Original Article

The effect of different levels of pomegranate kernels and kernel oil on the performance of laying hens and egg quality traits

Alireza Gharagozloo[1*], Farshid Khairi[2]

[1]Faculty of Agriculture, Ph.D. in Animal Sciences, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. [2]Faculty of Agriculture, Department of Animal Science, Islamic Azad University, Shahrekord Branch, Shahrekord Iran

Correspondence: Alireza Gharagozlou, Faculty of Agriculture, Ph.D. in Animal Sciences, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran, Alireza_gh514@yahoo.com

ABSTRACT

This experiment was designed and implemented to investigate the effect of different levels of pomegranate kernel and kernel oil on the egg quality traits and performance of 144 laying hens from commercial mixed white Leghorn breed (Hy-Line W-80) in nine treatments, four replicates, and four pieces of hens distributed in each replicate. This experiment with a completely random design of pomegranate kernels at four levels (0.1, 0.2, 0.3, and 0.4%), four levels of pomegranate kernel oil (0.1, 0.2, 0.3, and 0.4%), and a control treatment was conducted for 10 weeks from the age of 25-35 weeks. The energy and protein levels were identical in the diets and were adjusted based on the NRC nutritional requirements for poultry (1994). The results showed that egg weight, egg mass weight, egg production percentage, feed conversion ratio, the Haugh unit, shell weight, and eggshell strength and thickness were not significantly affected by different levels of pomegranate kernel and kernel oil. However, the egg white index, yolk index, and egg specific weight were significantly affected by the treatments ($P < 0.05$). According to our findings and no reduction of production, it is recommended to use pomegranate kernels and kernel oil in the feed of laying hens to improve the health of human society, reduce production costs, and reduce environmental pollution.

Keywords: Pomegranate kernel, Pomegranate kernel oil, Performance of laying hens, Egg quality

Introduction

A huge part of the world's human population suffers from malnutrition. The world's population is on the rise, leading to increased people's food expectations, including the need for animal protein sources. To respond to these needs, the poultry industry has witnessed dramatic growth in recent years, and this increase in production is necessary to meet human nutritional needs (Hernandez, Madrid, Garcia, Orengo, & Megias, 2004). Since the cost of feed accounts for approx. 60-70% of the total production cost of laying hens (Rezaj & A, 2005), it is scientifically necessary to replace the conventional feed ingredients in poultry feeding with agricultural, livestock, and industrial waste. The use of waste will reduce environmental pollution besides reducing diet costs (Ghaznavi, Vashan, Javad, Afzali, Nazar, Ghiashi, & Ehsan, 2018). The pomegranate kernel pulp falls into the wastes of agricultural processing industries. The pomegranate plant belongs to the Punicaceae family. The annual production of pomegranate is 990,000 tons in Iran, which is one of the major countries producing pomegranate in the world (Ghaznavi, Vashan, Javad, Afzali, Nazar, Ghiasi, & Ehsan, 2018). The industrial pomegranate processing and the production process of products, such as pomegranate juice, sauce, concentrate, and syrup, produce a large volume of waste, and pomegranate kernel pulp includes the kernel and outer shell (Ghaznavi, Vashan, Javad, Afzali, Nazar, Ghiasi, & Ehsan, 2018). Pomegranate skin and pulp comprise about 40-50% of the fruit's weight (Abbasi, Rezaei, & Rashidi, 2008). Pomegranate contains polyphenolic compounds, including ellagic acid and its derivatives punicallgin and punicallin, which possess antioxidant properties (Brown, Rosner, Willett, & Sacks, 1999). Punicallgin has antioxidant, antifungal, and antimicrobial properties (T. Rababah, K. Ereifej, M. Al-Mahasneh, & M. Al-Rababah, 2006; Ashoush, El-Batawy, & El-Shourbagy, 2013). The increase in toxic effects and cancer diseases caused by the use of antibiotics and synthetic antioxidants has redoubled the use of medicinal plants and plant antioxidant compounds to reduce or delay the oxidation of fats and improve the immune system of birds (T. Rababah, K. Ereifej, M. Al-Mahasneh, & M.J.P.S. Al-Rababah, 2006). Accordingly, this research aimed to investigate the effect of different levels of pomegranate kernel and kernel oil on the egg quality traits and the performance of laying hens.

