P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.089

Effects of Pomegranate Juice Inclusion in Drinking Water on Growth Performance, Carcass Quality, Intestinal Microbiota and Physiological Parameters of Broiler Chickens

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Article History: 25-153 Received: 22-Jun-25 Revised: 11-Aug-25 Accepted: 13-Aug-25 Online First: 31-Aug-25

ABSTRACT

This study evaluates broiler productivity, carcass characteristics, blood biochemicals, intestinal microbiota, antioxidant status, and immune function associated with pomegranate juice (PJ) supplementation in drinking water. Three hundred, one-day-old Cobb-500 chicks were randomly and equally distributed into five treatment groups (four replicates × 15 birds each). The treatments were a negative control group receiving no-treatment; a group supplemented with 500 mg/L lincomycin (a conventional antibiotic) served as a positive control; and three groups receiving drinking water supplemented with 10, 20, or 30mL/L of PJ, respectively. The experimental period lasted until the birds reached five weeks of age. The findings outlined that the highest level of PJ addition (30mL/L) significantly improved birds' growth and carcass characteristics compared to the negative and positive control groups. PJ supplementation also significantly reduced (P<0.05) intestinal populations of *Salmonella* as well as *Escherichia coli*, comparable to the effects of lincomycin. Moreover, high PJ inclusion notably enhanced (P<0.05) blood biochemical and hematological parameters as well as antioxidant biomarkers. In conclusion, supplementing broiler drinking water with 30mL/L of PJ proved to be a potent natural alternative to conventional antibiotics, offering antimicrobial and antioxidant benefits while supporting growth, physiological health, and immune function.

Key words: Pomegranate juice, Growth performance, Immunological markers, Broiler chickens.

INTRODUCTION

Antibiotics have long been employed in poultry production, at sub-therapeutic levels, to suppress harmful bacteria and stimulate growth (Jazi et al. 2018). However, growing concerns over the development of antibiotic-resistant microbes and the presence of antibiotic residues in poultry products have raised serious public health issues (Shirani et al. 2019; Ali et al. 2025). In response, several countries have imposed bans on the use of antibiotics as a growth stimulant agent in animal feed (Abreu et al. 2023). This has prompted a global exploration for safe, effective, and economically feasible alternatives that can support poultry health and productivity (Abd El-Ghany 2023; Chen et al. 2025). Current research efforts are focused on identifying natural compounds with antimicrobial properties, the ability to modulate gut microbiota, and growth-enhancing potential (Gadde et al. 2017).

Pomegranate (*Punica granatum*) has garnered increasing attention in poultry nutrition due to its potential health-promoting and growth-enhancing properties (Akuru et al. 2021). Several components of pomegranate fruit are rich with bioactive compounds, including ascorbic acid, retinol, folic acid, omega-5 punicic acid, potassium, phenolics, alkaloids, triterpenes, and sterols (Zaouay et al. 2012; Sreekumar et al. 2014; Giménez-Bastida et al. 2021). Such constituents have demonstrated antioxidant, immunomodulatory, and anticancer effects in both animal (Ghasemi-Sadabadi et al. 2022; Younis et al. 2025) and human (Esmaeilinezhad et al. 2019; Akhtar et al. 2019) studies. Additionally, pomegranate antimicrobial activity against bacteria, fungi, and yeasts has been demonstrated, thereby supporting gastrointestinal health (Abd El-Ghany 2023; Chen et al. 2025). Previous studies have informed

Cite This Article as: Al-Suwailem NK, Abbas AO, Kamel NN and Moustafa ES, 2025. Effects of pomegranate juice inclusion in drinking water on growth performance, carcass quality, intestinal microbiota, and physiological parameters of broiler chickens. International Journal of Veterinary Science 14(6): 1069-1077. https://doi.org/10.47278/journal.ijvs/2025.089

that dietary inclusion of pomegranate by-products improves the performance of poultry, including quails (Abbas et al. 2017) and broilers (Sharifian et al. 2019; Ghasemi-Sadabadi et al. 2021).

