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Egg production, egg quality, blood lipids, and ovarian related indices in laying hens fed pomegranate by-products

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ABSTRACT: The present experiment was conducted to investigate effects of pomegranate seed (PS) on egg production variables, egg quality traits, blood biochemical attributes, and ovarian related indices of laying hens. A total of 360 Hy-line laying hens (w-80), at 25 weeks of age were randomly allotted to 5 dietary treatments in a completely randomized design during a ten-week period. Experimental treatments consisted of a basal diet (CTL) or supplementation of 1 g, 2 g, 3 g, and 4 g PS/kg to basal diet. Supplementation of 4 g PS/kg feed increased egg production rate of laying hens by 2.6% ($p<0.05$) while the other production variables were not affected by dietary treatments. Dietary inclusion of 3 or 4 g PS/kg tended to increase yolk color by 10.7% compared to layers fed on CTL treatment. The greatest large and small yellow follicles count and ovary weight observed when laying hens received 4 g PS/kg feeds compared to those fed on the other dietary treatments ($p<0.05$). Blood cholesterol content decreased in response to feeding 4 g/kg PS supplemented diets ($p<0.05$). Furthermore, blood triacylglycerol concentration decreased in layers fed diets containing 3 or 4 g PS/kg ($p<0.05$). In conclusion, dietary supplementation of 4 g/kg PS seems suitable to improve egg production rate and decrease blood cholesterol and triacylglycerol contents.

***Keywords*:** Laying hen, Pomegranate seed, Egg production, Blood lipids, Ovary

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INTRODUCTION

Nowadays, the world's population has been growing and human food expectations such as the need for animal protein resources have increased (Hernández et al., 2004). Also, with expectations for increasing human population by 2050 (Bongaarts, 2009), higher meat production by 73% is required to meet the human demand (Bruce, 2016). On the other side, the approach of increasing poultry products might impose greater metabolic rate and favors increased production of the reactive oxygen species over the antioxidant capacity of them. There is many information in the literature, indicating that oxidative stress severely affects poultry performance and health (Rafiei and Khajali, 2021; Surai et al., 2019). Oxidative stress stimulates apoptosis in follicular cells and results in decreased follicle numbers and consequently reduces egg production (Li et al., 2020). Moreover, stress has detrimental effect on egg quality traits and cholesterol concentrations (Scanes, 2016). As such, advanced strategies are needed to increase the production rate without any sever effects on laying hen.

The use of agricultural by-products in poultry diet has been common for a long time. In this regard, appropriate processing of these materials make a suitable feed ingredient, with reducing effects on the environmental pollution (Azizi et al., 2018). At the same time, nutritional value of some by-products may let the nutritionists to use them as feed additives. Pomegranate belongs to the *Punicaceae* family and is the native fruit of the Iran and Mediterranean (Azizi et al., 2018). Some phenolic contents of pomegranate (ellagic acid and punicalagin) influence its antibiotic activity (Sarkhosh et al., 2007) and some of the others (punicalagin, punicalin, gallic acid and particularly ellagic acid) was reported to affect serum lipid concentrations of rabbits (Rajabian et al., 2001). In pomegranate processing factories, large amounts of by-products including outer peel and pomegranate seed (PS) pulp are produced. The PS are rich in sugars, vitamins, polysaccharides, polyphenols, minerals and poly-unsaturated fatty acids (Miguel et al., 2004). Accordingly, pomegranate by-products have shown to possess antioxidant (Dhar, 2006; Louba, 2007), and immune progressive (Yamasaki et al., 2006) properties when applied in animal diet. Recently, researchers have shown the key role of pomegranate active components on genes associated with metabolism of lipids and body fat reduction properties. (Koba et al., 2002). Also, isoflavones as the main phyto-estrogen compound available in pomegranate (Oguike et al., 2005) may influence ovary follicles and subsequently affect egg production of laying hens. However, existing findings on pomegranate by-products in laying hens are contradictory. It has been shown that egg production, egg mass, and daily feed intake (DFI) of laying hen (Kostogrys et al., 2017) and laying quail (Abbas et al., 2017) improved in response to feeding dietary supplemental pomegranate peel powder and PS oil, respectively. Moreover, Eid et al. (2021) demonstrated the ameliorating impact of pomegranate peel powder on production rate of laying hens exposed to oxidative stress. Nevertheless, the other researchers did not show similar results (Gultepe et al., 2022; Saki et al., 2014). On the other hand, although egg qualitative traits including eggshell thickness, egg shell breaking strength and, haugh unit were not affected by pomegranate by-products (ÇEtİNgÜL et al., 2019; Saki et al., 2014), yolk index improved in the work of Kostogrys et al. (2017). Thus, more investigations are warranted to explore the effect of pomegranate by-products on laying hens. Furthermore, to our knowledge, this is the first experiment studding the effect of pomegranate by-products on ovarian related indices of laying hens. It was hypothesized that dietary supplementation of PS may affect egg production rate through modifications in ovarian features or change in quality of egg through variations in blood attributes. Thus, the present experiment was designed to investigate effects of supplemental 1 g, 2 g, 3 g, and 4 g PS/kg feed on egg production variables, egg qualitative indices, serum biochemical attributes and ovarian morphology in laying hens.

