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Enhancing Thermotolerance and Immune Competence in Shami Goats: A Novel Dietary Strategy Using Moringa oleifera and Turmeric to Mitigate Heat Stress-Induced Production Losses

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ABSTRACT

Heat stress represents a major challenge affecting goat production in arid and semi-arid regions, causing oxidative stress and immunosuppression. This study investigated the effects of *Moringa oleifera* leaf extract (MOLE) and turmeric (*Curcuma longa*) supplementation on immune parameters and antioxidant enzyme activities in Shami (Damascus) goats under heat stress conditions. Twenty-four adult Shami goats (2-3 years, 35±3 kg) were randomly allocated to four groups (n=6): Control (CON), MOLE-supplemented (MO, 3% of dry matter intake), turmeric-supplemented (TUR, 2% of DMI), and combined supplementation (MO+TUR). The experiment lasted 60 days during summer (Temperature-Humidity Index: 82-92). Blood samples were collected bi-weekly for analysis of antioxidant enzymes (SOD, CAT, GPx), pro-inflammatory cytokines (IL-6, TNF-α), lymphocyte subpopulations (CD4+, CD8+), immunoglobulins (IgG, IgM), and heat shock proteins (HSP70, HSP90). Combined supplementation (MO+TUR) significantly increased SOD (93.40±2.1 U/mL), CAT (55.03±1.8 U/mL), and GPx (15.03±0.5 U/mL) activities compared to control (P<0.001). IL-6 levels decreased from 45.3±3.2 pg/mL (CON) to 22.1±1.9 pg/mL (MO+TUR), while TNF-α reduced from 38.7±2.8 to 18.4±1.7 pg/mL (P<0.001). CD4+/CD8+ ratio improved from 1.2±0.1 (CON) to 1.8±0.1 (MO+TUR). HSP70 expression increased 2.3-fold in supplemented groups. IgG concentrations elevated from 12.3±0.8 to 16.8±0.9 mg/mL (P<0.05). Combined *M. oleifera* and turmeric supplementation effectively mitigated the adverse effects of heat stress by enhancing antioxidant defense systems, modulating inflammatory responses, and improving cellular and humoral immunity in Shami goats. This natural intervention strategy presents a sustainable approach for maintaining goat health and productivity under heat stress conditions.

1.Introduction

Global livestock production faces an escalating challenge from climate change, with heat stress emerging as a critical factor compromising animal health, welfare, and productivity. Ruminants, in particular, are highly susceptible, as heat stress disrupts their homeothermy, leading to suppressed feed intake, altered metabolism, and significant economic losses for producers [1]. The Shami goat, a prized breed for high-quality meat and milk, is no exception. Beyond production metrics, heat stress induces a state of physiological dysregulation characterized by oxidative damage and immunosuppression [2]. The resultant increase in disease susceptibility not only raises animal welfare concerns but also drives the prophylactic use of antibiotics, which in turn contributes to the global threat of antimicrobial resistance [3].

Therefore, identifying natural, sustainable, and effective strategies to bolster an animal's intrinsic defense mechanisms against heat stress is a paramount goal for the modern food industry [4]. In this context, phytogenic feed additives (PFAs) derived from medicinal plants offer a promising alternative to conventional interventions [5]. Moringa oleifera leaf extract is renowned for its dense profile of vitamins, minerals, and potent flavonoids, exhibiting strong antioxidative and immunomodulatory properties. Similarly, turmeric, containing the bioactive curcuminoid complex, is a well-established anti-inflammatory and antioxidant agent [6].

While the individual benefits of these botanicals are documented in vitro, their synergistic application as a dietary supplement to modulate the specific physiological responses of goats under realistic heat stress conditions remains underexplored [7]. Current literature often lacks a simultaneous investigation of both the cellular and humoral arms of the immune system alongside key antioxidant enzyme activities [8].

This study posits that a combined supplementation of Moringa oleifera and turmeric will act synergistically to enhance the antioxidant defense system and fortify immune function in heat-stressed Shami goats. The objectives of this research were to: (1) evaluate the effects of dietary Moringa oleifera leaf extract and turmeric supplementation on the cellular (lymphocyte proliferation, phagocytic activity) and humoral (antibody titers) immune responses; and (2) quantify the activities of crucial antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), in Shami goats subjected to cyclical heat stress. The findings aim to provide a scientific basis for a natural, feed-based strategy to enhance thermotolerance, improve animal resilience, and secure the supply chain for high-quality goat-derived food products.

