Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Thermotolerance and Immunity in Stressed Shami Goats through Supplementation of Moringa oleifera and Turmeric in the Presence of Heat Stress.

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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.

ARTICLE INFO

Article History:

Received: 2025/12/29
Accepted: 2026/2/5

Keywords:

Phytogenic feed additives,
oxidative stress,
heat shock,
livestock sustainability,
innate immunity,
glutathione peroxidase,
superoxide dismutase.

DOI: 10.48311/fsct.2026.118568.83012.

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1.Introduction

Climate change is mounting pressure on global livestock production, and heat stress is becoming a highly important concern in facilitating deteriorated animal health, welfare, and productivity. Ruminants, specifically, are very vulnerable, as heat stress impairs their homeothermy, which results in stunted feed consumption, altered metabolism and massive losses of money to producers [1]. Not an exception is the Shami goat which is a highly valued animal in producing quality meat and milk. In addition to production measures, heat stress results in a physiological dysregulation condition that is marked by oxidative injury and immunosuppression [2]. The ensuing susceptibility to diseases not only creates animal welfare issues, but also leads to the prophylactic use of antibiotics, which subsequently leads to the worldwide risk of antimicrobial resistance [3]. It thus becomes a priority to the contemporary food industry to find natural, sustainable and efficient ways of enhancing inherent defenses of an animal against heat stress [4]. In this respect, phytogenic feed additives (PFAs) of medicinal plants can provide an alternative to traditional interventions [5]. The extract of Moringa oleifera leaves is characterized by a high concentration of vitamins, minerals, and potent flavonoids and has a high antioxidative and immunomodulatory activity. On the same note, turmeric which has the bioactive complex of curcuminoid is an established anti-inflammatory and antioxidant [6]. Although the benefits of these botanicals on the individual are reported in vitro, their.

synergistic use as a dietary supplement to regulate the targeted physiological responses of goats in the real heat Stress situations is under-researched [7]. Nevertheless, a concomitant study of the cellular and humoral components of the immune system and the main antioxidant enzyme actions is rarely performed in the current literature [8].

The research hypothesis is that a joint supplementation of Moringa oleifera and turmeric would have a synergistic effect on the action of antioxidant defense system and immune performance of heat-stressed Shami goats. This research set out to achieve the following objectives:

(1) assess the impacts of dietary supplements of Moringa oleifera leaf extract and turmeric on the cellular (lymphocyte proliferation, phagocytic activity) and humoral (antibody titers) immune responses; and (2) determine the activities of important antioxidant enzymes, such as the superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), in Shami goats that are subjected to cyclical heat stress. The research will be useful and align with a scientific foundation of a natural, feed-based approach to increase thermotolerance, animal resilience, and supply chain of high-quality goat-based food products.

2. Materials and Methods

2.1 Experimental Animals and Management

A total of 24 healthy adult female Shami (Damascus) goats (30 months of age and an average weight of 35kg/kg) were selected in a commercial farm in Salah ad Din Province, Iraq. Animals were confirmed non-pregnant by way of ultrasound examination and did not have any metabolic or infectious disease history. The experimental goats were subjected to 14 days of adaptation before the experiment. The experiment was carried out in the summer (June-August 2023) when the natural heat pressure was caused by the environmental conditions. The housing of animals was in semi open sheds that had concrete floors and good ventilation. There were 3 goats in each pen (4×5 m) with ad libitum access to fresh water. Automated data loggers were used to record the environmental parameters such as the ambient temperature, relative humidity, Temperature-Humidity Index (THI) on an hourly basis (HOBO U12-012, Onset Computer Corporation, USA). The Institutional Animal Ethics Committee gave approval to the experimental protocol (Protocol #2023-045) and performed it according to international standards on animal welfare.

1.1 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 basic diet was composed of alfalfa hay (40 percent), wheat straw (20 percent), and concentrate mixture (40 percent) as the diet to ensure the requirements of maintenance and production as per the NRC (2007). The basal diet chemical composition was: crude protein 14.2, neutral. detergent fiber 42.5%, acid detergent fiber 28.3%, and metabolizable energy 10.8 MJ/kg DM.

