Scientific Research

Dietary Supplementation with *Moringa oleifera* and Turmeric Synergistically Enhances Immuno-Antioxidant Status and Alleviates Heat Stress in Shami Goats

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ARTICLE INFO	ABSTRACT
	Heat stress poses a significant problem to goat production in the tropical and subtropical areas especially during the summer; when temperatures are above the thermoneutral zone. The current study aimed at determining whether dietary supplementation with the Moringa oleifera leaf extract (MOLE) and turmeric would mitigate against the adverse effects of heat stress on immune functions and the oxidative status of Shami goats. Twenty-four adults female Shami goats Shami goats were randomly assigned four equal groups and exposed to a natural heat stress (THI: 82-92) over 60 days. The control group was served with the basal diet whereas the treatment groups were served with the basal diet supplemented with 3% MOLE, 2% turmeric and 1.5% MOLE plus 1% turmeric. Antioxidant enzymes, inflammatory cytokines, lymphocyte populations, immunoglobulins and heat shock proteins were measured by taking blood samples at biweekly intervals. The combined supplementation group had significant improvements on all the measured parameters. The antioxidant enzyme activities were higher and the SOD activity was higher (93.40 U/mL) compared to controls (68.25 U/mL). The pro inflammatory cytokines were reduced significantly -IL-6 reduced to 22.1 pg/mL and TNF-a reduced to 18.4 pg/mL. The proportion of CD4+/CD8+ increased to 1.8, which is a good sign of cellular immunity. The expression of heat shock protein 70 was 2.3-fold larger, whereas the concentration of IgG was raised by 12.3 to 16.8 mg/mL. These results indicate that Moringa and turmeric supplementation can be effective as a natural approach to beat heat stress in goats through improvement of antioxidant defenses, attenuation of inflammation and maintenance of immune functions. This strategy could help sustain both productivity and health in goat herds exposed to the increasing temperature as a result of climate change.

1.Introduction

The issue of climate change is a big threat to the livestock production worldwide with heat stress being one of the most dominant factors affecting the health, welfare and productivity of animals. Ruminants are especially prone to it, such as the much-prized breed of the Shami goats [1]. Having temperatures outside the thermoneutral range leads to a series of physiological imbalances to change the levels of feed consumption and metabolic changes that directly inhibit growth and milk production. Even more importantly, heat stress leads to a condition of systemic oxidative stress because of over production of reactive oxygen species (ROS), which overburdens the intrinsic antioxidant defense system [2]. This oxidative stress is inherently associated with suppression of both cellular and humoral immune responses and making an animal more susceptible to infections and becoming a matter of serious animal welfare concerns. This immunosuppression can lead to increased antimicrobial use, and this adds to the challenge of antimicrobial resistance in the world [3].

In that regard, the research of natural, sustainable and efficient ways to replace synthetic additives has turned out to be one of the most important in the field of animal nutrition. A potential solution to the problem is the phytogenic feed additives (PFAs) obtained out of the medicinal plants because of their multifunctional bioactivity [4]. Recently, the leaf extract of the moringa oleifera has been shown to possess a powerful profile of flavonoids, phenolic acids, and vitamins, and has been shown to have an antioxidant and immunostimulatory effects in other species. On the same note, turmeric with most of its efficacy explained by its curcuminoid components is a proven anti-inflammatory and free radical-scavenging agent [5].

The single effects of Moringa oleifera and turmeric are reported but there is no comprehensive study on the potential of the two to reduce the complicated immuno-oxidative sequelae of heat stress in ruminants. The present literature pays attention to individual parameters and does not involve a comprehensive evaluation of the immune-antioxidant axis in practical heat stress conditions [6]. We hypothesize that the synergistic effects of Moringa oleifera and turmeric will lead to the improvement of antioxidant defense system and immune competence of heat-stressed Shami goats, and future enhance the resilience of the latter [7].

Thus, the aim of the study was to examine the effects of dietary supplementation of a combination of Moringa oleifera leaf extract and turmeric on: (1) important plasma antioxidant enzyme functions (superoxide dismutase, SOD; glutathione peroxidase, GPx, and catalases, CAT); (2) immune functions at the cellular level (proliferation of lymphocytes, phagocytic activity); and (3) humoral immune system response (titers of antigen-specific antibod The results are to have a scientific foundation of a new nutritional approach to boost animal thermotolerance, animal welfare, and sustainability of goat farming business under warm climate.

2.Materials and Methods

2.1 Study Location and Animals

This study was carried out in a commercial goat farm in Salah ad Din Province in Iraq, at the hottest months of the year (June to August 2023). The area has drastic summer temperature conditions that are characteristic of arid Middle Eastern weather which presents natural heat stress environments of the study.

A very particular criteria was used to select twenty-four healthy adults female Shami goats: they had to be aged between 2-3 years, with a body weight of 35+-3 kg, their non-pregnancy was ensured through ultrasound, and no history of disease within the last six months. A rigorous veterinary check of all the animals was done prior to the commencement of the study. They were medicated against internal and external parasites and given two weeks to adjust and acclimatize themselves to the experimental facilities and procedures of handling. The goats were kept in semi-open sheds which would not keep the goats out of direct sunlight but would allow the goats to ventilate. The size of each pen was 4x5 meters and three animals were placed there, which was enough to move around and socialize. There were also automatic waterers that supplied fresh, clean water all the time and feed was supplied twice a day at 07:00 and 16:00 hours.

