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ANIMAL SCIENCE

The potential of lemon peel powder as an additive in layer quails (*Coturnix coturnix Japonica*): An experimental study

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Abstract: The current research intended to examine the impact of dietary lemon peel powder (LPP) on laying quail performance, egg quality criteria, and the antioxidant capacity of the yolk. A total of 120 female Japanese quails (272.6±9.3 g), aged 21 weeks, were allotted to 6 trial groups, each with 5 replicates of 4 quails. Additions of 0, 1, 2, 3, 4, or 5 g/kg of LPP to the basal diet were used to create the treatment groups. Quails were fed *ad libitum* for 70 days. Neither performance parameters nor egg production was affected by LPP. However, eggshell-breaking strength improved by adding 2 g/kg LPP to the diet, but worsened at 5 g/kg. Moreover, the relative weight of eggshell and yolk L* value decreased with the treatments. Dietary LPP enhanced oxidative stability, reducing malondialdehyde (MDA) and increasing 1,1-diphenyl-2-picrylhydrazyl (DPPH) yolk values. The current study demonstrated that LPP, a safe and easily accessible agricultural by-product, enhanced eggshell quality when it was included in the diet of laying quails at doses of 2 g/kg. In contrast, improvement of yolk antioxidant capacity required increased amounts of LPP (4 g/kg). LPP could be advantageous to animal nutrition as an adequate substitute to reduce waste by-products

Key words: Antioxidant capacity, egg quality, lemon peel powder, performance, quail.

INTRODUCTION

In developing countries, producing and processing vegetables and fruits results in a significant amount of waste by-products, which harms the environment (Domínguez et al. 2020). Even though reducing food waste is a priority, its reuse and entry into the food chain are essential for the circular economy (Sabater et al. 2020). Food waste may be recognized as an ingredient due to its nutritional content., which plays a relevant role in animal feed production worldwide (Sabater et al. 2020). Food waste is a cheap source of high-value components for the food and meat industries (Domínguez et al. 2020).

Lemon (*Citrus lemon* L.), belonging to the *Rutaceae* family, is the third most produced and consumed citrus fruit worldwide after orange and mandarin (Rahman et al. 2019), which is processed to obtain juice (Alnaimy et al. 2017) generating a large number of peel residues (Papoutsis et al. 2017). Lemon is rich in some valuable bioactive substances including flavonoids, anthocyanins, flavones, isoflavones, and isocatechins (Rahman et al. 2019) which have antimicrobial and anti-inflammatory properties (Youssef et al. 2020, Alagawany et al. 2021) and could improve meat shelf life (Khalifah et al. 2021).

Most of the available studies have evaluated lemon in feed such as essential oil (Alagawany et

al. 2021, Sevim et al. 2021), lemon juice (Gültepe et al. 2019) or leaves (Alzawqari et al. 2016), and results about the effects of lemon parts on development Akbarian et al. 2013, Salehifar et al. 2017, Sevim et al. 2021) and egg quality (Gültepe et al. 2019, Sevim et al. 2021, Wan et al. 2021) are unclear. Nevertheless, information on the use of dried lemon peel in animal feed is scarce. Dried lemon peel powder is a potential ingredient in animal feed because it is easier to store, transport, and add to the diet than traditional lemon products (Chen et al. 2019). To the best of our information, there is no detailed study on the effect of LPP on the laying quail performance and egg quality, specifically, on its antioxidant capacity in yolks. Hence, the current research was undertaken to find out the effect of LPP in the diet on laying quails' productivity and egg quality. Moreover, this experiment aims to evaluate the effect of LPP supplementation in diets for laying quails on the antioxidant capacity of egg yolks.

MATERIALS AND METHODS

The study was carried out at an animal farm, so no special certification was required for breeding laboratory animals. Nevertheless, criteria specified by European policy for protecting animals were applied during the experimental period (The European Parliament and the Council of the European Union 2010). The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page. The European National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

Animals and management

A total of 120 female quails (*Coturnix coturnix japonica*) of 21 weeks with similar body weights (272.6±9.3 g) were obtained from a commercial farm and used for this experiment. This trial was performed using a completely randomized design in a rural farm at Selçuklu, Konya, Türkiye (38°1'36", 32°30'45"). The quails were randomly allocated to six experimental groups. Each group included 5 subgroups with 4 females each.

Animals were randomly allocated to 6 identical and disinfected battery cages (30 cm wide and 45 cm long) corresponding to experimental diets. Quail were all reared under controlled environmental conditions at the same house, and the illumination schedule was 16 hours of light and 8 hours of darkness. Each pen was arranged at a temperature and relative humidity of 20±2.0 °C and 55±5 %, respectively. Individual feeders and drinkers were placed in each pen to enable *ad libitum* intake.

