Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 90: …… July 2026
DOI: 10.5455/ajvs.316720

Effect of Pomegranate Peel and Grape Seed Extract on Carcass Traits, Biochemical Parameters, and Immune-Related Gene Expression in Rabbits

Salwa S. Shalan[1], Sherif I. Ramadan[1], Hadeel S. El-Qaliouby[1], Basant Mohamed Shafik[1], Eman Ahmad Sallam[1]
[1]Animal and poultry production, Department of Animal Wealth Development, Faculty of Veterinary, Benha University, Moshtohor, Qalyubia, Egypt

Key words:
Grape seed extract, Growth performance, Immune response, Pomegranate peel extract, Rabbits.

***Correspondence to**:
salwasafwat5@gmail.com

Article History
Received: 07 Apr 2026
Accepted: 07 May 2026

ABSTRACT

This study assessed the effects of dietary supplementation with pomegranate peel extract (PPE) and grape seed extract (GSE) on carcass traits, biochemical parameters, hematological indices, and immune gene expression in Alexandria and V-line rabbits. A total of 108 weaned male rabbits (54 per breed) were randomly allocated to three dietary treatments: control, PPE (250 mg/kg), and GSE (250 mg/kg) for 56 days. GSE supplementation significantly increased live body weight in V-line rabbits (2030 g) compared with the control (1525 g), while PPE showed moderate improvements (1772 g). organ weights remained unaffected, with no adverse effects observed on blood biochemical parameters or hematological indices, further confirming the safety of both extracts. PPE improved lipid profiles in V-line rabbits by increasing HDL (26.60 mg/dL) and reducing LDL cholesterol, though triglycerides were elevated (P = 0.00) in Alexandria rabbits. Hematological analysis showed reduced (P = 0.02) white blood cell counts in supplemented groups, indicating decreased systemic inflammation. Gene expression analysis revealed that both extracts significantly upregulated anti-inflammatory *IL-10* (4.170 in V-line GSE) and pro-inflammatory *TNF-α* (3.048 in V-line GSE) compared to control (≈1.000), suggesting balanced immunomodulatory effects. In conclusion, dietary supplementation with PPE and GSE at 250 mg/kg enhanced growth performance and modulated immune responses in rabbits with breed-specific variations, supporting their potential as functional feed additives in sustainable rabbit production systems.

1. INTRUDUCTION

The rabbit industry represents a valuable sector in livestock production. It contributes in the developing countries significantly to the provision of high-quality animal protein for human consumption (Lebas et al., 1997). The use of natural and sustainable substitutes for artificial additives to improve rabbit health and productivity had gained popularity in recent years. Phytogenic feed additives derived from plants have garnered significant attention among these alternatives. The nutritional value of these additives is due to their high content of bioactive compounds such polyphenols, tannins, and flavonoids that have potent antibacterial, anti-inflammatory, and antioxidant properties (Ghasemzadeh and Ghasemzadeh, 2011).

Two well-known examples of agro-industrial by-products with significant potential as functional feed ingredients are pomegranate (Punica granatum L.) peel and grape (Vitis vinifera) seeds (Molina-Alcaide and Yáñez-Ruiz, 2008). Traditionally considered waste, these materials are now recognized as abundant and cost-effective sources of valuable phytochemicals. Pomegranate peel is exceptionally rich in ellagitannins, particularly punicalagins, demonstrating a strong antioxidant capacity superior to that of the pulp (Li et al., 2006). Similarly, grape seed extract is renowned for its high concentration of proanthocyanidins, a class of condensed tannins known for their powerful biological activities (Shi et al., 2003).

The inclusion of these extracts in animal diets is hypothesized to exert multifaceted benefits. Primarily, their antioxidant activity can mitigate oxidative stress, a condition that impairs cellular integrity and overall animal health (López-Alarcón and Denicola, 2013). By reducing oxidative damage, these compounds may improve metabolic efficiency and nutrient utilization, potentially leading to enhanced growth performance and better carcass traits, including dressing percentage and meat quality in various meat-producing species (Jiang and Xiong, 2016). Additionally, the modulation of biochemical parameters, such as lipid profile (cholesterol, triglycerides), liver and kidney function markers (ALT, AST, creatinine), can be indicative of improved metabolic health and organ function in livestock species (Olagaray and Bradford, 2019).

The complete blood count (CBC) serves as a crucial diagnostic tool to assess the overall health status, reflecting potential effects on the hematopoietic system and immune response (Thrall et al., 2004). Beyond these systemic effects, the immunomodulatory potential of polyphenols is of particular interest. By altering the expression of important immune-related genes, such as cytokines (such as interleukin-1β (*IL-1β*), interleukin-6 (*IL-6*), tumor necrosis factor-alpha (*TNF-α*), and immunoglobulins, which are essential to the inflammatory and antibody-mediated immune responses, these substances can affect the immune system (Middleton et al., 2000).

The dietary supplementation with pomegranate peel and grape seed extract orchestrates a favorable shift in the immune-related gene expression profile in rabbits. It mainly inhibits the *NF-κB* signaling pathway to create an anti-inflammatory state marked by the downregulation of the pro-inflammatory *TNF-α* gene (Aggarwal and Shishodia, 2006; Basu and Penugonda, 2009), and the anti-inflammatory *IL-10* gene's overexpression or supportive modulation. This is essential for reducing inflammation and preserving immunological homeostasis (Terra et al., 2009). This rebalancing of the cytokine milieu contributes significantly to enhanced immune status, reduced metabolic stress, and improved health outcomes observed in animals fed these natural phytogenic additives (Middleton et al., 2000; Jiang and Xiong, 2016).

Therefore, the purpose of this study is to thoroughly assess how dietary supplementation with pomegranate peel and grape seed extract affects growing rabbits' carcass features, some serum biochemical parameters, complete blood count, and the expression of immune-related genes. The results should offer scientific proof that these natural byproducts can be valued as useful feed additives to support healthy and sustainable rabbit production.