2.2 Experimental Design

There were four treatment groups, each consisting of six goats chosen as animals with the random number generator.

Group 1 - Control (CON): Was fed on the basal diet without any supplementation Group

2 - Moringa (MO): Basal diet combined with 3per cent Moringa Oleifera leaf extract (DM basis) Group.

3 - Turmeric (TUR): Basic diet combined 2 percent turmeric powder (dry matter basis) Group.

4 - Combined (MO+TUR): 1.5 percent Moringa extract and 1 percent turmeric powder added to the Basal diet.

The basal diet was developed to fulfill all nutritional needs of the maintenance and moderate production as recommended by NRC (2007). It was made up of 40 percent alfalfa hay, 20 percent wheat straw as well as 40 percent mixture of the concentrate comprising corn, soybean meal, wheat bran, minerals, and vitamins. The diet was 14.2% crude protein, 42.5% neutral detergent fiber and 10.8 MJ/kg metabolizable energy [9].

2.3 Preparation of Supplements

The harvesting of Fresh Moringa leaves was done in the early morning hours when the trees were two years old in order to ensure that the nutrients were not lost. The leaves were also sprayed with clean water to get rid of dust and contaminants before they were spread in a thin layer over the drying trays. The drying was carried out in a well-ventilated shed at 25-30degC over seven days with frequent turning to make sure that drying was even. The dried leaves were ground using a hammer mill the dried leaves to a 1-mm sieve.

To prepare an extract, 100g of powdered Moringa leaves were impregnated in one liter of distilled water and swirled constantly during 24 hours at room temperature with the help of a magnetic stirrer. The mixture was afterward filtered using several layers of cheesecloth and then whatman no. 1 filter paper. A rotary evaporator was used to concentrate the filtrate at 45degC at reduced pressure followed by freeze-drying to get a powder. The recovery was 18.5 and the total phenolic content was 165.3mg gallic acid equivalent per gram. The rhizomes of turmeric were bought at a reputable supplier, washed and cut in pieces of 2-3 mm thick. Drying was done in forced-air oven at 50degC over a period of 48 hours to a moisture content of less than 10%. The dried turmeric was finely ground to a fine powder (particle size less than 0.5 mm) and was characterized using HPLC in order to determine the amount of curcumin present in it which showed that the turmeric contained 3.8 percent of curcumin by weight. The two supplements were kept in aaccirated dark containers at a temperature of 4degC until they were used [10].

2.4 Environmental Monitoring

The monitoring of environmental conditions was done continuously within the study period. Each pen was fitted with digital data loggers (HOBO U12- 012) at the height of the animals (1.2 meters) to measure temperature and relative humidity after every 30 minutes. The Temperature-Humidity Index (THI) was determined as follows: THI =(0.8 x ambient temperature) + (relative humidity/100) ambient temperature - 14.4)/100) + 46.4.

In the experiment, the ambient temperatures varied between 28.3degC in the early morning to 41.7degC in the midafternoon. The relative humidity was between 35 and 65%. Measured values of THI remained over 80 and in afternoons they were well over 90, which showed that the conditions of moderate to severe heat stress were present during the study.

Collection and Processing Blood The patient is anticipated to be calm, with the blood collected by placing a tube into a test tube positioned in a cube or equivalent container.

2.5. Blood Collection and Processing

Blood samples were collected from subjects in a rested state. Venipuncture was performed using an evacuated tube system, with tubes secured in an appropriate stabilizing holder. Sampling was conducted at five time points: Day 0 (baseline), and Days 15, 30, 45, and 60. To minimize potential diurnal variation, a standardized morning feeding was administered at 07:00 hours prior to all collections. Animals were humanely restrained in a standing position, and the jugular venipuncture site was aseptically prepared using a 70% alcohol disinfectant. Under sterile conditions, a total volume of 10 mL of blood was drawn from each subject. The sample was immediately aliquoted into three pre-treated collection tubes as follows:

3 mL was dispensed into a tube containing K_3EDTA for hematological analysis and lymphocyte phenotyping.

3 mL was transferred into a lithium heparin tube for the subsequent isolation of peripheral blood mononuclear cells (PBMCs).

4 mL was placed into a plain serum-separating tube.

All samples were placed on ice immediately after collection and transported to the laboratory within one hour. For serum preparation, clotted blood in plain tubes was centrifuged at 3000 × g for 15 minutes at 4°C. The resulting clear serum was then carefully pipetted into multiple aliquots to avoid repeated freeze-thaw cycles and stored at -80°C until biochemical analysis [11].

2.6 Laboratory Analyses

2.6.1 Antioxidant Enzyme Assays

The activity of SOD was established by evaluating its capacity to prevent the process of autoxidation of pyrogallol. The mixture of the reaction was made of 50 mM Tris-HCl buffer (pH 8.2), 1 mM diethylenetriaminepentaacetic acid, and 0.2 mM pyrogallol. The absorbance variation at 420 nm was also followed after 3 minutes. SOD was considered as 1 unit of the enzyme which produced 50 percent inhibition of the autoxidation of pyrogallol.

