Effects of Sumac fruit (*Rhus coriaria*) powder on laying performance and stored egg quality traits under different storage conditions

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Abstract

Introduction: Natural sources of antioxidants have been gained an intense interest in poultry nutrition studies due to their potential impacts on birds' performance, blood parameters, and immune system (Peschel *et al.*, 2006). These beneficial impacts, which have mainly been addressed to the improvements in the health and function of the gastrointestinal tract and nutrients digestion, are attributed to the bioactive compounds found in herbal plants (Simitzis *et al.*, 2008). Sumac (Rhus coriaria) which belongs to the *Anacardiaceae* family is an herbal plant that contains a vast range of beneficial compounds, including myricetin, phenolic acids, quercetin, L-ascorbic acid, benzoic acid, gallic acid, and other organic acids (Kheiri *et al.*, 2015, Özcan and Haciseferogullari, 2004). Also, Sumac fruit has also been reported to contain high fiber (14.6%, fresh content) (Ozcan and Haciseferogullari, 2004) and fat (18.74% dry matter (DM)) accompanied by low levels of crude protein (4.69% DM) and ash (2.93%) (Raodah *et al.*, 2014). The internal quality of an egg, the most potential, safe, and accessible source of protein worldwide, losses over the storage time. Thus, it is assumed that Sumac fruit powder (SFP) may help to improve egg quality for long storage duration. It has been proven that storing eggs at high ambient temperature reduces their internal quality by accelerating CO2 emission through the eggshell (Chukwuka et al. 2011). Moreover, it has been shown that the CO2 emission increases the albumin pH significantly, which subsequently decreases the internal quality traits of the egg by weakening the protein bonds between lysozyme and ovomucin (Ahn et al. 1999). The effects of non-herbal antioxidants and, in some cases, the inorganic antioxidants, such as selenium, on these parameters have been well studied. However, there are limited studies that have investigated the effects of herbal antioxidants, especially Sumac fruit powder.

Material and Methods: One hundred-twenty laying hens (Hy-line W-36), 53 weeks of age, were distributed in three experimental groups with five replicates, and eight birds per replicate with similar body weights (1394 ± 42g) by employing a completely randomized design. Corn-soybean meal-based diets were formulated according to Hy-line W-36 nutritional management guide (2020) and fed for eight weeks. Experimental diets included a) zero sumac (control), b) 0.25% sumac, and c) 0.5% sumac supplemented treatments. Performance parameters, including egg yield, egg weight, egg mass, feed conversion ratio, and feed intake, were recorded throughout the experiment. Egg quality traits were evaluated in two experiments (the first phase, at the end of the experiment, and the second phase, after being stored in different conditions). In the first experiment, internal and external quality traits, were evaluated after storing the eggs under different conditions. Total phenolic compounds were measured using Gallic acid as the standard by a spectrophotometer (model M501; Unicosh Co, China) in a wavelength of 765 nm light absorbance as described by (Golzadeh *et al.*, 2012). Total phenolic compounds were determined using collected supernatants in the colorimetric method by sodium carbonate (Na2CO3) 20% in Folin-Ciocalteau reagent (Merck Co. Germany) and measuring wavelength absorbance at 720 nm. The results showed that SFP used in the present study contained 45.61 mg/g total phenolic compounds. The data obtained at the present study were statistically analyzed using GLM PROC of SAS software Ver. 9.2 (SAS, 2009). The significance of the differences among means were also determined by Tukey test, where the P-Values below 0.05 were considered as significant. A completely randomized design one, and a 3×2×2 factorial arrangements was used for experiment one and two, respectively.

