and malondialdehyde (MDA) according to Koracevic et al. (2001) were

determined by colorimetric procedures with 2-thiobarbituric acid (2-TBA); monitoring the change of absorbance at 532 nm with the spectrophotometer. Glutathione peroxidase (GP) was measured by a direct spectrophotometric procedure according to Paglia and Valentine (1967). Antioxidants levels were measured by using commercial kits purchased from Biodiagnostic Company (29 El-Tahrir St. Dokki, Giza, Egypt) and a spectrophotometer (Shimadzu UV–VIS Recording 2401, PC, Japan).

### Microbiological analysis and gut health

From 5 chickens randomly selected from each treatment, after slaughtering, 10 g of the caecal content were weighed in a sterile stomacher bag, the sample was well mixed using a stomacher machine (ATSB-400, Athena, Majiwada, Mumbai, India). Samples were transferred separately to a 250 ml Erlenmeyer flask consisting of 90 ml of sterile peptone saline solution (0.1% peptone and 0.85% NaCl; Ready MED, Scieno-Chem LLP, Thane West, Thane, India) as a recovery diluent, well combined, after that up to $10^{-7}$ serial dilutions (if needed) by a mean of 1 ml pipette transferred into two Petri dishes, and then the media were poured. Appropriate dilutions prepared from caecal content samples were used from inoculating different nutrient and selective media. The bacteriological examinations of caecal content samples included total bacterial counts, total coliform, faecal coliform, *Salmonella* spp., toal lactic acid bacteria and *Shigella* spp, total *Listeria* sp. and total *Staphylococcus* ssp. The identification and enumeration procedures were carried out in the laboratory of the Department of Microbiology, Faculty of Agriculture, Ain Shams University, Egypt. Using the Harrigan et al. (1990) procedure, the total bacterial count and the number of lactic acid bacteria were enumerated. For total bacterial count enumeration a ten-fold serial dilution of the bacterial suspension was made. This was done until $10^{-7}$ dilution was achieved. Then, 0.1 ml was pipetted from the $10^{-7}$ dilution onto the surface of each of two Petri dishes containing 15 ml of a solidified and sterile plate count agar (PCA, Oxiod, Basingstoke, Hampshire, UK), and then spread evenly with a sterile glass spreader. The plates were then incubated for a maximum of 24–72 h at 30 °C (including control plates). Total lactic acid bacteria were enumerated on MRS agar (Difco, Fisher Scientific GTF AB, Göteborg, Sweden) by serial dilutions ($10^{-5}$ and $10^{-7}$). Plates were incubated in anaerobic conditions by using the pouring plate technique at 37 °C for 24–72 h. Counting of *Escherichia coli* was performed using Indole, methyl red, Voges-Proskauer (Merck SA, an affiliate of Merck KGaA, Darmstadt, Germany), and citrate reaction biochemical assays. Total coliforms and faecal coliform were estimated on a MaConkey agar (Oxiod, Basingstoke, Hampshire, UK) using pouring plate technique by serial dilution ($10^{-3}$–$10^{-4}$). Plates were incubated aerobically at 37 °C for total coliforms or 44.5 °C for faecal coliform for 24–48 h for coliform and faecal coliform, respectively. *Salmonella* has been detected and enumerated using S.S. agar (Oxide CM 99, Basingstoke, Hampshire, UK), considering that black and pink colonies in this agar are typical settlements (colonies) of *Salmonella* spp. Ileal sample (25 g) was weighed in a sterile stomacher bag or flask, and then 225 ml of buffer peptone water was added, then 1 ml was plated onto XLD plates and incubated at 37 °C for 24 and 48 h. Typical colonies of Salmonella in XLD were red with a black centre. Biochemical reaction (triple sugar iron agar, lysine iron agar, citrate agar and urea agar) (Oxiod, Basingstoke, Hampshire, UK) was used for confirmation of *Salmonella* typical colonies. Yeasts and molds were counted at 28 °C for 72 h using the potato dextrose agar medium (Oxiod, Basingstoke, Hampshire, UK) following the procedure of Kurtzman et al. (2011).

### Statistical analysis

Data were examined for normality using the Shapiro–Wilk test prior to the analysis and all percentages and data on the caecum microbial count were subjected to arcsine and logarithmic transformation, respectively. According to the following statistical model, data were analyzed using one-way analysis of variance:

$$Y_{ij} - \mu + T_i + e_{ij}$$

where: $Y_{ij}$ – observation, $\mu$ – overall mean, $T_i$ – treatment fixed effect $T_1$ to $T_5$, and $e_{ij}$ – residual of the model. Tukey's Studentized Range (HSD) Test checked the significance ($P < 0.05$) of the differences between treatment means. Statistical analyses were performed using software (SAS, Version 9.4. SAS Institute Inc., Cary, NC, 2014).

