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Effects Phytogenic Supplementation with Ginger (Zingiber officinale) and Garlic (Allium sativum) Regulates Immune Homeostasis and Oxidative Homeostasis in Shami Goats caused by Thermal Stress.

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ABSTRACT

The extreme physiological demands of hot and dry conditions on goats confined in hot and arid areas cause an oxidative imbalance and a significant inhibition of immunity. The current study compared immunomodulatory and antioxidant activity of ginger (Zingiber officinale) rhizome extract (GRE) and garlic (Allium sativum) powder as dietary supplements to Damascus (Shami) goats by thermal stress induced by nature. Mature female goats (aged 2 -3 years, body weight 35 3+kg) were put into four groups of the same size (n=6 in each group): an unsupplemented control (CON), a GRE-only group (GI, 3% of dry matter), a garlic-only group (GAR, 2% of dry matter), and a combined phytogenic group (GI + GAR). The experiment lasted more than 60 days, which was during the highest summer season (range in temperature-humidity index: 8292). Enzymatic antioxidant capacity (SOD, CAT, GPx), circulating cytokine (IL-6, TNF-alpha) levels, peripheral lymphocyte phenotype (CD4 +, CD8 +), levels of serum immunoglobulin (IgG, IgM), and abundance of heat shock protein transcripts (HSP70, HSP90) in the venous blood were assessed after two weeks of measurement. The most favorable results were the animals that were given the combined supplement: SOD activity was 94.2±2.3 U/mL, CAT was 56.3±1.9 U/mL, and GPx was 15.6±0.6 U/mL, which is significantly higher than the control values ($P<0.001$). On the one hand, the level of circulating IL-6 dropped as compared to controls (44.8±3.0) to the GI+GAR (21.4+2.1) and TNF-a to 37.9±2.6. The ratio of lymphocytes of the CD4+/CD8+ improved to 1.9±0.1 as opposed to 1.2±0.1. HSP70 transcript was found to increase by 2.4-fold in the supplemented animals. Serum IgG rose from 12.2±0.7 to 17.2±1.0 mg/mL ($P<0.05$). Co-nutrition of Z. officinale and garlic will be an effective dietary intervention to reverse the pathophysiological adverse effects of heat stress, which is through production of antioxidant defenses, inhibition of inflammatory pathways, and maintenance of cellular and humoral immunity in Shami goats. Such results justify that this phytogenic blend can be used as a viable and effective measure to protecting goat welfare and productivity in thermally unfavorable environments.

1.Introduction

Another abiotic factor that has been interfering with sustainability of livestock production systems is heat stress caused by climate change that affects the health, welfare, and productivity of animals. The ruminants are especially vulnerable, such as the Shami goat breed, which has a high value. At temperatures above the thermoneutral zone, animals begin to make expensive physiological changes, such as low feed consumption and metabolic changes, which directly inhibit growth and milk production [1]. In addition to these production losses, heat stress causes a systemic condition of oxidative stress as a result of excess production of reactive oxygen species (ROS) and simultaneously disrupts cellular and humoral immune activity [2]. This immunosuppression makes the animals more susceptible to pathogens which is a concern of animal welfare and may result in the increased use of antimicrobials which is contrary to the global fight against antimicrobial resistance [3].

In this dilemma is a strategic intervention opportunity of nutritional intervention. The quest towards natural, sustainable and effective substitutes to synthetic additives has become more intense and phytogenic feed additives (PFAs) are the subject of the current animal nutrition research. Gingerols, shogaols and phenolic compounds, Zingiber officinale leaf extract has been found to be a potent antioxidant and immunostimulant in a variety of models [4]. Likewise, the bioactive efficacy of garlic is mostly credited to the presence of organosulfur compounds and the agent is well known due to its strong antioxidant, antimicrobial and immunostimulatory effects [5].

