Archives of Razi Institute, Vol. 76, No. 2 (2021) 359-369

DOI: 10.22092/ari.2020.342143.1454

Original Article

Enhancement of Productive Performance, Bone Physical Characteristics, and Mineralization of Laying Hens during the Post-Peak Period by Genistein

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Received 28 February 2020; Accepted 29 March 2020
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Abstract

Genistein (GEN), a soybean isoflavone, is structurally and functionally similar to endogenous estrogen; therefore, it has the potential to enhance estradiol properties. This study aimed to evaluate the effects of GEN on the reproductive performance and bone status of laying hens. In total, 80 Hy-line W-36 (40 weeks old, the late stage of egg production cycle) with an initial body weight of 1,230±15.8 g (Mean±S.E.M), similar egg production, and egg weight were randomly assigned into two groups with 10 replicates and 4 birds in each replicate (40 laying hens per group). Laying hen diets had 0 (control) and 20 mg/kg GEN (white powder, Sichuan Guanghan co. Ltd., purity of 98.5%) for 6 weeks (41 to 46). At the end of the experiment, 20 hens (one hen from each replicate) were slaughtered, and the samples of bone and shell gland (approximately 50 mg) were surgically taken immediately after slaughter for Real-time PCR. The results indicated that dietary GEN increased egg production, feed intake, and egg mass; however, it decreased egg weight ($P<0.05$). Furthermore, the feed conversion ratio was greater in birds received GEN, compared to those in the control group ($P<0.05$). GEN enhanced egg quality indices included eggshell strength, thickness, and percentage ($P<0.05$). Mechanical properties of the tibia, such as weight, length, and breaking strength were also increased by GEN ($P<0.05$). Moreover, dietary GEN increased the calcium content of the tibia ($P<0.05$). The mRNA expression of Calbindin-D28k (CaBP-D28k) and transient receptor potential vanilloid channel type 6 (TRPV6) upregulated in eggshell glands of hens treated with GEN paralleled to the controls ($P<0.05$). In conclusion, the findings of the present study showed that GEN had the potential to improve the bone physical characteristics, mineralization, and the productive performance of Hy-line W-36 laying hens in their post-peak period.

Keywords: Bone, CaBP-D28k, Genistein, Productive performance, TRPV6

Amélioration de la Performance Productive, des Caractéristiques Physiques Osseuses et de la Minéralisation des Poules Pondeuses Pendant la Période Après-Sommet par la Génistéine

Résumé: La génistéine (GEN), une isoflavone de soja, est structurellement et fonctionnellement similaire à l'œstrogène endogène; par conséquent, elle a le potentiel d'améliorer les propriétés de l'œstradiol. Cette étude visait à évaluer les effets de GEN sur les performances de reproduction et l'état osseux des poules pondeuses. Au total, 80 Hy-line W-36 (40 semaines, le stade avancé du cycle de production d'œufs) avec un poids corporel initial de 1,230 ± 15.8 g (moyenne ± SEM), une production d'œufs similaire et un poids d'œuf ont été répartis au hasard en deux groupes avec 10 répétitions et 4 oiseaux dans chaque répétition (40 poules pondeuses par groupe). Les régimes pour poules pondeuses avaient 0 (témoin) et 20 mg/kg de GEN (poudre blanche, Sichuan Guanghan Co. Ltd., pureté de 98,5%) pendant 6 semaines (41 à 46). À la fin de l'expérience, 20 poules (une

