




Effects of *Pistacia terebinthus* seed meal and different storage times on egg quality of laying hens

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ABSTRACT - The present study examined the effect of supplementing the diets of laying hens with *Pistacia terebinthus* seed meal on egg quality during different storage intervals. A total of 192 laying hens (Babcock) were divided into six groups, and each group was further divided into four subgroups with eight hens each. *Pistacia terebinthus* seed meal was added to the diets of laying hens in the experimental groups at a rate of 0, 10, 20, 30, 40, and 50 g kg⁻¹ of feed. At the end of the study (56 days), a total of 288 eggs (48 eggs from each group) were collected randomly. Seventy-two eggs were analyzed on day zero of storage, while the other eggs were stored at a temperature of 4 °C. The remaining eggs were analyzed after 10, 20, and 30 days of storage. The result revealed that, at 30 days of storage, the supplementation of terebinthus had a significant effect on the Haugh unit on inclusion levels of 20 and 40 g kg⁻¹. Likewise, the inclusion level of 30 g kg⁻¹ manifested a significant impact on yolk color at 20 days of storage. Eggshell breaking strength and egg weight remained unaffected. Terebinthus seed could be used to extend the storage time of eggs without any adverse effect on egg quality.

Key Words: egg, laying hen, yolk pigmentation

Introduction

Table eggs are the product of the commercial layer industry, and the distribution of eggs from the production unit to end consumers takes some time. Before distribution to the end consumer, the eggs are stored and then transported to different markets. During the storage and transportation process, the quality of eggs may deteriorate due to unexpected environmental conditions and prolonged storage time (Samli et al., 2005). Genetic factors can also affect egg quality (Johnson and Merritt, 1955; Williams, 1992). A decrease in egg quality may incur a major loss to the layer hen industry and also to farmers. Numerous efforts have been made to improve the quality and shelf life of edible eggs by using herbal powder, essential oils, and plant products (Aji et al., 2011; Rahimi et al., 2011; Khan et al., 2012).

Pistacia terebinthus belongs to the *Anacardiaceae* family and grows in humid cold and hot climates. It is found mostly on the eastern Mediterranean coast, Syria, Iran, and Turkey. The terebinth extract contains flavonoids, phenolics, triterpenoids, and essential oils (Monaco et al., 1973; Yalpani and Tyman, 1983; Boelens and Jimenez, 1991; De Pooter et al., 1991; Shobha et al., 1992; Ansari et al., 1994; Magiatis et al., 1999; Kawashty et al., 2000). Terebinth is used to produce turpentine and baking bread, and its shoots are consumed as vegetables (Schultes, 1991). Terebinth is rich in tannins and also contains a resinous substance. It has many medicinal and pharmacological uses as an antispasmodic, expectorant, antiseptic, cytostatic, and is also used to treat streptococcal infections and cancer (Polunin, 1969; Brown, 1995).

Very few studies have been carried out regarding the effect of terebinth in poultry diets to explore its implications on the performance parameters of broilers as well as the performance of laying hens and its effect on egg quality parameters. It is important to ensure the uninterrupted supply of quality eggs and to improve the storage duration of eggs; some solutions must be described which can safely improve the egg quality during different storage times. No literature is available regarding the use of terebinth in poultry diets and its effects especially on egg quality. Taking this into

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consideration, the present study was designed to examine the effect of dietary terebinth (*Pistacia terebinthus*) seed meal on the egg quality parameters in laying hens during different storage times.

Material and Methods

This study was conducted in Afyonkarahisar, Turkey (38°45'24.7896" N latitude, 30°32'19.3344" E longitude, and 826 m altitude). Research on animals was conducted according to the institutional committee on animal use (case no.: 49533702-114 of 07/09/2016). For this study, 192 Babcock white laying hens (30 weeks old) were used. Treatment groups were divided into six groups. Each group was further subdivided into four subgroups with eight birds each. All six treatment group diets were supplemented with terebinth (*Pistacia terebinthus*) at an inclusion level of 0, 10, 20, 30, 40, and 50 g kg⁻¹ as feed, respectively. The control group diet was not supplemented with terebinth (*Pistacia terebinthus*). Terebinth seeds were procured from the market and included in the feed formulation after grinding. Feed was formulated to meet the nutrient requirement of the birds as recommended by NRC (1994)

(Table 1). *Ad libitum* feed and fresh drinking water was available during the trial.

