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Bay Laurel (*Laurus nobilis* L.) in Japanese Quails Feeding 1. Performance and Egg Quality Parameters

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Abstract: The aim of this study was to evaluate the effect of supplemented bay laurel (*Laurus nobilis* L.) leaves to a conventional diet of laying Japanese quail on the performance and some egg quality. A total number of 60 and 54 days old laying type quails were distributed into individual cages for three different treatments. The control diet did not contain *Laurus nobilis* (LN) leaves (LN0) and the other diets were supplemented with 2 g (LN2) and 4 g (LN4) leaves kg^{-1}, respectively. The trial lasted for a total of 10 weeks. Supplemented leaves of LN (2 or 4 g kg^{-1} feed) had no effect on any performance parameters, external and internal egg quality traits of quails. The diets have not changed the amount of egg yolk total cholesterol at 121 days old. However, egg yolk triglyceride concentration of quails fed LN4 diet was lower ($p<0.01$) than quails fed with the other diets at 121 days old. Besides, the amount of total cholesterol and triglyceride of egg yolk increased ($p<0.01$) in all of the groups with age. In egg yolk in the group receiving diet LN4, the level of palmitic acid was lower ($p<0.05$) and oleic acid was higher ($p<0.05$) than those of the other 2 groups at 118 days. Besides at that age, the level of palmitic acid was lower and oleic acid was >55, 76 and 97 days. There was no change in the other fatty acids. The changes in total saturated and total monounsaturated fatty acids were parallel with palmitic acid and oleic acid, respectively. Total polyunsaturated fatty acid content in the present study did not change with diet and age. The results of this study demonstrated that supplemented a conventional diet with LN could change some biochemical parameters of quail egg yolk.

Key words: *Laurus nobilis* leaves, Japenese quail, performance, some egg quailty traits, polyunsaturated, Turkey

INTRODUCTION

Stress factors such as transportation, electric shocks, fasting, low or high ambient temperature, etc. are one of the limiting factors which affect the welfare and the production of poultry. Rapid growth and high egg production are themselves a source of stress (chronic stress). In contrast, lower performance, traditional, backyard or village poultry are less likely to be affected in the same way by stress factors (Nadia *et al*., 2008).

Stress induces free radicals formation and develops oxidative stress, initiating lipid peroxidation in tissues followed by abnormalities in lipid metabolism, growth and production in general (Eid *et al*., 2006). Lipid oxidation products are related to cardiovascular and other diseases. Synthetic or natural antioxidants have been widely used in feed industry in order to prevent such undesirable oxidative effects. Because synthetic antioxidants such as butylated hydroxytoluene and butylated hyrdoxyanisole are considered to have carcinogenic potential, natural antioxidants have received considerable attention in human and animal nutrition (Imaida *et al*., 1983; Elmastas *et al*., 2006).

Much research has been done to enrich eggs with n-3 fatty acids (Galobart *et al*., 2001) and to prevent lipid oxidation in these products. For this purpose, researchers tend to examine the effects of aromatic plant extracts on lipid oxidation of bird products (Galobart *et al*., 2001; Botsoglou *et al*., 2005) . However in practice, the amount of produced traditional commercial feed is so much

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more that it is incomparable with enriched feed and stress continue continuously in birds having high egg production. If the plant does not contain toxic substance (s) and these do not affect absorption of nutrient (s) negatively, the plant may be more favourable according to its extract.

Numerous spices and aromatic herbs have been examined for their antioxidant/antiradical activity. Bay laurel *(Laurus nobilis* L. (LN)), a species held in high esteem since, ancient times and evergreen tree is cultivated in many warm parts of the world, particularly in the Mediterranean area is natively cultivated on the coastal up to an altitude of 600-800 m (Derwich *et al*., 2009). Methanolic extracts of the plant contain polar compounds (such are phenols, flavones and flavonols) and show antioxidative activity against lipid peroxidation (Simic *et al*., 2003). The antioxidant activity, reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities of ethanol and water extracts of LN were carried out by the group. The total antioxidant capacity of both extracts exhibited strong *in vitro* antioxidant capacity (Elmastas *et al*., 2006). The essential oil from the leaves (0.8-3%) contains mostly 1, 8-cineol (up to 50%) also eugenol, acetyl and methyl eugenol, α- and β-pinene, phellandrene, linalool, geraniol and terpineol (Zekovic *et al*., 2009). Conforti *et al*. (2006) determined that the amounts of phenolics in LN leaves were 210 and 219 mg g^{-1} for wild and cultivated plant extracts, respectively.

