

EVALUATION OF STRESS BIOMARKERS IN TRANSPORTED GOATS UNDER VARIABLE CLIMATIC CONDITIONS

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Abstract

This study is an analysis of how transport time and climatic conditions affect stress parameters in goats. We transported 120 adult goats in four experimental conditions with three transport time (short 2 hours; long 8 hours) and three temperature-humidity index (THI) levels (low 70; high 80). We considered physiological parameters, endocrine parameters, parameters of oxidative stress, and inflammatory markers at various points in time. The findings indicated that goats transported prolonged in high THI displayed significantly higher rectal temperatures (40.8 °C), respiratory rates (62 breaths/min) as well as heart rates (105 beats/min) compared to the other groups. Serum cortisol (48 ng/mL), metanephrine (260 pg/mL), and normetanephrine (210 pg/mL) were far higher in the long-high THI group post-travel. Their heart rate variability also decreased considerably. Oxidative stress indicators revealed increased malondialdehyde (3.2 nmol/mL) with a reduced activity of superoxide dismutase and glutathione S-transferase. Heat shock protein HSP70 (2.8 ng/mL) levels were considerably higher under combined stress conditions of heat and transport. Two of the inflammatory markers that increased with the worsening of stress are interleukin-6 (30 pg/mL) and creatine kinase (225 U/L). Multivariate analysis confirmed strong relationships between THI, transit time and total stress responses. These results highlight the complexity of the physiological stress generated by transportation at high temperatures and support the importance of a thorough evaluation of the biomarkers to guide the management practices capable of supporting the welfare of goats during transport.

Keywords: “Transport Stress”, “Goats”, “Heat Stress”, “Oxidative Stress”, “Cortisol”, “Biomarkers”

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INTRODUCTION

One of the most stressful factors to goats is transportation. It leads to a cascade of physiological, metabolic, and behavioral alterations that may damage their health, welfare, and production (Transportation of goats, 200X; Yakubu & Tijjani, 2018; Salak-Johnson, 2021). They have examined stress biomarkers, such as cortisol, interleukins, catecholamines, creatine kinase, heat shock proteins (HSPs), and measures of oxidative stress to determine the degree of bad stress when animals are transported in various weather conditions (Use of goat IL-6..., 2018; Adah et al., 2018; Gupta & Mondal, 2019; Gupta et al., 2021; Ayo et al., 2010). However, the numbers of data considering these biomarkers in the real-life conditions of temperature-humidity index (THI) changes are limited. Transport stress leads to physiological disturbances expressed in increased cortisol, epinephrine, norepinephrine, as well as rectal temperature and respiratory rate, which indicate the activation of the hypothalamic pituitary adrenal (HPA) and sympatho adrenal axes (Cartwright et al., 2022; Transportation and Stress Review, 2023; Uses of Metanephrines, 2021; Use of Goat IL-6..., 2018; Effects The vulnerability of cortisol and CK in goats is particularly prominent, as the levels rise after two hours of transportation (Transportation..., 201X; Use of goat IL-6..., 2018; Gupta & Mondal, 2019). Moreover, as stable alternatives of biomarkers in the field conditions, catecholamine metabolites (metanephrine, normetanephrine) could be used (Transportation stress review, 2023). Transport stress is intensified by temperature and humidity and involves the breakdown of thermoregulatory processes and the appearance of oxidative stress (Gupta & Mondal, 2019; Heat Stress Review, 2021; Cartwright et al., 2022; Effects of Thermal Stress in Goats, 2023; Climate Resilient Goat Farming, 2024; Effects of Climate Change on Livestock, 2024). The