Dietary treatment

Supplementation of the experimental treatments involved the use of lemon peels. Lemon (*Citrus lemon*) peel powder (LPP) was supplied by a regional market (Dağinciri Ltd. Şti., Aydın, Türkiye). LPP was analysed according to the procedure described by Singleton & Rossi (1965) to estimate phenolic content and reducing power (%). In addition, the half maximal inhibitory concentration (IC_{50}) was determined as proposed by Singh et al. (2002) (Table I).

Treatments were designed by adding LPP to the basal diet in mash form at levels of 0

Table I. Chemical composition, reducing power and total phenolic content of lemon peel powder.

Parameters	Level
Crude protein (%)	7.62
Crude fibre (%)	53.31
Crude fat (%)	4.36
DPPH (%)	7.66
Total phenolic content (g/kg GAE)	14.60
IC_{50}	5.894

DPPH: 2,2 diphenyl-1-picrylhydrazyl, GAE: Gallic acid equivalent, IC_{50}: The half maximal inhibitory concentration.

(control), 1, 2, 3, 4, and 5 g/kg. The basal diet was designed following the recommendations of the National Research Council (1994) to supply the requirements of laying quail. All diets were administered for 70 days and were isocaloric and isonitrogenous, so no differences were found in terms of energy or protein between experimental diets. The AOAC (2006) proceedings were used to analyse the chemical composition of the LPP (Table I) and the basal diet (Table II).

Determination of performance parameters

The quails were randomly divided into six trial groups at the start of the trial. Initial and final weighing were determined with a precision weighing balance (±0.01 g) to evaluate body weight changes. Trial diets were given by weighing each subgroup, and then, feed intake was calculated using the difference between the quantity supplied and the leftovers from each experimental unit as shown by Sarmiento-García et al. (2022). The total was divided by the number of quails and the days consumed the feed. The feed intake was expressed in g/bird/day. Then, average body weight gain (g/day) was calculated by subtracting initial body weight (g) from final body weight (g) over the study period.

At the same time, eggs were collected and recorded each day (at 10:00 am). Egg production was calculated by dividing the egg number obtained in a day by the total quails and multiplying by 100. This value was expressed as a percentage (%). The obtained eggs (n=300) were individually weighed utilizing a calibrated precision weighing balance over the course of the final three days of the treatment (±0.01 g) to eliminate time-dependent differences. Equation (1) was used to compute egg mass using these data:

$$\text{Egg mass} = (\text{egg production (\%)} \times \text{egg weight (g)}) / 100 \quad (1)$$

Finally, the feed conversion ratio was calculated according to the following equation (2):

Table II. Basal diet and nutrient composition.

Ingredient	g/kg	Nutrient composition	g/kg
Corn	570.0	Metabolizable energy (kcal ME/kg)[2]	2900
Soybean meal (460 g/kg CP)	261.0	Crude protein[3]	200.0
Meat-bone meal (450 g/kg CP)	27.6	Crude fat[3]	73.5
Full-fat soybean	65.0	Crude cellulose[3]	39.7
Limestone	52.0	Moisture[3]	125.2
Sunflowers oil	17.1	Calcium[2]	25.0
Salt	3.0	Available phosphorus[2]	3.5
Premix[1]	2.5	Lysine[2]	10.6
DL methionine	1.8	Methionine[2]	4.6
Total	1000.0	Cystine[2]	4.1
		Methionine+Cystine[2]	8.7

[1]CP: Crude Protein. [1]Premix provides 80 mg manganese (manganese oxide), 60 mg iron (iron carbonate), 5 mg copper (copper sulphate pentahydrate), 1 mg iodine, 0.15 mg selenium, 8800 IU vitamin A (trans-retinol acetate), 2200 IU vitamin D3 (cholecalciferol), 11 mg vitamin E (tocopherol), 44 mg nicotinic acid, 8.8 mg Cal-D-Pan, 4.4 mg Vitamin B2 (riboflavin), 2.5 mg thiamine, 6.6 mg vitamin B12 (cyanocobalamin), 1 mg folic acid, 0.11 mg biotin, 220 mg choline to per kg of diet.
[2]Calculated values
[3]Analysed values

Feed conversion ratio = feed intake (g feed) / egg mass (g mass) (2)

Evaluation of egg quality characteristics

The analyses described were conducted at the Egg Quality Laboratory (Faculty of Agriculture, Selcuk University, Konya, Türkiye). The external and internal quality characteristics of all eggs (n=300) harvested in the last three days of the experiment were examined at environmental temperature. Ten eggs were analyzed for each experimental treatment.

Damaged, cracked, and broken eggs were quantified throughout the study and measured as a % of the total number of eggs. The resistance of the eggs to eggshell breaking was checked by applying a rising pressure with the Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel). A digital micrometer (Mitutoyo, 0.01 mm, Japan) was employed to determine the thickness of the eggshell (μm) by taking the average of the values of its three parts (pointed, equatorial, and blunt).