Results and Discussion: The results indicated that egg production (EP) and egg mass (EM) were decreased in the groups of birds fed with SFP supplemented diets ($P<0.05$), however, feed conversion ratio (FCR) increased significantly ($P<0.05$). These responses were in line with the previous studies where the researchers believed that a monocyclic monoterpene component and polyphenolic compounds found in sumac fruit contents are responsible for low lipid absorption and biliary acid reabsorption from the gut, respectively (Gumus *et al.*, 2018, Ikeda *et al.*, 1992). Evaluating of internal and external quality egg traits in experiment one showed that Haugh unit (HU) and egg density (ED) decreased ($P<0.05$); while, the yolk height (YH), yolk index (YI), yolk pH (YpH), and albumen pH (ApH) significantly increased by SFP supplementation ($P<0.05$). These responses are generally attributed to the impacts of polyphenolic compounds, since previous studies have found a strong relationship between these compounds and the protein bonds of egg white, and therefore, ApH, HU, egg density (ED), YH, etc. (Ahn *et al.*, 1999). These results were in agreement with previous studies (Arpášová *et al.*, 2014). Regarding the internal quality traits of the eggs stored in different conditions, the main effects of SFP supplementation on HU and ApH of the stored eggs were significant (P<0.05 and increased HU and reduced ApH. Moreover, the main effects of storage time and temperature on the internal quality traits of the eggs were significant in all measured parameters, except for YpH ($P<0.05$). A significant interaction was found between the SFP levels and storage time on YI and YH ($P<0.05$). Furthermore, significant interactions were observed between SFP levels and storage temperature on ApH and YpH ($P<0.05$). The storage time and the storage temperature interactions on HU and YpH were also significant ($P<0.05$); briefly, the results indicated that the more storage time and temperature increased, HU unit and YpH decreased. These observations confirmed previous findings (Mirghelenj *et al.*, 2017b) regarding capability of natural sources of antioxidants in preventing internal quality reduction of eggs under different storage conditions; probably by inhibiting lipid peroxidation in the vitelline membrane of yolk, and therefore, pH alteration (Ahn *et al.*, 1999). Finally, no significant interactions were detected among all three independent variables in internal quality traits of the egg.

Conclusion: According to the results obtained in this study, SFP supplementation in laying hens' diet significantly reduced production performance and egg quality traits throughout the experiment; however, it was capable of maintaining the internal quality of the eggs during different storage conditions.

Keywords: Egg quality, herbal plant, laying hen, natural antioxidant, production performance, sumac

Introduction

The egg is a safe and cheap source of protein that is easily accessible in any local market worldwide. However, the quality of this protein source reduces due to different conditions of storage. Therefore, eggs need to be protected against the factors that decrease their internal quality. Several studies have investigated the effects of different antioxidant supplements with properties on quality traits of eggs stored in various conditions (Mirghelenj *et al.*, 2017a, Mirghelenj *et al.*, 2017b, Pappas *et al.*, 2005, Skřivan *et al.*, 2010). The factors affecting on internal quality trait of the eggs, especially during long-term storage, have not been clearly known. However, studies have reported that egg quality reduces due to increased ApH through storing for a long time (Silversides *et al.*, 2001). It has been proven that storing eggs at high ambient temperature reduces their internal quality by accelerating CO_2 emission through the eggshell (Chukwuka *et al.*, 2011). Also, it has been shown that CO_2 emission increases ApH significantly, which subsequently decreases internal quality traits of the egg by weakening the protein bonds between lysozyme and ovomucin (Ahn *et al.*, 1999). The effects of non-herbal antioxidants and, in some cases, the inorganic antioxidants, such as selenium, on these parameters have been well studied (Liu *et al.*, 2020). However, there are limited studies that have investigated the effects of herbal antioxidants (Pappas *et al.*, 2005). In this regard, Mirghelenj *et al.* 2017a have reported that using 4.5% grape pomace (one of the natural antioxidants sources) in laying hens' diet significantly restricted the changes in the ApH and, therefore, HU value during the storage time. This would consequently maintain the internal quality of the eggs. Besides, the same authors reported that 15% tomato pulp supplementation in laying hens, have also similar results on mentioned parameters (Mirghelenj *et al.*, 2017b).

Due to the concerns about using antibiotics, medicinal plants attracted considerable attention and interest as an alternative to antibiotics and synthetic antioxidants (Gurbuz and Ismael, 2016, Hertrampf, 2001). On the other hand, there have been growing demands for natural food preservation agents and natural antioxidants in poultry diets to improve meat and egg quality (Peschel *et al.*, 2006). The beneficial properties of herbal plants, including antioxidant, antiviral, and antibiotic activities, have been proven in previous study (Nouzarian *et al.*, 2011). The beneficial impacts of the bioactive compounds found in herbal plants on the gastrointestinal tract and nutrient digestion have also been reported (Ali *et al.*, 2021, Patra *et al.*, 2019). Therefore, it is assumed that supplementing laying hens' diet with these herbal plants might improve the egg production rate and the internal quality traits of the eggs (Brenes and Roura, 2010, Golzadeh *et al.*, 2012). Previous investigations have reported the antioxidant activity of medicinal plants, such as rosemary, grape pomace, and oregano, due to their phenolic compounds (Simitzis *et al.*, 2008). SFP (*Rhus coriaria*) is also a potential alternative to synthetic antioxidants due to its bioactive compounds. Sumac is a native fruit in subtropical and temperate countries, especially North America, Africa, and Asian countries. This herbal plant is belonged to the *Anacardiaceae* family and contains myricetin, phenolic acids, quercetin, and anthocyanins (Nasar-Abbas *et al.*, 2004). Additionally, it has been shown that sumac fruit is a rich source of L-ascorbic, benzoic, and Gallic acids (Kheiri *et al.*, 2015) and contains organic acids, such as tartaric, malic, and citric acids (Özcan and Haciseferogullari, 2004). Furthermore, in an earlier study, it has been reported that the sumac fruit contains 14.6% (as-fed) crude fiber (Ozcan and Haciseferogullari, 2004), 18.74% (DM) crude fat, 4.69% (DM) protein, and 2.93% (DM) ash (Raodah *et al.*, 2014).