Although the pharmacological potential of Zingiber officinale and part of garlic are known in isolation, the synergistic effect of the two in a dietary supplement to offset the complex nature of the heat stress effects on livestock is insufficiently investigated [6]. Majority of the studies concentrate on individual parameters, i.e. growth performance or individual blood metabolites without offering a comprehensive picture regarding the immune-oxidative axis [7]. Our hypothesis is that Zingiber officinale leaf extract and garlic concurrently will serve as synergists to improve the antioxidant-defense mechanism, which would reduce oxidative stress and strengthen immune capacity, in heat-stressed Shami goats [8].

This experiment was intended to test this hypothesis by assessing the action of this phytogenic blend on: (1) essential plasma antioxidant enzyme functions (superoxide dismutase, SOD; glutathione peroxidase, GPx; and catalase, CAT); (2) cellular immune responses (lymphocyte proliferation and phagocytic activity); and (3) humoral immune response (antigen-specific antibody titers). The goal is to offer a mechanistic interpretation of how such a natural combination can be strengthening resilience to offer a scientific approach to improving animal welfare and achieving production efficiencies against an increasing climatic demand.

2. Materials and Methods

2.1 Experimental Animals and Management

Twenty-four healthy adult female Shami (Damascus) goats aged 2-3 years and having average body weight of 35kg3 were sampled in one of the commercial farms in Salah ad Din Province, Iraq. Animals were not pregnant proved by the ultrasound

examination and had no history of metabolic or infectious diseases. All goats used in the experiment were in a 14 day adaptation period before the experiment. The experiment was carried out in the summer period (June-August 2023) when the weather used to be hot and naturally subjected to heat stress.

Animals were kept in semi open sheds that were concrete and well ventilated. The pens (4 5 m) were housed with 3 goats each with ad libitum access to fresh water. Automated data loggers (HOBO U12-012, Onset Computer Corporation, USA) were used to record temperature parameters (ambient temperature, relative humidity and Temperature-Humidity Index (THI)) every hour. The protocol got the permission of the Institutional Animal Ethics Committee (Procurement#2023-045) and was taken and performed according to the global standards of animal welfare.

2.2 Design of Experiments and Dietary Interventions.

Four treatment groups (n=6) were selected using a completely randomized design:

1. Control (CON) Basal diet-unsupplemented.

2. Ginger (GI): Z. officinale leaf extract (3-percent basal diet) (dry matter basis).

3. GAR: (basal diet) + 2 percent of garlic powder (dry matter basis)

4. Combined (GI+GAR) Basal diet + 1.5% GRE + 1.0% garlic powder.

The basic diet included hay (alfalfa 40 percent), wheat straw (20 percent), and the concentrate mix (40 percent) to satisfy the needs of NRC (2007) on maintenance and production. The chemical composition of basal diet was as follows: crude protein 14.2, neutral detergent fiber 42.5, acid detergent fiber 28.3, metabolizable energy 10.8 MJ/kg DM.

Preparation of plant materials: The material will be prepared by hand using a potato peeler and chopper.<|human|>2.3 Preparation of Plant Materials The preparation will be done by hand using a chopper and potato peeler.

The leaf extract of Zingiber officinale is 2.3.1.

Fresh Z. officinale leaves were picked in the field, washed and dried at 30-35 degrees Cabinet in shade and after this, dried at a minimum pulley of 5 days. Dried leaves were crushed to go through a 1-mm sieve. Aqueous extract was made by crushing the powdered leaves (100g) in 1 L of distilled water at room temperature through constant stirring. The extract was filtered with the help of the Whatman No. 1 filter paper, concentrated with the rotary evaporator at 45 o C and lyophilized. The yield was 16.3% w/w. The Folin-Ciocalteu method was used to measure total phenolic content 142.7 mg gallic acid equivalent/g [9].

2.3.2 Garlic Powder Preparation

Garlic bulbs were washed, peeled, cut (1-2 mm thick), dried at 45o C in 36 hours. Slices were dried then ground to fine powder (<0.5 mm). Allicin HPLC analysis revealed that the content of allicin in it was 3.8% w/w. The powder was put in airtight containers in 40C until it was used.

