



Impact of Pomegranate Molasses and Peel on Antioxidant Enzyme Activity and Lipid Peroxidation in Laying Hens

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ABSTRACT

Background: Oxidative stress impairs laying hen performance by disrupting the balance between reactive oxygen species and endogenous antioxidant defenses. Pomegranate by-products (molasses and peel) are rich in polyphenols and may strengthen antioxidant capacity in layers.

Methods: One hundred eighty Lohmann Sandy hens (52 wk) were randomly allotted to nine diets: control (T1), molasses at 0.5% (T2) and 1.0% (T3), peel at 1.0% (T4) and 2.0% (T5), and combinations (T6-T9). Diets were iso-nitrogenous/iso-caloric (17.5% CP; 2750 kcal/kg ME; 3.5% Ca). Blood was sampled at Weeks 52, 55 and 58 for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) by ELISA. Data were analyzed by two-way ANOVA; means separated by Duncan's test at $P < 0.01$.

Result: Relative to control (T1), 1.0% molasses (T3) produced the greatest improvements: SOD 101.85 ± 2.10 vs 47.18 ± 5.83 U·mL⁻¹; CAT 32.66 ± 4.92 vs 13.73 ± 1.99 U·mL⁻¹; GPx 40.82 ± 0.85 vs 15.50 ± 3.23 U·mL⁻¹ and MDA 10.56 ± 0.89 vs 23.96 ± 1.36 nmol/mL (all $P < 0.01$). Peel alone yielded moderate effects; combination diets showed intermediate responses.

Key words: Antioxidant enzymes, Laying hens, Malondialdehyde, Oxidative stress, Pomegranate molasses, Pomegranate peel.

INTRODUCTION

Oxidative stress is a major constraint in poultry production, as it disrupts the balance between reactive oxygen species and the antioxidant defense system, thereby impairing health and productivity (Surai *et al.*, 2019). High-yielding laying hens are especially vulnerable during late production stages due to their elevated metabolic activity and reproductive demands, which increase oxidative load and accelerate cellular damage (Aryal *et al.*, 2025; Oke *et al.*, 2024). Nutritional interventions that improve antioxidant resilience are therefore crucial for sustaining performance and welfare in layers.

Among natural feed additives, pomegranate (*Punica granatum* L.) by-products such as molasses and peel are rich sources of phenolic compounds including ellagitannins, anthocyanins and punicalagins, which exhibit strong antioxidant and anti-inflammatory activities (Husain *et al.*, 2018; Kang *et al.*, 2019). Recent syntheses also highlight the consistent benefits of plant polyphenols for oxidative balance, immunity and performance in poultry (Lioliopoulou *et al.*, 2024). Recent studies have confirmed that pomegranate peel supplementation improves egg quality, antioxidant enzyme activity and health status in laying hens (Xu *et al.*, 2025; Ghasemi-Sadabadi *et al.*, 2022), while polyphenol-rich extracts enhance growth, immunity and oxidative resilience in broilers exposed to heat stress (Safari *et al.*, 2018; Ahmadipour *et al.*, 2021). Reviews have also emphasized the nutraceutical potential of fruit by-products, including pomegranate, in supporting sustainable livestock production systems (Jain *et al.*, 2024; Siddiqui *et al.*, 2024).

Furthermore, valorization of pomegranate peel and molasses as functional feed ingredients provides both

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nutritional and environmental benefits, reducing agro-industrial waste while supplying low-cost antioxidants for animal diets (Srinontong *et al.*, 2024; Ciampi *et al.*, 2022). However, limited information is available on the combined use of pomegranate molasses and peel in layer diets, particularly under late production stages when oxidative stress is more pronounced.

The present study was therefore undertaken to investigate the effects of dietary supplementation with pomegranate molasses, peel and their combinations on antioxidant enzyme activities and lipid peroxidation in Lohmann Sandy laying hens during 52-58 weeks of age.

MATERIALS AND METHODS

Experimental site and period

The study was conducted at the Poultry Experimental Unit, Department of Animal Science, College of Agricultural

Engineering Sciences, University of Sulaimani, Kurdistan Region, Iraq. The experiment began on October 3, 2024 and continued for 60 days, concluding on December 2, 2024.

