

Egg Iron Enrichment in Response to Various Levels of Pomegranate by-Product in Laying Hen Diet

Research Article

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ABSTRACT

The present study was conducted to investigate the effects of different levels of pomegranate by-product (PBP) on performance, egg quality and blood parameters in laying hens. A total of 144 Bobze layer hens from 44 to 56 weeks during 12 weeks were randomly assigned into 4 treatments including 0 (control, corn and soy bean meal), 4, 8 and 12% of PBP, with 4 replicates and 9 layers in each. Results have indicated that there was a significant increase in egg mass, egg production the decrease in feed intake and feed conversion ratio by 4% PBP in comparison to other treatments ($P < 0.05$). Significantly increased egg weight by inclusion of 8% PBP compared with 0 and 12% PBP. There was no significant effect of PBP levels on external and internal egg characteristics. The lowest cholesterol and the higher high-density lipoprotein and malondialdehyde have shown by 4% PBP ($P < 0.05$). The highest amount of iron in egg yolk was observed respectively by treatments 4 and 12% PBP. It was concluded that egg yolk iron and production rate were increased by 4% PBP supplementation in diet.

KEY WORDS egg, iron enrichment, laying hen, performance, pomegranate by-product.

INTRODUCTION

Eggs bring up as a good source of the three principal nutritional requirements: energy, protein and essential ancillary factors (vitamins, carotenoids, minerals and certain fatty acids). In terms of this fact eggs are economical feed and near the perfect food (Ramadan *et al.* 2010). Today nutritionists have close attention to increasing the nutritive value of eggs through altering the nutritional composition of the layer hens diet. Many types of minerals can also be enriched in designer eggs. This could be achieved by the dietary manipulation of hen's diet. These trace minerals are very important for human health because the deficiency of these components are leading to development of certain deficiency disease (Al-Massad *et al.* 2011).

Lack of iron is one of the most prevalent nutritional disorders affecting human health and being recognized as causing 2.4% of the total global burden of diseases (Ezzati *et al.* 2004; Rodgers *et al.* 2004). It is well known that the nutritive composition of egg can be changed by the nutritional composition of diet fed (Ambikadevi and Lalithambika, 2000; Paik *et al.* 2009).

Enriched eggs with iron will resulted in eggs with superior biological and nutritional value for human consumption (Park *et al.* 2004). In humans, iron is an essential component of proteins involved in oxygen transport (Ramadan *et al.* 2010; Varmaghany *et al.* 2013). Park *et al.* (2004) have reported Fe content of egg could be enriched by Fe supplementation and organic forms (Fe-Met or Availa-Fe) are more effective than sulfate form.

Researches have demonstrated that iron content of eggs could be increased by organic iron supplemented (Paik *et al.* 2009). The total pomegranate production in Iran was estimated to be approximately seven hundred thousand (700000) tons annually (FAO, 2009) which accounted for half of the global production (Abbasi *et al.* 2008; Eikani *et al.* 2012). Increasing agro-industrial units for producing pomegranate juice has led to increased amounts of by-products including peels and seeds (Shabtay *et al.* 2008; Oliveira *et al.* 2010).

Annual production of pomegranate by-products (peels and seeds) exceeds one hundred and twenty thousand (120000) tons in Iran (Afshar, 2011; Taher-Maddah *et al.* 2012).

In spite of sufficient knowledge of the biological effects of pomegranate by-product in human and animal health, there is scant information on its nutritive value for poultry (Viuda Martos *et al.* 2010). Pomegranate peels are characterized by an interior network of membranes comprising almost 26-30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit (Negi *et al.* 2003; Afaq *et al.* 2005; Zahin *et al.* 2010).

Iron and magnesium are present in large quantities in the peel as compared with comestible part, the pomegranate contains 47.5 mg/g (Nasr *et al.* 1996). According to the Iran situation in facing the anemia crisis especially in children, in this study an attempt has been made to enrich egg yolk iron content in respect to different levels of pomegranate by-product (PBP) iron in layers diet.