## Results

### Growth performance

Growth traits (BW, BWG, FI, FCR) of broiler chickens are shown in Table 3. Birds from CO groups had better growth performance traits than control and antibiotic groups regarding BW, BWG and FCR and consumed less feed during different experimental periods.

**Table 3.** Growth traits of broiler chickens in different ages as influenced by dietary supplementation with different cinnamon oil levels

| Trait[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| Body weight, g | | | | | | | |
| day | | | | | | | |
| 1 | 38.50 | 38.58 | 38.58 | 38.33 | 38.54 | 0.433 | 0.9930 |
| 14 | 324.58$^{b}$ | 331.33$^{ab}$ | 340.42$^{a}$ | 332.54$^{ab}$ | 338.71$^{a}$ | 2.901 | 0.0271 |
| 28 | 1112.30$^{c}$ | 1121.06$^{c}$ | 1162.77$^{a}$ | 1162.77$^{a}$ | 1146.25$^{b}$ | 3.004 | <.0001 |
| 42 | 2104.30$^{d}$ | 2139.78$^{c}$ | 2245.53$^{a}$ | 2183.45$^{b}$ | 2145.73$^{c}$ | 4.567 | <.0001 |
| Body weight gain, g/day | | | | | | | |
| days | | | | | | | |
| 1–14 | 20.44$^{b}$ | 20.91$^{ab}$ | 21.56$^{a}$ | 21.01$^{ab}$ | 21.44$^{ab}$ | 0.226 | 0.0441 |
| 14–28 | 56.26$^{b}$ | 56.41$^{b}$ | 58.74$^{a}$ | 59.30$^{a}$ | 57.68$^{ab}$ | 0.312 | 0.0003 |
| 28–42 | 70.86$^{c}$ | 72.77$^{b}$ | 77.34$^{a}$ | 72.91$^{b}$ | 71.39$^{c}$ | 0.250 | <.0001 |
| 1–42 | 49.19$^{d}$ | 50.03$^{c}$ | 52.54$^{a}$ | 51.08$^{b}$ | 50.17$^{c}$ | 0.109 | <.0001 |
| Feed intake, g/day | | | | | | | |
| days | | | | | | | |
| 1–14 | 33.73 | 31.67 | 30.66 | 31.55 | 32.08 | 0.638 | 0.1323 |
| 14–28 | 97.62$^{a}$ | 95.18$^{ab}$ | 92.98$^{b}$ | 97.05$^{ab}$ | 95.06$^{ab}$ | 0.792 | 0.0274 |
| 28–42 | 146.08$^{a}$ | 141.93$^{b}$ | 139.40$^{c}$ | 143.49$^{b}$ | 142.33$^{b}$ | 0.539 | 0.0004 |
| 1–42 | 92.48$^{a}$ | 89.59$^{bc}$ | 87.68$^{c}$ | 90.69$^{ab}$ | 89.82$^{b}$ | 0.380 | 0.0002 |
| Feed conversion ratio, g/g | | | | | | | |
| days | | | | | | | |
| 1–14 | 1.65$^{a}$ | 1.51$^{ab}$ | 1.42$^{b}$ | 1.50$^{b}$ | 1.50$^{b}$ | 0.024 | 0.0036 |
| 14–28 | 1.74$^{a}$ | 1.69$^{ab}$ | 1.58$^{c}$ | 1.64$^{bc}$ | 1.65$^{abc}$ | 0.017 | 0.0030 |
| 28–42 | 2.06$^{a}$ | 1.95$^{b}$ | 1.80$^{c}$ | 1.97$^{b}$ | 1.99$^{b}$ | 0.010 | <.0001 |
| 1–42 | 1.88$^{a}$ | 1.79$^{b}$ | 1.67$^{c}$ | 1.78$^{b}$ | 1.79$^{b}$ | 0.010 | <.0001 |

[1] number of chicks at hatch, 14, 28 and 42 days is 300 (Group feeding, N = 5); SEM – standard error of the mean; $^{abc}$ – means within a row with different superscripts are significantly different at $P < 0.05$

Supplementation of CO significantly increased chicken BW on days 14, 28 and 42 in comparison with control and the antibiotic-treated groups. Moreover, BWG calculated during the different growth intervals was significantly improved as a consequence of CO supplementation. The highest values of BW and BWG were observed in the group fed 500 mg/kg CO. FI and FCR were significantly decreased in response to CO supplementation during all periods, except for FI during the first two weeks of age. The lowset (desired) FCR from hatch to the end of the experiment was observed in the group fed 500 mg/kg CO with the significant difference with the control group. This effect was more pronounced for the 500 mg/kg CO level in comparison with the other CO levels and even the antibiotic treatment.