2. MATERIALS AND METHODS

2.1. Ethical Statement and Experimental Location:

From January to March 2024, the lab animal research unit at Benha University's Faculty of Veterinary Medicine, in the Department of Animal Wealth Development to conduct the experimental procedures. The Institutional Animal Care and Use Committee (IACUC) fully approved the study protocol under permission number BUFVTM 23-09-23, guaranteeing compliance with animal research ethics.

2.2. Preparation of Phytogenic Extracts:

Pomegranate Peel Extract (PPE) was made with fresh peels that were cleaned well, chopped into little pieces, and oven-dried for 48 hours at 40°C. After being dried, the material was ground into a fine powder using an electric grinder and kept in sealed containers until it was incorporated into the diet (Sandhya et al., 2018).

Grape Seed Extract (GSE) was produced by cleaning and drying grape seeds at 45°C. The coarsely powdered seeds were macerated with 70% ethanol to defatting them. After being re-dried, the residue was finely crushed into a powder and kept in airtight containers (Mirkarimi et al., 2013).

Based on previous studies both extracts were supplemented to the basal diet at a concentration of 250 mg per kg of feed (Eid et al., 2020; Gómez et al., 2022).

2.3. Experimental Animals, Housing, and Design:

At one month of age, 108 weaned male rabbits with an average starting body weight of 500g from two breeds (V-line and Alexandria, n=54 each) were employed. The experiment lasted for 56 days after a week of adaptation. The rabbits were kept apart in clean, well-ventilated wire cages in a controlled environment with unlimited access to food and drink. A factorial design (2 breeds × 3 diets) was employed. Rabbits from each breed were randomly distributed into three dietary groups, each with three replicates (pens) containing six rabbits. The dietary treatments were:

- Control group: Fed the basal diet.
- PPE group: Fed the basal diet supplemented with 250 mg/kg pomegranate peel extract.
- GSE group: Fed the basal diet supplemented with 250 mg/kg grape seed extract.

The basal diet was formulated according to NRC (1977) recommendations for growing rabbits are presented in Table (1). The basal diet was formulated to meet the nutritional requirements of growing rabbits, with the calculated nutrient levels indicating a balanced ration suitable as a control diet for the experimental period. The proximate and phytochemical composition of PPE and GSE are presented in Table (2).

2.4. Data Collection and Analytical Procedures:

2.4.1. Carcass Traits Evaluation:

At the termination of the experiment (day 56), initial and final weight of rabbits (representative sample of rabbits selected randomly from each group) were taken and statistically analysis as growth performance parameters. The weight of the hot carcass was noted (5 rabbits per group were slaughtered for carcass evaluation), and the weights of internal organs (liver, spleen, heart, kidneys, and gastrointestinal tract) were measured. Organ weights were represented as a percentage of live weight, and the dressing percentage was computed in relation to the live body weight (Wang et al., 2016).

2.4.2. Hematological and Biochemical Analyses:

At the time of slaughter, two tubes were filled with blood samples. Using commercial kits and a

spectrophotometer, serum that had been collected in the first tubes without anticoagulant and separated by centrifugation was examined for liver function markers (alanine aminotransferase, ALT; aspartate aminotransferase, AST), kidney function markers, total protein, and albumin. Albumin was subtracted from total protein to get the globulin concentration (Doumas et al., 1971). Using conventional hematological procedures, a second blood sample was obtained in EDTA tubes and utilized for a complete blood count (CBC), which included the total leukocyte count (TLC), erythrocyte count (RBC), hemoglobin (Hb) concentration, and packed cell volume (PCV) (Campbell, 1995). Lipid profile (TG, VLDL, HDL, LDL) was measured using commercial kits and results are presented in Table (4).

2.4.3. Gene Expression Analysis via qRT-PCR:

Spleen samples were collected from 5 rabbits per group after slaughter and kept at -80°C. GENEzol^{TM} Reagent was used to extract total RNA, and spectrophotometric analysis was used to determine its quantity and quality. One microgram of total RNA was used to create complementary DNA (cDNA).

Gene-specific primers for the target genes Tumor necrotic factor- α (*TNF-α*) and Interleukin-10 (*IL-10*) were used in quantitative real-time PCR (qRT-PCR), with GAPDH acting as the housekeeping gene for normalization (Vandesompele et al., 2002). The control group served as the calibrator for the $2^{(-\Delta\Delta Ct)}$ approach used to determine the relative mRNA expression levels (Livak and Schmittgen, 2001).

2.5. Statistical Analysis:

SPSS software (Version 21, Chicago, IL, USA) was used to statistically analyze all data. The primary impacts of diet, breed, and their interaction were identified using a two-way analysis of variance (ANOVA). Tukey's Honest Significant Difference (HSD) post hoc test was utilized for mean separation in cases where significant effects were discovered. A P-value of less than 0.05 was used to establish statistical significance, and the data are displayed as mean ± standard error (SE). Before analysis, the Shapiro-Wilk Test was used to confirm the assumptions of normality and homogeneity of variances.

3. RESULTS

The effects of dietary additives (PPE and GSE) and rabbit breed (Alexandria and V-Line) on carcass traits, biochemical, hematological, and immune parameters are presented in Tables (4, 5 and 6) and figures (1and 2) respectively.

3.1. Carcass Characteristics and Organ Weights:

Carcass characteristics showed varied responses to dietary supplementation (Table 4). Initial body weight was recorded and confirmed non-significant among groups (P>0.05), ensuring that final weight demonstrated significant treatment (P = 0.007) and breed effects (P = 0.01), with the V-line GSE group exhibiting the highest weight (2030 ± 8.86 g), significantly exceeding control groups in both breeds (V-line: 1525 ± 7.07 g; ALEX: 1558 ± 6.32 g). Dressing percentage remained unaffected by treatments (P > 0.05), ranging from 52.22% to 56.15%. Liver weight showed a significant breed effect (P = 0.001), with V-line breed displaying heavier livers (0.030-0.032 g) compared to ALEX (0.020-0.029g). Other organ weights, including spleen, intestine, heart, stomach, and kidney showed no significant differences among treatments or breeds.

