

Dietary supplementation of Eucalyptus leaves enhances eggshell quality and immune response in two varieties of Japanese quails under tropical condition

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ABSTRACT The effect of dietary supplementation of Eucalyptus leaves (EL) powder on productive performance and immune response in 2 varieties of Japanese quail was investigated. A total of 180 twelve-week-old laying Japanese quails from 2 color varieties (gray and white) were randomly assigned and distributed according to a completely randomized design in a 3 × 2 factorial arrangement (dietary treatment × variety) forming 6 subgroups (30 each). EL were mixed with the diet in 3 levels (0, 0.1, and 0.2%). Each hen was individually housed in a wire cage of laying batteries and kept in an open house under hot environmental temperature. Productive traits were determined for an experimental period of 6 wk. Egg quality, carcass traits, blood parameters, and immune response were also determined. The results indicated that the productive traits were not significantly

affected by EL supplementation. Shell quality and broken eggs significantly improved in quails fed a diet containing EL compared with those in the control. The quails fed a diet supplemented with 0.1% EL exhibited significantly higher cellular mediated and humoral immune responses than those in the other treatment groups. Glutathione peroxidase activity tended to be significantly increased by the dietary administration of EL at the level of 0.2%. Concerning quail varieties, it could be noticed that the gray quails exhibited higher productive performance, shell quality, and cellular immunity than the white counterparts. It could be concluded that supplementing a diet with 0.1 EL as a natural feed additive greatly enhances eggshell quality and immunocompetence and reduces number of broken eggs of Japanese quails raised under high environmental temperature.

Key words: eucalyptus leaf, Japanese quail, immunity, egg quality, antioxidant status

2020 Poultry Science 99:879–885

<https://doi.org/10.1016/j.psj.2019.09.001>

INTRODUCTION

Poultry farmers turned their attention to natural feed additives for improving productive performance and enhancing the health status of their flocks, in particular that kept under heat stress conditions. Owing to changes in consumers' concern and ban on antibiotics usage since 2006, most producers supplement ration with one or more natural additives as a safe and inexpensive alternative source. The feed manufacturers are adopting new

forms of natural feed additives such as probiotics, prebiotics, yeast, organic acids, phytochemicals, and zeolites. However, many natural feed additives can be derived from plant sources. It is well documented that some plant extracts can affect the secretion of digestive enzymes and act as antibacterial, antiviral, anti-oxidizing agents and immunostimulatory (Ertas et al., 2005; Cross et al., 2007; Sedaghat and Torshizi, 2017; Alagawany et al., 2018). Eucalyptus (*Eucalyptus camaldulensis*) is a tall evergreen tree and now extensively cultivated in many regions worldwide. It contains several vital compounds including p-cymene, 1, 8-cineole, β-phellandrene, spathulenol, cryptone aldehydes, cuminal, phellandral, and α-phellandrene leading to multifunctional characteristics such as antimicrobial, anti-inflammatory, and antioxidative properties (Barra et al., 2010). In humans, Eucalyptus is used to reduce

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Received March 24, 2019.

Accepted September 29, 2019.

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nasal congestion in common cold during cold winter months (Sadlon and Lamson, 2010). Some researchers reported that feeding poultry a diet supplemented with eucalyptus improves productive traits, antioxidant status, and immune response in laying hens and broilers (Abd El-Motaal et al., 2008; Farhadi et al., 2017; Chen et al., 2018). It was reported that the diet supplemented with 0.1% eucalyptus resulted in best performance and highest income in chicken among eucalyptus, pomegranate, tilia, and thyme (Osman et al., 2007).

The quail is characterized by rapid alternation of generations, high egg production, and lower susceptibility to diseases than domestic chicken (Knaga et al., 2018). In Middle East, Japanese quails are reared for both meat and egg production at family-type small-scale enterprises. Generally, many regions suffer from harsh environmental conditions (high temperature and humidity percentage) during a long summer season. Accordingly, a poor production and negative impact particularly in eggshell quality were expected from flocks reared under uncomfortable environmental conditions. However, changes in external and internal quality characteristics of eggs obtained from quails with different plumage colors have previously been reported (Yilmaz et al., 2011; Sari et al., 2012; Bagh et al., 2016). Studies on productive performance of different varieties or lines reared as well as feeding Eucalyptus leaves (EL) in quails at high environmental conditions are rare. Therefore, the present study was conducted to determine the productive performance of 2 varieties of quails fed a supplemented diet with EL to attenuate the negative effect of heat stress and to enhance the immunity status.