Although, there is a report of LN extract on blood lipid profile of quail (Al-Attar, 2006), we could not find any report about whether LN leaves are natural antioxidant on performance and egg parameters in birds. Therefore, the aim of this study was to evaluate the effect of supplemented LN leaves to a conventional diet of laying Japanese quail on the performance and some egg quality.

MATERIALS AND METHODS

Animals, diets and husbandry: A total number of 60 quails, 54 days old were distributed one by one into individual cages (20×25×25 cm) for three different treatments. Each manger was covered with 1×1 cm sized intermittent wire for prevention of feed scattering. All birds were kept under similar and standard hygienic, environmental and managerial conditions according to the Turkey Experimental Animal Care Guidelines. Dry matter, crude protein, crude fibre, total ash and ether extract contents of corn, soybean meal and wheat ingredients were determined according to the AOAC (1990) and metabolizable energy values were calculated using formulas described by Janssen (1989). Diets of the 3 groups had 185 g crude protein and 2800 kcal ME kg^{-1} (Table 1). The control diet did not contain LN leaves (LN0) and the other diets were supplemented with 2 g leaves (LN2) and 4 g leaves (LN4) kg^{-1}, respectively. The leaves harvested from Black sea region of Turkey were dried in the shade and ground into a fine powder in a mill. During the feeding period that lasted 70 days, feed and water were offered *ad libitum* whereas photoperiod was 17 h daily. All birds were weighed at intervals of 7 days and feed consumption was recorded weekly while egg production was recorded daily.

Egg characteristics: Ten eggs were chosen randomly from each treatment for all of experimental measurements and biochemical analysis.

Exterior and interior egg characteristics: Egg weight, egg specific gravity, egg shape index, egg surface area, shell weight per unit surface area, shell thickness, shell ratio, yolk index, yolk content, albumen index, albumen content were measured at the last week of the experiment (123 days).

Some biochemical characteristics of quail egg yolk: The lipid fractions of egg yolk samples were extracted according to Folch *et al*. (1957) for analysis of total cholesterol, triglyceride and fatty acid methyl esters.

Table 1: Ingredient and chemical composition of basal diet

Ingredients	Values (g kg^{-1})
Maize	484.57
Soybean meal	275.85
Wheat	150.00
Sunflower oil	10.00
Limestone	60.45
Dicalcium phosphate	11.45
NaCl	3.50
Vitamin premix[1]	1.00
Mineral premix[2]	1.00
DL-methionine	1.74
L-Lysine HCl	0.44
Calculated contents (per kg)	
Metabolizable energy kcal	2,800.00
Crude protein (g)	185.00
Methionine (g)	4.50
Lysine (g)	10.00
Threonine (g)	6.90
Ca (g)	26.00
Available P (g)	3.50
Na (g)	1.60

[1]Vitamin premix provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 1,500 IU; vitamin E, 50 mg; vitamin K_3, 5 mg; vitamin B1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; niacin, 25 mg; Ca-D-pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apocarotenoic acid ester, 2.5 mg; choline chloride, 400 mg. [2]Trace mineral premix provided per kilogram of diet: Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; CO, 0.20 mg; I, 1 mg; Se, 0.15 mg

Total cholesterol and triglyceride of quail egg yolk: These analyses were performed at the start (56 days) and end (121 days) of the experiment. Triglyceride and total cholesterol amounts of egg yolk samples were directly measured with commercial kit (Beckman Coulter, USA) using UNICEL D×C 800 (Beckman Coulter, USA) automatic analyzer system.

