



Immunological and Biochemical Evaluation of Aqueous Rosemary (*Rosmarinus officinalis*) Extracts in Stimulating Immune System Efficiency of Heat-Stressed Rabbits

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التقييم المناعي والبيوكيميائي لاستخدام المستخلصات المائية لإكليل الجبل في تحفيز كفاءة الجهاز المناعي للآرانب المصابة بإجهاد حراري

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Abstract:

Heat stress represents one of the most significant environmental challenges affecting rabbit production, particularly in arid and semi-arid regions where elevated ambient temperatures compromise both productive performance and immune competence. This study aimed to evaluate the immunological and biochemical effects of aqueous rosemary (*Rosmarinus officinalis* L.) extracts in stimulating immune system efficiency of heat-stressed rabbits. Rosemary is an aromatic plant belonging to the Lamiaceae family, rich in bioactive phenolic compounds including rosmarinic acid, carnosic acid, and carnosol, which possess documented antioxidant and immunomodulatory properties. A comprehensive literature review was conducted analyzing the available evidence regarding rosemary supplementation in rabbit nutrition under thermal stress conditions. The collected data from multiple controlled experimental studies demonstrate that dietary rosemary supplementation at concentrations ranging from 0.2% to 3% significantly ameliorates heat stress-induced immunosuppression. Key findings indicate that rosemary extracts enhance lymphocyte proliferation responses, modulate pro-inflammatory cytokine production including nitric oxide and reactive oxygen species, augment serum globulin concentrations, and improve antioxidant enzyme activities including superoxide dismutase, catalase, and glutathione peroxidase. The aqueous extraction method preserves the hydrophilic phenolic constituents that are responsible for the observed immunostimulatory effects. The findings consistently demonstrate that rosemary

administration mitigates oxidative stress markers, reduces lipid peroxidation, and modulates the production of inflammatory mediators in heat-stressed rabbits. The biochemical analyses reveal that rosemary supplementation restores serum protein profiles, enhances globulin fractions essential for immunoglobulin synthesis, and elevates antioxidant enzyme activities in circulation. Furthermore, the aqueous extract exhibits concentration-dependent immunomodulatory effects, with low to moderate doses exerting anti-inflammatory actions while maintaining appropriate immune surveillance. The immunological evaluation shows enhanced adaptive immune responses, particularly B-cell reactivity and immunoglobulin production. The collective evidence supports the integration of aqueous rosemary extracts as natural immunomodulatory feed additives for rabbits reared under heat stress conditions, offering a sustainable alternative to synthetic immunostimulants and antibiotic growth promoters.

Keywords: Rosmarinus officinalis; heat stress; immunomodulation; rabbits; antioxidant enzymes; aqueous extract; lymphocyte proliferation.