## Carcass traits

The impact of dietary CO supplementation on carcass traits of broiler chickens relative to control and antibiotic-treated groups is presented in Table 4. All carcass traits (%) did not significantly differ among experimental groups except for gizzard percentage. Treatment with CO at different levels

**Table 4**. Salughter body weight, giblets and carcass traits of broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Trait[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| Body weight at slaughter, g | 2100.50$^{d}$ | 2145.00$^{c}$ | 2275.50$^{a}$ | 2195.50$^{b}$ | 2150.50$^{c}$ | 6.075 | <.0001 |
| Carcass, % | 73.24 | 72.93 | 74.67 | 74.88 | 72.19 | 0.654 | 0.1477 |
| Liver, % | 2.19 | 2.27 | 2.39 | 2.36 | 2.21 | 0.100 | 0.8030 |
| Gizzard, % | 3.40$^{b}$ | 3.71$^{ab}$ | 3.92$^{ab}$ | 4.28$^{a}$ | 3.95$^{ab}$ | 0.128 | 0.0254 |
| Heart, % | 0.51 | 0.54 | 0.51 | 0.55 | 0.49 | 0.016 | 0.1903 |
| Giblets, % | 6.09 | 6.52 | 6.82 | 7.19 | 6.65 | 0.218 | 0.1266 |
| Dressing, % | 79.33 | 79.46 | 81.49 | 82.07 | 78.84 | 0.697 | 0.0647 |

[1] total number of slaughtered chickens was 25 (5 birds/treatment); giblet weight = liver + heart + gizzard; dressing weight = carcass weight+giblets weight; SEM – standard error of the mean; $^{ab}$ – means within a row with different superscripts are significantly different at $P < 0.05$; all the ratios of carcass traits and giblets are related to the live body weight at slaughter; the carcass is included the neck and wingstips

increased gizzard percentage in comparison with the control group. The highest gizzard % was observed in the group fed 1000 mg/kg CO with significant difference with the control group.

## Haematological parameters

No significant differences among experimental groups concerning WBCs and haemoglobin values were noted (Table 5). However, the differences among treatments were significant for RBCs and PCV, since avilamycin supplementation significantly elevated RBCs in comparison with the group supplemented with the highest level of CO (1500 mg/kg), but when compared with the control group, CO supplementations did not significantly affect RBCs. High levels of CO (1000 and 1500 mg/kg) significantly increased PCV in comparison with the low level of CO supplementation (500 mg/kg).

**Table 5.** Haematological parameters of broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Parameter[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| Red blood cells | 2.80$^{ab}$ | 3.00$^{a}$ | 2.79$^{ab}$ | 2.79$^{ab}$ | 2.59$^{b}$ | 0.062 | 0.0261 |
| White blood cells | 26.39 | 28.58 | 28.27 | 27.36 | 29.01 | 1.241 | 0.6426 |
| Haemoglobin | 10.97 | 11.42 | 11.33 | 11.40 | 11.08 | 0.116 | 0.1058 |
| Packed cell volume, % | 35.00$^{ab}$ | 35.09$^{ab}$ | 34.43$^{b}$ | 35.48$^{a}$ | 35.66$^{a}$ | 0.156 | 0.0207 |

[1] total number of whole blood samples was 25 (5 samples/treatment); SEM – standard error of the mean; $^{ab}$ – means within a row with different superscripts are significantly different at $P < 0.05$

## Serum proteins and lipids profile

The effect of diet supplemented with CO on the blood serum content of proteins and lipid profile of broiler chickens is shown in Table 6. Birds fed 1000 and 1500 mg/kg CO were found to have higher blood levels of total protein, albumin and globulin than those in the control group, however, no significant differences were observed between 500 mg/kg CO and control groups. No significant changes were recorded in the albumin/globulin (A/G) ratio in response to different treatments. Regarding lipid profile, the CO supplemented groups had lower TC, TG, LDL and VLDL than the control group. The levels of TC and LDL in all CO groups were also lower than those in the antibiotic group. Furthermore, the group treated with 500 mg/kg CO had the lowest values of TC, TG, LDL and VLDL in comaprison with the other groups, and showed significant decreases versus the control group. Nevertheless, there were no significant differences between all experimental groups in HDL concentrations.