poule de chaque réplique) ont été abattus et les échantillons d'os et de glande coquille (environ 50 mg) ont été prélevés chirurgicalement immédiatement après l'abattage pour une PCR en temps réel. Les résultats ont indiqué que le GEN alimentaire augmentait la production d'œufs, la consommation d'aliments et la masse d'œufs; cependant, il a diminué le poids des œufs (P<0.05). De plus, le taux de conversion alimentaire était plus élevé chez les oiseaux recevant du GEN, comparé à ceux du groupe témoin (P<0.05). Les indices de qualité des œufs améliorés par GEN comprenaient la force, l'épaisseur et le pourcentage de la coquille (P<0.05). Les propriétés mécaniques du tibia, comme le poids, la longueur et la résistance à la rupture ont également augmenté de GEN (P<0.05). De plus, le GEN alimentaire a augmenté la teneur en calcium du tibia (P<0.05). L'expression de l'ARNm de la calbindine-D28k (CaBP-D28k) et du canal vanilloïde potentiel du récepteur transitoire de type 6 (TRPV6) régulée à la hausse dans les glandes de coquille d'œuf de poules traitées avec GEN a été parallèle aux témoins (P<0.05). En conclusion, les résultats de la présente étude a montré que GEN avait le potentiel d'améliorer les caractéristiques physiques des os, la minéralisation et la performance productive des poules pondeuses Hy-line W-36 dans leur période après-sommet.
Mots-clés: Os, CaBP-D28k, Génistéine, Performances Productives, TRPV6

1. Introduction

The eggshell gland (ESG) in hens is a biologically exclusive organ that has an important role in the calcification of eggshell by delivering a large amount of Ca^{2+} ions from the bloodstream to the lumen of the uterine during the eggshell formation (Yang et al., 2013). Changes in proteins responsible for calcium-regulating, such as Calbindin D28K (CaBP-D28k) and transient receptor potential vanilloid channel type 6 (TRPV6), are thought to happen due to the alterations of estrogen levels (Beck and Hansen, 2004). The time course of estrogen synthesis has been well documented over the hen's productive life, which is characterized by increasing estrogen in circulation, accompanying the commence of sexual maturity, and decreasing prior to a molt resulting in a reduction in egg production. Soybean isoflavones (ISF) are estrogenic compounds with no steroid structure that is extracted from grass, such as legumes (Cao et al., 2015). The ISF derived from plants especially soybeans have nowadays become an interesting subject to scientific studies due to their antibacterial, antifungal, and estrogenic activities. Genistein (4, 5, 7-trihydroxyisoflavone, GEN) in products derived from soy is a type of phytoestrogen that accounts for about two-thirds of ISF (Price and Fenwick, 1985; Patel et al., 2016). The structural similarity allows GEN to bind to sex hormone-binding proteins and estrogen receptors. Furthermore, the GEN can declare both estrogenic and anti-estrogenic activities through binding the specific receptors (Dixon and Ferreira, 2002). Studies on animals have revealed that supplying diets with soy protein could reportedly promote the reproductive performance, growth, and quality of livestock production (Sahin et al., 2007; Steinshamn, 2010; Shin et al., 2012; Retana-Marquez et al., 2016).

The duration of laying-peak is short; furthermore, egg production and quality decrease rapidly during the post-peak. In the process of eggshell and bone formation, calcium (Ca) and phosphorus (P) have vital roles and are two key minerals. The use of estrogen-like compounds has been offered to improve the absorption of Ca (Arjmandi et al., 2005). It has been revealed that the levels of ISF supplementation can directly affect the content of Ca in ovariectomized mice (Fonseca and Ward, 2004). In recent years, the use of GEN as a dietary supplement has become common, especially for post-peak hens that desire a natural replacement to traditional hormone therapies. Additionally, it has been used in poultry for helping them pass this stressful period which can affect egg production and quality.

The amount of mineral contents and adequacy is a common reflector for bone status in poultry diets (Rath et al., 1999). In fact, bone strength will be affected by the rate of bone mineralization (Reichmann and

Connor, 1977); therefore, the possibility of fractures will be increased due to fragile bones (Blake and Fogelman, 2002; Molnár, 2010). Moreover, the reduction of feed intake is negatively associated with brittle legs and often concludes a drop in the number of eggs laid and weight gain (Orban et al., 1999). Studies have shown that food rich in ISF demonstrate effects of bone-preserving and osteoporosis eases in women who are in the postmenopausal period (Arjmandi et al., 1996). The utilization of different levels of GEN resulted in different physiological responses in birds; moreover, the period of supplementation is another factor that affected response; accordingly, there are contradictions in the results of previous studies. This study aimed to investigate the effects of GEN for six weeks on productive performance and bone case of Hy-line W-36 laying hens at their post-peak period.