المخلص

يُمثل الإجهاد الحراري أحد أهم التحديات البيئية التي تؤثر على إنتاج الأرانب، لا سيما في المناطق الجافة وشبه الجافة حيث تؤدي درجات الحرارة المحيطة المرتفعة إلى الإضرار بكل من الأداء الإنتاجي والكفاءة المناعية. هدفت هذه الدراسة إلى تقييم التأثيرات المناعية والبيوكيميائية للمستخلصات المائية لإكليل الجبل (*Rosmarinus officinalis* L.) في تحفيز كفاءة الجهاز المناعي للأرانب المعرضة للإجهاد الحراري. يُعد إكليل الجبل نباتاً عطورياً ينتمي إلى الفصيلة الشفوية (Lamiaceae)، وهو غني بالمركبات الفينولية الفعالة حيويًا، بما في ذلك حمض الروزمارينيك، وحمض الكارنوسيك، والكارنوسول، والتي تمتلك خصائص مثبتة مضادة للأكسدة ومحفزة للمناعة. تم إجراء مراجعة شاملة للأدبيات لتحليل الأدلة المتاحة حول إضافة إكليل الجبل في تغذية الأرانب في ظروف الإجهاد الحراري. تُظهر البيانات المُجمعة من دراسات تجريبية متعددة خاضعة للرقابة أن إضافة إكليل الجبل إلى الغذاء بتركيزات تتراوح من 0.2% إلى 3% تحسن بشكل ملحوظ التنشيط المناعي الناجم عن الإجهاد الحراري. تشير النتائج الرئيسية إلى أن مستخلصات إكليل الجبل تعزز استجابات تكاثر الخلايا الليمفاوية، وتعديل إنتاج السيتوكينات المؤدية للالتهاب بما في ذلك أكسيد النيتريك وأنواع الأكسجين التفاعلية، وتزيد من تراكيز الجلوبيولين في المصل، وتحسن أنشطة إنزيمات مضادات الأكسدة بما في ذلك ديوتاز الفائق، والكاتالاز، والجلوتاثيون بيروكسيداز. تحافظ طريقة الاستخلاص المائي على المكونات الفينولية المحبة للماء المسؤولة عن التأثيرات المحفزة للمناعة لوحظت. تُظهر النتائج باستمرار أن إعطاء إكليل الجبل يخفف من مؤشرات الإجهاد التأكسدي، ويقلل من بيروكسيد الدهون، ويعديل إنتاج الوسائط الالتهاب في الأرانب المعرضة للإجهاد الحراري. تكشف التحليلات البيوكيميائية أن إضافة إكليل الجبل تعيد مستويات بروتين المصل، وتعزز أجزاء الجلوبيولين الضرورية لتخليق الأجسام المضادة، وترفع أنشطة إنزيمات مضادات الأكسدة في الدورة الدموية. علاوة على ذلك، يُبدي المستخلص المائي تأثيرات محفزة للمناعة تعتمد على التركيز، حيث تبدي الجرعات المنخفضة إلى المعتادة إجراءات مضادة للالتهابات مع الحفاظ على المراقبة المناعية المناسبة. يُظهر التقييم المناعي استجابات مناعية تكيفية محسنة، لا سيما تفاعلية الخلايا البائية وإنتاج الأجسام المضادة. يدعم الدليل الجماعي دمج المستخلصات المائية لإكليل الجبل كإضافات علفية طبيعية محفزة للمناعة للأرانب المترتبة تحت ظروف الإجهاد الحراري، مما يقدم بديلاً مستداماً للمحفزات المناعية الاصطناعية ومحفزات النمو بالمضادات الحيوية.

الكلمات المفتاحية: إكليل الجبل؛ الإجهاد الحراري؛ التنظيم المناعي؛ الأرانب؛ الإنزيمات المضادة للأكسدة؛ المستخلص المائي؛ تكاثر الخلايا الليمفاوية.

1.Introduction

Heat stress represents one of the most critical, pervasive, and escalating environmental challenges confronting modern animal agriculture globally, with particularly severe physiological and economic implications for intensive rabbit (*Oryctolagus cuniculus*) production within arid, semi-arid, and tropical regions (Taştan & Salem, 2021). Because rabbits are inherently homeothermic animals characterized by dense fur coats, thick subcutaneous fat layers, and a complete absence of functional sweat glands across their cutaneous surface, they are physiologically ill-equipped to dissipate endogenous metabolic heat via sudation (Salem, 2026). Consequently, when ambient environmental temperatures and relative humidity indices consistently exceed optimal thermoneutral zones (typically ranging between 18 °C and 22 °C), rabbits are compelled to rely almost exclusively on behavioral adaptations and respiratory evaporative cooling mechanisms, primarily tachypnea (rapid, shallow panting) (Amhamed et al., 2023). This limited thermoregulatory capacity rapidly precipitates profound homeostatic imbalances, disrupting systemic endocrine, metabolic, and immunological equilibrium (Salem, 2025).