2. Materials and Methods

2.1 Experimental Animals and Management

Twenty-four healthy adults female Shami (Damascus) goats, aged 2-3 years with an average body weight of 35±3 kg, were selected from a commercial farm in Salah ad Din Province, Iraq. Animals were confirmed non-pregnant through ultrasound examination and had no history of metabolic or infectious diseases. Prior to the experiment, all goats underwent a 14-day adaptation period. The study was conducted during the summer season (June-August 2023) when environmental conditions imposed natural heat stress.

Animals were housed in semi-open sheds with concrete floors and adequate ventilation. Each pen (4×5 m) accommodated 3 goats with ad libitum access to fresh water. Environmental parameters including ambient temperature, relative humidity, and Temperature-Humidity Index (THI) were recorded hourly using automated data loggers (HOBO U12-012, Onset Computer Corporation, USA). The experimental protocol was approved by the Institutional Animal Ethics Committee (Protocol #2023-045) and conducted following international guidelines for animal welfare.

2.2 Experimental Design and Dietary Treatments

In this study, goats were to four treatment groups (n=6) using a completely randomized design:

1. Control (CON): Basal diet without supplementation
2. Moringa (MO): Basal diet + 3% *M. oleifera* leaf extract (dry matter basis)
3. Turmeric (TUR): Basal diet + 2% turmeric powder (dry matter basis)
4. Combined (MO+TUR): Basal diet + 1.5% MOLE + 1% turmeric powder

The basal diet consisted of alfalfa hay (40%), wheat straw (20%), and concentrate mixture (40%) formulated to meet NRC (2007) requirements for maintenance and production. Chemical composition of the basal diet was: crude protein 14.2%, neutral detergent fiber 42.5%, acid detergent fiber 28.3%, and metabolizable energy 10.8 MJ/kg DM.

2.3 Preparation of Plant Materials

2.3.1 Moringa oleifera Leaf Extract

Fresh *M. oleifera* leaves were collected from 2-year-old trees, washed, and shade-dried at 25-30°C for 7 days. Dried leaves were ground to pass through a 1-mm sieve. Aqueous extract was prepared by macerating 100 g of leaf powder in 1 L distilled water for 24 h at room temperature with continuous stirring. The extract was filtered through Whatman No. 1 filter paper, concentrated using rotary evaporator at 45°C, and lyophilized. The yield was 18.5% w/w. Total phenolic content was determined as 165.3 mg gallic acid equivalent/g using Folin-Ciocalteu method [9].

2.3.2 Turmeric Powder Preparation

Turmeric rhizomes were washed, sliced (2-3 mm thickness), and dried at 50°C for 48 h. Dried slices were ground to fine powder (<0.5 mm). Curcumin content was analyzed by HPLC and found to be 3.8% w/w. The powder was stored in airtight containers at 4°C until use.

2.4 Blood Sampling and Processing

Blood samples (10 mL) were collected from jugular vein at 0, 15, 30, 45, and 60 days of the experiment at 07:00 h before morning feeding. Samples were divided into three aliquots:

1. EDTA tubes (3 mL) for hematological analysis and lymphocyte phenotyping

2. Heparinized tubes (3 mL) for peripheral blood mononuclear cell (PBMC) isolation
3. Plain tubes (4 mL) for serum separation

Serum was obtained by centrifugation at 3000×g for 15 min at 4°C and stored at -80°C until analysis.

2.5 Analytical Procedures

2.5.1 Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity was measured using the method of Marklund and Marklund (1974) based on pyrogallol autoxidation inhibition. Catalase (CAT) activity was determined following Aebi (1984) by monitoring H_2O_2 decomposition at 240 nm [10]. Glutathione peroxidase (GPx) activity was assayed according to Paglia and Valentine (1967) using cumene hydroperoxide as substrate [11].

2.5.2 Cytokine Analysis

Serum concentrations of IL-6, TNF-α, IL-1β, and IL-10 were quantified using goat-specific ELISA kits (Cusabio Biotech, China) following manufacturer's protocols. Optical density was measured at 450 nm using microplate reader (BioTek ELx800, USA). Inter- and intra-assay coefficients of variation were <10% and <8%, respectively.

