Effect of ellagic acid and mesocarp extract of *Punica granatum* on productive and reproductive performance of Gallus gallus

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Abstract

In the present study, we determined the potential effects of ellagic acid and mesocarp extract of *Punica granatum* on the productive and reproduction performance of laying hens. Five treatments groups were setup: (1) control group (without ellagic acid), (2) 50 mg of ellagic acid, (3) 100 mg of ellagic acid, (4) 200 mg of ellagic acid, and (5) mesocarp extract of *P. granatum*. All the groups were investigated for feed intake, body weight, egg production, egg quality, fertility, hatchability, antioxidant status of serum and liver, lipid peroxidation, and antibacterial activities. Egg production, feed intake and body weight were significantly increased ($p < 0.05$) with 100 mg of ellagic acid and *P. granatum* extract while no significant effect was observed on albumen and yolk weight, yolk index, yolk color, egg-shape index, and Hough unit. Both ellagic acid and *P. granatum* extract significantly improved hatchability while 100 and 200 mg/kg of ellagic acid numerically decrease fertility. Besides, ellagic acid and *P. granatum* extract significantly decreased malondialdehyde concentration and increased total antioxidant capacity, glutathione peroxidase, and total superoxide dismutase in serum and liver samples of laying hens ($p < 0.05$). The lipid peroxidation was decreased among the treatments groups, with 100 mg of ellagic acid and *P. granatum* extract showed the best activity. Moreover, ellagic acid showed strong killing activity against *Escherichia coli* and *Staphylococcus aureus* while it was ineffective against methicillin-resistant *S. aureus*. Our results conclude that ellagic acid and *P. granatum* promotes egg production, hatchability, and antioxidant enzymes activities of the Gallus gallus.

Introduction

The modern chicken has been the focus of research for egg production, meat quality and growth performance. Hens provide eggs and meat globally and various dietary supplements have been used to increase egg production, meat quality and hen performance (Al-Harthi 2014; Brewer 2011). Several plants derived product such as grape seed, oregano, rosemary, garlic, onion, and plum resins contain high levels of natural antioxidants (Brewer 2011; Mielnik et al. 2006; Ri et al. 2017; Yashin et al. 2017). These antioxidant compounds are secondary metabolites of plants that are synthesized in response to environmental stresses and pathogen attack. The use of these antioxidants are highly beneficial to the animal diet because of their advantageous effects on animal health (Pagare et al. 2015). When poultry feed is supplemented with phenolic compounds such as antioxidant agents, the majority of them showed beneficial effects on bird health. Therefore, it is always intriguing to find new antioxidant and determined their potential activities that can enhance hen performance. Among the well-known antioxidant compounds, ellagic acid is a well-characterized and multi-functional polyphenolic compound. Ellagic acid is a condensed dimer of gallic acid and present abundantly in pomegranate, cranberries, strawberries, blackberries, raspberries, and walnuts (Tsao 2010). Pomegranate (*P. granatum*) is known as super-fruit because of high antioxidant activity and is rich source of polyphenols such as punicic acid, ellagitannins and ellagic acid (Fernandes et al. 2015). In fact pomegranate extracts surpassed the antioxidant potential of green tea that had been tested for treatment of prostatic disease (Bell and Hawthorne 2008). The dietary benefits of pomegranate have been largely associated to its ellagitannins and ellagic acid content

(Long et al. 2019). Ellagic acid acts as an antioxidant by countering the deleterious effects of oxidative stresses and showed anticarcinogenic, antibacterial, antiproliferative, and anti-inflammatory properties in different hosts such as humans, rats, and bacteria (Rahal et al. 2014; Shahidi and Yeo 2018). In present work, we explored the effect of ellagic acid and *P. granatum* extract in lying hens and revealed the changes in egg quality parameters and reproductive performance after feeding the ellagic acid supplemented diet.

Materials And Methods

Experimental design

Birds handling and experimental protocols were followed as previously described (Gul et al. 2017). A total number of 150-birds + 15-cocks of local yellow-brown laying hens of 28-weeks old were equally divided into 5-groups. Five experimental diets were formulated to meet the nutrient recommendation of laying hen's management guide which met or exceeded the Nutrient Requirements of Poultry (1994) recommendation (Table 1). The experimental diet was offered from 28 to 44 week of age as follows; Group A was control diet without ellagic acid while Groups B, C, and D had 50, 100, and 200 mg/kg of ellagic acid and Group E has 200 mg/kg mesocarp extract of *P. granatum*. All hens were raised under a 16 h light and 8 h darkness cycle and allowed free access to feed and water.

Table 1
Ingredient and nutrient level composition analysis of the control basal diet.