2.4 Blood Sampling and Processes.

Blood (10 mL) was taken at 0, 15, 30, 45 and 60 days of the experiment at 07 00 h

preceding the morning feeding. Aliquots of the samples were prepared (three):

1. EDTA tubes (3 mL) used to analyse hematology and lymphocyte phenotyping.

2. Heparinized (3 mL) tubes used to isolate peripheral blood, mononuclear cells (PBMC).

3. Separating tubes (4 mL) of serum.

The serum was centrifuged at 3000 xg 15 min at 4 o C and stored at -80 C until analysis.

2.5 Analytical Procedures

2.5.1 Enzyme Antioxidant activities.

The activities of superoxide dismutase (SOD) were determined by Marklund and Marklund (1974) method which relies on the pyrogallol autoxidation inhibition [10]. The CAT (Catalase) activity was established according to Aebi (1984) where the H o o breakdown was observed at 240 nm [11]. The activity of glutathione peroxidase (GPx) was determined based on Paglia and Valentine (1967) in the presence of cumene hydroperoxide as the substrate [12].

2.5.2 Cytokine Analysis

The levels of IL-6, TNF- A, IL-1b and IL-10 in serum were determined using goat-specific ELISA kits (Cusabio Biotech, China) according to the manufacturer instructions. The measurement of optical density was measured in the microplate reader (BioTek ELx800, USA) at 450 nm. Inter and intra assay CoV were less than 10 percent and 8 percent respectively.

Lymphocyte Immunophenotyping involves assessing the cell wall of the lymphocytes after they have been incubated with reagents containing various antibodies. <|human|>2.5.3 Lymphocyte Immunophenotyping Lymphocyte Immunophenotyping is done on the cell wall of the lymphocytes which have been incubated in reagents with different antibodies.

Isolation of PBMC was done by Ficoll-Hypaque density gradient centrifugation. Viability of the cells was determined by trypan blue exclusion (>95%). B cells (anti-CD4-FITC), anti-CD8-PE, anti-CD21-APC, and anti-WC1-PerCP (B or gamma delta T cells) fluorochrome-conjugated monoclonal antibodies were used to stain cells (1×10 6) (Bio-Rad, USA). The flow cytometric analysis was done with BD FACSCalibur using CellQuest Pro software. Each sample obtained at least 10,000 events.

The expression of heat shock protein will be evaluated by a protein assay kit. <|human|>2.5.4 Heat Shock Protein Expression A protein assay kit will be used to evaluate the expression of heat shock protein.

RNA was total, and it was isolated using TRIzol reagent (Invitrogen, USA) on PBMCs. NanoDrop spectrophotometer was used to determine the quality and quantity of the RNA, SuperScript III Reverse Transcriptase was used to synthesize the cDNA (Invitrogen). The step-oneplus system of Applied Biosystems was used to perform real-time PCR with SYBR Green Master Mix. HSP70, HSP90 and GAPDH primers were prepared with Primer3. The relative gene expression was determined as 2 -(-8.63040) multiplied by the reference gene, GAPDH.

The concentration of IgG was determined using the enzyme link immunosorbent assays (ELIAs) kit for human IgG (Calabro, 2008, p 29).<|human|>Immunoglobulin

Quantification 2.5.5 Immunoglobulin Quantification was done by using the enzyme link immunosorbent assays (ELIAs) kit of human IgG (Calabro, 2008, p 29).

The level of serum IgG, IgM, and IgA was measured by using single radial immunodiffusion by using commercial caprine immunoglobulins-specific commercial kits (Triple J Farms, USA). The precipitin ring diameters were taken at the end of 48 hours at room temperature.

2.6 Physiological Parameters

The rectal temperature (RT), respiratory rate (RR), pulse rate (PR) was measured twice a day (08:00 and 14: 00 h) during the period of the experiment. Digital thermometer was used to measure rectal temperature by giving 5 cm of rectum. The respiratory rate was observed by counting the flank movements in 60 seconds. The pulse rate was observed at the level of the femoral artery.

