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The Effects of Different Levels of Pomegranate Seed Pulp with Multi-Enzyme on Performance, Egg Quality and Serum Antioxidant in Laying Hens

Research Article

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ABSTRACT

This study was conducted to investigate the effect of supplementation different levels of pomegranate seed pulp (PSP) on performance and blood parameters in laying hens. A total of 96 layers hens (Hy-line W-36) at 24 week of age were randomly assigned into 4 treatments including 0 (control), 5, 10 and 15% of PSP, with 4 replicates containing 6 layers in each. Results showed no significant effect of PSP levels on feed intake, egg mass, egg weight, feed conversion ratio and body weight gain. Supplementation of PSP at 5% increased egg production and is significantly different ($P<0.05$) to 15% PSP but not in control group, suggesting negative effect of high level PSP in layers diet .Haugh unit, yolk and albumen indexes as well as eggshell weight, eggshell ratio and breaking strength were not significantly affected by dietary PSP. There was no significant effect of PSP on serum triglycerides, high-density lipoprotein and total antioxidant. In contrast, the serum malondialdehyde was significantly ($P<0.05$) increased using 5% PSP in diet, with cholesterol level significantly ($P<0.05$) increased in all inclusion level of PSP compared to the control. The results shown that supplementation of PSP up to 15% improve egg production but higher concentration has detrimental effects on laying performance. Supplementation of PSP increased ($P<0.05$) cholesterol in the layers blood.

KEY WORDS antioxidant, egg quality, laying hen, performance, pomegranate seed pulp.

INTRODUCTION

The utilization of agro-industrial by-products in animal nutrition has lately received particular attention because of decreasing dependence of animal on cereals that are used in human nutrition (Afsharhamidi and Razeghi, 2010). In terms of economics, it can be considered to decrease feed costs at an optimal production (Samli *et al*. 2006; Amoah and Martin, 2010) and also an innovative outlet for waste reduction.In this regard, the use of plant residue juicer became attractive in many developing countries due to its nutritional properties and mass volume production. Pomegranate (*Punicagranatum*) is one of the oldest edible fruits and is widely grown in many tropical and subtropical countries (Salaheddin and Kader, 1984). The total pomegranate production in Iran was reported approximately 700000 tons for the year 2009 (FAO, 2009) which accounted for half of the global production (Abbasi *et al*. 2008). Edible parts of pomegranate fruit comprise 78% juice and 22% seed. Pomegranate by-products constitute approximately 52% of the total weight of fruit (Kullkarni and Aradhya, 2005) which remain after the processing of pomegranate for juice, sauce and concentrate. Pomegranate seeds are rich in sugars, vitamins, polysaccharides, polyphenols, minerals and polyunsaturated fatty acids (PUFA) (Miguel *et al*. 2004). Pomegranate seed oil consists of 65-80% conjugated fatty acids

(Hora, 2003) in which the most predominant fatty acid (up to 72%) is a cis-9, trans-11 and cis-13 linoleic acid, octadecatrienoic acid, so-called punicic acid (El-Shaarawy and Nahapetian, 1983; El-nemr *et al.* 1990; Hernandez *et al.* 2002; Melgarejo and Arte, 2000). Antioxidant effects and eicosanoid enzyme (cyclo-oxygenase and lipo-oxygenase) inhibition properties of pomegranate seed oil (Schubert *et al.* 1999; Singh *et al.* 2002) has been observed. Due to the presence of phenolic compounds (punicalagin, punicalin, gallic acid and particularly ellagic acid), it may affect serum lipid concentrations and other blood parameters (Rajabian *et al.* 2007). Moreover, some investigations showed phenolic compounds particularly ellagic acid and punicalagin that are responsible for antibiotic activity of pomegranate (Sarkhosh *et al.* 2007).

There is a relatively high level of fiber (approximately 32.3%) (Afsharhamidi and Razeghi, 2010) and tannin (approximately 2.7%) in pomegranate seed. Therefore, antinutritional effects may limit the use of PSP in poultry diets. Making supplementation of multi-enzyme seems to be necessary.

In this respect, the benefits of supplementing poultry diets with enzymes are well documented. Investigations since 1960 have shown that supplementing enzymes to high viscous diets resulted in improvement on metabolized energy, feed intake and performance in broilers (Annison, 1991). There is limited information on the effect of PSP content in layer diets.