Ingredient (%)	Basal diet amount
Corn	61.50
Soybean meal (46% CP)	26.23
Wheat bran	2.0
Limestone	8.33
Di-calcium phosphate	1.34
Salt	0.30
Premix	0.30
Total	100
Nutritional composition (%)	
Metabolizable energy (kcal/kg)	2750.360
Crude protein	16.5
Crude fiber	2.67
Ca	3.72
Phosphorus	0.57
Lysine	0.85
Methionine	0.40
Met + Cys	0.69
Sodium	0.34
The premix provided the following /kg of the diet: IU Vit. A 9500, vit. D3 1600mg; vit.E 10IU; vit.K3.5mg; thiamine 1mg; riboflavin, 7.6mg; biotin, 2mg; folic acid, 6mg; vit B12 ,2mg; Mn,62mg; I, 1.5mg; F,6mg Cu, 5mg; Zn, 54mg; Se,.2mg; calcium pantothenate,10mg.	

Extraction of mesocarp extract of P. granatum

Broken and unwanted *P. granatum* were obtained from the local market. The outer coat was removed from fruits, washed with water and juice was extracted. The extract was lyophilized and *P. granatum* powder (200 mg / 100 ml) was dissolved in boiled water. The solution was cooled at room temperature, filtered by Whatman No. 1 filter paper and added in feed.

Data collection and layers performance

The feed provided to the treatments groups was observed daily. The feed intake was determined as follows; Feed consumed = Feed offered – Feed refused

Eggs were collected from each group daily. Average hen-day egg production was determined as follows; Egg produced (%) = (No of eggs produced in a single day) / (No of live birds in that groups on that day) x 100

Bodyweight was calculated as the difference between the initial body weight and final body weight during the experimental period, while feed conversion ratio (FCR) was measured as the egg mass value divided by the amount of feed consumed. The egg mass was calculated as the weight of individual egg in gram per day per hen.

Egg quality parameter

Eggs were examined thoroughly for exterior and interior quality. Egg composition was weekly determined using three egg per replicate and egg weight, length, and width were measured. The egg was cautiously broken on a glass plate and the yolk was separated from albumen and eggshell. Albumen weight was calculated from the whole egg by subtracting yolk weight and shell weight. Egg shape index and yolk index were calculated as follows;

Egg shape index (%) = (height/diameter) x 100, yolk index (%) = (width/length) x 100

Eggshell thickness was measured at the three regions on the egg (air cell, equator, and sharp end). The Haugh unit was calculated using egg weight and albumen height which was obtained by using a micrometer while the yolk visual color was measured by matching the yolk with 15 bands of the Roche improved yolk color fan.

Hatching performance

Semen approximately 2.5 ml was collected from 15-pedigree cock and gradually mixed with an equal amount of diluent and fertilized 30 µl per hen. At the end of the experiment, all fertilized eggs were collected and hatching performance was evaluated. The total number of eggs in the incubator, unfertilized eggs, infertile eggs, and mortality were recorded to assess the fertility and hatchability of fertile eggs.

Antioxidant capacity measurement

At the end of the experiment, 2-birds per replicate were randomly chosen to collect blood from the wing vein and centrifuged at 958 g for 10-min to isolate serum from the blood while liver sample collected after 12 h fasting from 15-layers (1 bird per replicate). The collected sample was washed twice with ice-cold phosphate buffer saline and dried with filter paper to keep away from blood contamination. For antioxidant parameter, the liver samples were homogenaized by mixing with ice-cold isotonic physiological saline at a ratio of 1g/ml and centrifuge (958 g, 10 min) to get supernatant. The quantity of

total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in serum and liver were measured with commercial kits according to the manufactures instructions (Nanjing Jiancheng Bioengineering Research Institute Nanjing, China). The ellagic acid and *P. granatum* extract antioxidant activities were determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging assay. The DPPH assay was performed as previously described (Lee et al. 2003). The mechanism of this assay is based on the inhibition of the DPPH free radical and transfer of hydrogen atom from an antioxidant compound to free radical DPPH.

Determination of thiobarbituric acid reactive substance (TBARS) level

Lipid peroxidation was determined by measuring TBARS concentration as described by Grau et al (2000). Malondialdehyde absorbance was determined at wavelength 521.5 nm against the blank. Tetraethoxypropane was used in the standard curve as a malondialdehyde precursor. TBARS level was expressed as malondialdehyde µg/kg of sample (Grau et al. 2000).

Determination of antimicrobial activities of ellagic acid

E. coli and S. aureus clinical strains were grown in tryptic soy broth and lysogeny broth media respectively for 3 h and approximately 2 x 10^9 CFU/ml cells were spread on Muller-Hinton agar plates and challenged with 1 mg of ellagic acid. Plates were incubated at 37°C and zones of inhibition were measured after 24 h as described previously (Habib et al. 2020).