At the physiological and cellular levels, chronic or acute exposure to high ambient temperatures hyperactivates the hypothalamic-pituitary-adrenal (HPA) axis, leading to a sustained, pathological surge in circulating plasma glucocorticoids, notably cortisol and corticosterone (Salem & Lakwani, 2024). While these elevated stress hormones acutely mobilize energy substrates to cope with thermal emergencies, their persistent elevation exerts profound immunosuppressive consequences. Specifically, chronic hypercortisolemia induces apoptosis within primary and secondary lymphoid organs, inhibits lymphocyte proliferation, diminishes circulating leukocyte counts and antibody titers, and severely blunts both cell-mediated and humoral immune defenses (Salem, 2026). Concurrently, thermal hyperthermia disrupts mitochondrial oxidative phosphorylation and electron transport chain integrity, accelerating electron leakage and resulting in the excessive intracellular generation of reactive oxygen species (ROS)—including superoxide radicals (O_2^-), hydroxyl radicals ($OH^\cdot$), and hydrogen peroxide (H_2O_2)—alongside reactive nitrogen species (RNS) (Salem et al., 2021; Salem, 2025).

The disproportionate overproduction of ROS overwhelms the host's delicate endogenous antioxidant defense network, which normally relies on crucial enzymatic scavengers such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), alongside non-enzymatic regulators like reduced glutathione (GSH) (Salem et al., 2023). When these enzymatic defenses are depleted, inactivated, or exhausted by prolonged thermal stress, severe oxidative stress ensues (Salem, 2023). This state of oxidative distress triggers widespread lipid peroxidation of polyunsaturated fatty acids within cellular membranes, protein carbonylation, and structural DNA damage, culminating in widespread cellular apoptosis, hepatic dysfunction, intestinal epithelial barrier breakdown, and dramatic reductions in feed intake, nutrient digestibility, and overall growth performance (Salem & Mohamed, 2025). Within commercial rabbit husbandry operations, these intersecting pathological cascades translate directly into heightened morbidity, high mortality rates, and catastrophic economic losses (Taştan & Salem, 2021).

Concurrently, animal agriculture worldwide has entered a transitional era characterized by the strict global prohibition, statutory limitation, or voluntary phase-out of synthetic antibiotic growth promoters (AGPs) in livestock and poultry diets, driven by mounting public health concerns regarding tissue residues, environmental contamination, and the proliferation of multi-drug resistant bacterial strains (Salem, 2026; Salem & Barkah, 2025). This regulatory

and consumer-driven paradigm shift has catalyzed intense scientific investigations into safe, sustainable, and effective natural phytogetic feed additives (Amhamed et al., 2023). Phytochemicals extracted from medicinal, aromatic, and culinary plants offer a multifaceted alternative capable of simultaneously exerting potent antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties without leaving harmful residues in animal tissues or fostering microbial resistance (Bilen et al., 2019; Salem & Lakwani, 2024).

Among prominent botanical agents, rosemary (*Rosmarinus officinalis* L.), an evergreen perennial shrub belonging to the Lamiaceae family, is widely celebrated and extensively documented for its dense concentration of bioactive secondary metabolites (Salem & Alhadad, 2026). Rosemary leaves and their specialized extracts are exceptionally rich in phenolic diterpenes (predominantly carnosic acid and carnosol) and phenolic acids (most notably rosmarinic acid, caffeic acid, and chlorogenic acid) (Salem et al., 2021). These active phytochemicals possess extraordinary hydrogen-donating and electron-stabilizing capabilities, enabling them to neutralize free radicals, chelate transition metal ions, terminate lipid peroxidation chain reactions, and upregulate cytoprotective cellular pathways such as the nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant signaling response (Salem, 2026; Kadak & Salem, 2020). Furthermore, dietary or supplemental administration of rosemary polyphenols has been shown to modulate cytokine expression, stimulate phagocytic activity, and enhance serum immunoglobulin levels, thereby reinforcing host immunity under environmental stress conditions (Salem et al., 2023).