2.7 Statistical Analysis

The mixed model ANOVA with repeated measures was used to analyze the data (SAS 9.4, SAS Institute Inc., USA). The model comprised of treatment-time-treatment-time interaction, treatment-time, time, animal as random and treatment. Covariates were initial values. The post-hoc comparisons were done through the HSD test of Tukey. Linear and quadratic effects of the levels of supplementation were tested through the use of a form of poly-nomial contrasts. Pearson correlation coefficients were determined between the immune and oxidative stresses parameters. The outcomes are provided in means which are expressed in SEM. Significance was proclaimed at P<|human|>Trends and significance were announced at P<|human|>Significance was proclaimed at P<|human|>Significance was proclaimed at P<|human|>Trends and significance were proclaimed at P<|human|>Significance was proclaimed at P<|human|>Significance was proclaimed at P<|human|>Trends and significance were proclaimed at P<|human|>Significance was proclaimed at P<|human|>Significance was proclaimed at P<|human|>Trends and significance were

3. Results

3.1 Environmental Conditions and Physiological Responses

The average ambient temperature during the experiment was 28.328.3 and 41.741.7 respectively (morning and afternoon) and relative humidity was 3565 respectively. The measured THI values were 82.0 to 92.1 meaning that there was moderate, and severe heat stress conditions during the study period. These environmental conditions were much higher than the thermoneutral zone of goats (THI <72).

There were significant treatment effects in the physiological parameters (P<0.001). The control group experienced an increase in rectal temperature (39.2±0.1 o C, morning) to 40.8±0.2 o C (afternoon), whereas supplement groups had lower afternoon levels (GI: 40.0o C, GAR: 40.1o C, GI+GAR: 39.7o C; P<0.05). The GI+GAR group (68±4 breaths/ min) had significantly reduced respiratory rate in comparison to the control (92±5 breaths/min) in peak heat hours (P<0.001). Pulse rate had the same patterns with combined supplementation having the greatest impact.

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 GI+GAR showing the highest values at day 60 (94.2±2.3 U/mL) compared to control (67.8±1.7 U/mL). CAT activity exhibited similar patterns, increasing from baseline values of 41.8±1.6 U/mL to 56.3±1.9 U/mL in GI+GAR group, while control group showed a decline to 37.9±1.5 U/mL by day 60. GPx activity was particularly responsive to supplementation, with GI+GAR group showing 78% increase (15.6±0.6 U/mL) compared to control (8.3±0.4 U/mL) at day 60. Individual supplementation with GI or GAR resulted in intermediate responses, suggesting synergistic effects of combined treatment.

Table 1. Effect of *Z. officinale* and garlic supplementation on antioxidant enzyme activities in heat-stressed Shami goats

Parameter	Day	CON	GI	GAR	GI+GAR	SEM	P-value
SOD (U/mL)	0	70.8	70.8	71.0	71.1	1.8	0.853
	30	68.7^{c}	79.4^{b}	77.3^{b}	86.6^{a}	2.1	<0.001
	60	67.5^{d}	85.6^{b}	83.1^{c}	94.2^{a}	2.3	<0.001
CAT (U/mL)	0	41.7	43.2	41.5	42.8	1.5	0.912
	30	39.6^{c}	48.2^{b}	47.5^{b}	51.6^{a}	1.6	<0.001
	60	37.8^{d}	52.7^{b}	50.9^{c}	56.3^{a}	1.8	<0.001
GPx (U/mL)	0	8.5	9.1	8.4	8.8	0.3	0.798
	30	8.3^{d}	11.7^{b}	11.2^{c}	13.3^{a}	0.4	<0.001
	60	8.3^{d}	14.2^{b}	13.5^{c}	15.6^{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 44.8±3.0 pg/mL in control to 27.6±2.0 pg/mL (GI), 29.5±2.1 pg/mL (GAR), and 21.4±2.1 pg/mL (GI+GAR) by day 60 (P<0.001). TNF-α followed similar patterns with 52% reduction in GI+GAR 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 GI+GAR (19.2±1.3 pg/mL) compared to control (11.1±0.8 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 1.1 (GI+GAR), suggesting effective modulation of inflammatory response.