Materials and Methods

The experimental diet and design

The present study was designed and conducted in the laying hen research farm of the Center for Education, Research, and Extension of Agriculture and Natural Resources in Hamadan Province. The effect of different levels of pomegranate kernel and kernel oil on the performance, and egg quantitative and qualitative traits in laying hens was investigated for 10 weeks. To this aim, 144 Hy-line (W-80) laying hens were assigned to nine treatments, four replications, and four birds per replication (n = 4 per 36 cages) from 25-35 weeks of age with similar average weight, average egg weight, and production percentage. The diets of the treatments with identical energy and protein levels were adjusted according to the NRC nutritional requirements of poultry (1994) (based on the corn-soybean meal). The percentages of dry matter, crude protein, lysine, arginine, methionine, crude fiber, crude fat, calcium, phosphorus, and sodium were equalized based on the NRC table recommendations (1994) using UFFDA diet formulation software. The percentages of diet ingredients are represented in Tables 1 and 2.

Experimental treatments consisted of 1) without using pomegranate kernels and oil (control group), using 0.1%, 0.2%, 0.3%, and 0.4% of pomegranate kernels, and using 0.1%, 0.2%, 0.3%, and 0.4% of pomegranate kernel oil. Food and water were freely available to the hens during the experiment. The light program of the hall included 16 h light and 8 h dark. The hens were assigned to nine experimental treatments with four replications each containing four birds (Fig. 1).

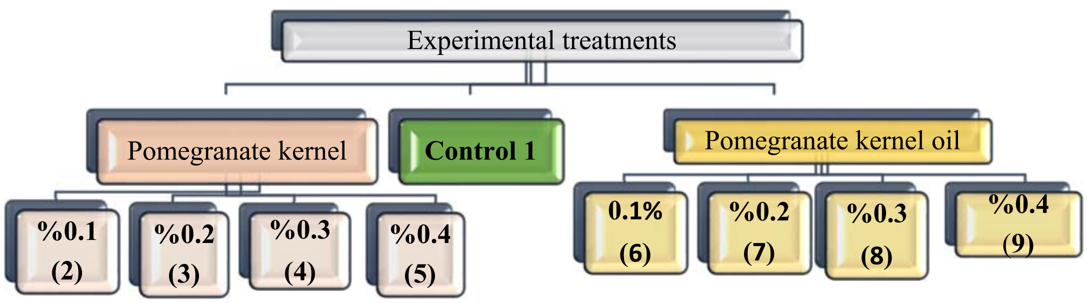

Figure 1. Feeding treatments to investigate the effect of different levels of pomegranate kernels and kernel oil on egg quality traits and performance of laying hens.

Evaluated variables

Plant materials

In this study, pomegranate samples were the subacid Shirin cultivar with an approximate weight of 200 g per fruit and a commercial ripeness index, obtained from the Saveh region.

Kernel separation from fruits

After washing and cutting the fruits into small pieces, the kernels were separated from the flesh by hand, and the extract was extracted through gentle rubbing on a metal net. To separate the remaining flesh particles, the kernels were washed with water and then dried on a wide clean cloth at room temperature (away from sunlight). The dried kernels were kept in linen bags and frozen (-18 °C) until use and oil extraction.

Table 1. Analysis of feed components of laying hens in 10 experimental weeks

Feed components	Control	Pomegranate kernel				Pomegranate kernel oil			
		0.1%	0.2%	0.3%	0.4%	0.1%	0.2%	0.3%	0.4%
Corn (8%)	599.95	606.75	613.45	620.45	627.55	599.55	599.45	599.35	599.25
Soybean (44%)	214	214.5	214.7	214.8	214.9	214.4	214.4	214.4	214.4
Wheat bran	36	36	36	36	36	36	36	36	36
Pomegranate kernel powder	0	1	2	3	4	0	0	0	0
Calcium carbonate	98.3	98.3	98.3	98.3	98.3	98.3	98.3	98.3	98.3
Di-calcium phosphate	21.1	21.1	21.1	21.1	21.1	21.1	21.1	21.1	21.1
Mineral supplement	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin supplement	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Lysine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Baking soda	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Oil (9400)	16.7	14.6	12.6	10.6	8.5	14.6	12.6	10.6	8.5

