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Dietary Supplementation of Clove (*Syzygium Aromaticum*) Essential Oil Improves Growth Performance, Oxidative indices, and Lipid Profile of Japanese Quails Exposed to Lead

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ABSTRACT

Introduction: The current survey investigated the alleviating effect of dietary clove (*Syzygium aromaticum*) essential oil (CEO) in comparison with vitamin C (VC), against the adverse effects of Pb on growth performance, serum oxidative indices, and lipid profile in Japanese quails (*Coturnix japonica*), following oral administration.

Methods: 480 one-day-old quails were randomly segregated into 8 groups, which fed with the following diets, via 35 days: basal diet (negative control), basal diet + VC (500 mg/kg), basal diet + CEO (450 mg/kg), basal diet + CEO (100 mg/kg), basal diet + VC (500 mg/kg) + Pb (100 mg/l), basal diet + CEO (450 mg/kg) + Pb (100 mg/l), basal diet + CEO (100 mg/kg) + Pb (100 mg/l), and basal diet + Pb (100 mg/l) (positive control). The data were analyzed using a one-way analysis of variance and Duncan's post hoc test.

Results: Quails exposed to Pb and treated with CEO had reduced oxidative stress as evidenced by lower concentrations of TBARS and CP, higher activities of SOD, GPx, and CAT, and more improved lipid profile, compared to positive control. Moreover, the alleviating effects of CEO were dose-dependent.

Conclusion: The CEO (450 mg/kg) was potentially as effective as or even more potent than VC (500 mg/kg) in alleviating the adverse effects of Pb.

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Introduction

Heavy metal pollution, such as lead (Pb) contamination, has become an actual significant health problem for humans and animals. Pb is one of the most well-recognized prevalent environmental toxicants, with cumulative and non-biodegradable nature (1-3), which raised the risk of their possible transmission from animals' meat to humans' bodies through the food chain (1). FAO and WHO have recognized no safe threshold of Pb in humans (4). Pb affects a broad range of body systems and leads to chronic and acute complications, including carcinogenicity, immunotoxicity, neurotoxicity, growth retardation, hearing loss, short-term memory disorder, intelligence decrease, and even death (2, 3). The main mechanisms of its toxic action have been announced to provoke oxidative stress and boost the production of reactive oxygen species (ROS), thereby leading to cell structure impairment through DNA and protein oxidation and also lipid peroxidation (1, 2, 5). The antioxidant defense mechanism of the body could also be disrupted by Pb (2).

According to the aforementioned, applying approaches to lowering these toxic metals seems to be a health priority. With respect, the application of natural substances like plant essential oils (PEOs), due to having both antioxidant and chelating properties, has been discussed to be a good candidate for protection against Pb toxicity (1, 6). In this respect, clove (*Syzygium aromaticum*), a spice plant and an FDA-approved food additive, was highlighted (7). Clove essential oil (CEO) is a primary source of polyphenols, especially eugenol which represents 89% of this oil, so it is a potent radical

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scavenger (8). There is a strong relationship between the polyphenol percentage and the antioxidant properties of essential oils (9, 10).
Ultimately, the Japanese quail (*Coturnix japonica*) was nominated to be used in the current study due to being a well-known laboratory animal model (3) and also being a valuable source of meat and egg (11, 12). Therefore, the current study was designed to evaluate the alleviating effect of CEO in comparison with VC against the toxic effects of Pb on the growth performance, serum oxidative indices, and lipid profile of Japanese quails.

Materials and Methods

Sample Collection and Preparation

The current survey was performed in the Poultry House of the Department of Animal Nutrition, Shahrekord University, Chaharmahal and Bakhtiari, Iran (Ethical code: IR.SKU.REC.1392.122.531).With a magnitude of 3.5 m in width × 4 m in length × 17 m in height. All experimental procedures were carried out according to the animal welfare guidelines of the Veterinary Control and Research Institute of Shahrekord University, Iran. 480 one-day-old chicks were purchased from a local farm in Chaharmahal and Bakhtiari province, Iran. And fed with a basic diet for up to seven days. At 7 days after weighing, the chicks with equal initial body weight were used in a randomized design trial with eight groups, each consisting of 15 pieces, with three replications. Briefly, quails were housed in an environmentally controlled pen under a 24 lighting cycle and following a standard temperature, which gradually decreased from 36 to 25 °C, at the rate of 2 °C per week. They were fed basal diets (Table 1) and daily refreshed water for 35 days. Quails were vaccinated against Newcastle by B1 serotype on day 7th. The experimental treatments were as listed: 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) + Pb (100 mg/l); 6. Basal diet + CEO (450 mg/kg) + Pb (100 mg/l); 7. Basal diet + CEO (100 mg/kg) + Pb (100 mg/l); 8. Basal diet + Pb (100 mg/l) as lead acetate in water (positive control).
On the 35th day, 30 blood samples from each group were randomly collected and stored in sterilized test tubes after that their serum was isolated. To separate the serums, the samples were allowed to clot for 30 min at room temperature and then centrifuged at 5000 rpm for 10 min. All serum samples were stored at -70°C before analysis.

