JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.2024006

Effects of Supplemented Coriander, Ajwain, and Dill Seed Essential Oils on Growth Performance, Carcass Characteristics, Gut Health, Meat Quality, and Immune Status in Broilers

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Numerous studies have assessed the benefits and optimal dosage of supplementation with essential oils (EOs), including extracts from plants of the *Apiaceae* family, as an alternative to antibiotic growth promoters (AGPs) in broilers. However, little consideration has been given to the actual chemical composition of the evaluated EOs when drawing critical conclusions, even though EO composition can vary with different extraction conditions and plant characteristics. Therefore, the present study was conducted to evaluate the effects of EOs from seeds of plants of the *Apiaceae* family: coriander (CEO), ajwain (AjEO), and dill (DEO), containing 56.8% linalool, 68.2% thymol, and 41.1% carvone, respectively, on the growth performance, gut health, and immune status of broilers. In total, 660 one-day-old broiler chicks were divided into 11 experimental diet groups and fed for 35 days with either the control diet, basal diet with added AGP (lincomycin, 500 mg/kg), or one of nine EO diets supplemented with CEO, AjEO, or DEO at 200, 400, and 600 mg/kg. Final body weights were improved by supplementation with not only AGP but also any EO except AjEO at 600 mg/kg; within each EO, supplementation of CEO at 400 mg/kg, AjEO at 200 mg/kg, and DEO at 200 mg/kg afforded the best growth performance. EO supplementation had beneficial effects on gut morphology, such as increased villus height in the duodenum, jejunum, and ileum, and against harmful microbiota, such as reduction of *Escherichia coli* and *Salmonella* spp. populations. Furthermore, EOs enhanced humoral immunity and improved meat quality by reducing drip loss, likely consequent to their antioxidant properties. Overall, this study presents evidence that CEO, AjEO, and DEO can each play a pivotal role in replacing AGPs, as well as providing information regarding optimal doses for broilers.

Key words: ajwain, coriander, dill, essential oil, growth performance, gut health

J. Poult. Sci., 61: jpsa.2024006, 2024

Introduction

Dietary subtherapeutic antibiotics play a significant role in improving the growth performance and gut health of broilers[1]. However, this practice was discouraged due to emergence of antibiotic resistance in birds and further transfer of antibiotic resistance genes to human. The European Union and China have banned the use of antibiotic growth promoters (AGPs) since 2006 and 2020, respectively, with numerous other countries considering similar measures, as human health can be directly affected by AGP residues in meat[2,3]. Consequently, broiler production faces significant challenges including decreased growth performance and increased disease prevalence, potentially leading to

Received: November 6, 2023, Accepted: January 15, 2024
Available online: February 23, 2024
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substantial economic losses. This necessitates the identification of a suitable replacement for AGPs that offers equivalent benefits without any harmful effects[4].

Alternatives to reduce and/or eliminate AGPs include prebiotics, probiotics, organic acids, antimicrobial peptides, bacteriophages, enzymes, and phytogenics such as essential oils (EOs) [5,6]. EOs have received considerable attention because they are natural, readily available, nontoxic, cost-effective, and residue-free. Notably, various EOs exhibit antimicrobial, antioxidant, anti-inflammatory, anti-stress, and growth-promoting effects in chickens[7]. EOs can alleviate oxidative stress through several mechanisms, including direct antioxidant action and expression of antioxidant enzymes[8]. Therefore, EOs with antimicrobial and antioxidant properties could be used to support broiler gut health and growth performance as possible replacements for AGPs.

EOs extracted from the seeds of plants belonging the *Apiaceae* family, such as coriander (*Coriandrum sativum*; CEO), ajwain (*Trachyspermum ammi*; AjEO), and dill (*Anethum graveolens*; DEO), exhibit antibacterial and antioxidant potential *in vitro* and are commonly available in South Asian countries[7]. Moreover, we previously demonstrated that these EOs exert considerable antibacterial effects against *Escherichia coli*, *Salmonella enteritidis*, and *Salmonella gallinarum* of poultry origin and have antioxidant properties[9]. In addition, several *in vivo* studies indicate the beneficial effects of CEO[10] and AjEO[11–13] on the growth performance and gut health of broilers; however, to our knowledge, no information is yet available regarding the effects of DEO on broiler production.

The bioactive compounds in an EO and their respective concentrations are key factors that determine its efficacy as an alternative to AGPs in broiler production. However, none of the *in vivo* studies reported the actual measured values of the chemical components of CEO or AjEO employed in their experiments, even though the qualitative and quantitative composition of these EOs likely differs owing to differences in the extraction conditions[14], genotype, climatic conditions, mode of reproduction, and morphological characteristics of the plant[15–18]. The bioactive compounds in an EO and their respective concentrations are the key factors to determine its efficacy as an alternative to AGPs in broiler production. Therefore, the present study was conducted using CEO, AjEO, and DEO with actual chemical composition to robustly establish the viability of each EO from the *Apiaceae* family as a substitute for AGPs, and to determine the optimal dosage for growth performance, intestinal health, and disease prevention in broilers.

Materials and Methods

Ethical approval

This study was conducted in accordance with the guidelines of the Organization of Research, Innovation and Commercialization, University of Veterinary and Animal Sciences, Lahore, Pakistan. All procedures involving birds were approved by this committee (No. DR/168).

Birds, experimental design, and dietary treatments

In total, 660 one-day-old broiler chicks (Cobb 500), with an average body weight of 42.5 g, were obtained from a commercial hatchery and randomly divided into 11 dietary groups. Each group was replicated in six pens with 10 chicks per pen. The experimental diets consisted of: 1) a basal diet having no antibiotic or EOs (control; CON); 2) the basal diet supplemented with AGP (lincomycin 500 mg/kg); 3–5) basal diet supplemented with three concentrations of CEO (200, 400, and 600 mg/kg); 6–8) basal diet supplemented with three concentrations of AjEO (200, 400, and 600 mg/kg); and 9–11) basal diet supplemented with three concentrations of DEO (200, 400, and 600 mg/kg). The EOs were extracted from the seeds of coriander, ajwain, and dill by hydro-distillation technique, and their bioactive constituents were identified using gas chromatography-mass spectrometry, as already described in our previous report[9], before using in current study. The major bioactive compounds in CEO, AjEO, and DEO were linalool (56.8%), thymol (68.2%), and carvone (41.1%) and limonene (19.9%), respectively (Fig. 1). The starter (1–14 d) and grower (15–35 d) basal diets were formulated to meet or exceed the nutrient requirements of the broilers, as shown in Table 1. The EOs were mixed with the basal diet via a post-pelleting spray. The prepared diets were packed and stored in polyethylene bags. Experimental diets and water were provided *ad libitum* throughout the experimental period. The birds were housed in floor pens (1.1 × 0.76 m) on fresh rice husks. All birds were vaccinated against Newcastle disease virus (NDV) at the 3rd and 19th day of age, infectious bronchitis (IBV) at the 3rd day of age, and infectious bursal disease at the 13th day of age.

Growth performance

Body weight (BW) of the birds was recorded weekly to calculate the average daily gain (ADG; g/d). Feed consumption was recorded to calculate average daily feed intake (ADFI; g/d). The feed conversion ratio (FCR) was calculated using feed intake and weight gain data.

Sample collection and carcass characteristics

Following experimental completion at 35 d, six birds per group with a near-average BW were randomly chosen and slaughtered by severing the jugular vein. The slaughtered birds were used for sample collection and carcass evaluation. Portions (approximately 2 to 3 cm) from the middle of duodenum, jejunum, and ileum were fixed in 10% buffered formaldehyde solution for morphological evaluation. The breast, leg quarters, gizzard, heart, liver, abdominal fat, spleen, bursa, and thymus were separated and individually weighed. Breast muscle samples were collected to evaluate the meat quality.

Ileal microbiota count

Abdominal cavities of the slaughtered birds were opened and the pre-cecal contents were collected under aseptic conditions in sterile plastic bags. Plastic bags containing ileal digesta samples were immediately placed in ice containers and transported to the laboratory for enumeration of *E. coli, Salmonella*, and *Lactobacillus* spp. populations. Samples were processed on the same day for bacteriological enumeration. Briefly, 1 g of ileal digesta was

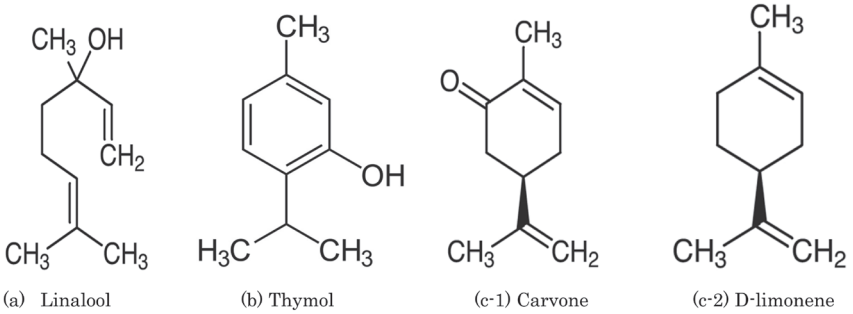

Fig. 1. **Chemical structures of the main bioactive compounds identified in coriander, ajwain, and dill seed essential oils.** (a) Linalool (56.8%) in CEO; (b) Thymol (68.2%) in AjEO; (c-1) Carvone (41.1%) and (c-2) Limonene (19.9%) in DEO. CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil.

serially diluted 10-fold with normal saline. An aliquot (10 µL) from each of the 10^{-3} to 10^{-5} dilutions was streaked on McConkey (for *E. coli*), Salmonella Shigella (for *Salmonella* spp.), and Lactobacilli agar (for *Lactobacillus* spp.). The bacterial colonies per gram of each sample were enumerated using a colony counter; the numbers are expressed as $\log_{10}$ colony-forming units (CFU) per gram[19].

