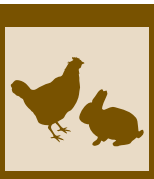
Effect of dietary supplementation of Panax ginseng leaf extract on production performance and egg quality of hens at the beginning of their laying period



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SUMMARY

A study was conducted to determine the effect of ginseng (*Panax ginseng* C.A. Meyer) leaf on egg production and egg quality characteristics of hens at the beginning of their laying period. Eighty commercial Atak-S brown layers at the age of 20 weeks were randomly allocated to one of four treatments with four replicates of five hens per treatment in a completely randomized design. The birds were fed standard layer diets control (0 mg/kg), 50 mg/kg, 100 mg/kg or 150 mg/kg Panax ginseng leaf extract (PGLE) for 12 weeks period. Laying performance was assessed by recording egg production, egg weight daily; feed intake and feed efficiency weekly and egg quality biweekly. Statistical analysis of the research results proved influence of PGLE supplementation on the weight of eggs (P <0.05), but they did not have any influence on body weight, feed intake and feed efficiency (P >0.05). Overall, there were no differences among groups in external egg quality parameters (P >0.05), while significant increases were observed in albumen and yolk index of internal egg quality. The trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and total phenolics concentration of PGLE were 606.3±0.948 mmol trolox/kg, 15.99±0.232 mmol TEAC/kg and 1.11±0.02 g gallic acid equivalents (GAE)/kg, respectively. As a result of research, it could be considered that up to 150 mg PGLE/kg in a laying hen's diet did not change the egg production performance of layers and there is only a positive effect on egg weight and eggshell redness increase which could contribute to profitability.

KEY WORDS

Atak-S, egg production, egg quality, laying hen, Panax ginseng.

INTRODUCTION

As a result of the prohibition of the use of antibiotics, a major problem has arisen in the egg industry in order to achieve effective production from healthy flocks such as improving the growth, feed utilization in poultry and reducing mortality[1]. Thus, alternative feed additives can be added to rations to improve poultry health, production performance and egg quality[2,3]. For this purpose, feed additives, which are used to increase yield or support animal health, should not have negative effects on both animal and consumer health. Indeed, the most suitable additives are natural additives derived from aromatic plants. Thus, herbal extracts, mixtures and essential oils are considered as alternative natural products and studies have been investigated on animal product performance and quality[4,5].

There are many bioactive compounds in the structures of plants. Essential oils derived from medicinal and aromatic plants give characteristics to plants which have antioxidant, antimicrobial, digestive and appetite enhancing properties. Their use in poultry compound feeds has recently become widespread due to their positive effects on performance in various animal species. But in practical terms, the use of herbal extracts is very limited in the feeding of modern-day chickens. There are many medicinal and aromatic plants and herbal extracts that may be an alternative to antibiotics as a natural growth promoter and one of them is the ginseng plant and its extract[6,7]. Ginseng has been used in China, Korea and Japan for about 2000 years and its usage area has spread all over the world in recent years. It is a plant belonging to the family of Araliaceae[8,9]. Saponin glycosides (ginsenosides) are the most important phytochemicals of ginseng and more than 60 ginsenosides were isolated in various ginseng species. Apart from these, essential oils, sterols, flavonoids, polysaccharides, polyacetylenes, vitamins (B and D group), enzymes and minerals are other active substances isolated[10,11]. The pharmacological effects of ginseng have been reported in the central nervous, endocrine, immune and cardiovascular system[12,13]. In addition, Panax ginseng is widely used as a physical, chemical and biological resistance enhancer[14], regulating blood lipid and decreasing blood sugar levels[15], cancer prevention[16], liver, kidney and heart protection and immunostimulatory effects[17].

Digestion in poultry is completed in a shorter period due to the high metabolic activity and short passage times of nutrients from the digestive system. Thus, ginseng may have many advantages, including the bioactive compounds contained in it, which could improve digestion effects in poultry. Panax gin-

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seng extract could be used as a natural feed additive in poultry feed due to the phenolic compounds contained in it because of its strong antioxidant, anti-radical, antimicrobial, mineral and high vitamin content[7].

There are many researches about botanical, chemical, biological, ecological characteristics and cultivation of ginseng in the world with its usability in animal husbandry, food and pharmaceutical industry. However, there are not enough studies about the use of Panax ginseng leaf extract in the diets of laying hens. The present research was conducted to determine the effects of supplementing graded levels of a commercial PGLE on egg production performance, egg quality and some blood serum biochemical parameters in laying hens at 20 weeks of age.

MATERIALS AND METHODS

Ethics statement and Location

This study was approved by the Animal Experimentation Ethics Committee (Process no. 309/2011 HADYEK-041) of Tokat Gaziosmanpa a University. Animals in this experiment were cared 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. The study was carried out at the research and application farm poultry unit of Tokat Gaziosmanpa a University, Turkey, situated at 40°19'51.95" N, 36°28'31.23" E and 856 m above sea level.