A total of 180 Lohmann Sandy laying hens, aged 52 weeks at the start of the trial, were used in this experiment. Birds were individually wing-banded, weighed and randomly assigned to dietary treatments under a completely randomized design.

Housing and management

Birds were housed in wire cages (55 cm × 60 cm) under controlled conditions: temperature 18-20°C, 14 h light: 10 h dark and good ventilation. Feed was offered at 110 g/hen/day; water was available *ad libitum*.

Experimental design and diets

The experiment followed a completely randomized design with nine dietary treatments. The control group (T1) received the basal diet without supplementation. Molasses was included at two levels: 0.5% (T2) and 1.0% (T3). Peel was included at two levels: 1.0% (T4) and 2.0% (T5). Four combination treatments were formulated: 0.5% molasses + 1.0% peel (T6), 0.5% molasses + 2.0% peel (T7), 1.0% molasses + 1.0% peel (T8) and 1.0% molasses + 2.0% peel (T9). Formulation followed standard practice in graded inclusion trials of pomegranate by-products in poultry diets (Li *et al.*, 2017).

All diets were formulated to be iso-nitrogenous and iso-caloric, meeting the nutrient requirements for laying hens (17.5% crude protein, 2750 kcal/kg ME and 3.5% calcium). Minor adjustments in soybean meal, wheat bran and wheat were made to maintain nutrient balance across treatments.

Feed ingredients and composition

The ingredient composition of the experimental diets is presented in Table 1. All rations were formulated using locally available feedstuffs to meet the nutrient

requirements of laying hens. Antifungal agents were included at 1% in all diets to maintain feed quality. The crude protein, metabolizable energy and calcium contents were kept constant across treatments.

Parameters measured

Blood samples were collected from hens at Weeks 52, 55 and 58. Approximately 3 mL of blood was drawn from the wing vein of two birds per replicate using sterile syringes. Samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 10 minutes to obtain serum, which was stored at -20°C until further analysis.

The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as the concentration of malondialdehyde (MDA), a biomarker of lipid peroxidation, were determined using commercial ELISA kits (BT Laboratory, China). All procedures were performed according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean±standard deviation (SD). A two-way analysis of variance (ANOVA) was performed using XLStat software (Addinsoft, France) to evaluate the effects of pomegranate molasses, pomegranate peel and their interaction across weeks. When significant differences were detected ($P<0.01$), Duncan's multiple range test (DMRT) was applied for mean separation among treatments.

RESULTS AND DISCUSSION

Antioxidant enzyme activities

The activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in laying hens are presented in Table 2-4. In the control group (T1), a progressive decline ($P<0.01$) was observed from Week 52 to 58, indicating reduced antioxidant capacity

Table 1: Ingredient composition (% as-fed) of experimental diets (T1-T9).

Ingredient	T1	T2	T3	T4	T5	T6	T7	T8	T9
M (Molasses)	0.0	0.5	1.0	0.0	0.0	0.5	0.5	1.0	1.0
P (Peel)	0.0	0.0	0.0	1.0	2.0	1.0	2.0	1.0	2.0
Antifungal	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Soybean meal	25.0	25.0	25.4	25.4	25.6	25.0	25.0	25.6	25.0
Primex	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Corn	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0
Wheat bran	5.0	4.0	3.0	3.0	2.0	3.0	2.0	2.0	2.0
Wheat	16.5	17.0	17.1	17.1	16.9	17.0	17.0	16.9	16.5
Oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Limestone	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Crude protein (%)	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
ME (kcal/kg)	2750	2750	2750	2750	2750	2750	2750	2750	2750
Calcium (%)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5

M= Pomegranate molasses; P= Pomegranate peel. Diets were formulated to be iso-nitrogenous (17.5% crude protein) and iso-caloric (2750 kcal/kg ME) by adjusting wheat bran and soybean meal levels while maintaining constant calcium (3.5%).

in hens not receiving dietary supplementation. For example, GPx activity in T1 decreased from 19.05 ± 0.29 at Week 52 to 12.10 ± 0.34 at Week 58, with an overall mean of 15.50 ± 3.23 . Similar reductions in antioxidant enzyme activity with advancing age have been reported in commercial layers (Surai *et al.*, 2019; Aryal *et al.*, 2025).