MATERIALS AND METHODS

Animals, management and sample collection

In total 144 Bovans laying hen with an initial age of 44 weeks were in completely randomized design (CRD) allocated into 4 dietary treatments, 4 replicates and 9 birds in each.

Four dietary treatments were included: a control diet (without PBP), and 2, 3 and 4 treatments by 4, 8 and 12% PBP levels respectively, during 12 weeks experiment. The experimental composition diets presented in Table 1. Feed intake (FI) was recorded weekly and egg production (EP) and egg weight (EW) were considered daily. Average daily feed intake (ADFI), egg mass (EM) hen-day egg production (HDP) and feed conversion ratio (FCR) were calculated by these information.

Egg mass was calculated by multiplying percentage egg production by egg weight for each replicate. Hen-day egg production was computed using following formula given by North (North and Bell, 1990):

Egg production (%) = (number of egg produced on each day/number of hens alive on each day) × 100

At 56 weeks of age, blood samples (5 mL) were collected from the brachial vein and centrifuged for 20 minutes at 3500 rpm to obtain plasma samples. Plasma samples were used for the determination of the cholesterol, triglyceride, high density lipoprotein (HDL), total antioxidant (TA) and malondialdehyde (MDA) contents. Cholesterol, triglyceride and HDL were measured by enzymatic method. Malondialdehyde was tested as an index of lipid peroxidation by the thiobarbituric acid (TBA) method according to Hong *et al.* (1989).

Total antioxidant was assayed by Randox kit according to Miller *et al.* (1993). Egg internal (albumen and yolk indexes, Haugh unit (HU)) and external (egg shell weight, ratio and breaking strength) quality parameters were determined in four randomly selected eggs in each replicate in every two weeks (16 eggs in treatment in 2 weeks). After weighting eggs individually, they were broken by sheer pressure to measure breaking strength, and then the shells were washed and dried in an oven for determination of shell weight (Harms and Russell, 2001).

Also, total lipid present in a homogenized sample of egg yolk were extracted by the method of Folch *et al.* (1957) and determination of egg yolk cholesterol according to Caston and Leeson (1990) and egg yolk iron according to Kiliç *et al.* (2002). Albumen and yolk heights and widths were measured for Haugh unit and yolk index respectively in each egg. Then Haugh unit was calculated using the following formula (Eikani *et al.* 2012):

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

Where:

AH: albumen height.

EW: egg weight (g).

Chemical analysis of feed

In order in formulating diet initially dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash and gross energy (GE) in feed analysis laboratory system by using AOAC (1990) were determined. Also, the amount of tannin based on the method (Schofield *et al.* 2001) and iron (AOAC, 2000) were measured by Spectrophotometry (Table 2).

Table 1 Composition and chemical analyses of experimental diets

Ingredients (%)	Pomegranate by-product (%)			
	0	4	8	12
Corn grain	54.31	51.11	47.91	44.97
Soybean meal (44%)	24.05	20.53	17.00	13.07
Wheat	5.00	5.00	5.00	5.00
Corn gluten (62%)	1.4	3.47	5.54	7.84
Pomegranate by-product	0	4	8	12
Soybean oil	2.50	3.00	3.50	3.90
Dicalcium phosphate	1.71	1.76	1.82	1.87
Oyster shell	9.92	9.92	9.92	9.92
Common salt	0.34	0.34	0.34	0.34
Vitamin premix ¹	0.25	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25	0.25
DL-methionine	0.26	0.26	0.25	0.24
L-lysine	0.01	0.11	0.22	0.35
Total	100	100	100	100
Chemical analysis (%)				
Metabolizable energy (kcal.kg)	2800	2800	2800	2800
Crude protein	16.8	16.8	16.8	16.8
Crude fiber	2.77	4.24	5.32	7.24
Ether extract	2.26	2.30	3.32	3.40
Calcium	4.22	4.22	4.22	4.22
Avail. phosphorus	0.42	0.42	0.42	0.42
Potassium	0.51	0.56	0.64	0.71
Chlorine	0.18	0.18	0.20	0.21
Sodium	0.17	0.17	0.17	0.17
DCAB	182.42	187.38	195.45	200.34
Arg				
Lys	0.82	0.82	0.82	0.82
Met	0.40	0.40	0.40	0.40
Met + Cys				
Thr	0.54	0.57	0.60	0.63
Dry matter	93.60	93.95	93.30	93.96
Ash	12.30	11.56	12.27	11.52

