

## ***Pistacia terebinthus* as a dietary supplement for laying hens**

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(Received 6 March 2019; Accepted 5 December 2019; First published online 16 February 2020)

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### **Abstract**

The aim of this study was to explore the potential of *Pistacia terebinthus* (terebinth) seed meal as a dietary supplement for laying hens. One hundred and ninety-two Babcock 30-week-old laying hens were assigned to one of six treatments (n = 32) with four replicates (n = 8). The hens were fed diets containing 0%, 1%, 2%, 3%, 4%, and 5% terebinth seed meal for eight weeks. Weekly egg production, feed consumption, egg weight, and egg mass were recorded. Egg quality was assessed at the beginning, middle and end of the study. Blood sampling was carried out on 12 birds from each treatment. Total antioxidant capacity, total oxidant status and oxidative stress index were determined. Egg production was greater from hens fed 3% and 4% terebinth than those in the other treatments. Egg weight was increased by supplementation with 2% or more terebinth. Feed consumption, feed conversion ratio, eggshell breaking strength, yolk colour, Haugh units, concentrations of glucose, total cholesterol, high density lipoprotein, low density lipoprotein, alanine aminotransferase, aspartate aminotransferase, total protein, phosphorus and calcium in serum, and total antioxidant capacity, total oxidant status, and the oxidative stress index did not differ across treatments. It is concluded that dietary terebinth seed supplementation generated positive effects on egg production and egg weight without adverse effects on egg quality or the metabolism of the hen.

**Keywords:** antioxidant, egg production, egg quality, feed consumption, serum biochemistry

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### **Introduction**

Poultry farming provides important sources of protein for most of the human population. Currently and in the future, food security will be a substantial challenge to sustainability and survival of the human population. Recent developments in poultry farming in hygiene and drug resistance have generated the need to discover alternative feed resources and additives, including phytobiotic herbal products that could replace costly feed resources and antibiotics without compromising the performance and economics of farm operations (Kucukkurt *et al.*, 2009).

Many plant products are known to possess antioxidant properties and have been shown to improve the antioxidant status in laying quail and their eggs (Kucukkurt *et al.*, 2009; Bulbul & Ulutas, 2015). Antioxidants are important to capture free radicals, mitigate problems with stress, and ultimately improve the health status of poultry. These antioxidants may be endogenous or exogenous (Kucukkurt *et al.*, 2009). Some non-conventional plant products have been identified as having potential to improve performance (Bayram *et al.*, 2007; Christaki *et al.*, 2011), possessing biochemical properties (Yazdi *et al.*, 2014), and antioxidant activity (Kucukkurt *et al.*, 2009; Bulbul & Ulutas, 2015).

*Pistacia terebinthus* is a flowering herb, which belongs to the Anacardiaceae family, and is also known as terebinth (Anonymous, 2016). It is found in the Canary Islands, Mediterranean region and Turkey. Terebinth has been used in medicine as treatment for some ailments, and in baking and cosmetics. Medicinally it has been used as an antispasmodic, expectorant, antiseptic, and cytostatic treatment, and to

treat bronchial, urinary, streptococcal infections, gall stones, haemorrhages, rheumatism, and tapeworm infestations. It has also been used in curing cancer patients (Anonymous, 2012). In Cyprus, it is used in baking bread and its shoots are consumed as a vegetable (Facciola, 1990). Despite these numerous medicinal effects, the impacts of terebinth on the performance and immune response of poultry have not been studied comprehensively. Therefore, this study was designed to explore the potential of *Pistacia terebinthus* seed meal as a dietary supplement for laying hens.

## Material and Methods

The current study was performed at the Animal Research Centre of Afyon Kocatepe University, Turkey, after the approval of the Ethics Committee on the Ethical Use of Animals (Approval no 49533702-114, dated 07/09/2016). A total of 192 white 30-week-old Babcock laying hens were used in this study. They were divided into six groups with four replicates in each group and eight birds in each. Six diets were formulated, which contained 0, 10, 20, 30, 40 and 50 g/kg terebinth (*Pistacia terebinthus*) to meet the nutrient requirements of the birds (NRC, 1994). The experiment lasted for 56 days. Terebinth seeds were procured from the market and included in feed formulation after grinding. Ad libitum feed and fresh drinking water were supplied during the trial. The ingredients that were contained in the diets and their nutrient profile are shown in Table 1.