In contrast, molasses-supplemented groups showed significant improvements. At 1.0% inclusion (T3), GPx

activity peaked at 40.82 ± 0.85 , SOD at 104.25 ± 0.72 and CAT at 36.62 ± 0.13 during Week 58, demonstrating strong enhancement of the antioxidant defense system. These results confirm that polyphenol-rich feed ingredients improve antioxidant enzyme activity by scavenging reactive oxygen species and stimulating the Nrf2/Keap1 pathway (Husain *et al.*, 2018; Kang *et al.*, 2019; Tornese *et al.*, 2024). In addition, greater GPx activity may reflect improved

Table 2: Effects of pomegranate molasses and peel on glutathione peroxidase (GPx) activity in laying hens (U·mL⁻¹ serum; Mean±SD).

Term	Week 52	Week 55	Week 58	Overall Mean±SD
T1	19.05 ± 0.29 n	15.35 ± 0.45 o	12.10 ± 0.34 p	15.50 ± 3.23 H
T2	31.45 ± 0.34 e	34.23 ± 0.55 d	37.88 ± 0.49 c	34.52 ± 3.23 B
T3	39.13 ± 0.45 b	40.25 ± 0.34 a	40.82 ± 0.60 a	40.82 ± 0.85 A
T4	20.18 ± 0.45 m	21.35 ± 0.26 d	21.73 ± 0.23 jk	21.09 ± 0.78 FG
T5	20.58 ± 0.38 lm	20.73 ± 0.49 lm	21.18 ± 0.43 d	20.83 ± 0.30 G
T6	26.15 ± 0.49 h	29.88 ± 0.70 f	30.25 ± 0.78 f	28.76 ± 2.23 C
T7	21.73 ± 0.30 j	21.38 ± 0.34 kl	21.43 ± 0.17 kl	21.51 ± 0.17 E
T8	24.95 ± 0.34 i	26.00 ± 0.18 h	27.38 ± 0.23 g	26.11 ± 1.23 D
T9	21.15 ± 0.34 kl	21.23 ± 0.55 klm	22.23 ± 0.38 j	21.54 ± 0.55 EF
Overall Mean±SD	24.98 ± 7.23 C	25.56 ± 7.45 B	26.10 ± 8.23 A	-

• a-p: Means within a column with different superscripts differ significantly (* $p < 0.01$, Duncan's test).

• A-H: Overall means with different superscripts differ significantly (* $p < 0.01$).

Table 3: Effects of pomegranate molasses and peel on superoxide dismutase (SOD) activity in laying hens (U·mL⁻¹ serum; Mean±SD).

Term	Week 52	Week 55	Week 58	Overall Mean±SD
T1	50.90 ± 0.47 o	50.40 ± 0.65 o	40.25 ± 0.61 p	47.18 ± 5.83 G
T2	97.72 ± 1.97 c	98.12 ± 0.64 c	101.27 ± 0.79 b	99.04 ± 2.0 B
T3	100.12 ± 0.84 b	101.20 ± 0.83 b	104.25 ± 0.72 a	101.85 ± 2.10 A
T4	75.37 ± 0.78 g	59.40 ± 0.76 k	51.05 ± 0.74 o	61.94 ± 11.23 EF
T5	71.07 ± 0.76 h	61.20 ± 0.57 j	51.40 ± 0.77 8o	61.22 ± 9.23 F
T6	61.62 ± 0.94 ij	80.27 ± 0.34 g	90.97 ± 0.65 d	77.62 ± 13.23 C
T7	71.45 ± 0.22 h	58.82 ± 0.83 kl	56.87 ± 0.64 m	62.38 ± 7.23 E
T8	63.07 ± 0.40 i	71.25 ± 0.43 h	86.20 ± 0.80 e	73.50 ± 10.23 D
T9	76.02 ± 0.30 g	57.25 ± 0.81 lm	53.45 ± 0.66 n	62.25 ± 10.17 E
Overall Mean±SD	74.15 ± 17.24 A	70.88 ± 18.23 B	70.63 ± 22.23 B	

• a-p: Means within a column with different superscripts differ significantly (* $p < 0.01$, Duncan's test).