¹ Vitamin premix supplied per kg of diet: vitamin A: 7.2 g; vitamin D₃: 7 g; vitamin E: 14.4 g; vitamin K₃: 1.6 g; vitamin B₁: 0.72 g; vitamin B₂: 3.3 g; vitamin B₃: 12.16 g; vitamin B₅: 12 g; vitamin B₆: 6.2 mg; vitamin B₁₂: 0.6 g; Biotin: 0.2 g and Cholin chloride: 440 mg.

² Mineral premix supplied per kg of diet: Manganese: 64 mg; Iron: 100 mg; Zinc: 44 mg; Copper: 16 mg; Iodine: 0.64 mg and Selenium: 8 mg.

Table 2 Chemical analysis of feed ingredients (as-fed basis, %)

Ingredients	Chemical composition							
	DM	CP	CF	EE	Ash	GE (kcal/g)	Tannins	Iron (mg/kg)
Corn grain	88.21	8.082	2.18	4.36	1.79	455	-	-
Soybean meal (44%)	91.87	44.19	6.20	1.43	5.94	461	-	-
Corn gluten (62%)	90.50	67.6	2.13	2.50	5.1	372	-	-
Pomegranate by-product	93.7	16.79	28.86	10.48	3.37	468	3.5	9.01
Wheat grain	92.74	11.85	1.69	2.44	1.53	-	-	-

DM: dry matter; CP: crude protein; CF: crude fibre; EE: ether extract and GE: gross energy.

Statistical analyses

Results compared using SAS 9.1 (SAS, 2008) software. For performance data the statistical model was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} : observations.

μ : overall mean.

T_i : effect of treatment.

e_{ij} : residual error.

Plasma and egg parameters data were compared using the following model:

$$Y_{ijk} = \mu + T_i + e_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} : observations.

μ : overall mean.

T_i : effect of treatment.
 e_{ij} : random error.
 ε_{ijk} : subsampling error.

Means were separated by Duncan's multiple range tests. Treatments differences were considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

The results of laying hens performance were presented in Table 3. There were the highest EM and EP and lowest FCR and FI by treatment 2 (4% PBP). Egg weight were increased by 8% of PBP in comparison to all treatments groups ($P < 0.05$). There were no significant effect of PBP levels on external and internal egg characteristics (Tables 4 and 5). Shell thickness significantly ($P < 0.05$) increased in control and 4% PBP compared to other treatments. Yolk color score was increased significantly ($P < 0.05$) with higher levels of dietary PBP. Plasma cholesterol decrease and plasma HDL increase by 4% PBP in diet compared with other treatments ($P < 0.05$).

The lowest levels of LDL were shown in hens feeding by basal diet. No significant differences were observed in triglyceride and total antioxidant among treatments (Table 6). As shown in Table 6, MDA concentration increased significantly by 4% PBP in comparison to other treatments ($P < 0.05$).

No differences were found on egg yolk cholesterol in controls and other PBP treatments, Table 7 ($P > 0.05$). Egg yolk iron levels significantly increased by 4 and 8% PBP treatments compared with control ($P < 0.05$). In contrast no significant difference has shown in plasma iron levels by increasing PBP in diet. It was clearly noted that increasing levels PBP in diet, enhance the levels of iron in the diet treatments. The highest amount of iron in egg yolk was observed respectively by treatments 4 and 12% PBP, whereas the control treatment was in the lowest levels of iron (Table 8).