To date, most of the researches have focused on the pomegranate peel inclusion into poultry diets (Ismail et al. 2012; Saleh et al. 2017; Younis et al. 2025), while the application of PJ remains largely unexplored. PJ contains high concentrations of ellagitannins such as punicalagin and ellagic acid in addition to vitamin C, which are strongly associated with antioxidant activity (Naveena et al. 2008; Ferrara et al. 2011; Ismail et al. 2012; Bonesi et al. 2019). We hypothesize that PJ may positively influence broilers' biological functions and serve as a natural growth promoter and antibiotic alternative. Hence, this study investigated the properties of PJ supplementation on broiler chickens' growth, carcass traits, intestinal microbiota, blood biochemical constituents, antioxidant status and immunity.

MATERIALS AND METHODS

Pomegranate juice preparation and examination

Ripe and healthy pomegranates (Egyptian variety) were procured weekly from a retail market in Al-Ahsa, Saudi Arabia. The fruits were thoroughly washed, manually cut and peeled. Seeds were separated manually, and then pressed using a hand-operated juicer to extract the pomegranate juice (PJ). The juice was subsequently filtered through a metal sieve to remove solid residues, and centrifuged at 1400 ×g for 10 minutes. The resulting supernatant was filtered, and stored at 4°C for no longer than 24 hours before being placed into the broiler drinking water.

Proximate composition of PJ including moisture, ash, crude protein, total lipids, and carbohydrate content as well as mineral contents (phosphorus, sodium, and potassium), were analyzed in triplicate samples using standard procedures of the AOAC (AOAC 1990). The pH was obtained using a digital pH meter (Hanna Instruments, Smithfield, RI, USA). Meanwhile, acidity was determined and expressed as the percentage of citric acid by titrating 10mL of PJ with 0.1N NaOH to a pH endpoint of 8.1. The PJ total phenols and total flavonoids content as well as antioxidant activity were assessed following the methods outlined by (Elfalleh et al. 2009). Proximate analysis of PJ was conducted weekly throughout the experiment to monitor consistency in its chemical composition. The detailed compositional data of PJ are presented in Table 1.

Experimental protocol

Three hundred one-day-old chicks (mixed sex Cobb-500 broiler; initial body weight: 45.6 ± 0.9 g) were randomly and equally allocated into five groups, each comprising four replicates × 15 chicks. The groups were a control receiving no treatment; a positive control receiving drinking water supplemented with 500mg/L of Lincoxin-N (Lincomycin Sulfate 10%, Rau Indore, Madhya Pradesh, India); and three groups receiving drinking water supplemented with pomegranate juice (PJ) at 10, 20, or 30mL/L, respectively. The experimental period extended to five weeks of age.

Birds were housed on a sawdust-covered floor in a climate-controlled facility. Brooding temperature was set at 32°C and reduced by approximately 3.5°C each week until it reached 24°C. A 24-hour light schedule was implemented on the first day, followed by a photoperiod of 23 hours and 1 hour dark for the remainder of the trial. All birds were offered a starter diet for 14 days, followed by a finisher diet from day 15 to day 35, in accordance with Cobb-500 management guidelines (Supplement 2022). The basal diet components and proximate analysis were determined according to AOAC procedures (A.O.A.C. 2005), are detailed in Table 2.

Table 1: The chemical analysis of pomegranate juice (PJ)

Item	Contents per 100mL juice
Moisture (g)	86.4±4.36
Carbohydrate (g)	14.02±2.231
Protein (g)	0.24±0.022
Total lipids (g)	0.05±0.001
Ash (g)	0.39±0.07
Phosphorus (mg)	9.47±1.015
Sodium (mg)	7.26±0.752
Potassium (mg)	271.94±60.52
pH	3.25±0.441
Acidity (g CA)[1]	0.29±0.063
Total phenols (g GAE)[1]	1.06±0.130
Total flavonoids (g RE)[1]	0.53±0.048
Antioxidant activity (DPPH, IC_{50} mL)[2]	1.94±0.321

[1]Values are calculated as citric acid (CA), gallic acid equivalent (GAE), and retinol equivalent (RE), respectively. [2]Values express the sample concentration at 50% inhibition (IC_{50}) of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals.

