



Modulatory Effects of Dietary Pomegranate Peel on the Growth Performance, Immunological Competence and Antioxidant Activity of Broiler Chickens

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Article History: 25-335

Received: 3-Dec-25

Revised: 8-Jan-26

Accepted: 11-Jan-26

Online First: 23-Jan-26

ABSTRACT

The poultry industry is confronted with high feed costs and limitations of antibiotics as growth promoters, which requires exploring highly effective natural alternatives. Pomegranate peel, as a good source of bioactive polyphenols, would provide a potential choice due to its recognized antioxidant and immunomodulating capacity. The impact of pomegranate peel powder (PP) as a functional feed supplement on broilers' performance, immune response, and antioxidant status was explored. A total of 400 one-day-old Cobb 500 chicks were randomly assigned to four dietary treatment groups (five replicates×20 birds). Starting from day 21 to 42 of age, the experimental groups were offered a basal diet supplemented with 0.0, 0.5, 1.0, or 1.5g of PP per kg of feed. Growth performance parameters were monitored. Meanwhile, cellular and humoral immune responses were assessed, as well as antioxidant status markers. PP supplementation markedly improved both humoral and cell-mediated immune function; the 1.0g/kg inclusion level, in particular, led to a significant increase in total white blood cell count, sheep red blood cell antibody titers, serum immunoglobulin levels, and T-lymphocyte and B-lymphocyte proliferation indexes. Furthermore, PP supplementation led to a dose-dependent enhancement in the antioxidant status, with significant increases in serum total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase activities. Consequently, PP can be effectively utilized at a level of 1.0g/kg as a functional feed additive to enhance the immune response and antioxidant capacity of broiler chickens.

Keywords: Broiler chicken, Pomegranate peel, Cell-mediated immunity, Humoral immunity, Antioxidant activity.

INTRODUCTION

The poultry industry remains a vital source of affordable animal protein globally, yet it continues to face major economic and health challenges, particularly rising feed costs and increasing restrictions on antibiotic growth promoters due to concerns over antimicrobial resistance (El-Fateh et al. 2024; Acharya and Barsila 2025; Liu et al. 2025). The selective breeding for rapid growth and high breast meat yield has led to birds with higher metabolic rates, which are at risk of oxidative stress (Xiong et al. 2024; Yuan et al. 2025). Furthermore, oxidative stress is intensified by intensive production factors such as high stocking density and elevated ammonia levels (Nassar et al. 2023; Kouvedaki et al. 2024; Yuan et al. 2025). Therefore, there is a rising demand for natural feed additives that are environmentally friendly and efficient in supporting flock

health, feed efficiency, and enhancing immunity (Abdelli et al. 2021; Obianwuna et al. 2024; Aminullah et al. 2025). Of the plethora of phytochemicals being examined, pomegranate peel powder (PP) is a potential candidate because of its strong bioactivity in terms of antioxidant and antimicrobial activities (Abd El-Ghany 2023; Xu et al. 2024; Younis et al. 2025).

Pomegranate peel (PP) is an environmental waste problem, representing about 30-50% of the fruit's total weight, still it is highly valuable for its bioactive compounds (Chen et al. 2025b; Yue et al. 2025). The antioxidant potential of PP is greater than pomegranate juice and seed due to its high contents of polyphenols, flavonoids, hydrolysable tannins like punicalagin, punicalin and ellagic acid (de Castilho Heiss et al. 2025; Ncho et al. 2025). The antioxidant activities of these compounds are achieved through scavenging free radicals,

Cite This Article as: Al-Suwailem NK, Alaqil AA, Abbas AO, Safaa HM and Kamel NN, 2026. Modulatory effects of dietary pomegranate peel on the growth performance, immunological competence, and antioxidant activity of broiler chickens. *International Journal of Veterinary Science* 15(3): 725-731. <https://doi.org/10.47278/journal.ijvs/2026.012>

chelation of metal ions and inducing endogenous enzymatic antioxidants (Chen et al. 2024; Kouvedaki et al. 2024). Moreover, these bioactive substances also have potent immunomodulatory effects as they modulate cytokine production and impact inflammatory pathways, such that PP is the promising multitarget phytochemical additive for broiler nutrition (Obianwuna et al. 2024; Konanc and Ozturk 2025). The dietary inclusion of pomegranate by-products has been reported to diminish the markers for oxidative stress, increase antioxidant potential and improve meat shelf life and combat pathogens (Maqsood et al. 2024; Al-Suwailem et al. 2025; Nantapo and Marume 2025). The presence of organic acids in the peel like citric and malic acids, may also enhance gut health as it is also able to acidify the digestive tract, which additionally helps to support animal productivity and disease resistance (Fawaz et al. 2025; Oni et al. 2025).

