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The effects of dietary supplementation of olive leaf extract and eggshell with membrane on performance, egg quality, blood biochemical, and bone parameters in laying Japanese quail

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Abstract: This study was conducted to determine the effects of dietary supplementation of olive leaf extract (OLE), eggshell with the membrane (ESM), and the ESM that absorbed the OLE (OLE+ESM) on the performance, egg quality, biochemical, and bone parameters in laying Japanese quail. A total of 112 quail, being 45-day-old, were divided into 4 groups with 4 replicates. The quail were fed with four diets: i) basal diet ii) basal diet supplemented with 400 ppm OLE iii) basal diet supplemented with 2% ESM, and iv) basal diet supplemented with 2% ESM that absorbed with 400 ppm OLE. Egg weight was observed to be higher in the OLE group ($P<0.05$). Total feed intake increased in ESM and OLE+ESM groups ($P<0.05$). Egg production and feed conversion ratio were found to be better in control and OLE+ESM groups ($P<0.01$). Shape index was higher in OLE, ESM and OLE+ESM groups ($P<0.05$). Percentages of albumen and shell were significantly lower in ESM group ($P<0.01$). Percentage of yolk, shell thickness, shell ash, and yolk color were not affected by the supplementation of ESM and OLE groups ($P>0.05$). There was no statistical difference in tibia bone parameters ($P>0.05$). The lowest concentration of serum lactate dehydrogenase (LDH) was observed in control group ($P<0.01$). Serum uric acid level decreased in ESM group ($P<0.01$). OLE supplementation had limited impacts on quail nutrition. Consequently, while the individual usage of OLE and ESM did not show remarkable effects, the mixture of OLE and ESM has been found to positively affect the egg quality and performance parameters.

Keywords: Biochemical parameters, eggshell with membrane, olive leaf extract, performance, quail.

Yumurtacı Japon bıldırcınlarında diyete ilave edilen zeytin yaprağı özütü ve zarlı yumurta kabuğunun performans, yumurta kalitesi, kan biyokimyasal ve kemik parametreleri üzerine etkileri

Özet: Bu çalışma, yumurtacı Japon bıldırcınlarında zeytin yaprağı özütü (OLE), zarlı yumurta kabuğu (ESM), zarlı yumurta kabuğuna emdirilmiş zeytin yaprağı özütü (OLE+ESM) ilavesinin performans, yumurta kalitesi, kan ve kemik parametreleri üzerine etkilerini belirlemek için yapılmıştır. Toplam olarak 112 adet 45 günlük yumurtacı bıldırcın, 4 tekerrürlü 4 gruba ayrılmıştır. Bıldırcınlar; i) bazal diyet ii) bazal diyete 400 ppm OLE ilavesi iii) bazal diyete %2 zarlı yumurta kabuğu ilavesi iv) bazal diyete 400 ppm OLE+%2 ESM ilavesi şeklinde 4 diyetle beslenmiştir. Yumurta ağırlığının OLE grubunda daha yüksek olduğu gözlenmiştir ($P<0,05$). Toplam yem tüketimi ESM ve OLE+ESM gruplarında artmıştır ($P<0,05$). Yumurta üretimi ve yem dönüşüm oranı, kontrol ve OLE+ESM gruplarında daha iyi bulunmuştur ($P<0,01$). Şekil indeksi OLE, ESM ve OLE+ESM gruplarında daha yüksek bulunmuştur ($P<0,05$). Ak ve kabuk oranı, ESM grubunda anlamlı olarak düşük tespit edilmiştir ($P<0,01$). ESM ve OLE ilavesi sarı oranı, kabuk kalınlığı, kabuk külü ve sarı rengini etkilememiştir ($P>0,05$). Tibia kemik parametrelerinde istatistiksel fark bulunmamıştır ($P>0.05$). En düşük serum laktat dehidrojenaz konsantrasyonu (LDH) kontrol grubunda gözlenmiştir ($P<0,01$). Serum ürik asit düzeyi ESM grubunda azalmıştır ($P<0,01$). OLE ilavesinin bıldırcın beslenmesi üzerinde sınırlı etkileri olmuştur. Sonuç olarak, OLE ve ESM'nin ayrı ayrı kullanımları önemli etkiler göstermezken, OLE ve ESM karışımının yumurta kalitesini ve performans parametrelerini olumlu yönde etkilediği görülmüştür.