Morphometric analysis

Villus height (VH), measured from the top of the villus to the villus-crypt junction, and crypt depth (CD), measured from the junction to the base of the crypt, in addition to the villus height-to-crypt depth ratio (VH:CD), were assessed to evaluate the duodenal, jejunal, and ileal morphology indicative of gut health in broilers, as suggested by Gopinger et al.[20]. Briefly, 1 cm cross sectional lengths of the intestinal samples were embedded in paraffin wax. Sections (5 µm thick) were cut using a microtome and then stained with hematoxylin–eosin. The VH and CD from six randomly selected villi and associated crypts in one section per chicken were measured. All measurements were performed using a microscope (Labomed T121100, Los Angeles, CA, USA). Images were captured using a digital camera (Euromex D C. 12355 F050, Berchem, Belgium), then calibrated using Labomed PixelPro™ software.

Meat quality parameters

The breast muscle samples were used to calculate the pH, color, drip loss, and thiobarbituric acid reactive substance (TBARS) values[21,22]. The pH of the breast muscles was measured using a pH meter with a meat-penetrating probe (WTW, pH 3210 SET2, Weilheim, Germany). The probe was calibrated with buffers at pH 4.00 and 7.00 prior to sample measurement. Color determination was conducted using the Minolta Colorimeter (Konica Minolta® CR-410, Osaka, Japan). Colors L* (lightness), a* (redness), and b* (yellowness) were recorded directly from the exposed surface of the breast fillets. A standard Minolta calibration plate was used to calibrate the chromatograph before testing. Drip loss of breast muscles was determined by weighing the samples before and after storage at 3 ± 1°C in a commercial retail cooler. The samples were stored in plastic Ziploc bags for 24 h. The samples were blotted with paper towels to remove excess moisture before weighing.

For TBARS measurement, breast muscle samples (2 g) were placed in 50 mL falcon tubes containing 50 µL 7.2% butylated hydroxytoluene along with 15 mL distilled water and then homogenized. Subsequently, 1 mL of the homogenate was transferred to a test tube containing 2 mL thiobarbituric acid/trichloroacetic acid solution and vortexed. The tubes were then incubated in a boiling water bath at 90°C for 15 min, cooled to 25°C, vortexed, and centrifuged at 2000 rpm (Neofuge, 15R, Heal Force, Shanghai, China) for 15 min at 4°C. The absorbance of the resulting supernatant was measured at 531 nm using a spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan) to calculate the TBARS. The results are expressed as mg of malondialdehyde (MDA) per kg of meat[23].

Humoral immunity and serum biochemical indices

Blood samples were collected from the wing vein of birds before slaughtering and serum was separated immediately by centrifugation at 1000 × *g* for 10 min at 4°C, and stored at −30°C until further processing. Serum samples were used to determine the immune response of birds by analyzing antibody titers against NDV and IBV using hemagglutination (HA) and hemagglutination-inhibition (HI) tests and enzyme-linked immunosorbent assay, respectively[24]. Serum total protein, glucose, triglyceride, and cholesterol levels were calorimetrically quantified using commercial kits (Merck Specialties Pvt., Ltd., Darmstadt, Germany).

Statistical analysis

Data were analyzed by one-way analysis of variance using JMP Pro 16 (SAS Institute, Cary, NC, USA). When significant ($P \leq 0.05$) treatment effects were observed, the means were separated using Tukey's honestly significant difference test.

Results

Growth performance

Table 2 shows the effects of CEO, AjEO, and DEO supple-

Table 1. **Ingredients (%) and nutrient composition of basal diets**

Ingredient (%)	Starter	Grower
Maize	57.8	57.8
Soybean meal	31.2	28.9
Canola meal	7.7	8.0
Dicalcium phosphate	1.0	1.0
Limestone	1.0	1.0
Lysine sulfate	0.4	0.37
DL methionine	0.3	0.3
L-threonine	0.1	0.1
L-valine	0.02	0.03
Minerals premix[a]	0.05	0.05
Vitamins premix[b]	0.03	0.03
Soda bicarbonate	0.1	0.1
Vegetable oil	-	2
Salt	0.3	0.3
Phytase[c]	0.01	0.01
Axtra enzyme[d]	0.01	0.01
Calculated nutrient composition (% as fed-basis)		
Metabolizable energy (kcal/kg)	2975	3100
Crude protein	22.0	20.5
Crude fat	2.85	4.75
Crude fiber	3.5	3.5
Calcium	0.9	0.84
Total phosphorus	0.79	0.77
Available phosphorus	0.45	0.4
Lysine	1.22	1.1
Methionine	0.46	0.43
Met+Cys	0.91	0.80
Threonine	0.83	0.73
Tryptophan	0.2	0.18
Arginine	1.28	1.15
Valine	0.89	0.8
Isoleucine	0.77	0.71
Analyzed nutrient composition (%)		
Crude protein	22.2	21.3
Ether extract	2.62	4.63
Crude fiber	3.41	3.43
Total ash	4.93	4.82
Calcium	0.9	0.86
Phosphorus	0.79	0.79

[a]80 mg Fe, 12 mg Cu, 85 mg Mn, 60 mg Zn, 0.8 mg I, 0.1 mg Se, 125 mg anti-oxidant mixture.
[b]12,000 IE retinol, 2,400 IE cholecalciferol, 50 mg DL-alpha-tocopherol, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine, 20 μg cyanocobalamin, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, and 0.2 mg biotin. [c]Ronozyme® HiPhos GT (Phytase 10,000 FTU/g); DSM Nutritional Products Ltd., Switzerland. [d]Axtra® XAP (endo-1,4-beta-xylanase, 20,000 U/g; alpha-amylase 2000 U/g; protease 40,000 U/g); Danisco Animal Nutrition/DuPont, Wilshire, UK.

mentation on the final BW, ADG, ADFI, and FCR of broiler chickens. Birds fed diets supplemented with AGP or any EO, except AjEO (600 mg/kg), were heavier ($P < 0.05$) than the control group, with CEO (400 mg) supplementation resulting in better performance than AGP supplementation. CEO (400 mg/kg) and AjEO (200 mg/kg) supplementation resulted in the highest final body weights ($P < 0.05$) in the CEO and AjEO groups. Supplementation with AGP or EOs affected ADG to different degrees, depending on the growth phase (starter: 1–14 d, early grower: 15–18 d, late grower: 29–35 d). In the starter phase, the ADG of birds receiving AGP or EO supplementation was higher ($P < 0.05$) than that for birds in the control group; in turn, the ADG ($P < 0.05$) from DEO (200 mg/kg) supplementation was higher than that from the AGP diet. Among the AjEO and DEO groups, AjEO (200 mg/kg) and DEO (200 mg/kg) supplementation yielded the highest ADG ($P < 0.05$). In the early grower phase, the ADG of birds supplemented with AGP or any EO, except for AjEO (400 or 600 mg/kg) and DEO (400 or 600 mg/kg), was higher ($P < 0.05$) than that of birds in the control group. In the late grower phase, the positive effects of AGP or EO supplementation disappeared, except in the CEO (400 mg/kg) group, in which the ADG remained higher than that of the control group.

In contrast, regardless of the growth phase, ADFI did not differ between the AGP or EO groups and the control group; moreover, EO concentration did not affect the within-group findings. Alternatively, responses of FCR to AGP or EO supplementation in all growth phases almost paralleled those of ADG. In the starter phase, the FCR of birds with AGP or any EO supplementation was improved ($P < 0.05$) in comparison with that in birds fed the CON diet, with DEO (200 mg/kg) and AjEO (200 mg/kg) supplementation affording better FCR ($P < 0.05$) than that from the AGP diet. In the early grower phase, the FCR of birds with AGP or any EO supplementation, except for AjEO (400 or 600 mg/kg) and DEO (400 or 600 mg/kg), was improved ($P < 0.05$) compared with that of birds in the control group. In the late grower phase, the positive effects of AGP or EO supplementation disappeared, except in the CEO (400 mg/kg), AjEO (200 mg/kg), and DEO (200 mg/kg and 600 mg/kg) groups, which maintained a better FCR than that of the control group.