2. Material and Methods

2.1. Experimental Design

All experimental procedures used in this experiment were approved by the Animal Care Committee of the Sari Agriculture and Natural Resources University, Sari, Iran. In total, 80 (40 weeks old, the late stage of egg production cycle) Hy-line W-36 with an initial body weight of 1,230±15.8 g, similar egg production, and egg weight were randomly assigned into two groups with 10 replicates and 4 birds in each replicate (40 laying hens per group). Following that, the laying hens' diets had 0 (control) and 20 mg/kg GEN (white powder, Sichuan Guanghan co. Ltd., purity of 98.5%) for 6 weeks (41 to 46). Every four hens were housed in a battery cage (40.6×45.7 cm) in a house with temperature maintained as close to 21°C as possible and a 16L:8D lighting program. It should be mentioned that the birds were supplied with feed and water *ad libitum*. The experimental diets were formulated according to the nutritional requirements suggested in the Hy-line W-36 Commercial Management Guide (Hy-Line International, 2009-2011). Table 1 tabulates the ingredients and the nutrient composition of the experimental diets.

2.2. Tissue Collection, RNA Extraction, and cDNA Synthesis

At the end of the experiment, 20 hens (one hen from each replicate) were slaughtered, and the samples of shell gland (approximately 50 mg) were surgically taken immediately after slaughter. Subsequently, the samples were washed with phosphate-buffered saline, immediately snap-frozen in liquid nitrogen, and stored at -80ºC until RNA extraction.

Total RNA was extracted from uterus samples using the RNeasy mini kit (Qiagen, Valencia, CA, USA) and reverse-transcribed with oligo-dT and Superscript II RNase H reverse transcription kit (Invitrogen, Taastrup, Denmark) according to the manufacturer's protocol. Electrophoresis through a 1.5 % agarose gel was used to confirm the RNA quality. Total RNA (1 µg) was treated with 1 U DNase (Invitrogen) to eliminate DNA contamination. For each sample, cDNA synthesis was carried out using the QuantiTec Reverse Transcription Kit (Cat. No. 205311; Qiagen, GmbH, Germany).

2.3. Real-time PCR

The Real-time polymerase chain reaction (PCR) was implemented to determine the relative transcripts of CaBP-D28k and TRPV6. Details of primer sequences are provided in Table 2. Expression of the β-actin transcript was used as an internal housekeeping gene. Real-time PCR reactions were carried out in a total volume of 15 µL with 1 µLcDNA (50 ng/mL), 7.5 µL SYBR Green master mix (QuantiNovaTM SYBR® Green PCR Kit, Qiagen Inc., Tehran, Iran), 1 µL forward and 1 µLreverse primers (20 ng of each), and 4.5 µL nuclease-free H_2O. Amplification was performed using a Corbett Rotor-GeneTM 3000 quantitative PCR system (Corbett Life Sciences, Sydney, Australia) with the following cycling parameters: 95 °C for 15 min and 40 cycles of 95 °C for 30 sec, 60 °C for 30 sec, 72 °C for 30 sec, followed by dissociation curves to verify amplification of single products (95 °C for 1 min, 50 °C for 45 sec, increasing 0.58/cycle until 95 °C was reached). Gene expression results were calculated using the ΔΔCt method with correction for amplification efficiency and were normalized to a calibrator sample.

Table 1. Ingredients and chemical composition of the experimental diets (% dry matter basis)

	xWeeks	
	41-42	**43-46**
Ingredients		
Corn gluten meal	5.00	5.00
Corn	60.01	60.75
Limestone	10.16	10.68
Soybean oil	2.91	2.60
Soybean meal	18.26	17.54
Di-calcium phosphate	2.24	2.08
Salt	0.36	0.37
$NaHco_3$	0.05	0.05
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL-Met	0.19	0.16
L-Lys	0.27	0.24
L-Threonine	0.05	0.03
Calculate nutrient analysis:		
Na (%)	0.18	0.18
CP (%)	16.35	16.05
ME (Kcal/kg)	2844	2822
Ca (%)	4.42	4.58
Available Phosphorus (%)	0.51	0.48
Threonine (%)	0.55	0.52
Argenin (%)	0.13	0.80
Lysine (%)	0.79	0.75
Met+Cys (%)	0.66	0.63