• A-H: Overall means with different superscripts differ significantly (* $p < 0.01$).

Table 4: Effects of pomegranate molasses and peel on catalase (CAT) activity in laying hens (U·mL⁻¹ serum; Mean±SD).

Term	Week 52	Week 55	Week 58	Overall Mean±SD
T1	15.62 ± 0.14 n	14.22 ± 0.21 o	11.35 ± 0.11 p	13.73 ± 1.99 I
T2	27.20 ± 0.22 e	28.45 ± 0.29 d	30.55 ± 0.40 c	28.73 ± 1.68 B
T3	26.80 ± 0.18 e	34.57 ± 0.14 b	36.62 ± 0.13 a	32.66 ± 4.92 A
T4	21.20 ± 0.18 j	19.55 ± 0.11 l	19.25 ± 0.12 l	20.00 ± 0.85 G
T5	20.62 ± 0.65 k	19.42 ± 0.08 l	18.67 ± 0.14 m	19.57 ± 0.95 H
T6	22.62 ± 0.14 h	23.30 ± 0.15 g	24.35 ± 0.20 f	23.42 ± 0.86 C
T7	22.00 ± 0.22 i	22.15 ± 0.18 hi	23.35 ± 0.33 g	22.50 ± 1.00 E
T8	21.50 ± 0.12 j	22.35 ± 0.13 hi	24.52 ± 0.24 f	22.79 ± 1.51 D
T9	22.55 ± 0.12 h	20.65 ± 0.14 k	20.47 ± 0.25 k	21.22 ± 0.98 F
Overall Mean±SD	22.23 ± 3.58 C	22.74 ± 5.03 B	23.23 ± 6.71 A	

• a-p: Means within a column with different superscripts differ significantly (* $p < 0.01$, Duncan's test).

• A-I: Overall means with different superscripts differ significantly (* $p < 0.01$).

Table 5: Effects of pomegranate molasses and peel on malondialdehyde (MDA) activity in laying hens (nmol/mL serum; Mean $\pm$ SD).

Term	Week 52	Week 55	Week 58	Overall Mean $\pm$ SD
T1	22.45 $\pm$ 0.29c	24.35 $\pm$ 0.32b	25.10 $\pm$ 0.30a	23.96 $\pm$ 1.36A
T2	13.37 $\pm$ 0.10l	10.72 $\pm$ 0.22o	9.65 $\pm$ 0.21p	11.25 $\pm$ 1.69G
T3	11.57 $\pm$ 0.21n	10.50 $\pm$ 0.18o	9.62 $\pm$ 0.14p	10.56 $\pm$ 0.89H
T4	15.47 $\pm$ 0.19e	14.35 $\pm$ 0.20hi	13.60 $\pm$ 0.15kl	14.47 $\pm$ 0.86C
T5	16.00 $\pm$ 0.15d	14.57 $\pm$ 0.19gh	14.52 $\pm$ 0.17gh	15.03 $\pm$ 0.80B
T6	13.87 $\pm$ 0.19ijkl	11.80 $\pm$ 0.15n	10.87 $\pm$ 0.17o	12.18 $\pm$ 1.36F
T7	13.62 $\pm$ 0.23jkl	14.57 $\pm$ 0.21gh	14.10 $\pm$ 0.27hijk	14.10 $\pm$ 0.49D
T8	14.90 $\pm$ 0.18fg	12.75 $\pm$ 0.11m	12.37 $\pm$ 0.19m	13.34 $\pm$ 1.20E
T9	15.25 $\pm$ 0.21ef	14.12 $\pm$ 0.24hij	13.65 $\pm$ 0.15jkl	14.35 $\pm$ 0.81CD
Overall mean $\pm$ SD	15.16 $\pm$ 3.58 A	14.19 $\pm$ 4.03 B	13.72 $\pm$ 4.71 C	

- a-p: Means within a column with different superscripts differ significantly (* p <0.01, Duncan's test).
- A-H: Overall means with different superscripts differ significantly (* p <0.01).

selenium utilization under polyphenol-rich diets (Malyugina *et al.*, 2021). This is consistent with evidence that dietary polyphenols can enhance trace-mineral bioavailability and antioxidant defenses (Andrés *et al.*, 2024). Comparable findings were reported by Xu *et al.* (2025) in laying hens and by Ahmadipour *et al.* (2021) in broilers, where pomegranate peel and extracts significantly elevated antioxidant enzyme activity. Comparable enzyme elevations with pomegranate peel were also reported in Japanese quails (Sharifian *et al.*, 2019).