Hence, the objective of the present study is to examine the effect of pomegranate seed pulp as supplement to layers diet on the production potential in poultry feeding with respect to feed intake, egg mass, egg production, egg internal and external parameters, feed conversion ratio, and blood parameters.

MATERIALS AND METHODS

A total of ninety-six 24-wk old Hy-line W-36 laying hens were randomly allocated into 4 dietary treatments, 4 replicates and 6 birds in each by a completely randomize design. Dietary treatments include: control (no addition of PSP) and 5, 10 and 15% PSP, respectively. Experiment lasted 12 weeks.

The ingredients and chemical composition of diets were shown in Table 1. Chemical analysis of PSP was carried out according to AOAC, 1990. Feed intake was recorded weekly. Egg production was measured daily and analyzed weekly. Egg mass, and feed conversion ratio were calculated from these data. At 32-wk of age, blood samples (5 mL) were collected from the brachial vein and centrifuged for 20 minutes at 3000 rpm to obtain plasma samples. Plasma samples were used for the determination of the cholesterol, triglyceride, high density lipoprotein (HDL), total antioxidant and malondialdehyde (MDA) contents. Cholesterol, triglyceride and HDL were measured by enzymatic method. Malondialdehyde was tested as an index of lipid peroxidation by the tiobarbitoric acid (TBA) method according to Hong *et al.* (1989).

Total antioxidant was assayed by Randox kite according to Miller (1993). Egg internal (albumen and yolk indexes, Haugh unit (HU)) and external (eggshell weight, ratio and breaking strength) quality parameters were determined on four randomly selected eggs from each replicate every two weeks (16 eggs/treatment per 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). Albumen and yolk heights and widths were measured for each egg. Then Haugh unit (HU), yolk and albumen indexes were calculated using the following formula:

$$AI = AH / [AL + AW] \times 100$$
$$YI = YH / YD \times 100$$
$$HU = 100 \log (AH + 7.57 - 1.7\, EW^{0.37})$$

Where:
AI: albumen index.
YI: yolk index.
AH: albumen height.
AL: albumen length
AW: albumen width.
YH: yolk height.
YD: yolk diameter.
HU: Haugh unit.
EW: egg weight.

Results compared using SAS (2004) software. For performance data the statistical model was $Yij = \mu + Ti + eij$. Plasma and egg parameters data were compared using the following model:

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

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

RESULTS AND DISCUSSION

The results of laying hens performance were presented in Table 2. No significant differences in egg weight, egg mass, feed intake, body weight gain and feed conversion ratio were observed among treatment groups.

Table 1 Diet composition and chemical analyses of experimental diets

Ingredients (%)	**Pomegranate seed pulp levels (%)** 0	5	10	15
Corn grain	60.15	55.87	51.89	46.65
Soybean meal (44%)	25.78	22.40	18.65	16.87
Corn gluten (62%)	0.62	2.78	4.97	6.00
Pomegranate seed pulp	0	5.00	10.00	15.00
Soybean oil	2.60	3.33	3.97	5.1
Dicalcium phosphate	2.04	2.00	2.01	1.99
Oyster shell	7.8	7.62	7.42	7.22
Common salt	0.28	0.27	0.25	0.25
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL- methionine	0.10	0.10	0.10	0.09
L-lysine	0.10	0.10	0.21	0.3
Multi enzyme kemin[3]	0.03	0.03	0.03	0.03
Total	100	100	100	100
Chemical analysis (%)				
Metabolizable energy (kcal/kg)	2850	2850	2850	2850
Protein	17.00	17.00	17.00	17.00
Calcium	3.5	3.5	3.5	3.5
Avail. phosphorus	0.50	0.50	0.50	0.50
Potassium	0.71	0.64	0.56	0.51
Chlorine	0.21	0.20	0.18	0.18
Sodium	0.14	0.14	0.14	0.14
Lys	0.93	0.85	0.85	0.86
Met	0.39	0.39	0.39	0.39
Tyr	0.69	0.68	0.66	0.64
Thr	0.63	0.60	0.57	0.54
Trp	0.23	0.21	0.18	0.17

[1] Vitamin premix supplied per kg of diet: vitamin A: 8800 IU; vitamin D_3: 2500 IU; vitamin E: 11 IU; vitamin B_1: 1.5 mg; vitamin B_2: 4.0 mg; vitamin B_3 (Calcium pantothenate): 8 mg; vitamin B_5 (Niacin): 35 mg; vitamin B_6: 2.5 mg; vitamin B_{12}: 0.01 mg; vitamin K_3: 2.2 mg; Biotin: 0.15 mg; Folic acid: 0.48 mg and Cholin chloride: 400 mg.
[2] Mineral premix supplied per kg of diet: Manganese: 75 mg; Iron: 75 mg; Zinc: 64.8 mg; Copper: 6.0 mg; Iodine: 0.87 mg and Selenium: 0.2 mg.
[3] Containing enzymes: xylanase, cellulase, hemi cellulase, pectinase, amylase, beta Glucunaz, lipase and protease. (The consumption: 300 g per ton).

