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Effect of *Lavandula stoechas* and hydrogen peroxide on quails in terms of egg production, liver function, blood parameters, and gene expression

Efecto de *Lavandula stoechas* y peróxido de hidrógeno en codornices sobre la producción de huevos, a nivel hepatico, sanguíneo y expresión genética

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ABSTRACT

The aim of this study was to evaluate the effects of supplementing *Lavandula stoechas* oil in the feed and hydrogen peroxide in the water on egg production, hatchability, and slaughter characteristics in quails aged 56–83 days (d) during the egg production period. Simultaneously, the study analyzed the expression levels of the GPx7 and NRF2 genes, blood serum biochemistry, and liver histopathological parameters to assess whether *L. stoechas* oil supplementation exhibits antioxidant effects in quails treated with hydrogen peroxide. In the study, each group consisted of 15 females and 6 males, with a total of 84 quails used. The study included four groups: a control group with basal feed; a *L. stoechas* group with 200 mg·kg^{-1} *L. stoechas* oil added to the basal feed; hydrogen peroxide group with a basal feed + 0.5% hydrogen peroxide added to the water; a *L. stoechas* + hydrogen peroxide group with a basal feed + 200 mg·kg^{-1} *L. stoechas* oil + 0.5% hydrogen peroxide added to the water. Each group was replicated three times. In the second 14 d (days 71 to 84), both the control and hydrogen peroxide groups exhibited lower average egg weights (P<0.05) compared to *L. stoechas* and *L. stoechas* + hydrogen peroxide groups. Additionally, *L. stoechas* and *L. stoechas* + hydrogen peroxide groups showed improved feed conversion efficiency compared to the control group (P>0.05). Furthermore, the fertilization rate and hatching yield were higher in the groups supplemented with *L. stoechas* oil compared to the control group (P>0.05). Conversely, the group with hydrogen peroxide added to the water exhibited lower yields than the other groups. Furthermore, it was observed that *L. stoechas* oil increased the GPx7 gene level regardless of gender, thus showing an antioxidant effect, but did not show a significant effect on the NRF2 gene expression level.

Key words: Gene expression; hatchability; liver characteristics; oxidative stress; quails

RESUMEN

El presente estudio tuvo como propósito evaluar los efectos de la suplementación con aceite de *Lavandula stoechas* (LSO) en el alimento y peróxido de hidrógeno (H_2O_2) en el agua de consumo sobre la producción de huevos, la incubabilidad y las características de la canal en codornices de entre 56 y 83 días (d) de edad durante el período de puesta. Al mismo tiempo, se analizaron los niveles de expresión de los genes GPx7 y NRF2, así como parámetros bioquímicos del suero sanguíneo y características histopatológicas del hígado, con el fin de determinar si la suplementación con LSO ejerce un efecto antioxidante en codornices expuestas a H_2O_2. En el estudio, se utilizaron 84 codornices, distribuidas en grupos compuestos por 15 hembras y 6 machos cada uno. Se establecieron cuatro grupos: un grupo control con alimento basal; un grupo LS con 200 mg·kg^{-1} de LSO añadido al alimento basal; un grupo H_2O_2 con alimento basal + 0,5 % de H_2O_2 en el agua; y un grupo LS + H_2O_2 con alimento basal + 200 mg·kg^{-1} de LSO + 0,5 % de H_2O_2 en el agua. Cada grupo fue replicado tres veces. Durante los segundos 14 d (días 71 a 84), tanto el grupo control como el grupo con H_2O_2 presentaron un menor peso promedio de los huevos (P<0,05) en comparación con los grupos LS y LS + H_2O_2. Además, los grupos LS y LS+H_2O_2 mostraron una mejor eficiencia de conversión alimenticia en comparación con el grupo control (P<0,05). Asimismo, la tasa de fertilización y el rendimiento de incubación fueron más altos en los grupos suplementados con LSO en comparación con el grupo control (P>0,05). Por el contrario, el grupo al que se añadió H_2O_2 en el agua mostró rendimientos más bajos que los demás grupos. Asimismo, se constató que el LSO incrementó la expresión del gen GPx7 en ambos sexos, indicando un posible efecto antioxidante, aunque no se detectaron cambios significativos en los niveles de expresión del gen NRF2.

Palabras clave: Expresión génica; incubabilidad; características hepáticas; estrés; estrés oxidativo

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INTRODUCTION

Quail (*Coturnix coturnix*) breeding produces meat and eggs, which are vital food sources supplying essential proteins, and its significance lies in the species' early onset of egg production and short time to slaughter age, making quails an attractive alternative to intensively raised chicken species for both meat and egg production [1]. Compared to chicken (*Gallus gallus domesticus*) eggs, quail eggs have lower fat content while maintaining similar cholesterol levels. They are also rich in essential amino acids and minerals such as calcium, phosphorus, and iron [2]. Fat and cholesterol levels vary between egg species, with both primarily synthesized in the liver and transported via the blood to be stored in eggs [3]. The conversion of feed energy into fat accumulation in meat and eggs is crucial because fat accumulation directly impacts production efficiency, profitability, and the health of both humans and animals [4, 5].