Carcass characteristics

The birds fed diets supplemented with AGP, or any EO except AjEO (600 mg/kg), were heavier ($P < 0.05$) at slaughter than birds fed the CON diet; however, the gizzard, liver, and abdominal fat weights did not differ ($P > 0.05$) between the various groups (Table 3). The breast weights of birds fed AGP were not significantly different from those of birds fed the CON diet. However, dietary supplementation with CEO (400 and 600 mg/kg), AjEO (200, 400, and 600 mg/kg), and DEO (200, 400, and 600 mg/kg) produced heavier breasts ($P < 0.05$) than those of birds fed the CON diet. Leg quarter weights did not differ between either the AGP or EO group and the control group. Heart weights were not significantly different between the AGP and control groups; however, supplementation with AjEO (200 and 400 mg/kg) and DEO (200 mg/kg) resulted in heavier hearts ($P <$

Table 2. **Effect of coriander, ajwain, and dill seed essential oil supplementation on final body weight, average daily gain, average daily feed intake, and feed conversion ratio of broiler chickens from 1 to 35 days of age**

Effect	Supplement (mg/kg) in diet											SEM	*P*-value
	CON	AGP	CEO (mg/kg)			AjEO (mg/kg)			DEO (mg/kg)				
			200	400	600	200	400	600	200	400	600		
Final BW (g)	1967^{d}	2153^{bc}	2167^{bc}	2299^{a}	2139^{bc}	2231^{ab}	2104^{c}	2068^{cd}	2244^{ab}	2079^{c}	2151^{bc}	16.4	<0.001
ADG (g/d)													
d 1 to 14	30.8^{e}	33.6^{bcd}	34.1^{abcd}	35.2^{abc}	33.1^{d}	35.4^{ab}	33.1^{cd}	33.2^{cd}	36.0^{a}	33.3^{cd}	33.6^{bcd}	0.25	<0.001
d 15 to 28	64.0^{e}	70.9^{abcd}	70.3^{abcd}	74.7^{a}	72.1^{abc}	73.3^{ab}	69.6^{abcde}	65.6^{de}	71.9^{abc}	67.3^{cde}	68.6^{bcde}	0.68	<0.001
d 29 to 35	73.2^{b}	80.6^{ab}	82.4^{ab}	90.6^{a}	77.2^{ab}	83.1^{ab}	76.9^{ab}	79.6^{ab}	86.7^{ab}	77.8^{ab}	84.9^{ab}	1.58	0.033
ADFI (g/d)													
d 1 to 14	43.7^{ab}	44.0^{ab}	44.0^{ab}	43.0^{b}	45.4^{a}	43.0^{ab}	42.9^{b}	43.4^{b}	43.6^{ab}	42.8^{b}	44.6^{ab}	0.15	<0.001
d 15 to 28	110.4	110.6	111.2	110.1	110.9	110.3	111.4	110.8	110.6	108.8	112.6	0.22	0.115
d 29 to 35	177.4^{ab}	179.4^{ab}	200.0^{a}	177.5^{ab}	190.5^{ab}	172.0^{b}	182.5^{ab}	175.4^{b}	180.2^{ab}	175.5^{b}	181.2^{ab}	1.71	0.021
FCR (FI/BW)													
d 1 to 14	1.42^{a}	1.31^{bc}	1.29^{bcd}	1.22^{cd}	1.38^{ab}	1.21^{d}	1.30^{bcd}	1.31^{bc}	1.21^{d}	1.29^{bcd}	1.33^{ab}	0.01	<0.001
d 15 to 28	1.73^{a}	1.56^{cde}	1.58^{bcde}	1.48^{e}	1.54^{cde}	1.51^{de}	1.61^{abcd}	1.69^{ab}	1.54^{cde}	1.62^{abcd}	1.64^{abc}	0.01	<0.001
d 29 to 35	2.44^{a}	2.23^{abcd}	2.42^{ab}	1.99^{d}	2.51^{a}	2.10^{cd}	2.40^{abc}	2.23^{abcd}	2.09^{d}	2.29^{abcd}	2.14^{bcd}	0.04	<0.018

CON, control; AGP, antibiotic growth promoter; CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
Values are expressed as means with pooled SEM values, n=6.
^{a,b,c,d,e}: Means sharing the same superscript in a row do not statistically significantly differ ($P > 0.05$).

0.05) than those of birds fed the CON diet.

Intestinal microbial population

Figure 2 shows the effects of CEO, AjEO, and DEO supplementation on the ileal microbiota, including *E. coli*, *Salmonella*, and *Lactobacillus* spp. The counts of *E. coli* in the ileum were significantly decreased ($P < 0.05$) by supplementation with AGP and all EOs, with all EO supplementation conditions except CEO (200 mg/kg) and DEO (200 mg/kg) yielding significantly lower counts ($P < 0.05$) than those following AGP supplementation. The *Salmonella* spp. counts were similar to those for *E. coli*. Supplementation with AGP and all EOs significantly decreased ($P < 0.05$) the *Salmonella* spp. count, with AjEO (400 and 600 mg/kg) and DEO (400 and 600 mg/kg) supplementation also affording lower counts ($P < 0.05$) than those following AGP supplementation. In contrast, supplementation with AGP or any EO did not alter ($P > 0.05$) the *Lactobacillus* spp. counts.

Intestinal morphology

Figure 3 shows the effects of CEO, AjEO, and DEO supplementation on VH, CD, and VH:CD in the duodenum, jejunum, and ileum in the small intestine of broilers. The duodenum exhibited increased VH for birds fed diets supplemented with AGP and all EOs ($P < 0.05$), except CEO (600 mg/kg) and AjEO (600 mg/kg), in comparison to that for birds fed the CON diet; however, the CD did not differ between either the AGP or EO group and the control group, and was not affected by the EO concentration within each group (Fig. 3a). VH:CD was significantly increased by CEO (200 and 400 mg/kg), AjEO (400 mg/kg), and DEO (400 mg/kg) supplementation ($P < 0.05$) but not by supplementation with AGP.

The jejunum showed increased VH for birds fed diets supplemented with any EO ($P < 0.05$), except CEO (600 mg/kg) and DEO (400 and 600 mg/kg), but not with AGP, compared to that for birds fed the CON diet; however, CD remained unaffected by any treatment ($P > 0.05$) (Fig. 3b). VH:CD increased ($P < 0.05$) in the CEO (200 and 400 mg/kg) groups compared to that in the control group. Increased VH was observed in the ileum only following CEO (200 mg/kg) and DEO (400 mg/kg) supplementation ($P < 0.05$) in comparison to that for birds fed the CON diet; the AGP diet had no effect. CD and VH:CD ratios remained unaffected (Fig. 3c).

Immune organ indices and humoral immunity

Table 4 shows the effects of CEO, AjEO, and DEO supplementation on immune organs such as the spleen, thymus, and bursa, as well as antibody titers against NDV and IBV. The spleen, thymus, and bursa indices, expressed as percentages of slaughter BW, were not affected by supplementation with AGP or any EO ($P > 0.05$), except for DEO (400 mg/kg) supplementation, in which the bursa index was higher ($P < 0.05$) than that in the CEO (600 mg/kg) group. In contrast, the antibody titer against NDV was not altered by AGP supplementation but was increased by CEO (600 mg/kg), AjEO (400 and 600 mg/kg), and DEO (600 mg/kg) ($P < 0.05$). Similarly, the antibody titers against IBV were also increased by EO supplementation: CEO (600 mg/kg), AjEO (600 mg/kg), and DEO (200, 400, and 600 mg/kg) ($P < 0.05$), but not by AGP supplementation. CEO (600 mg/kg) yielded the highest antibody titer within the CEO groups.