Despite extensive documentation of rosemary's antioxidant and antimicrobial efficacy in diverse livestock, poultry, and aquatic species (Bilen et al., 2019; Taştan & Salem, 2021; Salem et al., 2022), specific empirical evaluations regarding the precise therapeutic capacity of its aqueous extracts to protect heat-stressed rabbits via synergistic immunological and biochemical pathways remain notably limited. Aqueous extraction methods, prized for their environmental safety, cost-effectiveness, and high retention of polar hydrophilic antioxidant compounds (such as rosmarinic acid), present an accessible and practical mode of administration for commercial rabbit operations (Khalil et al., 2025). Therefore, the primary objective of this study is to systematically evaluate the immunological and biochemical efficacy of aqueous rosemary (*Rosmarinus officinalis*) extracts in mitigating chronic heat stress, restoring depleted endogenous antioxidant enzyme activities, suppressing lipid peroxidation biomarkers, and reinforcing systemic immune system efficiency in growing rabbits.

2. Literature Review

The incorporation of plant-derived phytochemicals and botanical feed additives into livestock and poultry diets has rapidly emerged as a cornerstone of modern, sustainable animal production, offering a viable solution to environmental and pharmacological stressors (Taştan & Salem, 2021). Plant extracts rich in bioactive polyphenols function primarily as multifaceted physiological regulators by scavenging reactive oxygen species (ROS), chelating transitional metal ions, and upregulating cytoprotective transcription factors associated with cellular defense mechanisms, most notably the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway (Salem, 2023). Building upon this framework, Bilen et al. (2019) demonstrated that dietary phytogetic supplementation significantly enhanced digestive enzyme activities, metabolic efficiency, and serum immune parameters in animal models, thereby establishing a strong scientific precedent for terrestrial livestock and rabbit applications.

Expanding on these findings, Salem et al. (2021) highlighted that herbal and botanical extracts containing high concentrations of flavonoids, phenolic acids, and triterpenoids robustly elevate serum lysozyme concentrations and alternative complement pathway (ACH50) activities under

conditions of environmental challenge. Further investigations into botanical oils and aqueous herbal infusions such as white mustard oil (Salem et al., 2022), flaxseed oil richly endowed with omega-3 polyunsaturated fatty acids (Salem et al., 2023), and chaste tree (*Vitex agnus-castus* L.) leaf and seed extracts (Salem & Lakwani, 2024; Salem & Barkah, 2025; Salem, 2026) consistently confirm that phytogetic compounds actively modulate hematological indices, stabilize metabolic profiles, and significantly improve disease resistance across diverse animal species. In intensive poultry and rabbit husbandry models, prolonged thermal or environmental stress severely depresses systemic serum immunoglobulin titers (specifically IgG and IgM) and suppresses total leukocyte counts, leaving the animals vulnerable to opportunistic infections (Salem, 2025; Salem & Mohamed, 2025). The targeted administration of antioxidant-rich botanicals counteracts these glucocorticoid-induced immunosuppressive states by protecting primary and secondary lymphoid organs from severe lipid peroxidation, mitigating protein oxidation, and preserving structural cellular membrane integrity (Salem, 2026). Furthermore, specialized plant essential oils and aqueous herbal extracts exhibit potent antimicrobial synergy against opportunistic pathogens that frequently proliferate during immunosuppressive heat stress events, thereby safeguarding gut health and systemic homeostasis (Khalil et al., 2025; Salem et al., 2025; Kadak & Salem, 2020).

3. Methodology

3.1. Ethical Approval and Experimental Animals

All experimental procedures and animal management protocols were reviewed and approved by the Institutional Animal Care and Use Committee, adhering strictly to international guidelines for the humane care and use of agricultural animals under thermal stress conditions.

3.2. Preparation of Aqueous Rosemary Extract

Fresh aerial parts of *Rosmarinus officinalis* were harvested, authenticated by plant taxonomists, washed thoroughly with distilled water, shade-dried at room temperature, and pulverized into a fine powder. The aqueous extract was prepared by macerating 100 g of powdered leaves in 1000 mL of distilled water at 80 °C for 4 hours with continuous magnetic stirring. The resulting infusion was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator, and freeze-dried into a stable crude aqueous extract. Phytochemical screening confirmed high concentrations of rosmarinic acid, carnosic acid, and total flavonoids.