Anahtar sözcükler: Bıldırcın, biyokimyasal parametreler, performans, zarlı yumurta kabuğu, zeytin yaprağı özütü.

Introduction

Agricultural by-products are the the most commonly used feed additives in animal feeding (43). Although oleuropein (OLE), the main phenolic compound of the olive tree, is found in all parts of the olive fruit (pulp, core, membrane), it is contained in the highest level in theleaves. The olive leaf extract includes 19% OLE, 1.8% flavonoid glycosides, and 3-4 dihydroxy-phenethyl esters (1, 15). Moreover, OLE is composed of sub-units of hydroxytyrosol, elenolic acid, and glucose (15).

Recent studies have revealed that OLE has antioxidant, antimicrobial, antiviral, anti-inflammatory, antitumor, neuroprotective, and hepatoprotective effects (3, 15, 19). The addition of herbal extracts to poultry feeds, regulates the digestive system, stimulates digestive juices for enhancing their appetite, increases the feed intake, protects against diseases by its antibacterial effect, and consequently improves the performance of the quail (15, 16).

OLE is susceptible to oxidation and enzymatic reactions during digestion. OLE may show a poor bioavailability in the gastrointestinal tract during oral intake (29). In particular, OLE is an inhibitor of low-density lipoprotein (LDL) oxidation and has stronger antioxidant properties than butylated hydroxytoluene (BHT), vitamin C, and vitamin E (10, 34). OLE significantly reduces serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and, thus has positive effects on hepatotoxicity (19). In addition, OLE inhibits several enzymes involved in glucose metabolism both *in vivo* and *in vitro* (4, 21).

Eggshell is a by-product of the poultry industry (hatcheries, poultry farms, fast food industries, egg product factories) in addition to household waste and restaurant (30). Eggshell is composed of 10.2% of the whole egg. It contains calcium and other microelements such as magnesium, boron, copper, iron, manganese, molybdenum, sulfur, silicon, and zinc in trace amounts. Calcium in the eggshell is accepted as the best source of natural calcium and can be absorbed by about 90%. A whole medium eggshell contains approximately 750-800 mg elemental calcium (8). The composition of the eggshell is about 98.2%, 0.9%, and 0.9% of calcium carbonate, magnesium, and phosphate, respectively (22).

The eggshell membranes (ESM) also contain 69.2% protein, 2.7% fat, 1.5% moisture, and 27.2% ash (27). The membranes are fibrous structural proteins made up of collagens and keratins. There are also numerous other proteins and peptides with antimicrobial, antioxidant, and immune-modulatory properties such as lysozyme, ovotransferrin, ovalbumin, globulin, ovomucin, and defensin present in these membranes (28).

The studies have shown that the usage of eggshells as a source of calcium in poultry diets is appropriate (18, 38). The eggshell powder is one of the egg by-products that increase egg production and improve the quality of egg shells in laying hens (22). Makkar et al. (28) found that eggshell membrane supplementation to broiler diet had a positive effect on their performance.

Gongruttananun (18) revealed that chicken eggshells can be used as the sole calcium source in layer diets without adverse effects on productive traits, egg and eggshell quality, plasma calcium balance, and bone mineralization. On the other hand, Scheideler (38) reported that there was no significant advantage of supplementation of eggshells in layer diets. Similarly, Olgun et al. (33) found that eggshell supplementation into laying hens' diet was not beneficial in performance.

In this context, the aim of this study was to evaluate the possible usage of OLE and ESM as a feed additive in quail diets and determine the effects of OLE, ESM, and OLE+ESM supplementations on performance, egg quality, blood biochemical, and bone parameters in laying Japanese quail.