Table 2: Ingredients and nutritional analysis of the broiler's starter and finisher basal diets

Ingredients (g)	Starter	Finisher
Corn	54.20	55.30
Soybean meal 48 %	42.00	40.90
Dicalcium phosphate	1.60	1.60
Ground limestone	1.45	1.45
Salt	0.25	0.25
Premix *	0.30	0.30
L-lysine	0.10	0.10
DL-methionine	0.10	0.10
Total	100	100
Nutritional analysis (%)		
Metabolizable energy (kcal kg^{-1}) **	2960	3050
Moisture	11.6	11.4
Ash	5.1	4.6
Crude protein	22.3	20.9
Crude fiber	3.3	2.9
Total lipids	2.2	3.4
Calcium	1.1	0.90
Available phosphorus	0.51	0.48

*Each 3kg comprises: Vit. A (12,000,000IU), Vit. D (2,000,000IU), Vit. E (10g), Vitamin B2 (5g), Vit. K3 (2g), Vit. B12 (10g), Vit. B1 (1g), Vit. B6 (1.5g), Biotin (50mg), Nicotinic acid (30g), Pantothenic acid (10g), Folic acid (1g), Choline chloride 50% (250mg), Manganese (60g), Zinc (50g), Iron (30g), Copper (10g), Iodine (1g), Selenium (0.1g), Cobalt (0.1g), and carrier limestone to complete 3kg. ** calculated (not analyzed).

Productive aspects

Birds' initial and final weight (BW) was recorded individually during the experimental period. Weight gain (BWG) was computed as the variation between initial and final BW. Feed intake was determined weekly and the total feed intake (FI) was calculated per group replicate throughout the study and the feed conversion ratio (FCR) was computed. The daily mortality was documented for mortality rate determination.

Carcass parameters

At the end of five weeks trial, 16 birds per group underwent overnight fasting, weighed, and then slaughtered to assess carcass characteristics. The weights of the breast and thigh were recorded to ascertain their percentages of the carcass weight. A digital scale (Kern, GmbH, Germany) was used to weigh the liver, gizzard, and heart (edible offal). The dressing rate (DR) was obtained by the calculation of the edible offal weight plus the eviscerated carcass weight relative to the preslaughter weight.

Microbiological analysis

At the time of slaughter, samples were harvested at random from the median area of the gastrointestinal system (16 birds per treatment group) and prepared to analyze the microbial population, as the methods described by Al-Khalaifah et al. (2022). Counts of *Escherichia coli* and *Salmonella* and were measured using standard methods identified by ISO 9308-2:2012 and ISO 6579-1:2017, respectively (ISO/TC 2012; ISO/TC 2017) (ISO/TC and SC 2007; ISO/TC 2012). *Proteus* count was detected according to Jamaluddin et al. (2018).

Blood sampling and analysis

At slaughter (week 5), 10mL of blood sample was taken from 16 birds per treatment group and divided into two equal portions. For hematological analysis, 5mL of the obtain sample was relocated into sterile EDTA containing tube. This portion was used to assess hemoglobin concentration (Hb), red blood cell count (RBCs), white blood cell count (WBCs), packed cell volume (PCV) and differential leukocyte counts, including lymphocytes (L) and heterophils (H). The remaining 5mL of blood was transferred into sterile plain tube and allowed to clot. Following coagulation and to separate serum, samples underwent centrifugation at 1800 ×g for 15 minutes. Serum was then obtained and stored at -20°C for further analysis.

Serum albumin, globulin, total protein, total cholesterol, triglycerides, high-density lipoproteins (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and malondialdehyde (MDA) were measured using colorimetric diagnostic kits (Diamond Diagnostics, Egypt).

Antioxidant and immunological biomarkers, including total antioxidant capacity (TAC), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), interleukin-10 (IL-10), interleukin-2 (IL-2) and interferon-gamma (IFN-γ) were measured using chicken-specific ELISA kits (MyBioSource Inc., San Diego, CA, USA).

Statistical tests

Data were tested employing SPSS software (Version 22.0; IBM Corp., Armonk, NY, USA). Data underwent one-way analysis of variance (ANOVA) to evaluate the treatment effect (control, antibiotic, or PJ at 10, 20, or 30mL/L), followed by Duncan's multiple range test for means comparisons. Significance was considered at P<0.05. Results are presented as means ± standard error (SE).

RESULTS

Birds' performance

The pomegranate juice (PJ) supplementation impact on broiler growth is presented in Table 3. The outcomes demonstrated that PJ inclusion at 30mL/L significantly improved body weight (BW), BWG, feed intake, and feed efficiency relative to both the control and lincomycin-treated groups. The group received PJ at 30mL/L showed the highest final BW, BW gain, and feed intake were recorded in, while the most favorable FCR was observed in birds supplemented with 10 and 20mL/L PJ (P<0.05). Meanwhile, the mortality rates did not differ among treatments.