To assess the internal quality of the eggs, they were broken and the residues were removed on a clean glass table. The relative weight of eggshells has been determined as a percentage of egg weight after the eggshells had been air-dried for three days at ambient temperature. Subsequently, the yolks and albumen were separated. Using a height gauge, albumen and yolk heights were determined, and a 0.01 mm digital caliper (Mitutoyo, Japan) was employed to determine width and length. From these values, the next parameters were estimated. To calculate the albumin index, the equation (3) was applied:

Albumen index = [albumen height (mm) / (albumen width (mm) + albumen length (mm) / 2)] x 100 (3)

Yolk index was determined using the following equation (4):

Yolk index = [height of yolk (mm) / diameter of yolk (mm)] x 100 (4)

The Haugh unit was obtained from egg weight and albumen height data based on equation 5 as described by Stadelman & Cotterill (1995).

Haught unit = 100 x log (albumen height + 7.57 – 1.7 x egg weight$^{0.37}$) (5)

Samples were laid on the flat side of Petri dishes for colorimetric analysis, and all processes were conducted to preserve the yolk integrity. To evaluate the parameters L*, (lightness), a* (redness), and b* (yellowness), yolks were measured using a digital colorimeter (Minolta Chroma Meter CR 400, Minolta Co., Osaka, Japan) previously calibrated as reported by Titcomb et al. (2019).

Determination of MDA and DPPH concentrations in the yolk

The DPPH and TBARS analyses were performed on 150 fresh eggs (5 per treatment). The thiobarbituric acid reactive substances (TBARS) test was performed with the modified procedure described by Sarmiento-García et al. (2021) in duplicate to evaluate lipid peroxidation. Two grams of each sample (2 samples per fresh yolk) were homogenized using an ultraturrax (IKA, USA) for 20 s with 12 mL of the TCA (trichloroacetic acid) solution (7.5% TCA, 0.1% EDTA, 0.1% Propyl gal-late). Filter paper nr 1 from Whatman (Maidstone, England) was applied as a separator of the mixture. The mixture was transferred into glass vials (3 mL) with the addition of 3 mL of thiobarbituric acid (TBA) solution (0.02 M) and vortexed. After, the mixture was incubated at 100ºC for 40 minutes to develop a pink color. Subsequently, the

mixture was cooled and applied centrifuge (2000 rpm) for 5 minutes. The blank containing 1 mL of TCA extraction solution and 1 mL of TBA solution was employed as a benchmark for the spectrophotometric measurement (Perkin Elmer, USA) of the supernatant at 530 nm wavelength. Malondialdehyde, a byproduct of tetraethoxypropane, was analyzed to provide a standard curve for calculating TBARS. TBA was expressed as µmol MDA/kg yolk using the equation (6) described by Kilic & Richards (2003).

$$\text{TBARS} = [((\text{absorbance}/k \times 2/1000) \times 6.8) / \text{sample weight})] \times 1000 \quad (6)$$

The antioxidant capacity of the obtained hydrolysates was tested using a modified technique based on the scavenging effect of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Sacchetti et al. 2005). Two grams of yolk sample had been mixed with 25 ml of methanol (100%) and subjected to an ultrasonic bath for 20 minutes for the extraction process. Whatman No. 1 filter paper (Maidstone, England) was utilized to separate the mixture and poured into 0.1 ml glass vials. 2.9 mL of DPPH solution (100 mL of Methanol (100%) + 0.0025 g of DPPH (97%)) was added to the mixture and vortexed for 25 seconds. Once the mixture was left for 30 minutes at room temperature, an absorbance measurement at 517 nm was read with a spectrophotometer (Perkin Elmer precisely UV/VIS Spectrometer). The monitoring solution was substituted with 95% ethanol. Every method was triplicated to establish the mean value. The DPPH was established using the formula (7) reported by Sacchetti et al. (2005):

$$\text{DPPH values} = [(\text{control absorbance} - \text{sample absorbance}) / \text{control absorbance}] \times 100 \quad (7)$$

Statistical analysis

SPSS 22.0 software program (SPSS Inc., Chicago, IL, USA), has been applied to analyze the variance of the data, considering the means of the cages as the test unit. Statistical significance was established as a probability value of $p < 0.05$, while a probability value of $p < 0.10$ was established as a trend. The robustness of linear and quadratic models for explaining the relationship of the dependent variable to an increasing level of LPP in the diet was assessed by orthogonal polynomial contrasts.