Grape seed extract (GSE) significantly improved live weight at slaughter, especially in V-line rabbits (2030 g), representing 33% increase over the control. Pomegranate peel extract (PPE) showed non-significant moderate improvement in V-line rabbits (1772 g, 16% increase). However, neither PPE nor GSE affected dressing percentage or organ weights, indicating that both natural antioxidants enhanced growth performance without causing adverse effects on organ development or function. V-line rabbits demonstrated better growth response to dietary supplementation compared to Alexandria rabbits.

3.2. Serum Biochemical Parameters:

Serum enzyme activities revealed significant treatment effects (Table 5). Glutamic oxaloacetic transaminase (GOT) activity showed highly significant treatment effects (P=0.00), with supplemented groups exhibiting elevated levels compared to controls. Glutamic pyruvic transaminase (GPT) activity demonstrated highly significant effects across treatment (P=0.00), breed (P=0.01), and interaction (P=0.001), with V-line PPE showing the highest activity (58.17 ± 3.32 U/L). Albumin concentration decreased significantly with supplementation (P=0.00), while total globulin remained stable (P=0.34).

Lipid profile showed pronounced effects with highly significant treatment, breed, and interaction effects (P=0.00). Triglycerides were highest in ALEX-PPE (155.22 ± 4.76 mg/dL) and lowest in controls. VLDL followed similar patterns, while high density lipoprotein (HDL) was highest in V-line PPE (26.60 ± 0.75 mg/dL). Low density lipoprotein (LDL) levels decreased with supplementation, with ALEX-PPE showing the lowest concentration (28.80 ±0.88

mg/dL). Urea concentration showed significant treatment effects (P=0.001) and interaction (P=0.01), with V-line GSE exhibiting the highest level (76.44±2.77 mg/dL). Creatinine showed significant interaction (P=0.005) but remained within normal ranges (1.12-1.52 mg/dL).

3.3. Hematological Parameters:

Hematological analysis revealed selective effects of dietary supplementation on blood cell parameters (Table 6). White Blood Cells (WBC) count showed significant breed-treatment interaction (P = 0.035), with ALEX control exhibiting the highest count (12.625±1.80 × 10^3/µL) and V-line GSE the lowest (5.150±0.794 × 10^3/µL). Red Blood Cells (RBC), Hemoglobin (HGB), and Mean Corpuscular Hemoglobin (MCH) remained unaffected by treatments (P>0.05). Hematocrit showed significant interaction (P=0.692), with V-line GSE displaying the highest value (48.175±0.640 %). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), platelet count, and lymphocyte percentage showed no significant differences among groups.

3.4. Immunological Parameters:

Cytokine expression revealed significant immunomodulatory effects (Figures 1 and 2). Moreover, *IL-10* expression showed highly significant treatment effects (P=0.00) and interaction (P = 0.020), with both extracts markedly upregulating expression. V-line GSE exhibited the highest IL-10 (4.170 ± 0.475), significantly exceeding controls (approximately 1.000). Also, TNF-α expression demonstrated highly significant effects across treatment (P = 0.00), breed (P=0.004), and interaction (P=0.003). V-line GSE showed the highest TNF-α (3.048±0.303), while control-maintained baseline levels (approximately 1.000), indicating differential immunomodulatory responses between breeds and treatments.

Table 1: Composition and Nutritional Content of the Experimental Basal Diet

Ingredient	%	Nutrient	
Berseem hay (16% CP)	29.64	**Digested energy (Kcal/Kg)**	2,601.53
Wheat bran	20.00	**Crude protein (%)**	18.01
Soya bean meal (44%)	18.90	**Crude fiber (%)**	13.78
Yellow corn	13.60	**Lysine (%)**	0.90
Barley grain	10.00	**Methionine + Cystine (%)**	0.60
Shmer straw	4.90	**Calcium (%)**	1.00
Di-calcium phosphate	1.54	**Total phosphorus (%)**	0.70
Limestone	0.37	**Chloride (%)**	0.23
Sodium chloride	0.37	**Sodium (%)**	0.20
Vit and min premix	0.30		
Sodium bicarbonate	0.15		
Antimycotoxin	0.10		
DL-methionine	0.09		
Anticoccidia	0.05		

The basal diet was formulated to meet the nutritional requirements of growing rabbits as recommended by NRC (1977). CP: Crude Protein; ME: Metabolizable Energy.

Table 2: Proximate and phytochemical composition of Pomegranate Peel Extract (PPE) and Grape Seed Extract (GSE)

Parameter	PPE	GSE
Proximate Composition (g/100g DM)		
Moisture (%)	7.27 ± 0.15	5.84 ± 0.19
Crude Protein (%)	3.74 ± 0.11	10.84 ± 0.42
Crude Fat (%)	0.85 ± 0.04	7.92 ± 0.31
Crude Fiber (%)	17.31 ± 0.72	16.83 ± 0.58
Ash (%)	4.32 ± 0.13	2.18 ± 0.08
Phytochemical Composition		
Total Polyphenols (mg GAE/g DW)	264.30 ± 7.2	392.58 ± 1.70
Total Flavonoids (mg QE/g DW)	38.60 ± 1.4	256.16 ± 1.60
Total Tannins (mg TAE/g DW)	264.30 ± 5.8	30.95 ± 0.17
Proanthocyanidins (mg/g DW)	22.40 ± 0.8	185.30 ± 4.2
Punicalagins (mg/g DW)	38.60 ± 1.1	ND
Antioxidant Activity		
DPPH Radical Scavenging (IC50, mg/mL)	0.21 ± 0.01	0.14 ± 0.01
FRAP (mmol Fe2+/g DW)	15.60 ± 0.5	22.30 ± 0.7

Values are expressed as mean ± SE. DM: Dry Matter; DW: Dry Weight; GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents; CE: Catechin Equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power.