Meat quality

Table 5 shows the effects of CEO, AjEO, and DEO supple-

Table 3. **Effect of coriander, ajwain, and dill seed essential oil supplementation on slaughter body weight and carcass characteristics of broiler chickens from 1 to 35 days of age**

Parameter	CON	AGP	Supplement (mg/kg) in diet									SEM	*P*-value
			CEO (mg/kg)			AjEO (mg/kg)			DEO (mg/kg)				
			200	400	600	200	400	600	200	400	600		
SBW (g)	1923^{d}	2179^{abc}	2159^{abc}	2293^{a}	2135^{bc}	2209^{ab}	2154^{abc}	2034^{cd}	2236^{ab}	2105^{bc}	2183^{ab}	21.2	0.016
Carcass characteristics													
Breast (g)	356^{c}	410^{bc}	461^{abc}	563^{a}	498^{ab}	520^{ab}	535^{ab}	526^{ab}	548^{a}	540^{a}	508^{ab}	10.6	<0.001
(% SBW)	(18.6)	(18.9)	(21.4)	(24.6)	(23.4)	(23.5)	(24.8)	(25.8)	(24.6)	(25.7)	(23.3)		
Leg quarter (g)	263^{ab}	321^{a}	261^{b}	278^{ab}	244^{b}	274^{ab}	270^{ab}	262^{b}	267^{ab}	241^{b}	257^{b}	4.2	0.004
(% SBW)	(13.75)	(14.73)	(12.07)	(12.12)	(11.45)	(12.43)	(12.57)	(12.92)	(11.97)	(11.45)	(11.75)		
Gizzard (g)	30.0	31.4	32.0	32.1	30.0	33.9	32.3	39.1	32.4	33.2	33.0	0.61	0.104
(% SBW)	(1.57)	(1.44)	(1.48)	(1.40)	(1.40)	(1.54)	(1.50)	(1.93)	(1.45)	(1.58)	(1.52)		
Heart (g)	9.77^{b}	12.3^{ab}	12.8^{ab}	12.6^{ab}	11.6^{ab}	14.5^{a}	13.1^{a}	11.8^{ab}	13.5^{a}	12.6^{ab}	12.3^{ab}	0.23	0.002
(% SBW)	(0.51)	(0.57)	(0.59)	(0.55)	(0.54)	(0.66)	(0.61)	(0.58)	(0.6)	(0.6)	(0.57)		
Liver (g)	56.2	58.9	60.6	51.0	52.3	61.2	58.6	55.4	57.2	56.7	53.0	0.92	0.290
(% SBW)	(2.93)	(2.71)	(2.81)	(2.23)	(2.46)	(2.78)	(2.73)	(2.73)	(2.57)	(2.7)	(2.43)		
Abdominal fat (g)	37.2	36.6	41.7	37.4	38.3	39.5	37.4	42.6	41.2	36.2	35.7	0.95	0.869
(% SBW)	(1.93)	(1.68)	(1.93)	(1.63)	(1.81)	(1.79)	(1.73)	(2.09)	(1.84)	(1.72)	(1.63)		

CON, control; AGP, antibiotic growth promoter; CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil; SBW, slaughter body weight; (% SBW), percentage of slaughterhouse body weight. Values are expressed as means with pooled SEM values, n=6.
[a,b,c,d]: Means sharing the same superscript in a row do not statistically significantly differ ($P > 0.05$).

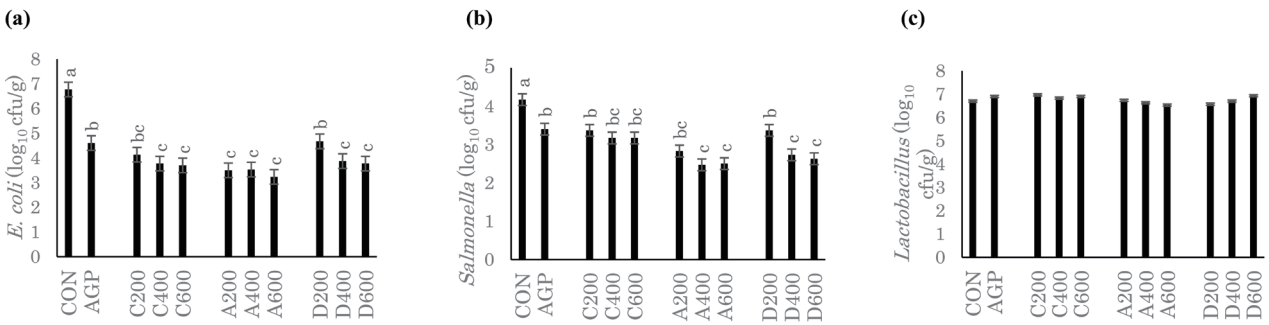

Fig. 2. **Effect of dietary supplementation with CEO, AjEO, and DEO on viable bacterial counts (log_{10} CFU/g) in the ileal digesta of broiler chickens.** (a) *Escherichia coli* population (log_{10} CFU/g), (b) *Salmonella* population (log_{10} CFU/g), and (c) *Lactobacillus* population (log_{10} CFU/g). CON, basal diet; AGP, lincomycin (500 mg/kg); C200, C400, and C600, CEO at 200, 400, and 600 mg/kg, respectively; A200, A400, and A600, AjEO at 200, 400, and 600 mg/kg, respectively; D200, D400, and D600, DEO at 200, 400, and 600 mg/kg, respectively. CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil; CFU/g, colony forming units per gram. One-way analysis of variance *E. coli* population: SEM = 0.12, $P < 0.001$; *Salmonella* population: SEM = 0.07, $P < 0.001$; *Lactobacillus* population: SEM = 0.05, $P = 0.632$. Tukey's honestly significant difference test [a,b,c]: Different letters show significant differences among treatments ($P < 0.05$). Values are the means ± standard error of the mean (n = 6).

mentation on the quality of breast muscles including pH, color (L*, a* and b* values), drip loss, and TBARS of broiler chickens. The pH and color (L*, a* and b*) of breast meat were not changed ($P > 0.05$) by supplementation with AGP or any EO. Drip loss was not affected by AGP supplementation but was significantly reduced ($P < 0.05$) by supplementation with CEO (600 mg/kg), AjEO (200 and 400 mg/kg), and DEO (200 and 600 mg/kg) than that of the CON group. CEO (600 mg/kg) supplementation caused the lowest drip loss within the CEO groups. EO (but not AGP) supplementation also improved the antioxidant capacity in breast muscles: dietary EO supplementation with CEO (400 and 600 mg/kg), AjEO (200, 400, and 600 mg/kg), and DEO (200 and 600 mg/kg) afforded decreased ($P < 0.05$) TBARS values. To clarify whether the drip loss of breast muscle is associ-

