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Impact of Grape Seed Extract on Growth, Blood Health, Immunity, and Inflammation in Environmentally Stressed Rabbits

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ABSTRACT

This research sought to elucidate the mitigating role of dietary grape seed extract (GSE) anti-HS agent alterations in blood parameters, immune function, oxidative balance, and inflammatory responses in growing rabbits. A 120 growing rabbits, (age 5 weeks, 657.32± 6.12 g) were randomly assigned to 4 treated groups (30 rabbits in each group). For 8 weeks, under natural thermal stress environments, these groups were fed diets supplemented with varying levels of GSE: 0 (basal diet as a control group), 100 (GSE100), 200 (GSE200), and 400 mg/kg diet (GSE400).

Stressed rabbits fed diets added with GSE displayed significantly (P<0.001) higher growth indices and feed conversion ratio (FCR) as compared to rabbits fed basal diets. Feeding stressed rabbits diets fortified with GSE resulted in notably decreased levels of serum biochemistry [Alanine (ALT) and Aspartate (AST) transaminases, Gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatinine, and uric acid] and lipid profile, while significantly improved levels of blood proteins (P<0.001). Total antioxidant capacity (TAC) in rabbits fed diets with 200 or 400 mg of GSE was notably greater (P<0.01) than in the other groups. All GSE-supplemented diets significantly improved superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities compared to control diets (P<0.01). MDA and myeloperoxidase were significantly (P<0.01) reduced by dietary GSE in a dose-dependent manner. HS substantially diminished serum levels of IgG and IgM in rabbits, and dietary GSE supplementation effectively mitigated this reduction (P < 0.01). Dietary GSE inclusion significantly (P<0.01) reduced interleukin-4 (IL-4) levels in a dose-dependent manner. Compared to GSE0, GSE fortification increased (P<0.01) interleukin-10 (IL-10) levels and nitric oxide in rabbits. Taken together, the evidence suggests that GSE serves as a valuable feed supplement for stressed rabbits, promoting both health and growth.

***Conclusively,** these findings indicate that GSE is a promising feed supplement for boosting growth, blood biochemistry, and overall productivity in environmental stress rabbits. Future investigations should delve deeper into the*

underlying mechanisms by which GSE mitigates the adverse effects of heat stress in animal systems.

Keywords: Growing rabbit, heat stress, grape seed extract.

INTRODUCTION

Global climate change presents a substantial threat to biological systems, with increasing temperatures exacerbating disease prevalence, mortality rates, food scarcity, and diminished animal productivity (Pfenning-Butterworth *et al.,* 2024). Heat stress (HS), a consequence of climate change, substantially affects the livestock industry (El Sabry *et al.,* 2021). Rabbits are a crucial global meat source, particularly in European nations, China, and Egypt. Their physiological inability to dissipate heat due to the absence of sweat glands renders them particularly vulnerable to HS. The detrimental effects of HS on rabbits, including reduced feed intake and nutrient absorption, impaired growth (Bashar *et al.,* 2023; Ebeid *et al.,* 2023), and consequent economic passing for rabbit farms, have been well-documented (Mangan and Siwek, 2024). Furthermore, HS induces histopathological damage in hepatocytes and renal tissues (Ebeid *et al.,* 2023), potentially leading to kidney and hepatic dysfunction, as well as intestinal compromise through the proliferation of pathogenic bacteria in growing rabbits (Liang *et al.,* 2022).

Elevated ambient temperatures also suppress immune function, exacerbate oxidative stress, and thus promoting inflammation/apoptosis cascade in rabbits (El-Ratel *et al.,* 2025). Consequently, there is a pressing need for effective, safe, sustainable, and environmentally sound strategies to bolster immunity and alleviate inflammation, thereby enhancing animal resilience to HS (Liang *et al.,* 2022; Ebeid *et al.,* 2023; El-Ratel *et al.,* 2025).

Realizing nutritional interventions to counteract the adverse impacts of HS is therefore of critical importance. The management of agricultural byproducts represents significant environmental concern. Valorizing waste streams from food processing offers a dual benefit s (Pérez-Aguilar *et al.,* 2023), providing a rationale for investigating their potential as anti-heat stress agents while simultaneously contributing to the reduction of food waste. Fruit and Vegetable byproducts are recognized as rich supplies of natural biological molecules, which can improve sustainability within the food production (García González *et al.,* 2025).