Animals and housing conditions

The eighty commercial Atak-S brown (Turkish native hybrid) layers at the age of 20 weeks were randomly allocated to one of four treatments with four replicates of five hens in each group. The current experiment was conducted with completely randomized design. The five birds per replicate were kept in a wire cages (450 cm^2 per bird) in a windowed poultry house at a light regimen of 16 h light and 8 h dark. Prior to the experiment, the birds were given a two week adaptation period before the trial began and the trial lasted 12 weeks. The treatments were a basal diet as control and three levels of supplementation, 50, 100 and 150 mg/kg 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. Laying hens had ad libitum access to water and diets in a mash form throughout the experimental period. The composition of feeds was analysed in the laboratory according to AOAC[18] procedures. The experimental basal diets were isonitrogenous and isoenergetic and were formulated to meet or slightly exceed the nutrient requirement of laying hens according to the NRC[19]. The ingredients and calculated nutrient level of the basal diet are shown in Table 1.

Three portable temperature and relative humidity loggers (HOBO U12-012 T/RH logger, temperature measuring range between -20 °C and 70 °C with ±0.35 °C accuracy; relative humidity range of 5%-95% with ±2.5% accuracy) were used to monitor indoor temperature and relative humidity at animal level. Data were recorded every 60 min and downloaded weekly throughout the experiment. The average temperature, relative humidity and light intensity of the entire experimental period were 15.74±0.129 °C, 48.15±6.410% and 14.76±3.828% lux, respectively.

Egg production performance and egg quality characteristics

Hens were weighed individually at the beginning and the end of the experiment. Feed consumption was recorded weekly and calculated as g per hen per day. Viability was observed visually and recorded daily throughout the entire experimental period. The value of feed efficiency was calculated as g feed per g egg. The first egg was laid within 23 weeks of age in all the groups. Eggs from each replicate were collected twice a day (09:00 - 11:00 and 14:00 - 16:00) and weighed with an electronic balance at the same time every day to calculate hen-day egg production ((Number egg produced / number live hens) x 100; HDP), hen-house production ((Number egg produced / number live hens in initial experiment) x 100; HHP), egg weight

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

Ingredients		Analysed nutrient composition	
Maize	294	Dry matter	898
Wheat	277	Crude protein (CP)	168
Soybean meal (47% CP)	92	Crude fat	36
Barley	30	Ash	136
Sunflower meal (36% CP)	80	Crude fibre	67
Full-fat soybean	100	**Calculated nutrient composition**	
Soybean oil	24	Calcium	36
Sodium bicarbonate	1	Available phosphorus	4.0
Dicalcium phosphate	14	Lysine	8.0
Marble powder	83	Methionine	4.5
Salt	2	Methionine+cystine	7.6
Vitamin-mineral premix*	3	ME (MJ/kg)**	11.72

* 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.

**Dry matter, crude protein and crude ash were analysed according to established procedures AOAC[18] and other nutrient compositions are calculated based on NRC[19] data of feedstuffs nutrient tables.

and egg mass ((weekly egg number in replicate x average egg weight)/100, EM) from 20 weeks to 32 weeks of age[20,21]. In addition, age at point-of-lay, first egg weight, age (day) at 10%, 20%, 30% and 50% egg production were recorded. Thirty two eggs were randomly collected from each experimental group on the last day of every two-week period to assess egg quality. Immediately after sampling, eggs were assayed for quality. An average of three measurements (3 times) taken at the equator, blunt edge and pointed edge of the egg were recorded for lightness (L*), redness (a*), and yellowness (b*) of the eggshell, using a Minolta CR400 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). The psychometric colour terms such as, Hue ($\tan^{-1}$ b*/a*; H), C* ($(a^{*2}+b^{*2})^{1/2}$) and the total colour difference over time ($(L^{*2}+a^{*2}+b^{*2})^{½}$; ΔE*) were used in order to evaluate the colour changes of the eggshell. The egg quality traits included specific gravity, eggshell breaking strength, shell thickness, yolk colour and albumen pH. All eggs were weighed individually. The egg shape index was calculated using the following formula: shape index=(width/length) × 100[22]. 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[23]. After that, shell breaking strength was measured using a shell strength device with a spiral pressure system (Fujihara, Saitama, Japan), and the values were expressed in kg/cm^2. 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. A Haugh unit was calculated according to the following formula: Haugh unit (HU) = 100 log [albumen height (mm) + 7.57- 1.7× egg weight$^{0.37}$ (g)][24]. Albumen index (%) = [Albumen height / (long diameter of albumen + short diameter of albumen / 2) × 100; AI]; yolk index (%) = (yolk height / yolk diameter) x 100; YI[22]. Egg surface area (ES) in cm^2 was calculated for each egg using the following equation suggested by Nordstrom and Ousterhout[25]: 3.9782 x egg weight$^{0.70}$. The shell weight (SW) was calculated with the following formula specified by Harms et al.[26]: (2.0341 x egg weight) - [(2.1014 x egg weight) / specific gravity]. Egg albumen pH values were measured with digital pH meters (Sartorious PP15, AG Weender Landstrasse 94 PP108, Goettingen, Germany). 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 yolk colour was determined with a DSM[27] yolk colour fan (DSM Nutritional Products Ltd., Basel, Switzerland), which ranges from a pale yellow at score 1 to a dark orange at score 15, according to the CIE standard colorimetric system.

