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Effect of different level of Pomegranate molasses on performance, egg quality trait, serological and hematological parameters in older laying hens

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Summary. The current study was planned to investigate the effects of pomegranate molasses (PM) on performance, egg quality and blood parameters in older laying hens. A total of 240 Babcock white laying hens (58 weeks old) were divided into 5 groups (n=48) with 8 subgroups having 6 hens in each. Pomegranate molasses was added in the drinking water to experimental groups with 0 %, 0.1%, 0.25 %, 0.5%, and 1%, respectively during 4 weeks. Results showed that egg weight values were same in all groups exclude 1% pomegranate molasses group over control however no significant (P>0.05) result were observed on feed consumption, egg production, FCR, egg mass, egg yolk cholesterol, live body weights, and water consumption. Similarly during mid-study analyses, egg quality parameters were remained not significant (P>0.05) by the supplementation of pomegranate molasses while during final analyses, egg yolk color had positively (P<0.05) effect in 0.5% pomegranate molasses supplemented groups, however, yolk index showed positive (P<0.05) effect in 0.1% pomegranate molasses supplemented groups over control. Pomegranate molasses did not show a positive effect on serum levels of total cholesterol (CHO), total protein (TP), low-density lipoprotein (LDL), high-density lipoprotein (HDL), gamma glutamyl transferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and glucose In hematology, only hemoglobin level was increased in all experimental groups over control while any other hematology parameters did not change. It is concluded that Pomegranate molasses showed positive effects on the egg yolk color, and egg weight of older laying hens without any adverse effects on egg traits. Moreover, pomegranate molasses may be used as an hemoglobin enhancer in water supplement in low hemoglobin level older laying hens. Further detailed studies with multiple dose levels need to be investigated.

Keywords: Hemoglobin, egg yield, egg yolk color, FCR, serum cholesterol

Introduction

The pomegranate *(Punica granatum)* is a deciduous shrub having family *Lythraceae* and sub family *Punicoideae* and it grows about 5 to 10 m tall. The fruit is typically grown from September to February (1). Aril is the edible part of pomegranate and it consists of 52% (w/w) of the total fruit and comprises of 78% juice and 22% seeds. The seed of pomegranate is rich in polyunsaturated fatty acids (PUFA), polyphenols, minerals, sugars, vitamins and polysaccharides (2). Therefore, pomegranate is investigated as a phytochemical source and it is used for various therapeutic purposes in medicine. Moreover, pomegranate peels abundant tannin and phenolic contents (3)

Traditional methods are still being used to produce pomegranate molasses (PM). It is concentrated

simply by boiling, without the addition of further sugar or other additives (4,5). Typical processing requires cleaning, crushing, extraction, filtration, clarification, and evaporation in open vessel or under vacuum, though a different type of fruits requires different production methods (6,7). Pomegranate molasses is a highly nutritive product because the product is a concentrate and especially the presence of high mineral contents makes it more nutritious and the strong antioxidant activity of it is also important for human health (8).

Although the previous research is limited on PM, large studies were conducted already for use of pomegranate juice as anti-inflammatory, antioxidant, anti-atherogenic, and antimicrobial effects (9). Concentrated pomegranate extract also increased antioxidant activity in the milk by 15-17% but at the same time, the somatic cells number decreased in animals (9). Similar with the PM used in the present study, Gültepe et al.(10) observed some positive effects of lemon juice as a water supplement on egg production during the late phase production cycle of laying hens. Furthermore, Çetingül et al.(26) reported that supplementation of PM with drinking water to laying hens may affect some quality parameters in eggs after 30 days of storage.Manters et al. (11) reported that pomegranate seed oil increased white blood cell number.Chalfoun-Mounayar et al. (12) found that the polyphenols bioactive compounds present in PM are approximately four times greater than those found in the pomegranate juice. Moreover, at a very low concentration (100 to 600 ml), PM contains strongest antioxidant characteristics in vitro as compared to pomegranate juice. This shows that high temperature does not change antioxidant activity of PM against reactive oxygen species (ROS). Limited studies are available on the use of PM regarding performance, egg quality trait and blood parameters in laying hens.