At the end of the trial (56th day), 48 eggs (total 288 eggs of six groups) were collected from each group (12 from each subgroup) on two consecutive days. From the first day of collection, 12 eggs from each group (three from each subgroup) to a total of 72 eggs, were analyzed on the same day without being kept in storage. The remaining eggs were stored at +4 °C. Seventy-two eggs per treatment in total were analyzed after every 10, 20, and 30 days of storage for egg quality parameters such as egg breaking strength (ORKA Egg Force Reader, EF 0468-2011), Haugh unit (HU), egg yolk color, and egg weight (SANOVO Engineering Egg Analyzer, EA0333, Denmark).

The Shapiro-Wilk test was performed to check the normal distribution of values (Shapiro and Wilk, 1965). Logarithmic transformation was performed on data which was not normally distributed. An ANOVA was used to determine the significant differences among the groups for independent variables. Subsequently, the Tukey-Kramer test was performed as post-hoc test on the mentioned variables. Repeated measures ANOVA was performed with single-factor for the determination of over-time changes on each

Table 1 - Ingredient and nutrient composition of the experimental diets and terebinth seed

Item	Inclusion levels of <i>Pistacia terebinthus</i> (g kg ⁻¹)					
	0	10	20	30	40	50
Ingredient (g kg ⁻¹ as fed)						
Corn	534.5	535.3	525.3	515.3	505.3	495.3
<i>Pistacia terebinthus</i>	0	10.0	20.0	30.0	40.0	50.0
Plant oil	8.3	8.0	8.0	8.0	8.0	8.0
Sunflower meal	173.5	150.7	150.7	150.7	150.7	150.7
Full fat soy	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal (44% CP)	73.6	85.8	85.8	85.8	85.8	85.8
Limestone	84.2	84.0	84.0	84.0	84.0	84.0
Dicalcium phosphate	17.3	17.5	17.5	17.5	17.5	17.5
Salt	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral mixture ¹	2.5	2.5	2.5	2.5	2.5	2.5
L-lysine	1.0	1.2	1.2	1.2	1.2	1.2
DL-methionine	1.0	1.0	1.0	1.0	1.0	1.0
Calculated analyses (as fed)						
CP (g kg ⁻¹)	170	170	170	170	170	170
ME (kcal kg ⁻¹)	2750	2772	2767	2761	2756	2750
Ca (g kg ⁻¹)	37.1	37.1	37.1	37.1	37.1	37.1
Available P (g kg ⁻¹)	3.8	3.8	3.8	3.8	3.8	3.8
Na (g kg ⁻¹)	2.0	2.1	2.2	2.3	2.4	2.5
Methionine + Cysteine (g kg ⁻¹)	7.1	7.2	7.1	7.1	7.1	7.1
Lysine (g kg ⁻¹)	8.3	8.4	8.3	8.3	8.3	8.3
Threonine (g kg ⁻¹)	6.1	6	6	6	6	5.9
Tryptophan (g kg ⁻¹)	2	2	2.1	2.1	2.1	2.1
Linoleic acid (g kg ⁻¹)	25.8	25.6	25.3	25.1	24.9	24.7
Composition of <i>Pistacia terebinthus</i> seed (as fed)						
Dry matter (g kg ⁻¹)	CP (g kg ⁻¹)	ME (kcal/kg)	Ca (g kg ⁻¹)	P (g kg ⁻¹)	-	-
900	102.9	3000	1.2	0.05	-	-

CP - crude protein; ME - metabolizable energy.

¹ Provided per kg of diet: vitamin A, 12,000,000 IU; vitamin D3, 3,000,000 IU; vitamin E, 35,000 IU; vitamin K3, 3,500 IU; vitamin B1, 2,750 IU; vitamin B2, 5,500 IU; nicotinamide, 30,000 IU; Ca-D-panthotenate, 10,000 IU; vitamin B6, 4,000 IU; vitamin B12, 15 IU; folic acid, 1,000 IU; D-biotin, 50 IU; choline chloride, 150,000 IU; manganese, 80,000 mg; iron, 60,000 mg; zinc, 60,000 mg; copper, 5,000 mg; iodine, 2,000 mg; cobalt, 500 mg; selenium, 150 mg; antioxidant, 15,000 mg.

group. This was followed by post-hoc Bonferroni corrected pairwise comparisons. In addition, trend analyses were applied on over-time trends (linear, quadratic, or cubic). The variables were expressed as mean \pm SEM. $P < 0.05$ was accepted as significant for all analyses. MedCalc Statistical Software version 17 (2017 MedCalc Software bvba, Ostend, Belgium) was used for all data analyses.

