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Original article

Changes in quality characteristics during storage time of eggs from layer hens fed diet supplemented with *Panax ginseng* Meyer leaf extract

Arda Yıldırım

Department of Animal Science, Faculty of Agriculture, Gaziosmanpaşa University, Tokat, Turkey - E-mail: arda.yildirim@gop.edu.tr

Summary. The objective of this study was to evaluate the changes in quality characteristics during storage time (ST) of eggs from layer hens fed diet supplemented with *Panax ginseng* Meyer leaf extract (PGLE). Four groups of commercial hens (Atak-S; Turkish native hybrid) were fed with diets containing 0 (control), 50, 100 and 150 mg PGLE/kg for 12 wk. A total of 128 eggs were obtained from hens, which were collected 2 times during the peak production (wk 31 to 32). The eggs were stored after being laid and subjected to storage of 1, 14 and 28 d at 10°C inside a refrigerator. The egg weight, shape index, shell strength, shell thickness and egg surface area were not influenced by dietary PGLE levels at different storage times. In an overall storage period, dietary PGLE addition to diet significantly decreased the specific gravity and shell weight. Likewise the effect of different levels PGLE on albumen index at 14 d, albumen pH at 14 and 28 d, haugh unit at 14 d was statistically significant, but this effect was not reflected in the overall ST. The use of dietary PGLE in laying hen diets had no a beneficial effect on the external and internal egg quality during ST.

Key words: *Panax ginseng* Meyer leaf extract, egg quality, storage time, laying hen, Atak-S

Introduction

Egg external and internal quality traits are the major important in the egg industry worldwide. These traits are influenced by many factors such as inherent characteristics of the bird strain and long- or short-term environmental conditions until (housing system, feeding systems and level, feed additives etc.) or immediately before consumption and at the time of purchase (egg handling and storage practices etc.) or ST and conditions of eggs until reaching consumers (1-3). Especially egg external and internal qualities traits can be affected negatively subject to increase in the storage times of eggs due to loss of moisture and CO_2 through the eggshell pores (4, 5). Eggs should stay fresh if marketed for not longer than 28 days after laying (6), and this period is shown on a package of eggs as the date of minimum durability (7).

External factors including cleanliness, freshness, egg weight and shell weight are important in consumer's acceptability of shell eggs (8, 9). The management and nutrition of the hen do play a role in internal egg quality, egg handling and storage practices; also have a significant impact on the quality of the egg reaching the consumer (10, 11). On the other hand, interior characteristics such as yolk index, Haugh unit, and chemical composition are also important in egg product industry as the demand for liquid egg, frozen egg, egg powder and yolk oil increases (9, 12).

P. ginseng Meyer (Araliaceae), also called Asian ginseng, is one of the most renowned herbal plants worldwide, but particularly in Asian countries, and has been used for thousands of years to maintain homeostasis of the body and enhance vital energy (13, 14). It is considered an adaptogenic agent that helps to enhance physical performance, promote vitality and stimulate metabolic function. It has previously been documented that bioactive components such as saponins (ginsenosides), antioxidants, peptides, polysaccharides, alkaloids, lignans and polyacetylenes are present in *P. ginseng* (15, 16). Among these, ginseng saponins (ginsenosides) are considered the principal bioactive ingredients in the total extracts of ginseng and over 30 ginsenosides have been identified in *P.ginseng* (15, 17-19).

Although the pharmaceutical effects of *P. ginseng* on physical, chemical, and biological stress (20, 21), systemic immune function (22) and glucose metabolism (23) are presented in animals, there is no scientific information on the quality traits of eggs from layer hens fed diet supplemented with PGLE. On the other hand, it has been known well having biologic activity of plant extracts (24, 25). Therefore, due to its antioxidant and antibacterial activity in laying hens (14, 23, 26, 27), dietary PGLE may improve the external and internal egg quality traits at the different storage times. The present study settled to use laying hens' egg to test this hypothesis since its quality characteristics is well known under experimental conditions. Therefore, the aim of the present study was to look whether different ST of eggs from layer hens fed diet supplemented with PGLE have influence on quality characteristics, so as to provide scientific evidence for the medicinal plant extract.