MATERIALS AND METHODS

Quail Housing and Husbandry

A total of 180 twelve-week-old Japanese quail laying hens from 2 color varieties (gray and white) were randomly assigned from a base population and transferred to battery cages. Each hen was individually housed in a wire cage (20 × 20 × 20 cm) supplied with individual feed trough in the front and a nipple drinker. The experimental design was completely randomized design in a 3 × 2 factorial arrangement (dietary treatment × variety). EL powder was mixed with diet in 3 levels (0, 0.1, and 0.2%). The quails were distributed into 3 dietary treatments within each variety forming 6 subgroups (30 individual records for each one). The quail received a laying ration containing 18% CP and 2,850 kcal/kg ME during the experimental period lasting 6 wk. Throughout the experiment, feed and water were available *ad libitum*. Birds were exposed to a lighting period of 16 h per day. All quails received uniform care and management practices throughout the whole experimental period. The average high and low ambient

temperatures recorded during the experimental period were 39.3°C and 23.8°C, respectively. No vaccination or medication was performed. The use and handling of quails were approved by the Ethical Committee of Qassim University.

Productive Traits

Body weight at the end of the experiment was individually recorded. Feed consumption was calculated in gram for each cage starting from 12 weeks of age. Egg production (weight and number) was daily recorded for each cage throughout the whole experimental period. Feed conversion ratio (FCR) was calculated at the end of the experiment on the basis of the amount of feed consumed in gram divided by egg mass in gram. Damaged eggs, including broken, cracked, and shell-less eggs, were recorded as they occurred. Broken eggs were then calculated.

Egg Quality Assessment

During the last week of the experiment, 90 intact eggs from each subgroup (540 in total) were collected to assess internal and external egg quality characteristics. Egg width and egg length were measured in mm using an electronic digital Vernier caliper (±0.01 mm). Egg shape was then calculated according to the following formula:

$$\text{Egg shape} = [\text{egg width} / \text{egg length}] \times 100$$

Breaking strength for intact eggs that had been freshly laid was determined in kg/cm² using Egg Force Reader, Orka Food Technology Ltd. The height of thick albumen and egg yolk was measured by placing the liquid content on a balanced surface using a tripod micrometer. Then, the yolk was separated and rolled on tissue papers to remove the residual albumen. Albumen weight was calculated by subtracting the yolk and shell weight from egg weight. The weight of eggshell, yolk, and albumen were expressed as a percentage of egg weight. Haugh unit was calculated according to the following formula of Sari et al., (2016):

$$\text{HU} = 100 \log(\text{H} - 1.7\text{W}^{0.37} + 7.57)$$

where H is the albumen height (mm) and W is the egg weight (g).

Yolk color was measured by comparing yolk color to the Roche yolk color fan. The liquid contents were put aside, and the shell plus membranes were washed under running water to remove adhering albumen. The wet eggshell was left for 24 h at room temperature for drying and then weighed to the nearest 0.01 g. The relative weight of dry eggshell was calculated on the basis of egg weight. To measure shell thickness, pieces from 3 different regions (2 poles and equator) of each eggshell

with intact membranes were measured with a dial gauge micrometer to the nearest 0.01 mm.

Carcass and Internal Organs

For evaluation of carcass yield, 90 birds were randomly selected (15/variety/dietary treatment) and subjected to preslaughter fasting for 4 h. Immediately afterward, the quails were weighed and slaughtered by cutting their jugular veins. After a 2-min bleeding time, each quail was dipped in a hot water bath at 60°C for 60 s and manually defeathered. Head and feet were removed. The carcass was eviscerated manually and weighed. Upon evisceration, the weight of eviscerated carcass, liver, heart, gizzard, and spleen was recorded and expressed as a percentage of live body weight. To minimize variations in the carcass procedure, all dissections were carried out by the same person.