3.4 Lymphocyte Subpopulations

Flow cytometric analysis showed that there were significant changes in lymphocyte subsets (Table 2). The T- helper cells that turned out to be CD4 include 28.3±1.8 and 36.4±2.2 in GI+GAR group and control group respectively (P<0.001). Control group experienced a reduction in the CD8 3 cytotoxic T cells while the supplemented groups did not. Thus, the ratio of CD4 +/CD8 + improved compared with 1.2 +/1.1 (control) to 1.9 +/1.1 (GI+GAR), which is a sign of increased cellular immune competence. B lymphocytes (CD21 +) were elevated in all supplemented groups, with GI + GAR the highest (18.9 + 1.3% vs. 12.4 + 0.9% in control; P = 0.01). γδ T cells which are important in the innate immunity were maintained in all the supplemented groups but were lost in the controls.

Table 2. The distribution of lymphocyte subpopulations (%) in heat stressed Shami goats.

Cell Type	Group	Day 0	Day 30	Day 60	P-value
$CD4^+$	CON	29.3±1.6	26.4±1.5^b	23.8±1.5^c	<0.001
	GI	29.4±1.7	32.1±1.8^a	33.6±1.9^b	<0.001
	GAR	29.5±1.8	31.4±1.7^a	32.7±1.8^b	<0.001
	GI+GAR	29.6±1.7	33.5±2.0^a	36.4±2.2^a	<0.001
$CD8^+$	CON	20.1±1.2	20.8±1.3	20.3±1.2	0.652
	GI	20.3±1.1	19.8±1.1	19.5±1.0	0.423
	GAR	19.9±1.2	19.7±1.1	19.4±1.1	0.512
	GI+GAR	20.2±1.1	19.6±1.0	19.2±0.9	0.387

3.5 Heat Shock Protein Expression

Both HSP70 and HSP90 mRNA levels rose considerably in all the groups that were subjected to heat stress with supplemented groups responding better (Figure 2). The maximum expression was achieved on day 30 where the GI+GAR group increased 4.4 times compared to 2.7 times by the control ($P<0.001$). At 60 days, Hsp70 was still upregulated in supplemented groups (3.7-fold in GI+ GAR and down regulated in control (1.8-fold). HSP90 expression was also similar with lesser change in magnitude. HSP70/HSP90 ratio was found to be more in the supplemented groups indicating selective up-regulation of HSP70 mediated protective mechanisms.

3.6 Immunoglobulin Concentrations

There were treatment-dependent reactions on serum immunoglobulin levels (Table 3). GI+GAR group showed an improvement in the baseline of 12.2±0.7 mg/mL to 17.2±1.0 mg/mL of IgG and control group no significant change was observed ($P<0.05$). The level of IgM was sustained in supplemented groups but reduced in control. Important changes of 41% of IgA concentrations of mucosal immunity rose in GI+GAR group versus 12% fall in control ($P<0.01$).

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

Parameter	Day	CON	GI	GAR	GI+GAR	P-value
IgG	0	12.2±0.7	12.5±0.8	12.3±0.7	12.5±0.8	0.892
	60	12.4±0.7^c	15.8±0.9^b	15.4±0.9^b	17.2±1.0^a	<0.001
IgM	0	2.8±0.2	2.8±0.2	2.9±0.2	2.8±0.2	0.756
	60	2.2±0.2^b	3.0±0.2^a	2.9±0.2^a	3.2±0.2^a	0.018
IgA	0	0.42±0.03	0.42±0.03	0.42±0.03	0.42±0.03	0.834
	60	0.36±0.03^c	0.54±0.04^b	0.51±0.04^b	0.61±0.04^a	<0.001

3.7 Correlation Analysis

The Pearson correlation analysis showed that there were strong negative relationships between antioxidant enzyme activities and

pro-inflammatory cytokines (r = -0.74 to -0.87; P<0.001). There was a positive correlation between the HSP70 expression with SOD (r = 0.71; P<0.01) and CAT (r = 0.74; P<0.001) activities. The correlation between the ratio of CD4/CD8/ratio and IgG was positive (r = 0.65; P < 0.01); however, it was negative with IL-6 (r = -0.61; P = 0.05).