Blood serum

At the age of 32 weeks, approximately 5 ml of blood were taken from the wing vein (*Vena cutanea ulnaris*) of each hen (32 in total) and were collected in a glass tube (16 mm x 100 mm), kept on ice and transferred to the laboratory. Blood samples were left to stand at room temperature for clotting, and then the serum was obtained by centrifugation at 3000 x g for 5 min. The resultant serum (supernatant) was collected in 1.5 mL eppendorf tubes and stored at -20 °C for biochemical assays. The concentrations of serum glucose, cholesterol and triglyceride were measured, using commercial kits (Cat. No. GLU0102, TC0102 and TG0102) on an auto-analyser (Mindray BS series analysers, Hamburg, Germany).

PGLE antioxidant activity and total phenolics assay

Total antioxidant activity was measured using Oyaizu's[28] ferric reducing antioxidant power (FRAP) assay method. The antioxidant capacity assay was carried out with a Spectronic Genesys 5 spectrophotometer by the improved ABTS$^{•+}$ method, as described by Re et al.[29]. A total phenolic constituent of PGLE was performed, employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard[30].

Statistical analysis

Data in the tables are presented as arithmetic means and standard error of means (SEM). The data were analysed by SPSS[31] 16.0 software for Windows (Inc. Chicago, IL. USA). The differences between groups were determined by one-way ANOVA test. Duncan's multiple-range tests were performed according to the following model:

$Yij = \mu + Ti + eij$

where Y denotes the dependent variable; μ denotes the mean; T is the effect of treatment; and e is the random residual error term. All values were presented as means and standard error mean; the significance levels were set at $P < 0.05$.

RESULTS

The total phenolic content, TEAC value and FRAP value of PGLE (dry matter) were 1.11±0.02 g gallic acid equivalents (GAE)/kg, 606.3±0.948 mmol trolox/kg and 15.99±0.232 mmol TEAC/kg, respectively.

The performance of the laying hens was not influenced by PGLE intake, as shown by the absence of differences in body weight, body weight gain, viability, feed intake, and feed efficiency among treatments in the Table 2 ($P > 0.05$).

The egg mass, egg production (hen-house and hen-day), morning and afternoon egg yield were also not influenced by feed intake containing different level PGLE when compared with the control (Table 3). No differences were observed in laying hens fed the experimental diets in external and internal egg quality (Table 4). The results demonstrated that levels of PGLE did not affect overall SI, shell breaking strength, egg shell thickness, shell weight and egg surface area, yolk colour, albumen pH and HU ($P > 0.05$) except for AI ($P < 0.05$) and YI ($P < 0.01$), which were higher when 50 mg PGLE/kg and 100 mg PGLE/kg were included in diets. The results of measurements of the eggshell colour L*, a*, b*, Hue, C* and ΔE* are presented in Table 5. Some serum blood parameters of laying hens fed dietary PGLE at 32 weeks of age are summarized in Table 6.

DISCUSSION

The total phenolic content, TEAC value and FRAP value of PGLE (dry matter) are higher than those reported by Hu and Kitts[32]; Saumya and Mahaboob Basha[33] and Yıldırım et al.[3]. Kim et al.[34] determined that the total phenolic concentration of ginseng extract was 7.78 g/kg in the ethyl acetate fraction. Deng et al.[35] have found that the total phenolic, FRAP and TEAC values of ginseng fresh leaf in lipolytic fraction were 9.25±0.17 mg/g GAE, 10.40±0.44 μmol/g Fe (II) and 10.57±0.17 μmol/g Trolox, respectively. The total phenolic content and antioxidant activity of ginseng may vary depending on the type of plant

Table 2 - Body weight, feed intake and feed efficiency of laying hens fed on diets supplemented with PGLE.

Parameters	PGLE[1] in diet (mg/kg)					
	0	50	100	150	SEM[2]	*P*
Initial body weight (g)	1374	1377	1377	1372	7.84	ns
Final body weight (g)	1753	1785	1810	1843	16.17	ns
Body weight gain (g)	369.5	408.2	449.7	471.9	23.30	ns
Viability (%)	85.0	100.0	100.0	100.0	2.72	ns
Feed intake (g/hen/day)						
0 - 4 weeks	84.9	96.6	92.7	94.9	1.72	ns
4 - 8 weeks	129.6	129.1	135.9	132.2	2.84	ns
8 - 12 weeks	132.8	138.8	139.3	138.1	2.51	ns
Overall	114.5	121.5	121.4	121.7	2.11	ns
Feed efficiency (g feed/g egg)						
0 - 4 weeks	6.62	7.27	6.37	7.22	0.70	ns
4 - 8 weeks	3.57	2.95	2.80	2.99	0.19	ns
8 - 12 weeks	2.41	2.53	2.46	2.54	0.06	ns
Overall	3.56	3.46	3.32	3.48	0.09	ns

[1]PGLE, Panax ginseng leaf extract; [2] Mean of standard error; $P > 0.05$.

and the extraction method used. In addition, the total phenolic content and antioxidant capacities among ginseng varieties affected by cultivation practices, cultivation conditions, climate and geographic origin[35,36]. The antioxidant capacity of ginseng extract may have the potential to be used as an effective dietary antioxidant to prevent oxidative stress-related diseases suggested by Oh et al.[37]. The results in the current study is similar to the results reported in previous research[3] for panax root extract and demonstrate the antioxidant potential of PGLE, evidenced by its free radical scavenging and ferric reducing abilities. This supports the use of the plant in traditional medicine. Moreover, when consumed as plant extract, PGLE may contribute to the total intake of dietary antioxidants like other panax ginseng extracts.