According to the studies, 0.5% SFP supplementation in laying hens' diet increased the egg weight numerically. However, FCR, egg production and feed intake were not affected (Arpášová *et al.*, 2014, Gumus *et al.*, 2018). Moreover, these authors have reported that HU, yolk color (YC), and yolk and albumen index parameters were only affected up to 1% by SFP supplementation in laying hens' diet (Sabir and Aydin, 2017). In the same study, the Japanese quail fed with 0.25% SFP supplemented diet showed increased body weight and egg mass. However, no significant impact was reported in other performance parameters (Sabir and Aydin, 2017). Most studies have reported that mainly blood and egg lipids are affected by SFP supplementation in the laying hens' diet. It has been shown that SFP inclusion in laying hens' diet significantly reduced High-density lipoprotein (HDL), low and very-low-density lipoprotein (LDL and VLDL, respectively), and cholesterol levels in their blood (Gurbuz and Salih, 2017). In the other study, 0.5% SFP supplementation significantly reduced serum and egg yolk lipids, especially cholesterol; however, serum protein increased (Gálik *et al.*, 2014).

Since the albumen and other egg component-producing cells are worn out by aging, their susceptibility to oxidative stress may increase. Though, this probably may result in reducing the internal quality traits of the eggs. Hence, the demand for antioxidants inclusion in aged laying hens' diet is increased. Due to the limited studies on the impacts of SFP on laying hens' diet, the present experiment designated to investigates the effects of SFP supplementation on the production performance and egg quality traits (especially during different storage times and temperatures) in laying hens.

Material and methods

The present study was conducted following all the procedures approved by the Animal Research Ethical Committee of the Animal Science Department of Urmia University under the ethical number of IR-UU-AEC 1344/PD/3 and paying close attention to the birds' welfare. The birds were maintained under precisely controlled environmental conditions in the experimental laying hen house of the Animal Science Department of Urmia University.

Birds and management

A total of 120 Hy-line W36 strain-laying hens (53 weeks of age) were purchased from a local commercial layer farm and assigned into three treatments, five replicates, and eight birds per replicate in a completely randomized design (with similar body weights ±5% and egg production rate ±1.5%).

Table 1- Ingredients and composition of experimental diets

Ingredients (%, as fed)	Basal diet (without SFP)
Corn	56.01
Soybean meal	24.62
Wheat bran	2.5
Soy oil	2.00
Dicalcium phosphate (DCP)	1.99
Oyster shell	11.43
Common salt	0.23
Sodium bicarbonate	0.15
DL-Methionine	0.16
L-Lysine HCl	0.005
Vitamin premix[1]	0.3
Mineral premix[2]	0.3
Calculated nutrient composition	
AMEn (kcal/kg)	2700
Crude protein (%)	16
Calcium (%)	4.4
Available phosphor (%)	0.5
Crude fiber (%)	3.2
Sodium (%)	0.17
Methionine (%)	0.45
Methionine + Cysteine (%)	0.72
Lysine (%)	0.85
Threonine (%)	0.54
Tryptophan (%)	0.19
DEB[3] (mEq/kg)	204

[1]Supplied vitamins per kilogram of diet: A, 10000 IU, D3 2500 IU, E 10 IU, B1 2.2 mg, B2 4 mg, B3 8mg, B6 2 mg, B9 0.56 mg, B12, 0.015 mg, and Choline 200mg.
[2]Supplied minerals per kilogram of diet: Mn, 80 mg, Fe 50 mg, Zn 60 mg, Cu 12 mg, and Sodium Selenite 0.3 mg.
[3]Dietary electrolyte balance

Corn-soybean meal-based diets were formulated according to the recommended layer hens' strain nutritional requirements and fed for eight weeks. Experimental diets included a) zero SFP (control), b) 0.25% SFP, and c) 0.5% SFP supplemented treatments.