Carcass characteristics

PJ supplementation significantly influenced carcass traits in broiler chickens (Table 4). Birds receiving 30mL/L of PJ showed an increased (P<0.05) dressing percentage relative to the control and lincomycin groups. Similarly, thigh yield was significantly greater (P<0.05) in broilers administered 30mL/L PJ compared to the other groups. Meanwhile, breast yield was notably greater (P<0.05) in the control and the 30mL/L PJ groups than in the lincomycin-treated and lower PJ supplemented levels.

Table 3: Growth performance of broiler supplemented with pomegranate juice (PJ) in drinking water

Indices	Control	Lincomycin 500 mg L^{-1}	PJ ml L^{-1}			P-value
			10	20	30	
Initial body weight (g)	45.9±1.48	45.7±1.26	45.6±1.24	45.5±1.34	45.3±1.15	0.346
Final body weight (g)	2045±14.5^{c}	2110±14.4^{b}	2148±4.8^{ab}	2151±4.8^{ab}	2184±19.6^{a}	<0.001
Body weight gain (g)	1999±16.2^{c}	2064±14.3^{b}	2102±5.7^{ab}	2105±4.4^{ab}	2139±17.0^{a}	0.033
Feed intake (g)	3048±13^{b}	3086±13^{b}	3077±17.54^{b}	3081±19^{b}	3150±21^{a}	<0.001
Feed/gain ratio	1.52±0.02^{a}	1.49±0.02^{b}	1.46±0.02^{c}	1.46±0.03^{c}	1.47±0.01^{ab}	0.003
Mortality (%)	2.89±0.09	2.88±0.04	3.01±0.09	2.90±0.03	2.85±0.07	0.756

Data express the means ± standard errors. Values with different superscripts within the same row differ significantly (P<0.05).

Table 4: Carcass traits of broiler supplemented with pomegranate juice (PJ) in drinking water

Indices (%)	Control	Lincomycin 500 mg L^{-1}	PJ ml L^{-1}			P-value
			10	20	30	
Dressing rate	74.4±2.45^{b}	73.0±2.74^{b}	74.7±2.54^{ab}	75.2±3.23^{ab}	77.7±1.87^{a}	<0.001
Breast weight	43.2±3.39^{a}	41.7±4.68^{ab}	35.9±6.28^{b}	41.2±6.56^{ab}	43.4±6.78^{a}	<0.001
Thighs weight	32.3±1.74^{b}	33.4±6.56^{b}	35.7±5.61^{b}	34.8±5.39^{b}	39.8±6.29^{a}	<0.001

Data express the means ± standard errors. Values with different superscripts within the same row differ significantly (P<0.05).

Intestinal bacteria

Controlling harmful gut microbiota is crucial for maintaining broiler chickens health and productivity. PJ supplementation significantly influenced the intestinal microbial profile (Table 5). Specifically, PJ supplementation markedly decreased (P<0.05) *Escherichia coli* counts relative to both the control and lincomycin-treated groups. Furthermore, the PJ-supplemented groups, as well as the lincomycin group, demonstrated significantly lower (P<0.05) counts of *Salmonella* and *Proteus* species relative to the control.

Haematological indices

The effects of PJ inclusion on the hematological profile of broiler chickens are detailed in Table 6. Hemoglobin level increased significantly (P<0.05) in broilers receiving PJ compared to both the control and the lincomycin-treated groups. Administration of PJ at 20 and 30mL/L also produced a significant elevation in RBC counts (P<0.05) relative to all other treatments. In contrast, total WBC counts were decreased (P<0.05) in both lincomycin- and PJ-treated birds versus controls. The relative proportion of heterophils declined in lincomycin- and PJ-supplemented groups, reaching its minimum in the 20 and 30mL/L PJ treatments. Similarly, lymphocyte percentages were significantly decreased (P<0.05) by PJ addition and lincomycin administration compared to controls. Meanwhile, PCV% showed no differences among treatments (P>0.05).