Statistical analysis

The results were expressed as means ± standard deviations and analyzed by SPSS version 25, IBM Corporation, USA. The significant difference was determined by ANOVA and followed by Tukey multiple range tests. Significant differences ($p < 0.05$) were represented by different alphabetical letters.

Results

Antioxidant activity of ellagic acid and P. granatum extract

In vitro antioxidant activities of ellagic acid and *P. granatum* extract were determined by DPPH radical scavenging assay. Ellagic acid showed a relatively higher radical scavenging activity than *P. granatum* extract. Ellagic acid scavenged approximately 78% while *P. granatum* extract 73% of DPPH radicals in the 0.8–100 µg/ml concentrations.

Bodyweight and feed intake

We determined the productivity parameters in isogeneic conditions in layers. The layers showed an increase in feed intake ($p < 0.05$) from week 33 to week 36 in treatment groups C (100 mg of ellagic acid) and E (*P. granatum* extract). Group B (50 mg of ellagic acid) also showed an increase in feed intake but not significantly ($p > 0.05$) while group D (200 mg of ellagic acid) has no increase in feed intake. During

the last week, the feed intake continuously increased in treatment groups C and E while groups B and D remained consistent (Table 2). The initial body masses of the birds in the various treatment groups were similar among the groups, however, after the end of the experiment, the groups fed 100 mg of ellagic acid was significantly heavier ($p < 0.05$) compared to the control and rest of the treatment groups (Table 3). We summarized that the supplementation of 100 mg of ellagic acid and *P. granatum* extract had a better effect on feed intake and bodyweight.

Table 2

Average feed intake values (in kg) per week of layers fed with different levels of ellagic acid and *P. granatum* extract during the whole experimental period. Values are given in means ± standard error. ^{abc}Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	Weeks 29–32	Weeks 33–36	Weeks 37–40	Weeks 41–44	Net feed intake/kg	ANOVA (p-values)
A	0.70 ± 0.02^{a}	0.74 ± 0.03^{a}	0.76 ± 0.01^{a}	0.74 ± 0.04^{a}	2.94 ± 0.025^{a}	0.10
B	0.72 ± 0.03^{a}	0.73 ± 0.01^{a}	0.77 ± 0.01^{a}	0.75 ± 0.04^{a}	2.97 ± 0.022^{a}	0.085
C	0.83 ± 0.01^{b}	0.85 ± 0.02^{b}	0.83 ± 0.02^{b}	0.85 ± 0.01^{b}	3.36 ± 0.015^{b}	0.025
D	0.80 ± 0.01^{a}	0.78 ± 0.02^{a}	0.72 ± 0.01^{a}	0.75 ± 0.02^{a}	3.01 ± 0.022^{a}	0.070
E	0.76 ± 0.04^{a}	0.78 ± 0.02^{c}	0.85 ± 0.04^{c}	0.83 ± 0.03^{c}	3.22 ± 0.032^{c}	0.040

Table 3

Average weekly bodyweight values (in kg) of layers fed with different levels of ellagic acid and *P. granatum* extract during the whole experimental period. Values are given in means ± standard error. [abc]Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	Weeks 29–32	Weeks 33–36	Weeks 37–40	Weeks 41–44	Net body-weight	ANOVA (p-values)
A	1.90 ± 0.02[a]	1.85 ± 0.04[a]	1.80 ± 0.02[a]	1.90 ± 0.04[a]	1.71 ± 0.03[a]	0.22
B	1.84 ± 0.03[a]	1.86 ± 0.05[a]	1.80 ± 0.04[a]	1.90 ± 0.03[a]	1.77 ± 0.03[a]	0.12
C	1.80 ± 0.04[a]	1.85 ± 0.05[a]	1.88 ± 0.01[b]	1.91 ± 0.04[b]	1.85 ± 0.03[b]	0.035
D	1.80 ± 0.04[a]	1.74 ± 0.05[a]	1.78 ± 0.05[a]	1.76 ± 0.04[a]	1.77 ± 0.04[a]	0.085
E	1.80 ± 0.04[a]	1.80 ± 0.03[a]	1.85 ± 0.02[a]	1.84 ± 0.03[a]	1.82 ± 0.03[a]	0.065

Feed conversion ratio

During the 29–32 and 33–36 weeks of the experiment, the feed conversion ratio was the lowest in group E and followed by group C, while group D showed the highest feed conversion ratio and followed by group B as shown in Table 4. In the last week of experiments, groups C and E showed better FCR compared to other treatment groups. Overall, the best FCR was observed in group C followed by groups E and B while the worst FCR was noticed in group D with 200 mg of ellagic acid.