A two-week adaptation period was applied before the experiment. During the experiment, performance parameters, including egg numbers and egg weights, were recorded daily, and egg mass, FCR, feed intake, and mortality rate were calculated weekly. The egg quality traits were evaluated in two phases: 1) the internal and external quality traits of the eggs were evaluated in the eighth week of the experiments, and 2) the egg internal quality traits were evaluated after being stored for 7 and 30 days at different storage temperatures. A total of three eggs were taken from each replicate for quality traits meaturments. The eggs were stored at room temperature for 24 hours to evaluate the internal and external quality traits in phase one. To evaluate the internal quality traits in phase two, we collected the eggs at the end of the experiment and stored them for seven and thirty days under 4 and 25°C ambient temperatures. Under precisely controlled conditions, the birds were housed in the experimental farm of laying hens in Urmia University. The birds had free access to water (provided by the nipple drinking system). Each bird was fed with a 100 g experimental diet in total twice daily; in the morning (7:00 am) and at noon (12:00 pm). A lightning program of 14L:10D was set with an intensity of 30 lux for light and a maximum of 5 lux for the dark period. House ambient temperature and moisture were maintained at 20-25°C and 40-50%, respectively.

Sumac fruit powder (SFP)

Sumac (Rhus coriaria) fruit was purchased from a local market in Urmia, Iran, and then was grounded before inclusion in the diets. The total phenolic compound of sumac was measured using Gallic acid as standard by a colorimetric method (McDonald *et al.*, 2001) using a spectrophotometer (model M501; Unicosh Co, China) in a wavelength of 765 nm as described by (Golzadeh *et al.*, 2012). SFP extract was obtained by mixing one gram of SFP with 10 ml methanol in the mortar and then filtering and centrifuging (at 1000×g for five minutes). Total phenolic compounds were determined using collected supernatants in the colorimetric method by sodium carbonate (Na_2CO_3) 20% in Folin-Ciocalteau reagent (Merck Co. Germany) and measuring wavelength absorbance at 720 nm. The results showed that SFP used in the present study contained 45.61 mg/g total phenolic compounds.

Production performance

To evaluate the effects of SFP on production performance parameters, the eggs were collected twice daily, and their number and mean weight were recorded. The egg mass was calculated by multiplying egg production percentage to mean egg weight (both as weekly) using the following equations:

(1) Hen day = number of live hens × days of experiment

(2) Egg production percentage = total egg production of each experimental unit/ hen day of related unit

Moreover, FCR was calculated using the equation mentioned below:

(3) FCR = Feed intake (g)/egg mass (g)

Feed intake was recorded at the end of each week by collecting the remained feed in the feeders. Bodyweight was measured at the beginning and end of the experiment. The mortality rate was determined at the end of the experiment.

Egg quality trait measurements (first experiment)

Three eggs were collected from each replicate and maintained at room temperature for 24 hours to chill. Initially, the eggs were weighed (0.01g; using a digital scale model KEB 60, China). Different amounts of salt were mixed with distilled water according to the Florida University method (Butcher and Miles, 1991) to evaluate the egg density. The eggshell-breaking strength (SHS; kg/cm^2) was measured using an egg force reader (Ogawa Seiki Co. Ltd. Tokyo, Japan). Egg yolk and albumen were weighed to calculate their percentage. Moreover, their height was measured using the Haugh meter (Analog

Baxlo Haugh Micrometer) wherever the Haugh meter ruler tip hit the albumen (1 cm around the yolk) and the top point of the egg yolk. The Haugh unit was calculated according to the following equation (Haugh, 1937):

$$HU = 100 \times \log (AH - 1.7 \times EW^{0.37} + 7.57) \quad (4)$$

$$YI = (YH/YD) \quad (5)$$

The yolk color index was evaluated using a Rosh color scale. Yolk index (YI) was calculated by dividing the yolk height (YH) to its diameter (YD) (Funk, 1948). Yolk and albumen were mixed with distilled water separately in a ratio of 1:9 until the foam was formed. The pH was measured by putting the pH meter (Microcontroller MTT65) sensor in the solutions after the foam had subsided (Funk, 1948). The eggshells were appropriately washed and dried for 12 hours at room temperature and for 72 hours in a 65 °C oven. Then, the eggshells were weighed (0.01g), and the thickness of three sections, including top, middle, and bottom of the eggshell, was measured using an outside micrometer (0.01mm; model YP001, Japan). The average of the three sections was considered as the shell thickness.

Egg quality trait measurements (second experiment)

Three eggs were collected from each replicate in the second experiment to be stored at 4 and 25 °C temperatures for seven and thirty days (12 eggs were collected from each replicate for the second phase). The Haugh unit, yolk and albumen height, and finally, the yolk and albumen pH were evaluated after the storage periods.