2.5.3 Lymphocyte Immunophenotyping

PBMCs were isolated using Ficoll-Hypaque density gradient centrifugation. Cell viability was assessed by trypan blue exclusion (>95%). Cells (1×10^6) were stained with fluorochrome-conjugated monoclonal antibodies: anti-CD4-FITC, anti-CD8-PE, anti-CD21-APC (B cells), and anti-WC1-PerCP (γδ T cells) (Bio-Rad, USA). Flow cytometric analysis was performed using BD FACSCalibur with CellQuest Pro software. A minimum of 10,000 events were acquired per sample.

2.5.4 Heat Shock Protein Expression

Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen, USA). RNA quality and quantity were assessed using NanoDrop spectrophotometer. cDNA synthesis was performed using SuperScript III Reverse Transcriptase (Invitrogen). Real-time PCR was conducted using SYBR Green Master Mix on StepOnePlus system (Applied Biosystems). Primers for HSP70, HSP90, and GAPDH were designed using Primer3 software. Relative gene expression was calculated using $2^{(-\Delta\Delta Ct)}$ method with GAPDH as reference gene.

2.5.5 Immunoglobulin Quantification

Serum IgG, IgM, and IgA concentrations were determined by single radial immunodiffusion using commercial kits (Triple J Farms, USA) specific for caprine immunoglobulins. Precipitin ring diameters were measured after 48 h incubation at room temperature.

2.6 Physiological Parameters

Rectal temperature (RT), respiratory rate (RR), and pulse rate (PR) were recorded twice daily (08:00 and 14:00 h) throughout the experimental period. Rectal temperature was measured using digital thermometer inserted 5 cm into rectum. Respiratory rate was determined by counting flank movements for 60 seconds. Pulse rate was measured from femoral artery.

2.7 Statistical Analysis

Data were analyzed using mixed model ANOVA with repeated measures (SAS 9.4, SAS Institute Inc., USA). The model included fixed effects of treatment, time, and treatment × time interaction, with animal as random effect. Initial values were used as covariates. Post-hoc comparisons were performed using Tukey's HSD test. Polynomial contrasts were used to test linear and quadratic effects of supplementation levels. Pearson correlation coefficients were calculated among immune and oxidative stress parameters. Results are presented as means ± SEM. Significance was declared at $P<0.05$ and trends at $P<0.10$.

3. Results

3.1 Environmental Conditions and Physiological Responses

During the experimental period, ambient temperature ranged from 28.3±1.2°C (morning) to 41.7±2.1°C (afternoon), with relative humidity varying from 35±5% to 65±8%. The calculated THI values ranged from 82.0 to 92.1, indicating moderate to severe heat stress conditions throughout the trial. These environmental conditions significantly exceeded the thermoneutral zone for goats (THI <72). Physiological parameters showed significant treatment effects ($P<0.001$). Rectal temperature in the control group increased from 39.2±0.1°C (morning) to 40.8±0.2°C (afternoon), while supplemented groups maintained lower afternoon values (MO: 40.1±0.1°C, TUR: 40.2±0.1°C, MO+TUR: 39.8±0.1°C; $P<0.05$). Respiratory rate was significantly reduced in MO+TUR group (68±4 breaths/min) compared to control (92±5 breaths/min) during peak heat hours ($P<0.001$). Pulse rate followed similar patterns with combined supplementation showing the most pronounced effect.

3.2 Antioxidant Enzyme Activities

Antioxidant enzyme activities demonstrated significant treatment × time interactions ($P<0.001$; Table 1). SOD activity progressively increased in supplemented groups, with MO+TUR showing the highest values at day 60 (93.40±2.1 U/mL) compared to control (68.25±1.8 U/mL). CAT activity exhibited similar patterns, increasing from baseline values of 42.3±1.5 U/mL to 55.03±1.8 U/mL in MO+TUR group, while control group showed a decline to 38.7±1.6 U/mL by day 60. GPx activity was particularly responsive to supplementation, with MO+TUR group showing 76% increase (15.03±0.5 U/mL) compared to control (8.52±0.4 U/mL) at day 60. Individual supplementation with MO or TUR resulted in intermediate responses, suggesting synergistic effects of combined treatment.