## Liver and kidney function indicators

No significant differences were observed among different treatments concerning serum levels of AST, ALT, creatinine, urea and uric acid (Table 7). However, serum ALP activity was significantly increased in the antibiotic treated group when compared with both groups treated with 500 or 1000 mg/kg CO.

## Immune function measurements

Treatment with CO at different levels increased the relative weight of broiler chicken lymphoid organs (spleen, thymus and bursa of Fabricius) and plasma content of IgM in comparison with control and antibiotic group. However, no effect of dietary CO supplementation on plasma levels of IgG and IgA (Table 8).

**Table 6.** Serum proteins and lipid profile of broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Component[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| Total protein, g/dl | 3.23$^{b}$ | 3.21$^{b}$ | 3.47$^{b}$ | 4.11$^{a}$ | 3.92$^{a}$ | 0.090 | 0.0002 |
| Albumin, g/dl | 1.22$^{b}$ | 1.23$^{b}$ | 1.34$^{ab}$ | 1.62$^{a}$ | 1.48$^{ab}$ | 0.054 | 0.0053 |
| Globulin, g/dl | 2.02$^{b}$ | 1.98$^{b}$ | 2.13$^{b}$ | 2.49$^{a}$ | 2.44$^{a}$ | 0.057 | 0.0007 |
| Albumin/Globulin, % | 0.60 | 0.62 | 0.63 | 0.65 | 0.61 | 0.034 | 0.8937 |
| Total cholesterol, mg/dl | 173.8$^{a}$ | 167.95$^{a}$ | 121.14$^{c}$ | 142.3$^{b}$ | 138.44$^{bc}$ | 5.800 | 0.0005 |
| Triglycerides, mg/dl | 71.50$^{a}$ | 65.08$^{ab}$ | 52.19$^{c}$ | 58.84$^{bc}$ | 65.81$^{ab}$ | 2.945 | 0.0094 |
| HDL, mg/dl | 43.50 | 44.25 | 48.50 | 52.45 | 54.83 | 4.190 | 0.3109 |
| LDL, mg/dl | 116.00$^{a}$ | 110.68$^{a}$ | 62.21$^{c}$ | 78.09$^{b}$ | 70.45$^{bc}$ | 2.187 | <.0001 |
| VLDL, mg/dl | 14.30$^{a}$ | 13.02$^{ab}$ | 10.44$^{b}$ | 11.77$^{ab}$ | 13.16$^{ab}$ | 0.589 | 0.0094 |

[1] total number of blood samples was 25 (5 samples/treatment); HDL – high-density lipoprotein, LDL – low-density lipoprotein, VLDL – very-low-density lipoprotein; SEM – standard error of the mean; $^{abc}$ – means within a row with different superscripts are significantly different at $P < 0.05$

**Table 7.** Liver and kidney function indicators in broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Parameter[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| AST, IU/l | 57.32 | 65.41 | 52.52 | 50.31 | 55.90 | 3.928 | 0.1539 |
| ALT, IU/l | 11.86 | 11.63 | 9.06 | 10.48 | 8.97 | 0.723 | 0.0617 |
| ALP, IU/l | 172.50$^{abc}$ | 209.55$^{a}$ | 146.5$^{c}$ | 152.85$^{bc}$ | 195.35$^{ab}$ | 10.167 | 0.0064 |
| Creatinine, mg/dl | 0.85 | 0.73 | 0.71 | 0.82 | 0.88 | 0.056 | 0.2802 |
| Urea, mg/dl | 13.20 | 11.80 | 11.00 | 10.30 | 12.00 | 0.768 | 0.2572 |
| Uric acid, mg/dl | 7.62 | 7.00 | 6.66 | 7.91 | 8.66 | 0.443 | 0.0900 |

[1] total number of blood samples was 25 (5 samples/treatment), parameters were measured in blood serum; AST – aspartate aminotransferase, ALT – alanine Aminotransferase, ALP – alkaline phosphatase; SEM – standard error of the mean; $^{abc}$ – means within a row with different superscripts are significantly different at $P < 0.05$

**Table 8.** Immune function measurements of broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Measurement | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| Spleen[1], % | 0.11$^{c}$ | 0.14$^{ab}$ | 0.18$^{a}$ | 0.15$^{ab}$ | 0.17$^{ab}$ | 0.008 | 0.0005 |
| Thymus[1], % | 0.25$^{c}$ | 0.30$^{ab}$ | 0.31$^{ab}$ | 0.28$^{bc}$ | 0.34$^{a}$ | 0.010 | 0.0020 |
| Bursa of Fabricius[1], % | 0.12$^{c}$ | 0.14$^{c}$ | 0.19$^{b}$ | 0.25$^{a}$ | 0.13$^{c}$ | 0.005 | <.0001 |
| Plasma IgG content[2], mg/ml | 3.20 | 3.16 | 4.05 | 3.61 | 3.59 | 0.204 | 0.0852 |
| Plasma IgM content[2], mg/ml | 1.50$^{ab}$ | 1.30$^{b}$ | 1.88$^{a}$ | 1.84$^{ab}$ | 2.01$^{a}$ | 0.111 | 0.0068 |
| Plasma IgA content[2], mg/ml | 0.96 | 1.38 | 1.18 | 1.18 | 1.30 | 0.136 | 0.3356 |