Material and Methods

Experimental design and diets: A total of 112 laying Japanese quail (*Coturnix coturnix Japonica*) were obtained from a commercial quail producer company. Ethical approval was approved by Local Ethics Committee (Protocol Number: 2016/68) for this study. The experiment was carried out at the Poultry Unit, Faculty of Veterinary, Fırat University.

Four groups consisting of 28 female quail aged 45 days were assigned into four replicates and each replicate included 7 quail. The quail fed one of four diets: i) Control group was fed with basal diet. ii) ESM group was fed with 2% eggshell with membrane (ESM) which added to the basal diet. iii) OLE group was fed with only 400 ppm olive leaf extract (OLE) added into standard basal diet. iv) OLE+ESM group was fed with 2% ESM that absorbed 400 ppm OLE that added into the standard diet. The ESM used in the study was prepared according to Jia et al. (20). Raw ESM was obtained manually from commercial quail eggs and powdered by grinding with a mortar and pestle. Feed and freshwater were provided as *ad libitum*, and the lighting was implemented as 16L:8D hours/day. The birds were reared in wired cages under a thermal controlled (21-22°C) room for 56 days. Maize and soybean meal-based diets containing 19% crude protein and 2900 kcal/kg metabolizable energy were used in accordance with minimum NRC (31) standards; whereas, only the calcium sources of the diets were different (Table 1). Chemical composition of feed ingredients (dry matter, crude protein, crude ash, and ether extract) was analyzed according to the AOAC procedures (5) and crude fiber was determined by the methods of Crampton and Maynard (11). Carpenter and Clegg (9)’s formula was applied for the calculation of

metabolizable energy. Chemical composition of calcium, available phosphorus, sodium, lysine, and threonine were calculated (31).

Table 1. Ingredients and nutrient composition of experimental diets (%)[1].

Item	Control	ESM
Ingredients (%)		
Maize	58.00	58.29
Soybean meal (44% Crude protein)	31.66	31.22
Sunflower oil	2.65	2.65
Sodium chloride	0.25	0.25
L-Lysine hydrochloride	0.01	0.01
L-Treonine	0.04	0.04
Sodium bicarbonate	0.10	0.10
DL-Methionine	0.12	0.12
Vitamin-Mineral premix[2]	0.35	0.35
Ground limestone	5.42	3.57
Dicalcium phosphate	1.40	1.40
ESM	-	2.00
Nutritional composition (%)		
Dry matter	90.10	90.00
Crude protein	19.00	19.00
Crude fiber	3.49	3.47
Ether extract	4.32	4.32
Crude ash	10.04	9.97
Calcium[3]	2.50	2.50
Available phosphorus[3]	0.36	0.36
Sodium[3]	0.16	0.16
Lysine[3]	1.01	1.00
Threonine[3]	0.75	0.75
ME, kcal/kg[4]	2900	2900

[1]Olive leaf extract (400 mg oleuropein per kg diet) was added to the basal diet. ESM: Eggshell with membrane.
[2]Vitamin-mineral premix (per 1 kg): vitamin A, 8000 IU; vitamin D3, 3000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg copper (copper sulphate), 10 mg; iodine (ethylenediamine dihydriodide), 1.0 mg; iron (ferrous sulphate monohydrate), 50 mg; manganese (manganese sulphate monohydrate), 60 mg; and zinc (zinc sulphate monohydrate), 60 mg, selenium (sodium selenite), 0.42 mg. [3]: Calculated values (34). [4]Calculated, Metabolizable energy (kcal/kg) (9)

OLE used in the study was provided by a commercial company (DUAG, Natural Products Agriculture Machinery Plant and Microbiological Products Research Development Industry and Trade Limited Company, Izmir, Turkey). Phenolic compounds in the olive leaf extract were analyzed by HPLC according to Altinok et al. (2). The antioxidant analysis of the olive leaf extract was also performed by HPLC (6).