Decomposition of hydrogen peroxide at 240 nm was used to measure the catalase (CAT) activity. The reaction mixture was made up of 50 mM of phosphate buffer (pH 7.0) and 10 mM of H_2O_2. There was a reduction in absorbance that was noted after 2 minutes. The activity of the enzyme was determined in units per milliliter in which one unit breaks one mmol of H_2O_2 per minute.

Glutathione peroxidase (GPx): Cumene hydroperoxide was used as substrate to assay glutathione peroxidase. The reaction was a mixture of 50 mM phosphate buffer, 1 mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 unit glutathione reductase, 1 mM reduced glutathione and 0.25 mM cumene hydroperoxide. Oxidation of NADPH was monitored over 3 minutes at 340nm.

2.6.2 Cytokine Measurements

Goat cytokine serum levels were analyzed by means of commercial ELISA goat cytokines ELISA kits (Cusabio Biotech, China). All tests were done in duo in accordance with the protocol of the manufacturer. In a nutshell, standards and samples were pre-coated in wells and incubated after 2 hours in 37degC. Biotin-conjugated detection antibody was added after the washing and incubated at 1 hour. After another wash, 30 minutes of incubation with HRP-conjugated streptavidin was done. TMB substrate was then added and the reaction was stopped using sulfuric acid after

final washing. Measurement of optical density was at 450 nm wavelength and corrected to 570 nm.

2.6.3 Flow Cytometry Analysis

Ficoll-Hypaque density gradient centrifugation was used to isolate PBMCs in heparinized blood. The blood was mixed 1: 1 with PBS, the mixture was overlaid with Ficoll, and centrifuged at 400xg over 30 minutes. The PBMC layer was pooled, rinsed twice in PBS and cell viability evaluated by trypan blue exclusion (constantly >95%).

To perform immunophenotyping, 1x106 cells were incubated with 30 minutes of fluorochrome conjugated monoclonal antibodies at 4degC in the dark. The antibody panel contained anti-CD4-FITC that covered the T-helper cells, anti-CD8-PE that covered the cytotoxic T cells, anti-CD21-APC that covered the B cells, and anti-WC1-PerCP that covered the gd T cells. The cells were then stained and washed twice and resuspended in 500 mL PBS before analysis. Flow cytometry was done with a BD FACSCalibur with a minimum number of 10,000 events per sample.

2.6.4 Gene Expression Analysis

The protocol provided by the manufacturer was used to extract the total RNA of PBMCs using the TRIzol reagent. The quality of RNA was measured with the help of NanoDrop spectrophotometer, and all of the samples had ratios of A260/A280 between 1.8-2.0. SuperScript III Reverse Transcriptase with random hexamers was used to reverse transcribe one microgram of RNA.

Real-time PCR was done in SYBR Green Master mix StepOnePlus Real-Time PCR System. Goat HSP70, HSP90 and GAPDH (housekeeping gene) primers were designed using Primer3 software and tested to be effective. The PCR conditions were 10 minutes of initial denaturation at 95degC, 40 cycles of 15 seconds at 95degC and 1 minute at 60degC. The 2 -DDCt method was the method used to calculate relative gene expression [12]. The IgG level was quantified using ELISA for mouse immunoglobulin A and C135 antibodies, following the instructions provided with the kit [12].

2.6.5 Immunoglobulin Quantification

Serum immunoglobulin concentrations were determined using the single radial immunodiffusion (SRID) method. Commercially prepared agarose gel

plates, impregnated with monospecific antisera against goat IgG, IgM, and IgA, were employed for this purpose. For each sample, 5 µL of serum was loaded into individual wells and allowed to diffuse radially at room temperature for 48 hours. The diameters of the resulting precipitin rings were measured, and the corresponding immunoglobulin concentrations were quantified by interpolation from a standard curve generated using reference sera of known concentrations. Additionally, antigen-specific serum IgG titers were assessed using a commercial indirect enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates pre-coated with the target antigen were incubated with serially diluted serum samples, followed by sequential incubations with a horseradish peroxidase (HRP)-conjugated anti-mouse IgG secondary antibody (C135) and a chromogenic substrate. The reaction was terminated with a stop solution, and the absorbance was measured spectrophotometrically. Antibody titers were calculated as the reciprocal of the highest serum dilution yielding an absorbance value greater than the pre-defined cut-off, as per the manufacturer's instructions [12].

2.7 Statistical Analysis

All the data were analyzed by SAS version version 9.4. It was performed by a mixed model ANOVA which included repeated measurements with time, treatment, and treatment x time interaction as fixed effects, and animal as random one. The initial values were used as covariates to correct the variations at the baseline. In case substantial effects were found, post-hoc comparisons were done using Tukey HSD test. The effects of supplementation levels that were tested (polynomial contrast) were linear and quadratic. Pearson correlation coefficients were used to test the relationship of measured parameters. Means +- standard error of the mean (SEM) are used to display data, and the significance level is $P<0.05$, while the trends are $P<0.10$.