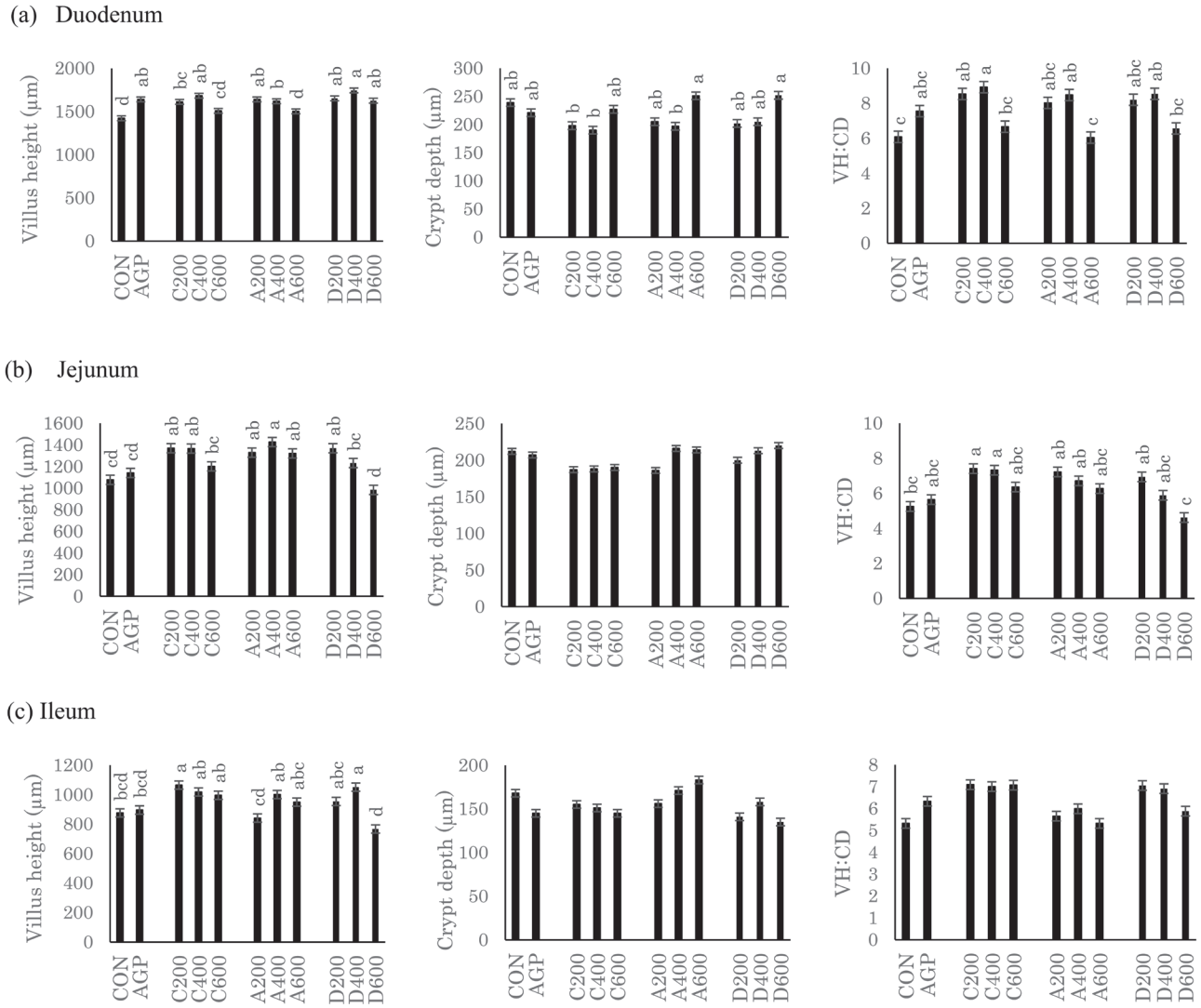

Fig. 3. **Effect of dietary supplementation of CEO, AjEO, and DEO on duodenal (a), jejunal (b) and ileal (c) morphology [villus height (VH), crypt depth (CD), VH:CD] of broiler chickens.** CON, basal diet; AGP, lincomycin (500 mg/kg); C200, C400, and C600, CEO at 200, 400, and 600 mg/kg, respectively; A200, A400, and A600, AjEO at 200, 400, and 600 mg/kg, respectively; D200, D400, and D600, DEO at 200, 400, and 600 mg/kg, respectively. CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil. One-way analysis of variance duodenum: VH (SEM = 13.06, $P < 0.001$), CD (SEM =3.96, $P < 0.001$), VH:CD (SEM = 0.18, $P < 0.001$); jejunum: VH (SEM = 19.62, $P < 0.001$), CD (SEM =3.13, $P = 0.09$), VH:CD (SEM = 0.16, $P < 0.001$); ileum: VH (SEM = 13.77, $P < 0.001$), CD (SEM =3.51, $P = 0.126$), VH:CD (SEM = 0.18, $P = 0.148$). Tukey's honestly significant difference test ^{a,b,c,d}: Different letters show significant differences among treatments ($P < 0.05$).

Values are the means ± standard error of the mean (n = 6).

ated with TBARS, the dependency of drip loss on TBARS was investigated. The results showed a positive correlation between drip loss and the TBARS value of breast meat of chickens fed the CON diet and the values of birds fed the nine diets supplemented with the three EOs (CEO, AjEO, and DEO) at 200, 400, and 600 mg/kg (Fig. 4).

Serum biochemical indices

Table 6 shows the effects of CEO, AjEO, and DEO supplementation on serum biochemical indices, total protein, glucose, triglycerides and cholesterol of broiler chickens. The serum total proteins, glucose, and triglycerides were not affected by supplementation with AGP or any EO ($P > 0.05$). In contrast, the serum cholesterol concentration was not changed by AGP supplementa-

Table 4. **Effects of coriander, ajwain, and dill seed essential oil supplementation on immune organ indices and humoral immunity in broiler chickens**

	Supplement (mg/kg) in diet												
	CON	AGP	CEO (mg/kg)			AjEO (mg/kg)			DEO (mg/kg)			SEM	*P*-value
			200	400	600	200	400	600	200	400	600		
Immune organs (% SBW)													
Spleen	0.15	0.13	0.17	0.15	0.15	0.19	0.12	0.18	0.16	0.17	0.15	0.01	0.683
Thymus	0.34	0.37	0.34	0.35	0.34	0.35	0.35	0.35	0.36	0.35	0.36	0.01	0.426
Bursa	0.15^{ab}	0.16^{ab}	0.17^{ab}	0.19^{ab}	0.14^{b}	0.18^{ab}	0.15^{ab}	0.18^{ab}	0.20^{ab}	0.23^{a}	0.20^{ab}	0.01	0.029
Humoral immunity													
NDV	3.00^{b}	3.33^{b}	3.67^{b}	5.00^{ab}	6.00^{a}	4.67^{ab}	6.33^{a}	6.33^{a}	3.67^{b}	4.00^{ab}	6.00^{a}	0.19	0.006
IBV	1477^{c}	1968^{abc}	1438^{c}	1762^{bc}	3046^{a}	2101^{abc}	2144^{abc}	2777^{ab}	2814^{ab}	2854^{ab}	2804^{ab}	96.1	0.014

CON, control; AGP, antibiotic growth promoter; CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil; NDV, Newcastle disease virus; IBV = infectious bronchitis virus; (% SBW), percentage of slaughterhouse body weight. Values are expressed as means with pooled SEM values, n=6. ^{a,b,c}: Means sharing the same superscript in a row do not statistically significantly differ ($P > 0.05$).

Table 5. **Effect of coriander, ajwain, and dill seed essential oil supplementation on the breast meat quality of broiler chickens**

Meat quality	Supplementation (mg/kg) in diet												
	CON	AGP	CEO (mg/kg)			AjEO (mg/kg)			DEO (mg/kg)			SEM	*P*-value
			200	400	600	200	400	600	200	400	600		
pH	5.77	5.8	5.85	5.69	5.83	5.68	5.74	5.75	5.88	5.83	5.84	0.01	0.296
L*	54.3	54.1	53.7	52.5	54.4	52.7	54.4	54.6	55.1	54.7	53.4	0.24	0.375
a*	11.8	12.4	11.4	11.7	12.5	12.6	11.5	11.5	12.5	11.8	12.6	0.12	0.165
b*	12.4	13.4	12.2	13.8	13	13.4	13.8	12.1	12.1	14.1	13.2	0.17	0.124
Drip loss (%)	3.19^{a}	2.96^{ab}	3.19^{a}	3.08^{a}	2.44^{cd}	2.36^{d}	2.59^{bcd}	2.89^{abc}	2.51^{bcd}	3.32^{a}	2.59^{bcd}	0.05	<0.001
TBARS (mg MDA/kg tissue)	0.207^{a}	0.172^{abc}	0.178^{ab}	0.136^{bcd}	0.115^{cd}	0.094^{d}	0.114^{cd}	0.133^{bcd}	0.103^{d}	0.180^{ab}	0.145^{bcd}	0.01	<0.001

CON, control; AGP, antibiotic growth promoter; CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil. Values are expressed as means with pooled SEM values, n=6.
^{a,b,c,d}: Means sharing the same superscript in a row do not statistically significantly differ ($P > 0.05$).

tion but was numerically reduced by supplementation with any EO, with several, including CEO (600 mg/kg), AjEO (600 mg/kg), and DEO (600 mg/kg), affording significant decreases ($P < 0.05$) compared to the value in birds fed the CON diet.

Discussion

Previous studies have demonstrated the effects of EOs of the *Apiaceae* family, especially CEO[10] and AjEO[11–13], on various performance parameters through *in vivo* chicken experiments, although data for DEO remains limited. However, none of the studies on CEO or AjEO utilized materials with defined analytical values, even for the major components, which could markedly influence the performance results. Therefore, the present study was conducted using EOs for which the chemical composition had been determined to robustly establish the viability of CEO, AjEO, and DEO as substitutes for AGPs, and to determine the optimal dosage for enhancing broiler growth performance, intestinal health, and immunity.

The results of this study showed that feeding with a diet supplemented with any concentration (200, 400, or 600 mg/kg) of CEO, which contained 56.8% linalool, significantly improved the final BW of broilers, similar to the results obtained using AGP. This finding is consistent with those obtained by Ghazanfari et al.[10], who reported increased BW gain in broilers fed a 0.03% CEO diet. Although the linalool content of CEO was not provided in the study[10], other reports mentioned values ranging from 66.1 to 75.3%[25–28], which are relatively close to the value obtained in our study. Supplementation with 200 and 400 mg/kg AjEO containing 68.2% thymol also significantly increased the final BW. This result aligns with those from Falaki et al.[11], who used a 0.015% AjEO diet, but differs from the results in Chowdhury et al.[12], obtained using a 0.04% AjEO diet. These discrepancies may be attributed to variations in thymol content in AjEO, which was reported to range from 15.5 to 67.4% in previous studies[14,29–31], implying that the feeding level of the AjEO diet in the latter experiment[12] may have resulted in underdosing of thymol. Furthermore, we found that feeding a diet supplemented with any concentration (200, 400, and 600 mg/kg) of DEO, containing 41.1% carvone and 19.9% d-limonene, significantly improved the final BW. To our knowl-

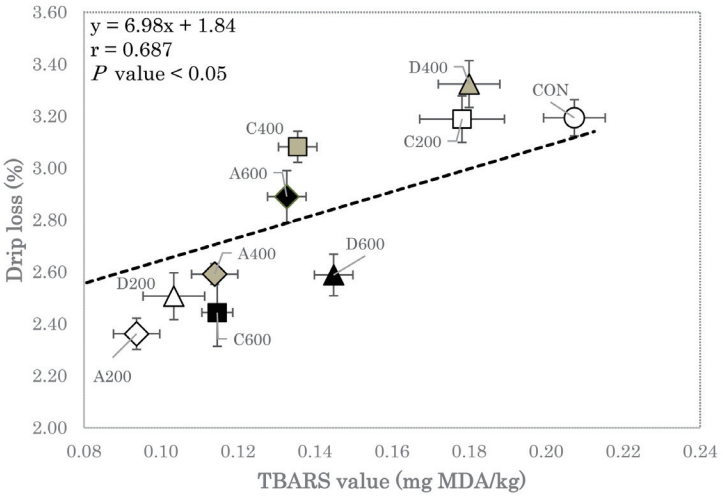

Fig. 4. **Dependency of drip loss on the TBARS value of breast meat in chickens fed CEO, AjEO, and DEO.** CON, basal diet; C200, C400, and C600, CEO at 200, 400, and 600 mg/kg, respectively; A200, A400, and A600, AjEO at 200, 400, and 600 mg/kg, respectively; D200, D400, and D600, DEO at 200, 400, and 600 mg/kg, respectively. TBARS, thiobarbituric acid reactive substance; CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil. Correlation analysis was conducted between the raw data of drip loss and TBARS using bivariate fit model in JMP Pro 16 (SAS Institute, Cary, NC, USA), and the treatment means were used to graph the data in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA).
Values are the means ± S.E. of data from six individual measurements in each group.