Table 2 The effects of PSP levels on performance of laying hen from 25 to 36 weeks of age

Parameters	Levels of PSP (%) 0	5	10	15	SEM	$P_{treatment}$	P_{linear}	$P_{quadratic}$
Feed intake (g/d)	97.46	98.65	97.87	97.93	0.8824	0.8137	0.7451	0.3795
Egg production (%)	84.87[ab]	87.50[a]	84.37[ab]	82.48[b]	1.2163	0.0795	0.7779	0.0774
Egg weight (g)	55.71	55.71	56.07	56.57	0.5597	0.4256	0.3190	0.2184
Egg mass (g)	97.03	48.84	47.51	47.74	0.8981	0.3989	0.7133	0.1792
FCR	2.17	2.06	2.14	2.14	0.0427	0.2916	0.6220	0.0740
Body weight gain (g)	69.5	129.62	105.42	99.63	26.5046	0.4836	0.3569	0.9473

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
PSP: pomegranate seed pulp and FCR: feed conversion ratio.
SEM: standard error of the means.

Improvement in egg production was observed in diet containing 5% of PSP in comparison to all treatment groups with no significant difference to the control and 10% PSP levels but significantly (P<0.05) different to 15% PSP in the diet. These results are in agreement with that of Rajani *et al.* (2011) who showed that body weight gain, feed conversion ratio and feed intake of broiler chickens is not affected by pomegranate peel treatment.

There is a lack of information regarding PSP in layer hen diet. In a research that was conducted to evaluate the effects of PSP on performance of cross-bred goats, Modarresi *et al.* (2010) showed that using pulp and pomegranate seed had no significant effect on body weight gain of goats (p=0.55). The insignificant effects of dietary PSP on egg weight might be due to similar body weight of hens, as well as similar crude protein contents in all dietary treatments. The increased egg production in the diet containing 5% of PSP could be explained by the presence of punicic acid as a conjugated linoleic acid (CLA) in PSP. It has been shown that CLA in layer hen diet (0.01 g CLA/kg diet) increased egg production (Jones *et al.* 2000).

Results showed that triglyceride, high density lipoprotein (HDL) and total antioxidant contents of plasma did not have any significant difference (Table 3). Previous reports

(Yamasaki *et al.* 2006; Rajabian *et al.* 2007; Modarresi *et al.* 2010) further supported these results. Serum cholesterol levels were significantly increased with PSP supplementation (P<0.05). In terms of serum cholesterol levels, the results of Rajabian *et al.* (2007) on serum lipids of hypercholesterolemia rabbits are in comparison with this study. They showed that feeding hypercholesterolemia rabbits with diets contain 1 and 2% of pomegranate seed oil had no significant effects on blood cholesterol. Also, Yamasaki *et al.* (2006) have shown that feeding rats with 0.12 and 1.2% pomegranate oil, led to significant increased of serum triacylglycerols and phospholipids, but did not have significant effect on total serum cholesterol. Increase in serum cholesterol of treatments containing PSP might be due to increased fat in the diets containing PSP to compensate energy inadequacy. Since crude fiber can enhance fecal excretion of cholesterol and bile cholesterol (Moundras *et al.* 1997), it is probably that increasing crude fiber digestibility by multi-enzyme could justify the increasing serum cholesterol in the current experiment. Pomegranate seed oil consists of 65-80% conjugated fatty acids which is the most important is 9-trans, 11-cis, 13-trans, called punicic acid (Abbasi *et al.* 2008). It has been shown that triglycerides and total cholesterol levels in plasma increased significantly with conjugated linoleic acid (CLA) in broilers (Du and Ahn, 2003) and pigs (Stangl *et al.* 1999) diets. So, the reason for the increased plasma cholesterol level in PSP treated layers in current study could be related to the changes in enzyme activities associated with lipid metabolism in the liver through increasing liver lipogenesis, as showed by Du and Ahn (2003). They suggested that there was a significant increase in fatty acid synthase and acetyl-CoA carboxylase (although not significant) activities, which were the main enzymes controlling fatty acid synthesis in the liver of broilers with CLA feeding.