The lack of information on the effects of Panax ginseng plant products, especially in laying hens, the possibility of comparing the findings of the study with other studies was limited. Catalan[38] reported that the commercial Panax ginseng added to the

Table 3 - Effects of dietary PGLE levels on egg productivity performance traits of laying hens.

Parameters	PGLE[1] in diet (mg/kg)					
	0	50	100	150	SEM[2]	*P*
Egg weight (g)						
0 - 4 weeks	47.7	51.4	50.6	49.9	0.55	ns
4 - 8 weeks	54.9[a]	56.2[ab]	55.1[a]	57.3[b]	0.33	*
8 - 12 weeks	58.8	59.8	58.5	60.7	0.35	ns
Overall	56.3[a]	57.7[ab]	56.3[a]	58.7[b]	0.38	*
Egg mass (g/hen/day)						
0 - 4 weeks	18.3	16.5	21.9	20.2	1.82	ns
4 - 8 weeks	40.1	44.4	48.7	45.1	2.11	ns
8 - 12 weeks	55.1	54.9	56.9	54.8	0.87	ns
Overall	39.1	43.9	44.7	44.3	1.40	ns
Oviposition time						
Morning egg weight (g)	57.3[a]	58.5[ab]	56.9[a]	59.5[b]	0.38	*
Afternoon egg weight (g)	53.0	55.8	53.8	54.7	0.54	ns
Hen-house egg production (%)						
0 - 4 weeks	36.8	32.3	43.2	39.4	3.76	ns
4 - 8 weeks	60.7	77.9	78.6	78.6	4.03	ns
8 - 12 weeks	79.1	91.8	86.9	90.2	3.03	ns
Overall	64.5	76.2	76.3	75.5	3.18	ns
Hen-day egg production (%)						
0 - 4 weeks	38.5	32.4	43.5	39.4	3.75	ns
4 - 8 weeks	73.1	79.0	88.3	78.6	3.74	ns
8 - 12 weeks	93.8	91.8	97.2	90.2	1.53	ns
Overall	69.5	76.2	79.6	75.5	2.59	ns
Morning hen-day egg yield (%)	53.8	54.0	66.3	62.0	2.89	ns
Afternoon hen-day egg yield (%)	15.7	22.2	13.4	13.4	1.37	ns

[1] PGLE: Panax ginseng leaf extract; [2] Mean of standard error; Means within a row followed by the different superscripts differ significantly. * $P < 0.05$.

Table 4 - Effects of dietary PGLE levels on means of external and internal egg quality characteristics.

Parameters	PGLE[1] in diet (mg/kg)					
	0	50	100	150	SEM[2]	*P*
Shape index	76.3	76.0	76.4	77.2	0.21	ns
Specific gravity (g/cm^3)	1.090^b	1.087^a	1.089^{ab}	1.090^b	0.00	*
Shell breaking strength (kg/cm^2)	2.51	2.13	2.24	2.28	0.07	ns
Egg shell thickness (µm)	351.3	343.7	339.7	352.4	2.00	ns
Shell weight (g)	6.38	6.61	6.42	6.61	0.07	ns
Surface area (cm^2)	66.98	67.77	66.84	67.81	0.31	ns
Yolk colour	10.59	9.75	9.57	10.32	0.15	ns
Albumen pH	8.69	8.71	8.71	8.69	0.01	ns
Albumen index	12.35^a	13.90^b	13.00^{ab}	12.78^{ab}	0.20	*
Yolk index	45.78^a	45.96^a	50.44^b	46.38^a	1.35	**
Haugh unit (score)	93.85	92.96	92.32	93.85	0.95	ns

[1] PGLE: Panax ginseng leaf extract; [2] Mean of standard error; Means within a row followed by the different superscripts differ significantly. * *P* <0.05; ** *P* <0.01

Table 5 - Change in colour of eggshell [lightness (L*), redness (a*), yellowness (b*)][1]

Parameters	PGLE[2] in diet (mg/kg)					
	0	50	100	150	SEM[3]	*P*
L*	66.94	65.79	65.67	66.38	0.27	ns
a*	12.50^a	13.62^b	13.78^b	13.20^{ab}	0.17	*
b*	25.29	25.11	26.27	24.70	0.31	ns
Hue	63.59	61.35	62.10	61.74	0.32	ns
C*	28.26	28.62	29.72	28.07	0.32	ns
ΔE*	72.75	71.87	72.23	72.24	0.20	ns

[1]A higher L* value means lighter colour; a higher a* value means a redder colour; a higher b* value means a more yellow colour. [2]PGLE, Panax ginseng leaf extract; [3]Mean of standard error; Means within a row followed by the different superscripts differ significantly. * *P* <0.05.

Table 6 - Effect of PGLE supplementation on some serum biochemical parameters in laying hens at end of experiment.