RESULTS

There was no mortality or illness symptom observed in any experimental group. The means in Table III demonstrated that LPP dietary did not affect performance regarding final body weight, weight gain, feed intake, and feed conversion ratio ($p > 0.05$) of laying quails throughout the experiment. No differences were observed in egg production, weight, or mass ($p > 0.05$) between experimental groups. The range values for the performance parameters and egg production were as follows: final body weight, 274.6-282.5 g; body weight gain, 0.50-8.90 g; egg production, 85.89-89.19%; egg weight, 12.30-12.85 g; egg mass, 10.67-11.46 g/day/quail; feed intake, 32.13-33.49 g/day/quail; feed conversion ratio, 2.93-3.03 g feed/g egg.

Table IV provides data on the features of egg quality with the administration of LPP to the laying quail diets. No significant effects on damaged egg rate and eggshell thickness were found among the six treatments ($p > 0.05$). Feeding 2 g/kg LPP to laying quails caused an increase in eggshell-breaking strength (1.69 kg) compared to the control and other ones. The lowest breaking strength (1.32 kg) value was found at a high LPP level (5 g/kg). The relative eggshell weight was linearly affected ($p < 0.01$)

Table III. Performance growth and egg production of laying quails (n=120) receiving dietary lemon peel powder.

Parameters	Lemon peel powder (g/kg)						S.E.M.	p-value		
	0	1	2	3	4	5		Anova	L	Q
Initial body weight (g)	265.7	272.1	269.8	280.4	276.9	270.6	6.83	0.718	0.403	0.295
Final body weight (g)	274.6	276.6	277.3	282.5	277.4	274.8	8.44	0.989	0.906	0.562
Body weight gain (g)	8.90	4.50	7.50	2.10	0.50	4.20	3.59	0.606	0.199	0.521
Feed intake (g/quail/day)	32.86	32.13	33.49	32.91	32.75	32.23	0.668	0.783	0.755	0.451
Egg production (%)	88.33	85.94	89.19	89.14	88.57	85.89	1.326	0.341	0.713	0.205
Egg weight (g)	12.30	12.42	12.85	12.65	12.46	12.71	0.206	0.568	0.316	0.400
Egg mass (g/quail/day)	10.87	10.67	11.46	11.27	11.04	10.94	0.255	0.465	0.610	0.192
FCR (g feed/g egg)	3.03	3.01	2.93	2.93	2.97	2.98	0.091	0.971	0.665	0.506

FCR: Feed conversion ratio; S.E.M.: Standard error means. L: Linear effect; Q: Quadratic effect.

by the LPP supplementation. As shown in Table IV, this value decreased as the concentration of LPP in the diet increased and this value was minimal at a 4 g/kg LPP level.

Dietary LPP did not affect the albumen, yolk indexes, and Haugh unit. For the yolk color parameters, redness (a*) and yellowness (b*) were not influenced by LPP dietary ($p > 0.05$). However, the yolk lightness value (L*) was affected by the supplementation with LPP in the diet ($p < 0.05$), and those values were substantially lower in the groups supplemented with 1, 3, and 5 g/kg LPP (50.46, 50.68, and 50.71, respectively) than in the control group (53.73).

Table V demonstrates the effect of using LPP in the diet on yolk DPPH and MDA values. Regarding yolk DPPH levels, considerable differences were described among the experimental groups ($p < 0.01$). Overall highest DPPH value (8.561 % reducing) was observed in the 5 g/kg LPP group, and the lowest DPPH was recorded in the control and 2 g/kg LPP groups. The TBARS procedure had been employed to assessed the inhibition ability of LPP on lipid peroxidation. Adding LPP to the diet was considerably significant on yolk MDA ($p < 0.01$). Laying quails fed supplemented diets showed a significant decrease in MDA value as compared to those fed control diets ($p < 0.01$). In addition, these values declined further when dietary LPP levels were above 3 g/kg.

DISCUSSION

In this experiment, the addition of LPP to the laying quails' diet did not affect the performance parameters or egg production. In close agreement with our findings, Sevim et al. (2021) claimed that adding lemon peel oil (300 mg/kg) to breeder quail diets did not affect performance except for feed intake. Similar results have been described in broilers, previous authors stated that the performance parameters were unaffected by the supplementation of 250 mg/kg dried lemon peel (Boumezrag et al. 2018), 0.2, 0.6 or 1.0% lemon pulp powder (Salehifar et al. 2017), or 300 and 400 mg/kg lemon peel extract (Akbarian et al. 2013). Contrary, Gültepe et al. (2019) observed that egg production improved in laying hens with the administration of 0.5 to 5% lemon juice to the drinking water, but the rest of the performance parameters were unaffected. These findings are partially similar to those described by Wan et al. (2021), who reported an improvement in feed intake and egg production in laying hens when 2 and 4% fresh lemon were added to the diet. On

Table IV. Effect of lemon peel powder on egg quality traits (n=300) in laying quails.