Table 3: Primer sequences used for quantitative real-time PCR analysis

Genes	Forward primer (5'→3')	Reverse primer (5'→3')	Size(bp)	Accession no.
IL10	TCAGCAAGCTGCAAGAGGAA	TGCCATCCTGGGGCTTTTAG	117	NM_001082045.1
TNF-α	ACCACGTAGCCGTGTTCAG	GCCTAGGTCTGGGTGACAAC	71	NM_001082263.1
GAPDH	GTCAAGGCTGAGAACGGGAA	CCAGCATCACCCCACTTGAT	95	NM_001082253.1

GAPDH was used as the reference gene for normalization of gene expression data.

Table 4: Effect of breed and dietary supplementation on carcass traits in rabbits

Parameter	ALEX breed			V-line breed			P Valu		
	Control mean ±SE	PPE mean ±SE	GSE mean ±SE	Control mean ±SE	PPE mean ±SE	GSE mean ±SE	Breed	Treat-ment	Breed and Treat-ment
Initial weight	506.11±2.66	502.353±3.61	501.429±3.29	505±1.88	505±2.53	507±4.89	0.39	0.80	0.61
Live weight (gm)	1558±6.32^{b}	1524±1.15^{b}	1681±9.47^{ab}	1525±7.07b	1772±1.03^{ab}	2030±8.86^{a}	0.01	0.007	**0.11**
Dressing%	56.15±.22	54.51±2.70	54.91±2.29	54.92±.59	55.17±1.28	52.22±1.54	0.69	0.50	**0.61**
Liver weight (gm)	0.025±.000	0.029±.001	0.02±.0008	0.031±.001	0.032±.001	0.03±.002	0.001	0.29	**0.54**
Spleen weight (gm)	0.0096±.0001	0.0008±.0001	.0008±.0001	0.001±.0001	0.010±.009	0.0009±.000 08	0.31	0.40	**0.39**
Intestine weight (gm)	0.15±.004	0.17±.018	0.16±.01	0.14±.01	0.14±.006	0.14±.01	0.13	0.56	**0.71**
Heart weight (gm)	0.003±.0003	0.003±.0002	0.003±.00009	0.003±.0003	0.003±.0002	0.004±.0003	0.04	0.57	**0.30-**
Stomach weight before cleaning (gm)	0.02±.004	0.03±.005	0.02±.002	0.02±.005	0.02±.002	0.02±.002	0.91	0.85	**0.88**
Stomach weight after cleaning (gm)	0.01±.0003	0.01±.0007	0.01±.0005	0.01±.0005	0.01±.0006	0.01±.0004	0.36	0.26	**0.43**
One kidney weight (gm)	0.003±.0004	0.003±.0003	0.003±.0001	0.004±.0004	0.003±.0003	0.003±.0001	0.32	0.08	**0.15**

Different superscript letters (a, b) indicate significant differences between means at P≤<0.05 significance level. PPE: Pomegranate Peel Extract / GSE: Grape Seed Extract.

Table 5: Effect of breed and dietary supplementation on serum biochemical parameters in rabbits

Parameter	ALEX breed			V-line breed			P Value		
	Control mean ±SE	PPE mean ±SE	GSE mean ±SE	Control mean ±SE	PPE mean ±SE	GSE mean ±SE	Breed	Treatment	Breed and Treat-ment
GOT	23.98±1.36^{c}	39.08±3.59^{ab}	44.20±3.56^{ab}	22.23±1.34^{c}	33.40±2.81^{bc}	47.25±2.84^{a}	0.52	0.00	**0.30**
GPT	28.11±2.18^{cd}	38.89±2.45bc	48.19±3.15^{ab}	26.97±1.49^{d}	58.17±3.32^{a}	46.49±2.83^{b}	0.01	0.00	**0.001**
Albumin	4.14±.06^{a}	3.55±.26^{ab}	3.19±.05^{b}	4.09±.12^{a}	3.63±.15^{ab}	3.24±.05^{b}	0.83	0.00	**0.87**
Total Globulin	4.02±.11	4.08±.26	3.85±.21	3.97±.03	3.51±.22	3.61±.15	0.07	0.34	**0.38**
Tg	66.93±3.09^{d}	155.22±4.76^{a}	134.67±4.13^{b}	63.81±1.95^{d}	87.78±4.24^{c}	93.54±2.86^{c}	0.00	0.00	**0.00**
VLDL	13.37±.61d	31.04±.95a	26.98±.82^{b}	13.01±.39^{d}	17.90±.54^{c}	18.75±.57^{c}	0.00	0.00	**0.00**
HDL	22.73±.55b^{c}	20.69±.63c^{d}	24.23±.74^{ab}	19.46±.59de	26.60±.75^{a}	16.68±.51^{e}	0.005	0.00	**0.00**
LDL	56.38±3.52^{a}	28.80±.88^{d}	43.18±1.32^{c}	56.94±1.74^{a}	54.20±1.66^{ab}	46.79±1.43^{bc}	0.00	0.00	**0.00**
UREA	68.62±4.24^{ab}	53.82±3.88^{bc}	59.23±4.02^{abc}	62.45±5.74^{abc}	47.92±2.41^{c}	76.44±2.77^{a}	0.60	0.001	**0.01**
Creatinine	1.18±.07	1.49±.12	1.52±.13	1.47±.06	1.12±.06	1.36±.05	0.30	0.28	**0.005**

Means within the same row bearing different superscript letters are significantly different (P≤0.05). GOT: Glutamic Oxaloacetic Transaminase; GPT: Glutamic Pyruvic Transaminase; Tg: Triglycerides; VLDL: Very Low-Density Lipoprotein; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

Table 6: Effect of breed and dietary supplementation on hematological parameters in rabbits