Table 6. **Effect of coriander, ajwain, and dill seed essential oil supplementation on the serum biochemical indices of broiler chickens**

Serum trait	CON	AGP	Supplement (mg/kg) in diet									SEM	*P*-value
			CEO (mg/kg)			AjEO (mg/kg)			DEO (mg/kg)				
			200	400	600	200	400	600	200	400	600		
Total proteins	3275	3050	3398	3067	3092	3432	3295	3167	3022	3039	3268	37.9	0.16
Glucose	212	217	227	240	226	244	216	218	228	212	207	3.86	0.625
Triglycerides	117	131	96.3	101	118	116	124	89.6	110	102	104	2.89	0.054
Cholesterol	194^{a}	189^{ab}	141^{abc}	144^{abc}	136^{bc}	165^{abc}	153^{abc}	132^{c}	163^{abc}	148^{abc}	139^{bc}	4.03	0.002

CON, control; AGP, antibiotic growth promoter; CEO, coriander essential oil; AjEO, ajwain essential oil; DEO, dill seed essential oil.
Values are expressed as means with pooled SEM values, n=6.
^{a,b,c}: Means sharing the same superscript in a row are not statistically significant ($P > 0.05$).

edge, this is the first evidence of the beneficial effects of dietary DEO. Considering that previous *in vivo* studies reported the toxic effects of carvone, regardless of DEO characteristics such as promising antioxidant, antimicrobial, anti-inflammatory, and antitumor properties[32,33], our finding provides new insights regarding potential ingredients for AGP alternatives.

Growth is a complex process involving not only metabolism but also the digestion and absorption of nutrients, which are closely related to gut health. Intestinal morphology and microbiota balance are key parameters for evaluating gut health. The small intestine, which comprises the duodenum, jejunum, and ileum, is the main absorption site and contains a series of finger-like projections, termed villi, that greatly increase the surface area available for nutrient absorption. The present results showed that AGP treatment did not improve the VH and VH:CD in the duodenum, jejunum, and ileum, except for the VH in the duodenum. However, CEO supplementation at 200 and 400 mg/kg significantly improved the VH and VH:CD in the duodenum and jejunum, and CEO supplementation at 200 mg/kg improved the VH in the ileum, suggesting that dietary CEO supplementation exerts positive effects on the gut morphology of broiler chickens.

In terms of microbiota balance, similar to the results following AGP treatment, any level of CEO supplementation prevented the growth of *E. coli* and *Salmonella*, whereas that of *Lactobacillus* remained unaffected. Our results regarding intestinal morphology and intestinal microbiota are consistent with the findings by Ghazanfari et al.[10], who reported improved duodenal, jejunal, and ileal morphology in broilers fed a 0.03% CEO diet, and re-

duced *E. coli* and unchanged *Lactobacillus* counts in broilers fed 0.02 and 0.03% CEO diets. Notably, although numerous studies have demonstrated antimicrobial efficacy *in vitro*[7,9], experimental *in vivo* evidence is limited. Using the same batch of CEO as employed in the present study, we have previously shown that CEO exerts antibacterial effects by measuring the zone of inhibition and minimum inhibitory concentration against *E. coli* and *S. gallinarum*[9]. The present findings of antibacterial activity *in vivo* confirmed those of Ghazanfari et al.[10] and extended them to include the possibility of extrapolation of *in vivo* effects from *in vitro* studies. These main effects of CEO on antibacterial activities and gut health may be attributed to linalool, which is a major bioactive component (56.8%) of CEO. In particular, linalool has been confirmed as a potent antimicrobial agent that causes damage to the membrane of pathogenic microbes leading to reduction in the bacterial count[34–36], and it can also enhance the VH in broiler intestine and the activity of digestive enzymes, possibly improving the digestibility and absorption of nutrients[34].

AjEO supplementation at 400 mg/kg improved VH:CD in duodenum. Moreover, AjEO supplementation reduced *E. coli* and *Salmonella* spp. counts in the intestine, although the *Lactobacillus* count remained unchanged, which is consistent with the previously reported *in vitro* antibacterial potential of AjEO[9]. Considering that thymol, a major bioactive component of AjEO, has been shown to exert antimicrobial activity against *E. coli* and *Salmonella*[37,38] through its lipophilic properties[37], this compound may exert significant effects on the gut health of broilers. However, the present results of morphological and antibacterial changes contrast with those reported by Chowdhury et al.[13], who also provided data on growth performance inconsistent with the results of the present study, possibly owing to a much lower thymol content than that (68.2%) of the AjEO evaluated herein.

Furthermore, DEO supplementation at all concentrations improved the VH, and at 400 mg/kg improved VH:CD in the duodenum. Additionally, DEO supplementation at 200 and 400 mg/kg, led to improvement in VH in the jejunum, and ileum, respectively. Similar to the results obtained for CEO and AjEO in the current study, all levels of DEO supplementation resulted in a reduction in *E. coli* and *Salmonella* spp. populations in the intestines of broilers but had no effect on the *Lactobacillus* count. These *in vivo* results parallel the findings of our previously published *in vitro* study[9], in which DEO significantly inhibited the growth of *E. coli* and *S. enteritidis*. These correlations between the *in vitro* and *in vivo* results are plausible based on the function of the major bioactive compound in DEO, carvone, which is an extremely potent antimicrobial agent that directly targets the cell membrane of bacterial species, disrupts its integrity, and increases its permeability[39]. As *in vivo* data on DEO are lacking in broiler chickens, the improvements in gut health observed in the present study in the context of accurate analytical values for DEO provide valuable insights regarding its potential as an alternative to AGPs.

In this study, we evaluated immune organ indices and serum antibody titer responses to NDV and IBV vaccine antigens to assess humoral immunity in broilers. The immune organ indices were not affected by either AGP or EO supplementation, except in the thymus of birds fed a diet containing 400 mg/kg DEO. AGP did not increase the specific antibody titers against either NDV or IBV compared to those in birds fed the CON diet. However, supplementation with 600 mg/kg CEO, AjEO, or DEO improved antibody titers against both viruses. Furthermore, even at lower doses, AjEO supplementation at 400 mg/kg and DEO supplementation at 200 and 400 mg/kg increased antibody titers against NDV and IBV, respectively. To our knowledge, virtually no data are available to confirm humoral immunity in response to CEO, AjEO, or DEO, except for those reported by Chowdhury et al.[13], who found an improved response against NDV following AjEO supplementation at 400 mg/kg in broilers. Our results confirmed the effectiveness of AjEO on humoral immunity, although some discrepancies exist in the growth performance and gut health between the two studies. As suggested by Nameghi et al.[40], bioactive compounds of EOs can act as strong antioxidants and may help maintain the structural integrity of immune cells; thus, the positive effects of EOs on humoral immunity observed in the present study may be ascribed to their antioxidant properties. This is further supported by our prior *in vitro* findings that the CEO, AjEO, and DEO used in the present study exhibit significant antioxidant activities including peroxide value, TBA, DPPH, and oxidative stability index[9]. Notably, antioxidant effects of the EOs *in vivo* are not only due to direct antioxidant action but also involve the induction of antioxidant enzymes, such as catalase and superoxide dismutase[8,41]. Given that the bioactive compounds linalool in CEO, thymol in AjEO, and carvone in DEO are responsible for the antioxidant properties of these EOs[42–44], further investigation at the molecular level is needed to elucidate the detailed mechanism by which each EO stimulates immune responses in broilers.