Results

Egg weight results revealed non-significant ($P > 0.05$) effect of terebinth in all inclusion groups during 0, 10, and 20 days of storage analysis. At 30 days of storage, the analysis revealed that egg weight significantly increased ($P < 0.05$) in the group that fed diet supplemented with 50 g kg⁻¹ terebinth compared with the other groups (Table 2).

Supplementation of terebinth had no significant ($P > 0.05$) effect on eggshell breaking strength and did not incur significant ($P > 0.05$) result in the terebinth seed meal treatment groups compared with the control group (Table 3).

Haugh unit results also remained unaffected ($P > 0.05$) with the supplementation of different levels of terebinth seed meal during 0, 10, and 20 days of storage. However, at 30 days storage, HU increased significantly ($P < 0.05$) in all terebinth-supplemented groups especially in 20 and 40 g kg⁻¹ treatments in comparison with the control group (non-supplemented group) (Table 4).

Terebinth supplementation results showed no effect ($P > 0.05$) on yolk color at 0, 10, and 30 days of storage except at 20 days of storage. The yolk colour index of the all groups increased especially with 30 g kg⁻¹ of terebinth, revealing significantly higher ($P < 0.05$) yolk colour index at 20 days of storage compared with the control group (Table 5).

Discussion

For egg weight, the group fed the diet supplemented with 10 g kg⁻¹ of terebinth displayed a decreasing pattern compared with the control group, while the other groups

Table 2 - Effect of dietary terebinth seed on egg weight at 0, 10, 20, and 30 days of storage

Group	0 day	10 days	20 days	30 days	P-value
Control	60.10 \pm 1.36	62.41 \pm 0.72	60.63 \pm 0.99	59.95 \pm 0.85AB	0.266
10 g kg ⁻¹	63.43 \pm 0.94b	60.92 \pm 1.01ab	59.91 \pm 1.02ab	59.54 \pm 1.11Ba	0.014
20 g kg ⁻¹	64.58 \pm 2.23	62.62 \pm 1.27	61.47 \pm 1.23	59.39 \pm 0.72B	0.083
30 g kg ⁻¹	61.63 \pm 1.55	61.75 \pm 1.16	59.89 \pm 1.07	62.02 \pm 1.23AB	0.650
40 g kg ⁻¹	61.73 \pm 1.61	60.86 \pm 0.94	60.56 \pm 1.02	60.07 \pm 0.83AB	0.795
50 g kg ⁻¹	62.58 \pm 1.48	61.89 \pm 1.01	62.51 \pm 0.89	63.74 \pm 1.10A	0.396
P-value	0.382	0.772	0.474	0.021	

a,b,c - Significant differences among the same group with respect to time.

A,B,C - Values with different uppercase letters in the same column differ significantly.

Table 3 - Effect of dietary terebinth seed on egg shell breaking strength (kg cm⁻²) at 0, 10, 20, and 30 days of storage

Group	0 day	10 days	20 days	30 days	P-value
Control	43.46 \pm 1.59	43.20 \pm 1.93	41.82 \pm 2.46	44.85 \pm 1.20	0.455
10 g kg ⁻¹	49.94 \pm 2.50	44.66 \pm 2.44	45.65 \pm 1.64	47.69 \pm 2.15	0.372
20 g kg ⁻¹	45.33 \pm 2.63	48.96 \pm 1.64	42.99 \pm 2.70	43.25 \pm 1.59	0.311
30 g kg ⁻¹	44.99 \pm 2.16	43.17 \pm 2.47	40.29 \pm 2.18	41.30 \pm 2.39	0.413
40 g kg ⁻¹	50.27 \pm 2.96a	44.80 \pm 1.13ab	42.90 \pm 2.27b	42.58 \pm 1.52b	0.046
50 g kg ⁻¹	46.10 \pm 2.32	48.19 \pm 3.13	44.68 \pm 2.87	43.97 \pm 2.54	0.885
P-value	0.302	0.341	0.700	0.283	

a,b,c - Significant differences among the same group with respect to time.