Parameter	ALEX breed			V-line breed			P Value		
	Control mean ±SE	PPE mean ±SE	GSE mean ±SE	Control mean ±SE	PPE mean ±SE	GSE mean ±SE	Breed	Treat-ment	Breed and Treat ment
WBC10^{3}/µL	6.42±.78^{b}	6.62±.41b	5.15±.79^{b}	12.62±1.80 a	7.20±.67^{b}	8.47±1.23^{ab}	0.001	0.02	**0.03**
RBC10^{6}/µL	6.02±.23	6.17±.10	6.56±.11	6.47±.39	6.45±.33	6.38±.26	0.41	0.70	**0.50**
HGB g/dl	10.84±.40	11.37±.30	11.92±.20	11.92±.79	12.04±.57	11.95±.68	0.19	0.59	**0.62**
HCT%	35.22±1.37^{b}	36.45±.34 b	48.17±.64 a	37.67±2.56 b	36.70±1.70 b	37.90±2.23 b	0.56	.59	**0.69**
MCVFL	58.46±.34 ab	57.85±.193^{b}	58.15±.75 b	58.10±.84^{b}	59.66±.43^{a}	59.22±1.53 ab	0.19	0.79	**0.36**
MCH pg	18±.23	17.67±.21	18.17±.34	18.40±.27	18.68±.40	18.70±.58	0.04	0.74	**0.67**
MCHC g/dl	30.78±.33	30.52±.34	31.25±.22	31.67±.07	31.32±.60	31.52±.35	0.05	0.49	**0.71**
PLT10^{3}/µL	240±42.03	273.25±43.79	251.25±20.09	328±123.73	458.40±32.11	346.75±68.66	0.02	0.37	**0.67**
LYMPH%	4.12±.41	4.30±.23	2.87±.72	4.27±1.06	3.44±.77	3.20±.53	0.82	0.24	**0.64**

Means within the same row bearing different superscript letters are significantly different (p < 0.05). WBC: White Blood Cells; RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelets; LYMPH: Lymphocytes.

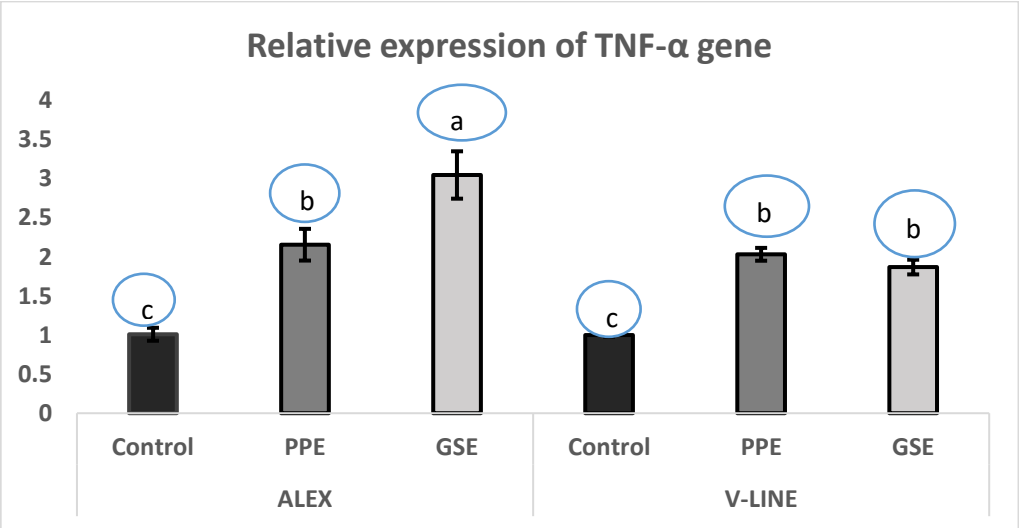

Figure 1: Effect of breed and dietary supplementation on serum *TNF-α* levels**.** Values are presented as mean±SE. Means with different superscript letters within the same chart differ significantly (p<0.05). (ALEX: Alexandria; GSE: Grape Seed Extract; PPE: Pomegranate Peel Extract).

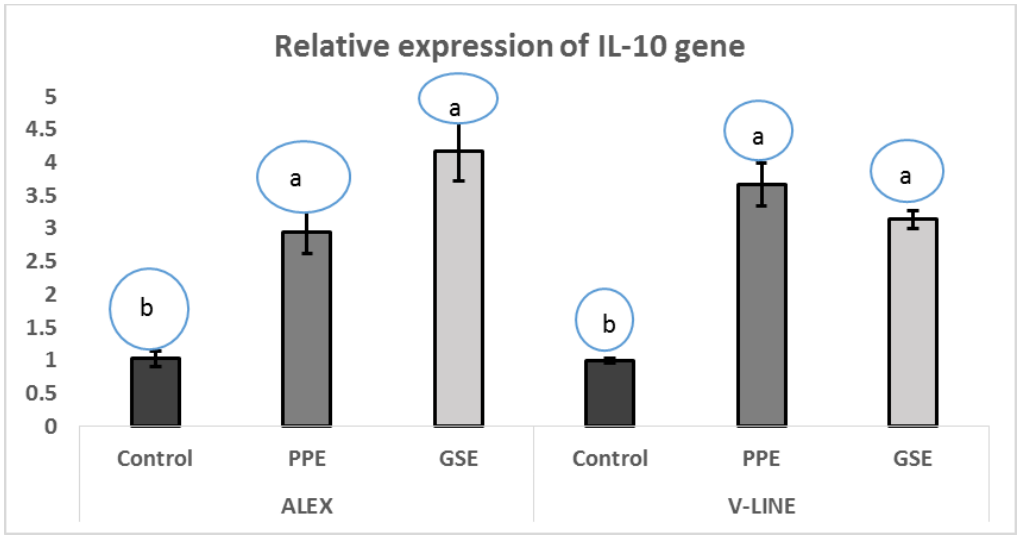

Figure 2: Effect of breed and dietary supplementation on serum *IL-10* levels. Values are presented as mean±SE. Means with different superscript letters (a, b, c) within the same chart differ significantly (p<0.05). (ALEX: Alexandria; GSE: Grape Seed Extract; PPE: Pomegranate Peel Extract).