Parameters	Lemon peel powder (g/kg)						S.E.M.	p-value		
	0	1	2	3	4	5		Anova	L	Q
Damaged egg (%)	0.40	1.03	0.32	0.16	0.34	1.01	0.340	0.436	0.802	0.289
Egg-breaking strength (kg)	1.50^{bc}	1.60^{ab}	1.69^{a}	1.52^{bc}	1.40^{cd}	1.32^{d}	0.049	0.001	0.001	0.002
Relative eggshell weight (%)	8.71	8.67	8.54	8.40	7.99	8.14	0.197	0.135	0.009	0.919
Eggshell thickness (µm)	222.3	221.3	223.4	215.6	216.7	220.2	3.36	0.625	0.307	0.599
Albumen index	2.63	2.70	2.58	2.84	2.84	2.81	0.148	0.753	0.240	0.984
Yolk index	49.18	49.26	49.98	48.24	48.14	48.89	0.970	0.867	0.511	0.665
Haugh unit	90.15	90.82	89.34	92.02	91.89	91.94	1.120	0.492	0.143	0.832
L*	53.73^{a}	50.46^{b}	52.04^{ab}	50.68^{b}	52.34^{ab}	50.71^{b}	0.654	0.017	0.072	0.189
a*	1.23	1.55	1.07	1.68	1.29	1.83	0.194	0.094	0.102	0.438
b*	35.48	31.34	33.43	32.72	34.57	32.58	0.995	0.189	0.578	0.367

L*: Lightness; a*: Redness; b*: Yellowness; S.E.M.: Standard error means. L: Linear effect; Q: Quadratic effect. ^{a,b,c,d}: Means with different superscripts in the same row were significantly different ($p < 0.05$).
Regression equation for eggshell breaking strength: 1.63-0.0482X,
Regression equation for relative eggshell weight: 8.77-0.144X,
Regression equation for yolk lightness: 52.4-0.309X.

the other hand, Goliomytis et al. (2018) described a decrease in performance parameters when the orange pulp (9%) was added to the laying hens' diet which was related to the presence of anti-nutritional substances, such as tannins and pectin. Several reasons might explain the inconsistencies between previous experiments. According to Salehifar et al. (2017), the part of the plant utilized, the doses, the concentration, and the profile of the active components present in the citrus could be for responsible the differences observed in performance parameters. Moreover, those authors suggest that the improvement in performance parameters could be more evident depending on the animal's physiological state, background nutrition, or housing conditions. Nevertheless, the results of this research have suggested that the inclusion of LPP in the diet of laying quails offers an excellent way to reuse lemon by-products without compromising performance and egg production parameters. It could also be an interesting approach to reducing feed costs.

In the current study, dietary LPP did not affect the damaged egg rate and eggshell thickness, however, significant differences were found in eggshell-breaking strength and relative eggshell weight. Doses of 2 g/kg LPP enhanced egg-breaking strength. Lemon peels are a valuable source of calcium and magnesium (Czech et al. 2020), which both play an important role in eggshell thickness (Olgun et al. 2022). The inclusion of LPP in the diet would increase mineral content, suggesting a higher bioavailability and absorption of these minerals at doses of 2 g/kg LPP compared to other levels. Similar results have been found in a study on breeder quails (Sevim et al. 2021), which revealed that 300 mg/kg of lemon peel essential oil improved eggshell-breaking strength. However, Wan et al. (2021) claimed that eggshell strength was unaffected in laying hens that received fresh lemon (2 or 4%) along with their diet. The eggshell-breaking strength was also reported to be unaffected when bioactive compounds of lemon such as hesperidin (Goliomytis et al. 2014), naringin (Li et

Table V. DPPH and MDA response of the fresh yolk (n=150) in laying quails receiving different doses of lemon peel powder.

Parameters	Lemon peel powder (g/kg)						S.E.M.	p-value		
	0	1	2	3	4	5		Anova	L	Q
DPPH (% reducing)	4.768^{c}	6.007^{bc}	5.251^{c}	5.862^{bc}	7.937^{ab}	8.561^{a}	0.6324	0.005	<0.001	0.222
MDA (µmol/kg)	3.781^{a}	2.611^{bc}	3.128^{b}	2.421^{c}	2.258^{c}	2.339^{c}	0.1223	<0.001	<0.001	0.048

S.E.M.: Standard error means, DPPH: 2,2 diphenyl-1-picrylhydrazyl, MDA: malondialdehyde, L: Linear effect; Q: Quadratic effect.
^{a,b,c}: Means with different superscripts in the same row were significantly different (p < 0.05).
Regression equation for DPPH: 4.59+0.725X,
Regression equation for MDA: 3.40-0.256X.

al. 2022), or both (İskender et al. 2017, Lien et al. 2008) were added to the diet. According to Czech et al. (2020), the calcium content of the peel of all citrus fruits is more than 50% compared to the pulp. Similarly, it has been reported that the magnesium content of lemon peel is 37% higher than that of its pulp. Therefore, removing this part of the fruit considerably decreases its nutritional value and consequently affects egg values, which could explain the differences between the studies reported.