The highest EM and EP and lowest FCR and FI were observed by 4% PBP in diet which may be due to palatability of this level of PBP in the diet. Improvement in EW was observed in diet containing 8% of PBP which could be due to increase of FI (Sunder *et al.* 2013). It was found that with the increased concentration of pomegranate by-product in the hens' diet, FI was significantly increased that agreement with (Kostogryś *et al.* 2017) reported the lowest consumption was observed at the highest concentration of pomegranate seed oil. In terms of performance, the results of Saki *et al.* (2014) on performance was in line with this study.

They have shown that body weight gain (BWG), FCR and FI of broiler chickens were not affected by pomegranate peel powder (PPP) treatments. Also these results were disagreement with Rajani *et al.* (2011) who have shown that BWG, FCR and FI of broiler chickens were not affected by pomegranate peel treatments. However, PPP had significant effects on yolk diameter and shell weight (Yassein *et al.* 2015). According to Hamady *et al.* (2015), the improvement in FCR and EP is attributed to the positive effect that plant feed (pomegranate peel extract) additives exert on gastro-intestinal enzymatic activity and thus enhancing nutrients absorption and digestibility. Pomegranate by-products are natural growth promoter attributed to the level of polyphenolic compounds, especially pomegranate peel have ellagitannins, gallagic acid, punicalagin and ellagic acid (Bostami *et al.* 2015). In addition, some investigations have shown antibiotic activity pomegranate by-products due to phenolic compounds particularly ellagic acid and punicalagin (Hassan *et al.* 2012; Saeed *et al.* 2018), which might improve the growth performance with reduce the microbial into the gastrointestinal tract. Consequently, it favors improvement of performance in the present study.

Supplementation of PBP levels in diet did not affect the external and internal egg characteristics. Kostogryś *et al.* (2017) reported that some parameters of egg quality (e.g. shell thickness, shell strength, yolk color, yolk index, egg diameter, Haugh units) were improved when hens were fed diet with pomegranate seed oil. Egg yolk color has always closed attention as an important egg quality characteristic.

In this study, yolk color index was significantly increased by increasing the amount of PBP in diet that could be related by pigments in pomegranate peel. The lowest plasma cholesterol and the highest HDL have achieved by 4% PBP. Low density lipoprotein (LDL) has increased with increasing dietary PBP levels. Reduction in plasma cholesterol by 4 and 8% PBP in the diet may be due to its high fiber content. Similar results to ours were reported by Abbas *et al.* (2017) who found a decrease in serum cholesterol, glucose and triglyceride by PPP, probably due to PPP phenolic compounds (punicalagin, punicalin, gallic acid and particularly ellagic acid).

Fiber by binding with bile salt in the intestinal tract, reduces intestinal transit time and increased excretion of sterols (increased secretion of bile) affects cholesterol metabolism and ultimately leads to the lower serum cholesterol levels (Jiménez-Moreno *et al.* 2009; Mateos *et al.* 2012).

Since, HDL content of plasma was affected by dietary treatments; it probably that low density lipoprotein (LDL) and very low density lipoprotein (VLDL) are reduced in current study (Ramírez *et al.* 2013).