Pomegranate oil (9000)	0	0	0	0	0	1	2	3	4

Mineral supplements (mg/kg of the diet) include copper (2.4 mg), iodine (0.34 mg), iron (30 mg), manganese (29.76 mg), selenium (25.87 mg), and zinc (25.87 mg). Vitamin supplements (per kg of the diet) include vitamin A (3520 IU), vitamin B1 (0.59 mg), 1 vitamin B2 (.6 mg), niacin (13.86 mg), pantothenic acid (3.13 mg), B6 (1 mg), biotin (0.06 mg), choline (80 mg), vitamin B12 (0.004 mg), B9 (0.19 mg), vitamin D3 (1000 IU), vitamin E (8.8 IU), and vitamin K (0.88 mg). All calculations are based on the NRC requirements for poultry (1994).

Table 2. Analysis of feed components of laying hens in 10 experimental weeks

Feed components	Control	Pomegranate kernel				Pomegranate kernel oil			
		0.1%	0.2%	0.3%	0.4%	0.1%	0.2%	0.3%	0.4%
Computational analysis									
Metabolizable energy	2711	2711	2711	2711	2711	2711	2711	2711	2711
Protein %	15.05	15.05	15.05	15.05	15.05	15.05	15.05	15.05	15.05
Lysine %	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Methionine + cysteine%	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Calcium%	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27
Available phosphorus %	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Sodium %	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Chlorine %	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Crude fiber%	3	3	3	3	3	3	3	3	3
Linolenic acid %	2.15	2.079	2.003	1.927	1.851	2.079	2.003	1.927	1.851

Oil extraction

Pomegranate kernel oil was extracted by the cold pressing method and refrigerated (+4 °C) until testing.

Examination of the fatty acid profile of extracted pomegranate kernel oil

Oil fatty acids were identified with a gas chromatographer (YOUNG LIN ACME, South Korea) with a column of 60 m, a diameter of 0.25 mm, and a particle size of 0.25 µm (Table 3).

Table 3. Structures of fatty acids of oil extracted from pomegranate kernels for testing

Fatty acids	C14:0	C16	C16:1n7	C18:0	C18:1n9	C18:2n6	C203n6	C20:4n6	C18:3n5
Percent	0.03	3.55	0.05	2	6.75	7.58	0.5	0.78	78.18

Functional traits

Record keeping

During the experimental period, egg production was recorded in all experimental units. The average laying percentage, egg mass production (day/bird/gram), feed intake, and conversion ratio were calculated and recorded weekly. The layers were weighed individually and their data were recorded at the end of the experimental period. The traits studied in this experiment were production performance traits including feed intake, egg weight, and egg laying rate. The feed intake was recorded weekly, and the weight and number of eggs were recorded daily. Then, the egg mass and feed conversion ratio (FCR) were calculated based on the obtained data. Data on the performance traits of the feed intake, egg laying rate, egg weight, produced egg mass, and FCR were measured and recorded weekly.

Daily feed was weighed in two steps and provided to the hens, and extra feed was collected, weighed, and recorded at the end of each week. The produced eggs were collected, weighed, and recorded daily at 15:00. The average weight of eggs, percentage of production, and FCR were calculated weekly basis. The percentage of laying was calculated by dividing the total eggs produced by each experimental unit per week by the day of the hen multiplied by 100. The produced egg mass multiplied by the percentage of production in the average egg weight and the weight of the experimental unit and the FCR obtained by dividing daily feed intake by the produced egg mass were calculated and recorded weekly (Vashan et al., 2009).

Record keeping

During the experimental period, egg production was recorded in all experimental units. The average laying percentage, egg mass production (day/bird/gram), feed intake, and FCR were calculated and recorded daily and weekly.