Table 1. Effect of *M. oleifera* and turmeric supplementation on antioxidant enzyme activities in heat-stressed Shami goats

Parameter	Day	CON	MO	TUR	MO+TUR	SEM	P-value
SOD (U/mL)	0	71.2	70.8	71.5	70.9	1.8	0.853
	30	69.3^{c}	78.5^{b}	76.2^{b}	85.3^{a}	2.1	<0.001
	60	68.3^{d}	84.2^{b}	81.7^{c}	93.4^{a}	2.3	<0.001
CAT (U/mL)	0	42.3	42.7	42.1	42.5	1.5	0.912
	30	40.2^{c}	47.3^{b}	46.8^{b}	50.2^{a}	1.6	<0.001
	60	38.7^{d}	51.2^{b}	49.8^{c}	55.0^{a}	1.8	<0.001
GPx (U/mL)	0	8.7	8.9	8.6	8.8	0.3	0.798
	30	8.5^{d}	11.2^{b}	10.8^{c}	12.8^{a}	0.4	<0.001
	60	8.5^{d}	13.7^{b}	12.9^{c}	15.0^{a}	0.5	<0.001

[a–d] Means within a row with different superscripts differ significantly (P<0.05)

3.3 Cytokine Profile

Pro-inflammatory cytokines showed marked reductions in supplemented groups (Figure 1). IL-6 concentrations decreased from 45.3±3.2 pg/mL in control to 28.4±2.1 pg/mL (MO), 30.2±2.3 pg/mL (TUR), and 22.1±1.9 pg/mL (MO+TUR) by day 60 (P<0.001). TNF-α followed similar patterns with 52% reduction in MO+TUR group compared to control. IL-1β levels were significantly suppressed in all supplemented groups (P<0.01). Conversely, anti-inflammatory IL-10 increased in supplemented groups, particularly in MO+TUR (18.7±1.2 pg/mL) compared to control (11.3±0.9 pg/mL) at day 60 (P<0.05). The IL-6/IL-10 ratio, an indicator of inflammatory balance, decreased from 4.0 (control) to 1.2 (MO+TUR), suggesting effective modulation of inflammatory response.

3.4 Lymphocyte Subpopulations

Flow cytometric analysis revealed significant alterations in lymphocyte subsets (Table 2). $CD4^+$ T-helper cells increased from 28.3±1.8% to 35.7±2.1% in MO+TUR group, while control group showed decline to 24.2±1.6% by day 60 (P<0.001). $CD8^+$ cytotoxic T cells decreased in control group but remained stable in supplemented groups. Consequently, $CD4^+/CD8^+$ ratio improved from 1.2±0.1 (control) to 1.8±0.1 (MO+TUR), indicating enhanced cellular immune competence. B lymphocytes ($CD21^+$) increased significantly in supplemented groups, with MO+TUR showing highest values (18.3±1.2% vs. 12.7±0.9% in control; P<0.01). γδ T cells, important for innate immunity, were preserved in supplemented groups while declining in controls.

Table 2. Lymphocyte subpopulation distribution (%) in heat-stressed Shami goats

Cell Type	Group	Day 0	Day 30	Day 60	P-value
$CD4^+$	CON	29.5±1.7	26.8±1.6^b	24.2±1.6^c	<0.001
	MO	29.2±1.8	31.3±1.7^a	32.8±1.8^b	<0.001
	TUR	29.7±1.7	30.8±1.6^a	31.9±1.7^b	<0.001
	MO+TUR	29.3±1.8	32.7±1.9^a	35.7±2.1^a	<0.001
$CD8^+$	CON	20.1±1.2	20.8±1.3	20.3±1.2	0.652
	MO	20.3±1.1	19.8±1.1	19.5±1.0	0.423
	TUR	19.9±1.2	19.7±1.1	19.4±1.1	0.512
	MO+TUR	20.2±1.1	19.6±1.0	19.2±0.9	0.387

P-values refer to the effect of time within each treatment group

3.5 Heat Shock Protein Expression

HSP70 and HSP90 mRNA expression increased significantly in all groups exposed to heat stress, with supplemented groups showing enhanced responses (Figure 2). HSP70 expression peaked at day 30 with 4.2-fold increase in MO+TUR group compared to 2.8-fold in control (P<0.001). By day 60, HSP70 expression remained elevated in supplemented groups (3.5-fold in MO+TUR) while declining in control (1.9-fold). HSP90 expression patterns were similar but with lower magnitude of change. The HSP70/HSP90 ratio was higher in supplemented groups, suggesting preferential upregulation of HSP70-mediated protective mechanisms.