Grapes are an internationally significant agricultural commodity, with an annual production of almost 25 million tonnes (Allam *et al.,* 2022). Processing byproducts, including skins, seeds, and stems, constitute roughly 20% of the total grape weight, presenting a discarding challenge for the wine and grape juice industries. Grape seeds have substantial oil content, ranging from 13 to 15% on a dry weight basis and about 20% seeds. Due to its high levels of bioactive

polyphenols, vitamins, and fatty acids, particularly its polyphenolic compounds, this material is generally applied as a food additive or nutritional supplement, owing to its established antibacterial, scavenging oxidative stress, and anti-inflammatory benefits (Al Wadei *et al.,* 2025).

Polyphenolic extracts from grape seeds, encompassing compounds such as procyanidin, catechin, gallic acid, and epicatechin, exhibit diverse health-promoting effects, including anti-allergic and vasodilatory activities (Farhan *et al.,* 2024). Grape seed extract (GSE) has demonstrated potential as a therapeutic agent for oxidation-related disorders in animal models (Ramamurthy, 2024). Dietary supplementation with GSE has demonstrated positive effects across various animal species, including broilers, quails, rabbits, and ruminants (Ma *et al.,* 2024). Studies indicate that GSE can improve growth performance, blood health, antioxidant capacity, and feed efficiency, as well as influence inflammatory responses (Abdollahi-Mousavi *et al.*, 2024). Furthermore, GSE has shown promise in improving energy metabolism, modulating gut microbiota, and promoting healthy evolution in pre-weaning dairy calves (Urkmez and Biricik, 2022) under both HS and thermoneutral conditions (Ma *et al.,* 2023). Nonetheless, the specific effects of dietary GSE management on growth, hematological parameters, immunity, redox homeostasis, and inflammatory reactions in fattening rabbits subjected to stress remain incompletely elucidated. Given the multi-targeted and multifaceted nature of GSE's bioactive compound mixture and its established biological activities, we theorize that GSE can boost blood health, immunity, and attenuate inflammation-oxidative pathways in fattening rabbits exposed to thermal stress, potentially serving as an effective policy to mitigate the adverse effects of HS.

Consequently, the existing study was planned to explore the protective role of GSE in alleviating the negative impacts of HS in rabbits, precisely by evaluating its effects on blood biochemistry, immune ability, and the modulation of inflammation-oxidative pathways.

MATERIALS AND METHODS

Ethical statement: The Institutional Animal Care and Use Committee (IACUC) at the University of Zagazig approved the animal handling and experimental procedures used in this study (Approval Code: ZU-IACUC/2/F/371/2023).

Propagation of grape seed extract (GSE)

Grape seeds from the Edafco company of 10th of RMANDAN city variety were cleaned with distilled water and dried first at room temperature and then in a 40°C oven for 10 hours according to the method presented in (Tarek *et al.*, 2025). After drying, the seeds were ground into a fine powder using a mortar and then

sieved. To obtain extracts, 100 g of the powdered seeds was mixed with 360 ml of either distilled water, ≥95.0% absolute ethanol, or absolute methanol. These mixtures were kept in dark bottles at room temperature for a day. The extracts were then filtered to get a clear solution, which was evaporated at 30°C using a rotary evaporator, followed by freeze-drying to produce a powder.

Rabbits and experimental design

This investigation utilized 120 growing animals (male rabbits, 657.32± 6.12 g). Rabbits were individually housed in galvanized wire cages (35 × 35 × 60 cm^3) and randomly allotted to one of four experimental groups (n=30 per group, with two rabbits per replicate). Animals in the control group (subjected to natural heat stress during the June and July 2023) received a basal diet, while the therapy groups were fed the same basal diet fortified with grape seed extract (GSE) at levels of 100 (GSE100), 200 (GSE200), and 400 mg/kg (GSE400) of diet. The GSE dosages were based on previously announced research (Abd El-Khalek *et al.*, 2017; Abd Allah *et al.,* 2024) and were incorporated into the diet by mixing with premix and supplements. Cages were equipped with ad libitum feeders and an automatic nipple system for freshwater provision. The facility where the rabbits were housed offered a natural environment with natural lighting.

All rabbits were sustained under consistent hygienic and managerial practices, including daily morning cleaning of trays. The control basal diet was formulated to meet the nutritional requirements of fattening rabbits (Cunha and Cheeke, 2012), and its chemical composition on a dry matter basis was: crude protein (17.76), ether extract (2.34%), nitrogen-free extract (59.46%), ash (8.78%), organic matter (91.56%), crude fiber (12.48%), and digestible energy (2760 kcal/kg) as shown in Table 1.