[1] total number of slaughtered chickens was 25 (5 birds/treatment); [2] total number of blood samples was 25 (5 samples/treatment); SEM – standard error of the mean; IgG - immunoglobulin G, IgM - immunoglobulin M, IgA - immunoglobulin A; $^{abc}$ – means within a row with different superscripts are significantly different at $P < 0.05$

**Table 9.** Plasma antioxidant parameters of broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Parameter[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| SOD, U/ml | 0.12$^{b}$ | 0.17$^{ab}$ | 0.21$^{a}$ | 0.20$^{ab}$ | 0.23$^{a}$ | 0.016 | 0.0105 |
| CAT, U/ml | 0.15$^{b}$ | 0.33$^{a}$ | 0.36$^{a}$ | 0.35$^{a}$ | 0.20$^{b}$ | 0.026 | 0.0007 |
| GP, U/ml | 0.12$^{b}$ | 0.17$^{ab}$ | 0.19$^{a}$ | 0.18$^{ab}$ | 0.15$^{ab}$ | 0.012 | 0.0203 |
| GSH, ng/dl | 0.17$^{b}$ | 0.25$^{a}$ | 0.29$^{a}$ | 0.26$^{a}$ | 0.23$^{a}$ | 0.012 | 0.0010 |
| TAC, mmol/l | 4.80$^{b}$ | 6.40$^{ab}$ | 6.80$^{a}$ | 6.60$^{ab}$ | 7.40$^{a}$ | 0.371 | 0.0090 |
| MDA, nmol/ml | 6.00$^{a}$ | 4.40$^{b}$ | 3.20$^{bc}$ | 2.80$^{c}$ | 3.40$^{bc}$ | 0.283 | 0.0001 |

[1] total number of blood samples was 25 (5 samples/treatment); SOD – super oxide dismutase, CAT – catalase, GP – glutathione peroxidase, GSH – reduced glutathione, TAC – total antioxidant capacity, MDA – malondialdehyde; SEM – standard error of the mean; $^{abc}$ – means within a row with different superscripts are significantly different at $P < 0.05$

## Antioxidant status

The values of super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), reduced glutathione (GSH) and total antioxidant capacity (TAC) were higher in CO treated groups than in the control group (Table 9). However, GP and GSH in 1500 mg/kg CO group were numerically higher in comparison with the control. The plasma content of MDA in all CO supplemented groups was substantially decreased in comparison with control and antibiotic groups.

## Caecal microbial count

Broiler chickens fed CO exhibited a significant decrease in the caecal total bacterial count (TBC), total yeast and molds count (TYMC), *E. coli*, and *Salmonella* in comparison with the control group.

**Table 10.** Caecum microbial count (*log* CFU/g) of broiler chickens as influenced by dietary supplementation with different cinnamon oil levels

| Caecal microbial count[1] | Control | Avilamycin, mg/kg | Cinnamon oil, mg/kg | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 500 | 1000 | 1500 | | |
| Total bacterial count | 5.59$^{a}$ | 5.21$^{c}$ | 5.24$^{bc}$ | 5.19$^{c}$ | 5.39$^{b}$ | 0.028 | <.0001 |
| Total yeast and molds count | 5.73$^{a}$ | 4.76$^{b}$ | 4.79$^{b}$ | 4.84$^{b}$ | 4.80$^{b}$ | 0.031 | <.0001 |
| *E. coli* | 5.82$^{a}$ | 4.64$^{b}$ | 4.62$^{b}$ | 4.72$^{b}$ | 4.81$^{b}$ | 0.042 | <.0001 |
| Lactic acid bacteria | 3.67$^{b}$ | 2.49$^{d}$ | 2.90$^{c}$ | 3.89$^{a}$ | 3.93$^{a}$ | 0.035 | <.0001 |
| *Salmonella* | 3.29$^{a}$ | 2.35$^{b}$ | 2.41$^{b}$ | 2.34$^{bc}$ | 2.23$^{c}$ | 0.024 | <.0001 |

[1] total number of slaughtered chickens was 25 (5 birds/treatment); SEM – standard error of the mean; $^{abc}$ – means within a row with different superscripts are significantly different at $P < 0.05$

This impact of CO supplementation was similar to the effect of antibiotic supplementation. The count of lactic acid bacteria has been increased linearly in the caecum of chicks fed CO in comaprison with control and antibiotic groups (Table 10).