MATERIALS AND METHODS

All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Faculty of Animal Science, Islamic Azad University of Shahrekord (approval ref. no. 2017-056). The experiment was conducted according to the regulations and guidelines established by this committee.

Preparation of pomegranate seed

The pomegranate was purchased from a local market as fresh Iranian pomegranate (Malas Saveh). The seeds were manually removed from arils and then air dried under ambient condition and finely ground in a grinder to pass 40-mesh. The seed powder was finally packed and stored at −18°C.

Pomegranate seed active components

Determination of total antioxidant activity, total phenolic content, and total flavonoids of pomegranate seed (Table 1) applied through the methods described in the work of Derakhshan et al. (2018).

Birds, management, and experiment design

A total of 360 Hy-line-laying hens (w-80), at 25 weeks of age were randomly distributed into 5 dietary treatments consisting of 6 replicates with 12 birds per replicate in a completely randomized design. Experimental treatments consisted of 5 groups of birds fed a basal diet (CTL) or basal diet supplemented with either 1 g, 2 g, 3 g, and 4 g PS/kg during the course of 10 weeks. Iso-energetic and iso-nitrogenous experimental diets were formulated to be used in this experiment. The feed formulation was performed according to Hy-Line W-80 breeder recommendation guideline and according to their production phase (Table 2). All birds were kept in cage production system in an environmentally controlled house and have had free access to water and mash feed in 4-level cages (120 cm × 60 cm × 50 cm) with controlled ventilation and lighting (16L: 8D). The troughs were filled up twice a day (6 AM and 3 PM), and the diets were added in the trough. Prior to trial commence, a 2-week pre-experiment period was carried out to ensure the eggs production rate is the same for each replicate.

Egg production variables and egg qualitative indices

Data on daily feed intake (DFI) for the whole period of the experiment were recorded for each treatment replicate. Collecting and weighting of eggs were done at the same time every afternoon. Egg production rate, egg weight, daily egg mass production (g egg/hen/day) and feed conversion ratio (FCR, g feed/ g egg) were calculated for the entire production period.

In total, 120 eggs (4 eggs/cage) were used for egg quality examination. After weighing the individual eggs, each egg was broken using an egg force reader (Orka Food Technology, Bountiful, UT) to measure eggshell breaking strength. Eggshell thickness is reported as an average value based on the thickness of eggshell measured at 3 different places (upper end, lower end, and the middle) using a micrometer screw gauge (Suce measuring instrument Co., Ltd., Nanjing, Jiangsu, China). An automatic egg quality analysis instrument was used to measure the haugh units and yolk color (EMT- 5200, Robotmation Co., Ltd., Tokyo, Japan). The albumen was removed from shells and shells plus membranes were weighed after 24 hours of air drying. Specific gravity of the egg was measured by dipping the eggs in a predetermined salt solutions specific gravity ranging from 1.060 to 1.100 (Peebles and McDaniel, 2004).

Ovarian related indices

At the end of the experimental period, 8 layer hens from each treatment were randomly chosen and the reproductive tract (from the infundibulum to the cloacae terminal) was detached and weighed, as was the oviduct. The ovary weight was recorded and the number of follicular hierarchies was counted. Ovarian follicles were separated from the stroma, classified as greater or less than 10 mm diameter, and counted.

Serum biochemical parameters

At the end of the experimental period, blood samples were taken from 2 birds of each cage and collected in non-heparinized tubes by brachial vein puncture. Serum was separated via 2000 × g centrifuge of blood samples for 15 minutes (SIGMA 4-15 Lab Centrifuge, Germany). Individual serum samples were analyzed for total cholesterol, triacylglycerol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium, phosphorus, iron, and magnesium with a spectrophotometer using the kit package (Pars Azmoon CO; Tehran, Iran).