Quality traits of eggs

At the end of the test period, two eggs from each cage were evaluated to determine the external quality traits (shell strength, thickness, and percentage) and internal quality traits (the Haugh unit, egg white height, yolk percentage, egg white percentage, yolk color, and yolk weight). The shell strength (kg/cm^2) was determined using a digital device through the Force Gauge method. The shell thickness was measured with a thickness gauge and by ultrasonic waves. The internal characteristics of the eggs were tested and calculated with an EMT-5200 device.

Results

Production and functional traits

The results (Table 3) revealed that the dietary pomegranate kernel and kernel did not significantly affect egg weight, egg mass weight, egg production, and FCR. These experimental results correspond to previous reports (Reed 1995; Sadeghi et al. 2009; Saki et al. 2014; Rabet et al. 2012; Zaman, et al., 2014) probably due to supplying the layers' requirements through the experimental diets. However, our results are not consistent with those of Ghaznavi et al. (Ghaznavi, Vashan, Javad, Afzali, Nazar, Ghiasi, & Dami, 2018). They used the pomegranate kernel pulp after processing with 29.2% raw fiber, and less fiber percentage in their experiments might have led to the positive effects of pomegranate pulp on the performance of laying hens.

The egg mass decreased and FCR increased by increasing the pomegranate kernel and oil content, but these differences were not significant (P > 0.05). The production percentage decreased in the 0.1, 0.2, 0.3, and 0.4% of the dietary pomegranate kernel by increasing the pomegranate kernel (P > 0.05). Nevertheless, this parameter increased in the treatments with 0.1, 0.2, 0.3, and 0.4% of pomegranate kernel oil with increasing oil in the diet, but this increase was not significant (P > 0.05). The feed intake increased with the increase in the pomegranate kernel and oil percentages (P < 0.05), which might have resulted from the palatability effect of pomegranate oil and oil-containing kernels.

Table 4. The effect of different levels of pomegranate kernel and kernel oil on production and functional traits of laying hens

Treatment		Production (%)	Feed per bird	Feed per cage	Mass weight (g/hen/day)	Mass weight (g/hen/week)	FCR	Egg produced per cage (g)	Produced egg (g)	Feed intake (g/hen/week)
Control		96.16	6340^{e}	25360.2^{ab}	58.258	407.79	1.565	16311.5	60.535	634.01^{b}
Pomegranate kernel (%)	0.1	96.788	6377^{d}	25509^{ab}	55.545	388.8	1.6425	15551.8	57.383	637.73^{b}
	0.2	97.468	6266^{f}	25063.1^{ab}	57/.26	400.82	1.6125	15598.9	58.76	645.38^{b}
	0.3	96.99	6202^{g}	24807.7^{b}	56.245	393.71	1.6725	15053.9	57.988	655.55^{ab}
	0.4	95.18	6452^{c}	25808.8^{ab}	55.245	368.7	1.6725	15468.1	58.035	645.22^{b}
Pomegranate kernel oil (%)	0.1	95.625	6455^{b}	25818.7^{ab}	59.143	414	1.565	16559.8	61.845	645.47^{b}
	0.2	96.875	6725^{a}	26900^{a}	57.85	404.95	1.665	16198	59.718	672.5^{ab}
	0.3	94.4	6725^{a}	25218.8^{ab}	56.093	392.65	1.695	14859	59.41	664.86^{ab}
	0.4	97.65	6725^{a}	26900^{a}	57.13	399.9	1.775	15297.5	58.503	709^{a}
SEM		1.95	0	448.3	3.06	21.4	0.14	1324.64	2.71	14.6
P-Value		0.31	0.8	0.004	0.67	0.67	0.52	0.63	0.41	0.0016

Quality traits of eggs

According to the obtained results, the combination of dietary pomegranate kernel and kernel oil affects the external quality traits of the egg (Table 4). The results of using pomegranate kernel and kernel oil on the eggshell traits (Table 4) indicate no significant effects on eggshell traits. Eggshell quality is one of the main quality traits of eggs in the laying hen industry, commercial laying hens, and mother hens. Undesirable eggshell thickness is not suitable for consumers and hatchery facilities because thin-shell eggs are broken during transportation and before reaching the supply and hatching center, thereby reducing the quality of healthy eggs. Moreover, an undesirable eggshell reduces the hatching efficiency of eggs. Several factors influence the shell traits, among which nutrition is one of the major factors affecting the shell thickness (A & J, 2015).