1.2 Preparation of Plant Materials

1.2.1 Moringa oleifera Leaf Extract

Fresh *M. oleifera* leaves were taken off 2-year-old trees, washed, and dried at 25-30 o C in shade after 7 days. The dried leaves were powdered using a 1-mm sieve. The maceration of 100 g of powdered leaves in 1 L of distilled water was done to prepare aqueous extract and was allowed to stir continuously during 24 h at room temperature. Whatman No. 1 filter paper was used to filter the extract, which was then concentrated by the use of rotary evaporator at 45 o C and lyophilized. The yield was 18.5% w/w. The total phenolic content was 165.3 mg gallic acid equivalent/g based on Folin-Ciocalteu method [9].

1.2.2 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.

1.3 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

1. Heparinized tubes (3 mL) for peripheral blood mononuclear cell (PBMC) isolation
2. 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.

1.4 Analytical Procedures

1.4.1 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].

1.4.2 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.

1.4.3 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.

1.4.4 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.

1.4.5 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.

1.5 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.

1.6 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

The environmental conditions that prevailed at the time of the study were in the morning that the ambient temperatures were 29.1+-1.4degC which increased to 42.3+-1.9degC in the afternoon. Relative humidity was 33+-6-63+-7. Obtained THI values ranged between 81.5 and 91.8, which ensured the animals used in the experiment were always subjected to moderate-severe thermal stress far beyond the thermoneutral temperature of caprine species (THI <72). Dietary treatments had a great impact on thermoregulatory indicators ($P<0.001$). The afternoon values were significantly different but, in the morning, there was no significant difference in the rectal temperature (39.1+-0.1degC). The rectal temperature of control animals at afternoon was the highest (40.9+-0.2degC), and the MO+TUR group had the most favorable state of thermoregulation (39.7+-0.1degC), followed by MO (40.0+-0.1degC) and TUR (40.1+-0.1degC) ones ($P<0.05$). In the peak thermal hours, respiratory rate in the control group came up to 94+-6 breaths/min, and the MO+TUR group had a significant decrease (66+-3 breaths/min; $P<0.001$). This trend was also reflected in pulse rate responses, and the combined supplementation had the most thermoprotective effect.

3.2 Antioxidant Enzyme Activities

Antioxidant Analysis of the enzymatic antioxidant defense system indicated very high significant treatment x time interactions with all of the biomarkers evaluated ($P<0.001$; Tables 1a, 1b and 1c). SOD activity demonstrated a consistent upward trajectory in all supplemented groups throughout the trial duration, with the MO+TUR group achieving peak values of 94.8±2.2 U/mL at day 60, representing a 40.4% improvement over the control group (67.5±1.7 U/mL). CAT activity followed an analogous trend, ascending from baseline concentrations of approximately 43.0 U/mL to 56.2±1.9 U/mL in the combined treatment group, in contrast to a notable deterioration observed in the control animals (37.9±1.4 U/mL) by trial conclusion. The most striking enzymatic response was recorded for GPx, where the MO+TUR group exhibited an 88% enhancement (15.6±0.5 U/mL) relative to control animals (8.3±0.3 U/mL) at the terminal sampling point. Individual administration of MO or TUR elicited moderate improvements that were intermediate between the combined treatment and control groups, providing compelling evidence for a synergistic interaction between these two phytogenic supplements in bolstering the antioxidant defense machinery under thermal stress.

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.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.

3.4 Lymphocyte Subpopulations

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.

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

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).

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 gradual improvement of antioxidant activity of enzymes in supplemented groups is one of the vital adaptive mechanisms against heat stress-induced oxidative pressure. The enhancement in SOD activity by 37% and an increase in CAT activity by 42% in MO + TUR group versus control shows powerful influence of the main antioxidants defense system. Such results support the hypothesis in the literature indicating that M. oleifera bioactive.

compounds, especially flavonoids and phenolic acids, increase the expression of antioxidant enzymes, because of the activation of Nrf2-ARE signaling pathways [16,17].

It is especially interesting that the synergistic enhancement of GPx activity (76% increase) in the combined supplementation group is essential because GPx is of paramount importance in the eradication of lipid peroxides and preserving cell membrane integrity in cases of heat stress [18]. The simultaneous upregulation of the three key antioxidant enzymes is an indication of a broad defenses against the various kinds of ROS, whereby the SOD transforms superoxide to hydrogen peroxide, which is then neutralized by CAT and GPx. The role of curcumin in this antioxidant effect may be a direct effect of curcumin on ROS scavenging and indirectly through the regulation of cellular signaling pathways. Recent mechanistic investigations have demonstrated that curcumin switches on the Keap1-Nrf2 mechanism, which results in transcriptional rise in antioxidant response factors [19]. The patterns of the activity of the observed enzymes indicate that the complementary actions of antioxidant protection are provided by combined supplementation, and *M. oleifera* contributes to direct antioxidant compounds and both supplements work to activate endogenous antioxidant mechanisms.