Serum biochemical components

The inclusion of PJ in broiler chickens' drinking water significantly altered serum biochemical parameters (Table 7). Birds receiving PJ supplementation exhibited markedly higher total protein, albumin, and globulin concentrations (P<0.05) than both the lincomycin-treated and control groups. In contrast, triglyceride levels were decreased (P<0.05) in the 30mL/L PJ supplemented group compared with all other PJ supplemented, the lincomycin-treated, and the control groups. Total cholesterol was also reduced (P<0.05) by both lincomycin and PJ supplementation relative to the control. Moreover, PJ addition significantly increased HDL cholesterol while simultaneously decreasing hepatic ALT activity compared with lincomycin and the control groups, suggesting a hepatoprotective effect.

Antioxidant capacity and immunological cytokines

The effect of PJ inclusion on broiler chickens redox status and immune response is presented in Table 8. It was found that PJ supplementation at 30mL/L significantly elevated (P<0.05) the TAC, glutathione level, and GPx activity in comparison with the other experimental groups. SOD activity was markedly elevated (P<0.05) in the 20 and 30mL/L PJ supplemented groups than that in the 10mL/L PJ supplemented, lincomycin, and the control groups. Moreover, PJ supplementation at 30mL/L substantially decreased (P<0.05) serum MDA level compared to the

Table 5: Intestinal microbiota count of broiler supplemented with pomegranate juice (PJ) in the drinking water

Indices*	Control	Lincomycin 500 mg L^{-1}	PJ ml L^{-1}			*p*-value
			10	20	30	
Salmonella	$1.30±0.06^a$	$1.05±0.03^b$	$1.06±0.07^b$	$1.05±0.04^b$	$0.96±0.05^b$	0.005
E. coli	$1.39±0.03^a$	$1.20±0.05^b$	$0.91±0.04^c$	$0.95±0.07^c$	$0.92±0.04^c$	<0.001
Proteus	$0.92±0.05^a$	$0.84±0.03^b$	$0.86±0.03^b$	$0.86±0.03^b$	$0.83±0.03^b$	<0.001

Data express the means ± standard errors. Values with different superscripts within the same row differ significantly (P<0.05). *Values are expressed as the log of microbial colony forming unit.

Table 6: Blood hematological parameters of broiler supplemented with pomegranate juice (PJ) in drinking water

Indices	Control	Lincomycin 500 mg L^{-1}	PJ ml L^{-1}			*p*-value
			10	20	30	
Hb (g dL^{-1})	$12.6±0.51^b$	$12.4±0.29^b$	$13.7±0.35^a$	$13.9±0.54^a$	$13.9±0.33^a$	0.009
RBCs (10^{12} L^{-1})	$1.88±0.04^c$	$2.08±0.04^{bc}$	$2.05±0.03^b$	$2.13±0.02^a$	$2.12±0.03^a$	0.007
WBCs (10^6 L^{-1})	$26.3±0.85^a$	$21.2±1.26^b$	$22.2±1.08^b$	$20.3±1.15^b$	$19.8±1.46^b$	0.013
PCV (%)	29.7±1.49	30.6±1.08	29.7±1.33	31.2±0.95	31.3±1.87	0.728
Heterophils (%)	$24.7±1.36^a$	$18.3±1.05^{bc}$	$20.7±1.31^b$	$16.6±1.21^c$	$16.3±1.10^c$	<0.001
Lymphocytes (%)	$51.7±1.12^b$	$58.3±1.14^a$	$59.3±1.24^a$	$61.2±1.14^a$	$61.5±0.83^a$	<0.001

Data express the means ± standard errors. Values with different superscripts within the same row differ significantly (P<0.05). HB, hemoglobin; RBCs, red blood cells; WBCs, white blood cells; and PCV, packed cell volume

Table 7: Serum biochemical indices of broiler supplemented with of pomegranate juice (PJ) in drinking water