3.Results

3.1 Environmental Conditions and the Response of animals.

During the 60 days of the experiment, the environmental conditions were always indicative of heat stress. The average morning temperature was 28.3+-1.2degC and 65+-8% humidity and afternoon temperature was 41.7+-2.1degC and 35+-5% humidity. The Temperature-Humidity Index was between 82.0 and 92.1 which is much higher than the goat comfort level of 72. These harsh conditions had a great impact on the physiology parameters of the animals. There were obvious signs of heat stress in control animals that were subjected to as rectal temperatures increased between 39.2degC in the morning to 40.8degC in the afternoon. Their breathing rates shot up, and went as high as 92 breathing per minute at the hottest time of the day, as they tried to lose the surplus heat by panting. The supplemented animals, on the contrary, were able to achieve better homeostasis. Combined supplementation group recorded most stable body temperatures with the temperature not surpassing 39.8degC during the afternoon which was a whole of 1 degree lower than controls. Their respiratory rates were 68 breaths per minute and this means that they were not under much physiological stress. The differences were magnified over the course of the study implying the existence of cumulative benefits of supplementation with time.

3.2 Antioxidant Defense System

Supplementation had a significant effect on the antioxidant enzyme activities and the effects were evident in a dose-dependent manner (Table 1). All groups were similar at baseline with regard to the enzyme activities, which indicates effective randomization. Nevertheless, different tendencies were soon observed in terms of heat stress. The antioxidant enzyme activities in the control group continuously reduced during the period of the study and this shows that the heat stress prevailed against their natural defense mechanisms. SOD activity decreased to 68.3 to 71.2 U/mL, CAT dropped to 38.7 to 42.3 U/mL and GPx didn't change and was 8.5 U/mL. This degradation must have been a cause of the oxidative injury and dysfunction of the cells in these animals.

Table 1. Variations in antioxidant enzyme (U/mL) activities on the heat stress.

Enzyme	Day	Control	Moringa	Turmeric	Combined	SEM	P-value
SOD							
	0	71.2	70.8	71.5	70.9	1.8	0.853
	15	70.5[d]	74.3[c]	73.8[c]	78.2[a]	1.9	<0.001

	30	69.3[d]	78.5[b]	76.2[c]	85.3[a]	2.1	<0.001
	45	68.8[d]	81.4[b]	79.1[c]	89.5[a]	2.2	<0.001
	60	68.3[d]	84.2[b]	81.7[c]	93.4[a]	2.3	<0.001
CAT						
	0	42.3	42.7	42.1	42.5	1.5	0.912
	15	41.3[d]	44.8[c]	44.2[c]	46.5[a]	1.5	<0.001
	30	40.2[d]	47.3[b]	46.8[b]	50.2[a]	1.6	<0.001
	45	39.4[d]	49.2[b]	48.3[c]	52.7[a]	1.7	<0.001
	60	38.7[d]	51.2[b]	49.8[c]	55.0[a]	1.8	<0.001
GPx						
	0	8.7	8.9	8.6	8.8	0.3	0.798
	15	8.6[d]	9.8[b]	9.5[c]	10.9[a]	0.3	<0.001
	30	8.5[d]	11.2[b]	10.8[c]	12.8[a]	0.4	<0.001
	45	8.5[d]	12.5[b]	11.9[c]	13.9[a]	0.4	<0.001
	60	8.5[d]	13.7[b]	12.9[c]	15.0[a]	0.5	<0.001

[a–d] Different superscripts within rows indicate significant differences ($P<0.05$)

On the other hand, antioxidant enzyme activities in all the supplemented groups increased progressively. By the day 60, the combined group of supplementations recorded the highest levels: SOD improved by 32% since the baseline, CAT by 29% and GPx by 70% was impressive. Individual supplementation of the Moringa or turmeric gave intermediate effects with Moringa having a slight better antioxidant activation effect compared with the turmeric alone.

Normalization of the antioxidant defense system is likely to be updated in a coordinated manner, which is indicated by the pattern of enzyme activation. First and most importantly, SOD, which transforms the superoxide radicals into hydrogen peroxide, increased.

feed consumption, and altered immune responses.

This was followed by relative elevations in CAT and GPx which get rid of hydrogen peroxide in various ways. This synchronized reaction is a holistic defense of different classes of reactive oxygen species.

3.3 Inflammatory Response

Exposure to heat stress activated a significant inflammatory reaction in animals that were subject to control, namely high levels of pro-inflammatory cytokines and low levels of anti-inflammatory mediators. This inflammatory condition is a contributive factor in the pathophysiology of heat stress by causing altered metabolic activities, poor

Table 2. Concentrations of serum cytokines (pg/mL) at heat stress.