The specific weight of eggs is one of the indirect methods to assess the quality of eggshells, and the shell weight is the main factor affecting this parameter. Eggs with a specific weight ≥ 1.088 have an acceptable shell quality (Rezaj & A, 2005). The utmost specific weights were measured in the control and treatments with 0.1, 0.2, and 0.3% of pomegranate kernels, and the treatment with 0.2% of pomegranate kernel oil presented the lowest specific weight. Based on the results of the statistical analysis of the data on the effect of different levels of pomegranate kernel oil on the internal quality traits of eggs (Table 4), pomegranate oil and kernels exerted no significant effects on the units and eggs. Although the Haugh unit was improved by increasing the pomegranate kernel oil in the diet, this increase was not significant (P > 0.05). These results agree with those of previous studies (Saki et al., 2014; Rabat, M. et al., 2013), suggesting no significant effect of pomegranate kernel pulp on egg white quality. This trait is largely linked to the strength or the structure of the white jelly so that the egg quality increases with increasing the white strength. This factor creates the jelly structure of the egg white, and the Haugh unit indicates the egg white quality; this unit increases with an increase in oosin (Leesons & Summers, 2001). Pomegranate kernel pulp probably increases the retention of nutrients and the egg white quality, due to its antioxidant compounds and pigments (Emami, 2016). The highest egg yolk color index was observed in the treatments fed with 0.3 and 0.4% pomegranate kernels while it was lowermost in the treatments fed with 0.3 and 0.4% pomegranate kernel oil. The egg yolk color directly reflects the xanthophyll level in the diet (Klasing, 2011). The diets with 0.3% and 0.4% of pomegranate kernels and those containing oil increase the absorption of carotenoids due to the increase of unsaturated fatty acids (Leesons & Summers, 2001), which may explain the highest yolk color values recorded in these treatments. Currently, carotenoid-rich medicinal plants are used instead of synthetic dyes to intensify the egg yolk color in laying hens. Consumption of those diets containing pigments (e.g., carotenoids and xanthophylls) makes the yolk more colorful (Belyavin, 1987); therefore, increasing carotenoid absorption by plants leads to an increase in the yolk color. These results are not in agreement with those reported previously (Rabet et al., 2012; Saki et al., 2014).

Table 5. Effects of different levels of pomegranate kernel and kernel oil on internal quality traits and eggshell traits of laying hens.

	Internal quality traits				Eggshell traits			
Treatment (%)	**Egg weight (g)**	**White index**	**Youlk color**	**Haugh unit**	**Specific weight (mg/mm^3)**	**Shell weight (g)**	**Strength (kg/cm^2)**	**Thickness (mm)**
Control	59.7	7.53^{ab}	7^{ab}	86.9	1.08^{a}	6.108	3.06	0.3
Kernel 0.1	57.99	7.3^{ab}	7.25^{ab}	86.06	1.08^{a}	5.71	3.12	0.31
Kernel 0.2	57.08	7.43^{ab}	6.75^{b}	86.7	1.08^{a}	5.72	2.18	0.3
Kernel 0.3	57.5	7.07^{b}	7.75^{a}	84.8	1.08^{a}	5.72	3.03	0.3
Kernel 0.4	59.9	7.7^{a}	7.75^{a}	87.74	1.075^{ab}	5.97	3.05	0.3
Oil 0.1	58.6	7.2^{ab}	7^{ab}	85.4	1.075^{ab}	5.72	3.06	0.3
Oil 0.2	57.2	7.25^{ab}	6.75^{b}	85.9	1.06^{b}	5.73	3.06	0.31
Oil 0.3	59.11	7.5^{ab}	7.25^{ab}	86.7	1.075^{ab}	5.91	3.01	0.3
Oil 0.4	56.79	7.5^{ab}	7^{ab}	87.5	1.078^{a}	5.9	3.05	0.31
SEM	2.6	0.36	0.5	2.6	0.007	0.3	0.095	0.021
P-Value	0.61	0.37	0.13	0.83	0.28	0.47	0.72	0.61

In the end, the data obtained from the research results were recorded in Excel software and analyzed with the analysis of variance (ANOVA) using SAS statistical software (version 6/1) with the GLM procedure. Then, the means were compared with Tukey's multiple-range test at a probability level of 5% (P < 0.05).