Cell-Mediated Immunity Assay

The cellular immune response was assessed by cutaneous basophilic hypersensitivity test using phytohemagglutinin (PHA-P, lectin from *Phaseolus vulgaris*). At the end of the experiment, 60 birds (10 from each subgroup) were randomly assigned to evaluate cellular immunity. Each quail was inoculated intradermally on day 120 with the mitogen PHA-P (Sigma Chemical Company, St. Louis, MO) into the left-wing web. Each bird received 50 µl of a suspension of 2-mg PHA-P/ml phosphate buffered saline. The wing web thickness was measured by a micrometer to the nearest 0.001 mm, before injection (as a control measurement). At 24, 48, and 72 h after injection, the swelling induced in wing web was measured to obtain the response to mitogen injection.

Humoral Immune Response

Newcastle disease virus vaccine (LaSota strain) was performed via drinking water at 16 weeks of age. At the end of the experiment, detection of antibodies against Newcastle Disease Virus (NDV) in serum of immunized quails was performed by ELISA using a Newcastle Disease (ND) antibody commercial test kit (Bio-Chek B.V., Reeuwijk, the Netherlands). The assay was carried out as described by the manufacturer's instructions. Briefly, serum samples were diluted to 1:500 and added to microtiter wells and incubated at room temperature for 30 min. Upon aspiration and washing, anti-chicken IgG labeled with enzyme alkaline phosphatase was then added to the wells and incubated at room temperature for additional 30 min. After another wash to remove unreacted conjugate, the substrate was added to the appropriate wells in the form of p-nitrophenyl phosphate chromogen and incubated at room temperature for 15 min. A stop solution was added to stop reaction. Finally, the absorbance of samples was recorded by microtiter plate reader at 405 nm.

Blood Hematology, Plasma Biochemistry, and Antioxidant Status

During slaughter, 90 blood samples were collected (15 birds/dietary treatment/variety) in heparinized tubes. The hematological parameters were determined in whole blood by using an automatic, fully digital, hematology analyzer (BC-3000 Plus; Shenzhen Mindray, Bio-Medical Electronics Co., Ltd.). These parameters were total count of red blood cells (RBC), hemoglobin, hematocrit, and thrombocytes. The remaining blood samples were centrifuged at 4,000 rpm for 10 min under cooling (4°C). The harvested plasma specimens were frozen at -20°C for further analysis. Total protein, albumen, total cholesterol, and triglycerides values were spectrophotometrically determined using commercial reagent kits (Stanbio Laboratory, L.P., Boerne, TX). The globulin value was calculated as the difference between the total protein and albumen.

Total antioxidant capacity (TAC, mmol/L) was determined using a commercial kit (Biodiagnostic for diagnostic and research reagents; Dokki, Giza, Egypt; www.bio-diagnostic.com). This method exploits the ability of antioxidants to reduce hydrogen peroxide (H₂O₂). Determination was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided H₂O₂. The antioxidants in the sample eliminate a certain amount of the provided H₂O₂. The residual of H₂O₂ is quantified colorimetrically by an enzymatic reaction that evolves the conversion of 3,5-dichloro-2-hydroxy benzenesulphonate to a colored product. Glutathione peroxidase (GSH-Px) was determined in erythrocytes. The RBC were collected from blood samples and washed with saline solution 3 times. Cold deionized water (4°C) was added to lyse cells. The resulting clarified supernatant was used in GSH-Px assay. The activity of GSH-Px enzyme (reduction of organic peroxide) was spectrophotometric monitored by decreasing absorbance at 340 nm. Malondialdehyde (MDA) level was determined from MDA equivalence standard. Samples and standards are first reacted with thiobarbituric acid in acidic medium at a high temperature (95°C) for 30 min to form a reactive pink product. The samples and standards were read spectrophotometrically at 534 nm.

Statistical Analysis

All data were subjected to a two-way ANOVA using JMP of the SAS software (SAS Institute Inc, 2013). The model included the main effects of EL dietary supplementation level, variety, and their interaction. The model was as follows:

$$Y^{ijk} = \mu + L_i + V_j + (LV)_{ij} + e_{ijk}$$

where Y_{ijk} = the observation taken on the k^{th} individual, μ = overall mean, L_i = the fixed effect of the i^{th} eucalyptus supplementation level, V_j = the fixed effect of the j^{th} variety of quails, $(LV)_{ij}$ = interaction between eucalyptus

Table 1. Effect of dietary EL treatment and variety on performance of Japanese quails.