Table 2. Primer sequences used for the analyses of the expression pattern TRPV6 and CaBP-D28k genes

Primer name	Product size (bp)	Primer sequence (5'-3')	Annealing temperature (°C)
β-actin	150	F-ACGGTGCTGTCTGGTGGTA R-TGTCTGACATGGGAGAGCAG	50
TRPV6	141	F-TGGAACGGACTAAGTCAGAAGTTG R- CGTTATGGCTG GGATGTTGTT	53
CaBP-D28k	297	F- TTAAATCTGCGTTGCTTCCATAC R- CAGCCCCAAGCAGGTAAAG	55

2.4. Sample Collection and Analytical Determination

Body weights of laying hens were determined at the beginning and end of the study. Feed consumption was recorded at weekly intervals for the whole experimental period. Daily egg production and egg weight were monitored during the study. Feed consumption, feed conversion, egg production, and egg quality were also examined in this study. Egg quality, including yolk height, albumen height, eggshell strength, eggshell thickness, and eggshell weight were measured. Eggshell strength was measured using an Egg Force Reader machine (Sanovoeng Co. Ltd., Tokyo, Japan), and eggshell thicknesses at three locations on the eggs (air cell, equator, and sharp end) was a mean value of measurements and were measured by a thickness Gauge (Seri 500, Mitutoyo, Tokyo, Japan). Egg shape index was measured using a Digital Caliper (Guilin Guanglu Measuring Instrument Co., Ltd. Guangxi, P.R. China), and the shape index was calculated according to this formula: shape index=(height/width)×100. The yolk that was separated from albumen was weighed, and the mass of albumen was calculated as the difference in egg weight minus yolk and shell weight. The percentage was calculated as a ratio of egg weight.

2.5. Mechanical Properties

After the birds were sacrificed, tibiae were dissected from the carcass, and the adherent tissues were removed and stored at -20°C for subsequent measurement. The three-point bending test was used to determine the mechanical properties of the tibia. The bones were thawed at room temperature, oven-dried at 105°C for 24 h, and defatted with diethyl ether for 48 h (Hosseini et al., 2016). Bone length and diameter at the center of the diaphysis were measured using a digital caliper. The mechanical properties of the tibia were determined by the three-point bending to failure with an Instron 1125 servo-controlled testing machine (Instron Corp., Canton, MA). The bones were held in identical positions. The distance between the two supporting ends was 5 cm, and the 10-mm diameter crosshead probe (50 kg) approached the bone at 5 mm/min until fracture occurred. Broken tibia samples were collected, dried overnight at 105°C in the oven, and ashed at 600°C for 16 h for mineral content measurement (Hosseini et al., 2016).

2.6. Chemical Analysis

The concentrations of Ca^2 and P in bones were determined by atomic absorption spectrophotometry (Varian SpectrAA 50B Atomic Absorption Spectrometer, Varian Ltd, USA) (AOAC, 2005 method 927.02).

2.7. Statistical Analysis

After the completion of Real-time quantitative PCR amplification, the data were obtained using Opticon Monitor software (Version 2.03, 2005). The exact copy number of constitutive transcript mRNA in tissue was derived from the threshold value according to the standard curve. The data presented as the fold change in tissues for CaBP-D28k and TRPV6 expression was normalized to *β-actin.* All experimental data were subjected to analysis of variance using the GLM procedure of SAS (SAS 9.1 for windows). A Duncan's multiple comparison test was used to separate different means among treatments, and a p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Laying Performance

Table 3 summarizes the feed intake, egg production, feed conversion, egg weight, and egg mass in the two groups. Egg production, egg mass, and feed intake were significantly influenced by dietary GEN and were greater in laying hens fed with 20 mg/kg GEN, compared to the controls. Furthermore, feed conversion ratio decreased in laying hens fed with 20 mg/kg GEN, compared to the control group, during the last week and whole experimental period ($P<0.05$). It is worth mentioning that the two groups were similar in terms of egg weight.