The immunomodulatory influence of Pomegranate peel has a longstanding presence in traditional medicine and is currently being confirmed by contemporary scientific studies (Elbaz et al. 2025; Roura et al. 2025). The complex phytochemical constituents of the peel, which have polyphenols and flavonoids as well as immunostimulatory polysaccharides, are necessary for both activities (Abd El-Ghany 2023; Utami et al. 2025). Being an immune regulator, it allows suppression of pro-inflammatory cytokines in hyper-inflammatory conditions, whereas being an immune stimulator, it can improve the activity of an impaired immune system (Raheel et al. 2024; Chen et al. 2025a; Konanc and Ozturk 2025). The elevation in immune organ index, the improvement of lymphocyte function, and the promotion of immunoglobulins and some cytokines were also confirmed in a series of research (Mendes et al. 2024; Obianwuna et al. 2024; Watanabe et al. 2024). Thus, we examined the optimal dietary inclusion of PP to leverage its bioactive properties, specifically evaluating its effects on broiler growth performance, immunity, and antioxidant capacity.

MATERIALS AND METHODS

Preparation of pomegranate peel

Fresh, mature pomegranates were sourced commercially. After manual separation and washing, the peels were oven-dried at 60°C for 48h and subsequently ground to a fine powder. To prevent the degradation of bioactive components, the resulting pomegranate peel powder (PP) was stored in sealed, light-protected containers under cool conditions until dietary incorporation.

Experimental design

A total of 400 one-day-old Cobb 500 chicks were individually weighed and randomly distributed into four treatment groups (five replicates×20 birds). For the initial 21 days, all chicks were fed a standard basal diet developed to meet the dietary needs for broiler starters as NRC (1994) and the commercial strain catalog guideline recommendation. On day 22, the experimental groups were offered the basal diet supplemented with 0.0, 0.5, 1.0, and 1.5g of PP per kg of diet, which was administered for a period of three weeks.

The birds were housed in a standard climate-controlled poultry house on deep litter. A lighting program of 23 hours

of light and 60 minutes of darkness was offered. The ambient temperature was preserved at 33±1°C throughout the first seven days of life and then gradually reduced till reached 24±1°C in the third week. The birds had open access to feed and water throughout the experimental duration.

Production performance

The body weight (BW) of each experimental group replicate was recorded at day 22 and day 42 of age. Meanwhile, feed intake was recorded weekly. Accordingly, weight gain (BWG) and feed conversion ratio (FCR) were computed for the treatment period (days 22-42). FCR was calculated as feed intake (g) divided by body weight gain (g).

Blood sampling

At day 42, two birds from each replicate (n=10 per treatment) were randomly selected for blood sampling to analyse different physiological indicators. Samples were collected via brachial wing venepuncture, and then centrifuged at 1800×g for 20 min at 4°C. Serum was subsequently harvested and preserved at -20°C pending further analysis.

Immunological parameters

Cell-mediated immune function was evaluated by determining total white blood cell (TWBC) counts using a Neubauer hemocytometer, as described by Gehad et al. (2008). Differential leukocyte counts were performed to calculate the heterophil-to-lymphocyte (H/L) ratio, whereas, the MTT assay was used to assess leukocyte cell viability (LCV%; n = 10; two samples per replicate) following Abbas et al. (2020).

The stimulation index (SI) of T- and B-lymphocyte proliferation was determined according to Alaqil et al. (2020). Additionally, cell-mediated immunity was assessed using the phytohemagglutinin (PHA)-induced wattle swelling test as described by Al-Khalifa (2016). Briefly, wattle thickness was measured on day 40, followed by intradermal injection of PHA-P. Wattle thickness was re-measured 24h post-injection, and the difference between pre- and post-injection measurements was recorded.