Trait	Eucalyptus level (L)			Variety (V)			P value		
	0%	0.1%	0.2%	Gray	White	SEM	L	V	L*V
Body weight, g	199.9	197.8	205.6	204.7	197.7	1.75	0.24	0.09	0.27
Production, %	95.9	95.7	95.6	96.8 ^a	94.6 ^b	0.33	0.89	<0.01	0.98
Egg weight, g	11.5	11.5	11.5	11.6 ^a	11.4 ^b	0.02	0.96	<0.01	0.67
Egg mass, g	465.1	461.8	462.4	470.3 ^a	455.9 ^b	1.72	0.44	<0.01	0.88
Feed consumption, g	907.5	943.6	925.3	945.4 ^a	910.5 ^b	10.34	0.39	0.03	0.15
Feed conversion ratio	1.96	2.05	2.00	2.02	2.00	0.02	0.43	0.42	0.18
Broken eggs, %	3.2 ^a	2.8 ^b	2.5 ^b	2.3 ^b	3.3 ^a	0.21	0.05	0.02	0.52

^{a,b}Means within a row with different superscript letters for each factor are significantly different. Abbreviations: EL, Eucalyptus leaves; SEM, standard error of mean.

supplementation level and quail variety, e_{ijk} = random error assumed to be independent normally distributed with mean = 0 and variance = σ^2 .

When significant differences between means were found, means were separated using Tukey's test.

RESULTS AND DISCUSSION

Table 1 shows the effect of EL dietary supplementation on laying performance and broken eggs percentage. As shown, the productive traits were not affected by the variation in EL levels. This result is in agreement with the study of Sedaghat and Torshizi (2017) in which a supplementation of camphor (Eucalyptus globules) did not have a significant effect on body weight gain, daily feed intake, and FCR. Similarly, no significant change in body weight was reported by the use of camphor derivatives in rats (Carou et al., 2009) and camphor (Eucalyptus globules) in quails (Abu-Taleb et al., 2003). As body weight did not change throughout the experimental period, it can be concluded that EL cannot be classified as a growth-promoting substance in Japanese quails. Based on the current results, it appears that the inclusion of EL declined the percentage of broken eggs (2.8 and 2.5%) when the added level was 0.1 and 0.2%, respectively, compared with control group (3.2%). Based on variety effect, it could be observed that the gray quails had a significant ($P < 0.01$) superiority in egg production, egg weight, and mass compared with the white variety. Also, the gray quails significantly ($P < 0.03$) had higher feed consumption, but this increase did not reflect on the feed conversion trait. Moreover, the

percentage of broken eggs significantly ($P = 0.05$) decreased in eggs produced from gray quails (2.3%) when compared with that of white siblings (3.3%). Regarding interaction between EL level and variety of quail, no significant difference was detected for all productive studied traits.

Effects of dietary EL levels on different internal and external egg quality traits within each quail variety are listed in Table 2. Egg shape index of supplemented groups showed a significant ($P < 0.03$) increase compared with the control. Addition of EL in low level (0.1) significantly ($P < 0.001$) increased shell thickness (297.2 μ) when compared with that in the other treatments (292.7 and 293.9 μ m for 0 and 0.2%, respectively). As a consequence of the improvement in shell thickness of eggs produced from treated quails, shell strength became more significantly ($P < 0.03$) stronger in groups that received EL. It is well known that the eggshell quality deteriorated under heat stress conditions. The improvement in eggshell strength may be due to a substance that exists in EL leading to increase in Ca metabolism under high temperatures, which was not measured in this study. Although the shell percentages were the same for all studied groups, the improvement in shell strength may be due to a good ultrastructure of mammillary bodies associated with EL inclusion. However, a further scanning investigation for eggshell ultrastructure is required to prove this hypothesis. These results are in good agreement with the findings of Abd El-Motaal et al. (2008). They found that the eggs produced from laying hens fed a diet containing 0.3 EL had a significantly higher breaking strength than other

Table 2. Effect of dietary EL treatment and variety on egg quality traits of Japanese quails.