Eggs quality, including taste, odor, color, and nutrient composition, is influenced by feed content, and recent approaches aim to enhance egg quality without harming human or animal health by replacing antibiotic growth promoters with plant–based feed additives; in this context, *Lavandula stoechas* (LS), a Mediterranean lavender species from the Lamiaceae family and native to Türkiye, is investigated [6, 7]. LS is considered an excellent feed additive and is reported to have antioxidant and antimicrobial properties [8, 9].

It is stated that the increase in reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) formed during cellular metabolism stimulates oxidative stress, disrupting the oxidative balance in the absence of antioxidants [10, 11]. The primary reactive oxygen species include singlet oxygen, H_2O_2, superoxide anion, and hydroxyl radicals. These radicals adversely affect normal cellular functions by causing significant damage to proteins, DNA, and lipids [12, 13]. H_2O_2 has been reported to decrease total antioxidant levels and significantly increase total oxidant levels in both blood serum and liver samples [14, 15].

The aim of this study was to determine the effect of supplementing *L. stoechas* oil (LSO) in feed and H_2O_2 in water on egg production, hatchability, and slaughter characteristics of quails over a four–week egg production period. Additionally, the study aimed to assess the expression levels of GPx7 and NRF2 genes, blood biochemistry, and liver histopathologic parameters to investigate whether LSO supplementation has an antioxidant effect in quails treated with H_2O_2.

MATERIAL AND METHODS

Formation of experimental groups

This study was accepted on 25/07/2024 and the decision numbered 2024/07–05 of Hatay Mustafa Kemal University Animal Experiments Local. The study was conducted at the Hatay Mustafa Kemal University Research and Application Center for Experimental Research Alternative Poultry Breeding Unit. Male and female quails, aged 56 days (d), were used in the study.

The study included four groups; a Control group with basal feed + 0% LSO, a LS group with 200 mg·kg^{-1} LSO added to the basal feed, H_2O_2 group with a basal feed + 0.5% H_2O_2 added to the water, and a LS + H_2O_2 group with a basal feed + 200 mg·kg^{-1} LSO + 0.5% H_2O_2 added to the water. Each group had three replicates, with each replicate consisting of 5 females and 2 males, totaling 21 animals per group. The study continued until the quails reached 84 d of age. The study ration information is provided in TABLE I. Feed and water were given to the animals *ad libitum* [16].

TABLE I
Nutrient content of the basic diet consumed by the Japanese quails in the study

Nutrient content determined by analysis	Quantity
Ingredients	**%**
Corn	55.64
Wheat bran	23.10
Sunflower Seed Meal	11.46
Soybean Meal	6.92
Marble Powder	1.74
Salt	0.37
Bone Meal	0.13
L–Lysine	0.09
DL–Methionine	0.35
Vitamin–Mineral premix*	0.20
Calculated composition**	
Metabolic energy (kcal·kg^{-1})	2767.91
Crude protein (%)	15.00
Phosphorus (available) (%)	0.40
Calcium (%)	0.98
Lysine (%)	0.68
Methionine + Cystine (%)	0.66

*1 kg of the premix provided: 15,000,000 IU of Vitamin A; 5,000,000 IU of Vitamin D3; 100,000 mg of Vitamin E; 3,000 mg of Vitamin K3; 5,000 mg of Vitamin B1; 8,000 mg of Vitamin B2; 60,000 mg of niacin; 15,000 mg of D–calcium pantothenate; 5,000 mg of Vitamin B6, 20 mg of Vitamin B12, 200 mg of D–biotin; 2,000 mg of Folic acid;100,000 mg of Vitamin C; 0,02 mg of Cyanocobalamin; 74 mg of Mn (from MnO); 45 mg of Zn (from ZnO); 4 mg of Cu (from CuO); 12,5 mg of Fe (from $FeSO_4$); 0,3 mg of I (from KI); 0,15 mg of Se (from NaSe); **Calculated according to NRC (1994)

Procedures related to egg production period characteristics

To determine live weight changes during the egg production period, the live weights of male and female quails were measured weekly (Ohaus NV622, USA). The eggs produced by the quails were collected daily, weighed individually, and recorded in the group record charts. Additionally, the amount of feed given to the animals in each group at the beginning of the week and the amount of feed left at the end of the week were recorded.

$$Chickens/coop\ (pcs) = \frac{Number\ of\ eggs\ collected\ in\ 14\ days}{Number\ of\ chickens\ on\ that\ day}$$

For percentage hen/poultry egg production

$$Hens\ /\ poultry\ house\ (\%) = \left(\frac{Number\ of\ eggs\ per\ hen}{Number\ of\ days}\right) \times 100$$

$$Ability\ to\ convert\ \ feed\ into\ eggs = \frac{Female\ quail\ weekly\ feed\ consumption}{Total\ weekly\ egg\ weight}$$

Incubation characteristics

Eggs collected over five consecutive days during the third and fourth weeks of the study were subjected to incubation. The eggs were stored at room temperature (24°C) with the blunt end facing up and the pointed end facing down until they were placed in the incubator. The average egg weight and hatching chick weight of the total eggs placed in the incubator were determined. Additionally, at the end of the incubation period (17 + 2 d), the eggs that did not hatch were visually inspected to determine the number of unfertilized eggs and those with embryonic death.