3.6 Immunoglobulin Concentrations

Serum immunoglobulin levels showed treatment-dependent responses (Table 3). IgG concentrations increased from baseline 12.5 ± 0.7 mg/mL to 16.8 ± 0.9 mg/mL in MO+TUR group, while control group showed no significant change (P<0.05). IgM levels were maintained in supplemented groups but declined in control. IgA concentrations, important for mucosal immunity, increased by 38% in MO+TUR group compared to 12% decline in control (P<0.01).

Table 3. Serum immunoglobulin concentrations (mg/mL) in heat-stressed Shami goats

Parameter	Day	CON	MO	TUR	MO+TUR	P-value
IgG	0	12.5±0.7	12.3±0.8	12.6±0.7	12.4±0.8	0.892
	60	12.8±0.8^c	15.2±0.8^b	14.9±0.9^b	16.8±0.9^a	<0.001
IgM	0	2.8±0.2	2.7±0.2	2.9±0.2	2.8±0.2	0.756
	60	2.3±0.2^b	2.9±0.2^a	2.8±0.2^a	3.1±0.2^a	0.018
IgA	0	0.42±0.03	0.41±0.03	0.43±0.03	0.42±0.03	0.834
	60	0.37±0.03^c	0.52±0.04^b	0.50±0.04^b	0.58±0.04^a	<0.001

3.7 Correlation Analysis

Pearson correlation analysis revealed strong negative correlations between antioxidant enzyme activities and pro-inflammatory cytokines (r = -0.72 to -0.85; P<0.001). HSP70 expression positively correlated with SOD (r = 0.68; P<0.01) and CAT (r = 0.71; P<0.001) activities. $CD4^+/CD8^+$ ratio showed positive correlation with IgG levels (r = 0.62; P<0.01) and negative correlation with IL-6 (r = -0.58; P<0.05).

4. Discussion

4.1 Physiological Adaptations to Heat Stress

The present study demonstrates that combined supplementation with *M. oleifera* and turmeric effectively mitigates heat stress-induced physiological perturbations in Shami goats. The THI values recorded (82-92) indicate severe heat stress conditions, consistent with previous reports in Middle Eastern goat production systems [12,13]. The observed reduction in rectal temperature and respiratory rate in supplemented groups suggests improved thermoregulatory efficiency, likely mediated through enhanced cellular heat stress response mechanisms. The superior performance of the combined supplementation group aligns with recent findings by Kholif et al. (2021) who reported synergistic effects of phytogenic compounds in ameliorating heat stress responses [14]. The maintenance of lower body temperature despite high environmental heat load indicates improved heat dissipation capacity and reduced metabolic heat production, potentially through modulation of thyroid hormone activity and mitochondrial uncoupling proteins [15].

4.2 Antioxidant Defense Mechanisms

The progressive enhancement of antioxidant enzyme activities in supplemented groups represents a crucial adaptive response to heat stress-induced oxidative challenge. The 37% increase in SOD activity and 42% elevation in CAT activity in the MO+TUR group compared to control demonstrates potent activation of the primary antioxidant defense system. These findings corroborate previous studies showing that *M. oleifera* bioactive compounds, particularly flavonoids and phenolic acids, upregulate antioxidant enzyme expression through Nrf2-ARE signaling pathway activation [16,17].

The synergistic elevation of GPx activity (76% increase) in the combined supplementation group is particularly noteworthy, as GPx plays a critical role in eliminating lipid peroxides and maintaining cellular membrane integrity during heat stress [18]. The coordinated upregulation of all three major antioxidant enzymes suggests comprehensive protection against different ROS species, with SOD converting superoxide to hydrogen peroxide, which is subsequently neutralized by CAT and GPx. Curcumin's contribution to this antioxidant response likely involves both direct ROS scavenging and indirect effects through modulation of cellular signaling pathways. Recent mechanistic studies have shown that curcumin activates the Keap1-Nrf2 system, leading to transcriptional upregulation of antioxidant response elements [19]. The observed enzyme activity patterns suggest that combined supplementation provides complementary mechanisms of antioxidant protection, with *M. oleifera* supplying direct antioxidant compounds and both supplements activating endogenous defense systems.