Table 4

Average FCR values were calculated weekly among layers fed with different levels of ellagic acid and *P. granatum* extract during the whole experimental period. Values are given in means ± standard error. [abc]Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	Weeks 29–32	Weeks 33–36	Weeks 37–40	Weeks 41–44	Total FCR	ANOVA (p-values)
A	2.82 ± 0.03[a]	2.80 ± 0.03[a]	2.84 ± 0.03[a]	2.82 ± 0.05[a]	2.82 ± 0.03[a]	0.10
B	2.85 ± 0.03[a]	2.80 ± 0.04[a]	2.87 ± 0.05[a]	2.80 ± 0.04[a]	2.83 ± 0.04[a]	0.090
C	2.76 ± 0.02[a]	2.74 ± 0.01[b]	2.72 ± 0.03[b]	2.70 ± 0.02[b]	2.73 ± 0.02[b]	0.040
D	2.84 ± 0.04[a]	2.84 ± 0.03[a]	2.86 ± 0.05[ab]	2.85 ± 0.04[ab]	2.84 ± 0.04[ab]	0.070
E	2.72 ± 0.03[a]	2.73 ± 0.02[a]	2.78 ± 0.04[c]	2.76 ± 0.03[c]	2.75 ± 0.03[c]	0.045

Egg characteristics parameters

During the whole 16-weeks feeding period, average egg weight among the treatment groups showed similarities and there was no significant difference between the groups while group D was the worse among the groups. The percentage of egg production in groups C and E were significantly increased ($p < 0.05$) and had less percentage of broken or cracked eggs. The ellagic acid and *P. granatum* extract significantly increased egg mass in groups C and E compared to the rest of the treatment groups while groups B and D showed decrease egg mass compared to the control group as shown in Table 5. The group D showed a high percentage of broken or cracked eggs, as well as some shell-less eggs, that might be the result of abnormal digestion, or birds fail to ingest a high amount of ellagic acid.

Table 5
Effects of different levels of ellagic acid and *P. granatum* extract on egg production, egg weight, egg mass and cracked egg during the whole experimental period.

Groups	Egg weight (g)	Egg mass (g/day/bird)	Egg production (%)	Broken/cracked egg (%)
A	58.76 ± 0.01^{a}	29.84 ± 0.08	78.24 ± 0.03^{a}	1.90 ± 0.04^{a}
B	58.21 ± 0.01^{a}	28.66 ± 0.19	78.14 ± 0.02^{a}	1.86 ± 0.04^{a}
C	58.28 ± 0.03^{a}	29.47 ± 0.02	83.50 ± 0.03^{b}	0.60 ± 0.02^{b}
D	57.13 ± 0.04^{a}	28.21 ± 0.20	76.75 ± 0.04^{a}	2.1 ± 0.03^{c}
E	58.78 ± 0.02^{a}	29.61 ± 0.024	81.25 ± 0.01^{c}	0.58 ± 0.01^{ab}

Data showed the average values ± standard error after 16-weeks of treatment. ^{abc}Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Egg quality parameters

The effect of ellagic acid and *P. granatum* extract on external and internal egg quality parameter of laying hens were shown in Tables 6 and 7. The whole egg quality parameters were not significantly affected ($p > 0.05$) by ellagic acid and *P. granatum* extract treatment groups compared to the control group except for shell weight and shell thickness that were increased significantly in group C and E ($p < 0.001$). The egg shape index, albumen weight (%), yolk weight (%), yolk index (%), and the color score did not change by adding ellagic acid and *P. granatum* extract in the diet. When we determined the Hough unit, which is the observed height of albumin correlated with egg weight, slightly increased in group E ($p > 0.05$) compared to the rest of the treatment groups as shown in Table 6.

Table 6

Effects of different levels of ellagic acid and *P. granatum* extract on egg external quality parameters during the whole experimental period. Values are given in means ± standard error. [abc]Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	Shell weight (g)	Shell thickness (mm)	Egg shape index (%)	Hough unit (%)
A	5.80 ± 0.07^{a}	0.374 ± 0.004^{a}	74.61 ± 0.02^{a}	73.61 ± 0.87^{a}
B	5.82 ± 0.06^{a}	0.375 ± 0.002^{a}	74.63 ± 0.03^{a}	73.63 ± 0.48^{a}
C	6.04 ± 0.02^{b}	0.403 ± 0.003^{b}	74.58 ± 0.31^{a}	73.86 ± 1.06^{a}
D	5.96 ± 0.03^{a}	0.371 ± 0.002^{a}	74.61 ± 0.02^{a}	73.62 ± 0.93^{a}
E	6.12 ± 0.02^{c}	0.397 ± 0.003^{c}	74.64 ± 0.32^{a}	74.20 ± 0.07^{a}