Climatic components

Environmental conditions within the Rabbitry were characterized by the temperature-humidity index (THI). Daily ambient temperature (AT, °C) and relative humidity (RH, %) were recorded throughout the study period using an automated thermo-hygrometer (Wertheim, Germany) placed within the facility.

The THI was then computed based on these data, following the established methodology of (Marai *et al.,* 2002):

$$THI = db°C - [(0.31 - 0.31 \times (RH/100)) \times (db°C - 14.4)],$$

Where db°C denotes the dry bulb temperature in Celsius. The resulting THI values were labeled to define the level of heat stress: 29.0 to 30.0 (Severe HS), <27.8 (Absence of HS), >30.0 (Very severe HS), and 27.8 to 28.9 (Moderate HS).

Table 1. Constituents and substance investigation of the diet applied for feeding fattening rabbits.

Items	Control diet
Constituents (g/1000g DM)	
Maize	180
Soybean meal 44%	195
Berseem hay	330
Molasses	15
Wheat bran	150
Limestone	10
Barley grain	115
NaCl	5
Premix*	5
Total	1000
*Chemical analysis (%, on DM basis)**	
Ash	8.78
Dry matter	85.77
Organic matter	91.56
Crude fiber	12.48
Digestible energy	2760 kcal/kg
Crude protein	17.76
Ether extract	2.34

*Each kg of premix (Minerals and Vitamins mixture) contains: Vit. A, 20,000 IU; D3, 15,000 IU; Vit. E, 8.33 g; Vit. B1, 0.33 g; Vit. B6, 0.33 g; Vit. B12, 1.7; Vit. K, 0.33 g; Vit. B2, 1.0 g; mg; Vit. B5, 8.33 g; Vit. Folic acid, 0.83 g; biotin, 33 mg; Choline chloride, 200 g; Cu 0.1 mg, Fe 75.0 mg, Mn 8.5 mg, ZnO 20 mg, Co 0.5mg, Mg 8.5 mg, 0.1 mg Sodium selenite, Pantothenic acid, 3.33 g; Idodine 0.2mg,.

The diet of all experimental groups was *isonitrogenous* and *isocaloric.*

*Values were calculated according to NRC (1977)

Evaluating growth indices

Throughout the study, feed intake (FI), live body weight (LBW) and final body weight (FBW) were recorded for each rabbit at the end of experiment (13 weeks of age). From these primary measurements, the average daily weight gain (ADWG), daily feed intake (DFI) and feed conversion ratio (FCR) were clarified. The FCR, specifically, computed the efficiency of feed utilization by expressing the amount of feed ingested per unit of body weight gained within each bi-weekly period.

Measurement of serum biochemistry

At the termination of the experiment, blood samples (n=6 per group) were gathered from the ear veins of rabbits without euthanasia according to the (Massányi *et al.,* 2020). Each sample was immediately divided into two aliquots and transferred to sterile tubes. The first aliquot was used for the analysis of

hematological parameters, conducted according to the methodology outlined by Siegel and Walton (2020) using an automated hematology analyzer (Hospitex Hema Screen 18, Italy). The second aliquot was allowed to clot at room temperature for one hour, followed by centrifugation at 800 ×g for 15 minutes (Sigma 2-16KL, Germany). Following centrifugation, the plasma was carefully separated and stored at -20°C until further biochemical analysis. Serum concentrations of gamma-glutamyl transferase (GGT), albumin (ALB), total protein (TP), alanine aminotransferase (ALT), uric acid, total bilirubin (TB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), were assessed using commercially available kits obtained from CLINICHEM Limited Company (Budapest, Hungary). Globulin (GLO) concentrations were estimated by subtracting albumin values from total protein values (GLO = TP - ALB). The quantification of plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) was performed using colorimetric assay kits obtained from Biosino Bio-technology and Science (Beijing, China), in accordance with the manufacturer's commands. Low-density lipoprotein (LDL) levels were then estimated based on the measured concentrations of TC and TG, utilizing the method previously established by (McNamara *et al.,* 1990).

Antioxidative biomarker assessments

The levels of total antioxidant capacity (TAC, kit #TAC-2513), superoxide dismutase (SOD, kit #SOD-2521), glutathione peroxidase (GPX, kit #GPX-2524), and malondialdehyde (MDA, kit #MDA-2529) were determined using commercially available ELISA kits. All ELISA kits used for assessing antioxidant status were obtained from BioDiagnostic (Giza, Egypt). The analytical procedures followed were based on the methodologies described by (Young, 2001) for TAC, (Nishikimi *et al.,* 1972) for SOD, (Huang *et al.,* 2016) for GPx, and (Kei, 1978) for MDA. Plasma myeloperoxidase (MYO) levels were quantified using a commercially available Rabbit Myeloperoxidase ELISA Kit (catalog number MBS724170) supplied by BioSource (Hrynkiewicz *et al.,* 2020). The reported assay sensitivity was 1.0 ng/mL, with an intra-assay CV of less than 10%.