Materials and Methods

Birds and experimental design

Eighty commercial Atak-S brown layers at the age of 20 weeks were randomly allotted to four experimental treatments with four replicates of five hens each group in a completely randomized design. The five birds per replicate were kept in standard battery cages (450 cm^2 per bird) in a windowed poultry house. A 16 h light and 8 h dark lighting program was applied during the experiment. Water and experimental diets were supplied *ad libitum* consumption.

Diets and dietary analysis

The experimental diets were isonitrogenous and isoenergetic and were formulated to meet or slightly exceed the nutrient requirement of laying hens according to the NRC (28). Dry matter (method 930.15), crude protein (method 954.01), crude fibre (962.09), ether extract (960.39) and ash (942.05) of dietary ingredients were determined according to AOAC (29). The ingredients and calculated nutrient level of the basal diet are given in Table 1.

Panax ginseng Meyer leaf extract

The treatments were a basal diet as control and three levels of supplementation, 50, 100 and 150 mg/kg

Table 1. Composition and nutrient content of basal diet (g/kg)

Ingredients	20 - 32 weeks	Calculated nutrients composition	
Maize	294	ME (MJ/kg)	11.7
Wheat	277	Crude protein	172.0
Soybean meal (47% CP)	92	Lysine	8.0
Barley	30	Methionine	4.5
Sunflower meal (36% CP)	80	Methionine+cystine	7.6
Full-fat soybean	100	Calcium	36.0
Soybean oil	24	Available phosphorus	4.0
Sodium bicarbonate	1	Analyzed nutrients composition	
Dicalcium phosphate	14	Dry matter	898.4
Marble powder	83	Crude protein	168.4
Salt	2	Crude fibre	67
Vitamin–mineral premix[1)]	3	Ether extract	36
		Ash	136

[1)] Each kg of vitamin–mineral premix contained: 4800000 IU vitamin A; 1200000 IU vitamin D_3; 12000000 IU vitamin E; 1600 mg vitamin K_3; 1200 mg vitamin B_1; 2400 mg vitamin B_2; 12000 mg vitamin B_3; 4000 mg vitamin B_5; 2000 mg vitamin B_6; 20000 mg vitamin C; 6 mg vitamin B_{12}; biotin; 400 mg folic acid; 120000 mg choline; 2000 mg Cu; 24000 mg Fe; 32000 mg Mn; 60 mg Se; 24000 mg Zn; 200 mg Co; 800 mg I.

diet of a commercial PGLE (Batch No: GRE–110508, Dried, 100% Natural), supplied by Changsha Herbway Biotech Co. Ltd. (China). The PGLE contained 80.7% ginsenosides, as determined by Changsha Herbway Biotech UV using spectrophotomeric analysis.

Ethics statement

This study was approved by the Committee on the Ethics of Animal Experiments of Gaziosmanpaşa University (Process no. 309/2011 HADYEK–041). Animals in this experiment were kept under the guidelines stated in the Guide for the Care and Use of Atak-S brown hybrid laying hens in the Poultry Research Institute, Ankara, Turkey. Prior to the experiment, the birds were given a two-week adaptation period.

Egg collection

A total of 128 eggs were collected (32/diet, 8 eggs/replicate) during the peak production period (wk 31 to 32) and were stored. The samples of 32 eggs each were stored for 1, 14 and 28 d at 10 °C in chambers in a refrigerator. Relative humidity was regulated at 55 to 65% for all treatments. Length of storage times was selected to simulate likely duration of consumer storage of eggs.

Egg quality analysis

On d 1, 14 and 28 of storage, 2 eggs were measured from each replicate, totaling 8 eggs/ treatment for external and internal egg quality. The egg quality traits included specific gravity, eggshell breaking strength, shell thickness and albumen pH. All eggs were weighed individually. Egg specific gravities were determined from graded salt solutions ranging from 1.069 to 1.099 with gradations of 0.003, as described by Hamilton (30). After that, shell breaking strength was measured using a shell strength device with a spiral pressure system (Fujihara, Saitama, Japan). Subsequently, the egg was broken on a glass plate with a waiting period of 5 min to measure the albumen and yolk heights using a tripod micrometer, the long and short diameters of albumen, and diameter of yolk using the digital calliper with a sensitivity of 0.001 mm. The formulas of some egg quality traits are presented in Table 2. Shell thickness was measured as an average of three measurements taken at the equator, blunt edge and pointed edge of the egg without membrane using the calliper. The pH values of the albumen were measured using a digital pH meter (Sartorious PP15, AG Weender Landstrasse 94–108, Goettingen, Germany).