$$Fertility\ rate\ \left(\%\right) = \left(\frac{Number\ of\ fertilized\ eggs}{Total\ number\ of\ eggs\ loaded\ in\ the\ incubator}\right) \times 100$$

$$Hatching\ rate\ \left(\%\right) = \left(\frac{Number\ of\ chicks\ hatched}{Total\ number\ of\ eggs\ loaded\ in\ the\ incubator}\right) \times 100$$

$$Output\ Power\ \left(\%\right) = \left(\frac{Number\ of\ hatched\ chicks}{Number\ of\ fertilized\ eggs}\right) \times 100$$

Operations related to slaughter properties

At the age of 84 d, 6 female and 6 male quails from each group were slaughtered, based on the wing number information to determine slaughter live weights. Slaughter carcass weight, as well as weights of the heart, liver, gizzard, and abdominal fat, were recorded for slaughter and organ characteristics. The full carcass weight was divided by the slaughter live weight and multiplied by 100 to determine the full carcass yield. Additionally, the oviduct weight in female quails and total testes weight in male quails were determined.

Processes related to blood properties

A total of 48 animals, six of each sex, were euthanized from the study groups using the decapitation method. During decapitation, 8 mL blood samples were collected into anticoagulant–free serum tubes (Vacutainer®, BD Diagnostics, Franklin Lakes, USA) for blood biochemistry analysis. The collected blood samples were left at room temperature until clot formation occurred (approximately 15 min). After clotting, the blood samples were centrifuged (NF 200, Nüve, Türkiye) at 1016.3 g for 10 min to obtain serum samples. Serum samples were analyzed for albumin (ALB), alkaline phosphatase (ALP), creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (UREA), total protein (TP), globulin (GLOB), and albumin/globulin ratio (ALB/GLOB) using an automatic biochemistry analyzer (Chem 200, Gesan, Italy) to assess the general health profile.

Operations related to gene expression characteristics

Molecular analysis

Total RNA isolation was performed using the Trizol method (TRIzol® Reagent, Ambion) following the manufacturer's instructions. A 50 mg tissue sample was homogenized in 1 mL Trizol. Next, 0.25 mL chloroform was added to the homogenate and vigorously shaken for 30 s. The mixture was then centrifuged at 4°C; 12,000 × g for 15 min (Hettich Universal 320 R, Germany). The resulting supernatant was carefully transferred to a new nuclease–free tube, and 0.5 mL isopropanol was added. After gently inverting the tube for 30 s, the sample was centrifuged at 4°C; 12,000 × g for 10 min. The RNA pellet was washed twice with 500 µL of 70% ethanol, centrifuging each time at 4°C, 7,500 × g for 5 min. Following this, the pellet was washed once with 500 µL of 99% ethanol and centrifuged at 4°C; 7,500 × g for 5 min. The RNA pellets were air–dried and then resuspended in nuclease–free water. The concentration and A260/A280 absorbance ratio of the isolated nucleic acids were measured using a nucleic acid meter (Thermo, Multiskan GO). The isolated samples were stored at -80°C until further user (Daihan Scientific Co. SimpleFreeze U500, Korea).

Complementary DNA (cDNA) synthesis: The total RNA samples obtained were converted into cDNA using the OneScript Plus cDNA Synthesis Kit (ABM Good, Canada). For this, according to the manufacturer's instructions, 1 µL of dNTP mix, 1 µL of reverse transcriptase, 4 µL of 5x RT buffer, 1 µL of oligo (dT) primer, 1 µL of random primer, 2 µg of total RNA, and 2 µL of nuclease–free water were combined to a total volume of 20 µL. The mixture was incubated in a thermal cycler at 25°C for 10 min, followed by 50°C for 50 min, and a final step at 85°C for 5 min (Bio–Rad T100, Bio–Rad Laboratories, Inc. USA). The resulting cDNA samples were stored at -20°C until further use.

Real–time quantitative PCR (RT–qPCR) analysis: The expression levels of glutathione peroxidase 7 (GPx7) [17] and nuclear factor erythroid 2-related factor 2 (NRF2) genes [18] in the antioxidant pathway were analyzed using RT–qPCR (Bio–Rad CFX96 Touch™, Bio–Rad Laboratories, Inc. USA). The primer sequences for these genes are provided in TABLE II. The GAPDH gene [19] was used as a reference gene. The RT–qPCR reactions were performed using the RealQ Plus 2X Master Mix Green kit (Ampliqon, Denmark). For each reaction, 12.5 µL of RealQ Plus 2X Master Mix, 150 nM of forward and reverse primers, 100 ng of cDNA, and nuclease–free water were combined to a total volume of 25 µL, following the manufacturer's instructions. The reaction conditions were as follows: initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 30 s, and annealing/extension at 60°C for 1 min. Each reaction was performed in duplicate, and the experimental results are reported as fold–change.