Keeping in view the above points, the present study was conducted to investigates the effects of different level of PM on performance, egg quality trait and blood parameters in older laying hens. The PM was preferred instead of the pomegranate juice because of its high concentration than juice.

Materials and Methods

The current study was performed at the Animal Research Center of Afyon Kocatepe University, Faculty of Veterinary Medicine after the approval of the Local Ethics Committee on the ethical use of animals under approval (Case No: 146-16, Date: 03/01/2016).

Experimental design and management

A total of 240 Babcock white laying hens (58 weeks old initial body weight 1.62 ± 0.18 kg) were divided into 5 groups (n=48) with 8 subgroups having 6 hens in each. Pomegranate molasses was added in the drinking water to experimental groups with 0%, 0.1%, 0.25 %, 0.5%, and 1%, respectively during 4 weeks. Total 16 hours light and 8 hours dark were applied and also feed and water were supplied *ad libitum*. In this study, all treatment groups were fed the basal diet, which was prepared according to the NRC (13) recommendation to meet the bird's requirement (Table.1).

Pomegranate molasses ingredients analyses were performed by HPLC in molasses obtained from the pomegranate as this method was reported by Angerosa et al. (15) (Table.2).

Data Collection and Analyses

Pomegranate molasses was poured on a daily bases in fresh drinking water. Hens were weighted at the beginning and at the end of the study to determine their live weights (initial body weight 1.62 ± 0.18 kg).

Egg production was recorded daily and was expressed percent of hen-day egg production (HDEP). Feed consumption of hens was recorded weekly. Feed conversion ratio (FCR) values were calculated from egg yield and feed consumption as follows:

FCR = feed consumption (g) / egg mass (g)

Eggs were weighed once a week. Egg mass calculated as follows:

EM = HDEP (%) × average egg weight (g).

Eggs were delivered to the laboratory at the end of 2nd and 4th week as three egg samples from each subgroup to determine egg quality parameters. Egg weight and eggshell thickness were determined in these eggs. Haugh Unit was calculated by measuring albumen height and egg yolk color was determined by

Table 1. Composition of the basal diet

Ingredients	**%, as-fed basis**
Corn	54.90
Vegetable oil	0.34
Sunflower meal (%32 CP)1	16.93
Full fat soya	10.00
Soybean meal (%44 CP)1	7.39
Limestone	7.87
Dicalcium phosphate	1.73
Common salt	0.40
Vitamin-mineral premix[2]	0.25
L-Lysine HCl	0.10
DL-methionine	0.10
Calculated values	
CP,%	17.00
ME,kcal/kg	2750
Ca	3,71
Av.P	0.38
Na,%	0,20
Met+Sis	0.71
Lysine,%	0.83
Treonin,%	0.61
Triptophane,%	0.20
Linoleic acid,%	2.36

1.Providedper kg of diet:Vitamin A:12.000.000 IU, Vitamin D3:3.000.000IU, Vitamin E:35.000, Vitamin K3:3.500,Vitamin B1:2.750IU, Vitamin B2:5.500IU, Nicotinamid: 30.000IU,Ca-D-Panthotenate:10.000IU,Vitamin B6: 4.000IU, Vitamin B12-15IU, Folic acid:1.000IU, D-Biotin: 50IU,Cholin clorid:150.000IU, Manganese: 80.000mg, Iron: 60.000 mg, Zinc:60.000 mg, Copper:5.000 mg, Iodine:2.000 mg, Cobalt: 500 mg, Selenium: 150 mg, Antioxidant:15.000 mg