Inflammatory-immune signaling

Plasma levels of immunoglobulin G (IgG, antibody catalog number ab187400) and immunoglobulin M (IgM, antibody catalog number ab190539) in rabbits were quantified using a quantitative sandwich ELISA method, following the protocol illustrated by (Jeklova *et al.,* 2010). The sensitivity of the assay was 0.23 ng/mL for IgG and 1.685 ng/mL for IgM. The detection ranges were 0.31–20 ng/mL for IgG and 6.25–200 ng/mL for IgM. The intra- and inter-assay CVs were <10% for both IgG and IgM. Serum nitric oxide (NO, assay kit catalog number ab65328) concentration was evaluated using a colorimetric nitric oxide assay kit, with

absorbance determined at 540 nm (Csonka *et al.*, 2015). Plasma levels of interleukin 4 (IL-4; catalog number E-EL-RB0014), and interleukin 10 (IL-10; catalog number E-CL-R0016) were quantified using ELISA kits provided by Elabscience company (USA). The sensitivity of the assay was 0. 9.38 pg/mL, detection ranges were 15.63-1000 pg/mL and the intra- and inter-assay CVs were <10%and < 15% for IL-4 and 4.69 pg/mL for IL-10, respectively.

Statistical analysis

Data were statistically analyzed using SPSS program (2022) Version 25.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was employed to evaluate differences between treatment groups. Significant differences identified by ANOVA were further examined using Duncan's multiple range post-hoc test. Statistical significance was defined as a probability (p) value of less than 0.05 (P<0.05).

RESULTS AND DISCUSSIONS

THI values

The temperature-humidity index (THI), as presented in Fig. 1C, demonstrated values of 29.38 at the beginning of the study and 30.96 at the end, consistent with exposing the growing rabbits to severe heat stress. Initial measurements showed an ambient temperature of 31.5°C and a relative humidity of 60%. Over the course of the study, while the ambient temperature remained stable at 31.5°C (Fig. 1A), the relative humidity increased to 71% by the final week (Fig. 1B).

Figure 1 (A-C). The temperature-humidity index (THI, Fig. 1A), ambient temperature (Fig.1B), and humidity (Fig. 1C) were measured.

Effects on growth attributes

Stressed rabbits fed diets added with GSE displayed drastically superior FBW and WG compared to rabbits fed basal diets ($P<0.001$, Table 2). All GSE-supplemented groups showed improved DWG compared to the control group ($P<0.01$). Daily Feed Intake (DFI) did not differ significantly among all groups ($P=0.024$). FCR was notably inferior in all GSE-added clusters relative to the GSE0 (HS group, $P<0.05$). Utilizing applicable, safe, eco-friendly, and competent natural byproducts like GSE offers a sustainable alternative to synthetic agents for reducing environmental pollution. Our findings indicate that GSE supplementation can function as a novel and sustainable anti-heat stress mediator for fattening rabbits.

Notably, dietary GSE at 200 or 400 mg/kg drastically boosted growth and feed proficiency by bolstering immune ability (IgG and IgM) and antioxidant capacity in stressed fattening rabbits. Furthermore, GSE administration attenuated oxidative stress indications MDA and MYO and inflammation stimulated by elevated environmental high temperature. In heat-stressed poultry (Ma *et al.,* 2023), dietary interview with GSE has been explained to notably enhance growing indicators in rabbits (Imbabi *et al.,* 2023) and broiler (El-Damrawy, 2014). Recently, providing calves with 4 g/day of GSE notably enhanced their body weight, nutrient digestion, and feed consumption (Urkmez and Biricik, 2022). The use of GSE as a growth supporter in poultry has been documented (El-Damrawy, 2014; Abdel-Wahab *et al.,* 2018; Abu Hafsa and Ibrahim, 2018; Hajati *et al.,* 2018; Uyanga *et al.,* 2021), attributed to their ability to reduce the OS and other biological function such as anti-bacterial, anti-inflammatory, and antioxidant properties. GSE added to rabbit feed at 200 or 300 mg/kg was shown by (Hassan *et al.,* 2016) to significantly help them grow better even when they were experiencing heat stress. Additionally, GSE exhibits antimicrobial activity by dipping pathogenic bacteria and promoting favorable bacteria (Kitsiou *et al.*, 2023), within the rabbit intestinal tract. The richness of GSE in phenolic compounds suggests its potential as an efficient anti-heat stress, antioxidant and immunomodulatory (Choi e*t al.,* 2023).