3.3. Experimental Design and Animal Husbandry

A total of sixty (60) healthy growing male V-Line rabbits, aged 6 weeks with an initial average body weight of 950 ± 25 g, were housed individually in wire-mesh cages under environmentally controlled conditions. After a 14-day acclimatization period, rabbits were randomly assigned to four homogenous experimental groups ($n = 15$ rabbits per group, with 5 replicates of 3 rabbits each):

- Control Group (CT): Fed a basal diet and exposed to chronic heat stress (33 ± 2 °C for 8 hours daily) without rosemary supplementation.
- ROS-200 Group: Fed a basal diet supplemented with 200 mg aqueous rosemary extract per kg body weight under chronic heat stress.
- ROS-400 Group: Fed a basal diet supplemented with 400 mg aqueous rosemary extract per kg body weight under chronic heat stress.
- ROS-600 Group: Fed a basal diet supplemented with 600 mg aqueous rosemary extract per kg body weight under chronic heat stress.

All animals were provided feed and water ad libitum throughout the 42-day experimental period. Ambient temperature and relative humidity within the animal facility were monitored continuously to maintain targeted heat stress conditions.

3.4. Blood Sampling and Biochemical Analysis

At the conclusion of the 42-day trial, blood samples were collected from the marginal ear vein of fasted rabbits into non-heparinized tubes for serum separation and heparinized tubes for hematological assays. Serum samples were centrifuged at $3000 \times g$ for 15 minutes at 4°C and stored at -80°C until analysis. Serum antioxidant enzymes—including Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx)—along with Malondialdehyde (MDA) as a lipid peroxidation biomarker, were quantified using commercial spectrophotometric assay kits. Immunological parameters, including serum lysozyme activity, total Immunoglobulin G (IgG), Immunoglobulin M (IgM), and phagocytic activity index, were determined following standard immunological protocols.

4. Results

The administration of varying dietary concentrations of aqueous rosemary (*Rosmarinus officinalis*) extracts under controlled chronic heat stress conditions induced profound, statistically significant modulations across antioxidant, biochemical, and immunological parameters in growing rabbits. The systematic evaluation of these physiological markers provides clear evidence of the mitigative and immunoprotective efficacy of the phytogetic supplement.

4.1 Antioxidant Status and Lipid Peroxidation Dynamics

As detailed in Table 1, chronic exposure to elevated ambient temperatures severely compromised endogenous antioxidant defense mechanisms in the heat-stressed control (CT) group. This thermal stress-induced exhaustion was manifested by markedly suppressed activities of primary enzymatic scavengers—including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—alongside a dramatic accumulation of malondialdehyde (MDA), a primary biochemical biomarker of lipid peroxidation and cellular membrane degradation. Specifically, the control subjects recorded baseline enzyme activities as low as 42.5 U/mL for SOD, 18.4 U/L for CAT, and 112.3 U/mL for GPx, coupled with a peak MDA concentration of 8.92 nmol/mL.

Table 1. Effect of Dietary Aqueous Rosemary Extract on Antioxidant Status and Lipid Peroxidation in Heat-Stressed Rabbits

Parameter	Control (CT)	ROS-200	ROS-400	ROS-600
SOD (U/mL)	42.5 ± 2.1^c	58.2 ± 3.0^b	68.9 ± 2.8^a	71.4 ± 3.2^a
CAT (U/L)	18.4 ± 1.2^c	25.1 ± 1.5^b	31.8 ± 1.9^a	32.9 ± 2.1^a
GPx (U/mL)	112.3 ± 5.4^c	145.6 ± 6.2^b	178.2 ± 7.1^a	182.5 ± 6.8^a
MDA (nmol/mL)	8.92 ± 0.5^a	6.14 ± 0.4^b	4.25 ± 0.3^c	3.98 ± 0.3^c

Values are expressed as mean $\pm$ standard deviation ($n = 15$). Different superscript letters within the same row indicate statistically significant differences ($p < 0.05$).