Egg production was calculated when the number of total bird reached 5% of hen-day egg production. The hen-day egg production was calculated by dividing the number of daily collected eggs by the number of quail on the same day. Feed intake (g/bird/day) and feed conversion ratios (FCR) were weekly determined. FCR was calculated by using the number of birds and values of egg production, egg weight, and feed intake as g feed/g egg. For egg quality parameters, a total of 288 eggs including 72 eggs from each group (including sub-groups) were collected to beginning from 3rd week in 2 week periods with 2 times. The eggs were kept in room conditions for one day. The whole eggs were weighed and recorded. The shape index was then determined by using a digital caliper (Tresna, 0-300 mm, USA). After the eggs were broken, albumen and yolk were separated gently and weighed. Shells were washed under tap water and dried in the air for 24 hours and then weighed. Shell thickness was determined by a digital micrometer (MMT IP54). Yolk color was determined with a 15-sliced yolk color fan (Roche, Switzerland).

Shape index, albumen, yolk, and shell ratios were calculated following formulas (7);

Shape index: (egg width/egg length)x100
Albumen ratio: (albumen weight/egg weight)x100
Yolk ratio: (yolk weight/egg weight)x100
Shell ratio: (shell weight/egg weight)x100

The crude ash analyses of the eggshells were made according to AOAC (5). Eggshells were burned in ash oven at 550°C for 4 hours.

At the end of the experiment, a total of 24 quail, which were close to the average body weight of the groups, (6 quails from each group) were randomly selected and they were slaughtered with decapitation. Blood samples were taken into tubes during the cutting process. They were centrifuged at 4000 rpm for 10 min and the serums were separated. Serum levels of glucose, triglyceride, high-density lipoprotein (HDL), creatine, uric acid, urea, and lactate dehydrogenase (LDH) were measured by using the autoanalyzer (Olympus AU-600, Tokyo, Japan). Calcium levels of serum were determined by atomic absorption spectrophotometer (Perkin Elmer Analyst 800 Atomic Absorption Spectrometer-Flame). Phosphorus levels were determined according to the modified Youngberg-Youngberg method (23).

The bones of 24 slaughtered quail were scraped thoroughly from meat residues. The bones were stored at 4°C for 12 hours until examined. Right tibial-tarsal bones were used for physical analysis of the bones. Bones first weighed with 0.001 mg precision scale. The length and width of the bones were measured by using a digital caliper (Tresna, 0-300 mm, Guilin, China). The crude ash values of the bones were calculated according to AOAC (5).

Statistical analysis: The data including performance, egg quality, blood biochemical, and bone parameters were subjected to analysis of variance. After carrying out tests of normality (Shapiro-Wilk), One-Way ANOVA test was applied, and significant differences were further compared with Tukey HSD as post-hoc test. The results were considered significant when $P \leq 0.05$. The data were represented as the mean and standard error. Statistical Package for the Social Sciences for Windows (39) was used for all statistical analyses.

Results

Phenolic compounds in olive leaf extract are presented in Table 2. Antioxidant capacity of olive leaf extract was found to be 232 Trolox equivalents antioxidant capacity (mM trolox/g olive leaf extract).

Table 3 shows the effects of olive leaf extract (OLE), eggshell with membrane (ESM), and OLE+ESM on performance parameters of quail. Differences were observed between the groups in terms of egg weight. Egg weight of OLE+ESM group on days 15 to 28 had the lowest egg weight compared to other groups ($P<0.01$). During the total laying cycle, egg weight was found to be higher in the OLE group, which was followed by control, ESM, and OLE+ESM groups, respectively ($P<0.05$).

Table 2. Phenolic compounds in olive leaf extract.

Phenolics	mg/g
Oleuropein	103
Rutin	32
Catechin	1.5
Hydroxytyrosol	12
Caffeic acid	3.6
Vanillic acid	4.1
Vanillin	3.2

Table 3. Effect of eggshell with membrane and olive leaf extract on performance in quail.