4. DISCUSSION

The current investigation showed that adding grape seed extract (GSE) to the diet considerably increased the live weight of V-line rabbits, while dressing percentage remained unaffected across all treatments. These results are consistent with earlier studies by Hassan et al. (2020), who revealed that supplementing New Zealand White rabbits with pomegranate by-product extract at a dose of 200 mg/kg considerably increased their final body weight and feed conversion ratio. Nassrallah et al. (2016) discovered that in male V-line growing rabbits, pomegranate peel extract (PPE) enhanced growth performance and carcass features. The growth-promoting effects observed with grape seed extract may be attributed to enhanced nutrient digestibility and improved antioxidant status, which collectively optimize metabolic efficiency and support muscle protein deposition in broiler (Chamorro et al., 2013; Hassan et al., 2016). V-line rabbits have bigger livers than ALEX rabbits, indicating a strong breed effect on liver weight, reflecting inherent genetic differences in organ development and metabolic capacity between these breeds. This result is keeping with the findings of Ayyat et al. (2024), who stated breed-specific responses in organ weights following dietary supplementation with pomegranate peel extract combined with trace minerals. The stability of other organ weights, including spleen, heart, kidney, and intestine, across all treatment groups suggests that neither PPE nor GSE adversely affected organ development or function at the supplementation levels

employed. This is reliable with the safety profile reported by Hassan et al. (2020) and Mpendulo et al. (2020), who found that polyphenol-rich extractsat appropriate supplementation levels did not compromise organ integrity or function in rabbits and broilers.

It is important to interpret the elevated serum liver enzymes (GOT and GPT) in supplemented groups carefully in light of cellular signaling and polyphenol metabolism. The study of Zamani et al. (2022) revealed that grape products typically showed neutral or modestly favorable effects on liver enzymes, with mean changes of -1.9 U/L for ALT and -1.1 U/L for AST compared to placebo, according to a comprehensive review and meta-analysis of randomized controlled studies. Elevated enzyme activities observed in our study may reflect increased metabolic activity rather than hepatotoxic injury, particularly given the absence of histopathological alterations and the maintained functional capacity of the liver.

Future studies should include histopathological examination of liver tissue to provide direct morphological evidence supporting the absence of hepatic damage. The reduction in serum albumin levels with polyphenol supplementation, while total globulin remained stable, represents an interesting metabolic adaptation that contrasts with the results of Hassan et al. (2020), who reported increased total protein and albumin concentrations in rabbits fed grape seed extract during heat stress. This variation may be explained by differences in environmental conditions, as heat stress can substantially alter protein metabolism, while in normal temperature conditions, polyphenols may modulate hepatic protein synthesis pathways differently, potentially redirecting amino acids toward immune protein production or other metabolic priorities The reduction in serum albumin observed in the current study may be attributed to the capacity of polyphenols to inhibit hepatic albumin synthesis through modulation of transcription factors, or alternatively, to the increased utilization of amino acids for acute-phase protein synthesis and immune function enhancement, as previously suggested by Olagaray and Bradford (2019).

The pronounced effects on lipid profile, with increased triglycerides and VLDL in ALEX rabbits supplemented with PPE, while LDL decreased substantially across treatments, reveal complex breed-dependent metabolic responses to polyphenolic compounds. Lupoli et al. (2020) found that grape products generally lower LDL and total cholesterol levels in a meta-analysis. However, the breed-specific elevation of triglycerides in ALEX rabbits may reflect variations in lipid metabolism gene expression, potentially involving SREBPs or PPARs. Polyphenols have been shown to modulate these key transcription factors through AMPK activation and subsequent regulation of lipogenic pathways (Sun et al., 2017; Liu et al., 2020). The reduction in LDL cholesterol, particularly pronounced in ALEX-PPE groups, represents a favorable cardiovascular outcome and aligns with the established mechanisms of polyphenol action on cholesterol metabolism. This by inhibiting the rate-limiting enzyme in cholesterol manufacture, hepatic HMG-CoA reductase, while concurrently increasing the expression of LDL receptors and encouraging the excretion of cholesterol through bile acid routes (Aviram and Dornfeld, 2001; Yin et al., 2015; Kaur et al., 2016). The differential HDL response, with elevated levels in V-line PPE but reduced levels in V-line GSE, suggests distinct effects of pomegranate versus grape polyphenols on reverse cholesterol transport mechanisms through modulation of ABCA1 (ATP- binding cassette transporter A1) and paraoxonase-1 activity (Rosenblat et al., 2006; Xu et al., 2016).

The selective effects on white blood cell counts, with significant breed-treatment interactions, indicate immunomodulatory properties of the polyphenolic extracts. While the stability of red blood cell parameters, hemoglobin, and mean corpuscular indices across treatments indicates that neither PPE nor GSE adversely affected erythropoiesis or red cell morphology. These results are according to the findings of Hassan et al. (2020), who stated maintained or improved hematological parameters in rabbits supplemented with grape seed extract under various environmental conditions. The significant interaction effect on hematocrit, with V-line GSE groups showing elevated values, may reflect enhanced erythrocyte production or reduced plasma volume. The obtained result given the antioxidant properties of grape polyphenols.The improved hematocrit could result from protection against oxidative hemolysis and enhanced red blood cell membrane stability, as demonstrated by Gong et al. (2010) who examined grape pomace effects on antioxidant defense systems in rabbits.

The marked upregulation of *IL-10* expression in both PPE and GSE supplemented groups represents a significant immunomodulatory effect with important implications for immune homeostasis and inflammatory regulation. Interleukin-10 (*IL-10*) is a potent anti-inflammatory cytokine produced by various immune cells, including macrophages, dendritic cells, and regulatory T cells. It plays a crucial role in limiting excessive inflammatory responses and maintaining immune homeostasis by suppressing the production of pro-inflammatory cytokines such as *TNF-α*, *IL-1β*, and *IL-6* (Moore et al., 2001). *IL-10* exerts its immunosuppressive effects through inhibition of the *NF-κB* signaling pathway and downregulation of antigen-presenting cell function, thereby preventing

tissue damage caused by prolonged inflammation (Couper et al., 2008). The upregulation of *IL-10* expression following dietary polyphenol supplementation has been reported as a key mechanism through which these bioactive compounds exert their anti-inflammatory and immunomodulatory effects in various animal models (Shakoor et al., 2021).