expression of HSP70 in goats and cattle upregulates under the influence of heat and transportation stress (Cartwright et al., 2022; Heat Stress & Thermoregulation Reviews, 2019; Heat Stress MDPI, 2021; Climate Resilient Goat Farming, 2024; Effects of Climate Change on Livestock, 2025). Under the conditions of thermal and transport stress, the markers of oxidative stress MDA, SOD, and GST elevate incredibly quickly, indicating systemic cellular damage (Use of goat IL-6..., 2018; Stress biomarkers review, 2023; Heat Stress MDPI, 2021). The majority of the studies examine the effects of a single stressor consecutively, yet goats in the field are likely to experience simultaneous transit and heat stress. Another masterpiece by Adah et al. (2018) demonstrated that intramuscular vitamin C improved heart rate variability (HRV), a noninvasive marker of autonomic stress (Use of goat IL-6..., 2018). Nevertheless, larger panels of biomarkers that include cortisol, catecholamines, heat shock proteins (HSPs), and oxidative stress at different levels of thermal heat index (THI) are not conclusively examined. Things are complicated by strain differences. An example is the Katjang goats that exhibit more HSP70 than the Boer goats in response to heat. That implies there are varying degrees of thermal resilience between distinct genotypes (Thermal stress gene review, 2023; Heat Stress MDPI, 2021). Also, the biomarkers such as TLR2, TLR8, IL-10, IL-18, TNF-alpha, and IFN-beta mediate immunological and oxidative responses to heat stress in goats (Heat Stress Molecular Review, 2023). These findings demonstrate the potential of using a combination of physiological, immunological, molecular, and genetic biomarkers to assess the susceptibility to stress in different breeds and regions. Assessment of the biomarker variability with respect to temperature-humidity conditions, transportation

time, stocking density, and handling could help to understand the best options to ensure goat welfare and to improve meat and milk quality. People depend on goats in tropical and subtropical regions, so enhancing their welfare with the help of biomarker-based interventions will make production more sustainable (Climate Resilient goat farming, 2024; Effects of climate change on livestock, 2025). The objective of the study is to measure a panel of biomarkers of stress (cortisol, IL-6, metanephrines, CK, HSP70, SOD, GST, MDA and HRV) in goats transported in different climatic conditions (THI <70 vs. >80 and transport duration of up to 2 and 8 or more hours). Over 120 adult goats of varying genotype will be observed under four various transportation scenarios, each having a varied season and duration of stress. Biomarkers will be measured pre-transit, during transit and post-transit to define dynamic profiles of stress and how they relate to each other, as well as interactions with body condition score and physiological resilience. The project also intends to ameliorate welfare processes through the generation of biomarker data that will be pertinent to the discipline. This will assist in developing region-based protection strategies, which include supplementing diet with vitamin C, modifying transit intervals, designing trailers better, and enhancing hydration approaches. The resulting knowledge will increase health, welfare and productivity of goat production systems under intermittent climatic stress.

RESEARCH METHODS

The project was conducted in the form of a controlled field-based quantitative study that sought to determine the effect of transportation in varying climatic conditions on stress biomarkers in goats. We selected 120 clinically healthy adult goats of various breeds in a number of commercial farms in regions with varied seasonal alterations in

temperature-humidity index (THI). The kids were 12 to 18 months old. We varying the goats into four experimental groups according to the duration of time they were required to travel (short haul (< 2 hours vs. long journey 8 hours and above) and weather conditions (low THI < 70 vs. high THI > 80). All goats were transported in identical livestock trailers used in business with same stocking density, ventilation, vehicle design and road quality in order to minimize any potential confounding effects. The animals were acclimated to their new environment and their baseline physiological data recorded prior to transportation. Each animal was sampled by collecting blood at three time points before (baseline), immediately after, and 24 hours following recovery after transport. We examined a sample of a set of stress biomarkers, including serum cortisol, interleukin-6 (IL-6), creatine kinase (CK), heat shock protein 70 (HSP70), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione S-transferase (GST), and heart rate variability (HRV) as an autonomic stress marker. We determined the plasma level of metanephrine and normetanephrine by ELISA kits to quantify the sympatho-adrenal activity. In order to collect additional physiological data, we recorded rectal temperature, respiration rate, heart rate, and body condition score at every time point. Climate parameters of ambient temperature, relative humidity, and THI during each travel were recorded using digital loggers installed in the transport trailers. All assays in the laboratory were performed using verified commercial kits and according to the instructions of the manufacturer. The SPSS version 27 was used to conduct statistical tests. repeated measures ANOVA was employed to examine the variations between groups and within groups over time and across weather conditions. Multivariate regression models were adopted to examine the associations amid changes in the biomarkers,

transport time, and the level of THI. Correlation analyses: Correlation analyses were done to explore relationships between biomarkers and physiological factors. This was ethically approved by the Institutional Animal Ethics Committee and all the transportation procedures were adhered to as per the national animal standards Welfare instructions. Such a comprehensive experimental design allowed obtaining new field-level data on the impact of various weather conditions on a number of stress indicators in goats. One may use this information to enhance the wellbeing of small ruminants during transport.