**Table 1** Ingredient and nutrient compositions of diets supplemented with varying levels of terebinth seed to be fed to laying hens

Ingredients	0% (Control)	1% Pistachia	2% Pistachia	3% Pistachia	4% Pistachia	5% Pistachia
Corn	53.45	53.53	52.53	51.53	50.53	49.53
<i>Pistacia terebinthus</i>	0.0	1.00	2.00	3.00	4.00	5.00
Sunflower oil	0.83	0.80	0.80	0.80	0.80	0.80
Sunflower meal	17.35	15.07	15.07	15.07	15.07	15.07
Full fat soya	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	7.36	8.58	8.58	8.58	8.58	8.58
Limestone	8.42	8.40	8.40	8.40	8.40	8.40
Dicalcium phosphate	1.73	1.75	1.75	1.75	1.75	1.75
Salt	0.40	0.40	0.40	0.40	0.40	0.40
vitamin-mineral mix <sup>1</sup>	0.25	0.25	0.25	0.25	0.25	0.25
L-lysine	0.10	0.12	0.12	0.12	0.12	0.12
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analyses (%)						
Crude protein	17.0	17.0	17.0	17.0	17.0	17.0
Metabolizable energy, Kcal/kg	2750	2772	2767	2761	2756	2750
Calcium	3.71	3.71	3.71	3.71	3.71	3.71
Available phosphorus	0.38	0.38	0.38	0.38	0.38	0.38
Sodium	0.20	0.21	0.22	0.23	0.24	0.25
Methionine + cysteine	0.71	0.72	0.71	0.71	0.71	0.71
Lysine	0.83	0.84	0.83	0.83	0.83	0.83
Threonine	0.61	0.60	0.60	0.60	0.60	0.59
Tryptophan	0.20	0.20	0.21	0.21	0.21	0.21
Linoleic acid	2.58	2.56	2.53	2.51	2.49	2.47

<sup>1</sup> vitamin A:12.000.000 IU, vitamin D3:3.000.000IU, vitamin E:35.000 IU, vitamin K: :3.500 IU, vitamin B<sub>1</sub>: 2.750 IU, vitamin B<sub>2</sub>: 5.500 IU, nicotinamide: 30.000 IU,Ca-D-Panthotenate:10.000 IU, vitamin B<sub>6</sub>: 4.000 IU, vitamin B<sub>12</sub>: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, per kg

Egg production and egg weight were recorded daily to calculate the average weekly egg production and weekly egg weight. Total egg mass was calculated from the average weekly egg production and egg weight. Feed consumption was tabulated weekly and expressed daily as g per bird. The data were used to calculate feed conversion ratio (FCR).

At the start of the study, at the end of the fourth week, and at the end of the trial, 72 eggs from all the six groups (12 from each treatment, three from each replicate) were collected (totalling 216 eggs in three collections) for the analysis of egg quality. Egg breaking strength was measured by using ORKA digital egg force reader (ORKA Food Technology, Wanchai, Hong Kong), working on the latest modified principle described by Tyler (1961). Haugh unit was calculated by measuring albumen height according to the method devised by Haugh (1937). Egg yolk colour was determined with the Roch improved yolk colour fan (ORKA Food Technology, Wanchai, Hong Kong), which compared the colours of yolks with 15 bands of the colour fan. These egg quality traits were assessed on the day the eggs were collected.

At the end of the trial, three birds from each replicate of each treatment were randomly selected for collection of blood samples for serological examination. Blood samples were placed in plain tubes and centrifuged at 7000 rpm for 10 minutes, after which time the serum was separated and stored at -20 °C until further analysis. For serological examination, glucose, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein (TP), serum phosphorus, serum calcium, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by automatic ELISA analyser (Chemwell 2910, Awareness Technology Inc., Palm City, Florida, USA). Physiological properties of the blood were determined with a haematology analyser (BC-5000Vet, Mindray, Shenzhen, PR China).