Discussion and conclusion

Production and functional traits

The obtained results (Table 4) revealed that the production percentage, FCR, gram of produced eggs, and mass weight/gram of hen/day were not significantly affected by the dietary pomegranate kernel and kernel oil. These experimental results agree with previous studies (Reed 1995, Sadeghi et al. 2009, Saki et al. 2014, and Rabet et al. 2012) probably due to supplying the layers' requirements through the experimental diets. However, our results are not consistent with those of Ghaznavi et al. (Ghaznavi, Vashan, Javad, Afzali, Nazar, Ghiasi, & Dami, 2018). They used the pomegranate kernel pulp after processing with 29.2% of raw fiber, and less fiber percentage in their experiments might have led to the positive effects of pomegranate pulp on the performance of laying hens. The egg mass decreased and FCR increased by increasing the pomegranate kernel and oil content, but these differences were not significant (P > 0.05). The production percentage decreased in the 0.1, 0.2, 0.3, and 0.4% of the dietary pomegranate kernel by increasing the pomegranate kernel (P > 0.05). Nevertheless, this parameter increased in the treatments with 0.1, 0.2, 0.3, and 0.4% of pomegranate kernel oil with increasing oil in the diet, but this increase was not significant (P > 0.05). The feed intake increased with the increase in the pomegranate kernel and oil percentages (P < 0.05), which might have resulted from the palatability effect of pomegranate oil and oil-containing kernels.

Internal quality traits and shell traits of eggs

Internal quality traits of eggs

Based on the statistical analysis results of the data for the effect of different levels of pomegranate kernel oil on egg quality traits (Table 5), the white index showed an upward trend with the increase in the dietary kernel percentage. The egg white quality is largely linked to the strength or the structure of the white jelly so that the egg quality increases with the increase of white strength, and the Haugh unit indicates the egg white quality; this unit increases with an increase in oosin (Leesons & Summers, 2001). Pomegranate kernel pulp probably increases the retention of nutrients and the egg white quality, due to its antioxidant compounds and pigments (Emami, 2016). The highest egg weight was obtained in the treatments fed with the control and 0.1% pomegranate kernel oil. Evidence shows that the yolk size in the egg is mainly influenced by genetics, age, production percentage, etc., and an increase in the egg size is mainly because of an increase in the egg white (Farkhoy, Sigharody, & Niknafas, 1994).

The effect of different levels of pomegranate kernel oil on the internal quality traits of eggs is presented in Table (5), which shows that pomegranate oil and kernels had no significant effects on egg Haugh units ($P > 0.05$). Although the Haugh unit was improved by increasing the pomegranate kernel oil in the diet, this increase was not significant ($P > 0.05$). These results are in agreement with those of previous studies (Saki et al., 2014; Rabat, M. et al., 2013), suggesting no significant effect of pomegranate kernel pulp on egg white quality. This trait is largely linked to the strength or the structure of the white jelly so that the egg quality increases with increasing the white strength. This factor creates the jelly structure of the egg white, and the Haugh unit indicates the egg white quality; this unit increases with an increase in oosin (Leesons & Summers, 2001). Pomegranate kernel pulp probably increases the retention of nutrients and the egg white quality, due to its antioxidant compounds and pigments (Emami, 2016).

The highest egg yolk color index was observed in the treatments fed with 0.3 and 0.4% pomegranate kernels while it was lowermost in the treatments fed with 0.3 and 0.4% pomegranate kernel oil. The egg yolk color index was significantly influenced by the different levels of pomegranate kernels and kernel oil ($P < 0.05$). The egg yolk color directly reflects the xanthophyll level in the diet (Klasing, 2011). The diet with 0.4% pomegranate kernels and those containing oil increase the absorption of carotenoids due to the increase of unsaturated fatty acids (Leesons & Summers, 2001), which may explain the highest yolk color values recorded in these treatments. Currently, carotenoid-rich medicinal plants are used instead of synthetic dyes to intensify the egg yolk color in laying hens. Consumption of those diets containing pigments (e.g., carotenoids and xanthophylls) makes the yolk more colorful (Belyavin, 1987); hence, increasing carotenoid absorption by plants results in an increase in the yolk color. These results are not in agreement with those reported previously (Rabet et al., 2012; Saki et al., 2014), but they are consistent with the findings of Rabet, et al. (2012).