Procedures related to histopathological features

Necropsy was performed on all animals following euthanasia. Liver tissues were collected and fixed in 10% buffered formalin. Subsequently, the tissues were dehydrated using alcohol and

TABLE II
Primer sets used in the study for the expression of the GPx7 and NRF2 genes in Quail

Genes	Primer sequences	Base pair (bp)
NRF2	F:5'–GACATGGACAGTTCTCCTGGAAGC-3' R:5'–AGTGCCTGGCTCCACAGAAGG-3'	99
GPx7	F:5'–TTGTAAACATCAGGGGCAAA-3' R:5'–TGGGCCAAGATCTTTCTGTAA-3'	140
GAPDH	F:5'–GAGGGGCCATCCACAGTCTTC-3' R:5'–CATCACCATCTTCCAGGAGCG-3'	357

NRF2: Nuclear factor erythroid 2-related factor 2, GPX7: Glutathione peroxidase 7, GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

Xylene according to standard procedures, embedded in paraffin, and sectioned into 5 μm thick slices. The sections were deparaffinized in xylene and then stained with Hematoxylin and Eosin (H&E) following dehydration through an alcohol series (100, 96, 80, and 70%). The stained sections were examined under a light microscope (Olympus CX31, Tokyo, Japan) and microphotographed using an Olympus DP12, Tokyo, Japan camera. Histopathologic findings in the liver were assessed using the following criteria: Grade 0 represented histopathologic changes affecting less than 5% of the total area. Grade 1 indicated mild histopathologic changes affecting between 5% and 33% of the total area. Grade 2 reflected moderate histopathologic changes affecting between 33% and 66% of the total area. Grade 3 denoted severe histopathologic changes affecting more than 66% of the total area [20, 21].

Statistical analysis

In the study, weekly live weight, slaughter traits, egg production traits, and blood parameters were analyzed using One–way ANOVA and Duncan test with IBM SPSS 22 package program. Chi–square test was applied for evaluating hatching results. Histopathological characteristics, specifically liver fat levels, were assessed using Kruskal–Wallis test, and Mann Whitney U test was used for between–group comparisons. Genetic characteristics were analyzed using GraphPad Prism 9.1.1 (GraphPad, San Diego, CA, USA). A significance level of $P<0.05$ was considered statistically significant. Expression levels of the genes were normalized to the reference gene and analyzed using the $2^{\Delta\Delta}$Ct method, with results presented as fold change.

RESULTS AND DISCUSSION

Weight and yield characteristics of egg producing quails

TABLE III presents the live weight of Japanese quails during the egg production period and their slaughter characteristics at the study's conclusion. Statistical analysis revealed that the numerical differences in initial, first 14 d, and second 14 d live weights between the control group and treatment groups (LS, H_2O_2, LS + H_2O_2) were not statistically significant ($P>0.05$). Moreover, male and female quails in the LS + H_2O_2 group showed higher live weights compared to the H_2O_2 group throughout the study period ($P>0.05$). Although the differences in live weight at slaughter between the control and treatment groups were not significant ($P>0.05$), significant differences ($P<0.05$) were observed in heart weight among female quails, with the control group exhibiting higher values compared to the treatment groups. Liver weight was found to be highest in the H_2O_2 group for males ($P>0.05$) and in the LS + H_2O_2 group for females ($P>0.05$). Additionally, egg duct weight was highest in the LS group, while testis weight was highest in the H_2O_2 group ($P>0.05$).

Antioxidants are compounds known for protecting organic substances from oxidative damage [22]. In the study, during the 28–d egg production period, male and female quails fed with feed supplemented with LSO exhibited higher body weights compared to other groups, whereas the addition of H_2O_2 to water resulted in lower body weights in both sexes, as show in TABLE III. This decrease in body weight could be attributed to oxidative stress induced by H_2O_2, which lacks antioxidant additives likeLSO. Prolonged oxidative stress from H_2O_2 can diminish health and productivity, alongside increasing malondialdehyde (MDA) levels in quails [15].

TABLE III
Weekly live weight and production performance of quails

Characteristics	Gender	Control	LS	H_2O_2	LS+H_2O_2	*P*
Start LW (56 days) (g)	Female	241.70	241.34	235.04	246.52	0.688
	Male	206.53	218.20	201.45	203.50	0.550
69-day LW (g)	Female	254.32	251.67	242.96	247.71	0.723
	Male	224.80	209.48	207.24	214.33	0.635
83 days LW (g)	Female	275.77	253.88	247.99	261.91	0.067
	Male	235.54	227.65	216.61	221.38	0.494
Slaughter and carcass characteristics						
Live weight at slaughter (g)	Male	231.02	224.01	213.23	219.90	0.568
	Female	265.25	258.32	252.71	258.94	0.878
Full carcass weight (g)	Male	174.79	170.09	162.13	165.34	0.646
	Female	209.23	209.36	197.75	204.52	0.806
Full carcass yield (%)	Male	75.62	75.90	75.91	75.18	0.629
	Female	78.87	80.96	78.18	78.98	0.423
Heart (g)	Male	1.93	2.00	1.76	1.88	0.523
	Female	2.20^{a}	1.78^{b}	1.82^{b}	1.72^{b}	0.048
Liver (g)	Male	3.19	2.94	3.93	3.18	0.505
	Female	7.22	7.07	6.84	8.27	0.299
Stoniness (g)	Male	3.93	3.72	4.25	4.05	0.439
	Female	4.71	4.66	4.80	4.67	0.982
Abdominal fat weight (g)	Male	6.01^{a}	4.61^{ab}	2.31^{b}	5.11^{a}	0.039
	Female	5.09	4.21	3.73	2.92	0.428
Right and left total testicular weight (g)	Male	6.10	6.40	7.27	5.92	0.156
Egg duct weight (g)	Female	7.12	8.20	7.37	7.17	0.093

LW: Live weight, LS: 200 mg·kg^{-1} *Lavandula stoechas* oil added to the basal feed, H_2O_2: 0.5% hydrogen peroxide added to the water, LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to the basal feed + 0.5% hydrogen peroxide added to the water, ^{a,b}: Different letters in the same row indicate different groups. *P*: Significance level ($P<0.05$)

On the other hand, essential oils from plants in the Lamiaceae family, such as LSO rich in phenols, are noted for their antioxidant and growth–promoting properties when added to feeds [23, 24]. In present study, the observed impact of feeds containing LSO on the body weight of male and female quails during the four–week egg production period likely stems from factors such as feed composition and concentrations of active substances.