Total antioxidant capacity (TAC) was tested using the total antioxidant power kit (Oxford Biomedical Research, Oxford, Michigan, USA) according to the manufacturer's protocol with the colourimetric microplate method. Total oxidant status (TOS) was determined using the TOS ELISA commercial kit (Sunredbio, Shanghai, PR China). The oxidative stress index (OSI) was calculated as:

$$OSI = \left( \frac{TOS}{TAC} \right) \times 100 \quad (\text{Verit } et al., (2006))$$

Statistical analyses were performed with MedCalc (MedCalc Software bvba, Oostend, Belgium, version 17.5). The assumptions of normality and homogeneity of variance that are necessary for robustness of the analysis of variance (ANOVA) were assessed using Shapiro-Wilk and Levene tests, respectively. One-way ANOVA was used to test for differences among treatments, followed by the Tukey-Kramer test for post-hoc comparison of the treatment means. An ANOVA for repeated measures was used to evaluate the egg quality traits that were recorded over time. Sphericity (Lecoutre, 1991) was used to test the sphericity of variances for traits that were recorded as repeated measures. When a significant difference was in the repeated measures ANOVA, Bonferroni corrected comparisons were carried out to determine the source of the difference. All data were expressed as mean  $\pm$  SE. The significance of effects was tested at  $P < 0.05$ .

## Results and Discussion

The nutrient composition of terebinth was approximately 10.29% crude protein, 3000 kcal/kg metabolizable energy, 0.12% calcium (Ca) and 0.005% phosphorus (P). Thus, terebinth seed meal has a good nutrient profile with reasonable protein and energy contents and the ability to replace other energy and protein sources to some extent.

Egg weight in the groups that were supplemented with 2% or more terebinth produced heavier eggs ( $P < 0.05$ ) compared with the unsupplemented control group and the group that was supplemented with 1% terebinth (Table 2). Similar findings have been reported by various researchers (Khan *et al.*, 2012; Abdel-Wareth *et al.*, 2013), who used herbs such as thyme and oregano and found an improved effect on egg weight and egg mass. However, other researchers have been unable to show a difference on egg weight with the supplementation of herb products (Christaki *et al.*, 2011; Bulbul & Ulutas, 2015). This difference may be attributable to the nature of the product or unique undiscovered properties of the herbs.

Overall egg production was greater ( $P < 0.05$ ) from the hens that were supplemented with 3% and 4% terebinth compared with the unsupplemented group (Table 3). However, the group that was supplemented with 5% terebinth produced eggs at a rate that was similar to the control. Dietary supplementation with herbs and their products has also been shown previously to improve egg production (Christaki *et al.*, 2011). As a consequence of the differences in egg production, egg mass was greatest ( $P < 0.05$ ) from the hens that were supplemented with 3% and 4% terebinth ( $58.1 \pm 0.4$  and  $57.9 \pm 0.5$  g, respectively) versus  $52.1 \pm 0.9$  g for the unsupplemented control. Positive effects of supplementation with plant products on performance have been well documented in previous research work (Bayram *et al.*, 2007; Christaki *et al.*, 2011).

**Table 2** Mean ( $\pm$  SE) weekly egg weights (g) produced by laying hens supplemented with terebinth over eight weeks