Eggshell traits

The obtained results indicate that the combination of dietary pomegranate kernel and kernel oil affects the external quality traits of eggs (Table 5). Some of the measured traits were significantly affected by the use of different levels of pomegranate kernels and kernel oil. The data on the effect of different levels of pomegranate kernel and kernel oil on the quality characteristics of eggshells, including shell weight, thickness, and strength, are presented in Table (5). The qualitative evaluation results of the eggs from hens fed with different levels of pomegranate kernels and kernel oil showed no significant difference between the experimental groups in terms of shell weight, shell thickness, and strength ($P > 0.05$). Indirect methods are used to examine eggshell quality, and shell weight is the major factor affecting this trait. Eggs with a specific weight ≥ 1.088 have an acceptable shell quality (Rezaj & A, 2005). The specific weights of the treatments fed with different levels of pomegranate kernel and kernel oil were significantly affected by the diets ($P < 0.05$). The highest specific weights of the eggs were measured in the control group and treatments with 0.1, 0.2, and 0.3% of pomegranate kernel, and the treatment with 0.2% of pomegranate kernel oil had the lowest value. This increase probably results from an increase in the oleic acid content in diets (Grobas, Mendez, Lazaro, De Blas, & Mateo 2001). This experimental result is in line with Grobas et al. (2015). The elevated specific weight is directly linked to eggshell strength, which was observed in the results of the experimental treatments, although these differences were not significant. The present results agree with those of Rahmani et al. (2012).), suggesting the improved shell strength with an increase in specific gravity. Eggshell quality is a major egg quality trait in the commercial laying hen and mother hen industry. Unfavorable eggshell thickness is not suitable for consumers and hatchery factories because thin-shelled eggs are broken during transportation and before reaching the supply and hatching center, thereby reducing the quality of intact eggs. Moreover, an undesirable eggshell reduces the hatching efficiency of eggs. The shell traits are affected by several factors, among which nutrition is one of the major factors affecting the shell thickness (A & J, 2014).

The shell quality was expected to be improved due to our analysis of pomegranate kernels used in the diets containing 3.1 (g/kg) calcium. Unprocessed pomegranate kernel pulp contains about 29.2% calcium, which can effectively improve the shell quality (Golian, Moeini, & Mazhari, 2009). The data in Table 2 indicate that this trait was not significantly affected by using different levels of pomegranate kernels and kernel oil ($P > 0.005$).

Conclusion

Based on the results of this research, the hens fed with different levels of pomegranate kernels and kernel oil used in the present experiment showed no negative effects and decreased performance. Therefore, the findings of this study demonstrate that pomegranate kernel and kernel oil can be used in laying hens to utilize agricultural wastes and increase the productivity of the potentially valuable wastes to reduce environmental pollutants.

Acknowledgments: The authors are grateful to Dr. Asadian, the former head of the Research, Education, and Extension Center for Agriculture and Natural Resources, Hamadan province, for assistance in implementing this project.

Conflict of interest: None

Financial support:. None

Ethics statement: None

References

Rezaj, P., & A, N. (2005). Breeding broiler chickens (2nd edition). Iran. Isfahan: Isfahan University of Technology.

Vashan, H., Javad, S., Afzali, Nazar, Molkaneh, Naseri, & Iran, R.J.P.A.d. (2009). Comparison of the effect of flaxseed and safflower on egg yolk fatty acid composition and antibody titer response of laying hens. 1(2).

Rabet, M., Saki, A., Zamani, P., Khah, H.M.V., & Mirzaei, S. (2012). Functional traits of laying hens in response to feeding pomegranate kernel pulp and multi-enzyme. A paper presented at the scientific-applied conference on the use of agricultural, urban, and industrial wastes in animal, poultry, and aquaculture diets, Tabriz, Iran.