4.3 Inflammatory Response Modulation

The marked reduction in pro-inflammatory cytokines (IL-6: 51%, TNF-α: 52%, IL-1β: 48%) in the MO+TUR group represents a significant attenuation of heat stress-induced inflammatory response. This anti-inflammatory effect is crucial as chronic elevation of these cytokines during heat stress leads to metabolic dysfunction, reduced feed intake, and compromised productivity [20,21]. The mechanism underlying this cytokine modulation likely involves inhibition of NF-κB signaling pathway, a master regulator of inflammatory gene expression. Both *M. oleifera* and curcumin have been shown to suppress IκB kinase activity, preventing NF-κB nuclear translocation and subsequent transcription of inflammatory mediators [22,23]. The simultaneous increase in anti-inflammatory IL-10 (65% elevation in MO+TUR) creates a favorable cytokine balance, as evidenced by the reduced IL-6/IL-10 ratio from 4.0 to 1.2. This shift toward an anti-inflammatory phenotype has important implications for immune function preservation during heat stress. Elevated pro-inflammatory cytokines have been associated with lymphocyte apoptosis, reduced antibody production, and impaired vaccine responses in heat-stressed ruminants [24]. The maintenance of cytokine homeostasis through phytogenic supplementation thus provides a foundation for preserved immune competence.

4.4 Cellular Immune Function

The preservation and enhancement of $CD4^+$ T-helper cell populations in supplemented groups contrast sharply with the decline observed in control animals. The 47% increase in $CD4^+$ cells and improved $CD4^+/CD8^+$ ratio (from 1.2 to 1.8) in the MO+TUR group indicates robust maintenance of cell-mediated immunity despite heat stress challenge. This finding is particularly significant given previous reports of heat stress-induced lymphocyte apoptosis and altered T-cell differentiation in ruminants [25,26]. The mechanism for preserved lymphocyte function likely involves multiple pathways. First, reduced oxidative stress in supplemented animals prevents ROS-mediated lymphocyte damage and apoptosis. Second, the anti-inflammatory cytokine environment promotes T-cell survival and proliferation. Third, bioactive compounds from *M. oleifera* and turmeric may directly stimulate lymphocyte proliferation through modulation of protein kinase C and MAP kinase signaling pathways [27,28]. The maintenance of γδ T cells, which constitute a significant proportion of circulating T cells in ruminants and play crucial roles in innate immunity and immunosurveillance, further supports the immunoprotective effects of supplementation. These cells are particularly sensitive to heat stress, and their preservation suggests comprehensive protection of diverse immune cell populations [29].

4.5 Heat Shock Protein Response

The enhanced HSP70 and HSP90 expression in supplemented groups represents an adaptive cellular stress response that provides cytoprotection during thermal challenge. The 2.3-fold greater HSP70 induction in MO+TUR group compared to control at day 30, with sustained elevation through day 60, indicates prolonged activation of cellular protective mechanisms. This finding aligns with previous studies showing that certain phytochemicals can act as hormetic stressors, priming cellular stress response pathways for enhanced protection against subsequent challenges [30,31]. The preferential upregulation of HSP70 over HSP90, as evidenced by the increased HSP70/HSP90 ratio, is particularly relevant for heat stress adaptation. HSP70 serves as the primary chaperone for preventing protein aggregation and facilitating protein refolding during thermal stress, while also inhibiting apoptotic pathways through interactions with Apaf-1 and caspase proteins [32,33]. The sustained HSP70 expression in supplemented groups, even as expression declined in controls, suggests that phytogenic compounds maintain cellular stress response capacity during chronic heat exposure. Recent evidence indicates that both *M. oleifera* and curcumin can modulate heat shock factor-1 (HSF1) activity, the master transcriptional regulator of heat shock response [34,35]. This modulation may involve post-translational modifications of HSF1, including phosphorylation and SUMOylation, which affect its DNA binding activity and transcriptional potency.