Week	Level of supplementation with <i>Pistacia terebinthus</i>						P-value
	0% (Control)	1%	2%	3%	4%	5%	
1	59.0 $\pm$ 1.2	58.7 $\pm$ 1.0	61.1 $\pm$ 1.7	61.2 $\pm$ 0.5	60.2 $\pm$ 0.7	59.1 $\pm$ 1.1	0.43
2	58.9 $\pm$ 0.8	58.5 $\pm$ 0.7	60.1 $\pm$ 0.9	60.5 $\pm$ 0.4	60.6 $\pm$ 0.7	60.2 $\pm$ 0.9	0.22
3	58.5 <sup>a</sup> $\pm$ 1.2	59.6 <sup>ab</sup> $\pm$ 0.7	61.4 <sup>ab</sup> $\pm$ 0.3	61.4 <sup>ab</sup> $\pm$ 0.3	61.5 <sup>ab</sup> $\pm$ 0.5	61.9 <sup>b</sup> $\pm$ 0.8	0.03
4	59.7 $\pm$ 0.8	59.5 $\pm$ 0.3	63.5 $\pm$ 2.2	62.3 $\pm$ 0.6	61.5 $\pm$ 1.0	61.6 $\pm$ 0.3	0.14
5	59.5 $\pm$ 1.1	60.2 $\pm$ 0.5	62.3 $\pm$ 1.9	62.4 $\pm$ 0.7	61.5 $\pm$ 0.5	62.9 $\pm$ 1.0	0.19
6	60.1 $\pm$ 0.9	61.2 $\pm$ 0.6	62.0 $\pm$ 1.1	62.2 $\pm$ 0.5	61.8 $\pm$ 0.9	62.0 $\pm$ 0.7	0.48
7	60.4 $\pm$ 0.6	60.5 $\pm$ 0.5	63.1 $\pm$ 2.4	63.1 $\pm$ 0.4	62.4 $\pm$ 1.0	62.1 $\pm$ 0.6	0.35
8	59.8 <sup>a</sup> $\pm$ 0.5	60.9 <sup>ab</sup> $\pm$ 1.1	61.3 <sup>ab</sup> $\pm$ 0.6	62.4 <sup>ab</sup> $\pm$ 0.5	63.3 <sup>b</sup> $\pm$ 1.1	62.0 <sup>ab</sup> $\pm$ 0.4	0.04
mean	59.5 <sup>a</sup> $\pm$ 0.2	59.9 <sup>a</sup> $\pm$ 0.3	61.9 <sup>b</sup> $\pm$ 0.4	61.9 <sup>b</sup> $\pm$ 0.3	61.6 <sup>b</sup> $\pm$ 0.3	61.5 <sup>b</sup> $\pm$ 0.4	<0.01
P-value	0.47	0.08	0.64	0.11	0.15	0.64	

<sup>a,b</sup> values within a row with the same superscript letter do not differ significantly ( $P < 0.05$ )

**Table 3** Mean ( $\pm$  SE) weekly egg production (%) of laying hens fed diets supplemented with terebinth over eight weeks

Week	Level of supplementation with <i>Pistacia terebinthus</i>						P-value
	0% (Control)	1%	2%	3%	4%	5%	
1	91.07 <sup>ab</sup> $\pm$ 3.50	93.30 <sup>ab</sup> $\pm$ 1.69	98.21 <sup>b</sup> $\pm$ 1.26	96.43 <sup>ab</sup> $\pm$ 2.52	98.21 <sup>b</sup> $\pm$ 0.73	88.39 <sup>a</sup> $\pm$ 2.25	0.02
2	94.20 $\pm$ 2.67	93.24 $\pm$ 1.63	95.54 $\pm$ 2.58	95.54 $\pm$ 2.68	96.43 $\pm$ 1.26	85.72 $\pm$ 3.34	0.06
3	88.72 $\pm$ 3.19	87.31 $\pm$ 3.40	94.20 $\pm$ 4.08	95.09 $\pm$ 1.34	91.96 $\pm$ 0.89	91.52 $\pm$ 2.23	0.37
4	90.18 $\pm$ 3.96	89.61 $\pm$ 2.69	95.54 $\pm$ 1.86	94.20 $\pm$ 1.12	95.09 $\pm$ 1.98	86.61 $\pm$ 4.03	0.20
5	88.02 $\pm$ 4.76	92.26 $\pm$ 2.81	85.94 $\pm$ 3.84	94.79 $\pm$ 2.76	95.83 $\pm$ 2.25	85.94 $\pm$ 1.97	0.15
6	82.81 $\pm$ 6.41	91.63 $\pm$ 3.72	89.96 $\pm$ 2.60	93.75 $\pm$ 2.71	95.32 $\pm$ 3.72	87.50 $\pm$ 3.19	0.27
7	86.10 $\pm$ 5.18	90.18 $\pm$ 2.3	90.63 $\pm$ 3.67	90.63 $\pm$ 1.12	90.07 $\pm$ 4.40	84.38 $\pm$ 4.02	0.71
8	79.58 $\pm$ 8.44	88.51 $\pm$ 2.12	84.38 $\pm$ 4.60	89.85 $\pm$ 1.50	89.06 $\pm$ 6.44	82.81 $\pm$ 2.99	0.62
mean	87.59 <sup>a</sup> $\pm$ 1.65	90.76 <sup>ab</sup> $\pm$ 0.78	91.80 <sup>ab</sup> $\pm$ 1.74	93.79 <sup>b</sup> $\pm$ 0.83	93.99 <sup>b</sup> $\pm$ 1.15	86.61 <sup>a</sup> $\pm$ 0.93	<0.01
P-value	0.19	0.21	0.15	0.21	0.27	0.20	