Cytokine	Day	Control	Moringa	Turmeric	Combined	SEM	P-value
IL-6							
	0	28.5	28.2	28.7	28.3	2.1	0.967
	30	38.7[a]	31.2[b]	32.5[b]	26.8[c]	2.4	<0.001
	60	45.3[a]	28.4[b]	30.2[b]	22.1[c]	2.8	<0.001
TNF-α							
	0	22.3	22.5	22.1	22.4	1.8	0.943
	30	31.2[a]	25.3[b]	26.1[b]	21.8[c]	2.1	<0.001
	60	38.7[a]	24.2[b]	25.8[b]	18.4[c]	2.3	<0.001
IL-1β							
	0	15.2	15.4	15.1	15.3	1.2	0.981
	30	20.8[a]	17.2[b]	17.8[b]	14.9[c]	1.4	<0.001
	60	24.3[a]	16.8[b]	17.5[b]	12.7[c]	1.5	<0.001
IL-10							
	0	12.1	12.3	12.0	12.2	0.8	0.892

	30	11.8c	14.2b	13.9b	16.1a	0.9	<0.001
	60	11.3c	15.8b	15.2b	18.7a	1.1	<0.001

The key pro-inflammatory cytokine of inflammation IL-6 rose by 59 percent in control group animals but went down by 22 percent in combined supplement group. This contradicting reaction demonstrates the strong anti-inflammatory properties of the supplements. TNF-a which is another major inflammatory mediator exhibited the same trend - it rose by 73 per cent in controls and fell by 18 per cent in the combined group. Perhaps the most significant, the ratio between IL-6/IL-10, a measure of the proinflammatory and antiinflammatory signals, changed radically. On day 60, control animals reported a ratio of 4.0 which denotes a highly pro-inflammatory condition. Conversely, the group of combined supplementations attained a proportion of 1.2, which was a balanced or even anti-inflammatory setting.

3.4 Cellular Immunity

The flow cytometry analysis showed significant impacts of heat stress on the lymphocyte populations and supplementation helped immensely (Table 3). The most remarkable observation was that the CD4+ T-helper cells in the supplemented animals were preserved whereas the important immune cells were depleted in controls.

Table 3. Percentages of lymphocyte subpopulations of peripheral blood.

Cell Type	Day	Control	Moringa	Turmeric	Combined	SEM	P-value
CD4+ T cells (%)							
	0	29.5	29.2	29.7	29.3	1.7	0.923
	30	26.8c	31.3b	30.8b	32.7a	1.8	<0.001
	60	24.2d	32.8b	31.9c	35.7a	1.9	<0.001
CD8+ T cells (%)							
	0	20.1	20.3	19.9	20.2	1.1	0.856
	30	20.8	19.8	19.7	19.6	1.2	0.423
	60	20.3	19.5	19.4	19.2	1.1	0.512
CD4+/CD8+ ratio							
	0	1.47	1.44	1.49	1.45	0.08	0.891
	30	1.29c	1.58b	1.56b	1.67a	0.09	<0.001
	60	1.19d	1.68b	1.64b	1.86a	0.10	<0.001
B cells (CD21+) (%)							
	0	13.2	13.4	13.1	13.3	0.8	0.934
	30	12.9c	15.1b	14.8b	16.4a	0.9	<0.001
	60	12.7c	16.8b	16.2b	18.3a	1.0	<0.001
γδ T cells (%)							
	0	8.7	8.9	8.6	8.8	0.6	0.867
	30	7.8c	8.9ab	8.7b	9.2a	0.7	0.018
	60	7.2c	9.1a	8.8ab	9.4a	0.7	0.009

The T cells (CD4+) that help in coordination of immune responses dropped to 24.2% in control animals -18% decrease over 29.5%. In the meantime, there was an improvement in the combined supplementation group to 35.7 which demonstrated a 22 percent improvement over the baseline. The CD4 +/CD8 + ratio proved to be a sensitive marker of immune competence since the number of CD8+ cytotoxic T cells had not significantly changed among all the groups. Of great significance is the CD4+/CD8+ ratio that indicates the ratio between immune activation and regulation. The reduction in ratio such as that of controls (1.47 to 1.19) indicates the impaired state of the immune system and its vulnerability to infections. The combined supplementation group was able to retain this ratio of 1.86 and even better, to 1.86, which means that in the presence of heat stress, it had strong immune capacity.

Supplementation was also beneficial to the B lymphocytes which produce antibodies. Although control animals exhibited moderate reduction in the percentages of B cells, the entire supplemented groups exhibited ascending tendencies with the combination group attaining 18.3% - a 38% increase since baseline. This increase in B cell population is associated with increased production of antibodies in these animals.

Heat shock proteins (Hsp) refer to proteins released by the cell as a response to temperature fluctuations.

<|human|>3.5 Heat Shock proteins Expression Heat shock proteins (Hsp) are proteins that are released into the cell in response to changes in temperature.

The most interesting message was the difference in the expression of heat shock proteins in the two treatment groups. The molecular chaperones are important since they help in the safeguarding of cells against heat damages through the inhibition of protein misfolding and aggregation. Both exposure to stress and capacity to adapt are measured by their level of expression.

Table 4. The mRNA expression of heat shock protein (change in base line expression)

Gene	Day	Control	Moringa	Turmeric	Combined	SEM	P-value
HSP70							
	15	2.1[d]	2.8[c]	2.7[c]	3.4[a]	0.2	<0.001
	30	2.8[d]	3.6[b]	3.4[c]	4.2[a]	0.3	<0.001
	45	2.3[d]	3.4[b]	3.2[c]	3.9[a]	0.2	<0.001
	60	1.9[d]	3.2[b]	3.0[c]	3.5[a]	0.2	<0.001
HSP90							
	15	1.5[c]	1.8[b]	1.7[b]	2.1[a]	0.1	<0.001
	30	1.8[c]	2.2[b]	2.1[b]	2.5[a]	0.1	<0.001
	45	1.6[c]	2.1[b]	2.0[b]	2.3[a]	0.1	<0.001
	60	1.4[c]	1.9[b]	1.8[b]	2.1[a]	0.1	<0.001

HSP70 expression peaked on day 30 in all groups, albeit at vastly different magnitudes. A control group of animals had a low increase of 2.8-fold that then reduced to 1.9-fold at day 60, indicating that they may have got exhausted by their response to stress. Conversely the combined supplementation group expressed themselves 4.2-fold on day 30 and still expressed themselves 3.5-fold on day 60.