Parameters	PGLE[1] in diet (mg/kg)					
	0	50	100	150	SEM[2]	*P*
Glucose (mg/dL)	306.8	302.2	294.2	298.9	2.83	ns
Cholesterol (mg/dL)	159.7	152.2	149.3	154.8	6.85	ns
Triglyceride (mg/dL)	1414.3	1687.6	1544.9	1709.1	67.63	ns

[1] PGLE: Panax ginseng leaf extract; [2] Mean of standard error; *P* >0.05

diet of the laying hens had no effect on the body weight, body weight gain and feed intake and was consistent with the present research finding. Albeit, in agreement with our previous study on Atak-S laying hens[3], the panax ginseng root extract supplementation no changed the values of body weight, body weight gain, feed intake, and feed efficiency during 12 weeks laying period. The current research displayed that viability was higher in laying hens fed diets supplemented with the PGLE than in those fed the control diet and it was similar with the finding of Yıldırım et al.[3]. This suggests that up to 150 mg PGLE/kg in a diet can be used as an ingredient without any adverse effect on production performance in laying hens or at 11.7 mg/bird/day or 100 mg/kg of body weight.

The lower egg production and egg mass (EM) between 0 and 4 weeks might be because that all hens unexpectedly laid during weeks 22 and 23. Similar to our previous research[3], all parameters were similar in the graded PGLE supplemented group compared with the control group, except that egg production of the 50 and 150 mg PGLE/kg groups was higher than the control, though did not reach statistical significance. These results are consistent with the findings of Ao et al.[39], who reported no positive effects on egg production and feed intake in laying hens fed different levels of fermented red ginseng extract. In disagreement with that, Jang et al.[40] observed that fermented wild ginseng culture by-product increased egg production which may be attributed to the improvement in health status of birds fed supplemented diets. This inconsistency might be due to the use of different ginseng sources, and different methods of preparation of the ginseng products and strains used in the experiment[3]. In addition, the level of PGLE supplementation might not have been enough to cause an improvement in egg productivity performance. Otherwise the PGLE supplementation

increased egg weight and morning (oviposition time) egg weight (57.6 *vs.* 56.3 g; $P<0.05$ and 58.3 *vs.* 57.3 g; $P<0.05$) as compared to laying hens fed the control diet in overall. This performance variable improved in linear fashion as dietary PGLE supplementation increased. Supplementation of PGLE increases egg weight which may be associated to its active constituents. It could be hypothesized that PGLE increased egg weight more than the control treatment. Therefore, the PGLE used in this work contains some components, like phenolic acids and antioxidant capacity, which have been implicated in other works as various pharmacological effects[14], physiological function and immunity[17]. The egg weight values in our experiment are in line with the finding of Jang et al.[40] and Osfor[41], who found that the significant weight increase observed in their trials. In contradiction, some researchers[3,38,39,42] illustrated that the PGLE supplementation to diets had no effect on the egg weight of the laying hens. Egg weight and egg shell quality characteristics vary according to the oviposition time. These results agree with previous results[3,43,44] that eggs laying early in the morning are heavier than those laid during the day. In addition, the morning henday egg yield was very high than the afternoon in all groups, but still heavy eggs were laid in the morning.

This finding agrees with Yıldırım et al.[3], who indicated that AI and YI parameters were not affected by Panax ginseng root extract groups in 20-32 week old laying hens. Therefore, it is thought that the difference detected in both parameters may be closely related to egg weight. Specific gravity significantly decreased when dietary 50 mg/kg PGLE supplementation to diets laying hens. In the current study, specific gravity data consistent with the findings of Yıldırım et al.[3] who determined the medium specific gravity ranged from 1.087 to 1.092 in 32 week old Atak-S laying hens. Hens fed with diet supplemented with PGLE had similar values of SI, eggshell thickness, shell weight, surface area, yolk colour and HU score, but lowest values of shell breaking strength compared to control group ($P>0.05$). SI is a method used to determine egg shape. The SI in ideal eggs is 74% in terms of commercial and hatching properties. If the SI value is greater than 76%, the eggs are round, 72-76% is normal, and <72% is longer[45]. In the experiment, the group which received 150 mg/kg PGLE was found to be more than 76% in terms of SI and the other groups were classified as normal SI. Yıldırım et al.[3] reported that the SI values (76.2-77.4) of the laying hens fed with Panax ginseng root extract supplement were similar to our findings. Eggs shorter and longer than normal eggs are not desirable by the consumer and they cause economic losses during transportation and marketing. To reduce the chances of breaking eggs at the marketing and transportation stages, the shell thickness of the eggs is expected to be at least 330 μm and more[46]. In this context, according to our findings related to the egg thickness close to each other in the groups (339.7-352.4 μm), thus it was among the desired minimum values. These results are consistent with those of several studies[3,38,39,40,42] who found that various ginseng supplemented to laying chicken diets had no significant effect on the egg shell weight. The findings agree with those reported by Ao et al.[39], who found no significant effect of dietary fermented red ginseng extract supplemented on HU, eggshell thickness and egg breaking strength parameters in ISA brown laying hens. By contrast, Yıldırım et al.[3] reported that the paler yolk at inclusions of 100 mg Panax ginseng root extract/kg and 150 mg Panax ginseng root extract/kg suggested xanthophylls interacted with ginsenosides of Panax ginseng root extract. Thus, this condition can be explained by ginsenoside content variability. Albumen quality, which is an important indicator of egg freshness, has a great significant in egg processing industry[47]. The pH is a useful means for describing changes in albumen quality over time during storage claimed by Silversides and Villeneuve[48]. However, albumen pH is not affected by PGLE groups and can be used to measure egg freshness. Overall albumen pH ranged from 8.69 to 8.71, which is in agreement with previous data, such as 8.67 for Atak-S laying hens[3] and 8.29 to 8.39 for 30 week old Dekalb white laying hens[2].