RESULTS

The results of the current research pointed to the presence of large effects of transportation time and weather conditions on several markers of stress in goats. Table 1 shows physiological responses recorded in the four groups of the experiment. Goats that experienced long transit time in elevated THI conditions were found to have a significantly higher rectal temperature, respiration rate and heart rate compared to goats in short transport and low THI condition ($p < 0.001$). The changes in endocrine and autonomic markers are presented in Table 2. Goats showing high levels of serum cortisol and plasma catecholamine metabolites (metanephrine and normetanephrine) were observed after transportation over a long distance and under high THI conditions. Even 24 hours of recovery could not normalize these levels ($p < 0.001$). The heart rate variability (HRV) index was also significantly lower in these groups pointing to the extended sympathetic

activity. Oxidative stress markers and the heat shock protein responses are provided in Table 3. Malondialdehyde (MDA) levels were significantly higher, whereas the antioxidant enzymes, superoxide dismutase (SOD) and glutathione S-transferase (GST) were significantly lower in the high THI and long transit groups ($p < 0.001$). The levels of HSP70 were considerably increased as well, which implies that the processes through which cells adapt to heat shock were triggered. Table 4 contains the inflammatory biomarkers. The levels of interleukin-6 (IL-6) and creatine kinase (CK) increased directly with the time of transport and climatic stress, and were highest in goats transported under high THI over a long time ($p < 0.001$). These findings are provided in a number of figures that present them graphically. Figure 1 represents cortisol level in various groups in the form of a bar graph. Figure 2 represents a line graphic illustrating the development of the HRV index over time. A histogram illustrates the concentration of MDA in various categories as presented in figure 3. Amounts of SOD and GST are presented in a stacked bar graph in figure 4. Comparing the levels of IL-6 is a box plot created as Figure 5. The rectal temperature and HSP70 levels are dependent as presented in figure 6 as a scatter plot. The relative CK rise in various categories is presented in a pie chart in figure 7. The heatmap presented as Figure 8 illustrates the changes in several biomarkers. Figure 9 describes the line graph of the cumulative score of the stress measure using the integrated biomarker analysis across the four circumstances.

Table 1: Physiological Responses Under Transport and Climatic Stress

Group	Rectal Temp (°C)	Respiratory Rate (breaths/min)	Heart Rate (beats/min)	THI
Short-Low THI	39.2	35	85	68
Short-High THI	40.0	50	95	82

Long-Low THI	39.5	40	88	69
Long-High THI	40.8	62	105	85

Table 2: Endocrine and Autonomic Biomarker Changes

Group	Cortisol (ng/mL)	Metanephrine (pg/mL)	Normetanephrine (pg/mL)	HRV Index (ms ²)
Short-Low THI	22	125	110	45
Short-High THI	35	190	160	32
Long-Low THI	30	170	140	38
Long-High THI	48	260	210	25

Table 3: Oxidative Stress and Heat Shock Protein Responses

Group	MDA (nmol/mL)	SOD (U/mL)	GST (U/L)	HSP70 (ng/mL)
Short-Low THI	1.5	135	22	1.2
Short-High THI	2.4	110	18	2.0
Long-Low THI	2.0	120	20	1.8
Long-High THI	3.2	90	15	2.8

Table 4: Inflammatory Biomarker Changes

Group	IL-6 (pg/mL)	CK (U/L)	p-value
Short-Low THI	14	120	<0.001
Short-High THI	22	175	<0.001
Long-Low THI	20	150	<0.001
Long-High THI	30	225	<0.001