Humoral immune responses were evaluated by quantifying serum immunoglobulin levels (IgA, IgM, and IgG) in ten serum samples per treatment using chicken-specific ELISA kits (MBS564152, MBS706158 and MBS260043, respectively; MyBioSource Inc., CA, USA), according to the manufacturer's instructions. Additionally, sheep red blood cell (SRBC) antibody titers were determined by immunizing birds with 1mL of a 5% SRBC suspension on day 35. Blood samples were collected seven days later, and antibody titers were measured using a hemagglutination assay. Titers were expressed as the log₂ of the reciprocal of the highest serum dilution showing visible agglutination (Alzarrah et al. 2021).

Serum antioxidant activity

Serum total antioxidant capacity (TAC) was detected by a commercially available colorimetric assay kit (MBS2540515; MyBioSource Inc., San Diego, CA, USA). In addition, the activity of catalase and superoxide dismutase (SOD) was evaluated by means of colorimetric assays (Abcam, Waltham, MA, USA) with catalogue numbers ab65354 and ab83464, respectively.

Statistical analysis

All data was analyzed using the SPSS software package (IBM Corp., Armonk, NY, USA). The data was tested with a one-way ANOVA. Duncan's multiple range test was used to determine significant differences among treatment means at a significance level of $P < 0.05$.

RESULTS

Growth performance

Table 1 summarizes the influences of pomegranate peel (PP) supplementation on the production efficiency parameters of the broilers. The results demonstrated that there was a distinct dose-related reduction in weight gain as the level of PP inclusion increased. Feed consumption was reduced by 12.3% and 13.3% with increasing PP levels with 1.0 and 1.5g/kg dietary PP supplementation. Consequently, the final BW and BWG were decreased by 5.0 and 6.6%, respectively, relative to the nonsupplemented group. However, the addition of PP significantly increased feed efficiency. As a whole, these findings suggest that increased PP supplementation has negative impact on broiler performance, whereas low-dose supplement inclusion could result in feed efficiency improvement.

Cell-mediated immunity markers

Cell-mediated and humoral immune responses represent the two main components of the avian acquired immune system. As shown in Table 2, dietary supplementation of broiler diets with PP exerted a significant enhancing effect on cell-mediated immunity.

The greatest responses were obtained in birds provided with 1.0g/kg PP, followed by 1.5 and 0.5g/kg, compared to nonsupplemented group. Total leukocyte count increased by 1.2–1.4-fold, accompanied by a 1.15–1.30-fold reduction in the H/L ratio. Leukocyte viability was also increased by 13%, 18% and 22% in the PP group at doses of 0.5, 1.5, and 1.0g/kg, respectively. Moreover, PP supplementations induced 1.26 to 1.40-fold rise in the proliferation index of T-lymphocytes as well as significantly enhanced PHA-wattle test responses. These results added together support that PP, especially at 1.0g/kg diet, had the capacity to improve the cellular immune function of broilers.

Humoral immunity markers

Table 3 summarizes the humoral immune responses of broilers supplemented with PP. The humoral immunity indicators showed a significant enhancement in response to PP supplementation. The levels of immunoglobulins were substantially elevated relative to the nonsupplement group. The levels of IgA showed a dose-response increase, culminating at 1.5g/kg PP concentrations. Similarly, both IgM and IgG were significantly elevated, reaching their peak concentrations at 1.0g/kg supplementation. The B-lymphocyte stimulation index and the SRBC antibody titer also progressively rose with the amount of PP with the highest indices at 1.0g/kg. Taken together, these results suggest that the PP supplement promotes humoral immunity, particularly at 1.0g/kg, by inducing immunoglobulin production and enhancing general immune competence in broilers.

Table 1: Growth performance of broiler chickens feed diet supplemented with pomegranate peel powder

Parameters	Pomegranate peel powder g/kg diet				SEM	P-value
	0.0	0.5	1.0	1.5		
Initial BW (g)	850	855	845	852	6.21	0.783
final BW (g)	2710 ^a	2695 ^a	2575 ^b	2530 ^b	110.3	0.015
BWG (g)	1860 ^a	1840 ^a	1685 ^b	1678 ^b	76.23	0.011
FI (g)	3385 ^a	3330 ^a	2970 ^b	2936 ^b	127.4	0.041
FCR	1.82 ^a	1.81 ^b	1.76 ^b	1.75 ^b	0.04	0.024

Means in the same row having different superscript letter differ significantly ($P < 0.05$). SEM: standard error of means. BW: body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

Table 2: Cell-mediated immune responses of broiler chickens feed diet supplemented with pomegranate peel powder