4.6 Humoral Immune Response

The significant elevation of immunoglobulin concentrations in supplemented groups demonstrates preservation of humoral immunity during heat stress. The 35% increase in IgG, 35% increase in IgM, and 38% increase in IgA levels in the MO+TUR group indicate enhanced B cell function and antibody production capacity. This contrasts with the well-documented suppression of antibody responses in heat-stressed ruminants [36,37]. The mechanism for improved immunoglobulin production likely involves both direct and indirect effects of supplementation. Direct effects include stimulation of B cell proliferation and differentiation through modulation of B cell receptor signaling and co-stimulatory molecules. Indirect effects include the favorable cytokine environment (increased IL-10, decreased IL-6) that promotes B cell survival and antibody class switching [38].

The particular enhancement of IgA production has important implications for mucosal immunity, which is often compromised during heat stress due to reduced gut barrier function and altered intestinal immune responses. The bioactive compounds in *M. oleifera* and turmeric have been shown to support intestinal epithelial integrity and promote IgA-producing plasma cell differentiation in gut-associated lymphoid tissue [39,40].

4.7 Integrated Stress Response and Practical Implications

The strong correlations observed between antioxidant enzymes, cytokines, and immune parameters suggest an integrated protective response orchestrated by phytogenic supplementation. The negative correlation between antioxidant enzyme activities and pro-inflammatory cytokines (r = -0.72 to -0.85) indicates that oxidative stress mitigation directly contributes to inflammatory response modulation. Similarly, the positive correlation between HSP70 expression and antioxidant enzymes suggests coordinated activation of cellular protective mechanisms. From a practical perspective, the superior performance of combined supplementation over individual treatments between the bioactive compounds of M. oleifera and turmeric. This synergy may result from complementary mechanisms of action, with *M. oleifera* providing diverse antioxidant compounds and nutritional cofactors, while curcumin offers potent anti-inflammatory and gene regulatory effects. The combination may also improve bioavailability and cellular uptake of active compounds through enhanced membrane permeability and transporter modulation [41,42].

The sustained effects observed throughout the 60-day experimental period, without evidence of tolerance development, support the feasibility of long-term supplementation during heat stress seasons. The absence of adverse effects on physiological parameters suggests good safety profiles for both supplements at the tested doses, consistent with their long history of use in traditional medicine and animal feeding practices [42,43].

4.8 Limitations and Future Directions

While this study provides comprehensive evidence for the beneficial effects of *M. oleifera* and turmeric supplementation, several limitations should be acknowledged. First, the study was conducted with female goats only, and sex-specific responses to supplementation during heat stress remain to be investigated. Second, the molecular mechanisms underlying the observed effects were inferred from functional outcomes rather than direct mechanistic studies. Future research should employ transcriptomic and proteomic approaches to elucidate specific signaling pathways and gene regulatory networks involved.

Additionally, dose-response relationships for optimal supplementation levels need further investigation, as the current study used fixed doses based on previous literature. The economic feasibility of supplementation strategies also requires evaluation, considering supplement costs, preparation methods, and potential impacts on milk yield and composition. Long-term studies examining effects on reproductive performance, offspring health, and transgenerational impacts would provide valuable insights for sustainable implementation in commercial goat production systems.

5. Conclusions

This study demonstrates that dietary supplementation with *M. oleifera* leaf extract and turmeric, particularly in combination, effectively mitigates heat stress-induced immunosuppression and oxidative damage in Shami goats. The protective effects are mediated through multiple complementary mechanisms including:

1. Enhancement of antioxidant enzyme activities (SOD, CAT, GPx)

providing comprehensive protection against oxidative stress

2. Modulation of inflammatory responses through suppression of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) and elevation of anti-inflammatory IL-10
3. Preservation of cellular immunity evidenced by maintained $CD4^+$ T cell populations and improved $CD4^+/CD8^+$ ratios
4. Upregulation of heat shock protein expression, particularly HSP70, providing cellular cytoprotection
5. Enhancement of humoral immunity through increased immunoglobulin production

The synergistic effects observed with combined supplementation suggest that integrating multiple phytogenic compounds may provide more comprehensive protection than single supplements. These findings have important implications for developing nutritional strategies to maintain goat health and productivity in heat-stressed environments, particularly relevant given climate change projections for increased frequency and severity of heat stress events.

Implementation of these supplementation strategies could contribute to sustainable intensification of small ruminant production systems in tropical and subtropical regions, supporting food security and rural livelihoods. Further research should focus on optimizing supplementation protocols, evaluating economic feasibility, and investigating long-term impacts on production performance and animal welfare.

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