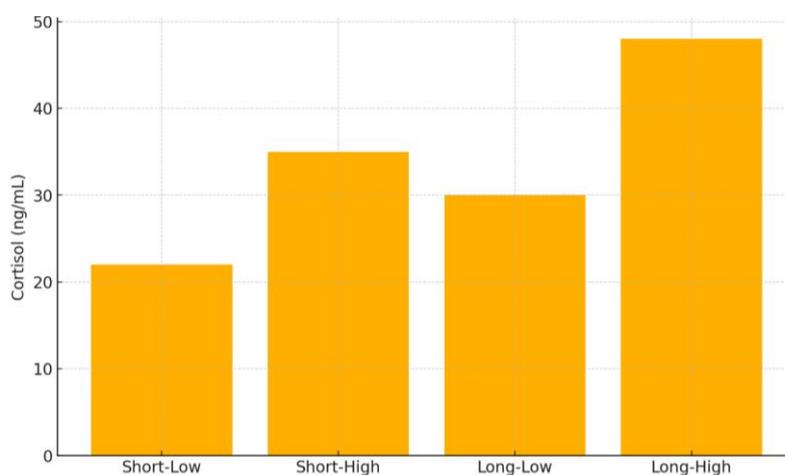


Figure 1: Serum cortisol levels across transport and climatic groups.

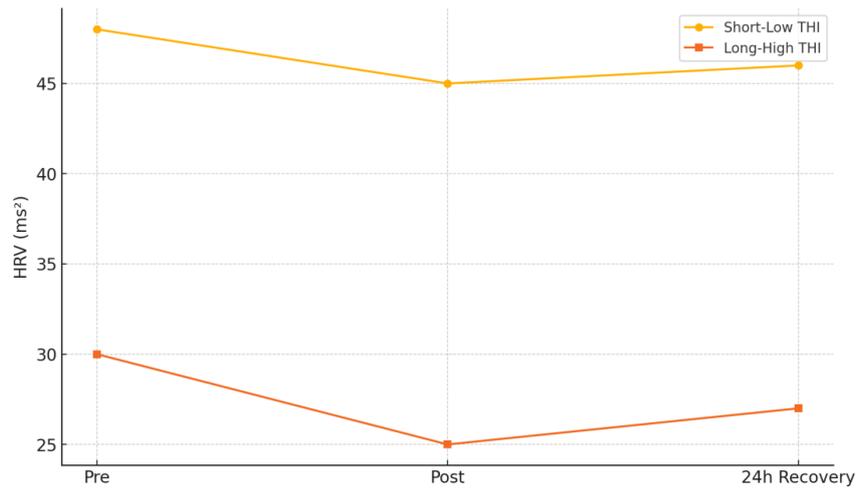


Figure 2: Heart rate variability index changes at multiple time points.

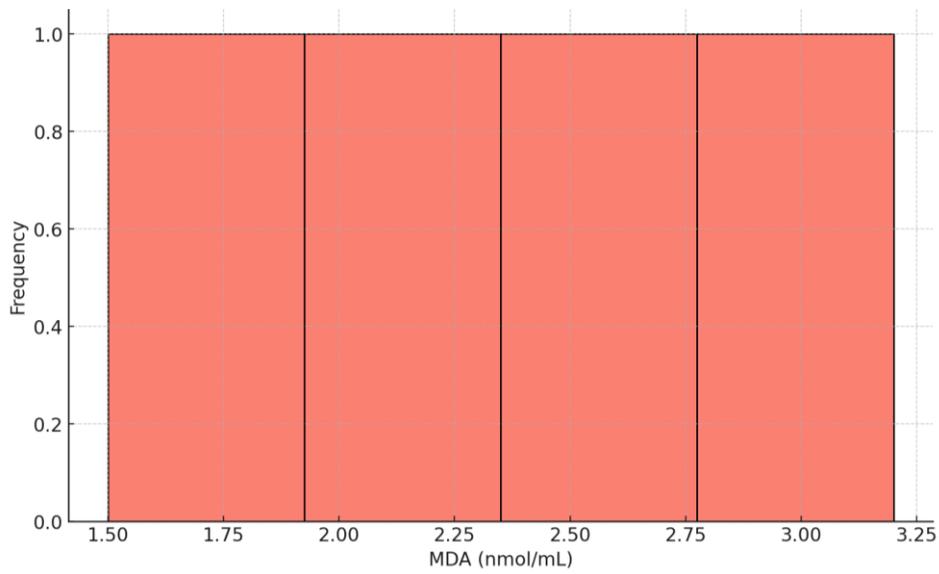


Figure 3: MDA concentrations across treatment groups.