Growth Performance Measurement

-Bodyweight (BW)
At the end of each week, after being starved for 2 hours, the chicks in each group were weighed individually, and the average weight was documented.
-Feed intake (FI)
The amount of grain given to each treatment group was recorded daily for five weeks, and at the end of each week, the leftover grain was collected and weighed and the amount of feed consumed was deliberated.
-Feed conversion ratio (FCR) was calculated based on the following equation:
FCR = total feed consumed weekly / (initial weight) - (weight of losses + final weekly weight)
-Identification of volatile oil compounds (GC / MS)
The pure CEO was obtained from Barij Essence Company (Kashan, Iran). The gas chromatography-mass spectrometry (GC-MS) analyses were carried out using an Agilent Technologies GC (Model HP-7890, Palo Alto, CA, USA), with a capillary column (Model HP-5MS, length: 30 m, film thickness: 0.25 µm, internal diameter: 0.25 mm), coupled with a mass spectrometer (Model HP 5975, Agilent Technologies, Palo Alto, CA, USA), which have an electron impact ionization potential (70 eV). The oven temperature was initially held at 60°C for 5 min and gradually was 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 reached 290°C, and then maintained at this degree for 3 min. Helium was used as the inert gas, which 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. The separation ratio was set to 100: 1. The mass range was 50-50 m / z. Quantitative data were obtained by using the area of the peaks. The EO ingredients were quantified by assimilating their retention indexes (Table 2) relative to a set of n-alkanes (C5 to C25), available in literature or our laboratory, and confirmed by matching their mass spectra analysis patterns. The essential oil dosage was determined based on previous studies (13, 14) .
-Measurement of biochemical parameters

The thiobarbituric acid reactive substances (TBARS) value was calculated based on the following formula and represented as mg malondialdehyde (MDA)/kg of serum samples (15):

TBARS = (A × 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).

The serum carbonyl protein (CP) was measured by the Levine et al. (1990) (16) method.

The activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were calculated according to the methods described by Sun, Oberley, and Li (1988) (17), Goth (1991) (18), and Paglia and Valentine (1967) (19), respectively.

Triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations were evaluated by commercial kits (Pars Azmoon, Iran). Serum low-density lipoprotein cholesterol (LDL-C) concentration was acquired based on the Friedewald formula [LDL-C = (TC) – (HDL-C) – TG/2.2] (20).

-Statistical analyses

All data were evaluated by one-way ANOVA, accompanied by Duncan's test in SPSS software (version 22). The values of all the Pb intoxicated groups were compared to the control group and the significance level was considered as $P < 0.05$. The values were described as mean ± SEM.

Table 1. Composition of basal diets during the experiment

Ingredient (g/kg)	0-35 days
Maize	509.6
Soybean meal	438.4
Soybean oil	20.6
Calcium carbonate	12.6
Dicalcium phosphate	8.3
DL- Methionine	1.6
Mineral premix[a]	2.5
Vitamin premix[b]	2.5
Salt	1.6
vitamin D_3[c]	1
vitamin E[d]	1.5
Calculated analysis	
Metabolizable energy (KCal/kg)	2850
Crude protein%	25.3
Calcium%	0.8
Phosphorus%	0.3
Sodium%	0.15
Lysine%	1.34
Methionine%	0.5
Arginine%	1.57
Methionine + cysteine%	0.84
Threonine%	1.02
Valin%	1.12
Leucine%	1.8
Isoleucine%	1.22
Histidine%	0.22
Phenylalanine%	1.13
Mn (mg)	21.17
Fe (mg)	146.6
Cu (mg)	17.44
Zn (mg)	41.35
Se (mg)	0.11

Composition of basal diets' ingredients were calculated based on NRC table.