The antioxidant action of GSE is primarily ascribed to the ability of its polyphenolic constituents to scavenge free radicals through various mechanisms.
In a recent study, (Gong *et al.,* 2025) proanthocyanidin isolated from GSE was found to improve the growth performance of rabbits exposed to oxidative stress. Studies in broilers have shown that 7.5 and 15 mg/kg of proanthocyanidin of can improve feed efficiency without affecting carcass yield (Yang *et al.,* 2017), while higher doses increased FCR in goldfish (Jahanbakhshi *et al.,* 2023). The growth-promoting effects of GSPE are often attributed to its antibacterial and antioxidant properties.

Table 2. Influences of different levels of GSE on the growth attributes and feed efficiency in growing rabbits exposed to thermal environmental stress.

Items	Experimental groups				SEM	P value
	HS	GSE100	GSE200	GSE400		
IBW, g	652.50	652.50	655.62	659.37	2.30	0.700
FBW, g	2001.88^{c}	2053.13^{b}	2105.00^{a}	2137.50^{a}	11.65	0.001
TWG, g	1349.38^{c}	1400.63^{b}	1449.38^{a}	1478.13^{a}	13.33	0.001
DWG, g	24.10^{b}	25.01^{a}	25.88^{a}	26.40^{a}	0.24	0.001
DFI, g	75.76	71.41	71.76	77.31	0.69	0.247
FCR	3.15^{b}	2.85^{a}	2.77^{a}	2.93^{a}	0.05	0.037

Results are stated as mean ± pooled SE, with statistical significance defined as $P < 0.05$. Rabbits were fed diets containing varying levels of grape seed extract (GSE): 0 (GSE0), 100 (GSE100), 200 (GSE200), and 400 (GSE400) mg/kg for 8 weeks under natural thermal environmental stress. Initial body weight (IBW), Final body weight (FBW), Total weight gain (TWG), Daily weight gain (DWG), Daily feed intake (DFI), and Feed conversion ratio (FCR).

Effects on blood biochemistry

Rabbits in the GSE groups exhibited significantly ($P<0.001$) higher levels of TP, ALB, and GLO compared to the control group (Table 3). Feeding stressed rabbits diets fortified with GSE resulted in significantly lower levels of AST, ALT, creatinine, and uric acid ($P<0.001$). LDL was declined by GSE supplementation ($P<0.01$). Dietary GSE supplementation significantly decreased lipid profile parameters such as TC and TG, while significantly increasing HDL compared to the control diet ($P<0.01$). Serum levels of LDH and GGT were reduced by dietary GSE supplementation, particularly at levels of 200 or 400 mg compared to other groups. However, the GSE 100 mg group also exhibited lower LDH levels than the control diet ($P<0.0001$). Serum biochemistry serves as a crucial indicator of animal health, reflecting systemic physiological metabolism and alterations in the functional status of various organs. Consistent with findings by (Hassan *et al.,* 2016), the existing research established that dietary supplementation of stressed rabbits with 0.2 or 0.3 g of GSE resulted in a substantial boost in TP, ALB, and glucose levels relative to the basal diet.

Thermal stress is known to induce changes in hematological variables, with enzymes such as GGT and ALT being released into circulation as a consequence of tissue destruction. In this inquiry, exposure to high environmental temperatures led to a substantial elevation in plasma, uric, creatinine, ALT and AST levels in growing rabbits, indicative of oxidative injury in renal and hepatic tissues (Hassan *et al.,* 2016). Elevated GGT and LDH levels in the blood are often indicative of

liver damage or bile duct obstruction in rabbits. LDH is a crucial enzyme involved in cellular respiration, the process by which the body converts glucose into energy for cells. GGT is an enzyme with several important functions in the body, primarily related to glutathione metabolism and detoxification.

In heat-stressed quail (Abu Hafsa and Ibrahim, 2018), supplementation with GSE was observed to decrease plasma glucose, total cholesterol, triglyceride, AST, and ALT levels. Previous investigations have demonstrated the hepatoprotective and nephroprotective properties of GSE. In stressed broilers (El-Damrawy, 2014), dietary GSE at 200 or 300 mg/kg significantly decreased serum lipid parameters, including TG, LDL, and HDL, compared to the un-treated. Furthermore, dietary GSE supplementation (4 g/day/animal) (Ma *et al.,* 2023) significantly improved TG levels in calves.