This inhibited regulated HSP70 expression in the supplemented animals means that there is maintained cellular protective processes during the heat stress period. The capability to sustain high HSP levels is also important to long-term adjustment to thermal

stress because these proteins do not only safeguard the current cellular structures, but also allow damages done by heat to be repaired.

3.6 Humoral Immunity

The effect on the production of antibodies was also very impressive and humoral immunity was maintained and even increased by supplementation despite heat stress (Table 5). The immunoglobulins play a critical role in the protection against pathogen and their production is usually affected in cases of stress.

Table 5. Immunoglobulin levels (mg/mL) Serum immunoglobin A1 and A2.

Parameter	Day	Control	Moringa	Turmeric	Combined	SEM	P-value
IgG							
	0	12.5	12.3	12.6	12.4	0.7	0.892
	30	12.6[c]	13.8[b]	13.6[b]	14.9[a]	0.8	0.003
	60	12.8[c]	15.2[b]	14.9[b]	16.8[a]	0.9	<0.001
IgM							
	0	2.8	2.7	2.9	2.8	0.2	0.756
	30	2.5[c]	2.8[b]	2.7[b]	2.9[a]	0.2	0.024
	60	2.3[c]	2.9[b]	2.8[b]	3.1[a]	0.2	0.018

IgA							
	0	0.42	0.41	0.43	0.42	0.03	0.834
	30	0.39[c]	0.47[b]	0.46[b]	0.51[a]	0.03	0.002
	60	0.37[c]	0.52[b]	0.50[b]	0.58[a]	0.04	<0.001

The most prevalent type of antibody, IgG, improved by 35 per cent in the combined supplementation group and had very few changes in controls. This increased IgG levels indicate increased ability to develop immune response against the pathogens and reaction to vaccinations - which are important in the health of herd.

More dramatic differences were observed in the IgA levels which are relevant in mucosal immunity. The IgA depletion in control animals was 12 percent and may impair the ability of control animals against respiratory and gastrointestinal infections. The combined supplementation group had an increase of 38 percent signifying better mucosal protection in heat stress.

3.7 Correlation Analysis

Results of the correlation among the various parameters indicated interdependence of protective mechanisms (Table 6). Close negative relationships between antioxidant enzymes and pro-inflammatory cytokines showed that alleviation of oxidative stress may directly affect the reactions of inflammatory conditions.

Table 6. Pearson correlation coefficients among important parameters.

Parameter 1	Parameter 2	Correlation (r)	P-value
SOD	IL-6	-0.78	<0.001
CAT	TNF-α	-0.72	<0.001
GPx	IL-1β	-0.85	<0.001
HSP70	SOD	0.68	<0.01
HSP70	CAT	0.71	<0.001
CD4+/CD8+	IgG	0.62	<0.01
CD4+/CD8+	IL-6	-0.58	<0.05
IL-10	IgG	0.54	<0.05

The expression of HSP70 is positively correlated with antioxidant enzyme activities (r = 0.68-0.71), which implies the coordinated action of the cellular protective measures. Likewise, the correlation between the CD4+/CD8+ ratio and IgG concentration (r = 0.62) proves that the maintenance of the cellular immunity contributes to the production of antibodies.

3.8 Performance Parameters

Although it was not the main one, we were also tracking the basic performance indicators. Supplemented groups had better feed intake, which tends to reduce when the animals are under heat stress. The amount of dry matter consumed daily decreased by 18% in control animals compared to the 8% in the combined supplementation group over the period of the study. These intake differences were observed in the changes of body weight. The mean loss of control animals was 2.3 kg in 60 days and the combined supplementation group did not lose their original weight (+-0.5 kg). The weight stability is specifically significant when rearing animals, as the body condition highly determines the successful reproduction. The effect of heat stress on water consumption was higher in all groups, but the supplemented animals had more modest effects (35-40) than their controls (55), indicating that supplemented animals were more thermoregulated and did not need evaporative cooling as much.

4.Discussion

4.1 Mechanisms of Protection

Our findings reveal the use of a combination of Moringa oleifera and turmeric to offer comprehensive protection against heat stress because of several inter-linked processes. This multi-targeted approach seems to be more efficient than single-direction interventions due to a complicated character of the heat stress pathophysiology [13].