Fatty acid methyl esters of quail yolk lipids: Fatty acid composition was determined at the 55, 76, 97 and 118 days old. One yolk of quail egg (about 2 g) was homogenized by ultra mixer in a chloroform/methanol (2:1 v/v) mixture using a high speed blender and filtered through Whatman paper. The filtrate was evaporated to dryness with a rotary evaporator (Heidolph EssLab, Essex, UK) at 50°C under reduced pressure and quail egg yolk lipids were obtained. Samples were analyzed in triplicate. Then the fatty acid methyl esters were prepared from extracted lipids from quail egg yolk by esterification reaction with 14% boron trifluoride-methanol complex according to the modified method of the AOAC (1990). About 0.5 N methanolic NaOH was added to the quail egg yolk lipids. The mixture was heated at 100°C during 5 min under refluxed conditions and cooled to 25°C. The cooled mixture was esterified with 14% boron trifluoride at 100°C during 15 min. After adding hexane, the hot solution was shaken and then saturated sodium chloride was added. The methyl ester phase was separated from the hexane phase in a separatory funnel and was placed in a vial. One microliter of fatty acid methyl esters from the extracted yolk lipids was injected to gas chromatography.

Sub-sequent fatty acid profiles were analyzed by gas chromatography. The fatty acid methyl esters were analysed using a 30 m (with 0.25 mm film thickness), Quadrex 30.00×250 μm 0.25 mm ID capillary GC column installed on a Perkin Elmer (Auto system) gas chromatograph with a Flame Ionization Detector (FID). The gas chromatograph temperature-programme was oven: Initial temprature. 100°C for 0 min, ramp 10°C min^{-1} to 180°C, hold 6 min, ramp 1.5°C min^{-1} to 220°C, hold 6 min, InjAauto = 250°C, Volume = 1 μL, Split = 30:1, Carrier Gas = He, Solvent Delay = 0.00 min, dedector Temp = 250°C.

Statistical analysis: The means of 3 groups were compared through Duncan's multiple-range test after all data were subjected to Analysis of Variance (one way-ANOVA). The means of two groups were compared by the Paired-samples t-test, using SPSS statistical package (Norusis, 2002).

RESULTS AND DISCUSSION

Performance parameters and exterior and interior egg characteristics: Supplemented LN leaves (2 or 4 g kg^{-1} feed) had no effect on performance parameters (body weight, feed intake, egg production and feed efficiency) of quails (Table 2). Direct comparison of our results with other studies is difficult because there is no report of *in vivo* antioxidant effects of LN. However, Nadia *et al*., (2008) reported that addition of herbs as natural antioxidants (1.0% thyme, rosemary, oregano or 0.5% curcuma longa) during the laying period improved egg production, egg mass and feed conversion, may be due to the phenolic compounds which considerably exhibit antimicrobial, antifungal and antioxidant activities and accordingly could improve the bird utilization of dietary nutrients.

Besides, they observed that addition of curcuma longa (1.0%) increased percentage of yolk weight and improved yolk colour index without affecting values of feed intake, shell weight and egg shape index. Moreover, addition of rosemary to diet had no significant effect on egg production. Some antioxidant components of plants may improve calcium deposition in uterus and consequently increase shell weight and shell thickness (Ramirez-Tortosa *et al*., 1999). In the present study, external egg quality traits (egg weight, egg specific gravity, egg shape index, egg surface area, shell weight per unit surface area, shell thickness and content) were not influenced significantly by any of the experimental treatments (Table 3). These results agree with Ali *et al*. (2007) and Nadia *et al*. (2008) who added 0.25% thyme and 0.5-1.0% thyme, rosemary, oregano or curcuma longa, respectively to hen's diets. There were also no significant differences in the present study among treatments in internal egg quality characteristics (ratio and index of albumen and yolk and Haugh unit; Table 3). Whereas, Nadia *et al*. (2008) reported that addition of 0.5% herbs in laying hens diets, insignificantly decreased albumen content with increased yolk content while 1.0% thyme, rosemary or curcuma longa showed significant effect.