Statistical analysis

Variance analysis of the data was performed using the GLM procedure of the SPSS (35) application package. The statistical model considered the effect of PGLE and ST and the interaction of PGLE and ST (PGLE × ST). The averages estimated were compared using Duncan's multiple–range tests. Data in the tables are presented as means ± SE (standard error).

Results and discussion

In the current experiment, external and internal quality characteristics of eggs during storage times are

Table 2. Formulas for measurement of egg quality traits

Criteria	Equation
[1] Shape index, %	(Egg width /Egg length) x 100
[1] Yolk index, %	(Yolk height/yolk diameter) x 100
[1] Albumen index, %	[Albumen height/(long diameter of albumen+short diameter of albumen /2)×100]
[2] Egg surface area (cm^2)	$3.9782 \times$ egg weight$^{0.70}$
[3] Shell weight (g)	(2.0341 x Egg weight) - [(2.1014 x Egg weight)/Specific gravity]
[4] Haugh unit (score)	100 log (Albumen height+7.57-1.7 $W^{0.37}$)

[1] Doyon et al [31], [2] Nordstrom and Ousterhout [32], [3] Harms et al [33], [4] Roush [34]

presented in Table 3 and 4. Dietary treatments had no effect on all parameters of external egg quality for 1, 14 and 28 d of storage at 10 °C (*p*> 0.05). Dietary PGLE at 100 mg/kg indicated that there was significant difference on overall shell weight value compared to control group in storage egg. There are no literature data relative to the effect dietary PGLE on egg stored of laying hens. Our previous report (36) pointed out that supplementation of laying hen (Atak-S) diet with PGLE at level of 50, 100 and 150 mg/kg did not affect body weight, feed intake, feed efficiency and egg production performance, but increased egg weight. As indicated in Table 3, there were no differences (*p*> 0.05) in egg weight, shape index, shell strength, shell thickness and egg surface area between the overall storage times. This result is in agreement with those found by Samli et al (4), Akyurek and Okur (37), Jin et al (1), Okur and Şamlı (38) indicating that egg weight was not affected by storage times in the egg of hens. Conversely, Scott and Silversides (39), Englmaierová and Tůmová (40), Raji et al (41) and Oliveira et al (42) claimed that the egg weight and surface area were adversely affected by storage times. Several authors found similar values between storage times in terms

Table 3. External egg quality traits at storage time of eggs from layer hens fed diet supplemented with *Panax ginseng* Meyer leaf extract (mean±SE)