<sup>a,b</sup> Values within a row with the same superscript letter do not differ significantly ( $P < 0.05$ )

Terebinth supplementation produced no significant difference in feed consumption (Table 4) or FCR over the trial. Feed conversion ranged from  $1.91 \pm 0.07$  g of feed per gram of egg produced by hens that were supplemented with 2% terebinth to  $2.11 \pm 0.08$  for the control hens. Previous studies have shown variable effects from the use of various herbal products on performance as measured by feed consumption, egg mass and FCR. It has been claimed that plant products generated improved feed consumption, egg mass and FCR (Abdel-Wareth *et al.*, 2013; Yazdi *et al.*, 2014). However, other studies contradict these results for effects on FCR (Christak *et al.*, 2011; Bulbul & Ulutas, 2015).

**Table 4** Mean ( $\pm$  SE) daily feed consumption (g/hen) of laying hens fed diets supplemented with terebinth over eight weeks

Week	Level of supplementation with <i>Pistacia terebinthus</i>						P-value
	0% (Control)	1%	2%	3%	4%	5%	
1	100 <sup>ab</sup> $\pm$ 6	97 <sup>b</sup> $\pm$ 3	102 $\pm$ 5	103 $\pm$ 2	101 $\pm$ 3	104 <sup>ab</sup> $\pm$ 3	0.83
2	102 <sup>ab</sup> $\pm$ 7	102 <sup>ab</sup> $\pm$ 2	105 $\pm$ 3	105 $\pm$ 3	106 $\pm$ 3	110 <sup>ab</sup> $\pm$ 2	0.70
3	108 <sup>a</sup> $\pm$ 6	111 <sup>ab</sup> $\pm$ 4	108 $\pm$ 3	115 $\pm$ 4	111 $\pm$ 3	114 <sup>ab</sup> $\pm$ 2	0.70
4	124 <sup>b</sup> $\pm$ 5	111 <sup>ab</sup> $\pm$ 3	112 $\pm$ 3	122 $\pm$ 2	122 $\pm$ 1	121 <sup>ab</sup> $\pm$ 5	0.06
5	129 <sup>ab</sup> $\pm$ 11	120 <sup>a</sup> $\pm$ 3	123 $\pm$ 2	127 $\pm$ 3	130 $\pm$ 6	122 <sup>a</sup> $\pm$ 2	0.83
6	107 <sup>ab</sup> $\pm$ 5	108 <sup>ab</sup> $\pm$ 2	103 $\pm$ 3	110 $\pm$ 5	113 $\pm$ 8	104 <sup>b</sup> $\pm$ 1	0.70
7	105 <sup>ab</sup> $\pm$ 4	109 <sup>ab</sup> $\pm$ 2	104 $\pm$ 3	111 $\pm$ 3	112 $\pm$ 8	106 <sup>b</sup> $\pm$ 1	0.70
8	105 <sup>ab</sup> $\pm$ 5	110 <sup>ab</sup> $\pm$ 6	109 $\pm$ 7	102 $\pm$ 6	109 $\pm$ 7	93 <sup>b</sup> $\pm$ 3	0.62
mean	110 $\pm$ 4	108 $\pm$ 2	108 $\pm$ 2	112 $\pm$ 3	113 $\pm$ 3	109 $\pm$ 3	0.87
P-value	0.01	<0.01	0.20	0.31	0.09	<0.01	

<sup>a,b</sup> Values within a column with the same superscript letter do not differ significantly ( $P < 0.05$ )

None of the terebinth-supplemented groups produced eggs with detectable differences in quality relative to the unsupplemented control. Egg breaking strength and HU were also unchanged throughout the trial ( $P > 0.05$ ). The yolk colour scores for the groups that were supplemented with 4% and 5% terebinth differed at the end of study, with the score for the 4% group being significantly greater than the score for the 5% group (Table 5). However, the scores for both groups did not differ from the intermediate scores that were achieved by the groups that were supplemented with less terebinth or from the unsupplemented control. Similarly, some studies have shown no effects from plant seed meal supplementation on egg quality parameters except for egg yolk colour (Christaki *et al.*, 2011; Bayram *et al.*, 2007; Bulbul & Ulutas, 2015), while other studies have asserted that the addition of herbs and plant products has no effect on egg quality (Nichol & Steiner, 2008; Navid *et al.*, 2014).