Indices	Control	Lincomycin 500 mg L^{-1}	PJ ml L^{-1}			*p*-value
			10	20	30	
Al (g dL^{-1})	$3.30±0.04^c$	$3.41±0.05^b$	$3.53±0.03^a$	$3.48±0.06^{ab}$	$3.47±0.03^b$	<0.001
Gl (g dL^{-1})	$2.07±0.07^c$	$2.39±0.08^b$	$2.74±0.04^a$	$2.75±0.06^a$	$2.72±0.03^a$	<0.001
TP (g dL^{-1})	$5.37±0.17^c$	$5.80±0.11^b$	$6.27±0.06^a$	$6.23±0.07^a$	$6.19±0.09^a$	<0.001
Trigly (mg dL^{-1})	$31.3±1.19^a$	$30.5±0.91^a$	$30.6±0.89^a$	$30.1±1.29^a$	$27.3±1.23^b$	0.005
Choles (mg dL^{-1})	$124±1.60^a$	$116±1.51^b$	$115±1.86^b$	$117±1.67^b$	$115±1.93^b$	0.042
HDL (mg dL^{-1})	$95.3±1.91^b$	$96.6±2.48^b$	$99.5±1.79^a$	$100.2±1.94^a$	$104.1±2.97^a$	0.013
AST (U dL^{-1})	69.6±1.14	68.5±1.15	65.9±1.36	66.6±1.61	68.2±1.49	0.346
ALT (U dL^{-1})	$51.7±1.38^a$	$50.7±1.2^a$	$47.5±1.13^b$	$48.3±1.10^b$	$46.7±1.39^b$	0.037

Data express the means ± standard errors. Values with different superscripts within the same row differ significantly (P<0.05). TP, total protein; Al, albumin; Gl, globulin; Trigly, triglycerides; Choles, cholesterols; HDL, high density lipoprotein; AST, aspartate aminotransferase; and ALT, alanine aminotransferase

Table 8: Antioxidant activity and immune response markers of broiler supplemented with pomegranate juice (PJ) in drinking water

Indices	Control	Lincomycin 500 mg L^{-1}	PJ ml L^{-1}			*p*-value
			10	20	30	
TAC (U mL^{-1})	$0.27±0.03^c$	$0.33±0.17^b$	$0.35±0.03^b$	$0.37±0.05^b$	$0.42±0.02^a$	<0.001
GSH (nmol mL^{-1})	$0.17±0.02^c$	$0.22±0.03^b$	$0.23±0.03^b$	$0.25±0.04^b$	$0.30±0.02^a$	0.002
GPx (U L^{-1})	$26.9±1.22^c$	$42.1±0.91^b$	$42.6±1.44^b$	$41.6±0.79^b$	$48.6±0.81^a$	<0.001
SOD (U L^{-1})	$0.24±0.02^c$	$0.25±0.01^c$	$0.29±0.03^b$	$0.32±0.02^a$	$0.34±0.02^a$	<0.001
MDA (µmol L^{-1})	$0.39±0.02^a$	$0.32±0.03^b$	$0.32±0.04^b$	$0.30±0.04^b$	$0.25±0.03^c$	0.002
IFNγ (ng L^{-1})	$3.58±0.11^c$	$4.21±0.08^a$	$4.09±0.04^b$	$4.23±0.04^a$	$4.27±0.10^a$	0.003
IL-2 (ng L^{-1})	$6.29±0.04^d$	$6.69±0.05^c$	$6.84±0.07^b$	$7.01±0.05^b$	$7.28±0.07^a$	0.002
IL-10 (ng L^{-1})	$16.5±1.27^b$	$17.6±0.84^{ab}$	$17.4±0.77^{ab}$	$17.9±0.54^{ab}$	$18.8±0.59^a$	0.021

Data express the means ± standard errors. Values with different superscripts within the same row differ significantly (P<0.05). TAC, total antioxidant capacity; GSH, glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; IFNγ, interferon gamma; IL-2, interleukin-2; and IL-10, interleukin-10.

other experimental groups. On the other hand, the IFNγ was significantly elevated (P<0.05) in the lincomycin and PJ treatment at 20 and 30mL/L than that in the 10mL/L PJ and the control groups. The IL-2 was significantly increased (P<0.05) with PJ supplementation at 30mL/L than that in the other experimental groups. Meanwhile, the IL-10 was significantly increased by the lincomycin and PJ treatments compared with the control, recording the highest level in the 30mL/L PJ supplemented group.