Statistical analysis

The data were statistically analyzed using SAS Ver. 9.2 (SAS, 2009), and the Tukey test was performed to determine the significance of the means. The P-values below 0.05 were considered significant, and the following statistical models were used:

For production performance and experiment one:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (6)$$

Y: observation; μ: mean observation; T_i: SFP level; ε_{ij}: experimental error

For experiment two, a 3×2×2 factorial arrangement was used according to the following statistical model:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (BC)_{jk} + (AC)_{ik} + (ABC)_{ijk} + \varepsilon_{ijkl} \quad (7)$$

Y_{ijkl}: The k^{th} observation of the j level of factor B and the i level of factor A in replicate l; A_i: effect of the i^{th} level of factor A (SFP); B_j: effect of the j^{th} level of the factor B (storage temperature); C_k: effect of the k^{th} level of the factor C (storage time); $(AB)_{ij}$: interaction of factor A and B; $(BC)_{jk}$: interaction of factor B and C; $(AC)_{ik}$: interaction of factor A and C; $(ABC)_{ijk}$: interaction of factor A, B, and C; ε_{ijkl}: experimental error with zero mean and variation.

Results

Production performance

The effects of SFP supplementation on the production performance of laying hens are shown in Table 2. As indicated, SFP inclusion in laying hens' diet significantly reduced the EP rate and the EM (P<0.05). However, FCR increased significantly in the birds fed with 0.25 and 0.5% SFP (P<0.05). EW, FI, BWG, and viability parameters of the birds were not affected by SFP supplementation.

Table 2- Effects of different levels of sumac fruit powder supplementation on production performance of laying hens (entire period)

Sumac (%)	Hen day Egg production* (%)	Egg Weight (g)	Egg Mass (g)	Feed Intake (g)	Feed Conversion Ratio (g:g)	Viability (%)
Control	67.19[a]	65.40	43.94[a]	99.00	2.25[b]	97.17
0.25	63.61[b]	64.52	41.04[b]	99.37	2.42[a]	99.02
0.5	63.47[b]	64.68	41.06[b]	99.50	2.42[a]	98.51
SEM	0.696	0.224	0.449	0.297	0.028	0.823
p-value	<0.01	0.074	<0.01	0.493	<0.01	0.349

[a,b] Means within same column with different letters differ significantly (P<0.05).
*Egg production, calculated according to the total egg production of each experimental unit per hen day of related unit. Viability, percentage of the birds remained alive to the end of the experiment.
SEM: standard error of means.

Internal and external quality traits of the eggs (first experiment)

The results obtained in the first experiment regarding the egg quality traits are shown in Table 3. As indicated, the HU and ED significantly reduced by 0.25 and 0.5% SFP supplementation (P<0.05). The YH, YI, and YpH parameters increased significantly only as the effect of 0.5% SFP (P<0.05). However, ApH was significantly increased by both levels of SFP (P<0.05).

Table 3- Effects of different levels of sumac fruit powder supplementation on internal and external quality traits of egg (entire period)

Sumac (%)	YP[1] (%)	AP (%)	SHP (%)	SHT (mm)	HU	YH (mm)	ApH	YpH	ED	SHS (kg/m²)	YC	YI
Control	28.40	62.90	8.68	0.457	86.26[a]	16.45[ab]	7.85[b]	6.81[b]	1.0875[a]	2.01	8.75	36.37[b]
0.25	26.77	64.42	8.79	0.429	77.20[b]	15.08[b]	8.26[a]	6.97[ab]	1.0818[b]	2.36	8.56	34.25[b]
0.5	2.97	64.16	8.85	0.455	78.33[b]	16.95[a]	8.20[a]	7.19[a]	1.0826[b]	2.71	8.12	38.93[a]
SEM	0.649	0.697	0.224	0.012	2.651	0.390	0.079	0.089	0.001	0.390	0.238	0.842
p-value	0.179	0.278	0.865	0.305	0.027	<0.01	<0.01	0.021	<0.01	0.466	0.189	<0.01

[1] Abbreviations, YP, Yolk percentage; AP, Albumen percentage; SHP, Shell percentage; SHT, Shell thickness; HU, Haugh unit; YH, Yolk height; ApH, Albumin pH; YpH, Yolk pH; ED, Egg density; SHS, Shell breaking Strength; YC, Yolk color index; YI, yolk index.
[a,b] Means within same column with different letters differ significantly (P<0.05).
SEM: standard error of means.