4.3 Inflammatory Response Modulation

The significant decrease in the pro-inflammatory cytokine levels (IL-6: 51%, TNF-a: 52%, IL-1b: 48) in the MO+TUR group is a strong reduction in the heat stress-induced inflammatory response. This anti-inflammatory action is essential because long-term heat stress in chronic elevation of these cytokines causes metabolic dysfunction, decrease in feed intake, and decrease in productivity [20,21]. The mechanism The mechanism involved in the underlying cytokine modulation probably includes the inhibition of NF-kB-signaling pathway, which is a global suppressor of the inflammatory gene expression. M. oleifera and curcumin were both reported to inhibit IkB kinase activity thereby preventing NF-kB nuclear translocation and ensuing transcription of inflammatory mediators [22,23]. The concomitant rise in the anti-inflammatory IL-10 (65% increase in MO + TUR) develops a good cytokine balance with the lowering of the IL-6 / IL-10 ratio of 4.0 to 1.2. This change of an anti-inflammatory phenotype is significant in terms of immune functions maintenance during heat stress. An increase in pro-inflammatory cytokines has been linked to lymphocyte apoptosis, decrease in antibody production and vaccine responses in heat stressed ruminants [24]. Homeostasis of cytokines use is then sustained by phytogenic supplementation hence, creating a basis of retained immune efficiency.

4.4 Cellular Immune Function

The maintenance of the populations of CD4+ T-helper cells and its improvement in the groups with supplements is a stark contrast to the decreasing amount of control animals. The 47 percent rise in CD4+ cells and the increase in CD4+/CD8+ ratio (1.2 to 1.8) of the MO+TUR group suggests that there is solid cell-mediated immunity even in the presence of heat stress. This observation is especially noteworthy considering that it has been earlier reported that heat stress led to the lymphocyte apoptosis and disturbed T-cell differentiation in ruminants [25,26]. The preserved lymphocyte functioning mechanism is probably characterized by a series of pathways. First, lower oxidative stress of supplemented animals averts ROS-induced lymphocyte damages and apoptosis. Second, T-cells survive and proliferate under the anti-inflammatory cytokine environment. Third, bioactive

M. oleifera and turmeric compounds directly can also induce lymphocyte proliferation by regulating protein kinase C and MAP kinase signaling pathways [27,28]. The immunoprotected actions of supplementation are further supported by the maintenance of gd T cells which are a major percentage of the circulating T cells in ruminants and also play important roles in innate immunity and immunosurveillance. These cells are especially vulnerable to the stress of heat, and their functionality is preserved indicating extensive defense of various populations of immune cells [29].

4.5 Heat Shock Protein Response

The supplemented groups had an improved HSP70 and HSP90 expression, which is an adaptive cellular stress response that offered cytoprotection during thermal challenge. The 2.3-fold increase in HSP70 in MO + TUR group over control at day 30 with the expression persisting till day 60 is an indicator of increased activation of cellular protective mechanisms. This observation is consistent with the earlier research that some phytochemicals may be hormetic stressors that trigger cellular pathways of stress response to be more protective to future challenges [30,31]. The selective upregulation of HSP70 in comparison to HSP90 which is indicated by the resulting higher level of HSP70/HSP90 ratio is especially pertinent to heat stress adaptation. HSP70 plays the major role of preventing aggregation of proteins and promoting folding of proteins during thermal stress, as well as inhibiting apoptotic pathways via interactions with Apaf-1 and caspase proteins [32,33]. The prolonged HSP70 expression in the supplemented groups despite the drop in expression in the control groups indicates that phytogenic compounds preserve the capability of the cells to respond to cellular stress during chronic heat exposure. Recent experience has shown that M. oleifera as well as The curcumin is able to regulate heat shock factor-1 (HSF1) activity, which is the master transcriptional regulator of heat shock response [34,35]. This change can be post-translational modification of HSF1, such as phosphorylation and SUMOylation, which can influence its transcriptional potency and DNA binding activity.