Total cholesterol and triglyceride of quail egg yolk: Cholesterol and fatty acids of egg yolk alter with dietary manipulation and drugs as well as genetics, age and production level of the bird (Baucells *et al*., 2000;

Table 2: Effect of dietary LN supplementation on performance traits of Japanese quails (N = 20)

Traits	LN0	LN2	LN4	SEM	p
Body weight (g)					
54 days	225.600	224.500	225.90	3.5.0	NS
124 days	220.200	221.900	216.40	3.7.0	NS
Feed intake, g hen^{-1} days	35.800	35.500	34.10	0.5.0	NS
Hen-day egg production (%)	92.800	93.700	89.40	1.3.0	NS
Egg weight (g)	11.400	11.100	10.90	0.2.0	NS
Feed efficiency, g egg g^{-1} feed	0.296	0.294	0.28	0.003	NS

*Quails were fed with the control diet from 42-56 days. SEM: Standard Error of the Mean; NS: Not Significant (p>0.05)

Table 3: Effect of dietary LN supplementation on exterior and interior egg characteristics of Japanese quails at 123 days (N = 10)

Traits	LN0	LN2	LN4	SEM	p-value
Egg weight (g)	11.40	11.3.00	10.900	0.200	NS
Egg specific gravity	1.075	1.076	1.076	0.001	NS
Egg shape index (%)	77.50	79.600	77.100	0.600	NS
Egg surface area (cm^2)	22.20	22.000	21.500	0.200	NS
SWUSA (mg cm^{-2})	51.40	51.400	51.500	0.600	NS
Shell thickness (mm)	0.202	0.201	0.198	0.006	NS
Shell content (%)	10.00	10.000	10.100	0.100	NS
Yolk index (%)	48.40	47.400	50.100	0.600	NS
Yolk content (%)	31.80	32.900	32.500	0.300	NS
Albumen index (%)	9.300	9.100	9.400	0.300	NS
Albumen content (%)	58.20	57.100	57.300	0.300	NS
Haugh unit	86.50	86.400	86.300	0.600	NS

*Quails were fed with the control diet from 42-56 days. SEM: Standard Error of the Mean; NS: Not Significant ($p>0.05$); SWUSA, Shell Weight per Unit Surface Area. Egg specific gravity = weight in air/(weight in air - weight in water); Egg shape index (%) = [width (cm)/length (cm)]×100. Egg surface area (cm^2) = $3.9782\times(\text{egg weight})^{0.7056}$; Shell weight per unit surface area (mg cm^{-2}) = shell weight (mg)/egg surface area (cm^2). Shell content (%) = (shell weight/egg weight)×100; Albumen weight = egg weight - (yolk weight+shell weight). Albumen content (%) = (albumen weight/egg weight) ×100; Albumen index (%) = Albumen height (mm)/[Albumen length (mm)+Albumen width (mm)/2]×100; Yolk index (%) = (yolk height/yolk diameter)×100; Yolk content (%) = (yolk weight/egg weight)×100; Haugh Unit (HU) = $100\times\log(\text{albumen height (mm)}+7.57 - 1.7\ \text{egg weight (g)}^{0.37})$

Grobas *et al*., 2001; Shafey *et al*., 2003; Guclu *et al*., 2008; Nowaczewski *et al*., 2010). Nadia *et al*. (2008) reported that thyme, rosemary or curcuma longa (at 1.0% concentration) significantly decreased egg yolk total lipid while total cholesterol content of egg yolk was not affected by plant supplementation. Effect of the diets contained LN (LN2 and 4) was not observed on the amount of egg yolk total cholesterol at 56 and 121 days old (Table 4).

The overwhelming evidence is that egg yolk cholesterol level is very resistant to change because of the particular mechanisms involved in yolk formation. Yolk precursors are synthesized in the liver of the laying hen and transported in the plasma to the ovary where they are taken up into the developing follicles by receptor-mediated endocytosis. As a consequence, the cholesterol content of the yolk is primarily dependent on the cholesterol content of triglyceride-rich lipoproteins (Shafey *et al*., 2003; Cabrera *et al*., 2005).

Finding data of this research demonstrated that triglyceride concentration was lower ($p<0.01$) in the egg yolk of the quails fed with LN4 diet than those fed the control diet at 121 days old (Table 4). The decrease of triglyceride may be due to the antioxidant compounds in *Laurus nobilis* on lipid metabolism. The results agree to a large extent with those obtained by Nadia *et al*. (2008) who reported that thyme, rosemary or curcuma longa (at 1.0%) significantly decreased egg yolk total lipid while total cholesterol content of egg yolk was not affected by plant supplementation.