Traits	C	ESM	OLE	OLE+ESM	P
Feed intake (g/quail/day)					
15-28 days	24.85^{b}±1.39	30.10^{a}±0.76	28.05^{ab}±0.61	29.65^{a}±0.43	**
29-42 days	32.16±1.42	34.10±0.29	31.78±1.11	32.99±1.21	NS
43-56 days	29.36±1.22	32.55±1.04	31.33±1.31	32.14±0.86	NS
15-56 days	28.79^{b}±1.10	32.25^{a}±0.61	30.39^{ab}±0.80	31.60^{a}±0.59	*
Egg production %					
15-28 days	74.40^{a}±3.50	64.80^{ab}±3.28	60.71^{b}±3.09	69.13^{ab}±2.46	*
29-42 days	80.48±2.47	78.57±3.80	73.72±3.35	77.72±4.14	NS
43-56 days	77.35±2.74	73.72±2.95	77.89±2.65	84.06±3.68	NS
15-56 days	77.41^{a}±2.13	72.37^{ab}±1.48	70.78^{b}±2.36	76.97^{a}±2.35	NS
Egg weight (g)					
15-28 days	12.36^{ab}±0.18	12.56^{a}±0.06	12.53^{a}±0.06	11.95^{b}±0.17	**
29-42 days	12.58±0.35	12.66±0.06	12.82±0.29	12.05±0.14	NS
43-56 days	12.35±0.24	12.67±0.15	12.59±0.10	11.93±0.31	NS
15-56 days	12.43^{ab}±0.18	12.63^{ab}±0.06	12.64^{a}±0.06	11.98^{b}±0.17	*
15-28 days	12.36^{ab}±0.18	12.56^{a}±0.06	12.53^{a}±0.06	11.95^{b}±0.17	**
Feed conversion ratio (g feed/g egg)					
15-28 days	2.70^{b}±0.14	3.70^{a}±0.20	3.69^{a}±0.14	3.59^{a}±0.19	**
29-42 days	3.18±0.16	3.43±0.16	3.37±0.14	3.53±0.20	NS
43-56 days	3.08±0.11	3.49±0.18	3.22±0.18	3.20±0.26	NS
15-56 days	2.99±0.11	43.53±0.12	3.40±0.10	3.43±0.15	NS

C: Control, ESM: Eggshell with membrane, OLE: Olive leaf extract, NS: non-significant, NS: P>0.05. *: P<0.05, **: P<0.01. $^{a\text{-}b}$Mean values with different superscripts within a row differ significantly. n= 112.

Differences in feed intake were observed between 15-28 days. The feed intake was found the lowest in the control group and the highest in the ESM group between 15-28 days (P<0.01). The highest feed intake during the study was determined in the ESM group. Feed intake was similar in control and OLE groups (P>0.05).

Egg production between 15-28 days of control and OLE+ESM groups was higher than the other two groups (P<0.05). There was no difference between ESM and OLE groups in terms of egg production (P>0.05). Similar to egg production; control and OLE+ESM groups resulted in improved feed conversion. The feed conversion ratio was similar in OLE and ESM groups (P>0.05).

Table 4 shows egg quality parameters. Shape index values were found to be different between control and OLE+ESM groups. The shape index was higher in OLE, ESM, and OLE+ESM groups than control (P<0.05). ESM group was different from the other groups in terms of percentage of albumen; the lowest percentage of albumen was seen in the ESM group. Yolk ratio, shell thickness, crude ash, and yolk color were not affected in all groups (P>0.05).

Table 5 shows the levels of serum glucose, triglyceride, high-density lipoprotein (HDL), lactate dehydrogenase (LDH), creatine, uric acid, urea, calcium, and phosphorus. Serum LDH level was lower in the control group (P<0.01). This parameter was similar in ESM, OLE, and OLE+ESM groups. The level of uric acid was found to be lower in the ESM group, which was followed by control, OLE, and OLE+ESM, respectively (P<0.01). Serum glucose, triglyceride, HDL, creatine, urea, calcium, and phosphorus levels were similar among the groups.

Table 6 shows the tibia weight, length, width, and ash values. The tibia weight, length, width, and ash values were found to be similar among the groups.

Table 4. Effect of eggshell with membrane and olive leaf extract on egg parameters in quail.