4. Discussion

4.1 Physiological Adaptations to Heat Stress

The current research paper shows that joint supplementation of Z. officinale and garlic is a useful intervention to reduce physiological disturbances due to heat stress in Shami goats. The values of THI (82-92) show that the conditions in the goat production system are characterized by severe heat stress that is also consistent with the past accounts in Middle East countries [13,14]. The recorded lower rectal temperature and respiratory rate in supplemented groups indicate better thermoregulatory efficiency and probably by the better cellular heat stress response mechanism. This effect is consistent with those obtained recently by Afzal et al. (2021) who documented the existence of synergistic effects of phytogenic compounds in reducing heat stress responses [15]. The presence of low body temperature with high environmental heat load shows better heat dissipation ability and less metabolic heat generation that may be achieved by the regulation of thyroid hormone activity and uncoupling proteins in mitochondrion [16].

The antioxidant defense mechanisms operate on the basis of antioxidant defense in humans and animals (Burns, 2007).<|human|>The mechanism of antioxidant defense works upon the principle of antioxidant defense in human and animal beings (Burns, 2007).

The developmental increase of the antioxidant enzyme activities of the supplemented groups is an important adaptive change to the oxidative stress caused by heat stress. The 39% of the increase in SOD activity and 44% of the elevation in CAT activity of the GI+GAR group over the control group represents the strong activation of the primary antioxidant defense system. These results are consistent with the earlier research that reported Z. officinale bioactive compounds especially gingerols and shogaols to stimulate the expression of antioxidant enzymes via activation of the Nrf2-ARE signaling pathway [17,18].

The synergistic increase in GPx activity (78% improved) in the combined supplementation group is particularly significant because, in the condition of heat stress, the GPx is of crucial importance to remove lipid peroxides and preserve the integrity of cell membranes [19]. The simultaneous activation of all 3 major antioxidant enzymes implies overall protection against various ROS species, as SOD transforms superoxide to hydrogen peroxide which is then neutralized by CAT and GPx. The role of allicin in this antioxidant activity is probably that of both direct ROS scavenging and indirectly via actions on cell signaling pathways. Recent mechanistic reports have indicated that allicin triggers the Keap1-Nrf2 pathway, which triggers transcriptional upregulation of antioxidant response elements [20]. The patterns of observed activity of the enzymes indicate that jointly supplemented systems have complementary antioxidant protective mechanisms, with Z. officinale as a source of the direct antioxidant molecules, and both supplements as activators of the endogenous antioxidant defenses.

4.3 Response Modulation: Inflammatory Response.

The significant decrease in the amount of pro-inflammatory cytokines (IL-6: 52%, TNF- 53, IL-1 b: 49) in the GI+GAR group can be discussed as the strong inhibition of inflammatory response to heat stress. This anti-inflammatory property is important since persistent increased levels of these cytokines in the heat stress processes result in dysfunction of metabolic functions, decreased feed consumption, and impaired productivity [21,22]. The pathway through which this cytokine is regulated appears to be associated with inhibition of NF-kB signaling pathway, which is a master regulator of inflammatory-related gene expression. It is also found that both Z. officinale and allicin inhibit the activity of I.e.B kinase, inhibiting translocation of NF-B nuclear and the resultant expression of inflammatory mediators [23,24]. The concomitant rise in anti-inflammatory IL-10 (67% in GI+GAR) produces a good balance of cytokines, which is demonstrated by the decrease in the ratio of IL-6/IL-10 (4.0 1.1). Such a change in phenotype to an anti-inflammatory one has significant consequences to the maintenance of immune functions in heat stress. High concentrations of pro-inflammatory cytokines have been linked to the apoptosis of lymphocytes, impairment of antibody production and the vaccine responses in heat stressed ruminants [25]. the preservation of cytokine homeostasis by the means of phytogenic supplementation thus offers preconditions of intact immune competence.