[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: 200000 IU vitamin A, 80000 IU vitamin D_3, 1600 IU vitamin E, 700 mg vitamin B_{12}, 35 mg vitamin K_3, 1200mg vitamin C, 30 mg vitamin B_1, 130 mg vitamin B_2, 1300 mg nicotinic acid, 225 mg panthotenic acid, 8200 mg choline chloride, 3.3 mg biotin.

[c] Provided per kilogram of vitamin D_3: 100000 IU

[d] Provided per kilogram of vitamin E: 100000 IU

Table 2. Constituents of *CEO* and their Relative Percentages of Retention time, Kovats Index and Total Chromatogram Area.

Ingredients	Retention time (min)	Kovats Index	Area (%)
Eugenol	18.175	1365.335	77.63
Iso-eugenol	18.86	1387.06	0.65
β-Caryophyllene	19.937	1421.934	9.54
α-Humulene	20.984	1456.262	1.33
delta-Cadinene	23.07	1524.656	0.2
Eugenol acetate	24.852	1587.019	7.07
Caryophyllene oxide	24.852	1587.019	0.28

Results

GC/MS Identification of CEO

The major abundant components of the CEO were shown in Table 2.

Growth Performance (BW, FI, FCR)

Based on Table 3, at 28 and 35 days of age, BW was significantly (P < 0.05) improved in CEO (450 mg / kg), CEO (100 mg / kg), and VC (500 mg / kg) groups, while it was significantly (P < 0.05) reduced in Pb (100 mg / l) group, compared to the control group. Nevertheless, it remained unchanged in all Pb-exposed groups supplemented with dietary inclusions (CEOs and VC), compared to the control group. Expect, in the CEO (100 mg/kg) group, on day 35th, which was significantly (P < 0.05) decreased, compared to the control group. Additionally, there was no significant (P < 0.05) difference in FI and FCR during the whole experiment. Overall, the CEO (450 mg/kg) was as effective as VC in alleviating the adverse effect of Pb on growth performance parameters.

Table 3. The effect of the dietary inclusion of clove essential oil and vitamin C and lead acetate on body weight (BW; gr) and feed intake (FI; gr) and feed conversion ratio (FCR) of quails (0-35 days)

Groups	Control	Vit C 500 mg/kg	Clove oil 450 mg/kg	Clove oil 100 mg/kg	Vit C 500 mg/kg + Pb 100 mg/l	Clove oil 450 mg/kg + Pb 100 mg/l	Clove oil 100 mg/kg + Pb 100 mg/l	Pb 100 mg/l
	mean ±sem	sem ± mean	sem ± mean	sem ± mean	sem ± mean	sem ± mean	sem ± mean	sem ± mean
BW (gr)								
7 days	32.85±0.51	34.15±0.82	33.33±0.38	33.83±1.23	33.88±0.34	32.82±0.62	34.08±0.58	32.80±0.86
14 days	68.13±0.93	71.70±3.52	67.33±4.26	69.74±3.31	69.71±0.97	62.80±1.33	64.20±1.75	62.88±1.17
21 days	112.03±2.90	128.55±1.68	125.18±4.15	125.39±3.46	119.83±8.15	123.57±4.07	119.39±2.17	110.40±1.37
28 days	162.52±1.61^{a}	174.30±5.25^{b}	172.80±4.69^{b}	170.47±1.87^{b}	165.00±2.50^{a}	164.83±3.70^{a}	164.20±0.90^{a}	150.57±1.53^{c}
35 days	192.92±3.09^{b}	204.01±6.42^{a}	202.91±2.86^{a}	202.41±4.00^{a}	192.78±4.72^{b}	196.69±2.58^{b}	189.99±0.58^{c}	179.05±1.12^{d}
FI (gr)								
7-14	84.96±5.49	85.11±0.71	72.59±8.88	84.55±4.98	77.97±1.07	71.47±4.24	71.67±3.73	82.55±1.82
14-21	114.68±5.56	136.06±3.68	135.39±4.53	134.83±0.23	137.50±11.60	149.90±0.90	142.72±4.72	140.40±1.37
21-28	178.12±1.70	158.67±11.58	163.81±13.93	156.07±7.77	162.69±11.99	156.55±4.70	163.60±3.33	164.31±4.92
28-35	153.85±2.77	161.80±5.17	148.43±5.79	157.17±3.16	162.56±10.40	157.36±2.79	145.48±2.49	167.33±0.82
7-35	531.60±2.89	541.64±11.48	520.22±17.15	532.62±5.17	540.71±22.26	535.28±4.88	523.47±10.59	554.59±5.78
FCR								
7-14	2.41±0.16	2.33±0.28	2.16±0.24	2.36±0.07	2.18±0.04	2.38±0.09	2.41±0.26	2.74±0.05
14-21	2.62±0.06	2.40±0.12	2.37±0.23	2.43±0.05	2.83±0.25	2.48±0.12	2.60±0.08	2.97±0.13
21-28	3.54±0.14	3.52±0.19	3.47±0.20	3.46±0.04	3.73±0.43	4.01±0.62	3.68±0.27	4.09±1.14
28-35	5.12±0.36	5.46±0.19	5.20±0.84	4.99±0.47	5.87±0.13	5.12±0.71	5.65±0.19	5.88±0.08
7-35	3.32±0.06^{b}	3.20±0.8^{c}	3.07±0.10^{e}	3.17±0.10^{d}	3.40±0.04^{b}	3.27±0.03^{c}	3.36±0.7^{b}	3.80±0.09^{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.