Contrary to eggshell-breaking strength, relative eggshell weight decreased linearly with dietary LPP (up to 4 g/kg). Goliomytis et al. (2018) described that eggshell relative weights were negatively affected when hens were fed with orange-dried pulp (9%) to the control. Those authors attributed these findings to the lowest feed intake in the orange-dried pulp group. In the current research, the maximum value for the eggshell weight was obtained for the control group with the highest feed intake value (32.91 g/quail/day). This outcome differs from the findings of the study conducted by Sevim et al. (2021), who found that adding 300 mg/kg lemon peel oil to breeder quails' diet did not affect the relative weight of the eggshell. Similar results were reported by Lien et al. (2008) who indicated that neither naringin nor hesperidin affected the relative eggshell weight. The aforementioned differences can probably be related to the dose as well as the portion of citric acid supplied.

The treatments did not affect the color traits (a*, b*) except for the lightness value (L*), which was declined when LPP was added to the diet. The control group obtained lighter yolk compared to the dietary LPP groups. Egg yolk color is an important trait for purchase decisions (Olgun et al. 2022), driven by intrinsic and extrinsic characteristics and sociocultural factors. However, a high percentage of the population associates darker yolks with improved quality, which could provide a potential market opportunity for eggs from quails receiving dietary LPP. The effect on the egg yolk color of including lemon and its byproducts in the layer diet is unclear. According to the current results, Goliomytis et al. (2018) demonstrated a reduced color L* value when orange peel at the 9% level was added to the hens´ diet. These authors described a reduction in feed intake when citrus was added, which could affect the color traits. On the other hand, Li et al. (2022) reported enhanced yolk color when naringin was added to the layer diet. These authors linked this finding with an increase in the activity of the antioxidant enzyme (neohesperidin dihydrochalcone) which could reduce antioxidant activity and lead to a major deposition of pigments. While Sevim et al. (2021) claimed that lemon peel oil did not affect the yolk L* value. Differences among studies could be explained by the dosage (300 mg/kg lemon peel oil vs 1-5 g/kg LPP) used as well as the lemon portion (peel oil *vs.* peel powder) involved.

The current study showed an improvement in oxidative yolk stability (DPPH and MDA value) when LPP was added to the diet. These results would indicate that the inclusion of LPP may decrease the vulnerability to lipid peroxidation of stored eggs related to the decrease of available cholesterol, and probably also increase their shelf life, as reported by Sarmiento-García et al. (2023). This fact is of great importance for consumers, making these findings a key point in the choice when buying eggs. Similarly, previous studies have reported the positive effect of different lemon sources such as lemon powder (Youssef et al. 2020), lemon leaf powder (Khalifah et al. 2021), lemon leaf oil (Alagawany et al. 2021), or powder concentrated (Alzawqari et al. 2016) on the total antioxidant capacity of plasma and meat. Goliomytis et al. (2014) reported that lemon components (specifically hesperidin) included in the hens´ diet enhanced the oxidative stability of the yolk. Those authors suggested that hesperidin, which is a bioactive compound found in lemons, reduces lipid peroxidation by binding the hydrogen ion to free radicals, similar to the action of other antioxidants such as vitamin E or C (Van Acker et al. 2000). In the same context, Lien et al. (2008) claimed that the addition of another lemon compound (in this case 0.5 g/kg naringin) to the diet decreased the value of MDA in serum. Li et al. (2022) declared that dietary naringin (1, 2, and 4 g/kg) improved the antioxidant capacity of the ovarian and serum, and reduced oxidative stress, which is consistent with the findings of Bao et al. (2022) in old hens. Bioactive chemical compounds in lemons are potent antioxidants and efficient removers of reactive oxygen species, suppressing the activity of oxygen free radicals that destroy normal cells. These substances could inhibit chain reactions during lipid peroxidation with beneficial effects on the products, their shelf life, and animal health (Gonzalez-Molina et al. 2010, Alagawany et al. 2021, Khalifah et al. 2021). These results suggest that the inclusion of LPP in the diet would not only have a negative effect but also could improve egg shelf life.

CONCLUSIONS

This research demonstrates that LPP can be incorporated as a feed ingredient without negatively compromising performance and egg production. Including a 2 g/kg level of LPP in the quail diet was effective in improving the resistance to eggshell breaking. However, to enhance the yolk's antioxidant capacity (MDA and DPPH value), it is necessary to use LPP at a level of 4 g/kg in the diet which may prolong shelf life and benefit consumers. These results suggest that incorporating LPP could be a potential strategy to reuse lemon by-products and improve some egg quality criteria.