3.2. Egg Quality

Table 4 tabulates the GEN effects on egg quality. As can be observed, GEN has improved calcium-related indices, such as eggshell thickness, weight, and strength ($P<0.05$), showing significant responses to

GEN dietary supplement; moreover, these indices are greater in laying hens fed with 20 mg/kg GEN, compared to the control group. However, there are no differences between the two groups regarding egg shape index, yolk weight, yolk and albumen height, as well as Haugh Unit (P>0.05).

3.3. TRPV6 and CaBP‑D28K mRNA Expression in ESG

TRPV6 quantification and CaBP-D28k mRNA expression were measured by Real-time PCR, and the results are shown in Figure 1. The mRNA expression of CaBP-D28k was greater in hens receiving GEN, compared to the controls (P<0.05). In addition, the mRNA expression of TRPV6 in the ESG of birds was greater in the GEN group, compared to the control group (P<0.05).

3.4. Bone Physical Characteristics and Mineralization

As shown in Table 5, GEN significantly affected the tibia length and weight of birds, compared to the control group. Furthermore, the volume of bone was increased when GEN was added to the bird's diet (P<0.05). Moreover, biomechanical properties, such as tibia breaking strength (TBS, kg/cm^2) in hens receiving 20 mg/Kg GEN was higher than that in the control group. The mineral content of Ca in the tibia was increased after GEN treatment (P<0.013), and the level of P in the tibia of GEN treatment was also significantly higher than that in the control group (P<0.05).

Table 3. Effect of dietary GEN on laying performance

	GEN (mg/kg)			
Variables	0	20	SEM	P-value
Egg production (%)	74.80	83.04	1.03	0.001
Feed intake (g/hen per day)	120.11x	129.75	1.63	0.041
Egg mass (g/hen per day)	48.20	51.49	0.68	0.020
Feed conversion (g feed /g egg)	2.49	2.22	0.04	0.001
Egg weight (g)	63.63	62.04	0.33	0.023

Table 4. Effect of dietary GEN on egg quality of laying hens

	GEN (mg/kg)			
Variables	0	20	SEM	P-value
Egg shape index	1.27	1.35	0.003	0.001
Eggshell thickness (mm)	0.42	0.54	0.030	0.037
Yolk height (mm)	1.76	1.73	0.007	0.138
Eggshell weight (%)	6.31	6.89	0.050	0.026
Eggshell strength (Kg/cm^2)	3.58	4.31	0.081	0.040
Albumen height (mm)	8.12	8.13	0.083	0.961

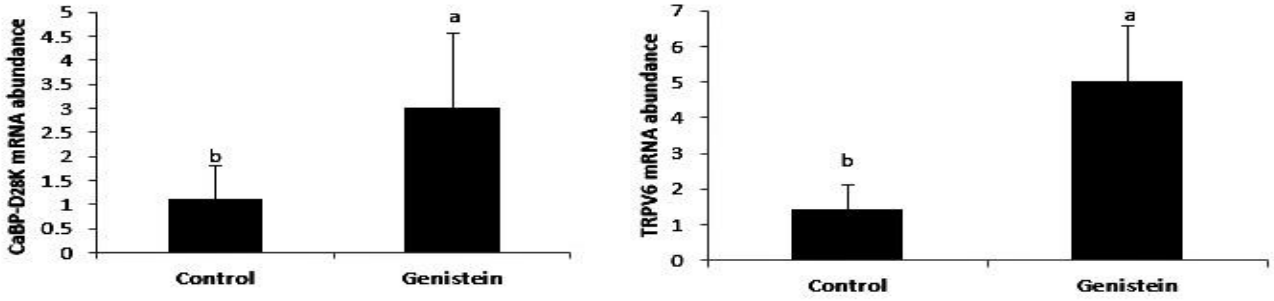

Figure 1. mRNA abundance (LSM±SEM) of CaBP-D28K and TRPV6 in the eggshell gland of laying hens