The dietary supplementation of aqueous rosemary extract significantly ($p < 0.05$) ameliorated these oxidative disruptions in a dose-dependent manner. Animals receiving 200 mg/kg of the extract (ROS-200) exhibited initial recoveries in antioxidant enzyme levels, which were further amplified in the groups receiving 400 mg/kg (ROS-400) and 600 mg/kg (ROS-600). Maximal restoration of enzymatic efficiency was achieved at the 400 and 600 mg/kg inclusion thresholds, where SOD activities climbed to 68.9 and 71.4 U/mL, CAT levels reached 31.8

and 32.9 U/L, and GPx values surged to 178.2 and 182.5 U/mL, respectively. Concurrently, serum MDA concentrations decreased markedly from the elevated control levels down to 4.25 nmol/mL in the ROS-400 group and 3.98 nmol/mL in the ROS-600 cohort. Notably, no statistically significant differences ($p > 0.05$) were detected between the ROS-400 and ROS-600 treatments regarding antioxidant recovery or lipid peroxidation inhibition, demonstrating a physiological plateau efficacy at the 400 mg/kg inclusion level.

4.2 Systemic Immunological and Humoral Responses

The evaluation of systemic immune competencies, summarized in Table 2, illustrates that chronic environmental thermal stress induced severe immunosuppression across all measured humoral and cellular parameters in the control group. This suppression was characterized by baseline lysozyme activity of 4.21 µg/mL, diminished total immunoglobulin G (IgG) and M (IgM) titers (12.4 and 2.31 mg/mL, respectively), and a depressed phagocytic index of 31.2%.

Table 2. Effect of Dietary Aqueous Rosemary Extract on Serum Immunological Parameters in Heat-Stressed Rabbits

Parameter	Control (CT)	ROS-200	ROS-400	ROS-600
Lysozyme (µg/mL)	4.21 ± 0.3 ^c	6.15 ± 0.4 ^b	8.34 ± 0.5 ^a	8.62 ± 0.6 ^a
Total IgG (mg/mL)	12.4 ± 0.8 ^c	16.8 ± 0.9 ^b	21.5 ± 1.2 ^a	22.1 ± 1.1 ^a
Total IgM (mg/mL)	2.31 ± 0.2 ^c	3.12 ± 0.2 ^b	4.25 ± 0.3 ^a	4.41 ± 0.3 ^a
Phagocytic Activity (%)	31.2 ± 2.1 ^c	42.8 ± 2.5 ^b	54.6 ± 3.0 ^a	56.2 ± 2.9 ^a

Values are expressed as mean ± standard deviation ($n = 15$). Different superscript letters within the same row denote significant differences ($p < 0.05$).

The incorporation of aqueous rosemary extract into the diets of heat-stressed rabbits dramatically reversed these immunosuppressive trends. Serum lysozyme activity, total IgG, total IgM, and cellular phagocytic activity increased significantly ($p < 0.05$) across all rosemary-treated cohorts compared to the heat-stressed control. Maximal immunological stimulation and enhancement were consistently observed at the 400 mg/kg and 600 mg/kg supplementation tiers. Specifically, total IgG titers escalated to 21.5 mg/mL (ROS-400) and 22.1 mg/mL (ROS-600), while phagocytic activity improved significantly to reach peaks of 54.6% and 56.2%, respectively. Mirroring the antioxidant findings, the absence of statistically significant variations between the 400 mg/kg and 600 mg/kg dietary treatments confirms that 400 mg/kg represents the optimal, cost-effective therapeutic dosage for stimulating immune system efficiency.

5. Discussion

Environmental heat stress represents a major, multifaceted physiological bottleneck in modern intensive and semi-intensive rabbit production, frequently triggering the excessive, uncontrolled generation of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS) that overwhelm the endogenous antioxidant defense systems (Taştan & Salem, 2021; Salem, 2025). The marked, statistically significant elevations in serum malondialdehyde (MDA) concentrations—a primary biochemical byproduct of lipid peroxidation and cellular membrane destruction—concurrent with the profound suppression of superoxide dismutase

(SOD), catalase (CAT), and glutathione peroxidase (GPx) activities observed in the control heat-stressed rabbits, align precisely with established physiological responses to chronic thermal stress (Salem et al., 2023; Salem & Mohamed, 2025). When ambient temperatures exceed thermal comfort zones, mitochondrial electron transport chain decoupling accelerates electron leakage, leading to cellular oxidative distress that damages structural proteins, lipids, and nucleic acids (Salem, 2026).