PGLE	ST	EW	SI	SG	SS	STh	SW	ESA
0	1	61.6±1.27	76.7±1.10	1.088±0.001	2.29±0.332	361.6±8.522	6.33±0.187	71.14±1.018
	14	60.1±1.05	76.5±0.80	1.082±0.002	2.24±0.321	360.4±8.624	5.52±0.228	69.92±0.847
	28	58.4±1.24	77.6±1.09	1.072±0.001	2.42±0.283	351.3±8.543	4.26±0.140	68.56±1.017
50	1	60.1±1.40	77.9±0.63	1.085±0.002	2.75±0.278	349.2±6.688	5.87±0.263	69.93±1.146
	14	60.6±1.26	76.5±0.78	1.081±0.001	2.09±0.356	357.5±7.526	5.43±0.211	70.37±1.023
	28	60.0±0.74	76.0±0.97	1.071±0.001	2.14±0.301	350.8±6.164	4.26±0.114	69.88±0.600
100	1	59.8±0.77	76.5±1.00	1.084±0.001	2.15±0.277	342.9±5.720	5.70±0.130	69.67±0.631
	14	61.1±1.49	77.1±0.75	1.081±0.001	2.49±0.159	359.2±4.532	5.54±0.135	70.73±1.205
	28	55.9±1.36	77.1±1.25	1.070±0.001	2.07±0.251	348.3±5.670	3.92±0.119	66.45±1.137
150	1	59.0±1.46	78.0±0.58	1.089±0.002	2.20±0.232	357.5±9.815	6.12±0.273	69.06±1.199
	14	58.9±0.57	74.5±0.99	1.081±0.001	2.40±0.444	352.1±7.097	5.30±0.124	68.99±0.468
	28	61.3±2.05	76.1±1.21	1.071±0.001	2.16±0.241	352.9±5.720	4.39±0.173	70.89±1.661
PGLE								
0		59.9±0.58	76.8±0.50	1.080±0.001	2.46±0.152	360.2±4.016	5.34±0.159 [a]	69.76±0.472
50		60.1±0.60	76.5±0.45	1.078±0.001	2.29±0.152	354.0±3.563	5.13±0.145 [ab]	69.93±0.486
100		58.9±0.67	76.8±0.47	1.078±0.001	2.28±0.114	353.5±3.216	4.95±0.149 [b]	68.94±0.550
150		60.6±0.78	76.5±0.48	1.079±0.001	2.34±0.163	357.1±3.985	5.22±0.152 [ab]	70.35±0.623
ST								
1		60.1±0.62	77.3±0.43	1.086±0.001 [a]	2.34±0.139	352.8±3.951	6.00±0.113 [a]	69.95±0.505
14		60.2±0.56	76.2±0.43	1.081±0.001 [b]	2.31±0.162	357.3±3.424	5.45±0.087 [b]	70.00±0.456
28		58.9±0.77	76.7±0.55	1.071±0.001 [c]	2.19±0.130	350.8±3.164	4.21±0.073 [c]	68.94±0.630
Main effects								
PGLE		ns	ns	ns	ns	ns	*	ns
ST		ns	ns	**	ns	ns	**	ns
PGLE×SD		ns	ns	ns	ns	ns	ns	ns

PGLE: Panax ginseng Meyer leaf extract (mg/kg), ST: Storage time (day), EW: Egg weight, SI: Shape index, SG: Specific gravity (g/cm³), SS: Shell strength (kg/cm²), STh: Shell thickness (µm), SW: Shell weight, ESA: Egg surface area (cm²).
*a,b,..: Means lacking a common letter within each column differ significantly at p < 0.05. ns: p>0.05, *: p< 0.05, **: p< 0.05.*

Table 4. Internal egg quality traits at storage time of eggs from layer hens fed diet supplemented with *Panax ginseng* meyer leaf extract (mean±SE)

PGLE	ST	YI	AI	ApH	HU
0	1	45.14±0.791	11.68±0.672	8.80±0.062	93.8±1.94
	14	44.91±0.848	9.72±0.435 a	9.20±0.017 c	89.3±1.50 a
	28	40.76±0.643	7.91±0.379	9.12±0.015 bc	84.5±1.64
50	1	44.81±1.022	11.85±0.901	8.83±0.056	95.2±2.60
	14	43.99±0.849	7.74±0.443 b	9.23±0.020 bc	82.8±1.88 b
	28	42.42±1.268	8.13±0.834	9.23±0.019 a	85.3±3.38
100	1	45.01±0.698	10.38±0.660	8.80±0.030	90.6±2.07
	14	43.58±0.527	9.23±0.506 a	9.28±0.011 a	88.2±1.91 a
	28	42.19±0.619	7.64±0.610	9.16±0.020 b	82.4±2.84
150	1	45.33±1.186	11.19±0.524	8.83±0.033	93.3±1.77
	14	43.34±0.895	9.28±0.477 a	9.25±0.016 ab	89.5±1.62 a
	28	43.31±0.957	7.62±0.473	9.09±0.027 c	81.2±2.49
PGLE					
0		42.78±0.592	9.27±0.367	9.09±0.034	87.5±1.16
50		43.76±0.496	9.01±0.467	9.10±0.034	87.2±1.54
100		43.75±0.385	8.75±0.340	9.10±0.035	85.8±1.33
150		43.33±0.517	8.86±0.357	9.08±0.031	85.8±1.38
ST					
1		45.07±0.449 a	11.28±0.349 a	8.82±0.023 c	93.2±1.05 a
14		43.95±0.392 a	8.99±0.259 b	9.24±0.009 a	87.4±0.96 b
28		42.17±0.463 b	7.83±0.287 c	9.15±0.014 b	83.3±1.30 c
Main effects					
PGLE		ns	ns	ns	ns
ST		**	**	**	**
PGLE×SD		ns	*	**	*