DISCUSSION

In the current trial, lincomycin was utilized as a common antibiotic therapy in a broiler group to attain simultaneous and supportive information regarding the effects of antibiotic therapy in broilers. The results revealed that lincomycin promoted broiler weight gain and improved feed conversion relative to the control group. The intestinal bacterial count, hematological and biochemical measurements, antioxidants, and immune activity were remarkably improved in the broilers received lincomycin relative to the control. Nevertheless, recent studies reported that administration of lincomycin for one week to broilers increased the antibiotic residues in the liver and kidney tissues to harmful levels (Wijayanti et al. 2024). The frequent therapy with antibiotic agents in poultry farming also elevated the occurrence of resistant populations of pathogenic bacteria like *E. coli* (Roth et al. 2019). Such harmful effects of antibiotic agents prompted researchers to seek natural alternatives possessing similar beneficial effects on growth promotion (Mehdi et al. 2018).

Recently, the pomegranate peel powder was successfully implemented in poultry feeds, demonstrating an improvement on growth and physiological performance of birds (Saleh et al. 2017; Sharifian et al. 2019; Ahmadipour et al. 2021; Akuru et al. 2021; Ghasemi-Sadabadi et al. 2021). Several nutritional and bioactive properties have been reported for pomegranate fruits and juices such as the high total antioxidant capacity and the high contents of total phenols, ellagitannins, and anthocyanins (Zaouay et al. 2012). The PJ is known to be used in human diets for its potential natural antioxidants, anti-inflammation, anticancer, and immune stimulation impacts (Lansky and Newman 2007; Viuda-Martos et al. 2010). Therefore, we assessed the effects of PJ addition on broiler chickens production and physiological parameters, and evaluated PJ's potential as a natural antibiotic alternative to lincomycin in poultry production.

The results indicated that broilers in the PJ groups had a better growth performance and carcass traits than that in the lincomycin and control groups. The best BW gain, final BW, carcass traits were obtained in the broilers treated with PJ at 30mL/L. Similar results were reported previously when evaluating the effect of including pomegranate peel or its extract to broiler diets on their growth performance and carcass characteristics (Ahmed et al. 2015; Kishawy et al. 2016). The possible growth-enhancing stimulus of pomegranate could be explained by its contents of essential oils that suppress intestinal pathogenic bacteria, strengthen beneficial flora, and enhance digestibility and energy usage in broilers (Qnais et al. 2007). Reddy et al. (2014) demonstrated that pomegranate by-products can augment the digestive enzymes activity of pancreas and intestine as well as inhibit pancreatic lipase, thereby improving nutrient assimilation, reducing dietary fat uptake, and protecting enterocytes from free-radical–induced damage. Moreover, supplementation with pomegranate peel extract in both broiler chickens and laying quails has been shown to enhance performance by increasing intestinal villus height and promoting lactic acid–producing bacterial populations, which together improve nutrient availability and absorption (Rao et al. 2018; Perricone et al. 2020).

The current findings displayed that PJ supplementation directly reduced the pathogenic bacterial count of *Salmonella*, *Escherichia coli*, and *Proteus*, relative to the control or lincomycin treatments (Table 5). The antibacterial potential of pomegranate against *Salmonella spp.*, *Escherichia coli*, and other pathogenic bacteria was previously reported *in vitro* (Nuamsetti et al. 2012; Pagliarulo et al. 2016; Wafa et al. 2017; Singh et al. 2019). It was suggested that elevated levels of phenolic acid, flavonoids, and hydrolysable tannins in pomegranate by-products are the primary contributors to its antibacterial efficacy (Singh et al. 2019). The phenols and flavonoids presence were also evidenced in the PJ utilized in our study (Table 1). Furthermore, it was shown that pomegranate tannins frustrated microbial enzymes, removed the substrates necessary for microbial proliferation, and obstructed the oxidative phosphorylation mechanism, leading to the disruption of membrane structure, metabolism, and function of gut bacteria populations (Viuda-Martos et al. 2010). The phenolic tannins can alter the intestinal pH, bind to pathogenic bacteria via the lectin–receptor process, and impede their attachment to the mucosa, and hence, reduce the count of pathogenic gut bacteria like *E. coli* and *Salmonella* (Vidanarachchi et al. 2005; Ahmed and Yang 2017). Also, pomegranate extracts seem to enhance the population of beneficial *Lactobacillus* *spp*. in the intestine which is often correlated with a decrease in gut harmful bacteria, hence, increasing digestion and absorption in broiler chickens (Sarıca and Ürkmez 2016).