## Discussion

The observed improvement in growth traits (BW, BWG and FCR) by adding CO (500 mg/kg) into broiler diets may be attributed to the effect of some active components (cinnamaldehyde and eugenol) existing in the cinnamon plant. These substances are considered as digestion stimulating factors that stimulate the secretion of endogenous digestive enzymes, protect the intestinal villi through intercellular antioxidant activity and beneficially affect the absorption of digested nutrients (Jamroz et al., 2005). The results of the present study are in agreement with those of Ahmed et al. (2019) who reported that BWG until 35 days of age was significantly increased when quail chicks were fed diets containing different levels of ginger (0.5 or 1.0 ml/kg diet) or CO (0.5 or 1.0 ml/kg diet) in comparison with chicks receiving dietary antibiotic (0.5 g colistine/kg diet). Mehdipour and Afsharmanesh (2018) observed that adding 200 mg/kg CO into quail feed improved BWG and FCR at day 35 of age in comparison with the control birds. Moreover, Toghyani et al. (2011) stated that the BW in broiler chickens at days 28 and 42 of age was higher in birds fed 2 g cinnamon powder/kg than in control one. Furthermore, Ciftci et al. (2009) observed that broiler chicks fed diet containing 500 mg/kg CO had higher BWG and FCR on day 35 of age than both control and antibiotic-supplemented groups. Contrarily, Symeon et al. (2014) did not detect significant effects of the dietary addition of different CO levels (0.5 or 1.0 ml/kg diet) on the growth performance of broiler chickens.

Except for gizzard percentage, which increased in line with the CO supplementation, all carcass traits were not influenced by the CO treatment. This improvement in gizzard percentages may be attributed to the cinnamon properties that could stimulate the digestive system in broilers, improve the function of the liver and increase the pancreatic digestive enzymes. Enhancement of the metabolism of oil, carbohydrates and proteins in the major organs (liver, heart and gizzard) would increase the growth rate of these organs (Langhout, 2000; Mellor, 2000). Results of the current study are in accordance with those reported by Gomathi et al. (2018) who observed that carcass traits such as relative weights of abdominal fat, liver, gizzard, heart and giblets were not significantly affected by the supplementation of 250 or 500 mg/kg CO into broiler diets. Accordingly, Symeon et al. (2014) reported no significant differences in the percentages of liver, heart and gizzard of broiler chicks due to the dietary supplementation of CO (0.5 or 1 ml/kg). Moreover, Hernández et al. (2004) observed that in broiler chickens that received diets supplemented with 200 mg/kg of CO no significant differences concerning gizzard, pancreas and liver weights at day 21 of age were observed in comparison with the control group. The significant effects on gizzard percentage are in agreement with those of Al-Kassie (2009) who observed that broiler chicks fed supplemented diets with 200 mg/kg of CO or thyme oil had significantly higher relative gizzard weight in comparison with the control group.

Results concerning haematological parameters are in accordance with those of Abo Ghanima et al. (2020) who found that layer chickens fed diet containing 300 mg/kg of CO showed no significant changes in RBCs, WBCs, Hb and PCV in comparison with the non-treated group. Contrarily, Al-Kassie (2009) reported that birds fed diets containing CO (100 or 200 ppm) or thyme essential oil (100 or 200 ppm) had significantly higher RBCs, WBCs, Hb and PCV in comparison with the control group.

The increase of total proteins, albumin and globulin at the serum of broilers fed the higher rates of cinnamon may be attributed to the fact that CO improves digestion and absorption of proteins allowing better use of protein in broiler chicken and thus an improvement of the weight gain (Bento et al., 2013; Krishan and Narang, 2014). The present results concerning serum proteins and lipids profile of broiler chickens are in agreement with those of Khafaji (2018) who observed that total protein and globulin revealed a significant increment in Ceylon cinnamon powder treated groups (500 and 1000 mg/kg), while albumin concentration was not significantly affected in comaprison with the control group. Furthermore, Al-Shuwaili et al. (2015) observed that adding 5% cinnamon powder to the growing turkey diet had no significant effect on plasma total protein. Conversely, Ali et al. (2018) indicated that the addition of 3% cinnamon powder to broiler chick diets significantly decreased plasma total protein, albumin and globulin at day 42 of age. Ahmed et al. (2019) observed that the addition of CO (0.5 or 1.0 ml/kg diet), ginger oil (0.5 or 1.0 ml/kg diet) and their combination (0.5 or 1 ml of the (1:1) mixture of ginger oil and CO/kg diet) into Japanese quail diet significantly decreased serum total protein.