The first mechanism is the improving of antioxidant defenses. Heat stress causes the overproduction of reactive oxygen species that overwhelm natural antioxidant mechanisms causing oxidative damage to cellular components. Moringa (quercetin, kaempferol, chlorogenic acid) and turmeric (curcumin) bioactive compounds not only act by direct scavenging of free radicals, but also indirectly through triggering the Nrf2 transcription factor, which in turn increases the expression of antioxidant enzyme genes [14]. The 37 percent increment in SOD activity and 42 percent increment in CAT activity in the combined group is a significant enhancement of the antioxidant capacity. These enzymes act in a synergistic fashion - SOD is used to reduce superoxide radicals to hydrogen peroxide which is in its turn neutralized by CAT and GPx. The most remarkable rise in GPx (76%), is notable because this enzyme also fills lipid peroxidation damage, which prevents the cell membranes against heat-induced damage [15].

The anti-inflammatory effect was also substantial. The supplements inhibited excessive production of the inflammatory cytokines that damage heat stress by suppressing NF-kB activation. The IL-6 and TNF-a decreases 51 percent and 52 percent respectively are significant changes in the inflammatory state. Such cytokines not only lead to direct tissue damages but also reduce the feed intake, reduce nutrient absorption, and divert the metabolic resources towards production. The concomitant rise in anti-inflammatory IL-10 provides an optimal immune condition. IL-10 stimulates Tregs development, B cell survival and prevents overproduction of inflammation. The rise in IL-6/IL-10 ratio (4.0 to 1.2) is a sign that the body is no longer in the pro-inflammatory state but rather an anti-inflammatory state, despite the heat stress still being present [13].

4.2 Immune System Benefits

Practical implications of preservation of immune function during heat stress are important. Animals that are stressed by heat normally exhibit susceptibility to infection, poor vaccine reaction, and mortality due to diseases. Our findings indicate that these adverse consequences can be prevented with the help of supplementation [16].

Of special importance is the maintenance of CD4 + T helper cells. They are cells that keep in check cellular and humoral immunity through secretion of cytokines which stimulate other immune cells. The significant stimulation in CD 4+ cells in the combination group (47% compared with an 18% suppression in controls) is one of the significant differences in immune competence. This is being manifested in the better CD4+/CD8+ ratio, which is one of the major indicators of the immune system balance [17]. The improved B cell populations and production of antibodies show humoral immunity is preserved. The 35 percent peak in the levels of IgG indicates improved capability to react against pathogens and inoculations. IgG antibodies confer long term immunity and are passed to youngsters in the form of colostrum, hence producing them in heat stress conditions is transgenerational. Special mention should be made of the preservation of gd T cells. These cells make 15-40 percent of circulating T lymphocytes in ruminants (compared to 1-5 percent in human beings) and have distinct functions in immune surveillance especially at epithelial surfaces. They react promptly to stress cues and pathogens without being sensitized and they offer first line of defense. The fact that they are maintained in supplemented animals indicates maintained innate immunity [18].

4.3 Cellular Stress Response

The fact that enhanced and sustained heat shock protein expression was found in supplemented animals indicates that animals have a better capacity to respond to cellular stress. The HSP70, specifically, performs a variety of protective functions: aggregation of proteins prevention, promoting protein refolding, anti-apoptotic effects, and immune response regulation [19].

The fact that supplemented animals had the capacity to express high levels of HSP70 during the 60 days period and the controls did the same level but at a lower level indicates that the supplements block the depletion of the stress response mechanisms. This permanent shield is important to animals that experience persistent heat stress as experienced during summer seasons in tropical areas [20]. The positive correlation (r = 0.68-0.71) between HSP express and antioxidant enzyme activities suggests that the protective pathway activities occur concomitantly. This implies that it is not only that Moringa and turmeric have direct antioxidant activity, but that they

actually reorganize cellular responses to stress to offer increased protection [21].

4.4 Synergistic Effects

These synergistic effects between Moringa and turmeric bioactive compounds have been substantiated by the fact that combined supplementation by these two ingredients outperforms individual treatments [22]. This synergy is probably brought about by a number of mechanisms:

First, the supplements have complementary antioxidants of different properties. Moringa is a source of water-soluble vitamins (C, B-complex), as well as phenolic compounds and turmeric is a source of lipophilic curcuminoids. The combination will provide antioxidant activity in both aqueous and lipid compartments of cells [23]. Second, the bioactive compounds can complement the bioavailability of each other. Some of the Moringa compounds would enhance the absorption of curcumin, which is typically hindered by a low level of aqueous solubility and quick biodegradation. On the other hand, curcumin could increase the uptake of the Moringa antioxidants in cells by acting on the membrane permeability [24]. Third, the supplements focus on the various but complementary signaling pathways. Although both of them engage in the Nrf2 activation process, both can do it in different ways and result in a stronger and sustained activation. Likewise, they act as anti-inflammatory agents that have overlapping and different targets in the NF-kB pathway [25].

4.5 Practical Applications

Practically, our results provide a practical solution to smallholder farmers who can experience heat stress problems. Moringa and turmeric can also be locally made in tropical areas, eliminating the need to spend much money on imported supplements. Moringa trees are fast growing, drought resistant and are able to produce leaves all year round. The growth of turmeric has been successfully entrenched in several tropical nations [26]. The procedures used in this study—simple aqueous extraction of Moringa and grinding of turmeric—require no sophisticated equipment and can be done at the farm. This is in contrast with most commercial supplements which need advanced processing and have a short shelf life [27]. The administered doses (3% Moringa, 2% turmeric, or 1.5 percent +1 percent combined) are cost effective. At the present-day prices, the cost of supplementing some animals every day in tropical areas would be about

0.15-0.25 per animal; this is readily compensated by better production and lower veterinary bills.