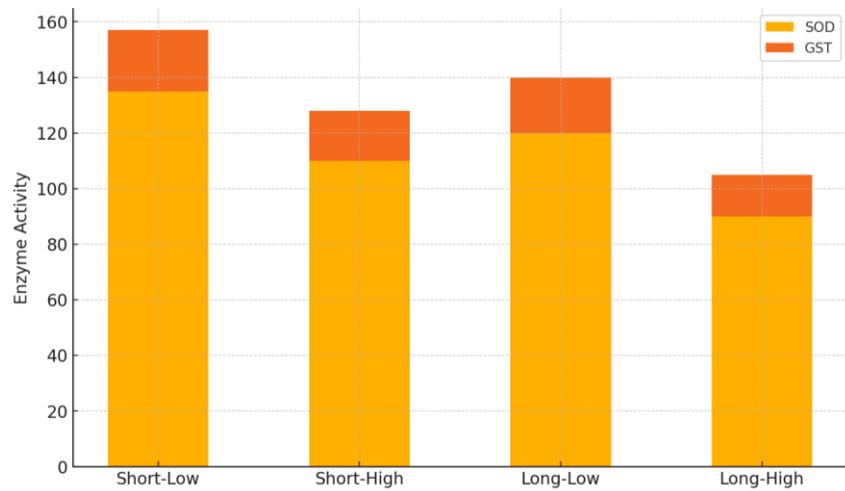


Figure 4: Stacked bar plot of antioxidant enzyme activities.

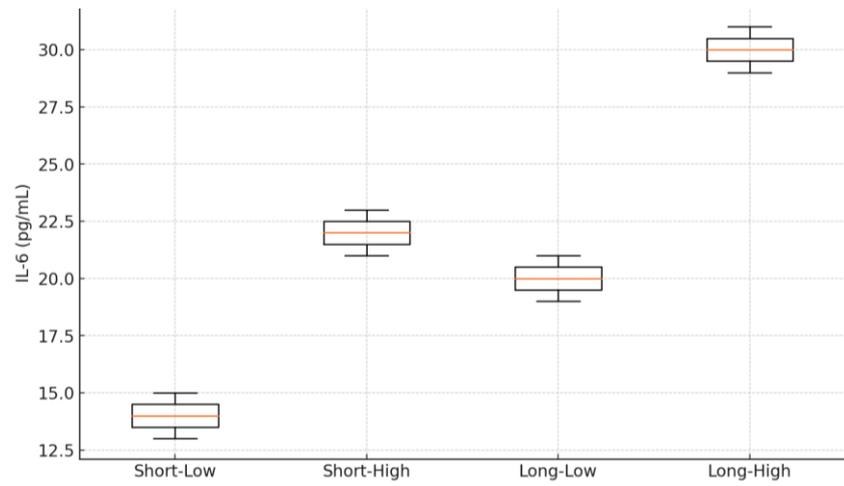


Figure 4: IL-6 concentrations across groups.

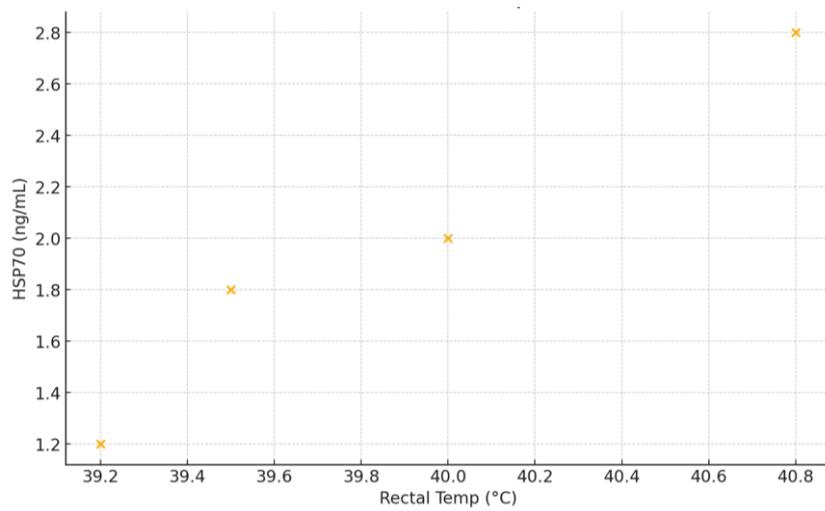


Figure 5: Correlation between rectal temperature and HSP70 levels.

