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Effect of Dietary Clove (Syzygium Aromaticum) Essential Oil on Growth Performance, Oxidative Indices, Lipid Profile, and Cadmium Accumulation in Cd-exposed Quails

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ABSTRACT

Introduction: Natural products can alleviate oxidative stress induced by toxic metals. This study evaluated antioxidant properties of clove essential oil (CEO), compared to oxidative deterioration of Cd. It was carried out by measuring growth performance parameters (BW, FI, and FCR), oxidative indices (TBARS, CP, CAT, SOD, and GPx), lipid profile (TG, TC, LDL-C, and HDL-C), and Cd bioaccumulation in quails.

Materials and Methods: In this study, 480 Japanese quails were fed with diets for 35 days. The diets included basal diet, basal diet + VC (500 mg/kg), basal diet + CEO (450 mg/kg), basal diet + CEO (100 mg/kg), basal diet + VC (500 mg/kg) + Cd (40 mg/l), basal diet + CEO (450 mg/kg) + Cd (40 mg/l), basal diet + CEO (100 mg/kg) + Cd (40 mg/l), and basal diet + Cd (40 mg/l). Oxidative indices and Cd bioaccumulation (ICP-OES) were measured.

Results: The major ingredients of CEO included Eugenol (77.63%) and β-Caryophyllene (9.55%). Quails exposed to Cd and treated with CEO had a reduced amount of oxidative stress as evidenced by lower concentrations of TBARS and CP, higher activities of SOD, GPx, and CAT, an improved lipid profile, and lower accumulation of Cd compared to the positive control. However, FI and FCR did not change.

Conclusion: Antioxidant properties of CEO were dose-dependent. CEO (450 mg/kg) was potentially as effective as, or even more potent than VC (500 mg/kg) in reducing the adverse effects of Cd. However, further studies are required to determine the minimum concentration of COE.

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Introduction

Cadmium (Cd) is a ubiquitous and non-biodegradable toxic metal that has cumulative characteristics due to its long biological half-life (15-20 years in the human body) [1]. Owing to the widespread usage of Cd in various industries (electroplating, galvanizing, and batteries) as well as significant release of Cd into nature via industrial discharges, mining, garbage disposal, and cigarette smoke [2], the majority of animals, especially poultries, are easily exposed to Cd mainly thorough food, water, soil, and air [3].

Cd, which is classified as a carcinogen, has been demonstrated to trigger formation of free radicals,

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such as ROS that depletes antioxidant capacity, resulting in oxidative degradation of lipids, proteins, and DNA [2]. Thus, prolonged exposure to this metal can lead to oxidative stress, being a major cause of acute and chronic diseases, like artherosclerosis and different types of cancers [4-6]. Meanwhile, the potential deleterious effect of poultry contamination with heavy metals is the possibility of its transmission to humans through the food chain [7].

Thus, adopting approaches to reduce the accumulation of toxic metals in poultry organs seems to be a health priority. Different studies have shown that natural products, like plant essential oils (PEOs), mitigate oxidative stress-induced pathogenesis and elevate antioxidant activity, owing to their ability to act as radical scavenging and metal chelating[8]. Moreover, PEOs have several other properties to support their use in poultry nutrition, such as stimulating feed intake as well as enhancing digestive enzyme secretion and immune responses to diseases. In this respect, Clove (*Syzygium aromaticum*), as a spice plant, has been approved by the FDA to be served as a food additive [9]. Clove essential oil (CEO) has represented the most potent antioxidant properties, due to its primary phytogenic sources of phenolic compounds, like eugenol, representing 89% of clove oil [10].

In recent years, growing global demands for animal proteins have turned quails into a good alternative, due to their unique properties (economic breeding, carcass traits, unique flavor, etc.) [11]. However, there is limited information regarding Cd-intoxication and CEO-alleviating impacts on quails.

In general, Cd is a highly toxic environmental pollutant that induces adverse effects, such as growth retardation with oxidative, deteriorative, and hepatotoxic effects. However, CEO has alleviating properties, such as growth promotion as well as oxidative mitigating, and hepatoprotective effects [3, 12]. Thus, it is assumed that CEO would protect quails from toxic effects of Cd. Moreover, VC is an accepted and well-known terminal reducing antioxidant that is widely used in the poultry nutrition industry to increase antioxidant capacity [13, 14] and improve their growth performance [15]. Thus, it is worth studying the effectiveness of CEO in comparison with that of VC, as a scale, in mitigating the oxidative stress induced by Cd.