4.6 Humoral Immune Response

The high level of immunoglobulin in supplemented groups compared to the control indicates the maintenance of humoral immunity in heat stress. The rise in IgG, IgM, and IgA levels in MO+TUR are 35% higher, 35% higher, and 38% higher which demonstrates an improvement in the B cell functions and the ability to produce antibodies. This is unlike the reported extensive inhibition of antibody reactions in heat-stressed ruminants [36,37]. The process of enhanced immunoglobulin synthesis is probably compounded of direct and indirect supplementation impact. Direct effects involve B cell proliferation and differentiation stimulation by the way of B cell receptor signaling and co-stimulatory molecule regulation. Among the indirect effects, there is the positive cytokine environment (higher IL-10, lower IL-6) that facilitates B cell survival and the switching of antibody classes [38].

The specific increase in the production of IgA has significant consequences on mucosal immunity that is usually impaired in heat stress because of a decreased gut barrier capacity and a distortion of intestinal immune reactions. The bioactive substances in M. oleifera and turmeric are demonstrated to sustain intestinal epithelial wellness and encourages IgA-generating plasma cells differentiation in the gut-associated lymphoid tissues [39,40].

4.7 Integrated Stress Response and Practical Implications

The significant and positive relationships that were found among antioxidant enzyme, cytokines, and immune parameters indicate a coordinated and protective response that was coordinated by phytogenic supplementation. The antioxidant enzyme activities are negatively correlated with the pro-inflammatory cytokines ($r = -0.72$ to -0.85), which suggests that the mitigation of oxidative stress is the direct cause of the changes in the inflammatory response. On the same note, the positive correlation between the expression of HSP70 and the antioxidant enzymes indicates the coordinated expression of cellular protective responses. Practically, the bioactive compounds of M. oleifera and turmeric are better than the individual treatments in terms of their superior performance in combined supplementation as opposed to individual treatments. This synergy can be due to complementary effect, whereby M. oleifera has a wide range of antioxidant substances and nutritional co-factors, whereas curcumin has a strong anti-inflammatory and gene-regulating effect. The combination can also increase bioavailability and cellular uptake of active compounds by increasing membrane permeability and regulation of transporters [41,42].

The long-term effects seen during the 60 days of experimental period without tolerance development is an indication that long term supplementation is possible during heat stress seasons. The fact that both supplements did not adversely affect the physiological parameters provides evidence that they have excellent safety profiles when used at the doses tested, as both have had extensive use over the years of both traditional medicine and animals as a feed.

4.8 Limitations and Future Directions

evidence of the positive impact of M. oleifera and turmeric supplementation, some limitations are to be considered. First, female goats were used in the study, and sex-specific reactions to supplementation during the heat stress are yet to be examined. Second, the mechanisms by which the effects were observed were deduced based on the functional outcomes as opposed to mechanistic studies. Future studies ought to use transcriptomic and proteomic methods to clarify certain signaling pathways and gene regulatory networks involved. Also, the dose-response optimization of the ideal level of supplementation requires further research, as the researcher in the present study was dealing with fixed doses, according to prior research. Economic viability of supplementation strategies also needs to be considered based on the prices of the supplements, the ways to prepare them and the possible effects they may have on the milk production and composition. The results of the effects on reproductive performance, offspring health, and transgenerational effects will be important in the context of sustainable implementation in commercial goat production systems, which could be studied in the long-term.

5. Conclusions

The current results indicate that M. oleifera leaf extract and turmeric combined dietary supplementation can effectively protect against immunosuppression and oxidative damage that are induced by heat stress in Shami goats by acting to reduce the effects through synergistic multi-target actions. They consist of positive changes in antioxidant enzyme activities (SOD, CAT, GPx), pro-inflammatory cytokines (IL-6, TNF-a, IL-1b) are suppressed and anti-inflammatory IL-10 is up-regulated, the maintenance of CD4+ T cell populations and enhanced CD4/CD8 ratios, HSP70, and enhancers of immunoglobulin production.

The higher effectiveness of the combined with the single finger supplementation highlights the prospects of the combination of multiple phytogenic compounds to have a total thermoprotective effect. The implications of these outcomes are great in the establishment of sustainable nutritional measures to maintain the health and productivity of goats in an ever harsh environment of heat stress due to climate changes especially in the tropical and subtropical production systems of small ruminants. The optimal protocols of supplementation, cost-effectiveness, and the impact of supplementation in the long run on animal production and welfare should be investigated in the future.

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