Table 7

Effects of different levels of ellagic acid and *P. granatum* extract on egg internal quality parameters during the whole experimental period. Data showed the average values ± standard error after 16-weeks of treatment. The egg internal quality parameters (albumen weight, yolk weight, yolk index, and yolk color) were not changed significantly ($p > 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	Albumen weight (%)	Yolk weight (%)	Yolk index (%)	Yolk color score
A	55.94 ± 0.36	35.10 ± 0.63	43.12 ± 0.51	7.15 ± 0.01
B	55.96 ± 0.32	35.19 ± 0.76	43.22 ± 0.51	7.15 ± 0.02
C	55.65 ± 0.26	35.17 ± 0.78	43.35 ± 0.32	7.40 ± 0.04
D	55.86 ± 0.34	35.14 ± 0.86	43.23 ± 0.52	7.23 ± 0.05
E	55.94 ± 0.36	35.19 ± 0.64	43.28 ± 0.64	7.16 ± 0.04

Reproductive performance of laying hens

Ellagic acid and *P. granatum* extract supplemented diet had significantly increased the hatchability of eggs. A diet containing 100 mg of ellagic acid and 200 mg/kg *P. granatum* extract had significantly increased hatchability ($p < 0.05$) as showed in Table 8. In group C and E, the hatchability percentage was 84.16%, 83.41% respectively. No significant effect was found in the percentage fertility among the different treatment groups while group C and D show low fertility between the groups. The *P. granatum*

extract had a positive effect on improving fertility while the highest fertility was observed in group A (control) and E in comparison to the rest of the group.

Table 8
Effects of different levels of ellagic acid and *P. granatum* extract on the reproductive performance of laying hens during the whole experimental period. Values are given in means ± standard error. [abc]Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	Fertility (%)	Hatchability (%)	Hatched chicks Bodyweight (g)	Mortality (%)
A	90.56 ± 0.85[a]	82.92 ± 1.07[a]	35.41 ± 0.18[a]	4.10 ± 0.01[a]
B	90.24 ± 0.64[a]	82.96 ± 0.34[a]	35.14 ± 0.16[a]	4.00 ± 0.23[a]
C	88.81 ± 0.82[a]	84.16 ± 1.32[b]	35.71 ± 0.14[a]	3.00 ± 0.12[b]
D	88.11 ± 0.62[a]	82.12 ± 0.08[a]	35.43 ± 0.52[a]	4.31 ± 0.31[a]
E	90.41 ± 0.71[a]	83.41 ± 1.23[c]	35.68 ± 0.26[a]	3.21 ± 0.04[c]

Hatched chicks bodyweight was not influenced by any of the treatment groups. The overall weight of hatched chicks was in the range of 35.14 to 35.71g among the groups. Mortality was also noticed during hatching period; mortality was significantly decreased in the treatment group C and E and their mortality percentage was 3.0% and 3.21% respectively compared to the rest of groups as mentioned in Table 8.

Serum antioxidant potential

Addition of ellagic acid and *P. granatum* extract to feed quadratically increases T-SOD, T-AOC, and GSH-Px ($p < 0.05$) potential in serum during the experimental period as shown in Table 9. The T-SOD concentration was higher in group E and followed by group D and C while T-AOC concentration was higher in the following order in groups; D > C > E > B > A. Highest GSH-Px activities were noticed in groups E and D while the lowest was observed in the control group. A significant decrease ($p < 0.01$) was detected in MDA level in ellagic acid treated groups C and D, and group E compared to control (Table 9).

Table 9

Effects of different levels of ellagic acid and *P. granatum* extract on serum antioxidant indices of laying hens during the whole experimental period. Data showed the average values ± standard error after 16-weeks of treatment. [abc]Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	T-SOD U/ml	T-AOC, U/ml	GSH-Px, U/ml	MDA, nmol/ml
A	417.2 ± 11.47^{a}	4.22 ± 0.14^{a}	1330.50 ± 0.41^{a}	9.85 ± 0.18^{a}
B	429.6 ± 10.06^{a}	4.26 ± 0.32^{a}	1376.10 ± 0.44^{a}	9.12 ± 0.46^{a}
C	480.6 ± 14.65^{b}	5.18 ± 0.02^{b}	1590.24 ± 0.89^{b}	7.64 ± 0.38^{b}
D	510.0 ± 8.56^{ab}	5.57 ± 0.38^{ab}	1620.57 ± 0.32^{ab}	7.36 ± 0.16^{a}
E	520.6 ± 12.43^{c}	5.14 ± 0.01^{c}	1660.52 ± 0.31^{c}	7.84 ± 0.36^{c}

Liver antioxidant potential

Antioxidant activity of ellagic acid and *P. granatum* extract are summarized in Table 10. The T-AOC and T-SOD were significantly increased in the treatment groups in the liver samples ($p < 0.05$) compared to the control group. The highest concentration of T-AOC and T-SOD was observed in group D followed by group C and E. Numerically, GSH-Px concentration increased in ellagic acid groups C and D, followed by group E while control and group B were similar. The MDA level was significantly decreased in groups C, D, and E ($p < 0.05$) compared to control group.