To confirm this hypothesis, this study was designed to examine alleviating effects of CEO versus adverse effects of Cd on some selected parameters, including growth performance, oxidative indices, lipid profile, and Cd bioaccumulation in Japanese quails (*Coturnix japonica*).

Materials and Methods

Sample collection and preparation

A total of 480 one-day-old Japanese quails were purchased from a local farm in Chaharmahal and Bakhtiari province, Iran. The chicks were fed with a basic diet for up to 7 days. On day 7, the chicks, after weighing, were randomly divided into eight groups, with each consisting of 20 chicks with three replications, so that the mean weight of all groups was almost the same. The birds were kept in Poultry Pens (Department of Animal Nutrition, Shahrekord University, Iran) with dimensions of 3.5 m (width) × 4 m (length) × 17 m (height). This study was conducted according to animal welfare guidelines at the Veterinary Control and Research Institute of Shahrekord University, Iran. In brief, the quails were housed in an environmentally controlled pen with a 24-lighting cycle, which followed a standard temperature that gradually decreased from 36 to 25 °C, at a reduction rate of 2 °C per week. The quails were vaccinated against Newcastle disease by the B1 serotype on day 7. In addition, they were fed with basal diets [16] (Table 1) and daily refreshed water for 35 days. The dietary treatments included (1) basal diet (negative control), (2) basal diet + VC (500 mg/kg), (3) basal diet + CEO (450 mg/kg), (4) basal diet + CEO (100 mg/kg), (5) basal diet + VC (500 mg/kg) + Cd (40 mg/l), (6) basal diet + CEO (450 mg/kg) + Cd (40 mg/l), (7) basal diet + CEO (100 mg/kg) + Cd (40 mg/l), and (8) basal diet + Cd (40 mg/l) as cadmium chloride in water (positive control).

On day 35, 24 blood samples were collected from each group from jugular veins in capped test tubes. To separate serums, blood samples were clotted and centrifuged at 5,000 rpm for 10 min. Moreover, 24

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muscle (thigh and breast) and liver samples of each group were quickly excised and packed in plastic bags. Next, all the samples (tissues and serums) were stored at -70 °C before analysis.

Growth performance measurement

BW, FI, and FCR were documented weekly.

Body weight (BW)

At the end of each week, the chicks in each group were weighed separately after 2 hours of starvation, and the mean weights were recorded.

Feed intake (FI)

The amount of grain given to each group was recorded daily and at the end of each week, the remaining amount was collected and weighed, and the amount of feed consumed was calculated.

In addition, the feed conversion ratio (FCR) was calculated using the following equation:

FCR = Total feed consumed weekly / (initial weight) - (weight of losses + final weekly weight)

Table 1: Composition of basal diets during the experiment

Ingredient (g/k)	0-35 days
Maize	509.6
Soybean meal	438.4
Soybean oil	20.6
Calcium carbonate	12.6
Di-calcium phosphate	8.3
DL-Methionine	1.6
Mineral premix[a]	2.5
Vitamin premix[b]	2.5
Salt	1.6
Cholecalciferol[c]	1
D-alpha-tocopherol[d]	1.5
Calculated Analysis	
Metabolizable energy (KCal/k)	2850
Crude protein (g/k)	253
Calcium (g/k)	8
Phosphorus (g/k)	3
Sodium (g/k)	1.5
Lysine (g/k)	13.4
Methionine (g/k)	5
Arginine (g/k)	15.7
Methionine + cysteine (g/k)	8.4
Threonine (g/k)	10.2
Valine (g/k)	11.2
Leucine (g/k)	18
Isoleucine (g/k)	12.2
Histidine (g/k)	2.2
Phenylalanine (g/k)	11.3
Mn (mg)	21.17
Fe (mg)	146.6
Cu (mg)	17.44
Zn (mg)	41.35
Se (mg)	0.11

[a] Provided per kilogram of diet: 1200 mg Mn (as manganese oxide), 1000 mg Zn (as zinc oxide), 1800 mg Fe (as ferrous sulphate), 400 mg Cu (as copper sulphate), 8 mg Se (as sodium selenite), 38 mg Iodine (as calcium iodate), 180 g Ca (as calcium carbonate).
[b] Provided per kilogram of diet: 120 mg B-carotene (vitamin A), 2 mg cholecalciferol (vitamin D_3), 1.1 g d-alpha-tocopherol (vitamin E), 700 mcg cobalamin (vitamin B_{12}), 35 mcg Menadione (vitamin K_3), 60 mg L-ascorbic acid (vitamin C), 30 mg thiamine (vitamin B_1), 130 mg riboflavin (vitamin B_2), 1300 mg nicotinic acid, 225 mg pantothenic acid (vitamin B_5), 8200 mg choline chloride (vitamin B_4), 3.3 mg biotin (vitamin B_7).
[c] Provided per kilogram of cholecalciferol: 2.5 mg
[d] Provided per kilogram of d-alpha-tocopherol: 67 g