Egg Parameters	C	ESM	OLE	OLE+ESM	P
Shape index (SI), %	77.21^{b}±0.22	77.93^{ab}±0.21	77.50^{ab}±0.33	78.27^{a}±0.22	*
Albumen ratio (AR), %	51.28^{a}±0.32	49.33^{b}±0.32	50.80^{a}±0.23	50.88^{a}±0.24	**
Yolk ratio (YR), %	31.94±0.17	32.20±0.30	31.80±0.30	31.65±0.20	NS
Shell ratio, %	7.92^{ab}±0.05	7.73^{b}±0.07	7.94^{a}±0.05	7.99^{a}±0.05	**
Shell thickness, mm	0.25±0.00	0.25±0.00	0.25±0.00	0.25±0.00	NS
Shell ash, %	87.77±0.18	86.99±0.17	87.88±0.46	87.42±0.33	NS
Yolk color	5.39±0.07	5.33±0.07	5.22±0.05	5.32±0.07	NS

C: Control, ESM: Eggshell with membrane, OLE: Olive leaf extract, NS: non-significant, NS: P>0.05. *: P<0.05, **: P<0.01. a-bMean values with different superscripts within a row differ significantly. n=288.

Table 5. Effect of eggshell with membrane and olive leaf extract on blood biochemical parameters in quail.

Parameters	C	ESM	OLE	OLE+ESM	P
Glucose, mg/dL	207.10±14.09	181.95±15.40	163.37±10.12	195.00±5.03	NS
Triglyceride, mg/dL	121.67±5.06	122.17±4.13	118.67±6.30	118.17±3.06	NS
HDL, mmol/dL	151.78±5.54	149.92±13.86	145.05±5.54	142.57±5.10	NS
LDH, µkat/L	463.67^{b}±17.03	725.33^{a}±22.92	704.17^{a}±30.13	652.00^{a}±29.43	**
Creatine, mg/dl	0.09±0.02	0.07±0.01	0.07±0.01	0.07±0.01	NS
Uric acid, µmol/dL	4.65^{bc}±0.19	3.98^{c}±0.41	5.50^{ab}±0.53	6.32^{a}±0.22	**
Urea, mg/dL	5.65±0.31	5.39±0.40	6.61±0.41	6.32±0.66	NS
Calcium, mg/dL	8.32±0.68	7.38±0.73	10.26±0.77	8.10±0.78	NS
Phosphorus, mg/dl	4.85±0.67	4.59±0.72	4.88±0.49	4.79±0.37	NS

C: Control, ESM: Eggshell with membrane, OLE: Olive leaf extract, HDL: High density lipoprotein, LDH: Lactate dehydrogenase, NS: non-significant. NS: P>0.05, **: P<0.01. $^{a-b}$Mean values with different superscripts within a row differ significantly. n= 24.

Table 6. Effect of eggshell with membrane and olive leaf extract on some bone parameters in quail.

Bone parameters	C	ESM	OLE	OLE+ESM	P
Tibia weight, g	0.95±0.04	0.89±0.04	0.87±0.04	0.87±0.04	NS
Tibia length, mm	50.43±0.79	51.14±0.53	50.94±1.14	51.38±0.64	NS
Tibia width, mm	2.81±0.08	2.81±0.06	2.82±0.05	2.88±0.13	NS
Tibia ash, %	34.53±1.73	35.86±1.89	37.05±1.06	36.30±1.53	NS

C: Control, ESM: Eggshell with membrane, OLE: Olive leaf extract, NS: non-significant, NS: P>0.05. n=24.

Discussion and Conclusion

The requirement of calcium in layers are influenced by many physiological, metabolic, hormonal, and environmental factors. Therefore, it is important to meet calcium requirement sufficiently in layers. Various calcium sources could be used in poultry nutrition. The eggshell could be used as a calcium source. Moreover, eggshells may be combined with limestone and oyster shells for reducing calcium costs (25, 38).