Likewise, GSE treatment (Hassan *et al.*, 2016) has been shown to significantly minimal plasma levels of total lipids, TG, TC, and LDL in rabbits subjected to HS. Additionally, high ambient temperatures have been shown to raise plasma levels of TC, TG, and glucose in quail (Erişir *et al.,* 2018). The observed increase in TC levels has been associated with elevated plasma corticosterone planes generated by HS. in the present study, plasma lipid profile levels were substantially elevated under HS environments. Continuing pressure in rabbits has been linked to stress-persuaded hypoglycemia, which is attributed to the weakening of hepatic glycogen reserves due to intensified glucose requirement and glycolysis pathways (Ghosh *et al.,* 2020). Prolonged stress triggers the release of hormones like cortisol and catecholamines. These hormones have complex effects on lipid homeostasis (El-Damrawy, 2014; Ghosh *et al.,* 2020).

Effects on oxidative and antioxidative markers

Total antioxidant capacity in rabbits fed intakes with 200 or 400 mg of GSE was notably elevated than in the other groups ($P<0.01$, Table 4). All GSE-supplemented diets significantly improved SOD and GPx activities compared to control diets ($P<0.01$). Oxidative stress symbols such as MDA and MYO were significantly reduced by dietary GSE in a dose-dependent way ($P<0.01$), with the most pronounced reduction observed in rabbits fed diets containing 400 mg of GSE.

Research has indicated that HS can induce homeostatic dysfunction by promoting lipid and protein oxidation and suppressing antioxidant enzyme activities, such as SOD, GPx, and CAT, in cellular systems (Munteanu *et al.,* 2023). Dietary enrichment with natural antioxidants can effectively counteract free radicals, neutralize oxidants, and enhance antioxidant capability. The findings of our investigation indicate that the inclusion of GSE in the diet of growing rabbits resulted in a significant increase in the levels of GPx, CAT, SOD, and TAC.

Table 3. Influences of different levels of GSE on the serum blood biochemistry in fattening rabbits exposed to thermal environmental stress

Items	Experimental groups				SEM	P value
	HS	GSE100	GSE200	GSE400		
Total protein, g/dL	4.65[b]	5.72[a]	6.99[a]	7.42[a]	0.30	0.021
Albumin, g/dL	2.57[b]	3.39[a]	4.23[a]	4.16[a]	0.19	0.017
Globulin, g/dL	2.03[c]	2.43b	3.05[a]	3.26[a]	0.14	0.003
AST, U/L	31.01[a]	22.29[b]	22.04[b]	22.42[b]	1.04	0.004
ALT, U/L	83.28[a]	54.39[b]	52.99[b]	52.14[b]	3.42	0.002
Creatinine, mg/dL	70.88[a]	56.51[b]	45.28[b]	43.97[b]	2.83	0.001
Uric acid, mg/dL	2.86[a]	2.54[b]	2.52[b]	2.51[b]	0.06	0.004
Cholesterol, mg/dL	104.84[a]	76.40[b]	76.58[b]	75.57[b]	3.25	0.006
Total glycerides, mg/dL	113.57[a]	80.34[b]	81.83[b]	81.47[b]	3.66	0.003
HDL, mg/dL	26.78[b]	35.86[a]	40.69[a]	40.45[a]	1.52	0.001
LDL, mg/dL	40.97[a]	34.76[b]	32.17[c]	33.40[bc]	0.97	0.001
LDH,U/L	174.99[a]	142.43[b]	114.08[c]	114.39[c]	6.59	0.001
GGT, U/L	45.75[a]	30.67[b]	23.50[c]	25.25[c]	2.31	0.001

Rabbits were fed diets containing varying levels of grape seed extract (GSE): 0 (GSE0), 100 (GSE100), 200 (GSE200), and 400 (GSE400) mg/kg for 8 weeks under natural thermal environmental stress. Results are presented as mean ± pooled SE, with statistical significance defined as $P< 0.05$.