Trait	Eucalyptus level (L)			Variety (V)			P value		
	0%	0.1%	0.2%	Gray	White	SEM	L	V	L*V
Egg shape	76.6 ^b	77.5 ^a	77.2 ^a	77.1	77.1	0.17	0.03	0.81	<0.001
Shell thickness, μ m	292.7 ^b	297.2 ^a	293.9 ^b	301.2 ^a	275.2 ^b	1.13	<0.001	<0.001	<0.001
Yolk index	49.0 ^a	48.5 ^b	48.5 ^b	48.5 ^b	48.8 ^a	0.11	0.01	0.04	0.10
Yolk color	5.62	5.64	5.80	5.9 ^a	5.5 ^b	0.038	0.07	<0.001	0.41
HU	58.1	58.5	58.6	58.4	58.4	0.12	0.09	0.59	0.39
Shell strength (kg/cm ²)	1.33 ^b	1.37 ^a	1.37 ^a	1.44 ^a	1.28 ^b	0.014	0.03	<0.001	0.06
Shell, %	11.7	11.9	11.8	11.74	11.85	0.11	0.51	0.69	0.004
Yolk, %	32.4	32.9	32.9	33.1 ^a	32.5 ^b	0.16	0.09	<0.001	0.13
Albumen, %	55.9	55.2	55.3	55.2 ^b	55.7 ^a	0.21	0.08	0.02	0.009

^{a,b}Means within a row with different superscript letters for each factor are significantly different. Abbreviations: EL, Eucalyptus leaves; SEM, standard error of mean.

Table 3. Effect of dietary EL treatment and quail variety on carcass and some internal organs.

Trait	Eucalyptus level (L)			Variety (V)			P value		
	0%	0.1%	0.2%	Gray	White	SEM	L	V	L*V
Carcass, %	67.5 ^a	64.3 ^b	64.8 ^b	65.5	65.6	0.38	0.001	0.95	0.42
Liver, %	2.37	2.68	2.51	2.49	2.55	0.06	0.12	0.63	0.23
Heart, %	0.79	0.75	0.81	0.78	0.79	0.01	0.17	0.66	0.46
Gizzard, %	1.58	1.72	1.74	1.69	1.67	0.03	0.07	0.62	0.76
Spleen, %	0.047	0.041	0.041	0.042	0.044	0.002	0.49	0.57	0.22

^{a,b}Means within a row with different superscript letters for each factor are significantly different. Abbreviations: EL, Eucalyptus leaves; SEM, standard error of mean.

treated groups. Likewise, [Chen et al. \(2018\)](#) reported that the addition of 0.8 g/kg polyphenols in EL improved the egg traits by increasing the eggshell thickness. The percentage of liquid components (yolk and albumen) did not significantly differ because of EL supplementation. Based on the variety effect, the performance of gray and white quails was the same for egg shape index, Haugh unit, and eggshell percentage. Similarly, [Bagh et al. \(2016\)](#) did not find a significant difference between gray and white lines for all physical properties of egg quality. In contrast to our results, egg shape index depends on plumage colors of the quails ([Yilmaz et al., 2011](#); [Sari et al., 2012](#)). They found that the mean of shape index obtained from gray plumage color was significantly lower than that of white plumage color. The mean value of shell thickness obtained from the quails with gray plumages was found to be significantly ($P < 0.001$) higher than that of white quails. This result is in agreement with the findings of [Sari et al. \(2012\)](#), who found a slight increase in shell thickness of gray quails compared with that of the white siblings. Logically and as a sequence of this advantage in eggshell thickness associated with gray variety, shell breaking strength recorded a significant ($P < 0.001$) increase (1.44 Kg/cm²) compared with that of white ones (1.28 Kg/cm²). In terms of yolk properties, it could be found that the relative weight of yolk and its color were significantly ($P < 0.001$) superior in gray quails compared with those in white variety. However, literature on the external and internal quality characteristics of eggs obtained from quails with different plumage

color lines is very limited. Studies on the shell quality of quail eggs are rare, and most studies are focused on chicken eggs. External egg quality of poultry species exhibits high variation because egg formation and subsequent egg shell quality is a complex process that depends on several biomechanisms, which can be determined by both genetic and environmental conditions such as species, strain, age of hen, feeding, and management ([Narinc et al., 2015](#)). Regarding interaction between EL level and variety of quail, there were significant differences for egg shape, shell thickness, and both shell and albumen percentages.

Supplementing feed with EL significantly ($P < 0.001$) decreased the dressed carcass (%) compared to the control group ([Table 3](#)). Meanwhile, there was no significant difference among EL levels for giblets or spleen percentages. However, a numerical increase in liver and gizzard (%) was detected in supplemented groups. Similar to our findings, no significant effects due to camphor dietary supplementation were observed on organ weights during the experimental period ([Sedaghat and Torshizi, 2017](#)). This reduction in carcass percentage associated with EL inclusion may be compensated by increasing productive organs (ovary and oviduct) to be more affective in egg production performance. However, the last hypothesis needs to be proven in a further study. Accordingly, it is not commended to supplement a diet with EL for growing quails. On the basis of variety effect, there was no significant difference between gray and white quails for all studied traits. Also, the interaction between EL level and variety was not significant.