REFERENCES

AKBARIAN A, GOLIAN A, GILANI A, KERMANSHAHI H, ZHALEH S, AKHAVAN A, DE SMET S & MICHIELS J. 2013. Feeding citrus peel extract affects growth performance, serum components, and intestinal morphology of broilers exposed to high ambient temperature during the finisher phase. Livest Sci 157: 490-497.

ALAGAWANY M, EL-SAADONY MT, ELNESR SS, FARAHAT M, ATTIA G, MADKOUR M & REDA FM. 2021. Use of lemongrass essential oil as a feed additive in quail's nutrition: Its effect on growth, carcass, blood biochemistry, antioxidant and immunological indices, digestive enzymes, and intestinal microbiota. Poult Sci 100: 101172.

ALNAIMY A, GAD AE, MUSTAFA MM, ATTA MAA & BASUONY HAM. 2017. Using of citrus by-products in farm animals feeding. Open Access J Sci 1: 58-67.

ALZAWQARI MH, AL-BADDANY AA, AL-BAADANI HH, ALHIDARY IA, KHAN RU, AQIL GM & ABDURAB A. 2016. Effect of feeding dried sweet orange (*Citrus sinensis*) peel and lemon grass (*Cymbopogon citratus*) leaves on growth performance, carcass traits, serum metabolites, and antioxidant status in broiler during the finisher phase. Environ Sci Pollut Res 23: 17077-17082.

AOAC. 2006. Official Methods of Analysis Association, 18th ed.; Association of Official Analytical Chemist: Washington, DC, USA.

BAO T, YAO J, ZHOU S, MA Y, DONG J, ZHANG C & MI Y. 2022. Naringin prevents follicular atresia by inhibiting oxidative stress in the aging chicken. Poult Sci 101: 101891.

BOUMEZRAG A, KHIATI B, BENARABA R, BOUKRAA L, HAMMOUDI SM, CHICOTEAU P & BENARBIA MEA. 2018. Modulation of broilers' productivity and blood biochemical parameters by Citrus elements dietary supplementation. Veterinaria 67: 129-137.

CHEN X, DING Y, FORREST B, OH J, BOUSSERT SM & HAMANN MT. 2019. Lemon yellow is a new highly stable, water-soluble food colorant from the peel of Citrus limon. Food Chem 270: 251-256.

CZECH A, ZARYCKA E, YANOVYCH D, ZASADNA Z, GRZEGORCZYK I & KŁYS S. 2020. The mineral content of the pulp and peel of various citrus fruit cultivars. Biol Trace Elem Res 193: 555-563.

DOMÍNGUEZ R, MUNEKATA PES, PATEIRO M, MAGGIOLINO A, BOHRER B & LORENZO JM. 2020. Red beetroot. A potential source of natural additives for the meat industry. Appl Sci: 10: 8340.

GOLIOMYTIS M, ORFANOU H, PETROU E, CHARISMIADOU MA, SIMITZIS PE & DELIGEORGIS SG. 2014. Effect of hesperidin dietary supplementation on hen performance, egg quality, and yolk oxidative stability. Brit Poult Sci 55: 98-104.

GOLIOMYTIS M, KOSTAKI A, AVGOULAS G, LANTZOURAKI DZ, SIAPI E, ZOUMPOULAKIS P, SIMITZIS P & DELIGEORGIS SG. 2018. Dietary supplementation with orange pulp (*Citrus sinensis*) improves egg yolk oxidative stability in laying hens. Anim Feed Sci Technol 244: 28-35.

GONZALEZ-MOLINA E, DOMÍNGUEZ-PERLES R, MORENO DA & GARCÍA-VIGUERA C. 2010. Natural bioactive compounds of *Citrus limon* for food and health. J Pharm Biomed Anal 51: 327-345.

GÜLTEPE EE, IQBAL A, ÇETINGÜL İS, UYARLAR C, ÖZÇINAR Ü & BAYRAM İ. 2019. Effects of lemon juice on performance, egg quality trait, and some blood parameters of laying hens in the late phase of production. Acta Vet Eur 45: 56-62.

İSKENDER H, YENICE G, DOKUMACIOGLU E, KAYNAR O, HAYIRLI A & KAYA A. 2017. Comparison of the effects of dietary supplementation of flavonoids on laying hen performance, egg quality, and egg nutrient profile. Brit Poult Sci 58: 550-556.

KHALIFAH AM, ABDALLA SA, DOSOKY WM, SHEHATA MG & KHALIFAH MM. 2021. Utilization of lemongrass essential oil supplementation on growth performance, meat quality, blood traits, and caecum microflora of growing quails. Ann Agric Sci 66: 169-175.

KILIC B & RICHARDS MP. 2003. Lipid Oxidation in poultry döner kebab: Pro-oxidative and anti-oxidative factors. J Food Sci 68: 686-689.