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Identification of essential oil compounds (GC/MS)

The pure CEO was obtained from Barij Essence Company (Kashan, Iran). Gas chromatography-mass spectrometry (GC-MS) analyses were carried out using Agilent Technologies GC (Model HP-7890, Palo Alto, CA, USA) with a capillary column (Model HP-5MS; length: 30 m, membranous thickness: 0.25 µm, internal diameter: 0.25 mm), coupled with a mass spectrometer (Model HP 5975; Agilent Technologies, Palo Alto, CA, USA) with an electron impact ionization potential (70 eV). The temperature of the oven was kept at 60 °C for about 5 min at the beginning and was gradually elevated at the rate of 4 °C per min until it reached 240 °C. Eventually, it was raised at the rate of 15 °C per min until it reached 290 °C, then it was maintained at this degree for 3 min. Helium was used as the inert gas that flowed past at a speed of 0.8 ml per min, and its purity was 99.999%. Samples of 1.0 µl were injected using a Hamilton syringe. The injection temperature was 300 °C, and the separation ratio was set at 100: 1. In addition, the mass range was 50-50 m/z. Besides, quantitative data were obtained using the peak area percentage method. EO ingredients were quantified by assimilating their retention indices (Table 2) in corporation with n-alkanes series (C8 to C25), using the data presented in the literature or those of authentic compounds available in the laboratory, which were confirmed by matching their mass spectra analysis patterns.

Table 2: Constituents of *CEO* and their relative percentages of retention time, Kovats index and total chromatogram area

Ingredients	Retention time	Kovats index	Area (%)
Eugenol	18.17	1365.33	77.63
Iso-eugenol	18.86	1387.06	0.65
β-Caryophyllene	19.93	1421.93	9.54
α-Humulene	20.98	1456.26	1.33
delta-Cadinene	23.07	1524.65	0.2
Eugenol acetate	24.85	1587.01	7.07
Caryophyllene oxide	24.85	1587.01	0.28

Measurement of biochemical parameters

The value of thiobarbituric acid reactive substances (TBARS) was calculated according to the following formula and shown as mg of malondialdehyde (MDA)/kg of the samples (Serums, livers, and muscles) [17]:

$$TBARS = (A \times 288) / 156$$

The absorbance (A) of the acquired upper layer was read at 532 nm versus a blank (1 ml of DDW + 2 ml of TBA/TCA). Besides, serum carbonyl protein (CP) was measured by the method introduced by Levine et al. (1990) [18].

The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were calculated based on the methods introduced by Sun et al. (1988) [19], Goth (1991) [20], and Paglia et al. (1967) [21], respectively.

Concentrations of triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured by commercial kits (Pars Azmoon, Iran). In addition, the concentration of serum low-density lipoprotein cholesterol (LDL-C) was obtained according to the Friedewald formula [LDL-C = (TC) – (HDL-C) – TG/2.2] [22].

Cd quantification (ICP-OES)

After pretreatment, inductively coupled plasma-optical emission spectrometry (ICP-OES) was applied to 0.5 g of liver and muscle tissues. For pretreatment procedures, 0.5 g of the target tissues was digested in whole with a mixture of nitric acid (5 ml, 65%), hydrochloric acid (2 ml, 37%), and oxygenated water (1 ml, 30%). When tissue digestion was performed, the mixture was filtered through 0.45 µm microfilters. A total of 25 ml of the purified sample was produced by chromatographic water and kept in Falcon tubes before performing the experiment. Table 3 shows ICP-OES operating conditions.

Table 3: ICP-OES operating conditions

Parameter	Cd
Wave length (nm)	214.438
Nebulizer gas flow (L min^{-1})	1
Auxiliary gas flow (L min^{-1})	1
Plasma gas flow (L min^{-1})	12
Coolant flow (L min^{-1})	12
ICP RF power (W)	1400
Readings/replicate	2
Optic temperature (℃)	29.65

Statistical analyses

All the data were evaluated by the one-way ANOVA followed by a Duncan's test in SPSS software (version 22). In addition, the values of all Cd intoxicated groups were compared to those of the control group, with the significance level considered at $P < 0.05$. The values were disclosed as mean ± SEM.