Table 10
Effects of different levels of ellagic acid and *P. granatum* extract on liver antioxidant indices of laying hens during the whole experimental period. Data showed the average values ± standard error after 16-weeks of treatment.
[abc]Means values within the same column with different superscript differ significantly ($p < 0.05$). Groups denoted: A; control (without ellagic acid), B; 50 mg/kg of ellagic acid, C; 100 mg/kg of ellagic acid, D; 200 mg/kg of ellagic acid, and E; 200 mg/kg of *P. granatum* extract.

Groups	T-AOC, U/ml	T-SOD (U/mg pro)	GSH-Px (U/ml)	MDA (nmol/mg pro)
A	1.75 ± 0.06[a]	98.36 ± 2.68[a]	33.46 ± 1.43[a]	0.76 ± 0.02[a]
B	1.82 ± 0.03[a]	97.52 ± 2.47[a]	33.48 ± 1.56[a]	0.68 ± 0.04[a]
C	2.10 ± 0.001[b]	102.52 ± 3.0[b]	35.96 ± 1.74[b]	0.54 ± 0.04[b]
D	2.20 ± 0.02[ab]	102.93 ± 3.0[ab]	35.46 ± 1.74[ab]	0.50 ± 0.03[c]
E	2.00 ± 0.02[c]	100.52 ± 2.2[c]	34.70 ± 1.03[c]	0.55 ± 0.02[ab]

Ellagic acid decreased lipid peroxidation in chicken meat

We evaluated the effects of ellagic acid and *P. granatum* extract supplementation on lipid oxidation in chicken meat by assessing the malondialdehyde level by measuring TBARS. We collected raw meat samples and determined TBARS values in chicken meat supplemented with ellagic acid and *P. granatum* extract. Ellagic acid fed group C showed lowest values of TBARS when compared to the rest of the groups (Fig. 1). Group E also showed lower TBARS values than group D and B, which suggested that lipid oxidation was decreased upon supplementation of ellagic acid and *P. granatum* extract in birds feed (Fig. 1).

Antimicrobial activities of ellagic acid

Ellagic acids showed strong killing activity against *E. coli* and *S. aureus* while it was ineffective against methicillin-resistant *S. aureus* (MRSA). The plates shown in Fig. 2 indicated a clear zone of inhibition against *E. coli* and *S. aureus* while no clear zone of inhibition was observed against MRSA which showed that ellagic acid is not active in killing MRSA.

Discussion

Antioxidants are considered very crucial for growth development in animals (Lobo et al. 2010). The supplementation of different antioxidant compounds in the diet is widely tested in poultry research such as essential oil, tannic acid, and coffee residues (Brewer 2011; Shahidi et al. 1992). Certain stresses such as heat, toxin, and pathogen can adversely affect poultry development while the supplementation of