Besides, the amount of total cholesterol and triglyceride of egg yolk increased in all of the groups with age ($p<0.05$). Shafey *et al*. (1998) reported that the yolk

Table 4: Effect of dietary LN supplementation on the total cholesterol and triglyceride of quail egg yolk (mg g^{-1}, N = 10)

Biochemical compounds	Age (days)	LN0	LN2	LN4	SEM	p-value
Total cholesterol	56	11.84	11.61	11.93	0.21	NS
	121	1391^{**}	13.79^{**}	13.49^{**}	0.13	NS
Triglyceride	56	196	189	192	2.66	NS
	121	234^{a**}	232^{a**}	219^{b**}	1.94	0.01

*Quails were fed with the control diet from 42-56 days.; SEM: Standard Error of the mean; P: Probability; NS: Not Significant ($p>0.05$). $^{a, b}$Means within a row with no common superscript differ significantly (Duncan, $p<0.05$). **Means within a column differ significantly $p<0.01$ (Paired-samples t-test)

cholesterol concentration produced by hens at the age of 52 weeks was significantly higher than those produced by hens <47 weeks.

Fatty acid methyl esters of quail yolk lipids: In this study, results clearly showed that palmitic and oleic acid levels in egg yolk were changed ($p<0.05$) only by diet LN4 at 118 days old (Table 5). In egg yolk, the level of palmitic acid, a saturated fatty acid was lower ($p<0.05$) and oleic acid, a monounsaturated fatty acid was higher ($p<0.05$) in the group receiving diet LN4 than those of the other 2 groups at 118 days old. However, it is thought that diet LN2 is insufficient and diet LN4 may be effective after only 2 months use on egg yolk's oleic acid.

Besides, quails at 118 days old, the level of egg yolk palmitic acid was lower and oleic acid was higher than those of the other ages ($p<0.01$) in all of the groups but the mechanism has not been known largely. In birds, lipogenesis is confined to the liver where it is particularly important in providing lipids for egg formation. Palmitate (C16:0), the principal product of the fatty acid synthase system in animal cells is the precursor of other long-chain saturated and unsaturated fatty acids. It may be lengthened to form stearate (18:0) or even longer saturated fatty acids by further additions of acetyl groups. Desaturase activities regulate the tissue concentrations of fatty acids, especially for polyunsaturated fatty acids or monounsaturated fatty acids. After palmitate lengthened to stearate (18:0), it can be converted to oleate (18:1) by desaturase.

These elongated and unsaturated metabolites are deposited in egg yolk (Collins *et al*., 1997; Ayerza and Coates, 2000). It can be concluded that while oleic acid content in egg yolk increased, palmitic acid content in egg yolk decreased during trial period at 118 days. With age, cell regeneration rate is increased and this process needs unsaturated fatty acid. Desaturase activities may be increased with age.

Therefore, oleat level in egg increased while palmitate level in egg decreased at 118 days. However, there was no change at the other fatty acids. The changes in total saturated and total monounsaturated fatty acids

Table 5: The effect of dietary LN supplementation on the fatty acid composition of quail egg yolk lipids percentage of fatty acid methyl esters, N=10) in different ages