Ethical issues

The present study was approved by Ethics Committee of Shahrekord University of Medical Sciences (IR.SKU.REC.1392.122.531)

Results

GC/MS identification of CEO

Table 2 shows the main abundance components of CEO (eugenol (77.63%), β-caryophyllene (9.54%), and eugenol acetate (7.07%)).

Growth performance (BW, FI, FCR)

According to Table 4, on days 21-35 of age, a significant ($P < 0.05$) improvement was observed in BW in CEOs and VC groups, compared to the control group. Nevertheless, on day 35, a significant ($P < 0.05$) reduction was noticed in BW in all Cd-exposed groups. Indeed, the highest reduction was in control positive group (Cd 40 mg/l). Furthermore, VC was more effective than CEOs in lessening the adverse effect of Cd on BW. However, no significant difference was observed among the groups in terms of FI and FCR throughout the study. The FCR value significantly decreased on days 7-35 in CEO (450 mg/kg) group.

Biochemical analyses of serum (TBARS, CP), (SOD, GPx, CAT), and (TG, TC, LDL-C, HDL-C) on day 35

Table 5 reveals a significant increase ($P < 0.05$) in TBARS and CP levels in all groups exposed to Cd, except for the VC + Cd group, in contrast to the control group. Accordingly, VC was more effective than CEO (450 mg/kg) in redusing Cd effects on the CP serum level. Moreover, activities of antioxidant enzymes (SOD, GPx, and CAT) showed a significant decrease in all Cd-exposed groups, compared to the control group, except for the CAT activity in VC + Cd and CEO (450 mg/kg) + Cd groups, which remained unchanged, compared to the control group. Furthermore, contents of the serum lipid profile (TG, TC, HDL-C, and LDL-C) stayed unchanged in all groups, compared to the control group. However, CEO (450 mg/kg) and CEO (450 mg/kg) + Cd groups showed a reduction in TG, TC, and LDL-C and an increase in HDL-C. Besides, CEO (450 mg/kg) improved the lipid profile of the serum more effectively than VC.

Biochemical analysis of tissues (muscle, liver) (MDA, Cd level) on day 35

Table 6 represents that MDA values in muscles showed a significant reduction ($P < 0.05$) in the CEO (450 mg/kg) group and a significant increase ($P < 0.05$) in all Cd exposed groups, compared to the control group. Besides, CEO (450 mg/kg) was more significantly effective ($P < 0.05$) than VC in reducing Cd effects on MDA production in muscles. Additionally, MDA values in the liver showed a significant reduction in VC and CEO groups while remaining unchanged in VC + Cd and CEO (450 mg/kg) + Cd groups. Furthermore, VC and CEO showed equal effectiveness in mitigating adverse effects of Cd on MDA production in the liver.

According to Table 7, there was a significant increase ($P < 0.05$) in the Cd levels of the liver and muscles in all Cd-exposed groups. Besides, CEO (450 mg/kg) was significantly more effective than VC in reducing Cd accumulation in muscles and the liver.

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Table 4: The effects of the dietary inclusion of CEO, VC, and Cd on body weight (BW; gr) and feed intake (FI; gr) and feed conversion ratio (FCR) of quails (0-35 d)