phenolic compound is effective against such kinds of stresses (Mishra and Jha 2019). Ellagic acid is a phenolic lactone compound naturally present in a variety of fruits (Beattie et al. 2005; Mielnik et al. 2006; Seeram 2007; Usta et al. 2013). Studies showed that ellagic acid has a number of roles such as; anti-mutagenic (Zahin et al. 2014), anti-carcinogenic (Zhang et al. 2014), anti-inflammatory (BenSaad et al. 2017), antibacterial (De et al. 2018; Panichayupakaranant 2010), and is a good chelator agent (Ahmed et al. 1999; Li et al. 2018; Saha et al. 2016). Dietary supplementation of ellagic acid has enhanced growth performance in rats and human but remain unexplored in poultry (Del Rio et al. 2013; Farzaei et al. 2015; Keservani et al. 2016; Rosillo et al. 2012; Usta et al. 2013). Our results summarized that birds supplemented with ellagic acid and *P. granatum* extract diet successfully increased egg production, bodyweight, egg mass, had better FCR and no depressing characteristics of birds were detected. The experimental diet did not affect the external and internal quality of eggs while increased shell weight and shell thickness. Our results showed that *P. granatum* extract had a positive effect on fertility while ellagic acid-treated groups showed decrease in fertility. Similar results were reported where ellagic acid improves overall health status of birds (Kratzer et al. 1975; Shanmugam and Rao 2015). In this study, we also found a significant increase in T-AOC, T-SOD, and GSH-Px concentration and a decrease in MDA level in the liver and serum samples of ellagic acid (100 and 200 mg/kg) and *P. granatum* extract treated groups. Our findings are in accordance with reports that reported ellagic acid as an agent that increases T-AOC, T-SOD, and GSH-Px and decreases the MDA concentrations in rats (Chen et al. 2018; Özkaya et al. 2010; Yüce et al. 2007) and trout fish (Mişe Yonar et al. 2014) in the liver, kidney and spleen samples. We also detected lipid peroxidation in raw chicken meat that was significantly decreased in ellagic acid (100 and 200 mg/kg) and *P. granatum* extract treated groups. Several reports provided evidence that ellagic acid can decrease lipid peroxidation in animals tissues (Bucak et al. 2019; Han et al. 2006; Majid et al. 1991; Nishigaki et al. 1993). Apart from the antioxidant function, ellagic acid showed antibacterial activities against *E. coli* and *S. aureus* while ineffective against MRSA. This result was consistent with the previous findings which showed the strong antibacterial activity of ellagic acid against different bacteria (Bindu et al. 2016; De et al. 2018). Altogether, we showed that ellagic acid and *P. granatum* extract have functional roles in better feed utilization, food digestion, productions performance of chickens, antioxidant potential, and antimicrobial activities. In summary, ellagic acid and *P. granatum* extract are safe to be supplemented in chicken feed and no undesirable effects were observed. Further study can increase understanding of their roles as antioxidants in egg-laying ducks and commercial broiler breeder.

Declarations

Funding

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Declaration of Competing Interest

The authors have declared no conflict of interest.

Code or data availability

Not applicable

Authors' contributions

Haji Gul, Zhaoyu Geng, Azam Hayat, and Gul Habib conceptualized the study. Haji Gul, Gul Habib, and Imran Khan performed the experiments. Haji Gul, Zhaoyu Geng, and Gul Habib received the funding. Gul Habib, Azam Hayat, Zhaoyu Geng and Mujaddad Ur Rehman revised and proofread the manuscript.

Ethical Approval

The animal management guidelines of the China Council on Animal Care were followed and the experimental protocols were approved by the Animal care committee of Anhui Agricultural University.

Consent to participate

Not applicable

Consent for publication

All authors read and approved the present version for publication.