Hen nutrition is an area that needs to be tested in relation to eggshell color[49]. Overall L*, b*, Hue, C* and ΔE* did not differ among treatments during the entire experiment. However, the present study demonstrates that the dietary PGLE supplementation significantly improved the a* values of egg in laying hens in both 50 and 100 mg/kg groups when compared to control group. Supplementing PGLE to diet, increased ($P<0.05$) the redness of the eggshell and came close the other among groups, but this effect was not reflected in the same way in the other parameters (Hue, C* and ΔE*). However, a possible mechanism of action could be due to any factor that promotes liver function, subsequent lipid metabolism, synthesis of protoporphyrin IX in the shell gland and deposition of pigment in the eggshell[49]. This fact agrees with the results of Odabasi et al.[50], who indicated that a decrease in pigmentation was associated with a decrease in the amount of redness (a*) in the eggshell. The lighter the shell in colour, the less redness it contains, and this negative correlation becomes stronger as the hen ages over the first 10 months of the laying cycle claimed by the same researchers. The present study, L* was numerically lower in PGLE groups than in the control. Hence, the lighter coloured eggshells (higher L*) would have less redness (a*). The averages of the overall measurements at three points on the same egg were also very close to those of the one point measurement. The results indicated high uniformity of eggshell pigmentation. Yıldırım et al.[3] determined the average L*, a* and b* values of brown eggshell from 32 weeks old hens to be between 65.0 and 66.4, 13.4 and 14.1, 24.9 and 26.8 for brown eggs, respectively. In the present study, the same parameter values were between 65.7 and 66.9; 12.5 and 13.8; 24.7 and 26.3, respectively for Atak-S brown egg. This is consistent with previous results, with same age effect. Shell colour is usually affected by genetic background, the season of lay, nutrition, housing systems, diseases, all kinds of stimulus, and drug, etc.[49,51].

While assessing the health status of poultry fed diets with unconventional feeds, serum biochemical parameters play an important role in providing useful information about the metabolic alterations of organs and tissues[52]. Melesse et al.[53] and Hussein et al.[54] reported that several factors cause a considerable influence on serum biochemical parameters of chickens such as feed additives, genotype and environmental temperature. The supplementation of PGLE to laying hens diets had no significant effect on the serum glucose, cholesterol, and triglyceride concentrations. However, serum cholesterol and glucose was numerically lower in PGLE groups than in the control. This consistent with Ao et al.[39], who observed that serum cholesterol concentrations varied from 176 mg/dL to 185 mg/dL and there were no significant differences when various levels of fermented red ginseng extract were supplemented. Muwalla and Abuirmeileh[55] and Yokozawa et al.[56] found that the addition of dietary ginseng decreased the level of blood lipids. On the other hand, total cholesterol concentration was decreased by fermented gin-

seng culture in laying hens demonstrated by Jang et al.[40]. However, this effect was not confirmed in the present study, probably because of the low levels of supplementation[3]. Overall, the data from the current study supported the contention that laying hen diets can contain up to 150 mg/kg PGLE without affecting measures of hen productivity or specific measures of egg and eggshell quality.

CONCLUSIONS

The results of this study showed that overall, dietary supplementation of PGLE to laying hens' diet improved the egg weight and eggshell redness at the beginning of the laying period, but it had no adverse effects on the health status of the hens, external egg quality and some serum biochemical measurements. Future research needed to explain the mechanism, significance and the use of PGLE as a source of bioactive substance and to determine the welfare of laying hens in different management conditions, including various stress factors such as environmental and physiological stresses, dietary ingredients and nutrient content for the poultry feed industry.

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AUTHOR'S CONTRIBUTION

AY conceived and designed the study, AY and MISM conducted the analyses and contributed to the data collection, drafted the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATMENT

The authors declare that they have no competing interests.