Table 4. Effect of dietary EL level and variety on immune response of Japanese quails.

Trait	Eucalyptus level (L)			Variety (V)			P value		
	0%	0.1%	0.2%	Gray	White	SEM	L	V	L*V
Cell-mediated immunity (swelling difference % ¹)									
After 24 h	33.9	38.2	34.0	40.6 ^a	30.1 ^b	0.027	0.47	<0.01	0.10
After 48 h	19.1	24.1	17.2	24.7 ^a	15.6 ^b	0.023	0.08	<0.01	0.14
After 72 h	7.7 ^b	12.1 ^a	8.8 ^b	12.0 ^a	7.1 ^b	0.019	0.05	<0.01	0.93
Humoral immunity									
NDV titer	5,257.5 ^b	6,768.5 ^a	5,461.8 ^b	5,355.6	6,302.9	286.5	0.04	0.07	<0.01

^{a,b}Means within a row with different superscript letters for each factor are significantly different. Abbreviations: EL, Eucalyptus leaves; SEM, standard error of mean; NDV, Newcastle Disease Virus.

$$^1\text{Swelling difference} = \frac{\text{swelling difference}}{\text{initial thickness of toe web}} \times 100$$

Table 5. Supplemental effects of EL and variety of quails on blood hematology, biochemistry, and antioxidant status.

Trait	Eucalyptus level (L)			Variety (V)			P value		
	0%	0.1%	0.2%	Gray	White	SEM	L	V	L*V
RBCs, 10 ⁶	3.20	3.16	3.21	3.17	3.22	0.05	0.94	0.68	0.81
Hemoglobin, g/dL	17.5	18.2	17.5	17.9	17.5	0.29	0.35	0.25	0.53
Hematocrit, %	43.6	41.9	42.7	43.2	42.7	0.72	0.83	0.75	0.82
Thrombocytes 10 ³ /μL	16.1	14.6	16.7	16.5	15.6	0.93	0.75	0.77	<0.001
Total protein	3.36	3.85	3.56	3.67 ^a	3.51 ^b	0.06	0.20	<0.01	0.96
Albumin, g/dL	2.36	2.82	2.64	2.58 ^b	2.63 ^a	0.06	0.61	<0.01	<0.01
Globulin, g/dL	1.00	1.03	0.92	1.09 ^a	0.88 ^b	0.07	0.10	0.02	0.79
Cholesterol, mg/dL	195.5	213.2	179.5	191.0	201.7	5.26	0.18	0.43	0.60
Triglycerides, mg/dL	159.6	158.2	149.2	153.4	158.2	4.48	0.59	0.57	0.32
TAC, mmol/L	0.92	0.99	0.89	0.99	0.87	0.045	0.65	0.19	0.69
GSH-Px, U/mL	53.5 ^b	58.0 ^{a,b}	63.2 ^a	59.1	57.3	1.61	0.05	0.59	0.90
MDA, nmol/mL	4.18	3.59	3.71	3.77	3.87	0.13	0.14	0.69	0.31

^{a,b}Means within a row with different superscript letters for each factor are significantly different.

Abbreviations: EL, Eucalyptus leaves; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; RBC, red blood cell; SEM, standard error of mean; TAC, total antioxidant capacity.