The significant decrease of cholesterol associated with CO supplementation may be due to its inhibition impact on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Elson et al., 1989). This coenzyme has a critical role in the process of cholesterol synthesis (Goldstein and Brown, 1995). Moreover, HDL forms a class of lipoproteins that vary somewhat in size. These lipoproteins carry fatty acids and cholesterol from the body's tissue to the liver. In the present study, the triglyceride level in blood decreased in response to treatment with CO. The dietary supplementation of CO in broiler diets caused a decrease in LDL and triglyceride, and did not reduce the HDL level. Triglycerides are secreted from the liver into the blood by triglyceride-rich lipoproteins; therefore, impaired hepatic lipogenesis results in decreased triglyceride concentrations in plasma (Zhou et al., 2009). In addition, CO contains cinnamic acid and some of its synthetic derivatives impaired the activity of hepatic HMG-CoA reductase and subsequently, the serum cholesterol is reduced (Lee et al. 2007). In layer quail, the effect of supplementing diets with herbal oils (0.2 ml rosemary oil or 0.15 ml dill essential oil or 0.25 ml chicory extract/kg diet) resulted in the reduction of both serum cholesterol and triglycerides (Torki et al., 2018). Moreover, Sarica et al. (2009) observed that dietary supplementation by oregano essential oil plus CO (500 mg oregano oil plus 500 mg CO/kg diet) or mannooligosaccharide (1 g Bio-Mos/kg diet) to quail diets reduced the levels of plasma total cholesterol and triglycerides. Conversely, Ali et al. (2018) reported that dietary supplementation of 3% cinnamon powder into broiler diet did not have any significant effect on plasma total cholesterol and triglycerides at day 42 of age.

Concerning liver and kidney function parameters, the current results are in line with those previously reported by Kanani et al. (2016) who stated that supplementing male broiler diets with 0.5% cinnamon powder did not significantly influence blood AST, urea and creatinine. Contrarily, Ahmed et al. (2019) observed that AST and urea values in growing Japanese quails were significantly lower in CO (0.5 or 1.0 ml/kg diet) and ginger oil (0.5 or 1.0 ml/kg diet) and its combination (0.5 or 1 ml of the (1:1) mixture of ginger oil and CO/kg diet) groups than both the control and antibiotic-supplemented groups. Abo Ghanima et al. (2020) found that plasma levels of ALT, AST, and urea of layer chickens were significantly lower in the CO treated group (300 mg/kg) than in the control group, while creatinine levels were not altered. Moreover, Ali et al. (2018) observed a significant increase in blood AST level for broiler chicks whose diet was supplemented with 3% cinnamon powder, however, the ALT level was not significantly affected.

Relative weight of lymphoid organs may be considered a measure of the state of the immune system; typically, greater weight of these organs is correlated significantly with strong immune functions (Ravis et al., 1988). In birds, bursa of Fabricius, thymus and spleen have important roles in producing antibodies to most avian diseases (Sang-Oh et al., 2013). Furthermore, cinnamon polyphenol extract can affect the immune response of the animal, which administers anti-inflammatory and proinflammatory effects and regulates the expression of the glucose transporter gene in mice macrophages (Cao et al., 2008). In the current study, heavier lymphoid organs were associated with the observed high levels of immunoglobulins. This result is in accordance with those of Sang-Oh et al. (2013) who observed that chicks supplemented with cinnamon powder at levels of 3, 5 and 7% had heavier lymphoid organs and higher levels of plasma IgG, IgM and IgA in comparison with the non-supplemented birds.

In addition, Yang et al. (2019) recorded that diets supplemented with CO at different levels (50, 100, 200, 400 or 800 mg/kg) or mixed with bamboo leaf flavonoids had major impacts on broiler chickens serum IgM content at day 42 of age. However, that study revealed that serum IgG and IgA and relative weights of thymus, spleen and bursa of Fabricius were not significantly influenced. In layer chickens, the dietary inclusion of 300 mg/kg of CO significantly induced the functions of the immune system (Abo Ghanima et al., 2020). Regarding the antioxidant status, desirable statuses were observed as a consequence of CO dietary supplementation. High SOD, CAT, GP, GSH and TAC activities and low MDA levels of birds fed diets supplemented with CO indicate that CO was capable of removing free radicals and could be considered a good natural antioxidant (Reda et al., 2020a). The protective characteristics of essential oils can boost their antioxidant defense mechanism by regulating and inducing the activity of antioxidant enzymes (Hsu and Liu, 2004). Phenolic admixtures of essential oils increase relevantly CAT activity, detoxifying hydrogen peroxide and converting lipid hydroperoxides into non-toxic compounds (Fki et al., 2005). Our results are in line with those of Ahmed et al.