References

1. Yuan L., Li W., Huo Q., Du C., Wang Z., Yi B., Wang M. (2018). Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. PeerJ, 6: e4435.
2. Olobatoke R.Y., Mulugeta S.D. (2011). Effect of dietary garlic powder on layer performance, fecal bacterial load, and egg quality. Poult Sci, 90: 665-670.
3. Yıldırım A., Sekeroglu A., Eleroglu H., en M.I., Duman M. (2013). Effects of Korean ginseng (*Panax ginseng* C.A. Meyer) root extract on egg production performance and egg quality of laying hens. South Afr J Anim Sci, 43: 194-207.
4. Botsoglou N.A., Florou-Paneri P., Christaki E., Fletouris D.J., Spais A.B. (2002). Effect of dietary oregano essential oils on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br Poult Sci, 43: 223-230.
5. Florou-Paneri P., Nikolakakis I., Giannenas I., Koidis A., Botsoglou E., Dot A.S.V., Mitsopoulos I. (2005). Hen performance and egg quality as affected by dietary oregano essential oils and tocopherol acetate supplementation. Int J Poult Sci, 4: 449-454.
6. Li T.S.C. (1995). Asian and American ginseng-A review. HortTechnology, 5: 27-34.
7. Yıldırım A., Erener G. (2010). The possibilities using of Ginseng (*Panax* spp.) in poultry nutrition. Hasad J Anim Sci, 26: 56-59 (*in Turkish*).
8. Choi H.J., Han H.S., Park J.H., Son J.H., Bae J.H., Seung T.S., Choi C. (2003). Antioxidantive, phospholipase A2 inhibiting, and anticancer effect of polyphenol rich fractions from *Panax ginseng* C. A. Meyer. J Korean Soc Agric Chem Biotechnol, 46: 251-256.
9. Park B.J., Lim Y.S., Lee H.J., Eum W.S., Park J., Han K.H., Choi S.Y., Lee K.S. (2009). Anti-oxidative effects of *Phellinus linteus* and red ginseng extracts on oxidative stress induced DNA damage. BMB rep, 42: 500-505.
10. Liu C., Xiao P. (1992). Recent advances on ginseng research in China. J. Ethnopharmacol, 36: 27-38.
11. Asci A., Baydar T., Sahin G. (2007). Evaluation of usage of herbal preparation and drug interactions in elderly people from toxicological aspect. Turk J Geriatr, 10: 203-214. (*in Turkish*)
12. Anoja S.A., Wu J.A., Yuan C. (1999). Ginseng pharmacology. multiple constituents and multiple actions. Biochem Pharmacol, 58: 1685-1693.
13. Shin H., Jeong H., An H., Hong S., Um J., Shin T., Kwon S., Jee S., Seo B., Shin S., Yang D., Kim H. (2006). The effect of *Panax ginseng* on forced immobility time & immune function in mice. Indian J Med Res, 124: 199-206.
14. Kiefer D., Traci Pantuso B.S. (2003). Panax ginseng. Am Fam Physician, 68: 1539-1542.
15. Qureshi A.A., Abuirmeileh N., Din Z.Z., Ahmad Y., Burger W.C., Elson C.E. (1983). Suppression of cholesterogenesis and reduction of LDL cholesterol by dietary ginseng and its fractions in chicken liver. Atherosclerosis, 48: 81-94.
16. Helms S. (2004). Cancer prevention and therapeutics: *Panax ginseng*. Altern Med Rev, 9: 259-274.
17. Sohn S.H., Jang I.S., Moon Y.S., Kim Y.J., Lee S.H., Ko Y.H., Kang S.Y., Kang H.K. (2008). Effect of dietary siberian ginseng and eucommia on broiler performance, serum biochemical profiles and telomere length. Korean J Poult Sci, 35: 283-290.
18. AOAC. (2000). Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Washington, DC, USA.
19. NRC. (1994). Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, D.C., USA.
20. North M.O. (1984). Breeder Management. In Commercial Chicken Production Manual. The Avi. Publishing Company. Inc. Westport, Connecticut, 240-321.
21. Um J.S., Paik I.K. (1999). Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poult Sci, 78: 75-79.
22. Doyon G., Bernier-Cardou M., Hamilton R.M.G., Castalgne F., Randall C.J. (1986). Egg quality 2. Albumen quality of eggs from five commercial strains of white leghorn hens during one year of lay. Poult Sci, 65: 63-66.
23. Hamilton R.M.G. (1982). Methods and factors that affect the measurement of egg shell quality. Poult. Sci. 61: 2022-2039.
24. Roush W.B.T. (1981). 159 calculator program for Haugh unit calculation. Poult Sci, 60: 1086-1088.
25. Nordstrom J.D., Ousterhout I.E. (1982). Estimation of shell weight and thickness from egg specific gravity and egg weight. Poult Sci, 61: 1991-1995.
26. Harms R.H., Rossi A.F., Sloan D.R., Miles R.D., Christmas R.B. (1990). A method for estimating shell weight and correcting specific gravity for egg weight in eggshell quality studies. Poult Sci, 69: 48-52.
27. DSM. (2013). Yolk color fan. Available at www.dsmnutritionalproducts.com (accessed: 10 February 2013).
28. Oyaizu M. (1986). Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr, 44: 307-315.
29. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C.A. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med, 26: 1231-1237.
30. Slinkard K., Singleton V.L. (1977). Total phenol analyses: automation and comparison with manual methods. Am J Enol Viticult, 28: 49-55.
31. SPSS. (2010). Statistical Package in Social Sciences for Windows. Statistical Innovations Inc., Chicago, USA.
32. Hu C., Kitts D.D. (2001). Free radical scavenging capacity as related to antioxidant activity and ginsenoside composition of Asian and North American ginseng extracts. J Am Oil Chem Soc, 78: 249-255.
33. Saumya S.M., Mahaboob Basha P. (2011). In vitro evaluation of free radical scavenging activities of *Panax ginseng* and *Lagerstroemia speciosa*: A comparative analysis. Int J Pharm Pharm Sci, 3: 165169.
34. Kim J.S., Moon G.S., Kim H.O., Lee Y.S. (2007). Antioxidant properties of ginseng (*P. ginseng* C.A. Meyer) extracts by organic solvent fraction-