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Figures

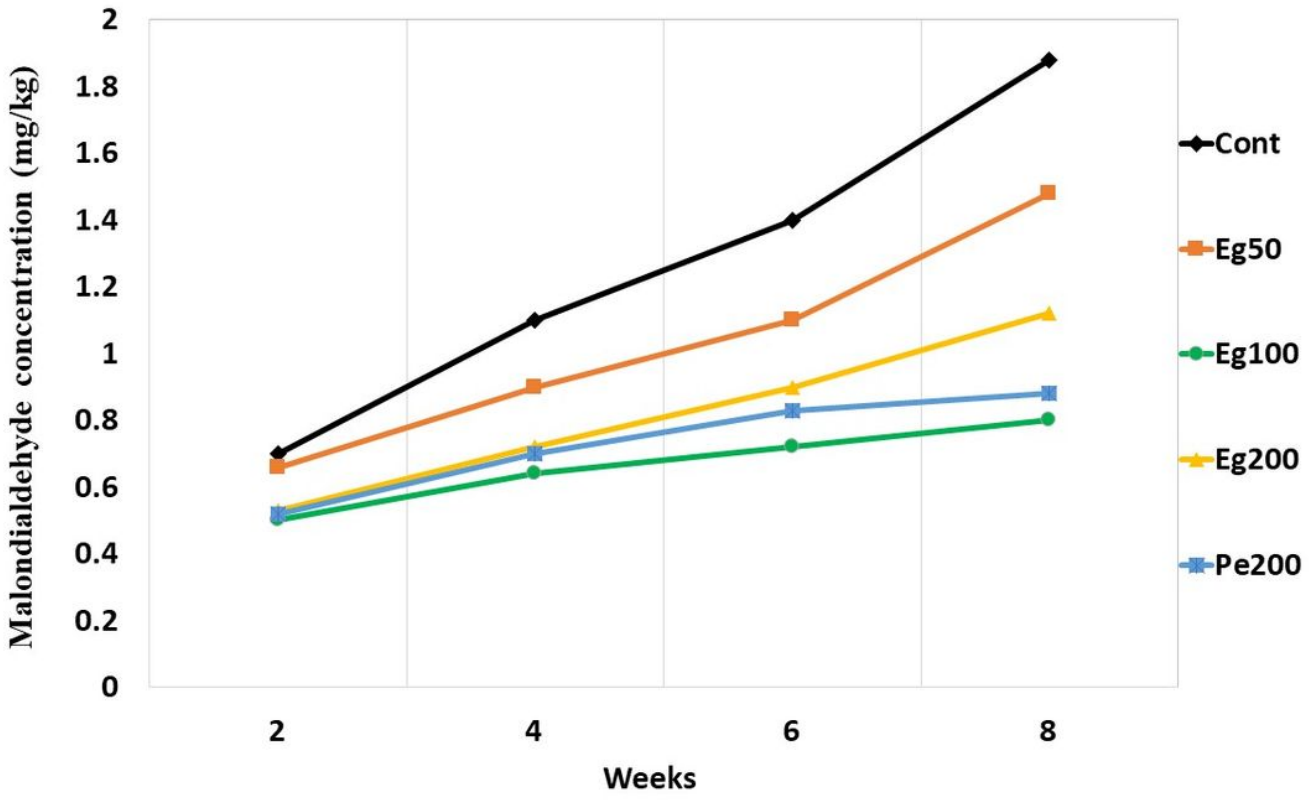

Figure 1

TBARS values in raw chicken meat were determined after 2, 4, 6, and 8 weeks of feeding ellagic acid and *P. granatum* extract containing diet. Cont.; without ellagic acid, Eg50; the group fed 50 mg ellagic acid,

Eg100; the group fed 100 mg ellagic acid, Eg200; the group fed 200 mg ellagic acid, Pe200; the group fed 200 mg *P. granatum* extract.

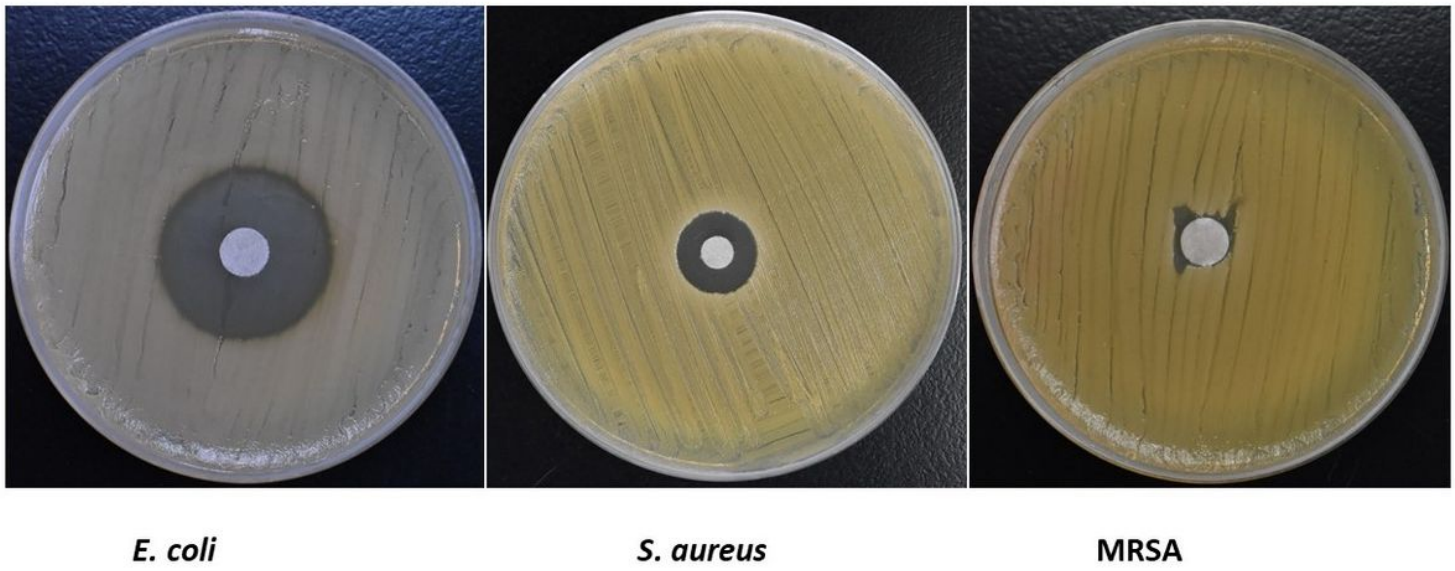

Figure 2

Ellagic acid (1mg) actively killed *E. coli* and *S. aureus* cells with an average of 8 mm and 3 mm zone of inhibition, respectively. Ellagic acid did not show any clear zone against MRSA.