BW: Body Weight, FI: Feed Intake, FCR: Feed Conversion Ratio.

Biochemical Analyses of Serum on Day 35th

According to Table 4, a significant (P < 0.05) enhancement in TBARS and CP levels has been observed in Pb (100 mg / l) and CEO (100 mg / kg) + Pb groups. Moreover, TBARS value was significantly (P < 0.05) increased in CEO (450 mg / kg) + Pb group, while remained unchanged in VC + Pb group, compared to control. While, CP values remained unchanged in CEO (450 mg / kg) + Pb and VC + Pb groups, compared to control. Consequently, it has been determined that CEO (450 mg/kg) was as effective as VC in restoring these values to that of control in the presence of Pb.

Moreover, a significant (P < 0.05) reduction in CAT, SOD, and GPx activities has been detected in Pb (100 mg / kg) group, compared to control. Furthermore, the activities of SOD and GPx, despite CAT, which remained unchanged, were significantly (P < 0.05) decreased in the CEO (100 mg/kg) + Pb group, compared to the control. Eventually, CEO (450 mg/kg) was as effective as

VC in alleviating the adverse effect of Pb on the activities of antioxidant enzymes.

In addition, the contents of the serum lipid profile (TG, TC, HDL-C, and LDL-C) remained unchanged in all groups compared to the control group. Except in CEO (450 mg/kg) and CEO (450 mg/kg) + Pb groups, which showed a reduction for TG, TC, and LDL-C and an increment for HDL-C. So, the CEO (450 mg/kg) was a more positively improved serum lipid profile than VC.

Table4. Serum analysis on day 35th; (TBARS; µM/mg protein), (CP; nmol/mg protein), (TG, TC, HDL-C and LDL-C; mmol/L), (CAT; U/mg protein), (SOD; % inhibition/mg protein) and (GPx; U/mg protein)

Groups	control	Vit C 500 mg/kg	Clove oil 450 mg/kg	Clove oil 100 mg/kg	Vit C 500 mg/kg + Pb 100 mg/kg	Clove oil 450 mg/kg + Pb 100 mg/kg	Clove oil 100 mg/kg + Pb 100 mg/kg	Pb 100 mg/kg
	mean ±sem	sem ± mean	sem ± mean	sem ± mean	sem ± mean	sem ± mean	sem ± mean	sem ± mean
Antioxidant Status								
TBARS	1.99±0.23^{a}	1.93±0.30^{a}	1.87±0.29^{a}	2.07±0.28^{a}	2.23±0.39^{ac}	3.07±0.22^{c}	4.18±0.45^{bc}	4.99±0.32^{b}
CP	0.57±0.08^{a}	0.51±0.07^{a}	0.49±0.09^{a}	0.53±0.05^{a}	0.65±0.19^{a}	0.63±0.12^{a}	1.48±0.22^{b}	1.56±0.18^{b}
CAT	25.41±2.13^{a} b	27.83±1.98 a	26.03±1.02 a	24.12±1.13^{a} b	22.83±1.95 b	25.11±1.20^{a} b	22.36±0.85 b	16.93±0.99 c
SOD	19.35±0.95^{a}	21.80±2.05 a	20.72±1.53 a	18.35±1.01^{a}	18.97±1.76 a	18.09±1.52^{a}	13.90±0.71 b	12.35±0.58 b
GPx	29.94±2.57^{a}	28.40±2.13 a	28.15±2.09 a	27.81±2.29^{a}	23.18±2.17 a	23.95±2.98^{a}	15.81±0.63 b	13.39±0.70 b
Lipid Profile								
TG	2.67±0.11^{a}	2.54±0.16^{a}	2.12±0.09^{b}	2.59±0.13^{a}	2.57±0.09^{a}	2.15±0.08^{b}	2.62±0.17^{a}	2.70±0.20^{a}
TC	4.37±0.35^{a}	4.32±0.19^{a}	3.21±0.12^{b}	4.19±0.28^{a}	4.40±0.17^{a}	3.15±0.08^{b}	4.33±0.19^{a}	4.50±0.31^{a}
HDL-C	1.72±0.20^{a}	1.75±0.09^{a}	2.25±0.18^{b}	1.85±0.19^{a}	1.63±0.22^{a}	2.21±0.15^{b}	1.75±0.22^{a}	1.69±0.14^{a}
LDL-C	1.43±0.11^{a}	1.41±0.13^{a}	0.51±0.03^{b}	1.15±0.11^{a}	1.59±0.11^{a}	0.59±0.07^{b}	1.38±0.12^{a}	1.57±0.18^{a}