This elevation aligns with extensive literature demonstrating the immunomodulatory properties of dietary polyphenols, as reviewed by Shakoor et al. (2021), who noted that these compounds can influence dendritic cell function, modulate macrophage responses, and regulate T cell populations while suppressing pro-inflammatory cytokine production. Tumor Necrosis Factor-alpha (*TNF-α*) is a pro-inflammatory cytokine produced primarily by macrophages and plays a central role in regulating immune responses, inflammation, and cell survival. Its upregulation is associated with enhanced immune surveillance (Aggarwal and Shishodia, 2006). The simultaneous upregulation of *TNF-α* expression, particularly pronounced in V-line GSE groups, initially appears contradictory to the anti-inflammatory reputation of polyphenols However this finding requires nuanced interpretation within the context of immune system complexity, as Abdel-Moneim et al. (2020) discussed that while polyphenols can inhibit *TNF-α* release via *NF-κB* pathway inhibition, the initial upregulation observed in our study may represent a hormetic response, where moderate activation of inflammatory pathways can enhance immune surveillance and preparation without progressing to pathological inflammation. The breed-specific differences in cytokine responses suggest genetic variation in pattern recognition receptors, signaling pathway components, or transcription factor expression that mediate polyphenol-immune interactions, Hussain et al. (2016) clarified the molecular processes behind the anti-inflammatory effects of polyphenols, such as the suppression of pro-inflammatory enzymes like Cyclooxygenase-2 (COX-2) and Inducible Nitric Oxide Synthase (Inos) and the regulation of transcription factors like Nuclear Factor Kappa B (NF-κB) and Activator Protein-1 (AP-1).

A particularly noteworthy finding is the consistent observation of significant breed-treatment interactions across multiple parameters, including liver enzymes, lipid profile, hematological indices, and cytokine expression, indicating that genetic background substantially influences the metabolic and immunological responses to dietary polyphenols.

6. CONCLUSIONS:

Dietary supplementation with pomegranate peel extract (PPE) and grape seed extract (GSE) significantly improved growth performance in rabbits, with breed-specific responses indicating genetic influence on treatment efficacy. PPE produced favorable lipid profiles, while GSE demonstrated superior effects on final body weight, particularly in V-line rabbits. Both supplements modulated immune responses by increasing anti-inflammatory cytokines while maintaining normal hematological parameters and organ weights. These findings confirming the safety and efficacy of pomegranate peel extract (PPE) and grape seed extract (GSE) as natural feed additives in sustainable rabbit production systems.

Acknowledgements

- Authors' declarations

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

- Data and material availability:

All data for this study is provided.

- Conflict of interests.

All authors have stated the absence of any conflicts of interest.

- Funding.

This research did not receive funding from any specific grant.

Authors' contributions

All authors contributed to the design and conception of the study. Ammar Abdul Ghafour: prepared the materials, collected, and revised the manuscript. Buthaina Abdul Hamid; wrote the first draft of the manuscript, and all authors commented on earlier versions. All authors read and approved of the final manuscript.

REFERENCES

Abdel-Moneim, A. M. E., Shehata, A. M., Khidr, R. E., Paswan, V. K., Ibrahim, N. S., El-Ghoul, A. A., Aldhumri, S. A., Gabr, S. A., Mesalam, N. M., Elbaz, A. M., Elsayed, M. A., Wakwak, M. M. 2020. Nutritional manipulation to combat heat stress in poultry - A comprehensive review. J. Therm. Biol. 98:102915.

Aggarwal, B. B., Shishodia, S. 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem. Pharmacol. 71(10):1397-1421.

Aviram, M., Dornfeld, L. 2001. Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. Atherosclerosis 158(1):195-198.

Ayyat, M. S., Al-Sagheer, A. A., El-Hais, A. M. A., Osman, R. M., Noreldin, A. E. 2024. Productive performance, carcass traits, and nutrient digestibility of growing rabbits as influenced by pomegranate peel powder and trace minerals supplementation: A sustainable agriculture approach. Trop. Anim. Health Prod. 56(1):28.

Basu, A., Penugonda, K. 2009. Pomegranate juice: A heart-healthy fruit juice. Nutr. Rev. 67(1):49-56.

Campbell, T. W. 1995. Avian Hematology and Cytology. 2nd ed. Iowa State University Press, Ames.

Chamorro, S., Viveros, A., Centeno, C., Romero, C., Arija, I., Brenes, A. 2013. Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma

lipids and mineral content in broiler chicks. Animal 7(4):555-561.

Couper, K. N., Blount, D. G., Riley, E. M. 2008. IL-10: The master regulator of immunity to infection. J. Immunol. 180(9):5771-5777.

Doumas, B. T., Watson, W. A., Biggs, H. G. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta 31(1):87-96.

Eid, Y. Z., Ohtsuka, A., Hayashi, K. 2020. Effect of dietary supplementation of pomegranate peel powder on growth performance, carcass characteristics and lipid metabolism in Japanese quail. Egypt. Poult. Sci. J. 40(1):133-150.

Ghasemzadeh, A., Ghasemzadeh, N. 2011. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J. Med. Plants Res. 5(31):6697-6703.

Gong, J., Forster, R. J., Yu, H., Chambers, J. R., Sabour, P. M., Wheatcroft, R., Chen, S. 2010. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. FEMS Microbiol. Lett. 208(1):1-7.

Gómez, M. A., Fernández, M., García, A. 2022. Effect of grape seed extract supplementation on growth performance and meat quality in rabbits. Anim. Feed Sci. Technol. 280:115068.