Statistical analysis

Data for recorded traits were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedure of SAS 9.2 (SAS Institute Inc., Cary, NC). For all statistical analyses, significance was declared at $p \leq 0.05$, unless otherwise stated. The tukey test was used for multiple treatment comparisons.

RESULTS

Egg production variables and egg qualitative indices

Effect of experimental treatments on egg production and egg qualitative variables are shown in Tables 3 and 4, respectively. Supplementation of 4 g PS/kg feed increased egg production rate by 2.6% compared to CTL diet ($p<0.05$). The other production variables were not affected by dietary treatments. On the other hand, laying hens fed 3 to 4 g PS/kg diet tended to have greater yolk color by 10.7% than birds fed CTL diet.

Ovarian related indices

Data related to ovarian attributes are shown in

Table 5. The ovary weight increased in birds dietary supplemented with 4 g PS/kg diet compared to the other dietary treatments ($p<0.05$). The greatest count of large and small yellow follicles observed when birds received 4 g PS/kg in their feeds ($p<0.05$). Also, dietary supplemental 2 and 3 g PS/kg tended to increase small yellow follicles compared to control diet and 1 g/kg PS supplementation to the feed.

Serum biochemical parameters

As indicated in Table 6, serum concentration of cholesterol decreased when birds fed 4 g/kg PS compared to CTL treatment or those fed 1 g/kg PS in the feed ($p<0.05$). Furthermore, concentration of triacylglycerol decreased in birds fed diets containing 3 and 4 g PS/kg than those in CTL treatment ($p<0.05$).

DISCUSSION

Pomegranate and its by-products present series of merits including antioxidant, lipotropic, and immune progressive features (Dhar et al., 2006; Koba et al., 2002; Louba, 2007; Yamasaki et al., 2006). These positive properties make expectations for their beneficial influence on performance and quality of poultry products. In the current experiment, basal diet supplemented with PS in different levels to study its effect on laying hen due to the controversial over the effect of pomegranate by-products on productive or qualitative traits of egg. The PS was evaluated for its antioxidant (38 %), total phenolic (72.8 mg GAE/g), and total flavonoid (25.35 mg rutin/g) contents. All these values fell within the physiological range similar to the other Iranian pomegranate cultivars as indicated in the work of Derakhshan et al. (2018).

The improved egg production rate of laying hen fed supplemental 4 g PS/kg might be associated with its antioxidant contents. These active components reduce the effect of free radicals on body organs and also may enhance the palatability of diets, as tended to improve DFI in this experiment. Similarly, supplementation of 25 to 75 g pomegranate peel powder/kg feed have shown to improve egg production rate of laying Japanese quail (Abbas et al., 2017). Also, Kostogrys et al. (2017) reported the increased egg production and egg mass of laying hen fed diets containing 5 to 15 g punicic acid/kg. Furthermore, Eid et al. (2021) indicated that inclusion of 4 g pomegranate peel powder/kg feed alleviated negative impact of Dexamethasone on production rate of laying hen. On the contrary, Habibi et al. (2019) and Gultepe et al. (2022) could not found any effect of pomegranate by-products on laying japanese quail and laying hen, respectively. The reason behind the variable aforementioned results might be explained by consumption of different pomegranate by-products and their variable contents of active components (Gözlekçi et al., 2011).

Eggshell thickness and egg specific gravity are important indicators for eggshell breaking strength during the storage and processing of table eggs (Wells, 1967). Nutrition is one of the influential factors on the shell thickness. In accord to our results, Saki et al. (2014) demonstrated that supplementation of 0 to 150 g PS pulp/kg diet of laying hen failed to affect eggshell breaking strength. Furthermore, graded levels of 0 to 10 g/kg pomegranate molasses to the feed did not affect eggshell thickness in laying hen (ÇEtİNgÜL et al., 2019). Also, Gultepe et al. (2022) could not find any effect of 1% to 10% pomegranate juice on egg breaking strength when added to drinking water. Additionally, Saki et al. (2019) reported that diet of laying hens added with pomegranate by-product did not affect the specific gravity of egg, however, supplementation of that beyond the 80 g/kg decreased eggshell thickness. Gil et al. (2000) believed that efficacy of pomegranate by-products is not only depending on its different antioxidant capacity and active compounds, but also on the processing of these products. It shows that type, amount, and processing of pomegranate by-products may affect eggshell breaking strength. In the current experiment, yolk color was darker in response to feeding diets supplemented with 3 g and 4 g PS/kg, suggesting the greater active content of them such as flavonoids (Gultepe et al., 2022), particularly compared to those fed 2 g PS/kg feed. Some of the active substances of phytogenic feed additives might reduce undesirable effects such as changing in odor, taste or color caused by oxidation. Similarly, Kostogrys et al. (2017) found that dietary 15 g/kg PS oil increased yolk color in laying hens. However, Gultepe et al. (2022) showed lighter yolk color when added pomegranate juice to drinking water. Further research is needed to find the underlying mechanism for the effect of pomegranate by-products on egg yolk color.