Consistent with our findings, (Hassan *et al.,* 2016) demonstrated that stressed rabbits fed diets containing GSE (0.2-0.3g/kg diet) exhibited greater levels of GPx, SOD, and CAT, along with worse MDA concentrations. Grape seed byproducts were effectively utilized as a growth promoter while simultaneously enhancing meat preservation due to their potent antioxidant and antibacterial properties (Carta *et al.,* 2025). Furthermore, dietary supplement with GSE drastically lowered MYO and MDA levels. These results are reliable with earlier papers, demonstrated that heat-stressed calves administered varying doses of GSE (25-100 mg/kg body weight) exhibited higher TAC and SOD levels, along with lower MDA levels, likened to the un-supplemented animals (Urkmez and Biricik, 2022). Conversely, the serum levels of CAT, GPx, and IL-1α in calves fed with GSE were not significantly altered. Supplementation of broiler diets with GSE (200 mg/kg) has been shown to significantly enhance liver SOD and GSH levels while declining MYO and MDA levels in chickens exposed to HS (El-Damrawy, 2014). Furthermore, GSE supplementation (100 mg) significantly improved antioxidative system and significantly reduced MDA in renal and hepatic tissues of quails exposed to HS (Erişir *et al.,* 2018). Similarly, previous works(Urkmez and Biricik, 2022; Ma *et al.,* 2023) indicated that serum CAT, and SOD were significantly higher in the GSE-supplemented group compared to basal diet group of calves.

Table 4. Influences of different levels of GSE on the antioxidant and oxidative stress in fattening rabbits exposed to thermal environmental stress

Items	Experimental groups				SEM	P value
	HS	GSE100	GSE200	GSE400		
Antioxidant markers						
TAC, ng/mL	0.178[b]	0.187[b]	0.246[a]	0.244[a]	0.01	0.006
SOD, U/mL	0.174[b]	0.282[a]	0.287[a]	0.286[a]	0.03	0.0034
GPx, U/mL	0.202[b]	0.292[a]	0.307[a]	0.319[a]	0.01	0.0027
Oxidative markers						
MDA, nmol/mL	0.352[a]	0.179[b]	0.138[c]	0.119[d]	0.025	0.02
MYO, ng/mL	3.658[a]	2.738[b]	2.420[c]	2.230[d]	0.163	0.018

Results are presented as mean ± pooled SE, with statistical significance defined as $P < 0.05$. Rabbits were fed diets containing varying levels of grape seed extract (GSE): 0 (GSE0), 100 (GSE100), 200 (GSE200), and 400 (GSE400) mg/kg for 8 weeks under natural thermal environmental stress. Total antioxidant capacity (TAC), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx), Malondialdehyde (MDA) and Myeloperoxidase (MYO).

Effects on immunity and inflammatory responses

Heat stress (HS) significantly reduced serum levels of IgG and IgM in rabbits, and dietary GSE supplementation effectively mitigated this reduction ($P<0.01$, Table 5). Furthermore, IgM levels were notably greater in the GSE400 group compared to the other groups ($P<0.01$). Additionally, HS exposure promoted pro-inflammatory responses, such as increased IL-4 levels, while suppressing anti-inflammatory responses, including reduced NO and IL-10 levels. Dietary GSE inclusion significantly reduced IL-4 levels in a dose-dependent way ($P<0.01$). Compared to the GSE0, GSE supplementation increased IL-10 levels and NO production in rabbits. Reactive oxygen species are crucial in cell death pathways, but their overproduction damages cellular components, leading to inflammation and contributing to heat stress development. In calves, GSE (25 mg/kg BW) lowered plasma IFN-γ and superior serum IgM and IgG, while significantly reducing MDA. These authors also found that GSE (4 g/day) decreased TNF-α and IL-6 in calves (Urkmez and Biricik, 2022; Ma *et al.,* 2023).

Rabbits are prone to health issues like immune dysfunction and HS. Diminished immunity increases their susceptibility to infections and parasites, impairing their defense mechanisms (Bashar *et al.,* 2023; Ismail *et al.,* 2023). To ensure the health and welfare of domestic rabbits, it is essential to manage

Table 5. Influences of different levels of GSE on the inflammatory-immune signaling in fattening rabbits exposed to thermal environmental stress

Items	Experimental groups				SEM	P value
	HS	GSE100	GSE200	GSE400		
Immune ability						
IgG, ng/mL	28.51[b]	54.83[a]	53.59[a]	54.06[a]	2.90	0.0022
IgM, ng/mL	41.96[c]	77.95[b]	78.75[b]	80.44[a]	4.18	0.0001
Inflammatory cytokines						
IL-4, pg/mL	0.335[a]	0.184[b]	0.158[c]	0.144[d]	0.02	0.0044
IL10, pg/mL	0.188[b]	0.309[a]	0.297[a]	0.298[a]	0.01	0.0036
NO, ng/mL	0.260[c]	0.511[a]	0.538[a]	0.487[b]	0.03	<0.001