Table 2. Ingredients of Pomegranate Molasses

Caffeic acid g/mol	4240.998
P-coumaric acid g/mol	0.799
Cinnamic acid g/mol	5.338
2.5 dihydroxy g/mol	819.052
Epicatechin g/mol	1356.114
Ellagic acid g/mol	883.340
Acetic acid (ppm)	273.60
Propionic acid (ppm)	624.72
Butyric acid (ppm)	5417.97
Composition of Molasses(8)	**Amount**
Water Soluble Dry Matter, %, minimum	68.0
Titration acidity (as citric acid), %, minimum	7.5
PH	3.0
Hydroxymethyl furfural (HMF), mg/100g, maximum	50

using Yolk Color Fan (DSM, Basel, Switzerland). Indexes of albumen and yolk calculated as follows (31):

Albumen index = Albumen height (mm) / [Albumen length (mm) + Albumen width (mm)] × 100

Yolk index = Yolk height (mm) / Yolk diameter (mm) × 100

At the end of the trial, 3 hens were randomly selected from each repetition group and blood was collected from the heart. Blood samples were drawn and placed in anti-coagulant containing tubes and without anticoagulant for serum separation. Complete blood cell count, serum glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein, aspartate transaminase (AST), alanine transaminase (ALT).

Statistics

The model assumptions of normality and homogeneity of variance were examined by Shapiro-Wilk and Levene tests, respectively. The statistical analysis was performed with MedCalc software (MedCalc Software bvba, Ostend, Belgium, version 17.5). One-way ANOVA was used for group comparison followed by Tukey-Kramer for post-hoc. All data were expressed as mean ± SEM. The significance level was considered as $p < 0.05$.

Results

The result of the present study indicates that egg weight did not change exclude 1% PM group over the control birds. Moreover, PM had no effect on feed consumption, HDEP, FCR, egg mass, egg yolk cholesterol, live body weights and water consumption (Table.3).

During mid-study analyses, egg quality parameters such as Haugh unit, eggshell thickness, albumen index, yolk index and egg yolk color were not significantly ($P>0.05$) affected by the supplementation of PM while during final study analyses, egg yolk color had significantly ($P<0.05$) increased in 0.5% PM supplemented groups, however, yolk index had significantly ($P<0.05$) increased in 0.1% PM supplemented groups over control while Haugh unit, eggshell thickness, and albumin index were not significantly ($P>0.05$) affected by the supplementation of PM (Table.4). The PM had

Table 3. Effects of different levels of Pomegranate molasses on performance and live weight parameters of laying hens for 4 weeks (Mean±SEM; n = 48)

Group	Daily Feed Consumption (g)	Mean Daily eggproduction (%)	FCR	Egg weight (g)	Egg Mass (g/hen/day)	Egg yolk cholesterol (mg/dL)	Water consumption (L/hen/day)
Control	110.93±2.58	58.83±3.63	3.46±0.35	57.26±2.29^{ab}	34.23±3.42	627.25±20.37	0.34±0.01
0.1%POM	106.87±3.49	50.80±4.42	4.74±0.68	48.96±2.62^{a}	25.28±2.81	611.87±27.99	0.32±0.00
0.25%POM	105.31±5.14	49.28±5.50	5.15±0.97	49.87±4.76^{a}	25.91±4.66	623.62±25.37	0.33±0.00
0.5% POM	107.81±5.55	58.21±6.31	4.22±0.57	48.90±2.64^{a}	29.47±4.57	586.87±9.23	0.35±0.00
1% POM	105.31±3.70	57.85±6.00	3.35±0.48	60.46±1.73^{b}	35.37±4.35	581.00±20.56	0.34±0.00
P	0.876	0.565	0.238	0.023	0.282	0 .447	

Live Weights

Groups	Initial weight	Final weight
Control	1637.72±43.98	1729.12±44.34
0.1%POM	1574.43±38.85	1647.40±71.49
0.25%POM	1615.81±40.35	1704.85±60.64
0.5% POM	1642.48±21.93	1716.74±28.99
1% POM	1651.87±40.29	1732.97±58.05
P	0.616	0.808