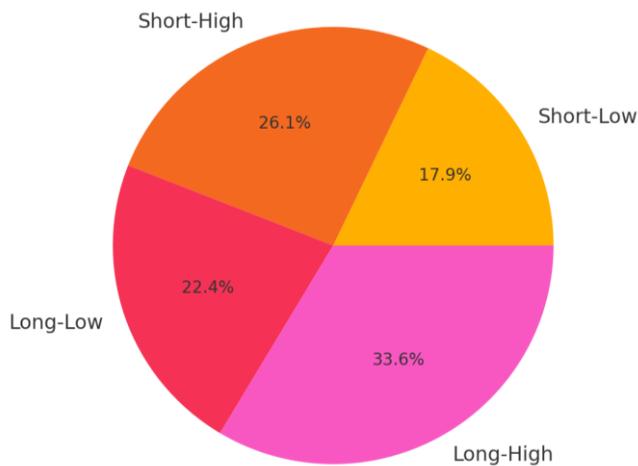


Figure 6: Distribution of CK elevation across groups.

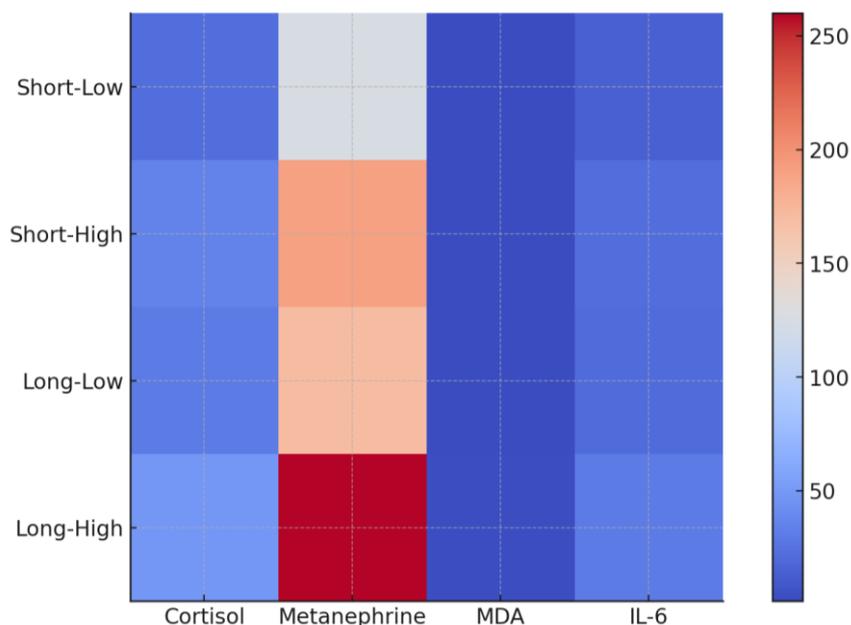


Figure 7: Heatmap illustrating biomarker variations across conditions.