Ghaznavi, Vashan, H., Javad, S., Afzali, Nazar, Ghiasi, & Dami, S.A.J.A. (2018). The effect of pomegranate kernel pulp processed with calcium hydroxide on egg quality traits, performance, cholesterol, blood biochemical parameters, and antioxidant status of laying hens. 30(117), 103-116.

A, L., & C, S. (2015). Nutrition of commercial poultry (T., H.R. & Tavakoli, M., Trans.). Iran. Tehran: Aeezh.

Emami, A. (2016). The effect of using pomegranate byproducts on performance, blood and rumen parameters, and acid composition of meat and milk of fattening and dairy goats. (Ph.D.), Birjand,

Clasing, K. (2002). Comparative feeding of birds (R. Sh., Trans.). Iran. Tehran: Tarbiat Modares University Press.

M, R., A, N., & Y, M.N. (2012). The effect of using different levels of a mixture of oregano, nettle, and chicory medicinal plants with and without enzymes on performance, egg quality, and blood parameters of laying hens. J. Livestock and Poultry Research. 1(2), 19-28. doi:10.22077/jlr.2012.164

Golian, A., Moeini, M., S., & Mazhari, M. (2009). In Poultry Nutrition. Tehran: Department of Education and Research of Agriculture Vice-Chancellor of Kousar Economic Organization.

Ghaznavi, Vashan, H., Javad, S., Afzali, Nazar, Ghiasi, & Ehsan, S. (2018). The effect of pomegranate kernel pulp processed with calcium hydroxide on egg yield, quality traits, cholesterol, blood biochemical parameters, and antioxidant status of laying hens. Animal Sciences (Research and Construction), 30(117), 103-116.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., & Megias, M. J. P. s. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. 83(2), 169-.174

Abbasi, H., Rezaei, K., & Rashidi, L. (2008). Extraction of essential oils from the seeds of pomegranate using organic solvents and supercritical CO2. *Journal of the American Oil Chemists' Society,* 85(1), .83-89

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. *The American journal of clinical nutrition,* 69(1), .30-42

Rababah, T., Ereifej, K., Al-Mahasneh, M., & Al-Rababah, M. (2006). Effect of plant extracts on physicochemical properties of chicken breast meat cooked using conventional electric oven or microwave. *Poultry science,* 85(1), .148-154

Ashoush, I. S., El-Batawy, O., & El-Shourbagy, G. A. J. A. o. A. S. (2013). Antioxidant activity and hepatoprotective effect of pomegranate peel and whey powders in rats. 58(1), .27-32

Rababah, T., Ereifej, K., Al-Mahasneh, M., & Al-Rababah, M. J. P. s. (2006). Effect of plant extracts on physicochemical properties of chicken breast meat cooked using conventional electric oven or microwave. 85(1), .148-154

Reed, J. D. J. J. o. a. s .(1995) .Nutritional toxicology of tannins and related polyphenols in forage legumes. 73(5), .1516-1528

Sadeghi, N., JANAT, B., OVEYSI, M., HAJI, M. M., & FOTOVAT, M. (2009). Antioxidant activity of Iranian pomegranate (Punica granatum L.) seed extracts .

Saki, A., Rabet, M., Zamani, P., & Yousefi, A. J. I. J. o. A. A. S. (2014). The effects of different levels of pomegranate seed pulp with multi-enzyme on performance, egg quality and serum antioxidant in laying hens. 4(4), .803-808

leesons, & Summers, J .D. (2001). In *scotts nutrition of the chicken*: guelph omtariocanada. (Reprinted from: 4 th ed.(

Belyavin, C. J. R. a. i. a. n. (1987). Natural products for egg yolk pigmentation .

Farkhoy, M., Sigharody, F., & Niknafas, F. (1994). Poultry breeding. Coasar Publication. pp: 150-266. In: Farsi.

Grobas, S., Mendez, J., Lazaro, R., De Blas, C., & Mateo, G. J. P. S. (2001). Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. 80(8), .1179-1171