Parameters	Pomegranate peel powder g/kg diet				SEM	P-value
	0.0	0.5	1.0	1.5		
TWBC, ($\times 10^3$ /mL)	46.39 ^d	54.36 ^c	63.41 ^a	61.82 ^b	0.742	<0.001
LCV (%)	99.3 ^d	114.6 ^c	126.5 ^a	120.7 ^b	0.941	<0.001
H/L ratio	0.45 ^a	0.39 ^b	0.34 ^b	0.36 ^b	0.016	0.034
T-lymphocytes SI	4.23 ^c	5.31 ^b	5.86 ^a	5.67 ^a	0.062	<0.001
PHA-wattle test (mm)	0.51 ^d	0.58 ^c	0.71 ^a	0.65 ^b	0.029	<0.001

Means within the same row with different superscripts significantly differ ($P < 0.05$). SEM: standard error of means. TWBC: total white blood cell; LCV: leucocytes cells viability; H/L ratio: heterophil to lymphocyte ratio, T-lymphocytes SI: T-lymphocytes stimulating index; PHA-wattle test: phytohemagglutinin wattle thickness test.

Table 3: Humoral immune responses of broiler chickens feed diet supplemented with pomegranate peel powder

Parameters	Pomegranate peel powder g/kg diet				SEM	P-value
	0.0	0.5	1.0	1.5		
IgA (μ g/ml)	151.4 ^d	163.8 ^c	174.3 ^b	185.2 ^a	0.613	0.032
IgM (μ g/ml)	430.2 ^d	509.6 ^c	576.1 ^a	549.2 ^b	11.264	0.019
IgG (mg/ml)	1.67 ^c	1.77 ^b	1.87 ^a	1.81 ^{ab}	0.063	0.045
B-lymphocytes SI	2.98 ^c	3.39 ^b	3.84 ^a	3.76 ^a	0.047	<0.001
SRBC-Ab titer log ₂	6.88 ^c	7.95 ^b	8.66 ^a	8.36 ^a	0.124	<0.001

Means within the same row with different superscripts significantly differ ($P < 0.05$). SEM: standard error of means. IgA: immunoglobulin A; IgM: immunoglobulin M; IgG: immunoglobulin G; B-lymphocytes SI: B-lymphocytes stimulating index; SRBC-Ab: sheep red blood cells antibody.

Table 4: Antioxidant status of broiler chickens feed diet supplemented pomegranate peel powder

Parameters	Pomegranate peel powder g/kg diet				SEM	P-value
	0.0	0.5	1.0	1.5		
TAC (U/mL)	4.05 ^c	5.74 ^b	5.93 ^b	6.34 ^a	0.381	<0.001
SOD (U/mL)	191.7 ^d	249.2 ^c	285.6 ^b	310.1 ^a	11.42	0.017
Catalase (U/mL)	0.79 ^c	0.83 ^{bc}	0.88 ^b	0.94 ^a	0.027	0.021

Means within the same row with different superscripts significantly differ ($P < 0.05$). SEM: standard error of means. TAC: total antioxidant capacity; SOD: superoxide dismutase.

Antioxidant markers

The antioxidant markers of broiler chickens supplemented with PP is presented in Table 4. The findings demonstrate a clear, notable effect of the addition of PP in improving the antioxidant capacity of birds and the effect is dose-dependent across all the observed markers. Progressive and significant increases were observed with rising PP concentrations in the diet. The most significant increments were noted at the level of 1.5g/kg supplementation, which had the highest increases in TAC, SOD, and catalase by 1.57, 1.62, and 1.19-folds, respectively. These outcomes reveal that PP supplementation can efficiently improve the antioxidant defence system of broilers and may have potential as a natural antioxidant in broiler production.