Chemical analysis of blood provides a sensitive tool for judging the effect of any feedstuff or additives on the health status of poultry and for diagnosis. Blood serves as the mainstream index for evaluating the overall physiological, nutritional and pathological status (Oleforuh-Okoleh *et al.*, 2015). In this study, the serological results (Table 6) indicated no difference in the serum constituents that were associated with the level of supplementation with terebinth ( $P > 0.05$ ). However, some studies indicated a decrease in cholesterol level of the blood of broilers fed with various plants products (Lanksy, 1993; Ghazalah & Ali, 2008; Alimohamadi *et al.*, 2014; Boka *et al.*, 2014). Paraskeuas *et al.* (2017) concluded that herbal additives improved plasma triglyceride levels and produced more favourable meat cholesterol levels. Contrary to these findings, other scientists found no effect of herbal supplementation on serum cholesterol, TP, HDL, LDL and glucose levels (Soltan *et al.*, 2008; Mansoub, 2011; Christaki *et al.*, 2011). Abdel-Wareth *et al.* (2013) also observed that serum calcium, phosphorus, glucose and alkaline phosphatase were unaltered in laying hens fed with herbal products.

**Table 5** Effects of level of supplementation with terebinth on egg quality traits (mean  $\pm$  SE) measured at the beginning, middle and end of the eight-week trial

	Week	Level of supplementation with <i>Pistacia terebinthus</i>						P-value
		0% (Control)	1%	2%	3%	4%	5%	
Egg breaking strength	0	43.0 $\pm$ 3.4	50.8 $\pm$ 2.4	44.3 $\pm$ 3.3	43.0 $\pm$ 2.1	50.1 $\pm$ 2.7	46.8 $\pm$ 1.6	0.20
	4	46.4 $\pm$ 2.1	50.0 $\pm$ 3.5	49.1 $\pm$ 2.9	45.5 $\pm$ 2.1	48.3 $\pm$ 5.6	48.0 $\pm$ 2.2	0.93
	8	46.1 $\pm$ 2.7	52.3 $\pm$ 2.6	44.2 $\pm$ 2.4	48.7 $\pm$ 3.0	49.9 $\pm$ 3.3	51.2 $\pm$ 2.6	0.49
	P-value	0.56	0.76	0.53	0.40	0.95	0.49	
Haugh unit	0	77.2 $\pm$ 1.9	74.8 $\pm$ 3.2	66.4 $\pm$ 5.5	71.5 $\pm$ 2.5	67.8 $\pm$ 5.9	75.6 $\pm$ 3.6	0.36
	4	62.7 $\pm$ 6.0	70.1 $\pm$ 4.1	65.2 $\pm$ 7.9	66.7 $\pm$ 5.3	71.5 $\pm$ 4.0	78.1 $\pm$ 2.0	0.37
	8	76.5 $\pm$ 2.4	81.4 $\pm$ 1.8	71.5 $\pm$ 5.9	76.0 $\pm$ 5.2	77.6 $\pm$ 4.6	76.8 $\pm$ 3.8	0.74
	P-value	0.07	0.07	0.69	0.34	0.48	0.76	
Yolk colour score	0	8.6 $\pm$ 0.5	9.4 $\pm$ 0.6	8.0 $\pm$ 0.4	9.3 $\pm$ 0.5	8.7 $\pm$ 0.5	8.5 $\pm$ 0.3	0.29
	4	8.5 $\pm$ 0.6	8.2 $\pm$ 0.4	8.6 $\pm$ 0.4	8.3 $\pm$ 0.3	8.3 $\pm$ 0.5	8.3 $\pm$ 0.4	0.99
	8	8.3 <sup>ab</sup> $\pm$ 0.4	8.0 <sup>ab</sup> $\pm$ 0.4	8.0 <sup>ab</sup> $\pm$ 0.3	9.2 <sup>ab</sup> $\pm$ 0.6	9.6 <sup>a</sup> $\pm$ 0.6	7.8 <sup>b</sup> $\pm$ 0.3	0.49
	P-value	0.96	0.07	0.52	0.24	0.15	0.34	