Table 4. Effects of different levels of Pomegranate molasses on Egg quality parameters of laying hens for 4 weeks (Mean±SEM; n = 48)

(MID after 15th days egg quality parameters)

Group	Shell Thick. (mm)	Haugh Unit	Yolk color (Roche fan)	Albumen index (%)	Yolk index (%)
Control	0.36±0.008	89.22±0.97	12.28±0.25	9.72±0.32	41.04±0.48
0.1%POM	0.37±0.006	89.13±1.63	11.84±0.28	9.78±0.45	42.04±0.61
0.25%POM	0.37±0.008	84.69±2.22	12.53±0.21	8.90±0.45	41.11±0.44
0.5% POM	0.39±0.006	86.61±1.98	11.40±0.68	9.06±0.49	39.80±0.66
1% POM	0.37±0.009	86.85±2.02	12.12±0.19	9.19±0.54	41.61±0.79
P	0.131	0.366	0.258	0.562	0.124

(Final after 30th days egg quality parameters)

Group	Shell Thick. (mm)	Haugh Unit	Yolk color (Roche fan)	Albumen index (%)	Yolk index (%)
Control	0.36±0.01	86.61±2.21	12.75±0.21^{ab}	9.01±0.49	41.54±0.63^{a}
0.1%POM	0.36±0.006	92.62±1.45	12.12±0.34^{a}	10.53±0.50	43.48±0.67^{b}
0.25%POM	0.36±0.008	88.93±1.55	12.40±0.27^{a}	9.68±0.50	41.62±0.4^{a}
0.5% POM	0.37±0.009	86.96±1.83	13.21±0.17^{b}	8.86±0.46	40.42±0.53^{a}
1% POM	0.38±0.005	86.63±1.94	12.65±0.15^{ab}	8.87±0.57	41.82±0.71^{ab}
P	0.139	0.105	0.030	0.101	0.015

no effect on serum HDL, GGT, LDL, TP, AST, ALT, CHO and glucose levels (Table.5).

Blood RBC, MCV, MCH, MCHC, platelet, RDWC and hematocrit level remained non-significant in all treatment groups while hemoglobin (He) increased in treatment over control (Table 6).

Discussion

Some studies showed that pomegranate had no significant effect on (FCR), feed intake, egg weight, and egg mass among the treatment group in laying hens 16). Similarly, in the present study we observed that egg

Table 5. Effects of different levels of Pomegranate molasses on Serum biochemical parameters of laying hens for 4 weeks (Mean±SEM; n = 48)

Groups	GLU (mg/d)	CHO (mg/d)	HDL (mg/d)	LDL (mg/d)	AST (U/L)	ALT (U/L)	GGT (U/L)	TP(mg/dL)
Control	215.37±10.5	104.50±16.64	21.50±0.65	46.50±10.43	176.87±11.20	2.37±0.41	27.62±1.3 [a]	6.55±0.49
0.1%POM	207.62±14.7	119.87±19.55	21.87±0.71	51.25±9.31	203.37±15.96	3.50±0.42	26.75±0.59 [a]	6.16±0.31
0.25%POM	222.25±10.5	110.37±18.90	22.37±1.33	49.25±11.54	191.12±11.90	2.75±0.36	30.25±0.77 [ab]	5.95±0.41
0.5% POM	206.62±18.1	100.00±6.54	22.75±0.97	44.75±4.94	194.37±11.53	2.12±0.29	31.87±1.34 [b]	6.66±0.45
1% POM	214.75±6.41	127.75±24.69	25.00±1.25	57.37±15.22	223.00±16.19	2.25±0.49	29.12±1.68 [ab]	7.07±0.50
P	0.181	0.819	0.153	0.186	0.201	0.135	0.037	0.425