Hassan, F. A., Mahrose, K. M., Basyony, M. M. 2020. Effects of grape seed extract as a natural antioxidant on growth performance, carcass characteristics and antioxidant status of rabbits during heat stress. Arch. Anim. Breed. 63(1):99-111.

Hassan, H. M. A., Mohamed, M. A., Youssef, A. W., Hassan, E. R. 2016. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Australas. J. Anim. Sci. 29(2):274-281.

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., Rahu, N. 2016. Oxidative stress and inflammation: What polyphenols can do for us? Oxid. Med. Cell. Longev. 2016:7432797.

Jiang, J., Xiong, Y. L. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. Meat Sci. 120:107-117.

Kaur, G., Chakraborty, S., Anand, V., Kumar, A. 2016. Grape seed extract: A potential dietary supplement in management of metabolic syndrome. PharmaNutrition 4(2):28-39.

Lebas, F., Coudert, P., de Rochambeau, H., Thébault, R. G. 1997. The Rabbit: Husbandry, Health and Production. FAO Animal Production and Health Series No. 21. FAO, Rome.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. 96(2):254-260.

Liu, Y., Zhang, L., Liang, J., Zhang, Z. 2020. Activation of AMPK stimulates fatty acid oxidation and inhibits triglyceride synthesis in HepG2 cells through PPAR-α and SREBP-1c. J. Food Biochem. 44(8):e13284.

Livak, K. J., Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25(4):402-408.

Lupoli, R., Lembo, E., Saldalamacchia, G., Avola, C. K., Angrisani, L., Capaldo, B. 2020. Bariatric surgery and long-term nutritional issues. World J. Diabetes 8(11):464-474.

López-Alarcón, C., Denicola, A. 2013. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Anal. Chim. Acta 763:1-10.

Middleton, E., Kandaswami, C., Theoharides, T. C. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52(4):673-751.

Mirkarimi, M., Tobianian, H., Eskandarion, S., Bahador, A., Tavakoli, M. 2013. The antimicrobial activity of grape seed extract against two important oral pathogens. Zahedan J. Res. Med. Sci. 15(1):43-47.

Molina-Alcaide, E., Yáñez-Ruiz, D. R. 2008. Potential use of olive by-products in ruminant feeding: A review. Anim. Feed Sci. Technol. 147(1-3):247-264.

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., O'Garra, A. 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19:683-765.

Mpendulo, C. T., Chimonyo, M., Maleleka, D., Hanson, S. 2020. Effects of dietary pomegranate peel meal on growth performance, carcass characteristics and meat quality of broiler chickens. S. Afr. J. Anim. Sci. 50(1):78-88.

Nassrallah, H. A., Abdel-Kafy, E. M., El-Adawy, M. M. 2016. Effect of dietary supplementation with pomegranate peel extract on growth performance, blood biochemical parameters and carcass traits of rabbits. Egypt. Poult. Sci. J. 36(2):493-507.

NRC (National Research Council). 1977. Nutrient Requirements of Rabbits. 2nd ed. National Academy Press, Washington, DC.

Olagaray, K. E., Bradford, B. J. 2019. Plant flavonoids to improve productivity of ruminants - A review. Anim. Feed Sci. Technol. 251:21-36.

Rosenblat, M., Hayek, T., Aviram, M. 2006. Anti-oxidative effects of pomegranate juice consumption by diabetic patients on serum and on macrophages. Atherosclerosis 187(2):363-371.

Sandhya, S., Khamrui, K., Prasad, W., Kumar, M. C. 2018. Preparation of pomegranate peel extract powder and evaluation of its effect on functional properties and shelf life of curd. LWT Food Sci. Technol. 92:416-421.

Shakoor, H., Feehan, J., Apostolopoulos, V., Platat, C., Al Dhaheri, A. S., Ali, H. I., Ismail, L. C., Bosevski, M., Stojanovska, L. 2021. Immunomodulatory effects of dietary polyphenols. Nutrients 13(3):728.

Shi, J., Yu, J., Pohorly, J. E., Kakuda, Y. 2003. Polyphenolics in grape seeds - Biochemistry and functionality. J. Med. Food 6(4):291-299.

Sun, L., Xu, G., Dong, Y., Li, M., Yang, L., Lu, W. 2017. Quercetin protects against lipid peroxidation and regulates the expression of lipid metabolism-related genes in skeletal muscle via AMPK pathway. Mol. Nutr. Food Res. 61(11):1700022.

Terra, X., Pallares, V., Ardevol, A., Blade, C., Fernandez-Larrea, J., Pujadas, G., Salvado, J., Arola, L., Blay, M. 2009. Modulatory effect of grape-seed procyanidins on local and systemic inflammation in diet-induced obesity rats. J. Nutr. Biochem. 20(3):210-218.

Thrall, M. A., Baker, D. C., Campbell, T. W., DeNicola, D., Fettman, M. J., Lassen, E. D., Rebar, A., Weiser, G. 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins, Philadelphia.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3(7):research0034.

Wang, C., Zhao, F., Li, Z., Jin, X., Zhang, C. 2016. Effects of dietary vitamin E and selenium supplementation on metabolic profiles and expression of selenium dependent glutathione peroxidase in rabbit muscle. Asian-Australas. J. Anim. Sci. 29(11):1583-1589.

Xu, Y., Zhang, M., Wu, T., Dai, S., Xu, J., Zhou, Z. 2016. The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Food Funct. 8(11):2985-2992.

Yin, W., Li, B., Li, X., Yu, F., Cai, Q., Zhang, Z., Cheng, M., Gao, H. 2015. Anti-inflammatory effects of grape seed procyanidin B2 on a diabetic pancreas. Food Funct. 6(9):3065-3071.

Zamani, H., Abedini, M. R., Emami, S. A., Karami, M., Forouzanfar, F., Sahebkar, A. 2022. Effects of grape products on liver enzymes: A systematic review and meta-analysis of randomized controlled trials. Phytother. Res. 36(10):3723-3738.