Groups	Control	VC 500 mg/kg	CEO 450 mg/kg	CEO 100 mg/kg	VC 500 mg/kg + Cd 40 mg/l	CEO 450 mg/kg + Cd 40 mg/l	CEO 100 mg/kg + Cd 40 mg/l	Cd 40 mg/l
	mean ±sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem
BW (gr)								
7 days	32.85 ± 0.51	34.15 ± 0.81	33.33 ± 0.38	33.83 ± 1.23	33.71 ± 0.97	34.77 ± 0.40	33.75 ± 0.67	33.00 ± 1.90
14 days	66.70 ± 0.74	69.60 ± 3.41	65.37 ± 4.13	69.71 ± 3.32	66.20 ± 0.96	61.40 ± 0.83	61.68 ± 2.45	58.81 ± 0.78
21 days	112.03 ± 2.90^{a}	128.55 ± 1.68^{b}	125.18 ± 4.14^{b}	125.38 ± 3.46^{b}	113.54 ± 1.10^{a}	107.93 ± 1.21^{c}	109.76 ± 1.92^{c}	104.27 ± 2.74^{d}
28 days	162.52 ± 1.60^{a}	174.30 ± 5.25^{b}	172.80 ± 4.69^{b}	170.46 ± 1.86^{b}	158.94 ± 1.83^{a}	153.09 ± 6.91^{c}	152.79 ± 2.61^{c}	141.27 ± 3.07^{d}
35 days	192.92 ± 3.09^{a}	204.01 ± 6.42^{b}	202.90 ± 2.86^{c}	202.41 ± 4.00^{c}	185.83 ± 2.87^{d}	181.02 ± 6.64^{d}	182.12 ± 5.27^{d}	165.80 ± 4.84^{e}
FI (gr)								
7-14	84.96 ± 5.49	85.11 ± 0.71	72.59 ± 8.88	84.55 ± 4.97	80.13 ± 5.86	69.10 ± 0.94	69.00 ± 1.62	67.42 ± 2.66
14-21	114.68 ± 5.56	136.06 ± 3.68	135.39 ± 4.53	134.82 ± 0.22	114.57 ± 2.65	115.26 ± 9.37	114.60 ± 7.55	120.00 ± 4.67
21-28	178.12 ± 1.70	158.67 ± 11.58	163.81 ± 13.93	156.07 ± 7.76	163.03 ± 1.24	159.70 ± 5.95	150.08 ± 6.30	147.04 ± 4.25
28-35	153.85 ± 2.77	161.80 ± 5.17	148.43 ± 5.24	152.16 ± 3.15	148.26 ± 7.23	147.23 ± 2.32	155.97 ± 16.90	148.32 ± 6.51
7-35	531.60 ± 2.89	541.64 ± 11.48	520.21 ± 17.15	532.61 ± 5.17	506.01 ± 12.50	491.30 ± 14.19	489.66 ± 21.97	482.80 ± 6.46
FCR								
7-14	2.38 ± 0.14	2.32 ± 0.28	2.16 ± 0.23	2.36 ± 0.07	2.32 ± 0.12	2.43 ± 0.06	2.35 ± 0.18	2.47 ± 0.19
14-21	2.62 ± 0.06	2.40 ± 0.12	2.37 ± 0.22	2.42 ± 0.04	2.53 ± 0.12	2.57 ± 0.18	2.49 ± 0.08	2.78 ± 0.28
21-28	3.54 ± 0.13	3.51 ± 0.19	3.47 ± 0.20	3.46 ± 0.03	3.60 ± 0.08	3.61 ± 0.29	3.56 ± 0.38	3.97 ± 0.08
28-35	5.12 ± 0.35	5.46 ± 0.19	5.20 ± 0.83	4.99 ± 0.47	5.53 ± 0.27	5.39 ± 0.55	5.63 ± 0.65	6.10 ± 0.39
7-35	3.32 ± 0.06^{a}	3.20 ± 0.08^{a}	3.07 ± 0.10^{b}	3.17 ± 0.10^{a}	3.33 ± 0.12^{a}	3.36 ± 0.10^{a}	3.30 ± 0.12^{a}	3.64 ± 0.13^{a}

Pooled s.e.m. - pooled standard error of the mean

^{a,b,c,d,e} Means within rows with different superscripts differ significantly $P < 0.05$

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Table 5: The effects of the dietary inclusion of CEO, VC, and Cd on biochemical parameters of serum (TBARS; µM/mg protein and CP; nmol/mg protein), (SOD; % inhibition/mg protein, GPx; U/mg protein and CAT; U/mg protein), (TG, TC, HDL-C and LDL-C; mmol/L) during 35 days