CHO: Cholesterol; HDL: High density lipoprotein, LDL: Low density lipoprotein, gamma glutamyl transferase (GGT), Aspartate Aminotransferase (AST); IgG: Immunoglobulin, TAS: total protein (TP), Total antioxidants species, TOS: Total oxidative species, Alanine Aminotransferase (ALT)

Table 6. Effects of different levels of Pomegranate molasses on Hematological parameters of laying hens for 4 weeks (Mean±SEM; n = 48)

Group	HCT (10^9/l)	RBC(10^{12}/l)	He (g/l)	MCV (fl)	MCH (pg)	MCHC (g/l)	PLT (10^9/l)	RDWC (pg)
Control	34.00±2.68	2.85±0.14	11.79±0.79	112.12±1.42	42.59±0,65	37.87±2.37[a]	16.83±2.58	11.96±0.34
0.1%POM	32.53±1.88	2.89±0.14	13.10±0.79	112.31±2.44	45.09±0.73	40.24±0.63[ab]	15.16±2.85	11.13±0.53
0.25%POM	41.50±7.36	2.82±0.11	13.14±0.58	108.75±5.98	43.34±1.37	37.61±1.34[a]	19.33±4.71	12.55±1.59
0.5% POM	31.84±0.83	2.82±0.05	13.09±0.25	112.62±1.21	46.38±0.47	41.23±0.68[ab]	16.91±3.46	11.39±0.57
1% POM	31.13±1.58	2.73±0.09	13.21±0.73	109.90±1.79	46.62±0.95	42.49±0.95[b]	11.41±2.18	12.73±1.01
P	0.280	0.916	0.514	0.874	0.209	0.059	0.533	0.688

RBC: Red blood cell count. He: Hemoglobin. MCV: Mean corpuscular volume. MCH: Mean corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin concentration. PLT:Platelet.MPV:Mean platelet volume

weights were remained same in all groups exclude 1% PM group over control. Also, we did not observe any significant effect of PM on egg mass, live body weight, water consumption, HDEP, yolk cholesterol and FCR.

For egg quality parameters such as eggshell weight, eggshell thickness, and eggshell breaking strength, dietary supplementation of pomegranate seed pulp did not show any significant result (17). Navid et al. (18) reported that dietary mixed herbal powder (*Thymus vulgaris*, Pennyroyal, Cumin, Alhagi, Garlic and Eucalyptus globules) had no significant effects on performance, egg quality (19)and immunity of laying hens. Similarly, during our mid-study analyses, the egg quality parameters such as eggshell thickness, Haugh unit, albumen index, and yolk index remained unchanged in PM supplemented groups.

Saki et al. (17) reported that pomegranate did not show any significant result on plasma levels of HDL, triglyceride, and total antioxidant contents. Similarly in the present study, we also did not observe any effect on TP, LDL, HDL, LDL, CHO, AST, ALT, glucose and cholesterol levels.

The amount of gamma glutamyl transferase in the bile is approximately 100 times greater which can be detected in pancreatic diseases, myocardial infarction, renal failure, diabetes mellitus, and chronic obstructive pulmonary disease (23). Gamma glutamyl transferase may also increase in the use of phenytoin, carbamazepine and barbiturate (24). Aboonabi et al. (25) reported significantly greater serum GGT levels in both pomegranate seed and juice supplemented rats than the control rats in agreement with our findings.

In the present study, we also did not observe a significant effect on different blood parameters such as HCT, RBC, MCV, MCH, MCHC, PLT, RDWC, and MPV. Although hemoglobin level was not statistically significantly increased in all experimental groups over control. Manthou et al. (28) concluded that pomegranate juice intake led to an increase in erythropoiesis or a decrease in degradation of red blood cell counts in the human model. The aforementioned findings may explain numerical hemoglobin trends in the PM groups of the present study.

Conclusion

It can be concluded that PM can be used in the process of homeopoesis. In this trial it did not show any significant effects on blood and serological parameters. However, some further trials can be performed to check its effectivity on different supplementation levels.

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