Fatty acids	Age (days)	LN0	LN2	LN4	SEM	p-value
C14:0 (Myristic acid)	55	0.43	0.44	0.44	0.02	NS
	76	0.55	0.49	0.54	0.02	NS
	97	0.55	0.54	0.56	0.01	NS
	118	0.27	0.23	0.24	0.02	NS
C16:0 (Palmitic acid)	55	27.22^{A}	27.86^{A}	27.27^{A}	0.23	NS
	76	28.06^{A}	29.03^{A}	28.47^{A}	0.26	NS
	97	27.43^{A}	27.81^{A}	27.67^{A}	0.23	NS
	118	22.07^{Ba}	22.24^{Ba}	$20.68^{B\,b}$	0.25	0.05
C16:1n7t (Trans palmitoleic acid)	55	0.59	0.59	0.57	0.03	NS
	76	0.44	0.41	0.38	0.02	NS
	97	0.76	0.72	0.64	0.02	NS
	118	0.30	0.32	0.28	0.02	NS
C16:1n9c (cis Palmitoleic acid)	55	3.63	3.63	3.60	0.13	NS
	76	3.46	3.78	3.82	0.14	NS
	97	3.86	4.10	4.30	0.11	NS
	118	2.49	2.76	2.83	0.08	NS
C18:0 (Stearic acid)	55	9.45	9.51	9.29	0.25	NS
	76	9.45	9.07	8.85	0.23	NS
	97	10.01	9.72	9.71	0.21	NS
	118	10.13	10.09	9.81	0.18	NS
C18:1n9c (cis Oleic acid)	55	47.27^{B}	46.62^{B}	47.44^{B}	0.44	NS
	76	47.43^{B}	46.57^{B}	47.71^{B}	0.42	NS
	97	46.46^{B}	46.24^{B}	46.43^{B}	0.36	NS
	118	52.74^{Ab}	52.38^{Ab}	54.30^{Ab}	0.32	0.05
C18:1n9t (Trans elaidic acid)	55	1.77	1.76	1.63	0.06	NS
	76	1.98	2.04	1.67	0.13	NS
	97	2.28	2.06	1.95	0.08	NS
	118	2.18	2.14	2.08	0.09	NS
C18:2n6 (Linoleic acid)	55	8.09	8.04	8.27	0.27	NS
	76	7.31	7.43	7.32	0.17	NS
	97	7.29	7.42	7.37	0.19	NS
	118	8.21	8.20	8.13	0.20	NS
C20:4n6 (Arachidonic acid)	55	1.16	1.17	1.11	0.05	NS
	76	0.92	0.85	0.93	0.05	NS
	97	1.08	1.07	1.04	0.03	NS
	118	1.20	1.22	1.24	0.03	NS
C22:6n3 (Docosahexaenoic acid)	55	0.38	0.38	0.38	0.02	NS
	76	0.40	0.33	0.32	0.02	NS
	97	0.29	0.32	0.33	0.02	NS
	118	0.42	0.42	0.40	0.02	NS
Total saturated	55	37.10^{A}	37.81^{A}	37.00^{A}	0.39	NS
	76	38.07^{A}	38.59^{A}	37.85^{A}	0.34	NS
	97	37.99^{A}	38.07^{A}	37.93^{A}	0.36	NS
	118	32.47^{Ba}	32.55^{Ba}	$30.73^{B\,b}$	0.32	0.05
SEM		0.44	0.58	0.56		
Probability		0.01	0.01	0.01		
Total monounsaturated	55	53.27^{B}	52.60^{B}	53.24^{B}	0.48	NS
	76	53.31^{B}	52.80^{B}	53.58^{B}	0.44	NS
	97	53.36^{B}	53.12^{B}	53.33^{B}	0.39	NS
	118	57.70^{Ab}	57.60^{Ab}	$59.49^{A,\,a}$	0.33	0.05
SEM		0.45	0.50	0.56		
Probability		0.01	0.01	0.01		
Total polyunsaturated	55	9.64	9.59	9.76	0.29	NS
	76	8.63	8.61	8.57	0.19	NS
	97	8.65	8.81	8.74	0.20	NS
	118	9.83	9.84	9.78	0.21	NS
SEM		0.22	0.20	0.24		
Probability		NS	NS	NS		

*Quails were fed with the control diet from 42-56 days; SEM: Standard Error of the Mean; P: Probability; NS: Not Significant ($p>0.05$). $^{a, b}$Means within a row with no common superscript differ significantly (Duncan, $p<0.05$). $^{A, B}$Means within a column with no common superscript differ significantly (Duncan, $p<0.05$)

have been associated with palmitic acid and oleic acid, respectively. Total polyunsaturated fatty acid content in the present study was not changed with diet and age but the reason of this is unknown. However, dietary LN levels (0.2 and 0.4%) could have been insufficient on the total polyunsaturated fatty acid content.

CONCLUSION

The results of the persent research demonstrated that addition of 4 g LN to a conventional diet could alter some biochemical parameters of egg yolk, especially triglyceride, palmitic and oleic fatty acids without affecting performance adversely and external and internal egg quality traits of quails. Further studies should be carried out to evaluate the possible effects of *Laurus nobilis* such as different levels and stress conditions in birds.

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