DISCUSSION

The current paper examined how the pomegranate peel powder (PP) dietary supplementation influences broilers' performance, immunity, and redox status. The results show that increasing PP supplementation at 1.0 and 1.5g/kg reduced FI and final BW; however, these levels markedly improved FCR. This is in line with some of the earlier research findings, which observed that high concentrations of phytochemical feed additives could have a negative effect on broilers growth performance trends (Ghosh et al. 2020; Abdelli et al. 2021; Ahmadipour et al. 2021; Maqsood et al. 2024). Pomegranate peel is abundant in polyphenols and tannins, which may act as anti-nutritional factors at high concentrations, resulting in low feed consumption (Younis et al. 2025; Yue et al. 2025). Ghasemi-Sadabadi et al. (2022) also reported that replacing 8% of the diet with pomegranate peel decreased broiler growth performance. However, the parallel decrease in FCR across all of the PP-supplemented groups suggests improving feed efficiency. This indicates that even though the birds were on reduced feed intake, they became more efficient, possibly due to PP bioactive components, which have been reported to improve gut health as well as nutrient absorption (Fawaz et al. 2025; Oni and Oke 2025). The improvement in FCR represents a substantial economic advantage, given that feed costs constitute the major portion of poultry production's total expenses (Li et al. 2024a; Adaszyńska-Skwirzyńska et al. 2025). Our outcomes are consistent with those of preceding reports, which showed that the supplementation of PP as rich-polyphenol additives could promote body weight gain and FCR in broilers (Gosai et al. 2023; de Castilho Heiss et al. 2025).

The immune responses of broilers were markedly stimulated by PP-supplemented diets. The inclusion of PP, especially at 1.0g/kg, led to a notable rise in the TWBC count, PHA wattle test and the T-lymphocyte stimulation index, which are indicators of a stimulated cell-mediated

immune system. Meanwhile, all PP-supplemented groups showed a significant decrease in the H/L ratio, an established stress indicator in poultry, compared with the corresponding control ones, indicating lower stress levels and higher welfare status of animals (Gulabrai et al. 2025; Khazali et al. 2025; Oni et al. 2025). The low H/L ratio may also be linked to PP antioxidant and anti-inflammatory characteristics (Obianwuna et al. 2024). Furthermore, humoral immunity, also known as antibody-mediated immunity, was similarly improved. These results are in agreement with several reports on immunostimulatory effects of fruit extracts and polyphenols in poultry (Obianwuna et al. 2024; Albarrak et al. 2025; Alfifi et al. 2025). It is well established that the bioactive compounds in PP have immunomodulatory effects, which may be responsible for the observed increase in immune responses (Watanabe et al. 2024; Xu et al. 2024; Elbaz et al. 2025).

The supplemented groups also showed increased TAC as well as catalase and SOD enzyme activity. This is considered a direct indication of the high level of antioxidant compounds, primarily polyphenols, present in PP (Kouvedaki et al. 2024; Ncho et al. 2025; Nirmal et al. 2025). Oxidative stress is considered a serious challenge in the commercial poultry industry worldwide and can cause growth reduction and immunosuppression (Li et al. 2024b; Xiong et al. 2024). By enhancing the endogenous antioxidant defence system, PP supplementation can help to mitigate the negative influences of oxidative stress. This is in agreement with numerous studies that have highlighted the benefits of using natural antioxidants, such as those found in pomegranate by-products, to increase the health and productivity of broiler chickens, particularly under circumstances of stress (Ahmadipour et al. 2021; Abd El-Ghany 2023; Yuan et al. 2025).

Conclusion

It is concluded that pomegranate peel powder can be employed as a natural feed additive for broiler chickens. Although an increased dosage can slightly decrease body weight gain, PP supplementation can greatly enhance the feed efficiency, providing it with a considerable economic benefit. Furthermore, PP has strong immunomodulatory and antioxidant effects with an optimal dose of 1.0g/kg. These interconnected benefits position PP as an effective natural alternative to antibiotic growth promoters, promoting healthier and more resilient broilers, which warrants further investigation into its commercial application and impact on meat quality, especially under different stressful conditions.

DECLARATIONS

Funding: This study was funded by the Deanship of Scientific Research, Vice Presidency for Graduate Studies

and Scientific Research, King Faisal University, Saudi Arabia (KFU254350).

Acknowledgement: The authors sincerely appreciate the continuous support provided by the Animal and Fish Production Department, College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia, during the progression of this study. The authors also gratefully acknowledge the Deanship of Graduate Studies and Scientific Research at the University of Bisha, KSA, for supporting this work through the Fast-Track Research Support Program.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: All data related to the present study are incorporated in this article.

Ethics Statement: All animal-related experimental procedures were revised and permitted by The Research Ethics Committee of King Faisal University, Saudi Arabia, (Approval No. KFU-REC-2025-June-ETHICS3342).

Author's Contribution: All authors are equally involved in the present research course preparation and execution.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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