*PGLE: Panax ginseng Meyer leaf extract (mg/kg), ST: Storage time (day), YI: Yolk index, AI: Albumen index, ApH: Albumen pH, HU: Haugh Unit (score), a,b,..: Means lacking a common letter within each column differ significantly at p < 0.05. ns: p>0.05, *: p< 0.05, **: p< 0.05.*

of shell thickness value, as was observed in the present study (4, 38, 40). Moreover, there were significant (p< 0.01) changes in specific gravity and shell weight between overall the storage times, which is in agreement with Samli et al (4), Jin et al (1), Englmaierová and Tůmová (40) who examined the storage times in laying hens. On the other hand, most recently Okur and Şamlı (38), who have found that shell weight does not change with storage.

It can be seen from the data in Table 4 that there was a significant effect of treatment groups supplemented with PGLE on albumen index at 14 d, albumen pH at 14, 28 d and haugh unit at 14 d of storage (p< 0.05). Surprisingly, yolk index values in 50 and 100 mg PGLE/kg groups were higher than in the control group. This effect was not reflected in the same way in the overall yolk index. Albumen index value in 50 mg PGLE/kg group was lower than the other groups at 14 d of storage.

Deteriorations in albumen pH were clearly shown in treatment and control group at 14 and 28 d of storage, respectively. This finding implies that reductions in egg quality occurred by supplementing PRGE to hen diets in a nonlinear manner. Surely, haugh unit value of 50 mg PGLE/kg significantly ($p< 0.05$) decreased with compared to control group. However, the parameters of overall internal egg quality did not affect by dietary supplementation of PGLE. It suggests that the activity of PGLE may be varied in the other species of laying hens or different laying period in Atak-S laying hens. No recent data were found regarding the study in quality characteristics during ST of eggs from layer hens fed diet supplemented with *P. ginseng*.

The data from the current study indicate that eggs from laying hens at peak production can have significant reductions of internal quality with increased overall ST ($p< 0.01$). Notably, dramatic reductions in yolk and albumen quality were become clear as from 14 d of ST. Kirunda and McKee (43) and Jones et al (44) stated that eggs which have been stored for a period of time therefore exhibit higher albumen and yolk pH, a lower haugh unit, a lower yolk index, a decreased albumen viscosity and have a larger air cell. In general, haugh unit, albumen index and yolk index declined and albumen pH increased during storage as expected. Water loss from the egg or movement of water from albumen to the yolk is the most likely reason this finding. Freshness is a major contribution to the egg quality. The internal quality of eggs begins to deteriorate after they have been laid due to loss of moisture and carbon dioxide via the eggshell pores. Refrigeration is very effective in preserving egg quality (5). These results are in agreement with many researchers who reported similar decreases of albumen index (37, 40), yolk index (4, 41) and haugh unit (1, 42) as ST increased.

An increase in albumen pH along ST has been reported by Jin et al (1), Samli et al (4), Scott and Silversides (39), Englmaierová and Tůmová (40) and Oliveira et al (42). According to our results, the fact is that the albumen pH and albumen quality changed with major differences as increasing ST. The gelatinous structure of the thick albumen gradually deteriorates during storage and making it thinner (5). The decrease in haugh unit may suggest an increase in albumen pH as a result of a loss of carbon dioxide via the eggshell pores (45). Based on haugh unit, albumen quality of eggs can be classified into 3 grades: AA (firm) = >72 haugh unit; A (reasonably firm) = 71 to 60 haugh unit; B (weak and watery) = <60 haugh unit (46). In this study, overall haugh unit in ST is ranged from 93.2 to 83.3 and egg grade was classified as AA (firm) when haugh unit was >72.

In conclusion, this experiment shows that the majority of parameters of egg quality were negatively influenced by the ST. PGLE supplementation could not be improved the decrease of quality during storage and PGLE in the layer diets should therefore not be used in practice.

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Correspondence:
Arda Yıldırım
Department of Animal Science, Faculty of
Agriculture, Gaziosmanpaşa University, Tokat, Turkey
E-mail: arda.yildirim@gop.edu.tr