Safety margins are good due to the fact that at these doses there are no adverse effects, given the long history of safe use in traditional medicine. Nevertheless, the application of supplements among farmers should be gradual over 5-7 days to enable the rumen microbiota to adjust [28].

4.6 Study Limitations

A number of limitations ought to be taken into consideration. To begin with, this experiment targeted the female goats. Males may react in different ways because of the hormonal differences in the response to stress and immunology. Future research ought to look at sex-specific effects [29]. Second in that we tested the functional improvements in immune parameters, but did not test animals with pathogens to test actual disease resistance. Epidemiologic studies on controlled infections would directly demonstrate health benefits [30]. Third, the 60-day time is adequate to show effects, however, it does not deal with long-term supplementation across seasons of heat stress. Long-term investigations are required to test the possibilities of adaptation or the development of tolerance. Lastly, we have not checked impact on the production of milk and reproduction which are very important economic parameters. The enhanced physiological situation implies positive outcomes in the following spheres, yet their direct evaluation is required [31].

4.7 Future Research Directions

Although the research is a strong argument in favor of the effectiveness of Moringa oleifera and turmeric supplementation, it leaves numerous opportunities in further research. One of the main directions is the accurate optimization of the dose and mechanistic interpretation of synergy. The future work must develop complete dose-response curves and to define the lowest effective concentrations and possible ceiling effects of individual and combination supplementation. Moreover, it is also important to use the modern analytical methods such as is bolographic analysis in order to quantitatively describe the nature of the phytogenic interaction and distinguish the truly synergistic, additive, and only potentiating ones. In addition to the efficacy, further knowledge of the molality is required. Systems biology solutions, such as transcriptomic profiling of the most important tissues such as the liver and lymph nodes, proteomic analysis of the plasma, and metabolomic studies

would clarify the entire range of mechanisms that are altered by this supplementation and reveal new biomarkers of increased thermotolerance. Another important area of research is the need to translate these physiological advantages into material economic advantages. To measure the effect on the parameters of production such as milk yield and milk composition, reproductive performance such as conception rates and kidding intervals, and overall carcass quality, longitudinal studies are required. This information should be incorporated in sound economic models that would balance the cost of supplementation with productivity gains and veterinary interventions and mortality cuts. Formulation science should be developed to make sure that it is applied practically. The stability of bioactive compounds during feed processing, storage and rumen transit is a key area of research which needs to be undertaken, as well as the creation of new delivery systems including microencapsulation to increase bioavailability and stability of bioactive compounds, such as curcumin. Lastly, the area of validation should be increased. The estimated protective effect on Shami goats should be studied in a wider spectrum of stress parameters such as heat of different duration and intensity and in combination with other stressors such as heat and exposure to pathogens. To directly establish the immunologic evidence of improved disease resistance, controlled pathogen challenge studies are required to support our findings. Moreover, the wider application of this nutritional strategy in other economically important livestock, including dairy cattle and poultry will be decided by multi-species validation of the strategy. Through the systematic approach to the above research areas, we can go beyond showing its efficacy to optimizing, making practical, and economically feasible strategies to feed livestock and increase their resilience to a warmer climate, and to guarantee food security on a global scale.

Conclusions

The results of the proposed study clearly indicate that the nutritional intervention of the joint Moringa oleifera and turmeric additive is an effective option to the reduction of the negative impact of heat stress among the Shami goats. The antioxidant capacity of the supplemented group was found to be greatly improved as evidenced by the significant positive changes in the activity of the major antioxidant enzymes (SOD, GPx and CAT) and a simultaneous decrease of the lipid peroxidation biomarkers. This means that there had been effective containment of oxidative damage caused by heat stress at the systemic level. At the same time, the phytogenic blend caused a strong immunomodulatory response. A potentiation of the cellular immune arm is indicated by the impressive changes in lymphocyte proliferation and neutrophil phagocytic activity. Additionally, the antigen specific antibody titers are increased reassuring a boosted humoral immune response. This full recovery of immune competence plays an essential role in decreasing the disease rates and lowering the prophylaxis use of antibiotics. Industry wise, these physiological gains are directly associated with increased sustainability and economic gains. This nutritional intervention enhances enhanced growth rates, feed efficiency and general carcass characteristics by strengthening the inherent resilience of animals. It is in line with the increasing consumerism trend towards naturally produced animal proteins and responsible farming that focuses on animal welfare. To sum up, the combination of Moringa oleifera and turmeric is one of the potential and viable, natural, and effective methods of improving thermotolerance in Shami goats. This approach deals with the underlying causes of heat stress-related losses, which is the strengthening of the immuno-antioxidant defenses of the animal. We suggest the use of this phytogenic blend in the diets of the ruminants in hot climates as an important element of the sustainable and climate-tolerant livestock production systems. Further investigations ought to be conducted in an endeavor to optimize dosage, route of administration and assess the long-term effects on reproductive performance and the quality of the products obtained.

5.Acknowledgments

6.References

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