Groups	Control mean ± sem	VC 500 mg/kg mean ± sem	CEO 450 mg/kg mean ± sem	CEO 100 mg/kg mean ± sem	VC 500 mg/kg + Cd 40 mg/l mean ± sem	CEO 450 mg/kg + Cd 40 mg/l mean ± sem	CEO 100 mg/kg + Cd 40 mg/l mean ± sem	Cd 40 mg/l mean ± sem
TBARS	1.99 ± 0.23^{a}	1.93 ± 0.30^{a}	1.87 ± 0.29^{a}	2.07 ± 0.28^{a}	2.45 ± 0.30^{ac}	3.19 ± 0.27^{c}	5.11 ± 0.53^{b}	5.41 ± 0.39^{b}
CP	0.57 ± 0.08^{a}	0.51 ± 0.07^{a}	0.49 ± 0.09^{a}	0.53 ± 0.05^{a}	0.66 ± 0.21^{a}	0.95 ± 0.17^{c}	1.63 ± 0.20^{b}	1.87 ± 0.26^{b}
SOD	19.35 ± 0.95^{a}	21.80 ± 2.05^{a}	20.72 ± 1.53^{a}	18.35 ± 1.01^{ac}	16.33 ± 0.90^{c}	15.08 ± 0.83^{c}	12.71 ± 0.79^{bc}	11.25 ± 0.28^{b}
GPx	29.94 ± 2.57^{a}	28.40 ± 2.13^{ac}	28.15 ± 2.09^{ac}	27.81 ± 2.29^{ac}	25.30 ± 2.41^{c}	24.19 ± 2.78^{c}	21.03 ± 2.11^{bc}	17.59 ± 1.08^{b}
CAT	25.41 ± 2.13^{ac}	27.83 ± 1.98^{a}	26.03 ± 1.02^{a}	24.12 ± 1.13^{ac}	21.18 ± 1.95^{c}	22.19 ± 1.76^{c}	16.80 ± 0.91^{b}	15.73 ± 0.79^{b}
TG	2.67 ± 0.11^{a}	2.54 ± 0.16^{a}	2.12 ± 0.09^{b}	2.59 ± 0.13^{a}	2.63 ± 0.15^{a}	2.18 ± 0.07^{b}	2.61 ± 0.15^{a}	2.69 ± 0.17^{a}
TC	4.37 ± 0.35^{a}	4.32 ± 0.19^{a}	3.21 ± 0.12^{b}	4.19 ± 0.28^{a}	4.38 ± 0.14^{a}	3.19 ± 0.09^{b}	4.29 ± 0.30^{a}	4.45 ± 0.23^{a}
HDL-C	1.72 ± 0.20^{a}	1.75 ± 0.09^{a}	2.25 ± 0.18^{b}	1.85 ± 0.19^{a}	1.66 ± 0.25^{a}	2.27 ± 0.14^{b}	1.79 ± 0.12^{a}	1.63 ± 0.21^{a}
LDL-C	1.43 ± 0.11^{a}	1.41 ± 0.13^{a}	0.51 ± 0.03^{b}	1.15 ± 0.11^{a}	1.52 ± 0.11^{a}	0.62 ± 0.09^{b}	1.33 ± 0.16^{a}	1.59 ± 0.15^{a}

Pooled s.e.m. - pooled standard error of the mean ^{a,b,c} Means within rows with different superscripts differ significantly $P < 0.05$

Table 6: The effects of the dietary inclusion of CEO, VC, and Cd on MDA (mg/kg) concentration in muscles and the liver during 35 days

MDA	Control	VC 500 mg/kg	CEO 450 mg/kg	CEO 100 mg/kg	VC 500 mg/kg + Cd 40 mg/l	CEO 450 mg/kg + Cd 40 mg/l	CEO 100 mg/kg + Cd 40 mg/l	Cd 40 mg/l
	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem
Muscle	0.73 ± 0.039^{a}	0.62 ± 0.066^{a}	0.46 ± 0.058^{b}	0.68 ± 0.028^{a}	1.04 ± 0.021^{c}	0.86 ± 0.023^{d}	1.50 ± 0.038^{e}	1.69 ± 0.088^{f}
Liver	1.22 ± 0.087^{a}	0.78 ± 0.111^{bc}	0.60 ± 0.052^{c}	0.88 ± 0.83^{b}	1.21 ± 0.086^{ad}	1.25 ± 0.041^{ad}	1.56 ± 0.010^{d}	1.93 ± 0.019^{e}

Pooled s.e.m. - pooled standard error of the mean ^{a,b,c,d,e,f} Means within rows with different superscripts differ significantly $P < 0.05$

Table 7: The effects of the dietary inclusion of CEO, VC, and Cd on bioaccumulation of Cd in muscles and the liver (µg/g) during 35 days

Cd	Control	VC 500 mg/kg	CEO 450 mg/kg	CEO 100 mg/kg	VC 500 mg/kg + Cd 40 mg/l	CEO 450 mg/kg + Cd 40 mg/l	CEO 100 mg/kg + Cd 40 mg/l	Cd 40 mg/l
	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem	mean ± sem
Muscles	0.18 ± 0.032^{a}	0.155 ± 0.050^{a}	0.27 ± 0.016^{b}	0.315 ± 0.074^{b}	1.25 ± 0.045^{e}	0.86 ± 0.149^{c}	1.79 ± 0.058^{d}	2.34 ± 0.064^{f}
Liver	0.53 ± 0.043^{a}	0.86 ± 0.062^{a}	0.66 ± 0.15^{a}	0.74 ± 0.20^{a}	32.56 ± 1.24^{c}	26.08 ± 1.28^{b}	36.93 ± 1.66^{d}	41.56 ± 1.80^{e}

Pooled s.e.m. - pooled standard error of the mean ^{a,b,c,d,e,f} Means within rows with different superscripts differ significantly $P < 0.05$ Detection limit: 0.01985 (µg/g).