Peel-supplemented diets (T4, T5) produced moderate increases, whereas combination treatments (T6-T9) showed intermediate responses, suggesting possible nutrient interactions or a ceiling effect of polyphenols at higher inclusion levels. High concentrations of tannins and ellagitannins in pomegranate peel, while antioxidant in nature, may also exert mild antinutritional effects by chelating trace minerals or reducing protein digestibility (Samtiya *et al.*, 2020; Tsegay *et al.*, 2025). Similar limitations were noted by Ghasemi-Sadabadi *et al.* (2022) when feeding higher levels of peel powder to layers.

These findings are in agreement with earlier studies where inclusion of fruit by-products in poultry diets improved oxidative resilience, reduced lipid peroxidation and supported productive performance (Srinontong *et al.*, 2024; Jain *et al.*, 2024; Safari *et al.*, 2018).

Lipid peroxidation (MDA levels)

The concentration of malondialdehyde (MDA), a biomarker of lipid peroxidation, is shown in Table 5. In the control group (T1), MDA levels increased steadily from 22.45 $\pm$ 0.29 at Week 52 to 25.10 $\pm$ 0.30 at Week 58, with an overall mean of 23.96 $\pm$ 1.36, confirming oxidative stress in unsupplemented hens. Similar increases in lipid peroxidation markers in aging or stressed birds have been reported previously (Surai *et al.*, 2019; Oke *et al.*, 2024).

In contrast, molasses supplementation significantly (P <0.01) reduced MDA levels, with the lowest values observed in T3 (1.0% molasses), which recorded 9.62 $\pm$ 0.14 at Week 58 and an overall mean of 10.56 $\pm$ 0.89.

Peel supplementation also reduced MDA concentration, though to a lesser degree than molasses. The combination treatments (T6-T9) yielded intermediate improvements, suggesting partial synergistic effects. These reductions can be attributed to potent polyphenols such as ellagic acid and punicalagins, which inhibit lipid peroxidation by neutralizing free radicals (Siddiqui *et al.*, 2024; Kang *et al.*, 2019).

Comparable outcomes have been reported in layers and broilers, where supplementation with pomegranate peel powder or extract lowered TBARS values and improved oxidative stability of meat and eggs (Xu *et al.*, 2025; Ghasemi-Sadabadi *et al.*, 2022; Ahmadipour *et al.*, 2021). Ciampi *et al.* (2022) further demonstrated that pomegranate extracts modulate oxidative stress at the cellular level, providing mechanistic support for the reductions observed in the present study.

Overall, the inclusion of pomegranate molasses and peel improved antioxidant enzyme activity and reduced lipid peroxidation, thereby enhancing oxidative stability in laying hens. These results support the utilization of pomegranate by-products as cost-effective feed additives in poultry diets, contributing both to improved bird health and to sustainable recycling of agro-industrial waste (Srinontong *et al.*, 2024; Jain *et al.*, 2024).

CONCLUSION

The present study demonstrated that dietary supplementation of pomegranate molasses and peel improved the antioxidant defense system and reduced lipid peroxidation in laying hens during late production. Molasses at 1.0% produced the most pronounced effects, significantly enhancing the activities of superoxide dismutase, catalase and glutathione peroxidase, while reducing malondialdehyde levels. Peel supplementation alone showed moderate benefits, whereas combination treatments yielded intermediate responses, suggesting possible nutrient interactions. These findings highlight the potential of pomegranate by-products as functional feed additives for enhancing oxidative stability and supporting the health and productivity of laying hens. The use of

molasses and peel also contributes to sustainable poultry production by valorizing agro-industrial by-products as cost-effective feed ingredients.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. The research was conducted independently and no financial or personal relationships influenced the outcomes of the study.

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