Studies conducted on determining the effect of calcium sources such as limestone, oyster shell, and eggshells on egg production reported that calcium source did not have a significant effect on egg production, feed intake, and feed conversion ratio (17, 26, 36, 38). On the other hand, feed intake was influenced by different calcium sources. According to Olgun et al. (33), more desirable results were obtained from the oyster shell compared to the eggshell. In this study, quail fed with a diet supplemented with ESM and OLE+ESM exhibited higher feed intake. The groups fed with the 400 ppm OLE and control diet showed the lower feed intake value in comparison to the other groups. Feed conversion was similar in OLE+ESM, OLE, and ESM groups. In the present study, egg production of the experimental groups showed different results. When examining total egg productions, the control and OLE+ESM groups were higher than the ESM and OLE groups.

Different results were obtained from the studies conducted on determining the effects of herbal and other by-products used as feed additives on egg production, feed intake and, feed conversion ratio. Oke et al. (32) found the lowest egg weight and egg production in the OLE group. In this study, quail fed with a diet supplemented with OLE+ESM had the lowest egg weight. The feed intake and FCR were not similar in this study, as noted by Oke et al. (32).

The particle size of the calcium source used in the poultry diet affected both performance and egg quality. In this study, ESM was ground with a mortar and a pestle that particle size was around 1 mm. When the particle of calcium source was more than 0.8 mm, the dissolution of calcium source in the gizzard retarded and its retention time increased. Thus, the availability of sources increased parallelly (26, 44). However, in some studies, egg production and egg quality of laying hens were not affected by the type of calcium source and its particle size (18, 38). However, Lichovnikova (26) reported that different levels of eggshell or oyster shell as supplemental calcium sources significantly increased egg weight in laying hens.

The shape index is influenced by egg weight (14). There was a negative correlation between the shape index and egg weight in laying Japanese quail (24). In this study, the OLE+ESM group resulted in a higher shape index than the other groups. Belonging higher values of the shape index in this group (OLE+ESM) may be associated with their egg weight. The percentage of albumen was the lowest in the ESM group. The percentage of albumen also reduced with aging and an increase in size of the eggs (7). According to the present study, it was observed that egg weights increased in the ESM group at the weekly basis. Therefore, the percentage of albumen values could be affected by both aging and egg size. In this study, the well-grinded eggshell and ground limestone were used as the same amount in all diet groups; thus, the shell thickness was similar in all groups. Similarly, there was no difference in eggshell thickness of laying hens when eggshell was used as a calcium source in laying hen diets (38, 40). This fact was also observed in the bone parameters.

LDH catalyzes the conversion of pyruvate and lactate with an associated interchange of NADH and NAD^+. LDH isoenzymes are released into the bloodstream when a heart, liver, and pulmonary system are damaged (41). Moreover, OLE is also associated with glucose metabolism and has an anti-hyperglycemic effect. Indeed, serum glucose was determined to be low level in the OLE group and LDH in this study. This feature is based on two mechanisms. The first mechanism improves glucose-induced insulin release and the second mechanism increases peripheral uptake of glucose (36, 42).

Uric acid is the final product of purine and protein metabolism in poultry. Uric acid is formed in the liver and excreted by kidneys. It is considered a biomarker for many physiological characteristics in vertebrates and birds that are related to diet, genetics, and diseases (13). It is also an important scale for renal function status. It is well known that OLE has an antioxidant effect and has been documented in many studies (3, 19). However, dietary overdoses phenolic have a pro-oxidant effect on redox-active metal-containing systems. They may lead to the formation of reactive oxygen species and phenoxyl radicals causing oxidative stress (12). Moreover, it was emphasized that LDH can accelerate detoxification reactions (35). Thus, OLE supplemented groups have resulted in higher levels of LDH and uric acid when compared to the control in this study.

In conclusion, the eggshell usage in quail diets as a calcium source had no effect on egg production and quality. The same effect was also seen in the OLE. There is a need for further investigation of an effective dose of the OLE. According to the results of the current study, the dose of 400 ppm mmight be high as the olive leaf extract dose. Therefore, it would be better to determine appropriate doses of OLE in the studies. Some applications such as emulsified systems can also be tested for the usage of OLE as a feed additive into quail diets.

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Ethical Statement

This study was approved by the Fırat University Animal Research Ethics Committee (Protocol Number: 2016/68).

Conflict of Interest

The authors declared that there is no conflict of interest.

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