TABLE IV presents the production performance characteristics of quails in the control and treatment groups throughout the study period. During the first 14 d, the average egg weight was significantly lower ($P<0.01$) in the H_2O_2 group compared to the control group. In the second 14 d, both the control and H_2O_2 groups exhibited lower average egg weights ($P<0.05$) compared to LS and LS + H_2O_2 groups. The LS group showed the best feed conversion into eggs during the second 14 d, which was significantly different ($P<0.05$) from the control group. Furthermore, in terms of egg production (number and percentage), the H_2O_2 group had significantly lower values ($P<0.01$) during the first 14 d, whereas the LS + H_2O_2 group had significantly higher values ($P<0.05$) during the second 14 d compared to the control group. There was no numerical difference

between the lavender oil added LS and LS + H_2O_2 groups (P>0.05), and the LS group was found to have the best feed to egg conversion rate. Meanwhile, the LS+H_2O_2 group exhibited higher (P<0.05) results in other egg production traits (Quail/poultry house, Quail/poultry egg yield). The parameters observed during the egg production period were lower in the group where H_2O_2 was added to the water, showing similarities to the control group. Similar to the result of the study, Torki *et al.* [25] reported that the addition of Lavandula angustifolia oil to the feed of laying hens improved FCR to a statistically insignificant degree compared to the control group. Additionally, Özbilgin and Kara [26] found that supplementing lavender (*L. angustifolia* Mill. subsp. angustifolia) oil to the feed of female quails during the egg production period (35–70 d of age) increased live weight, egg production, egg mass, and egg weight values. Moreover, adding 250 mg of lavender oil to the feed resulted in a numerical improvement in feed utilization. Phytogenic feed additives, categorized as natural growth promoters, are generally known to enhance feed conversion rates, thereby increasing nutrient absorption efficiency in the intestine [27].

TABLE IV
Production performance characteristics of laying quails

Periods[a]	Control	LS	H_2O_2	LS+H_2O_2	*P*
	Average egg weight (g)				
Days 57 to 70	521.06^{a}	530.15^{a}	380.90^{b}	561.88^{a}	0.003
Days 71 to 84	574.61^{b}	694.67^{a}	531.52^{b}	699.63^{a}	0.011
	Average feed consumption of female quail (g)				
Days 57 to 70	2366.67	2047.62	1904.76	2447.62	0.523
Days 71 to 84	2395.24	2028.57	1904.79	2257.77	0.123
	Feed conversion to eggs (g/g)				
Days 57 to 70	4.54	3.87	5.01	4.38	0.735
Days 71 to 84	4.20^{a}	2.91^{b}	3.60^{ab}	3.24^{b}	0.043
	Quail / poultry house (pcs)				
Days 57 to 70	8.73^{a}	8.67^{a}	6.73^{b}	9.40^{a}	0.001
Days 71 to 84	9.33^{bc}	10.73^{ab}	8.93^{c}	11.53^{a}	0.018
	Quail / poultry egg yield (%)				
Days 57 to 70	62.38^{a}	61.91^{a}	48.10^{b}	67.14^{a}	0.001
Days 71 to 84	66.67^{bc}	76.67^{ab}	63.81^{c}	82.38^{a}	0.019

[a]: first 14 d: between 57-70 d; Second 14 days: 71-84 d; LS: 200 mg·kg^{-1} *Lavandula stoechas* oil added to the basal feed; H_2O_2: 0.5% hydrogen peroxide added to the water; LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to basal feed + 0.5% hydrogen peroxide added to the water; ^{a,b,c}: Different letters in the same row indicate different groups; *P*: Significance level (P<0.05, P<0.01)

TABLE V presents the hatching results of eggs used in the incubation process across the study groups. Regarding hatching efficiency, hatching power, and fertility rate, the groups supplemented with lavender oil in feed and H_2O_2 in water showed no significant differences compared to the control group (P>0.05). However, the LS group exhibited the highest hatching parameters, while the H_2O_2 group showed the lowest results. Furthermore, in terms of total egg weight loaded into the incubator and chick hatching weight, the LS group demonstrated significantly higher values (P<0.01) compared to the control group.