LI H, HOU Y, CHEN J, WU H, HUANG L, HU J, ZHANG Z, LU Y & LIU X. 2022. Dietary naringin supplementation on laying performance and antioxidant capacity of three-yellow breeder hens during the late laying period. Poult Sci 101: 102023.

LIEN TF, YEH HS & SU WT. 2008. Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits, and antioxidant activity in laying hens. Arch Anim Nutr 62: 33-43.

NATIONAL RESEARCH COUNCIL. 1994. Nutrient Requirements of Poultry. National Academies Press: Washington, DC, USA.

OLGUN O, GÜL ET, KILINÇ G, YILDIZ A, ÇOLAK A & SARMIENTO-GARCÍA A. 2022. Performance, egg quality, and yolk antioxidant capacity of the laying quail in response to dietary choline levels. Animals 12: 3361.

PAPOUTSIS K, PRISTIJONO P, GOLDING JB, STATHOPOULOS CE, BOWYER MC, SCARLETT CJ & VUONG QV. 2017. Enhancement of the total phenolic compounds and antioxidant activity of aqueous Citrus limon L. pomace extract using microwave pre-treatment on the dry powder. J Food Process Preserv 41: e13152.

RAHMAN ANA, EL-HADY M & SHALABY SI. 2019. Efficacy of the dehydrated lemon peels on the immunity, enzymatic antioxidant capacity, and growth of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). Aquaculture 505: 92-97.

SABATER C, RUIZ L, DELGADO S, RUAS-MADIEDO P & MARGOLLES A. 2020. Valorization of vegetable food waste and by-products through fermentation processes. Front Microbiol 11: 581997.

SACCHETTI G, MAIETTI S, MUZZOLI M, SCAGLIANTI M, MANFREDINI S, RADICE M & BRUNI R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 91: 621-632.

SALEHIFAR E, KASHANI NN & NAMEGHI AH. 2017. Effect of lemon pulp powder on growth performance, serum components and intestinal morphology of broilers exposed to high ambient temperature. J Livest Sci 8: 155-162.

SARMIENTO-GARCÍA A, GÖKMEN SA, SEVIM B & OLGUN O. 2022. A novel source of calcium: Effects of calcium pidolate

concentration on egg quality in aged laying quails (*Coturnix coturnix Japonica*). J Agric Sci 160: 551-556.

SARMIENTO-GARCÍA A, OLGUN O, KILINC G, SEVIM B & GÖKMEN SA. 2023. The use of purple carrot powder in the diet of laying quails improved some egg quality characteristics, including antioxidant capacity. Trop Anim Health Prod 55: 220.

SARMIENTO-GARCÍA A, PALACIOS C, GONZÁLEZ-MARTÍN I & REVILLA I. 2021. Evaluation of the production performance and the meat quality of chickens reared in organic system. as affected by the inclusion of *Calliphora sp*. in the diet. Animals 11: 324.

SEVIM B, GÖKMEN SA, CURABAY B, CUFADAR Y & BAHTIYARCA Y. 2021. The effect of the addition of orange and lemon peel essential oils and mixtures to breeder quail rations on performance, egg quality and hatch parameters. Turk J Agric Food Sci Technol 9: 1988-1992.

SINGH RP, MURTHY KNC & JAYAPRAKASHA GK. 2002. Studies on the antioxidant activity of pomegranate (*Punicagranatum*) peel and seed extracts using in vitro models. J Agric Food Chem 50: 81-86.

SINGLETON VL & ROSSI JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents Am J Enol Viticul 16: 144-158.

STADELMAN WJ & COTTERILL OJ. 1995. Egg Science and Technology. Nueva York, EEUU: The Haworth Press, p. 590.

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION. 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes; European Parliament: Strasbourg, France, p. 1-47.

TITCOMB TJ, KAEPPLER MS, COOK ME, SIMON PW & TANUMIHARDJO SA. 2019. Carrot leaves improve color and xanthophyll content of egg yolk in laying hens but are not as effective as a commercially available marigold fortification. Poult Sci 98: 5208-5213.

VAN ACKER FAA, SCHOUTEN O, HAENEN GRMM, VAN DER VIJGH WJF & BAST A. 2000. Flavonoids can replace a-tocopheryl as an antioxidant. FEBS Lett 473: 145-148.

WAN Y, MA R, QI R, LI Y, LIU W, LI J, LIU L, ZHENG J, YUE J & ZHAN K. 2021. Dietary fresh lemon improves the albumen quality, immune status, and lipid metabolism of Jingfen laying hens during the late laying period. Ital J Anim Sci 20: 834-841.

YOUSSEF SFF, ABDEL LATIF HA & ABDEL-KAFY ESM. 2020. Supplementing fresh lemon to growing Japanese quail diet as a safe alternative to antibiotic, synthetic vitamins and prebiotic under hot climate. Egypt Poult Sci J 40: 867-881.

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