Since, HDL content of plasma was not affected by dietary treatments; it is probable that low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol are increased in current study.

As shown in Table 3, in contrary to expectations, there is no significant difference in total antioxidant content of plasma, whereas malondialdehyde concentration increased significantly in group with 5% PSP (P<0.05). However, Rajani *et al.* (2011) have shown that pomegranate peel treatment reduced MDA levels of frozen stored meat samples of broilers.

Malondialdehide is a marker for oxidative stress and indicates the degree of lipid peroxidation. One possible explanation for increasing MDA concentration might be the fact that the extracts of pomegranate peel exhibited higher antioxidant activity *in vitro* compared to the seed extracts (Singh *et al.* 2002; Guo *et al.* 2003). Also, it has been shown that total PUFA in pomegranate seed oil content exceeds 88.0% (Abbasi *et al.* 2008). Dietary long chain n-3 PUFA are highly vulnerable to oxidation (Esterbauer *et al.* 1991; Guichardant *et al.* 2006) so the higher susceptibility of PUFA in PSP to oxidation might be responsible for high amounts of MDA contents of plasma in the current study.

Table 4 presents results of egg external quality parameters. Eggshell weight, eggshell ratio and breaking strength did not show any significant difference in dietary treatments. These results indicated that probably total calcium, phosphorus, and vitamin D_3 contents and their bioavailability in all experimental diets were similar and not affected by dietary PSP level.

Current results might be due to the effect of multi-enzyme to reducing the anti-nutritive properties associated with the incorporation of PSP in laying hen diets. Table 5 represents the effects of PSP levels on egg internal quality parameters.

Table 3 The Effect of different levels of PSP on blood factors at 32 weeks of age

Parameters	Levels of PSP (%)				SEM	$P_{treatment}$	P_{linear}	$P_{quadratic}$
	0	5	10	15				
Cholesterol (mg/dL)	110.87[b]	158.00[a]	162.62[a]	160.00[a]	13.2373	0.0499	0.0171	0.2145
Triglycerides (mg/dL)	136.35	136.35	162.51	151.77	18.6823	0.2953	0.3416	0.1130
HDL (mg/dL)	15.50	16.87	16.12	16.00	1.6097	0.9437	0.7883	0.5998
Total antioxidant (mg/dL)	0.45	0.50	0.54	0.55	0.0460	0.5020	0.2229	0.3987
Malondialdehyde (mg/dL)	8.12[b]	9.83[a]	7.68[b]	6.60[b]	0.5484	0.0096	0.5831	0.0140

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
PSP: pomegranate seed pulp and HDL: high density lipoprotein.
SEM: standard error of the means.

Table 4 The effects of PSP levels on egg external quality parameters of laying hens 25 to 36 weeks of age

Parameters	Levels of PSP (%)				SEM	$P_{treatment}$	P_{linear}	$P_{quadratic}$
	0	5	10	15				
Shell weight (g)	5.46	5.36	5.33	5.46	0.0747	0.4105	0.8567	0.1095
Shell ratio (%)	9.60	9.50	9.55	9.60	0.091	0.2239	0.2036	0.0975
Breaking strenght	1.95	2.27	1.94	1.95	0.3799	0.6407	0.4070	0.7142

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

Table 5 The effects of PSP levels on egg internal quality parameters of layinh hens 25 to 36 weeks of age

Parameters	Levels of PSP (%)				SEM	$P_{treatment}$	P_{linear}	$P_{quadratic}$
	15	10	5	0				
Albomen index (%)	6.73	6.87	7.35	7.09	0.1658	0.0995	0.0577	0.2619
Yolk index (%)	44.06	44.28	44.93	44.44	0.4651	0.6016	0.4009	0.4555
Haugh unit (%)	95.68	96.25	98.21	97.18	0.6718	0.0903	0.0528	0.2524

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

Feeding layers with PSP had no significant effects on yolk and albumen indexes. Also Haugh unit was not affected by dietary treatments, which could confirm the low effect of nutrition on Haugh unit.

CONCLUSION

In conclusion, pomegranate seed pulp can be a potential feed supplement when added to the diets of laying hens up to 5% without negative effects on performance and egg production and quality. Higher levels will have a negative effect on egg production and quality in layers as shown by increased cholesterol level.

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