TABLE V
Characteristics of evaluating the hatching results of the eggs obtained

Characteristics	Control	LS	H_2O_2	LS + H_2O_2	X^2 / *P*
Hatching rate (%)	84.91	88.24	80.20	84.80	2.732 / 0.435
Fertility rate (%)	91.51	94.96	88.12	94.40	4.608 / 0.203
Output power (%)	92.78	92.92	91.01	89.89	0.954 / 0.812
Hatching egg characteristics					
Total egg weight loaded in the incubator (g)	12.30^{b}	12.97^{a}	11.90^{c}	12.06^{bc}	0.001
Chick emergence weight (g)	8.91^{b}	9.40^{a}	8.55^{c}	8.62^{c}	0.001

LS: 200 mg·kg^{-1} *Lavandula stoechas* oil added to the basal feed, H_2O_2: 0.5% hydrogen peroxide added to the water, LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to the basal feed + 0.5% hydrogen peroxide added to the water, ^{a,b,c}: Different letters in the same row indicate different groups, X^2: Pearson Chi Square; *P*: Significance level (P<0.01)

The addition of lavender oil to the diets of quails during the egg production period positively affected total egg weight loaded into the incubator and chick hatching weight, as show in TABLE V (P<0.01). However, its impact on hatching performance was only numerical, suggesting that LSO mitigated the effects of oxidative stress in the group where H_2O_2 was added to their water. Similarly, Radwan–Nadia *et al.* [28] found that various herbal additives (thyme (*Thymus vulgaris*), rosemary (*Salvia rosmarinus*), oregano (*Origanum vulgare*), and turmeric (*Curcuma longa*)) improved hatchability and fertility rates in laying hens, highlighting their potential as important natural antioxidants.

Blood biochemistry parameters

TABLE VI presents the blood biochemistry parameters of quails in the study groups. Blood serum GOT/AST levels in male and mixed–sex quails from the LS+H_2O_2 group were significantly lower (P<0.05) compared to the other groups. However, in females, although the GOT/AST levels were numerically lower, the difference was not statistically significant (P>0.05) compared to the other groups.

γ–Glutamyltransferase (γ–GT) and AST are enzymes involved in the transamination of glucogenic amino acids to produce glucose [29]. Elevated AST concentrations in the blood may indicate hepatocellular disease in birds [30]. Similarly, high serum γ–GT levels in birds are typically regarded as indicators of liver, bile duct, and kidney epithelium damage. However, in female birds, increased γ–GT levels may also reflect heightened liver metabolism associated with ovulation. AST is primarily associated with liver parenchymal cells but is also present in red blood cells, cardiac muscle, and skeletal muscle. Elevated AST levels in female birds, similar to γ–GT, can be associated with the ovulation process rather than indicating liver damage [29]. It has been noted that there may be gender differences in liver enzyme activities in quails [29].

The hepatoprotective effects of vegetable oil supplementation are contingent upon the high levels of antioxidant and anti–inflammatory compounds present in the oils being supplemented [31]. LSO, for instance, primarily comprises d–fenkone, α–pinene, and camphor, which belong to the group of oxygenated monoterpenes known for their antioxidant and free radical scavenging effects [32]. Studies investigating the *in vivo* effects of LSO, recognized for its antioxidant activities [32, 33], have shown

TABLE VI
Blood parameters in quail in the study groups

Characteristics	Gender	Control	LS	H_2O_2	LS+H_2O_2	*P*
ALB	Male	1.26	1.22	1.34	1.22	0.671
	Female	1.69	1.79	1.71	1.70	0.875
	Mixed	1.48	1.50	1.52	1.46	0.960
ALP	Male	847.50	872.50	761.83	1011.00	0.590
	Female	844.83	825.00	1074.17	777.83	0.231
	Mixed	846.17	848.75	918.00	894.42	0.913
CRE	Male	0.72	0.77	0.76	0.78	0.734
	Female	0.61	0.72	0.63	0.61	0.389
	Mixed	0.66	0.74	0.70	0.70	0.488
GOT/AST	Male	257.35^{a}	252.68^{a}	267.30^{a}	205.38^{b}	0.035
	Female	236.32	225.37	213.85	192.63	0.344
	Mixed	246.83^{a}	239.03^{a}	240.58^{a}	199.01^{b}	0.026
GPT/AST	Male	11.00	10.83	10.33	8.50	0.391
	Female	11.67	9.50	9.83	9.50	0.157
	Mixed	11.33	10.17	10.08	9.00	0.112
UREA	Male	9.83	10.33	11.00	10.33	0.895
	Female	8.33	9.50	12.50	10.17	0.055
	Mixed	9.08	9.92	11.75	10.25	0.083
TP	Male	2.90	2.73	3.15	2.83	0.555
	Female	4.13	4.47	4.23	4.42	0.778
	Mixed	3.52	3.60	3.69	3.63	0.974
GLOB	Male	1.64	1.51	1.81	1.62	0.521
	Female	2.44	2.68	2.52	2.72	0.745
	Mixed	2.04	2.10	2.17	2.17	0.956
ALB/GLOB	Male	0.79	0.82	0.75	0.77	0.640
	Female	0.70	0.69	0.69	0.63	0.633
	Mixed	0.74	0.75	0.72	0.70	0.614

LS: 200 mg·kg^{-1} *Lavandula stoechas* oil added to the basal feed, H_2O_2: 0.5% hydrogen peroxide added to the water, LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to the basal feed + 0.5% hydrogen peroxide added to the water, ALB: Albumin, ALP: Alkaline Phosphatase, CRE: Creatine, GOT/AST: Glutamic Oxaloacetic Transaminase/Aspartate Aminotransferase, GPT/AST: Glutamate–Pyruvate Transaminase/Alanine Aminotransferase, TP: Total Protein, GLOB: Globulin, ALB/GLOB: Albumin to Globulin ratio, ^{a,b}: Different letters in the same row indicate different groups. *P*: Significance level (*P*<0.05)

The use of cinnamon (*Cinnamomum verum*) and ginger (*Zingiber officinale*) oils in quails has also been shown to decrease serum AST levels [36]. In the current study, it was observed that serum AST levels (U/L) were generally lower in the group where hydrogen peroxide and LSO were used together compared to the other groups, with the difference being statistically significant in males, as show in TABLE VI. It has been reported that lavender (*Lavandula angustifolia*) oil, which shares similar essential content with LSO, did not alter serum AST levels when added alone to the diet of quails [37]. The lower AST levels observed in the group where hydrogen peroxide and *L. stoechas* oil were combined may be attributed to the potential hepatoprotective effects of LSO, which exhibits antioxidant properties depending on the dosage, as discussed in the literature. The differences observed in male quails could be influenced by gender–related variations in liver metabolism and individual physiological differences.