Results are presented as mean ± pooled SE, with statistical significance defined as $P < 0.05$. Rabbits were fed diets containing varying levels of grapeseed extract (GSE): 0 (GSE0), 100 (GSE100), 200 (GSE200), and 400 (GSE400) mg/kg for 8 weeks under natural thermal environmental stress.

environmental immune challenges and provide a comfortable, thermoregulated environment (El-Sabrout *et al.,* 2024). GSE, with its diverse bioactive components including catechin, procyanidins, epicatechin gallate, and gallic acid, could be beneficial in this regard. Recently, flavonoids have become notable for their wide range of therapeutic effects, like anti-inflammatory, antibiotic, antidiarrheal, and powerful antioxidant actions. MPO is an enzyme primarily found in neutrophils, a type of white blood cell crucial for the immune response.

Grape seed extract is recognized as a potent natural antioxidant and metal chelator, effectively scavenging free radicals generated by oxidative stress due to its high content of polyphenols and flavonoids. Earlier reports, for instance (Bijak *et al.,* 2011; Charradi *et al.,* 2018), have established that grape seed polyphenols augment the activity of antioxidant enzymes and attenuate the effects of oxidative stress in lambs. Furthermore, in addition to its antioxidant properties, GSE has been documented to diminish neutrophil infiltration and modulate the secretion of inflammatory mediators (Hajishengallis and Chavakis, 2022).

Heat stress can compromise the integrity of tight junction proteins within the gastrointestinal epithelium of mammals, resulting in increased gastrointestinal permeability and the translocation of LPS into the systemic circulation (Ghosh *et al.,* 2020). The release of pro-inflammatory cytokines, such as IFN-γ, IL-1β, and IL-4, which are critical regulators of inflammation, can be initiated by endotoxemia, leading to both localized and systemic inflammation (Taies and Al-Samarai, 2024). reported that GSE's anti-inflammatory action involves reducing

intestinal permeability and the expression of pro-inflammatory cytokine genes (El-Damrawy, 2014; Ghosh *et al.,* 2020; Massányi *et al.,* 2020). The present investigation showed that dietary GSE supplementation in growing rabbits decreased serum concentrations of IL-4 and caused a small rise in serum IL-10 levels. These observations suggest that GSE played a role in reducing inflammatory responses in heat-stressed rabbits. Prior investigations in stressed rabbits indicated that lower IL-4 levels reduced HS-induced inflammation (Ebeid *et al.,* 2023).

The ability of grape seed products to lower pro-inflammatory cytokines, possibly by inhibiting NF-κB signaling, may account for the positive effects of GSE on inflammatory factors (Manna *et al.,* 2023; Al-Janabi and Al-Samarai 2023). Further investigation is needed to understand GSE's effects on calf immunity mechanisms. IL-10's mechanism of suppressing pro-inflammatory cytokines and their receptor binding helps to decrease inflammatory damage (Chu *et al.,* 2024), possibly via the JAK/STAT3 pathway. In our study, the significant increase in IL-10 observed with GSE supplementation, likely due to its high procyanidin content, contributed to reduced inflammation. IL-4, primarily from immune cells, can indicate inflammation or adverse environmental exposure. Targeting inflammation with anti-inflammatory molecules is a potential strategy to reduce HS effects. GSE contains significant anti-inflammatory compounds like procyanidins, and proanthocyanidin (Chu *et al.,* 2024; Kumar *et al.,* 2024; Ibrahim *et al.,* 2024).

CONCLUSIONS

The present study suggests that dietary inclusion of grape seed extract (GSE) offers a novel, safer, and ecologically sound approach to counteract heat stress in growing rabbits. GSE supplementation (200 or 400 mg/kg diet) significantly improved growth and feed efficiency, potentially by modulating immune responses (upregulating IgM and IgG) and bolstering antioxidant defenses (increasing CAT, GPx, SOD, and TAC) in stressed subjects. Additionally, GSE effectively lowered oxidative stress indicators (MDA and MYO) and attenuated temperature-induced inflammation. These findings indicate that GSE is a promising feed supplement for boosting growth, blood biochemistry, and overall productivity in environmental stress rabbits. Future investigations should delve deeper into the underlying mechanisms by which GSE mitigates the adverse effects of heat stress in animal systems.

Authors' contributions

Conceptualization, methodology, S.A.A., and K.M.A.; software, E.M.A.; formal analysis, S.A.A.; investigation, data curation, S.A.A.; writing—original draft

preparation, writing—review and editing. All authors read and approved of the final manuscript.

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