<sup>a,b</sup> Values within a row with the same superscript letter do not differ significantly ( $P < 0.05$ )

**Table 6** Means ( $\pm$  SE) for constituents of serum from laying hens that had been supplemented with terebinth for eight weeks

Serum constituent	Level of supplementation with <i>Pistacia terebinthus</i>						P-value
	0% (Control)	1%	2%	3%	4%	5%	
Glucose (mg/dL)	203 $\pm$ 5	207 $\pm$ 8	215 $\pm$ 5	218 $\pm$ 11	235 $\pm$ 4	209 $\pm$ 12	0.07
Cholesterol (mg/dL)	111 $\pm$ 22	126 $\pm$ 18	98 $\pm$ 6	116 $\pm$ 19	95 $\pm$ 4	123 $\pm$ 26	0.78
HDL (mg/dL)	26.1 $\pm$ 1.6	21.1 $\pm$ 2.3	24.0 $\pm$ 1.8	22.8 $\pm$ 2.6	25.6 $\pm$ 4.7	22.5 $\pm$ 2.0	0.61
LDL (mg/dL)	31.1 $\pm$ 4.6	30.9 $\pm$ 1.9	28.3 $\pm$ 1.1	28.4 $\pm$ 4.4	28.0 $\pm$ 1.1	33.3 $\pm$ 4.2	0.92
AST (U/L)	287 $\pm$ 23	230 $\pm$ 11	305 $\pm$ 37	268 $\pm$ 40	362 $\pm$ 14	235 $\pm$ 6	0.43
ALT (U/L)	4.70 $\pm$ 1.36	4.57 $\pm$ 0.90	3.00 $\pm$ 0.58	3.38 $\pm$ 0.89	4.00 $\pm$ 0.71	2.00 $\pm$ 0.58	0.67
Protein (g/dL)	6.09 $\pm$ 0.33	5.77 $\pm$ 0.25	5.45 $\pm$ 0.15	5.46 $\pm$ 0.12	5.62 $\pm$ 0.28	5.47 $\pm$ 0.09	0.53
(mg/dL)	6.46 $\pm$ 0.58	7.41 $\pm$ 0.58	6.75 $\pm$ 0.69	6.96 $\pm$ 0.61	6.62 $\pm$ 0.68	5.17 $\pm$ 0.78	0.40
Calcium (mg/dL)	23.1 $\pm$ 1.2	25.2 $\pm$ 1.1	24.5 $\pm$ 1.2	25.0 $\pm$ 2.2	24.6 $\pm$ 0.4	20.2 $\pm$ 5.2	0.34

HDL: high density lipoprotein, LDL: low density lipoprotein, AST: aspartate aminotransferase, ALT: alanine aminotransferase

Total antioxidant capacity, TOS and OSI also remained unaffected after supplementing the diet of laying hens with terebinth seed meal (Table 7). Some researchers reported that plants and herbal products improved immunity, mitigated stress, and increased antioxidant activity (Allen *et al.*, 1998; Agarwal, 1999; Diwanay *et al.*, 2004; Wheeler, 1994; Ziauddin *et al.*, 1996). Both TAC and TOS contribute to the overall health status of the animals and are indicative of their physiological status (Bulbul & Ulutas, 2015).

**Table 7** Oxidant and antioxidant status of laying hens fed diets supplemented with various levels of terebinth

Supplement Level	Total antioxidant capacity (TEAC <sup>1</sup> /L)	Total oxidant status (pg/mL)	Oxidant status index
Control	2.69 ± 0.17	0.0129 ± 0.0063	0.51 ± 0.25
1% Pistachia	3.35 ± 0.53	0.0052 ± 0.0010	0.17 ± 0.03
2% Pistachia	2.68 ± 0.45	0.0053 ± 0.0015	0.27 ± 0.14
3% Pistachia	2.65 ± 0.80	0.0035 ± 0.0005	0.17 ± 0.04
4% Pistachia	1.92 ± 0.45	0.0049 ± 0.0014	0.25 ± 0.03
% Pistachia	2.28 ± 0.59	0.0207 ± 0.0132	0.88 ± 0.52
<i>P</i> -value	0.34	0.23	0.17

<sup>1</sup> Trolox equivalent antioxidant capacity

The haematology analyses revealed no differences that were associated with the level of terebinth supplementation with the exception of haemoglobin (Table 8). Similar to the above studies, the present study found the inclusion of various levels of *Pistacia terebinthus* in the diet of laying hens produced no adverse effect or sudden changes in the blood profile.