TABLE VII presents the expression levels of GPx7 and NRF2 genes analyzed to assess the antioxidant effects of LSO supplementation in quails treated with H_2O_2. While no significant difference was observed in NRF2 gene levels between the groups (as show in FIG. 1b), the GPx7 gene level was significantly increased (*P*<0.05) in the LS ♀ group compared to the control ♀ and control ♂ groups (as show in FIG. 1a). Furthermore, a significant decrease (*P*<0.05) in GPx7 gene level was observed in the LS+H_2O_2 ♀ and LS+H_2O_2 ♂ groups compared to the LS ♀ group (as show in FIG. 1a).

TABLE VII
Evaluation of relative expression result of GPx7 and NRF2 genes in groups

Factors / Groups	N	GPx7	NRF2
Control	12	1.10^{b}	1.04
LS	12	2.05^{a}	1.17
H_2O_2	11	1.76^{a}	1.04
LS+H_2O_2	11	0.96^{b}	1.19
P		0.008	0.446

LS: 200 mg·kg^{-1} *L. stoechas* oil added to the basal feed; H_2O_2: 0.5% hydrogen peroxide added to the water; LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to the basal feed + 0.5% hydrogen peroxide added to the water; NRF2: Nuclear factor erythroid 2-related factor 2; GPX7: Glutathione peroxidase 7; ^{a,b}: Different letters in the same column indicate different groups; P: Significance level (*P*<0.01)

promising results in mitigating malathion–induced liver damage in mice (*Mus musculus)* [34]. In these studies, mice were administered various doses of essential oil along with malathion over a 30–d period. In malathion–induced hepatotoxicity, a decrease in plasma albumin levels and a significant increase in bilirubin, AST, ALT, ALP, ACP, LDH, and γ–GT levels have been observed. Interestingly, it has been reported that these parameters decrease in a dose–dependent manner when animals are co–administered essential oil with malathion. It has been suggested that essential oils exhibit protective effects against malathion–induced liver damage in rats, attributed in part to their antioxidant properties [32].

Similarly, vegetable oils derived from rosemary have been reported to significantly reduce blood AST levels in broilers [35].

L. stoechas is an aromatic plant rich in therapeutic active compounds [32]. In this study, LSO was observed to increase the expression level of GPx7, an antioxidant gene, in quail liver tissue compared to the control ♀ and control ♂ groups, as show in Table 7. Some phenolic compounds present in *L. stoechas* exhibit antioxidant properties [33]. For instance, Selmi *et al.* [38] demonstrated in a mouse study that LSO enhanced the activity of GPx, an antioxidant enzyme, under conditions of oxidative stress. Bastos *et al.* [18] reported that cinnamon, known for its antioxidant properties, increased hepatic GPx7 gene expression levels in quails. Similarly, Li *et al.* [39] showed that selenium supplementation in quails increased GPx enzyme activity and GPx4 gene expression levels. These findings suggest that LSO may exert hepatoprotective effects by enhancing antioxidant gene expression. However, the