Table 4 - Effect of dietary terebinth seed on Haugh unit at 0, 10, 20, and 30 days of storage

Group	0 day	10 days	20 days	30 days	P-value
Control	76.66 \pm 4.15a	72.96 \pm 3.29a	73.38 \pm 1.81a	56.10 \pm 5.68Ab	0.009
10 g kg ⁻¹	73.06 \pm 2.91ab	78.01 \pm 1.51a	70.87 \pm 1.41b	65.91 \pm 1.53ABb	0.001
20 g kg ⁻¹	71.30 \pm 7.67	77.58 \pm 1.66	74.49 \pm 1.57	69.30 \pm 1.84B	0.426
30 g kg ⁻¹	81.61 \pm 1.59a	76.56 \pm 1.78ac	71.63 \pm 1.57bc	66.36 \pm 2.75ABb	0.001
40 g kg ⁻¹	76.80 \pm 3.83	74.72 \pm 1.62	73.24 \pm 1.12	68.73 \pm 2.79B	0.282
50 g kg ⁻¹	76.18 \pm 4.34	73.93 \pm 1.13	69.16 \pm 2.32	64.45 \pm 2.47AB	0.128
P-value	0.570	0.377	0.265	0.026	

a,b,c - Significant differences among the same group with respect to time.

A,B,C - Values with different uppercase letters in the same column differ significantly.

Table 5 - Effect of dietary terebinth seed on yolk color index at 0, 10, 20, and 30 days of storage

Group	0 day	10 days	20 days	30 days	P-value
Control	8.00±0.39b	8.83±0.49b	8.42±0.60Ab	10.36±0.56a	0.007
10 g kg ⁻¹	9.67±0.67	8.25±0.70	10.08±0.80AB	10.83±0.59	0.123
20 g kg ⁻¹	8.09±0.44a	9.08±0.76ab	10.08±0.58ABb	11.00±0.62b	0.023
30 g kg ⁻¹	10.00±0.58	10.50±0.65	11.50±0.51B	11.42±0.57	0.217
40 g kg ⁻¹	9.33±0.59a	8.83±0.93ab	11.08±0.66ABab	11.45±0.58b	0.006
50 g kg ⁻¹	8.30±0.40a	10.58±0.56b	10.00±0.43ABab	11.27±0.51b	0.005
P-value	0.062	0.130	0.023	0.808	

a,b,c - Significant differences among the same group with respect to time.

A,B,C - Values with different uppercase letters in the same column differ significantly.

did not display any significant effect with extending storage time. Our results are supported by the findings of Samli et al. (2005) and Akyurek and Okur (2009), who reported no egg weight loss at 5 °C temperature during 10 days of storage.

Silversides and Scott (2001) observed that supplementation of terebinth in the diet of layer hens had no effect on egg breaking strength with different storage time. On the contrary, some researchers have demonstrated that with the supplementation of terebinth in the diets of laying hens, egg shell weight and shell thickness decrease with extended storage time (Samli et al., 2005; Jin et al., 2011). Likewise, in our study, the groups fed diet supplemented with terebinth did not display any positive effect in terms of eggshell breaking strength at 0, 10, 20, and 30 days of storage compared with the control group. A gradual decrease in egg breaking strength was observed in the group fed diet supplemented with 40 g kg⁻¹ of terebinth.

Regarding HU, Jin et al. (2011) observed that it did not change at 5 °C temperature during different storage durations, while some other researchers reported that HU decreased dramatically at 5 °C with extended storage time (Tona et al., 2004; Samli et al., 2005; Akyurek and Okur, 2009). In our study, we observed that groups fed diets supplemented with 20 and 40 g kg⁻¹ of terebinth manifested significantly higher HU values at 30 days of storage compared with the other treatment groups and the control group. In contrast, HU decreased in the group fed diet supplemented with 10 g kg⁻¹ of terebinth compared with the other treatment groups and control group. Changes in the egg quality parameters such as HU have a direct relation with moisture loss through shell pores by evaporation and also escape of carbon dioxide from egg albumen (Shenstone 1968). Inclusion of 20 and 40 g kg⁻¹ of terebinth have proper number of active components which can significantly reduce the microbial and chemical activity inside eggs up to 30 days of egg storage, which further leads to increased HU compared with the other treatment and control groups.

For egg yolk color, Jin et al. (2011) observed that yolk color gradually decreases with the increase in storage time. Some other researchers observed that plant seed meal supplementation in the diet of laying hens have positive effects on egg yolk color (Christaki et al., 2011). In our study, we observed that the group fed the diet supplemented with 30 g kg⁻¹ of terebinth showed a maximum value of yolk color compared with the control group at 20 days of storage, while the other groups fed diet supplemented with terebinth remained unaffected. The groups fed diet supplemented with 40 and 50 g kg⁻¹ of terebinth did not display any positive effects in terms of yolk color at different storage times.

Conclusions

Pistacia terebinthus seeds at 20 and 40 g kg⁻¹ supplementation levels could be used to extend the shelf life of eggs without any adverse effect on egg quality.

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