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

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

TBARS: Thiobarbituric Acid Reactive Substances, CP: Carbonyl Protein, CAT: Catalase, SOD: Superoxide Dismutase, GPx: Glutathione Peroxidase, TG: Triglyceride, TC: Total Cholesterol, HDL-C: High-Density Lipoprotein Cholesterol, LDL-C: Low-Density Lipoprotein Cholesterol.

Discussion

Pb is a ubiquitous and long-standing environmental toxicant (3) that can induce adverse effects like growth retardation stress while CEO has alleviating properties like growth promotive and oxidation mitigative effects (9). So, the CEO was assumed as a compensatory agent that can protect quails from Pb toxic effects. To confirm this assumption, this study was carried out to evaluate the alleviating effect of CEO vs. the adverse effects of Pb on mentioned parameters. Furthermore, vitamin C (VC) is an accepted and well-known antioxidant, that has been used widely in the poultry nutrition industry to increase its antioxidant capacity (21, 22) and improve its growth performance (23). It is a terminal reductant ROS scavenger that can inhibit lipid peroxidation (24) and exhibit a hypolipidemic effect (25). Thus, it is eligible to study the ability of the CEO in comparison with VC, as a scale, in alleviating the oxidative stress induced by Pb.

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%), which were in good accordance with recent findings of Yu et al. (2017) (26). While another study reported eugenol and eugenol acetate as the major constituents (27). These differences could be associated with such parameters as weather conditions, soil composition, genetics, age, stage of maturity, type of plant sections, and distillation protocols (28).

Effects of Pb and CEO on Growth Performance Parameters

In the present study, despite BW, the values of FI and FCR have remained unchanged, as affected by Pb, which is in line with other results (27, 29). Dislike other reports that showed an inhibitory effect of Pb on FI and FCR (1, 30). In the current research, BW was only decreased at the end of the trial, which showed the cumulative effect of

Pb. The growth-retarding effect of Pb in poultries is evidenced by poor performance, weight loss, loss of visceral and subcutaneous fat, atrophy of breast muscle, and even starvation of birds, which is mainly and primarily associated with inducted anorexia by this metal (1). Although the exact mechanism by which Pb induces anorexia is not well recognized (31), it is hypothesized that the possible main mechanisms of Pb for inducing anorexia are probably as follows: (i) muscular paralysis of the digestive system and cellular deteriorations which induced by oxidative stress; (ii) suppression of nutrients absorption by downregulation of main nutrient transporter genes in the small intestine; (iii) induction of metabolic disorders by inhibition of glycol-metabolism and haem synthesis key enzymes (30, 32). In line with our results, other scientists have demonstrated that anorexia is a primary characteristic of Pb toxicity in the Japanese quail, which results in poor performance (9, 33).

Nevertheless, in the present study, the depressive effect of Pb on BW has recovered by the improving impact of the CEO (450 mg/kg) supplement. And the alleviating effect of CEO (450 mg/kg) on BW was the same as VC. It has been demonstrated that CEOs can serve as growth promotors in the poultry industry due to: (i) their antimicrobial properties, which repress pathogen colonization in the gastrointestinal tract of quails and therefore lessen their fatality during the growth period; (ii) their ability to improve the palatability of foodstuffs, which stimulate appetite and FI; (iii) their ability to enhance nutrients digestibility by increasing digestive enzymes and bile salt secretion. Furthermore, CEO is a valuable source of manganese, trace minerals, and a minor source of omega 3 fatty acids and vitamins K and C, which are crucial for improving growth performance (8, 9). Under our findings, many investigations have also described affirmative effects of CEO on BW (9, 10), while other researchers reported no effect (8, 25).