Internal quality traits of the eggs (second experiment)

The results of factorial arrangement (3×2×2) to evaluate the main and interaction effects of SFP levels, storage times, and storage temperatures on YI, HU, ApH, YpH, and YH parameters are shown in Tables 4 to 8. As indicated in Table 4, the main effects of SFP levels on HU and YpH were significant, where SFP supplementation increased HU, and decreased ApH (P<0.05). Significant differences were observed as the main effects of storage time on all parameters, except for YpH. In this regard, enhancing the storage time increased the YI, ApH, and YH and decreased the HU (P<0.05). The main effects of storage temperature on all parameters, except for YpH, were significant. As indicated, increasing the storage temperature significantly decreased the YI, HU, and YH and increased the ApH (P<0.05).

Table 4- Main effects of sumac fruit powder supplementation, storage time and storage temperature on the internal quality traits of eggs

P-values	YI[1]	HU	ApH	YpH	YH (mm)
SFP level	0.356	<0.01	<0.01	0.121	0.469
Storage time	<0.01	<0.01	<0.01	0.234	<0.01
Storage temperature	<0.01	<0.01	<0.01	0.625	<0.01
Sumac (%) × storage time (days)	0.041	0.676	0.194	0.585	0.042
Sumac (%) × temperature (°C)	0.865	0.516	<0.01	<0.01	0.834
Storage time (days) × temperature (°C)	0.553	<0.01	0.258	<0.01	0.554
Sumac (%) × storage time (days) × temperature (°C)	0.920	0.129	0.823	0.775	0.921

[1]Abbreviations, YI, Yolk index; HU, Haugh unit; ApH, Albumen pH; YpH, Yolk pH; YH, Yolk height; SFP, sumac fruit powder.
[a,b] Means within same column with different letters differ significantly (P<0.05).
SEM: standard error of means.

The interaction between SFP levels and storage time on the internal quality traits are shown in Table 5. As reported, YI and YH were significantly increased after 30 days of storage in both zero and 0.5% SFP-supplemented groups (P<0.05). No significant interaction was found on the remaining parameters.

The data reported in Table 6 represents the interaction between SFP levels and storage temperature. As indicated, ApH and YpH were significantly affected by both SFP levels and storage temperature, where ApH and YpH had the lowest values at 4°C of storage temperature in the 0.5% SFP supplemented groups (P<0.05).

The interaction effects of storage time and storage temperature on the internal quality traits of the eggs are shown in Table 7. As shown, the HU reduced significantly after storing the eggs for 30 days, where the lowest HU belonged to the eggs stored at 25°C for 30 days and the highest to the eggs stored at 4°C for seven days (P<0.05). Furthermore, increasing the storage temperature had significantly reduced YpH (P<0.05). Regardless of the storage time, the highest YpH was observed in the eggs stored at 4 °C.

Interaction effects of the SFP levels, storage time, and storage temperature on the internal quality traits of eggs are shown in Table 8. No significant interaction was observed among the main factors on the internal quality traits of the eggs.