ation. J Food Sci Nutr, 12: 267-272.
35. Deng G.F., Lin X., Xu X.R., Gao L.L., Xie J.F., Li H.B. (2012). Antioxidant capacities and total phenolic contents of 56 vegetables. J Funct Foods, 5: 260-266.
36. Chen C.Y.O., Ribaya-Mercado J.D., McKay D.L., Croom E., Blumberg J.B. (2010). Differential antioxidant and quinone reductase inducing activity of American, Asian, and Siberian ginseng. Food Chem, 119: 445-451.
37. Oh C.H., Kim G.N., Lee S.H., Lee J.S., Jang H.D. (2010). Effects of heat processing time on total phenolic content and antioxidant capacity of ginseng Jung Kwa. J Ginseng Res, 34: 198-204.
38. Catalan A.A.S. (2011). Panax Ginseng in Diets of Commercial Laying Hens. 67F. Thesis (Master Degree)-Animal Sciences Graduate Program. Federal University of Pelotas-Pelotas City-Rio Grande do Sul State, Brazil.
39. Ao X., Zhou T.X., Kim H.J., Hong S.M., Kim I.H. (2011). Influence of fermented Red Ginseng extract on broilers and laying hens. Asian-Austral J Anim Sci, 24: 993-1000.
40. Jang H.D., Kim H.J., Cho J.H., Chen Y.J., Yoo J.S., Min B.J., Park J.C., Kim I.H. (2007). Effect of dietary supplementation of fermented wild ginseng culture by products on egg productivity, egg quality, blood characteristics and ginsenoside concentration of yolk in laying hens. Korean J Anim Sci, 34: 271-278.
41. Osfor M.H. (1995). Some biochemical and nutritional studies on the effect of *Panax ginseng* powder on adult Japanese quails. Pol J Food Nutr Sci 45: 73-79.
42. Yan L., Meng Q.W., Lee J.H., Wang J.P., Kim I.H. (2011). Evaluation of dietary wild-ginseng adventitious root meal on egg production, egg quality, hematological profiles and egg yolk fatty acid composition in laying hens. Livest Sci, 140: 201-205.
43. Tumová E., Zita L., Hubený M., Sk ivan M., Ledvinka Z. (2007). The effect of oviposition time and genotype on egg quality characteristics in egg type hens. Czech J Anim Sci, 52: 26-30.
44. Gumulka M., Kapkowska E., Maj D. (2010). Laying pattern parameters in broiler breeder hens and intrasequence changes in egg composition. Czech J Anim Sci, 55: 428-435.
45. Turkoglu M., Arda M., Yetisir R., Sarıca M., Altan A., Erensayın C. (2004). Poultry Science. Ankara, Turkey (*in Turkish*).
46. Stadelman W.J. (1995). Quality Identification of Shell Eggs. In: Egg Science and Technology. Ed. Stadelman, W. J. and Cotterill, O. J. The Haworth Press, Inc., New York, London, 39-66.
47. Jin Y.H., Lee K.T., Lee W.I., Han Y.K. (2011). Effects of storage temperature and time on the quality of eggs from laying hens at peak production. Asian-Austral J Anim Sci, 24: 279-284.
48. Silversides F.G., Villeneuve P. (1994). Is the Haugh unit correction for egg weight valid for eggs stored at room temperature? Poult Sci, 73: 50-55.
49. Samiullah S., Roberts J.R., Chousalkar K. (2015). Eggshell color in brown-egg laying hens-a review. Poult Sci, 94: 2566-2575.
50. Odabasi A.Z., Miles R.D., Balaban M.O., Portier K.M. (2007). Changes in brown eggshell color as the hen ages. Poult Sci, 86: 356-363.
51. Yang H.M., Wang Z.Y., Lu J. (2009). Study on the relationship between eggshell colors and egg quality as well as shell ultrastructure in Yangzhou chicken. Afr J Biotechnol, 8: 2898-2902.
52. Kudair I.M., Al-Hussary N.A.J. (2010). Effect of vaccination on some biochemical parameters in broiler chickens. Iraqi J Vet Sci, 24: 59e64.
53. Melesse A., Maak S., Schmidt R., Vonlengerken G. (2011). Effect of long term heat stress on some performance traits and plasma enzyme activities in Naked-neck chickens and their F1 crosses with commercial layer breeds. Livest Sci, 141: 227e31.
54. Hussein A.S., Ayoub M.A., Elhwetiy A.Y., Ghurair J.A., Sulaiman M., Habib H.M. (2018). Effect of dietary inclusion of sugar syrup on production performance, egg quality and blood biochemical parameters in laying hens. Anim Nutr, 4: 59-64.
55. Muwalla M.M., Abuirmeileh N.M. (1990). Suppression of avian hepatic cholesterogenesis by dietary ginseng. J Nutr Biochem, 1: 518-521.
56. Yokozawa T., Satoh A., Cho E.J. (2004). Ginsenoside-Rd attenuates oxidative damage related to aging in senescence-accelerated mice. J Pharm Pharmacol 56: 107-113.