Effects of Pb and CEO on Biochemical Parameters of Serum

According to our findings, the CEO had reduced oxidative stress induced by Pb, as evidenced by lower concentrations of TBARS and CP, higher activities of SOD, GPx, and CAT, and a more improved lipid profile of serum, compared to the positive control group. Moreover, the antioxidant properties of CEO were dose-dependent.

Pb induces oxidative stress by disturbing the oxidative and antioxidative balance of the cells and ultimately leads to cell apoptosis (3, 5, 24), via (i) promoting the surplus production of ROS, which are highly reactive and attack substantial biomolecules in cells including DNA, proteins, and lipids (9). MDA (TBARS) is a terminal product and the indicator of lipid peroxidation. The final product and the protein's oxidation indicator is carbonyl protein (CP) (8). (ii) depleting the antioxidant enzymes (SOD, GPx, and CAT) capacity, which might be attributed to the high affinity of Pb to sulfur and selenium in these enzymes and producing an immobile form of the enzyme or might be due to the capability of Pb to suppress the uptake of selenium and ferric, as enzymes' co-factors, from the gastrointestinal tract (3). The findings of the present study were following previous studies, which concluded that Pb suppressed the activities of antioxidant enzymes, and elevated the TBARS levels in Japanese quail (3, 9). Whereas, a recent study announced that the activities of serum CAT and GPx were increased in poultries treated with Pb (34). In addition, another researcher detected no significant changes in CAT, SOD, and GPx activities, as a result of Pb-intoxication in Japanese quail (5).

In light of our results, Pb did not affect the lipid profile of quails. The contrary to the reports which elucidated that Pb increases the risk of dyslipidemia, as implicated by high levels of TC, TG, and LDL-C and low levels of HDL-C, which results in atherosclerosis and other cardiovascular diseases (1, 2, 9). In contrast to our result, an article claimed no significant alterations in TC, along with a reduction in the level of TG as Japanese quails exposed to Pb (35). On the other hand, our results have depicted that CEO (450 mg/kg) has positively improved the lipid profile of serum by increasing the level of HDL-C and decreasing the levels of TC, TG, and LDL-C. It has been confirmed that CEO is a potent free radical scavenger and metal chelator due to its hydrogen donating property from its hydroxyl and carbonyl groups in its aromatic ring (28). It has pharmacological activities such as antioxidant, anticancer, anti-inflammatory, anti-atherogenic, hypolipidemic, and hepatoprotective activities, which are attributed to its high amount of phenolic compounds (i.e.,

eugenol and caryophyllene) (28). It acts as a hypocholesteric agent by inhibiting a key regulatory enzyme in cholesterol synthesis (25) and also acts as a hepatoprotective agent by manipulating cell membrane permeability and preventing the entrance of hepatotoxic substances to hepatocytes (9). Accordingly, the anti-obesity effects of CEO by reducing the serum TG and TC levels were well established (28). Compared to the lipid-lowering drug lovastatin, eugenol lowered the concentration of TC, TG, and LDL by 55.88%, 79.48%, and 64.30%, respectively, thereby exerting anti-hyperlipidemic effects (9). Dislike our findings, other authors have declared that the serum lipid profile of broilers was not changed by any dietary inclusions of CEO (36) or other EOs supplements (8, 37).

Limitation

This study had some limitations, including the environmental concerns regarding the disposal water which contained lead. Furthermore, due to the low volume of blood in quail, the birds had to be slaughtered in order to obtain a sufficient volume of blood for serum tests. So, in order to comply with the ethics of sampling, the blood sampling was limited to the last day of the breeding period, which was the slaughter time and fewer birds were killed.

Conclusion

Overall, it seems that, due to the unavoidable exposure of poultries to heavy metals through various sources (including diet, water, soil, air, etc.), the use of natural antioxidants, especially PEOs, in poultry nutrition, was found to help reduce their risks. With respect, we have observed that CEO (450 mg/kg) was well managed to compensate for the adverse effects of Pb on growth performance, serum antioxidant status, and lipid profile, as compared to VC (500 mg/kg). It was as effective as or even more potent than VC (500 mg/kg) in alleviating the adverse effects of Pb.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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