Discussion

This study investigated the effects of different levels of SFP supplementation on production performance and egg quality traits, especially after storing the eggs for different periods of times (7 and 30 days) and at varying temperatures (4 and 25 °C). Limited studies have examined the effects of SFP powder supplementation on the laying hens' productive performance and egg quality traits, especially for the eggs stored in different conditions. As indicated in Table 2, EP, EM, and FCR were adversely affected by SFP inclusion in laying hens' diet. Previous studies have shown that the SFP contains various bioactive compounds, including phenolic acids, myricetin, quercetin, and anthocyanins (Nasar-Abbas *et al.*, 2004). Moreover, it has been shown that using herbal plants similar to SFP such as field beans (Vicia faba L.) and sorghum (Nyachoti *et al.*, 1997) has also adverse effects on production performance of laying hens, due to their phenolic compounds. Furthermore, it has been reported that protein production and secretion in laying hens' oviduct reduced due to the disruption in protein digestion and absorption processes in the small intestine caused by polyphenolic compounds (Goñí *et al.*, 2007). Moreover, there is a protein in the cell membrane of the enterocytes, called "Niemann-Pick protein C1-Like 1; NPC1L1", that is responsible for cholesterol and biliary lipids absorption and reabsorption. The bioactive compounds found in medicinal plants, such as curcumin in turmeric, could potentially disrupt this protein's function (Altmann *et al.*, 2004). Gumus et al. (2018) noted that reduced total cholesterol level of serum might be due to the hypocholesterolemic effects of the sumac fruit caused by a monocyclic monoterpene component called D-limonene (l-methyl-4-(1-methylethenyl)-cyclohexane). Therefore, it might be one of the probable reasons for the decreased productive performance of laying hens fed with diets containing SFP. Thus, due to the high levels of polyphenolic compounds in the SFP used in this study (45.61 mg/g dry matter) for a relatively long time (eight weeks), the production performance reduced similar to previously mentioned studies. Additionally, studies on the effects of green tea, which contains high polyphenolic compounds (similar to SFP), on production performance showed that the egg production percentage reduced probably due to those compounds, such as flavonoids and catechins (Kanani *et al.*, 2020, Kojima and Yoshida, 2008). The egg yolk consists of high lipids, and therefore, any inhibition of lipid absorption and biliary lipids reabsorption would reduce egg production performance (Ikeda *et al.*, 1992). Thus, it can be suggested that reduced protein production and lipid absorption caused by SFP supplementation is responsible for the decreased production performance of old laying hens and the quality traits of their eggs. Yolk production and its weight are highly dependent on lipid transportation from the liver to the ovary (Sun *et al.*, 2015, Tam *et al.*, 1985). As it has been proven, egg weight is mainly affected by its yolk weight. Therefore, any inhibition in the lipid digestion and absorption processes in the small intestine would reduce yolk weight and thus the egg weight (Ikeda *et al.*, 1992). In the present study, the egg production percentage reduced, and the mean egg weight remained unaffected. Since the egg mass is directly related to both egg production and average egg weight, the egg mass was expected to reduce due to SFP supplementation and a reduction in egg production percentage. Thus, it could also affect the FCR that is a parameter directly related to EM. As feed intake remained unaffected, a reduction in EM would increase FCR. Previous studies have reported no significant impacts of SFP supplementation on laying performance (Arpášová *et al.*, 2014, Gumus *et al.*, 2018). Hence, the findings of the present study were not completely in line with the previous ones. According to our previous study (Mosayyeb Zadeh *et al.*, 2021), sumac supplementation could significantly reduce serum lipids and yolk triglyceride contents. Therefore, the reduced production performance might be potentially addressed to decreased serum lipids, and probably low steroid sex hormones in the circulating system. Since the VLDL, especially VLDLy, is responsible for lipid transportation from the liver to the ovary (Montorzi *et al.*, 1995, Ratna *et al.*, 2016), thus, low lipid transportation (due to low VLDL) for yolk production in the ovary might be another probable reason for the reduction in egg production percentage of SFP supplemented birds.

Regarding the internal and external quality evaluations of the eggs, yolk-related parameters were mainly affected by SFP supplementation, probably due to the same reasons mentioned above. As stated, inhibition of lipid absorption might cause a reduction in yolk weight and height, but it is still not clear how the SFP or its bioactive compounds could alter yolk pH.

It has been noted that the protein bonds between ovomucin and lysozyme weaken as the albumen pH increases (Ahn *et al.*, 1999). Thus, it might affect the albumen height and subsequently the Haugh unit (Ahn *et al.*, 1999). The Haugh unit is a parameter directly related to albumen height, and the albumen itself is directly related to the egg's white thick protein production. Therefore, the low Haugh unit might be caused by increased pH and reduced egg's white thick protein secretion in the laying hens' oviduct probably due to the polyphenolic compounds of SFP. As indicated, both Haugh unit and egg density decreased in this study, while the ApH increased. Thus, it can be posited that the Haugh unit reduction due to low thick protein secretion in the oviduct might be one of the probable reasons for the decreased egg density. These findings are in agreement with a previous study (Arpášová *et al.*, 2014). It has been well documented that the internal quality of the eggs reduces with increasing the time and temperature of the storage (Pappas *et al.*, 2005). There are promising reports on the effects of the natural antioxidants, especially those from the herbal plant, on the internal quality of the eggs stored at different temperatures and duration times. However, there is almost no available study investigated the effects of SFP supplementation in laying hens' diet on the internal quality of the eggs stored under different conditions. Therefore, the studies on the effects of the polyphenolic compounds of other herbal plants on the internal quality of the eggs stored under different conditions were used to compare the present results. Previously, (Mirghelenj *et al.*, 2017a, Mirghelenj *et al.*, 2017b) investigated the effects of dried tomato and grape pomaces on the internal quality of the eggs stored in different conditions. The researchers stated that 15% of the dried tomato pomace and 4.5% of the grape pomace could significantly maintain the internal quality traits of the eggs by inhibiting the ApH increment, which subsequently reduced the HU. Those findings are in complete agreement with the results obtained in the present study. The exact mechanisms behind the factors affecting the albumen height have not been clearly recognized yet. Nonetheless, previous study have indicated that the internal quality of the eggs reduces by increasing the albumen pH and storage duration time (Silversides *et al.*, 2001). Ahn et al. (Ahn *et al.*, 1999) believe that one of the probable reasons for increased ApH and subsequent decreased HU might be the oxidation of the lipids in the vitelline membrane of egg yolk, which results in ion exchanges between yolk and albumen and thus weakens the protein bonds between ovomucin and lysozyme by pH alteration. Other study has pointed out the effects of the storage time and temperature on ovomucin proteolysis and weakening the disulfide bonds in albumen protein and then suggested that 4.5% grape pomace could potentially maintain the egg quality traits (Mirghelenj *et al.*, 2017a). The results of the present study showed that supplementing the laying hens' diet with the different levels of SFP significantly improved the internal quality traits of the eggs stored in different conditions. It is assumed that the increased Haugh unit due to the antioxidant properties of the polyphenolic compounds in SFP was responsible for inhibiting the vitelline membrane oxidation and maintaining the internal quality traits of the eggs. The main effects of the SFP supplementation were not significant on YI, YpH, and YH. Furthermore, the results showed that increasing the storage time and temperature significantly impaired internal quality parameters, and using both levels of SFP supplementation potentially inhibited severe quality reductions, which might be due to its antioxidant property.