Table 5. Effect of dietary GEN on mechanical properties of tibia bone of laying hens

	GEN (mg/kg)			
Variables	0	20	SEM	P-value
Weight (g)	6.82	7.83	0.12	0.049
Length (mm)	112.14	115.61	0.02	0.042
Diameter (mm)	7.44	7.69	0.07	0.109
Volume (cm^2)	8.96	9.35	0.21	0.019
TBS[1] (kg/cm^2)	377	496	6.5	0.010
Ca (% ash)	28.29	30.56	0.22	0.010
P (% ash)	65.22	96.35	4.32	0.036

[1]Tibia Breaking Strength, Calcium, and Phosphorus in tibia (% ash)

4. Discussion

Evidence provided by the present study showed that GEN diet noticeably affected the feed efficiency, egg mass, and egg production during the post-peak period of egg-laying Hy-line W-36 that was consistent with the results of previous studies (Saitoh et al., 2001; Zhao et al., 2005; Sahin et al., 2007; Akdemir and Sahin, 2009). Jiang et al. (2007) reported that the addition of ISFs 10 or 20 mg per kilogram to the diet increased feed intake, weight gain, and bodyweight of male broilers significantly, which was in line with the results of this study. In addition, daidzein supplementation (another ISF existing in soy) dose-dependently refined the egg production in post-peak period of laying hens (Ni et al., 2007) and Shaoxing ducks (Zhao et al., 2005).

Additionally, GEN could overrun blank binding sites of estrogen receptors and act as an estrogen agonist that concluded total raise in systemic estrogenic effect. Moreover, mRNA expression of gonadotropin receptors upregulated by agonistic actions of GEN will improve follicle development in chicken that would result in redeveloping follicles and laying performance at post-peak (Cassidy, 2003; Liu and Zhang, 2008). The increased egg weight can be referred to both arising feed intake and phytoestrogen effects of GEN.

The results of the present study showed greater eggshell strength and thickness in laying hens fed with GEN, compared to controls that were consistent with the findings of the previous studies (Akdemir and Sahin, 2009). The important role of calcium in the formation of eggshell is well known; in addition, it has been revealed that soy isoflavones can lessen the concentrations of intracellular calcium in osteoclasts (Gao and Yamaguchi, 1998; Kajiya et al., 2000) providing more calcium for the formation of eggshell (de Matos, 2008). Furthermore, a study reported that GEN could increase Ca levels in the shell gland (Gao and Yamaguchi, 1998). Moreover, the upregulation of the expression of the Ca pump which is ATP-dependent is another action of estrogen in the plasma membrane of the human uterus (Yang et al., 2011);

accordingly, GEN as an estrogen agonist could possibly demonstrate the same impact on Ca amount and increase it in the ESG. As was expected, the improvement in egg quality in response to the enhancement of calcium either from absorption or retention using GEN supplementation was observed in the results. Therefore, it was indicated that the improvement of eggshell quality can be prospective by amending the metabolism of Ca using GEN. Furthermore, it has been shown that the shell thickness, egg weight, and Haugh unit of quails can be increased using dietary GEN at 800 mg/kg (Akdemir and Sahin, 2009).