Our results indicate that PJ supplementation exerts significant hypocholesterolemic and hypolipidemic effects as previously reported (Yaseen et al. 2014; Hou Chen et al. 2019). It was proposed that the anti-hyperlipidemic action of pomegranate may be facilitated via the low expression of peroxisome proliferator-activated receptor alpha (Ahmadipour et al. 2021) and the regulation of the HMG-CoA reductase activity (Esmaillzadeh et al. 2006). Meanwhile, Lv et al. (2016) attributed enhanced cholesterol catabolism to the high ellagic acid content of pomegranate, which promotes bile acid secretion from hepatocytes. Furthermore, the present study, along with several prior investigations demonstrated that dietary pomegranate by-products inclusion in broiler rations reduces hepatic enzymes activities (AST and ALT) (Sharifian et al. 2019; Ghasemi-Sadabadi et al. 2021; Abdel Baset et al. 2022; Elnaggar et al. 2022). This hepatoprotective effect is largely attributed to pomegranate's abundant phenolic compounds. Moreover, administration of PJ significantly increased RBCs count as well as Hb concentration in broilers (Table 5). Similarly, pomegranate peel powder, extract, or juice supplementation has demonstrated beneficial effects on the broiler hematological profile (Elnaggar et al. 2022), in rodent (Attia et al. 2014) and in humans (Manthou et al. 2017).

Presenting the impact of PJ on the redox and immune parameters, the results revealed the improvement of several investigated parameters in the groups supplemented with 20 and 30mL/L PJ, relative to the other experimental groups. The antioxidant bioactivity of pomegranate by-products may stem from their high levels of polyphenolic components, including tannins, anthocyanins, catechins, gallic acids, ellagic acids and caffeic acids (Seeram et al. 2005; Johanningsmeier and Harris 2011; Singh et al. 2018; Ghosh et al. 2020). Pomegranate supplementation has been stated to reduce serum malondialdehyde, mitigate protein and DNA damage, and suppress reactive oxygen species generation, thus protecting the liver and other organs from oxidative damage (Rao et al. 2018; Saleh et al. 2018; Akuru et al. 2020; Akuru et al. 2021). Besides, pomegranate by-products can stimulate the immunological response in broilers (Kishawy et al. 2016; Rezvani and Rahimi 2017). In the present investigation, PJ supplementation increased the IFNγ, IL-2, and IL-10 as a main indicator for immune capacity in broilers. The IFNγ is a key cytokine for stimulating immune response against any microbial invasion (Schoenborn and Wilson 2007; Ng et al. 2023). The IL-2 is another essential cytokine for the regulation of white blood cell's function, especially the lymphocytes, emerging the natural response to the microbial infection (Arenas-Ramirez et al. 2015). The IL-10 plays an essential role in immunoregulation, B-cell lymphocyte proliferation, and anti-inflammation (Mosser and Zhang 2008). It was found that feeding pomegranate peel to broilers enlarged their primary and secondary lymphoid organs (i.e. thymus, bursa and spleen) (Rao et al. 2018; Elnaggar et al. 2022). Moreover, it was suggested that pomegranate seeds are enriched with polyunsaturated fatty acids (Fadavi et al. 2006; Carvalho Filho 2014), which contribute to the immune organs' enlargement and stimulation (Carvalho Filho 2014).

Conclusion

In summary, the results indicate that supplementing broiler drinking water with PJ at 30mL/L may represent a promising natural alternative to conventional antibiotics, with beneficial effects on broiler chickens growth, physiological parameters, and overall health. Additional investigation is needed to assess the efficacy of PJ supplementation under challenging environmental and infectious conditions.

DECLARATIONS

Funding: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (KFU251957).

Acknowledgement: The authors would like to acknowledge the Department of Animal and Fish production, College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia for their valuable logistical and financial support.

Conflict of Interest: The authors declare no competing interests.

Data Availability: All data generated and analyzed during this study are included in the published article; no additional datasets were produced.

Ethics Statement: The experimental protocols involving animals were approved by the Research Ethics Committee of the King Faisal University, Saudi Arabia, with approval number (KFU-REC-2023-OCT-ETHICS1482).

Author's Contribution: Conceptualization: AOA, ESM and NNK; Data curation: NKA and ESM; Formal analysis: NNK, NKA and ESM; Investigation: AOA and ESM, Methodology: AOA, NKA and ESM; Writing– original draft: NNK and ESM; Writing– review & editing: Ahmed O Abbas All authors discussed the results and commented on the manuscript.

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

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