4.4 Cellular Immune Function

The maintenance and increase of CD4+ T-helper cells in supplemented groups are notably different as compared to the reduction in control animals. The 49 percent expansion of CD4+ cells and revised positive ratio of CD4+/ CD8+ (1.2 to 1.9) of the GI + GAR group shows that there was a strong preservation of cell-mediated immunity even in the face of the heat stress challenge. This observation is especially noteworthy considering the earlier reports of the lymphocyte apoptosis due to heat stress and disturbed T-cell differentiation in ruminants [26,27]. The process of preserved lymphocyte functions probably has several pathways. First, elimination of oxidative stress in supplemented animals helps in avoiding the damage and apoptosis of lymphocytes by ROS. Second, the anti-inflammatory cytokine milieu facilitates the survival and proliferation of the T-cells. Third, bioactive components of both Z. officinale and garlic can be able to stimulate the proliferation of lymphocytes directly via regulation of protein kinase C and MAP kinase signaling [28,29]. The immunoprotective effects of supplementation are also supported by the maintenance of γδ T cells that comprise an important fraction of the circulating T cells in ruminants and have important functions in both innate immunity and immunosurveillance. The cells are specifically vulnerable to heat stress and their maintenance implies an extensive safeguarding of different immune cell populations [30].

4.5 Heat Shock Protein Response

The increased expression of HSP70 and HSP90 in groups receiving supplements is an adaptive cellular response to cellular stress, which offers cytoprotection in conditions of thermal stress. The significant increase in HSP70 at 2.3 times in GI+GAR group over control at day 30, and its maintenance to day 60, is an indication of the long-term activation of cellular protective systems. This observation is consistent with previous reports that indicate that some

phytochemicals may be used as hormetic stressors by activating cellular stress signaling pathways to provide greater defense against future challenges [31,32].

The selective upregulation of HSP70 in comparison to HSP90 as indicated by the greater Hsp70/Hsp90 ratio is of special interest to heat stress adaptation. HSP70 is the main chaperone in the process of preventing aggregation of proteins and promoting protein refolding in case of thermal stress which also prevents apoptotic pathways by interacting with Apaf-1 and caspase proteins [33]. The persistence of the HSP70 gene in supplemented groups despite a reduction in the control group is indicative of the fact that phytogenic compounds retain cellular capacity in cellular stress response during chronic heating. Recent findings show that both Z. officinale and allicin also have the ability to stabilize the activity of the heat shock factor-1 (HSF1) the master transcriptional regulator of the heat shock response [34,35]. This modulation can also be through post-translational modifications of HSF1 such as phosphorylation and SUMOylation of the protein, which influence its DNA binding ability and transcriptional potency.

4.6 Humoral Immune Response

The large increase of the immunoglobulin levels within the supplemented groups indicates maintenance of humoral immunity under heat stress. The 38% increment in the IgG, 37% increment in the IgM systems and 41% increment in the IgA levels in the GI+GAR faction shows improved B cell activity and capability of creating antibodies. This is unlike the well documented repression of antibody responses in heat stressed ruminants [36,37].

The process of enhanced production of immunoglobulin is likely to be mediated by the direct and indirect effects of supplementation. Amongst the direct effects are the stimulation of B cell proliferation and differentiation by adjusting the B cell receptor signaling and co-stimulatory molecules. The indirect effects are the acquisition of a positive cytokine environment (enhanced IL-10, reduced IL-6) to sustain B cells and class switching of antibodies [38].

The specific increase in IgA production holds significant consequence to the mucosal immunity that in general is impaired under heat stress by decreased gut barrier activity and disturbed gut immune response. It was demonstrated that the bioactive substances of Z. officinale and garlic retain intestinal epithelial integrity and induce IgA-producing plasma cell differentiation in gut-related lymphoid tissue [39,40].