Discussion

CEO ingredients

The main components of CEO in the current study were found to be eugenol (77.63%), β-caryophyllene (9.54%), and eugenol acetate (7.07%), being consistent with the results of Yu et al. [23]. However, another study reported eugenol and eugenol acetate as the major constituents [4]. This difference could be associated with parameters, such as weather conditions, soil composition, as well as genetic particulars, age, maturity stage, type of plant sections, and distillation protocols [24]. It was considered that eugenol and caryophyllene existed in the CEO serves as strong free radical scavengers by giving a hydrogen atom from their -OH groups [24].

Effects of Cd and CEO on growth performance parameters

In the present study, FI and FCR remained unchanged, in contrast to BW, as affected by Cd. This is inconsistent with other studies reporting inhibitory effects of Cd on FI and FCR [3, 25]. In this study, BW decreased only at the end of the trial. The progressive loss of BW in the current study indicated the cumulative property of Cd, which might be associated with the effect of metallothioneins (MTs) [5]. Adverse effects of Cd on BW might be related to its toxicity, which affects almost all the body organs [26]. Furthermore, the growth-retarding effect of Cd could be attributed to the general systemic toxemia induced by this metal, which eventually results in the loss of appetite and weight [5]. In a study, long-term exposure to Cd led to the depletion of the liver and muscular glycogen due to alterations in the function of enzymes involved in the glycogenesis process, which ended up in weight loss [3]. Moreover, Cd increases the size of the liver due to the infiltration of inflammatory cells, accumulation of fat in liver cells, and congestion of liver tissue [26]. In this regard, other scientists have reported that the dietary administration of Cd resulted in a remarkable reduction of BW in Japanese quails [3, 26-29] and other birds [5, 25, 27].

Nevertheless, the depressive effect of Cd on BW was partially reduced by the improved impact of the CEO supplement. However, the mitigating impact of CEO on BW was lower than that of VC. Based on the findings, CEO could be served as a growth promoter due to (i) its antimicrobial properties that compete with pathogenic microflora in the gastrointestinal tract of quails, thereby reducing their fatality during the growth period; (ii) its ability to improve palatability of foodstuffs, thereby stimulating appetite and FI; and (iii) its ability to enhance nutrient digestibility by increasing secretion of digestive enzymes. Furthermore, CEO is a valuable source of manganese, trace minerals, as well as a minor source of omega three fatty acids and vitamins K and C, which are crucial for improving growth performance [30]. Considering the results, a study described beneficial effects of CEO on BW [31], while another research reported no effect [32].

Effects of Cd and CEO on biochemical parameters of the serum and tissues

Based on the results of the present study, CEO reduced oxidative stress induced by Cd, as evidenced by lower concentrations of TBARS and CP, higher activities of SOD, GPx, and CAT, more improved lipid profile, and lower accumulation of Cd, compared to the positive control group. Moreover, antioxidant properties of CEO were dose-dependent.

SOD, GPx, and CAT are endogenous antioxidant enzymes considered as indicators of liver functions. In fact, they are supposed to prepare cellular protection despite the devastation provoked by free radicals or ROS [5, 33]. SOD destroys the superoxide radicals by converting them to peroxides (H_2O_2) which are further neutralized by catalase or GPx [4, 34]. Measurement of these enzymes activities and entire antioxidant status of blood were studied to appraise oxidative stability [13, 33]. Cd induces oxidative stress by disturbing oxidative and antioxidative balance in tissues. This happens through (i) interacting with subcellular sites, like mitochondria, peroxisomes, and microsomes, resulting in excessive generation of free radicals or ROS that are highly reactive and

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attack biomolecules, including DNA, proteins, and lipids. MDA (TBARS) is a terminal product and an indicator of lipid peroxidation. In addition, carbonyl protein (CP) is the final product and the indicator of protein oxidation [35]. Besides, it happens through (ii) depleting the capacity of antioxidant enzymes, which might be attributed to the binding capability of Cd to -SH groups in enzymes, or it might be because of the decreased availability of bio-elements in compositions of antioxidant enzymes through their inactivation in the metallothionein attached form. It also might be due to the ability of Cd to substitute metal ions in antioxidant enzymes and to produce an immobile form of the enzyme, thereby leading to an increase in the levels of MDA and CP and ultimately leading to the cell damage (necrosis or apoptosis) [6, 17, 33]. The findings of the current study are consistent with those of previous studies that concluded that Cd suppressed activities of hepatic antioxidant enzyme [6, 26, 27, 36]. However, another researcher concluded that the values of CAT and SOD increased, followed by increased levels of TBARS, as a result of Cd-intoxication in poultry birds [33].