Table 3 Effects of different dietary levels of pomegranate by-product (PBP) on the performance of laying hen between 44-56 weeks of age

Productive performance	Dietary PBP levels (%)				SEM	P-value
	0	4	8	12		
Feed intake (g/bird)	108.61 ^b	107.13 ^c	109.83 ^a	110.42 ^a	0.326	< 0.0001
Egg mass (g)	53.48 ^a	55.29 ^a	54.04 ^b	52.2 ^c	0.262	< 0.0001
Production rate (%)	86.59 ^b	89.92 ^a	86.71 ^b	85.24 ^c	0.370	< 0.0001
Feed conversion ratio	2.04 ^b	1.94 ^c	2.04 ^b	2.13 ^a	0.011	< 0.0001
Egg weight (g)	61.78 ^b	61.35 ^{bc}	62.35 ^a	61.24 ^c	0.143	< 0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of different dietary levels of pomegranate by-product (PBP, %) on external egg characteristics (EEC) of laying hen between 44-56 weeks of age

External egg characteristics (EEC)	Dietary PBP levels (%)				SEM	P-value
	0	4	8	12		
Egg length	57.89	57.89	58.11	58.12	0.229	0.9515
Egg width	44.56	44.18	44.29	44.33	0.169	0.3247
Shape index (%)	77.04	76.36	76.33	76.33	0.330	0.7137
Egg surface (cm)	89.77	88.56	88.75	89.60	1.164	0.5634
Specific gravity (g/mm)	1.0835	1.0837	1.0842	1.0832	0.002	0.7445
Egg weight/shell surface	0.7079	0.7046	0.7050	0.7074	0.002	0.5623
Shell weight (g)	5.97	5.92	5.97	6.01	0.070	0.7446
Shell ratio (%)	9.42	9.51	9.40	9.48	0.184	0.7720
Shell thickness	0.3842 ^a	0.3855 ^a	0.3735 ^b	0.3735 ^b	0.003	0.0435

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of different dietary levels of pomegranate by-product (PBP, %) on internal egg characteristics (IEC) of laying hen between 44-56 weeks of age

Internal egg characteristics	Dietary PBP levels (%)				SEM	P-value
	0	4	8	12		
Thick albumen length	69.80	69.17	69.24	69.86	0.274	0.1204
Thick albumen width	50.17	49.77	50.20	50.07	0.260	0.278
Albumen height	6.62	6.66	6.18	6.31	0.182	0.6513
Albumen index (%)	5.52	5.62	5.18	5.27	0.160	0.6245
Yolk diameter (mm)	42.87	43.51	42.31	42.14	0.320	0.4235
Yolk height	17.50	17.31	17.23	19.93	0.112	0.4762
Yolk index (%)	40.16	41.12	39.69	39.63	0.506	0.7083
Haugh unit (%)	92.95	92.36	91.37	90.92	0.648	0.7209
Yolk color	6.48 ^d	9.81 ^c	10.60 ^b	11.34 ^a	0.086	0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Furthermore, polyphony's of pomegranate may stimulate and promote cholesterol metabolism by modifying cholesterol transport by HDL (Esmailzadeh *et al.* 2004). In an earlier experiment conducted by Saki *et al.* (2014), there were no significant differences between the control and supplementation of pomegranate seed pulp (PSP) in the hematological parameters of laying hens. Decreases in plasma cholesterol, HDL and LDL were shown by PPP supplementation in diets (Yassein *et al.* 2015). On the other hand, Hossin, (2009) have demonstrated significant decrease in total cholesterol and HDL in hypercholesterolemic rats (positive control) administrated with PPP (5, 10 and 15%) and its extracts (1, 2 and 3%), although there were no significant effect of dietary PBP on plasma triglyceride and TA concentrations in the current study.

Some researchers have reported that the pomegranate juice, pomegranate rind powder extract treatment substantially inhibited lipid oxidation in cooked chicken to a much greater extent than butylated hydroxyl toluene (BHT) treatment. Results by Yamasaki *et al.* (2006) have shown that feeding rats with 0.12 and 1.2% pomegranate oil, led to significant increase of serum triglycerides and phospholipids, but no significant response was found on total serum cholesterol. Higher MDA have shown by 4% PBP in comparison to other treatments. Previous reports (Rajabian *et al.* 2001; Saki *et al.* 2014) further have supported these results. Malondialdehyde can generally be used as a biomarker for free radical induced damage and endogenous lipid peroxidation (Singh *et al.* 2002; Rosenblat *et al.* 2006; Li *et al.* 2016).