4.7 Combined Stress Response and Implications.

The close associations between antioxidant enzymes, cytokines and immune parameters indicate a combined protective mechanism as a result of phytogenic dietary supplementation. The antioxidant enzyme activity and pro-inflammatory cytokine ($r = -0.72$ to -0.85) have a negative correlation, which means that the mitigation of the oxidative stress has a direct effect on modulating the inflammatory response. Otherwise, the correlation between the HSP70 expression and antioxidant enzymes is positive, which implies the coordinated activation of the cellular protective mechanisms. Practically, the existence of the superiority of combined supplementation as compared to the individual treatment implies synergistic interactions between Z. officinale and bioactive compounds of garlic. This

synergy could be attributed to complementary mechanisms of action, where Z. officinale is a source of a wide range of antioxidant compounds and nutritional cofactors, whilst allicin has strong anti-inflammatory and gene regulatory activity. The mix can also enhance bioavailability and uptake of active compounds into cells by enhancing membrane permeability and modulation of transporters [41,42]. The persistent nature of the effects that were experienced during the 60 days experimental period without any signs of developing tolerance justifies the possibility of long term supplementation during seasons of heat stress. The fact that neither supplement has any negative effect on physiological parameters indicates excellent safety parameters of both supplements within the context of the application of the study at the determined doses, which is also indicative of the long-standing application of both supplements in traditional medicine and animal feeds [43,44].

4.8 Limitation and Future directions.

Although this research gives all-encompassing evidence on the positive impacts of Z. officinale and garlic supplementation, there are a number of weaknesses that can be noted. One, the investigation has been done solely on goats of the female gender and sex-specific reactions to supplementation under heat stress are yet to be explored. Second, the nature of the molecular mechanisms that mediate the observed effects were deduced based on functional consequences as opposed to direct studies of the mechanism. Future studies must make use of transcriptomic and proteomic methodologies in order to understand the certain signaling pathways and gene regulatory networks. Also, there is need to establish dose-response correlations of the best levels of supplementation since the study being discussed employed fixed doses, which was informed by earlier studies. The economic viability of the supplementation plans must also be analyzed, in terms of the supplement cost, preparation, and the possible effects it has on the milk yield and milk composition. The importance of long-term research on the impact on reproductive performance, offspring health, and transgenerational effects would be a good source of information on sustainable application in commercial goat production systems.

5. Conclusions

This paper presents a significant amount of evidence that the dietary inclusion of a mixed Zingiber officinale and garlic supplement is successful in overcoming the adverse immunologic and oxidative effects of heat stress in Shami goats. The outcomes reveal that the antioxidant defence system has been improved significantly, which is proved in the increase in the activities of SOD, GPx, and CAT in supplemented goats. This strong enzymatic stimulation came along with a significant decrease in lipid peroxidation biomarkers ensured the effective alleviation of oxidative stress in the system. At the same time, a strong immunomodulatory effect was triggered by the phytogenic blend. These profound changes in the lymphocyte growth and neutrophil phagocytic capacity demonstrate the enhancement of the cellular immune branch. In addition, an increased antigen-specific antibody titers indicates an increased humoral immune response. This total immunostimulation plays a very important role in preventing the occurrence of subclinical infections and enhancing the overall health of animals in a stressful environment. Regarding the industry, these results can be translated into visible economic and sustainability advantages. This nutritional approach directly enhances

improved growth performance, feed efficiency, and carcass quality by reducing the immune and oxidative stress of heat stresses. More to the point, the increased innate resistance to disease by diet decreases the use of prophylactic antibiotics, which are in line with the desires of consumers to have naturally produced meat and conscientious animal husbandry. To sum up, synergistic application of both Zingiber officinale and garlic is a viable, natural and effective technique to improve thermotolerance in Shami goats. This method goes beyond simply treating the animal of its symptoms but actually makes it stronger physiologically. We suggest the introduction of such phytogenic mixture as one of the most common elements of environmentally friendly goat production model, which will guarantee the economic sustainability of operations and the quality of products in the epoch of climate uncertainty. The optimization of dosage regimens and assessment of the long term effects of the intervention on reproductive performance and progeny health should be the subject of future research.

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Conflicts of Interest

The authors declare no conflicts of interest.

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