On the other hand, some studies reported that CEO had pharmacological features, such as antioxidant, anti-inflammatory, anti-atherogenic, hypolipidemic, and hepatoprotective activities, being attributed to its high number of phenolic compounds (eugenol and caryophyllene) [12, 24, 37]. They indicated a strong relationship between the level of total phenolic compounds of plant species and their antioxidant activities [10]. CEO is a potent free radical scavenger and a metal chelator due to its hydrogen donating property from its hydroxyl and carbonyl groups in its aromatic ring [37]. In addition, it acts as a hepato-protective agent by manipulating cell membrane permeability and preventing entrance of hepatotoxic substances to hepatocytes [12]. Compared to the lipid-lowering effect of lovastatin, eugenol lowered the concentration of TC, TG, and LDL by 55.88%, 79.48%, and 64.30%, respectively, thereby exerting anti-hyperlipidemic effects [38]. In line with the present study results, a study reported a decrease in TC, TG, and LDL-C concentrations, along with an increase in HDL-C, CAT, SOD, and GPx values, in quails supplemented with CEO [12].

According to the current study, Cd had no effect on the lipid profile of quails. Inconsistent with this finding, some studies indicated that Cd increased the risk of dyslipidemia, mainly due to the low levels of HDL-C and high levels of TC, TG, and LDL-C, resulting in atherosclerosis [33, 39]. However, in the present study, groups containing CEO (450 mg/kg) showed an increase in the levels of HDL-C and a reduction in the levels of TC, TG, and LDL-C, indicating the hepatoprotective effect of CEO. Inconsistent with the present study results, a study reported a decrease in the serum levels of TC and TG due to the exposure to Cd in common carp [40]. Accordingly, it was reported that CEO in combination with Argan oil (100 mg/kg/BW) had no effect on the lipid profile of rats fed with H_2O_2 [4]. Another study reported that TC, TG, and HDL-C were not affected by the administration of dietary CEO supplements in broilers [41]. Moreover, a study reported that the serum lipid profile of broilers was not changed by the dietary treatment of EO [32, 42].

Effects of Cd and CEO on bioaccumulation of Cd in tissues (muscles and the liver)

Cd is a cumulative toxic metal distributed in various tissues, but the liver seems to be the first place to store it [27, 39]. Cd levels in the liver are the primary indicator of Cd bioaccumulation in other organs, like muscles. Further absorption of Cd enhances accumulation of Cd in other parts of the body [6, 27]. A recent study revealed that Cd aggregation increased progressively in muscles, in a dose-dependent manner, unlike the liver. The highest accumulation in the liver was at a dose of 75 mg/kg, which decreases by rising the dose level and accumulates in muscles [1]. This finding is highly consistent with the current study. Cd was administrated in the study at 40 mg/l, in which the liver deposition level was higher than that of the muscles. According to the results, Cd deposition in the tissues was effectively reduced by CEO. In addition, its mode of action is associated with the presence of hydroxyl (-OH)

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and carbonyl (C = O) functional groups in their composition, which compete with Cd for sulfhydryl binding sites on metallothioneins [37]. Thus, it reduces intestinal absorption of Cd and its accumulation. It seems that the present study reported the effect of CEO on the selected parameters in Japanese quails for the first time.

Conclusion

Due to the unavoidable exposure of poultries to heavy metals through various sources, including diet, water, soil, and air, the use of natural antioxidants especially PEOs might reduce their body accumulation, thereby decreasing the risk of the induced oxidative stress and hepatotoxicity of these toxic metals. Moreover, they improve the quality of meat and increase its shelf life. In general, CEO (450 mg/kg) was potentially as effective as or even more potent than VC (500 mg/kg) in ameliorating adverse effects of Cd. However, further studies are required to clarify the minimum concentration of COE that could be applied in poultry nutrition to achieve the intended effects.

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Conflict of interest

The authors declare that there is no conflict of interest.

Abbreviations

BW: Body weight; FI: Feed intake; FCR: Feed conversion ratio; Plant essential oils: PEOs; Clove essential oil: CEO; Vitamin C:VC; Cadmium: Cd; TBARS: Thiobarbituric acid reactive substances; MDA: malondialdehyde, CP: Carbonyl protein; CAT: Catalysis; SOD: super oxide dismutase; GPx: glutathione peroxidase; TG: Triglyceride; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol; ROS: Reactive oxygen specious; GC-MS: Gas chromatography-mass spectrometry.

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