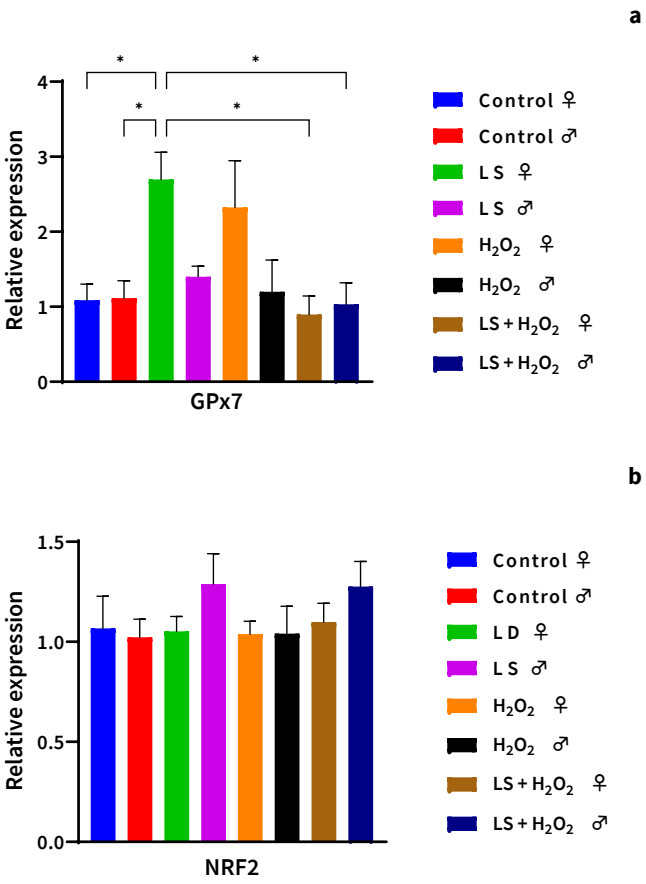

FIGURE 1. Analysis of relative expression of GPx7 (a) and NRF2 (b) genes in quail liver tissue by RT-qPCR. LS: 200 mg·kg^{-1} *Lavandula stoechas* oil added to the basal feed; H_2O_2: 0.5% hydrogen peroxide added to the water; LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to the basal feed + 0.5% hydrogen peroxide added to the water; GPX7: Glutathione peroxidase 7, NRF2: Nuclear factor erythroid 2-related factor 2; ♀: Female quail; ♂: Male quail

lack of significant difference observed in NRF2 gene expression levels between the groups may be attributed to the dosage of LSO used or its potential inability to activate the NRF2 pathway.

Ahmed–Farid *et al.* [40] demonstrated that H_2O_2 suppressed GPx and NRF2 gene expression levels in rat liver tissue in a dose–dependent manner, with a lesser effect at lower doses. However, in this study, it was observed that H_2O_2 did not significantly affect GPx7 and NRF2 gene expression levels, as show in TABLE VII. This discrepancy may be attributed to the specific dose of H_2O_2 used in present experiment. Oxidative stress is a complex process influenced by various factors, and any beneficial dietary change likely involves multiple mechanisms.

In the study, the numerical differences observed in terms of fatty liver score among mixed–sex and male quails in the groups were not statistically significant (as show in TABLE VIII; as show in FIG. 2 A, B). However, in female quails, the fatty liver score was significantly higher (P<0.05) in the H_2O_2 group compared to the control group (as show in FIG. 2 C, D).

TABLE VIII
Fatty liver score in quails

Gender	Control	LS	H_2O_2	LS+H_2O_2	*P*
Mixed	1.42 (0 – 2)	1.33 (0 – 3)	2.00 (1 – 3)	1.50 (0 – 2)	0.247
Male	1.00 (0 – 2)	0.67 (0 – 1)	1.33 (1 – 2)	1.00 (0 – 2)	0.280
Female	1.83^{b} (1 – 2)	2.00^{ab} (1 – 3)	2.67^{a} (2 – 3)	2.00^{ab} (2 – 2)	0.030

LS: 200 mg·kg^{-1} *Lavandula stoechas* oil added to the basal feed, H_2O_2: 0.5% hydrogen peroxide added to the water, LS+H_2O_2: 200 mg·kg^{-1} *L. stoechas* oil to the basal feed + 0.5% hydrogen peroxide added to the water, ^{a,b}: Different letters in the same row indicate different groups. *P*: Significance level (P<0.05)

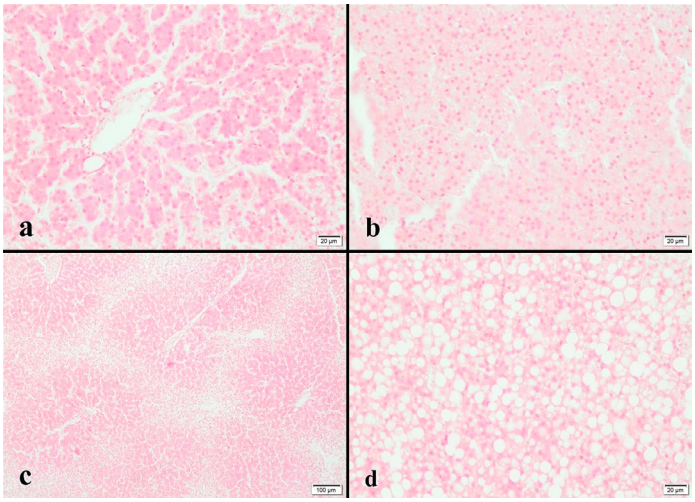

FIGURE 2. Histopathologic findings in the liver. a) Control male; mild fat vacuoles in the liver, H&E. b) Blackhead male; few fat vacuoles in the liver, H&E. c) Control female; moderate fat vacuoles in hepatocytes, H&E. d) H_2O_2 female; diffuse fat vacuoles in the liver, H&E

L. stoechas extracts are known for their use as painkillers, antimicrobials, sedatives, and for relieving urinary tract inflammation, as well as for their heart–strengthening and vascular occlusion properties [32]. However, in this study, it was found that the use of LSO alone or in combination with H_2O_2 did not significantly affect fatty liver in male and female quails. Conversely, it was observed that H_2O_2 significantly increased fatty liver in female quails.

CONCLUSION

The addition of *L. stoechas* to the feed was found to reduce feed intake and improve egg weight, egg number, and feed conversion rate in quails aged 56–84 d during the egg production period. Additionally, it increased hatchability, fertility rate, and chick hatching weight. It was observed that the oxidative stress induced by hydrogen peroxide added to the water decreased yield in quails, but this effect was mitigated by the antioxidant properties of *L. stoechas* oil added to the feed. At the gene expression level, it was observed that adding LSO to the quail feed increased antioxidant gene levels, alleviated stress caused by hydrogen peroxide, and demonstrated a hepatoprotective effect.

Conflict of interests

The authors have no conflict of interest to declare in regard to this publication.

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