Ovarian features were evaluated to clarify if there is any influence of PS on egg production rate through the change in ovarian follicles. As laying hens become older, egg production decreases in conjunction with weakness in the process of recruitment of follicles into the hierarchy and reduction in ovarian steroids and gonadotropins (Williams and Sharp, 1978).

Moreover, exposure of laying hens to oxidative stress stimulates apoptosis in follicular cells and results in reduced follicle numbers and consequently egg production (Eid et al., 2001). It has been shown that injecting follicle-stimulating hormone (FSH) to old hens, subcutaneously, increased estrogen concentrations, increased number of small follicles in their ovaries, and then stimulated rapid growth phase of small follicles in laying hens (Johnston and Gous, 2003). Thus, administration of exogenous substances may positively affect ovarian features in laying hen. Isoflavones are the main phyto-estrogen compounds available in natural plants such as pomegranate (Oguike et al., 2005). In the current study, weight of ovary and numbers of small and large follicles were influenced by supplementation of PS, in line with improvement in the rate of egg production. Saki et al. (2014) stated that feeding graded levels of a phytogenic feed additive increased ovary weight and number of small follicle in laying hens. Zhao et al. (2005) reported that ovary weight or the numbers of hierarchical follicles were not affected by supplementation of Daidzein as a type of isoflavones, but oviduct weight was increased. Source of phyto-estrogens, dose, duration of use, and intrinsic oestrogenic state are probable reasons for variable results in birds received phytogenic active components (Cassidy, 2003; Price and Fenwick, 1985).

For many years, there has been a concern over the use of eggs due to cholesterol content of them and risk of coronary disease. As such, lower cholesterol content of egg might be a favorable trait for human health. The arrangement of lipids formed in the liver for yolk synthesis can be affected by dietary modifications (Walzem, 1996). In the current study, blood cholesterol and triacylglycerol concentration decreased by increasing supplemental levels of PS in the feed. Pomegranate presents beneficial effects on metabolism of lipids (Hou et al., 2019). It has been demonstrated that pomegranate peel polyphenols, punicalagin, and pomegranate ellagic improve metabolism of cholesterol by increasing total bile acid production in the liver cells (Lv et al 2016). Additionally, PS is a rich source of naturally occurring compounds such as B-sitosterol, campesterol, and stigmasterol with partially similar structures to the cholesterol (Prakash and Prakash, 2011). These steroids compete with absorption of dietary cholesterol, and also inhibit the re-absorption of endogenous cholesterol in the gastrointestinal tract (John et al., 2007). It may be the reason for decreasing effect of pomegranate by-products on blood cholesterol content in laying hens. In line with the results of this experiment, Abbas et al. (2017) stated the reduction in blood cholesterol and triglyceride concentrations in response to supplementation of pomegranate peel powder in diet of laying quail. Also, Ahmed et al. (2015) found lower meat cholesterol content in broilers fed diets containing pomegranate by-products. Furthermore, blood cholesterol, triacylglycerol and LDL decreased when diet of broiler chickens added with pomegranate by-products compared with un-supplemented treatment (Yaseen et al., 2014). Nevertheless, Gultepe et al. (2022) could not find any impact of pomegranate juice on blood cholesterol content of laying hen despite their indirect response of their liver enzymes. More investigations on the effects of dietary PS on egg lipid contents are warranted.

CONCLUSION

Dietary supplementation of PS improved egg production rate while did not affect the other production variables and eggshell breaking strength. Also supplemental 4 g/kg PS numerically increased egg yolk color. The increased number of large and small ovary follicles observed after supplementation of pomegranate by-products in the feed, supporting the improvement in egg laying rate. Furthermore, blood cholesterol and triacylglycerol concentrations decreased following dietary inclusion of pomegranate by-products, suggesting lipotropic effects of PS. It seems that 4 g supplemental PS/kg is a recommendable level for supplementation in diet of laying hen.

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