Table 6 Effect of different dietary levels of pomegranate by-product (PBP, %) on some hematological parameters of laying hens (%)

Hematological parameters	Dietary PBP levels (%)				SEM	P-value
	0	4	8	12		
Cholesterol (mg/dL)	126.75 ^b	109.5 ^b	124.25 ^b	150 ^a	5.239	0.003
Triglyceride (mg/dL)	772.75	763.75	896.25	837.00	28.206	0.3635
High density lipoprotein (mg/dL)	25.75 ^a	32.25 ^a	16.75 ^b	25.25 ^a	2.786	0.0007
Low density lipoprotein (mg/dL)	20.22 ^b	51.97 ^a	47.85 ^a	57.55 ^a	4.097	0.0015
Total antioxidant (mmole/L)	0.4562	0.4951	0.5225	0.5315	0.007	0.3610
Malondialdehyde (nmole/L)	8.15 ^b	9.74 ^a	7.65 ^c	6.68 ^d	0.086	0.0001

The means within the same row with at least one common letter, do not have significant difference ($P > 0.05$).
SEM: standard error of the means.

Table 7 Effects of different dietary levels of pomegranate by-product (PBP, %) on egg yolk cholesterol

Dietary PBP levels (%)	Egg cholesterol (mg/g)
0	187.49
4	142.35
8	136.35
12	204.38
SEM	9.157
P-value	0.0697

SEM: standard error of the means.

Table 8 Effects of different dietary levels of pomegranate by-product (PBP, %) on plasma and egg yolk iron contents

Item	Dietary PBP levels (%)				SEM	P-value
	0	4	8	12		
Diets iron (mg/kg)	17.980 ^d	19.327 ^c	21.977 ^b	24.495 ^a	0.137	< 0.0001
Plasma iron ($\mu\text{g/mL}$)	5.125	5.052	5.017	4.660	0.444	0.8812
Egg yolk iron (mg/g)	1.857 ^c	2.430 ^a	2.085 ^b	2.172 ^{ab}	0.146	0.0021

The means within the same row with at least one common letter, do not have significant difference ($P > 0.05$).
SEM: standard error of the means.

Most of the cholesterol found in the egg yolk is synthesized in the liver of the hen, and transported by the blood in the form of lipoproteins and deposited in developing follicles. The concentration of plasma cholesterol is closely associated with the egg yolk cholesterol concentration (El-Bagir *et al.* 2006; Tang *et al.* 2015).

Increased egg yolk iron levels by 4 and 8% PBP in diet could lead to the enrichment of eggs iron. It is well known that diet-origin iron can be incorporated into the egg yolk (Seo *et al.* 2010; Bess *et al.* 2012). In contrast decrease egg yolk iron levels were indicated by increasing 8 and 12% PBP in diet. Iron percentage in vegetable sources such as pomegranate pulp have a low absorption rate and this may decreased absorption of the treatments includes 8 and 12% PBP (Cediél *et al.* 2012; Pizarro *et al.* 2016). The other factor which could reduce the iron absorption in this respect, may be related to PBP tannins content. Since these tannins could be complications by mineral and lead to mineral decrease absorption (Mahmood *et al.* 2014; Batonon-Alavo *et al.* 2015). Decreases cholesterol and triglyceride concentrations in serum and egg-yolk as well as increasing HDL concentration in serum and markedly improving yolk color in all dietary were shown in diet supplemented by PBP.

CONCLUSION

In conclusion, the supplementation of pomegranate by-product in layer diets under the present study did not appear to cause any adverse effects on egg production and egg quality compared with the control in laying hens. Using of 4% PBP in diet can improve egg production rate and increased iron in egg yolk.

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