(2019) who reported higher values of TAC, SOD, GP and GSH in growing quails fed diets containing CO (0.5 or 1.0 ml/kg diet), ginger oil (0.5 or 1.0 ml/kg diet) or their combination (0.5 or 1 ml of the (1:1) mixture of ginger oil and CO/kg diet) than in those from control and antibiotic-supplemented groups. In addition, Ciftci et al. (2010) observed significant increases in CAT and GP levels, while MDA levels in broiler chicks fed CO (500 or 1000 ppm) supplemented diets significantly decreased in comparison with control and antibiotic (10 ppm avilamycin) groups. In addition, Yang et al. (2019) reported a significant decrease in liver MDA content at day 21 of age when studying the impact of mixing diets with different levels (50, 100, 200, 400 or 800 mg/kg diet) of CO on MDA and TAC in the blood serum and broiler chicken liver at days 21 and 42 of age. In layer chickens, a significant decrease in the levels of SOD and MDA as a consequence of feeding diets containing 300 mg/kg of CO was observed (Abo Ghanima et al., 2020).

The small intestine is the principal part of the digestive tract in which nutrients are digested and absorbed (Reda et al., 2020a). CO exhibits antimicrobial properties against most intestinal bacteria; limiting the expansion and colonization of numerous pathogenic and non-pathogenic species in the digestive tract (Dorman and Deans, 2000). Many studies have reported that these active substances extracted from aromatic and medicinal plants may modify the structure of cellular membranes of the entero pathogenic strains leading to ion leakage from the cell and finally lead to pathogen damage (Tiwari et al., 2018). Moreover, these characteristics can be mainly due to their phenolic substances that affect bacterial cells negatively (Peñalver et al., 2005). These substances also demonstrate notable antimicrobial and antifungal properties (Basílico and Basílico, 1999). The main phenolic component of cinnamon plants is cinnamaldehyde which has antibacterial properties. Cinnamaldehyde is known to cause disintegration of the bacterial cell membrane, the release of cell contents, break through the cell membrane to facilitate degradation of the enzyme system, reduce intracellular pH causing adenosine triphosphate reduction (Alagawany et al., 2017) and cause damage to the integrity of the membrane, affecting pH homeostasis and inorganic equilibrium (Oussalah et al., 2006). Cinnamaldehyde, eugenol, and carvacrol antimicrobial activity has been reported because of their preventive effects on pathogenic microorganisms (Hernández et al., 2004). Tiihonen et al. (2010) attributed the desirable effects of CO on reducing the expansion of *E. coli* populations due to its ability to destroy the bacterial cellular membrane. In addition, essential oils promote mucus secretion inside the digestive tract that decreases the adhesion of pathogenic bacteria to the gut epithelium (Jamroz et al., 2006). Our findings are in line with those reported by Ahmed et al. (2019) who observed an increase in the total number of lactic acid bacteria (useful bacteria) and a decrease in other caecum strains of bacteria, fungi, yeast and salmonella as a result of raising the CO concentration from 0.5 to 1 ml/kg diet in growing quail in comparison with control and antibiotic-treated groups. Chowdhury et al. (2018) found that the addition of CO at 300 ml/kg diet level decreased the amount of *E. coli* in broiler chicks pre-caecal content. Moreover, Jamroz et al. (2005) demonstrated that the use of a mixture of cinnamaldehyde, carvacrol and capsaicin extract (100 mg/kg diet) increased the total lactobacillus count and decreased the total *E. coli* count in broiler chicken intestinal tract.

## Conclusions

The supplementation of broiler diets with cinnamon oil (CO) has had beneficial effects on growth and carcass characteristics, immune function, antioxidant status and caecal microbial count. So, CO could be used as a natural alternative feed additive to promote gut health and consequently improve growth performance of broiler chickens instead of antibiotic growth promoters.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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## Ethical approval

This study was conducted according to the suggestions and guidelines of the advisory committee on the ethics of animal experiments, Poultry Department, Zagazig University, Egypt. The laboratory analyses, experiments and protocols were handled in accordance with the ethical standards as stated in the guidelines represented by the Committee of Animal Care and Welfare, Benha University, Egypt; ethical approval No. 2020-4.

## Data availability statement

Data used and analyzed are available from the corresponding author upon reasonable request.

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