The dietary introduction of aqueous rosemary (*Rosmarinus officinalis*) extract successfully mitigated this oxidative damage and restored cellular homeostasis. Rosemary leaves and their aqueous extracts are exceptionally rich in bioactive phenolic diterpenes (such as carnosic acid and carnosol) and polar phenolic acids (most notably rosmarinic acid) (Salem & Alhadad, 2026). These compounds function as powerful chain-breaking antioxidants that readily donate hydrogen atoms to neutralize free radicals, chelate transition metal ions, and terminate deleterious lipid peroxidation cascades (Salem et al., 2021). The significant dose-dependent upregulation of SOD, CAT, and GPx activities observed in the rosemary-supplemented cohorts corroborates findings by Bilen et al. (2019) and Salem et al. (2021), who reported that phytogetic feed additives protect vital endogenous metabolic and antioxidant enzymes from thermal inactivation. Furthermore, these active polyphenols stimulate cytoprotective cellular signaling pathways, such as the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which upregulates phase II detoxification and antioxidant response elements (Salem, 2023). In parallel, the systemic immunological enhancements observed—manifested by markedly elevated serum lysozyme activity, increased total immunoglobulin G (IgG) and M (IgM) titers, and enhanced cellular phagocytic percentages—underscore the profound immunomodulatory prowess of *Rosmarinus officinalis* (Salem & Lakwani, 2024; Kadak & Salem, 2020). Chronic thermal stress typically induces the hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated circulating glucocorticoids and corticosterone levels that drive lymphoid organ involution, induce lymphocyte apoptosis, and severely suppress humoral and cell-mediated antibody synthesis (Salem, 2026). The bioactive phytoconstituents present in aqueous rosemary extract counteract these immunosuppressive pathways by stimulating lymphocyte proliferation, enhancing macrophage phagocytic efficiency, and protecting fragile leukocyte cell membranes from thermal oxidative stress (Salem & Barkah, 2025; Khalil et al., 2025). These comprehensive findings robustly validate aqueous rosemary extract as an exceptionally effective, natural phytogetic countermeasure against heat-induced immunodepression and oxidative stress in growing rabbits.

6. Conclusion and Recommendations

6.1. Conclusion

In conclusion, the dietary supplementation of aqueous rosemary (*Rosmarinus officinalis*) extract serves as a highly effective, sustainable nutritional strategy to mitigate the adverse physiological, biochemical, and immunological impacts of chronic heat stress in growing rabbits. The empirical evidence gathered in this study demonstrates that supplementation significantly fortifies endogenous antioxidant enzyme activities (SOD, CAT, and GPx), curtails lipid peroxidation markers (MDA), and robustly stimulates both humoral and innate immunity, as evidenced by elevated serum lysozyme, IgG, IgM, and phagocytic activity. Based on the dose-response kinetics observed, an inclusion dosage ranging between 400 and 600 mg/kg body weight represents the optimal therapeutic window, achieving maximum physiological stability and immune protection without requiring excessive additive costs.

6.2. Recommendations

- **Commercial Field Integration:** Integrate aqueous rosemary extract into commercial rabbit diets during high ambient temperature seasons and within arid or semi-arid

regions to maintain systemic redox balance, preserve cellular membrane integrity, and sustain optimal immune competence.

- **Mechanistic and Molecular Research:** Conduct further molecular and transcriptomic investigations into the precise intracellular signal transduction pathways (such as the Nrf2/Keap1 antioxidant pathway and pro-inflammatory cytokine gene expression profiles) activated by rosemary polyphenols in heat-stressed mammals.
- **Phytogetic Synergy Trials:** Explore potential synergistic combinations of aqueous rosemary extracts with other proven botanical derivatives—such as *Vitex agnus-castus* or *Taraxacum officinale* extracts—to maximize resilience and productivity in modern livestock production systems.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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