**Table 8** Means (± SE) from haematological analyses of blood from laying hens fed diets supplemented with terebinth for eight weeks

Characteristic <sup>1</sup>	Level of supplementation with <i>Pistacia terebinthus</i>						<i>P</i> -value
	0% (Control)	1%	2%	3%	4%	5%	
WBC	2.90 ± 0.19	2.20 ± 0.21	2.14 ± 0.23	2.35 ± 0.27	2.68 ± 0.22	2.45 ± 0.25	0.18
LC	1.74 ± 0.04	1.72 ± 0.05	1.70 ± 0.04	1.81 ± 0.04	1.73 ± 0.04	1.68 ± 0.04	0.31
NC	0.73 ± 0.03	0.68 ± 0.05	0.81 ± 0.04	0.79 ± 0.05	0.67 ± 0.03	0.74 ± 0.04	0.14
MC	0.044 ± 0.019	0.043 ± 0.007	0.043 ± 0.001	0.043 ± 0.001	0.044 ± 0.001	0.044 ± 0.008	0.88
RBS	2.70 ± 0.05	2.59 ± 0.05	2.61 ± 0.05	2.62 ± 0.04	2.60 ± 0.04	2.63 ± 0.05	0.62
Hb	10.43 <sup>ab</sup> ± 0.24	11.25 <sup>a</sup> ± 0.21	10.85 <sup>ab</sup> ± 0.25	10.25 <sup>b</sup> ± 0.20	10.76 <sup>ab</sup> ± 0.25	10.27 <sup>b</sup> ± 0.16	0.02
Ht	34.75 ± 0.38	34.17 ± 0.39	34.42 ± 0.41	34.80 ± 0.40	34.81 ± 0.40	34.34 ± 0.50	0.82
MCV	108.58 ± 0.49	108.09 ± 0.44	108.36 ± 0.64	108.60 ± 0.60	108.56 ± 0.68	107.51 ± 0.47	0.73
MCHC	30.86 ± 0.43	30.80 ± 0.50	31.21 ± 0.51	30.69 ± 0.48	31.45 ± 0.42	30.84 ± 0.46	0.86
PLT	27.03 ± 0.63	25.89 ± 0.53	26.98 ± 0.68	27.90 ± 0.58	26.59 ± 0.60	26.39 ± 0.40	0.25
MPV	6.53 ± 0.07	6.55 ± 0.08	6.47 ± 0.09	6.53 ± 0.09	6.35 ± 0.08	6.35 ± 0.07	0.26

<sup>1</sup> WBC: white blood cell count x10<sup>3</sup>/uL, LC: lymphocyte count x10<sup>3</sup>/uL, NC: neutrophil count x10<sup>m</sup>, MCV: mean corpuscular volume fL, MCHC: mean corpuscular haemoglobin count g/dL, PLT: platelet x10<sup>3</sup>/L, MPV: mean platelet volume fL

<sup>a, b</sup> values within a row with the same superscript letter do not differ significantly (*P* < 0.05)

## Conclusions

Terebinth apparently has no adverse effects on the blood chemistry or oxidant-antioxidant status of poultry. Thus, it can be used safely. Because supplementation of the diet of laying hens with terebinth seed meal improved egg production, it has potential for use as an alternative non-conventional feed resource in poultry farming.

### Acknowledgement

This study was supported by Scientific Research Projects Coordination Unit of Afyon Kocatepe University (Project Number 16.KARIYER.94).

### Authors' Contributions

ISC and IB conceived the idea, AI; HS and UO conducted the trial and collected the samples; CU performed analysis; EEG and AR analysed the data and wrote the article.

### Conflict of Interest Declaration

Authors declared there was no conflict of interest regarding this article.

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