The effects of dietary EL on cellular mediated and humoral immune responses are given in Table 4. Cellular immunity was unaffected by the EL treatment at 24 and 48 h after PHA-P inoculation. However, the quails fed a diet containing 0.1% EL exhibited significantly ($P < 0.05$) higher swelling response 72 h after immunization. As the same trend occurred in cellular mediated immunity, humoral immune response was affected by EL supplementation in which the greatest titer against NDV was significantly seen in 0.1% of EL compared with the other groups ($P < 0.04$). This result is in agreement with the quail trial of Sedaghat and Torshizi (2017). They demonstrated that cellular responses to the PHA-P and 2,4-dinitro 1-chlorobenzene skin test were not influenced by dietary camphor (250, 500, 750, 1,000, 5,000, or 10,000 ppm). In addition, humoral responses to secondary sheep RBC, avian influenza virus, and NDV immunizations were positively improved by camphor supplementation, in which greater secondary response to sheep erythrocytes belonged to 750 and 1,000 ppm of camphor groups. Evidence suggests that camphor acts as an antimicrobial, antiviral, anticoccidial, analgesic, anticancer, and antitussive agent (Chen et al., 2013). It has also been found that camphor extract modulates the immune function in rats (Lee et al., 2006). Broilers fed a diet supplemented with EL powder (0.1 or 0.3%) had a greater primary antibody response to sheep RBC compared with the control (Farhadi et al., 2017). It has been reported that the laying hens fed diets supplemented with 0.2 or 0.3 EL were significantly hyperresponsive to PHA-P injection compared with other groups (Abd El-Motaal et al., 2008). Furthermore, eucalyptus essential oil from *Eucalyptus globulus* is able to stimulate the innate cell-mediated immune response and provide scientific support for an additional use of this plant extract, besides those concerning its known antiseptic and anti-inflammatory properties in rats (Serafino et al., 2008). Regarding variety of quails, the results revealed that the gray quails had a significant ($P < 0.01$) increase in swelling response for PHA-P injection at all times studied compared with the white siblings. However, no significant difference between

varieties was detected for NDV titer. The interaction was not significant for cell-mediated response, while it was significant ($P < 0.01$) for humoral immunity.

All traits of hematology and blood biochemistry were not affected by EL supplementation (Table 5). In discordance with the current results, Bello (2015) found a significant increase in hemoglobin and thrombocytes levels in groups given different levels of *Eucalyptus globules* leaves. This improvement can be attributed to the fact that Eucalyptus contains and serves as a good source of iron, beta-carotene, and vitamin C. This conflict results may be attributed to use a different variety of EL in the present study. Considering the quail variety, it could be noticed that the gray one had significantly ($P < 0.02$) higher total protein and globulin concentrations than the white group. The last observation may be an approach for supporting immune response of gray quails. TAC level was not affected by dietary EL administration. However, GSH-Px activity tended to be significantly ($P < 0.05$) affected by the dietary administration of EL. There was a linear increase associated with the EL level. It is suggested that antioxidant agents in EL enhance GSH-Px activity to minimize oxidative stress in chicken by inhibiting the oxygen free radical production. In agreement with our results, supplementation of 0.8 g/kg polyphenols in EL in laying hens' diet enhanced the serum antioxidant status by increasing enzymatic activities (GSH-Px, T-SOD, TAC), inhibited oxidative damage, and provided protective effect to liver tissue (Chen et al., 2018). The MDA level, an indicator of lipid peroxidation, increases with increases in lipid mobilization and oxidation of lipids. Insignificant decrease in MDA was noticed in quails fed a diet containing EL (Table 5). It is stated that camphor (a substance synthetically produced from *Cinnamomum camphora*) can act as an antioxidant by reducing MDA levels (Lee et al., 2006), which partially supports the present findings. The observed decrease in MDA level in the groups supplemented with EL might have been due to the inhibition of lipid peroxidation in erythrocyte membranes due to the antioxidant effect of EL. These results were in agreement with the findings of the study by

Shata et al. (2014), which reported a significant increase in superoxide dismutase activity and oxidative stress by using camphor. It is stated that camphor can act as an antioxidant by reducing MDA levels (Lee et al., 2006; Sedaghat et al., 2016). Likewise, Eucalyptus administration for 4 wk in rats caused a significant decrease in liver MDA (Kumar and Laxmidhar, 2011). Furthermore, it was reported that polyphenols in EL contained several compounds such as gemin D, pedunculagin, tellimagrandin I, tellimagrandin II, pentagalloyl glucose, oenothien B, and others, which possessed antioxidant activity (Chen et al., 2014a) or even antiaging effect (Chen et al., 2014b). Concerning antioxidant activity of quail variety, there was no significant difference between the gray and white quails. In conclusion, EL at the level of 0.1 could be used as an effective feed additive in Japanese quails' diet to improve eggshell traits and health performance under high environmental temperature. Also, the gray quails exhibited higher productive performance and sustainable cellular mediated immunity than the white quails.

ACKNOWLEDGMENTS

Qassim University must be thanked for providing the farm facilities and battery cages.

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