In conjunction with the antioxidant properties of the EOs, the TBARS value of breast meat decreased in all EO supplementation groups, except CEO supplementation at 200 mg/kg and DEO supplementation at 400 mg/kg, whereas it remained unaffected by AGP. The changes in TBARS values caused by EO supplementation were reflected in the drip loss, although significant reduction in drip loss was not obtained in some groups despite a significant reduction in TBARS (Table 5). With regard to whether oxidative damage to muscles is involved in the degree of tissue drip loss, Adeyemi et al.[45] and Hazrati et al.[46] suggested that drip loss in breast meat can be improved by enhancing antioxidant stability, as higher drip loss is associated with protein and lipid deterioration owing to oxidative processes. Consistent with this, our study showed a positive correlation between the TBARS value and drip loss levels (Fig. 4), suggesting that the antioxidant properties of the tested EOs play an important role in the downregulation of drip loss in the muscle of chickens. To the best of our knowledge, these findings provide the first evidence that these EOs can improve meat quality, likely by reducing oxidative damage. It should be further noted that reduced serum cholesterol concentration consequent to supplementation with

any EO at high concentration could help reduce cardiovascular risk and lower cholesterol content in meat if the concentration of cholesterol mainly reflects low-density lipoprotein levels. Given that linalool in CEO, thymol in AjEO, and carvone in DEO act as inhibitors of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, thereby affording cholesterol-lowering properties[44,47,48], the use of these EOs may be desirable as feed additives for both producers and consumers to provide healthy, resilient chickens.

Thus, this study demonstrated that feeding diets supplemented with EOs for 35 days generally led to improvements in growth performance, intestinal health, meat quality, and humoral immunity in broilers. Detailed review of growth performance revealed that the ADG was also improved by supplementation with any EO, albeit in a manner dependent on the growth phase (Table 2); in particular, the ADG of birds in the later phase was less profoundly affected by EO supplementation, implying that the beneficial effects on feed efficiency of any EO would be reduced in older chickens. One possible reason for the limited effects of EO supplementation on ADG in older birds could be the extreme deficiency of antioxidant agents in supplemented EOs. However, the lack of observed improvement in ADG in older birds that were fed EOs at higher concentrations does not support this possibility. Alternatively, considering that the fractional turnover rate of proteins in the body is much higher in the early stages than in the later stages[49], it can be hypothesized that younger chicks require more resilient intestines to efficiently absorb nutrients and/or a more efficient process of producing antioxidant molecules to scavenge the superoxide produced by protein turnover-related oxidative metabolism. These requirements in younger birds may be fulfilled by EO supplementation. Further studies focusing on the effect of EO supplementation on the age-dependent growth performance of chicks are needed to support this hypothesis. Additional studies are also needed to clarify the detailed mechanism underlying the growth-enhancing effect of EO supplementation in young chicks.

In conclusion, final BW was improved by supplementation with not only AGP but also any EO except AjEO at 600 mg/kg; in particular, supplementation with CEO at 400 mg/kg, AjEO at 200 mg/kg, and DEO at 200 mg/kg yielded the best growth performance within each EO group. EO supplementation exerted beneficial effects on gut morphology, such as increased VH and VH:CD in the duodenum, increased VH in the jejunum and ileum, and against harmful microbiota, such as the reduction of *E. coli* and *Salmonella* spp. populations. Furthermore, EO supplementation enhanced not only humoral immunity but also meat quality, as evidenced by reduced drip loss, likely owing to the associated antioxidant properties. Therefore, it can be concluded that any of the tested EOs may play a pivotal role in replacing AGPs in broilers. However, additional studies are needed to assess the mechanism of action of each EO on the growth performance of broilers, as greater improvement in feed efficiency is obtained when younger chicks are provided EO–supplemented feed.

Acknowledgements

This research was funded by the Higher Education Commission (HEC), Pakistan, under the Indigenous 5000 PhD Fellowship Program (to Mr. Usman Ali). The authors gratefully acknowledge the financial support from the HEC.

Author Contributions

Usman Ali, Saima, and Masaaki Toyomizu designed the study. Usman Ali, Saima, Shafqat Nawaz Qaisrani, Athar Mahmud, and Zafar Hayat conducted the experiments, collected samples, and performed all analyses. Usman Ali and Masaaki Toyomizu performed the statistical analysis, interpreted the data, and prepared the figures and tables. Usman Ali, Saima, and Masaaki Toyomizu drafted the manuscript. Saima and Masaaki Toyomizu co-supervised the work, including concept development, all procedures, and preparation of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Kairmi SH, Taha-Abdelaziz K, Yitbarek A, Sargolzaei M, Spahany H, Astill J, Shojadoost B, Alizadeh M, Kulkarni RR, Parkinson J and Sharif S. Effects of therapeutic levels of dietary antibiotics on the cecal microbiome composition of broiler chickens. Poult Sci, **101**: 101864. 2022. https://doi.org/10.1016/j.psj.2022.101864, PMID:35477134

[2] Zhang LY, Peng QY, Liu YR, Ma QG, Zhang JY, Guo YP, Xue Z and Zhao LH. Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. Poult Sci, **100**: 101163. 2021. https://doi.org/10.1016/j.psj.2021.101163, PMID:34082177

[3] Khan K and Ahmad N. Using cinnamon (*Cinnamomum zeylanicum*) and turmeric (*Curcuma longa* L) powders as an antibiotic growth promoter substitutions in broiler chicken's diets. Anim Biotechnol, **34**: 4466–4473. 2023. https://doi.org/10.1080/10495398.2022.2157282, PMID:36576077

[4] Scicutella F, Mannelli F, Daghio M, Viti C and Buccioni A. Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: a review. Antibiotics (Basel), **10**: 1010. 2021. https://doi.org/10.3390/antibiotics10081010, PMID:34439059

[5] Sugiharto S. Role of nutraceuticals in gut health and growth performance of poultry. J Saudi Soc Agric Sci, **15**: 99–111. 2016. https://doi.org/10.1016/j.jssas.2014.06.001

[6] Gadde U, Kim WH, Oh ST and Lillehoj HS. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim Health Res Rev, **18**: 26–45. 2017. https://doi.org/10.1017/S1466252316000207, PMID:28485263

[7] Ali U, Naveed S, Qaisrani SN, Mahmud A, Hayat Z, Abdullah M, Kikusato M and Toyomizu M. Characteristics of essential oils of *apiaceae* family: their chemical compositions, in vitro properties and effects on broiler production. J Poult Sci, **59**: 16–37. 2022. https://doi.org/10.2141/jpsa.0210042,

PMID:35125910

[8] Kikusato M. Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. Anim Biosci, **34**: 345–353. 2021. https://doi.org/10.5713/ab.20.0842, PMID:33705621

[9] Ali U. Saima, Toyomizu M, Qaisrani SN, Mahmud A, et al. Implication of chemical compositions and in vitro properties of coriander, ajwain and dill seed essential oils as potential replacement of antibiotic growth promoters in broilers. Large Anim Rev, 29:27-34, 2023. https://www.largeanimalreview.com/index.php/lar/article/view/609/214

[10] Ghazanfari S, Mohammadi Z and Adib Moradi M. Effects of coriander essential oil on the performance, blood characteristics, intestinal microbiota and histological of broilers. Rev Bras Cienc Avic, **17**: 419–426. 2015. https://doi.org/10.1590/1516-635X1704419-426

[11] Falaki M, Shargh MS, Dastar B, Hashemi S and Mahoonak AS. Growth performance, carcass characteristics and intestinal microflora of broiler chickens fed diets containing Carum copticum essential oil. Poult Sci J, 4:37-46, 2016. https://www.cabdirect.org/cabdirect/abstract/20183059670

[12] Chowdhury S, Mandal GP and Patra AK. Different essential oils in diets of chickens: 1. Growth performance, nutrient utilisation, nitrogen excretion, carcass traits and chemical composition of meat. Anim Feed Sci Technol, **236**: 86–97. 2018. https://doi.org/10.1016/j.anifeedsci.2017.12.002

[13] Chowdhury S, Mandal GP, Patra AK, Kumar P, Samanta I, Pradhan S and Samanta AK. Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. Anim Feed Sci Technol, **236**: 39–47. 2018. https://doi.org/10.1016/j.anifeedsci.2017.12.003

[14] Patil SD, Maknikar PP, Wankhade SJ, Ukesh CS and Rai MK. Chemical composition, antimicrobial and antioxidant activity ofessential oils from cumin and ajowan. Nusantara Bioscience, **8**: 60–65. 2016. https://doi.org/10.13057/nusbiosci/n080111

[15] Applegate TJ, Klose V, Steiner T, Ganner A and Schatzmayr G. Probiotics and phytogenics for poultry: myth or reality? J Appl Poult Res, **19**: 194–210. 2010. https://doi.org/10.3382/japr.2010-00168

[16] Grashorn M. Use of phytobiotics in broiler nutrition – an alternative to infeed antibiotics? J Anim Feed Sci, **19**: 338–347. 2010. https://doi.org/10.22358/jafs/66297/2010

[17] Kiczorowska B, Kiczorowska B, Klebaniuk R, Bąkowski M and Al-Yasiry ARMH. Culinary herbs – the nutritive value and content of minerals. J Elem, **20**: 599–608. 2015. https://doi.org/10.5601/jelem.2014.19.4.739

[18] Al-Yasiry ARM and Kiczorowska B. Frankincense–therapeutic properties. Advances in Hygiene and Experimental Medicine, **70**: 380–391, 2016. https://doi.org/10.5604/17322693.1200553

[19] Koç F, Samli H, Okur A, Ozduven M, Akyurek H and Senkoylu N. Effects of Saccharomyces cerevisiae and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulg J Agric Sci, **16**: 643–650. 2010. https://agrojournal.org/16/05-15-10.pdf

[20] Gopinger E, Xavier EG, Elias MC, Catalan AAS, Castro MLS, Nunes AP and Roll VFB. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poult Sci, **93**: 1130–1136. 2014. https://doi.org/10.3382/ps.2013-03426, PMID:24795305

[21] Jaspal MH, Ijaz M, Haq HA, Yar MK, Asghar B, Manzoor A, Badar IH, Ullah S, Islam MS and Hussain J. Effect of oregano essential oil or lactic acid treatments combined with air and modified atmosphere packaging on the quality and storage properties of chicken breast meat. Lebensm Wiss Technol, **146**: 111459. 2021. https://doi.org/10.1016/j.lwt.2021.111459

[22] Khan IA, Parker NB, Löhr CV and Cherian G. Docosahexaenoic acid (22:6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy. Poult Sci, **100**: 865–874. 2021. https://doi.org/10.1016/j.psj.2020.10.069, PMID:33518140

[23] Ohkawa H, Ohishi W and Yagi K. Colorimetric method for determination of MDA activity. Biochemistry, **95**: 90738–3. 1979.