The capacity of the shell gland to deliver large amounts of Ca to the uterine is of significant importance for the formation of eggshell during the calcification process. As the results showed, the TRPV6 and CaBP-D28K expression increased significantly during GEN in the ESG, compared to the control group, which demonstrated their importance during eggshell formation (Jonchere et al., 2012). The large volumes of Calbindin in the intestine and ESG of avian have been found along with tissues that play vital roles in transport huge amounts of Ca^{+2} (Taylor and Wasserman, 1967; Sugiyama et al., 2007; Bar, 2009). Ca^{2+} transport and Calbindin are related in both tissues (Bar et al., 1992; Bar, 2009), and Calbindin concentration in the ESG and the Ca^{2+} deposition in shell are correlative (Bar et al., 1992; Yosefi et al., 2003). Moreover, researchers have revealed that Calbindin-D28k synthesis is induced by estrogen in the ESG of avian (Bar et al., 1992, 1999). Therefore, it is probable that the receded Calbindin expression in the ESG during post-peak could be related to decreased estrogen secretion. In the present study, increased expression of CaBP-D28K in the GEN group indicates that the use of supplemental GEN can compensate the estrogen shortage and eventually improves Ca^{2+} transport to the lumen of the shell gland and finally ameliorates eggshell quality and strength. On the other hand, TRPV6 which is a Ca^{2+} channel located in the apical part of the epithelium of ESG and involved in the active Ca^{2+} transport from the epithelial cells into the uterine lumen rich in calcium fluid is significantly influenced after GEN supplementation. Increased levels of TRPV6 expression and improved shell quality and thickness demonstrate that GEN, as a soybean ISF, could supply adequate estrogen. This is important in the Ca^{2+} provision through the pattern of shell calcification by TRPV6 as a transcellular Ca^{2+} transport and CaBP-D28K in the ESG, which is consistent with the findings of the previous studies (Yang et al., 2012; Yang et al., 2013). It suggests an analogue of TRPV6 mRNA expression in ESG which is also observed in the duodenum of the intestine and plays a key role in shell formation.

Laying hens that are raised in cages are disclosed to the bone metabolic diseases during post-peak period (Sahin et al., 2007), which are the reasons for economic costs to the poultry industry. The results of this study showed that in the tibial bone, the supplementation of GEN not only raised the Ca and P levels noticeably well but also enhanced the strength of it that was in line with the results of a study performed by Lv et al. (2019). They reported that 400 mg/kg GEN significantly improved the TBS index, compared to the control group of laying hens; moreover, the Ca level in laying hens that received 40 and 400 mg/kg GEN were dependent on the dosage of supplemental GEN in diets. It has also been proved that GEN in the human intestine can affect positively calcium absorption by its estrogenic effects (Arjmandi et al., 2005). Another study by Gjorgovska et al. (2016) revealed similar results; accordingly, beneficial effects ($P<0.05$) on aged laying hens were noted using the high supplementation of ISF (1800 mg/kg of feed). Furthermore, some bone indices showing its quality, such as volume, ash/tibia calcium, and weight, were improved; however, they could not affect the phosphorus content in the tibial bone ($P>0.05$). This suggests that the ISFs supplements will be effective to improve the bone calcium content and body weight during post-peak. In another study carried out by Sahin et al. (2007), the use of soy isoflavones as supplements obviously improved

mineral density in bones of Japanese quail. It has been demonstrated that the secretion of acidic materials can be inhibited from osteoclasts by the GEN and finally conduces to form brittle bones (Barnes and Blair, 1996). In addition, the concentration of intracellular Ca can be decreased in osteoclasts using ISFs (Zhao et al., 2016). Therefore, according to the recent results, dietary GEN enhanced Ca metabolism through the regulation of Ca and P deposition and absorption, which eventually caused improvements in bone strength of Hy-line W-36 hens during post-peak.

In conclusion, the obtained data in the present study showed that the addition of 20 mg/kg of GEN to the diets of laying hens at the post-peak period could improve feed efficiency, egg mass, egg production, and laying performance. Moreover, the enhancement of the egg quality associated with calcium metabolism is another effect of the main ISF (GEN). Finally, it was indicated that the use of 20 mg/kg of GEN could be an effective additive to improve bone mechanical properties and density of Hy-line W-36 laying hens at the post-peak period.

Authors' Contribution

Study concept and design: F. S.
Acquisition of data: T. S.
Analysis and interpretation of data: T. S., M. K. and F. G.
Drafting of the manuscript: T. S., F. S. and M. K.
Critical revision of the manuscript for important intellectual content: T. S., M. K. and F. S.
Statistical analysis: S. H., M. K. and T. S.
Administrative, technical, and material support: S. H., M. K. and T. S.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

This study was granted by Gorgan University of agricultural sciences and natural resources.

Acknowledgment

We would like to thank Dr. Essa Dirandeh for his help in all parts of experiment.

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