Conclusion

In conclusion, even though SFP supplementation reduced production performance and egg quality traits, it potentially inhibited the reduction of the quality of the eggs stored at high environmental temperatures for a long time. It is assumed that the antioxidant properties of the polyphenolic compounds found in SFP are responsible for these impacts, which prevented the oxidation of the yolk vitelline membrane lipids and then the Haugh unit reduction. In addition, these phenolic compounds probably had lowered the levels of blood total lipids and the lipid content of the egg, which might reduce the overall lipid peroxidation in laying hens. Thus, we recommended using 0.5% SFP supplementation in laying hens' diet to maintain the internal egg quality for a long time.

Conflict of interest

No potential conflict of interest relevant to this article was reported

Data availability

The data supporting this study's findings are available from the corresponding author, Seyyed Ali Mirghelenj, upon reasonable request.

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اثرات استفاده از سطوح مختلف پودر سماق در جیره مرغ‌های تخمگذار بر عملکرد تولید و صفات کیفی تخم مرغ تحت شرایط مختلف نگهداری

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چکیده

زمینه مطالعاتی: اثرات سطوح مختلف جیره‌ای پودر سماق بر عملکرد تولیدی و صفات کیفی تخم مرغ‌ها در مرغ‌های تخمگذار مُسن مورد بررسی قرار گرفت. **هدف:** هدف از مطالعه حاضر ارزیابی اثرات سطوح جیره‌ای مختلف پودر سماق در جیره بر عملکرد تولیدی و صفات کیفی تخم مرغ‌ها تحت شرایط مختلف نگهداری بود. **روش کار:** تعداد ۱۲۰ قطعه مرغ تخمگذار سویه Hy-line W36 با سن ۵۳ هفتگی و وزن مشابه (گرم ۴۲ ± ۱۳۹۵) بین سه تیمار، پنج تکرار و ۸ پرنده در هر تکرار بصورت کاملاً تصادفی توزیع شدند. تیمارهای آزمایشی شامل: ۱) تیمار شاهد (بدون سماق)، ۲) ۰/۲۵ درصد و ۳) ۰/۵ درصد پودر سماق در جیره بودند. **نتایج:** نتایج بدست آمده در مطالعه حاضر نشان داد که درصد تولید و توده تخم مرغ تولیدی با افزودن هر دو سطح پود سماق به جیره کاهش یافت (P<۰/۰۵). در حالیکه، ضریب تبدیل خوراک در تمام دوره‌های آزمایشی بطور معنی‌داری افزایش یافت (P<۰/۰۵). ارزیابی خصوصیات کیفی تخم مرغ‌ها در انتهای دوره آزمایش نشان داد که در تخم مرغ‌های تولید شده توسط پرندگان تغذیه شده با هر دو سطح سماق واحد هاو و چگالی تخم مرغ‌ها کاهش یافته (P<۰/۰۵) و در مقابل، ارتفاع زرده، شاخص زرده، pH زرده و pH سفیده بطور قابل توجهی افزایش یافت (P<۰/۰۵). هیچ اثر متقابل معنی‌داری بین سطح سماق، زمان و دمای نگهداری مشاهده نشد. **نتیجه‌گیری کلی:** افزودن پودر سماق به جیره خوراکی مرغ‌های تخمگذار موجب کاهش در عملکرد تولید و صفات کیفی داخلی و خارجی تخم مرغ شد، در حالیکه کیفیت داخلی تخم مرغ‌ها طی دوره نگهداری در شرایط محیطی مختلف به خوبی حفظ شد.

واژگان کلیدی: آنتی اکسیدان طبیعی، سماق، عملکرد تولید، کیفیت تخم مرغ، مرغ تخمگذار.