[24] El-Shall NA, Shewita RS, Abd El-Hack ME, AlKahtane A, Alarifi S, Alkahtani S, Abdel-Daim MM and Sedeik ME. Effect of essential oils on the immune response to some viral vaccines in broiler chickens, with special reference to Newcastle disease virus. Poult Sci, **99**: 2944–2954. 2020. https://doi.org/10.1016/j.psj.2020.03.008, PMID:32475429

[25] Baratta MT, Dorman HJD, Deans SG, Biondi DM and Ruberto G. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. J Essent Oil Res, **10**: 618–627. 1998. https://doi.org/10.1080/10412905.1998.9700989

[26] Delaquis P, Stanich K, Girard B and Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol, **74**: 101–109. 2002. https://doi.org/10.1016/S0168-1605(01)00734-6, PMID:11929164

[27] Singh G, Maurya S, de Lampasona MP and Catalan CAN. Studies on essential oils, Part 41. Chemical composition, antifungal, antioxidant and sprout suppressant activities of coriander (*Coriandrum sativum*) essential oil and its oleoresin. Flavour Fragrance J, **21**: 472–479. 2006. https://doi.org/10.1002/ffj.1608

[28] Kačániová M, Galovičová L, Ivanišová E, Vukovic NL, Štefániková J, Valková V, Borotová P, Žiarovská J, Terentjeva M, Felšöciová S and Tvrdá E. Antioxidant, antimicrobial and antibiofilm activity of coriander (*Coriandrum sativum* L.) essential oil for its application in foods. Foods, **9**: 282. 2020. https://doi.org/10.3390/foods9030282, PMID:32143314

[29] Singh G, Maurya S, Catalan C and de Lampasona MP. Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract. J Agric Food Chem, **52**: 3292–3296. 2004. https://doi.org/10.1021/jf035211c, PMID:15161185

[30] Vitali LA, Beghelli D, Biapa Nya PC, Bistoni O, Cappellacci L, Damiano S, Lupidi G, Maggi F, Orsomando G, Papa F, Petrelli D, Petrelli R, Quassinti L, Sorci L, Zadeh MM and Bramucci M. Diverse biological effects of the essential oil from Iranian Trachyspermum ammi. Arab J Chem, **9**: 775–786. 2016. https://doi.org/10.1016/j.arabjc.2015.06.002

[31] Grădinaru AC, Trifan A, Şpac A, Brebu M, Miron A and Aprotosoaie AC. Antibacterial activity of traditional spices against lower respiratory tract pathogens: combinatorial effects of *Trachyspermum ammi* essential oil with conventional antibiotics. Lett Appl Microbiol, **67**: 449–457. 2018. https://doi.org/10.1111/lam.13069, PMID:30187508

[32] Mills MG, Ramsden R, Ma EY, Corrales J, Kristofco LA, Steele WB, Saari GN, Melnikov F, Kostal J, Kavanagh TJ, Zimmerman JB, Voutchkova-Kostal AM, Brooks BW, Coish P, Anastas PT and Gallagher E. CRISPR-generated Nrf2a loss- and gain-of-function mutants facilitate mechanistic analysis of chemical oxidative stress-mediated toxicity in zebrafish. Chem Res Toxicol, **33**: 426–435. 2020. https://doi.org/10.1021/acs.chemrestox.9b00346, PMID:31858786

[33] Pina LTS, Serafini MR, Oliveira MA, Sampaio LA, Guimarães JO and Guimarães AG. Carvone and its pharmacological activities: A systematic review. Phytochemistry, **196**: 113080. 2022. https://doi.org/10.1016/j.phytochem.2021.113080, PMID:34999510

[34] Cabuk M, Alcicek A, Bozkurt M and Imre N. Antimicrobial properties of the essential oils isolated from aromatic plants and using possibility as alternative feed additives. National Animal Nutrition Congress, 184–187, 2003.

[35] Lee KW, Everts H and Beynen A. Essential oils in broiler nutrition. Int J Poult Sci, **3**: 738–752. 2004. https://doi.org/10.3923/ijps.2004.738.752

[36] Silva F, Ferreira S, Queiroz JA and Domingues FC. Coriander (Coriandrum sativum L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. J Med Microbiol, **60**: 1479–1486. 2011. https://doi.org/10.1099/jmm.0.034157-0, PMID:21862758

[37] Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM and von Wright A. Characterization of the action of selected essential oil components on Gram-negative bacteria. J Agric Food Chem, **46**: 3590–3595. 1998. https://doi.org/10.1021/jf980154m

[38] Dorman HJD and Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol, **88**: 308–316. 2000. https://doi.org/10.1046/j.1365-2672.2000.00969.x, PMID:10736000

[39] Mutlu-Ingok A and Karbancioglu-Guler F. Cardamom, cumin, and dill weed essential oils: chemical compositions, antimicrobial activities, and mechanisms of action against Campylobacter spp. Molecules, **22**: 1191. 2017. https://doi.org/10.3390/molecules22071191, PMID:28714890

[40] Hesabi Nameghi A, Edalatian O and Bakhshalinejad R. Effects of a blend of thyme, peppermint and eucalyptus essential oils on growth performance, serum lipid and hepatic enzyme indices, immune response and ileal morphology and microflora in broilers. J Anim Physiol Anim Nutr (Berl), **103**: 1388–1398. 2019. https://doi.org/10.1111/jpn.13122, PMID:31106919

[41] Windisch W, Schedle K, Plitzner C and Kroismayr A. Use of phytogenic products as feed additives for swine and poultry1. J Anim Sci, **86**: E140–E148. 2008. https://doi.org/10.2527/jas.2007-0459, PMID:18073277

[42] Duarte A, Luís Â, Oleastro M and Domingues FC. Antioxidant properties of coriander essential oil and linalool and their potential to control Campylobacter spp. Food Control, **61**: 115–122. 2016. https://doi.org/10.1016/j.foodcont.2015.09.033

[43] Luna A, Lábaque MC, Zygadlo JA and Marin RH. Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. Poult Sci, **89**: 366–370. 2010. https://doi.org/10.3382/ps.2009-00130, PMID:20075292

[44] Mohammadi F. Chemical composition and dietary effects of pennyroyal and dill on biochemical, hematological, and oxidative stress biomarkers in broiler chickens. J Agric Sci Technol, **22**: 401–413. 2020. http://jast.modares.ac.ir/article-23-25698-en.html

[45] Adeyemi KD, Sabow AB, Shittu RM, Karim R, Karsani SA and Sazili AQ. Impact of chill storage on antioxidant status, lipid and protein oxidation, color, drip loss and fatty acids of semimembranosus muscle in goats. CYTA J Food, **14**: 1–10. 2015. https://doi.org/10.1080/19476337.2015.1114974

[46] Hazrati S, Rezaeipour V and Asadzadeh S. Effects of phytogenic feed additives, probiotic and mannan-oligosaccharides on performance, blood metabolites, meat quality, intestinal morphology, and microbial population of Japanese quail. Br Poult Sci, **61**: 132–139. 2020. https://doi.org/10.1080/00071668.2019.1686122, PMID:31661976

[47] Yarmohammadi Barbarestani S, Jazi V, Mohebodini H, Ashayerizadeh A, Shabani A and Toghyani M. Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens. Livest Sci, **233**: 103958. 2020. https://doi.org/10.1016/j.livsci.2020.103958

[48] Lee KW, Everts H, Kappert HJ, Frehner M, Losa R and Beynen AC. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br Poult Sci, **44**: 450–457. 2003. https://doi.org/10.1080/0007166031000085508, PMID:12964629

[49] Muramatsu T and Okumura JI. Whole-body protein turnover in chicks at early stages of growth. J Nutr, **115**: 483–490. 1985. https://doi.org/10.1093/jn/115.4.483, PMID:3981267