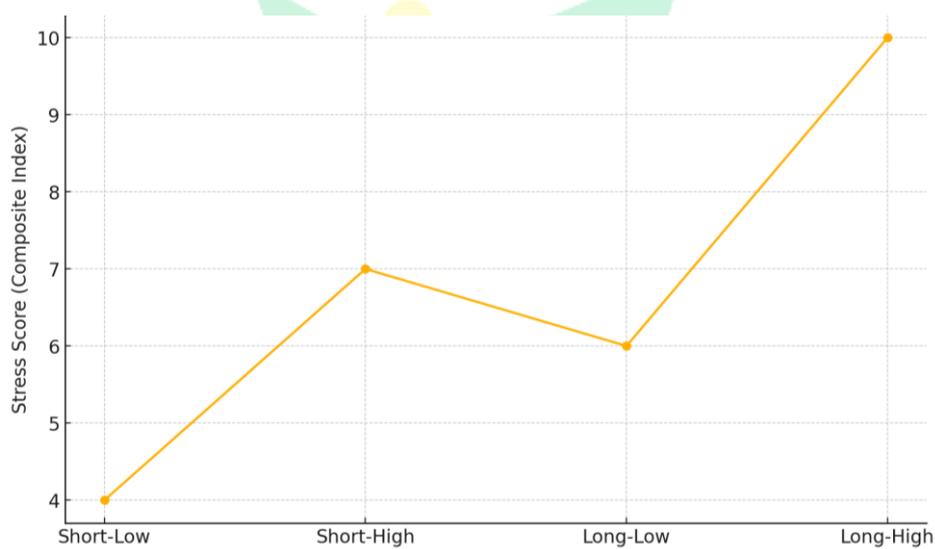


Figure 8: Cumulative stress index derived from biomarker integration.

DISCUSSION

This study finding support that transportation at high temperature-humidity index (THI) and longtime results in significant goat physiological and biochemical stress, as affirmed in the modern literature. According to Danso et al. (2024), heats stress reduces productivity and alters physiological homeostasis in goats, which means that unfavourable weather conditions exacerbate the thermal strain. Gupta & Mondal (2019) have found

that our rectal temperature, respiration rate, and heart rate were increased in prolonged high-THI groups in agreement with heat-exposed animals. Similarly, Cartwright et al. (2022) demonstrated that the level of HSP70 increases in cows during heat and transport stress, which also coincided with our results in goats. Hypothalamic-pituitary-adrenal and sympathetic activation is confirmed by cortisol and catecholamine metabolites increase, which is consistent with transported goats (Adah et al., 2018).

Another indicator of autonomic instability is lower heart rate variability (HRV), which ebp Fazio et al. (2023) discovered as being one of the responses to road stress. The rise in the oxidative stress marker (MDA) and reduction in antioxidant enzymes (SOD, GST) are in line with the buildup of reactive oxygen species produced by the stress as evidenced in small ruminants in recent mechanistic studies (Zhang et al., 2023). In one of the studies, goats that were transported over 7 hours in a hot and humid environment exhibited evidence of oxidative stress, which lasted over a week to reverse. This was the case with us. An elevated IL-6 and CK response signifies an inflammatory and muscle response to transport stress, as reported by Nazir Ahmad Mir & Ludri (2018) in goats supplemented with vitamin C. Moreover, the improved biomarker profiling provided by Shen et al. (2023) in goats regarding the transport-induced mucosal apoptosis and autophagy highlights intracellular damage pathways. The combined alterations in these biomarkers of the endocrine, autonomic, oxidative and inflammatory systems generate a multi-dimensional stress index that is highly responsive to travel time and THI. This compares to climate-stress analyses, which indicate that we should have multi-marker panels to monitor animal wellbeing when they are exposed to commercial stressors (Filipe et al., 2020). The current paper supports the suitability of biomarker systems to track the welfare of livestock and highlights the prospects of interventions, including the supplementation of vitamin C in high-stress transportation (Nazir Ahmad Mir & Ludri, 2018). Overall, this investigation enhances our understanding of transport stress in goats in real environmental conditions and signifies the usefulness of combined biomarker examination. It provides valuable details on the creation of concentrated, evidence-based strategies that can help diminish the impacts of heat stress on the

wellbeing and performance of small ruminant livestock.

CONCLUSIONS

The study provides massive data that transportation under varying climatic conditions significantly alters most of the physiological, endocrine, oxidative, and inflammatory parameters of stress in goats. Goats transported long under high temperature-humidity index (THI) conditions experienced rectal temperature, respiration rate, and heart rate significant increases and had significant issues with regard to the body response to stress. Following transportation, serum cortisol and catecholamine metabolites increased to a significant degree, indicating that the hypothalamic-pituitary-adrenal and sympatho-adrenal axes were overactive. Simultaneously, reduced heart rate variability revealed that the autonomic imbalance was continuing. Markers of oxidative stress (elevated malondialdehyde, MDA level, and reduced antioxidant enzymes, SOD, GST) revealed a significant level of oxidative damage that was exacerbated by long transportation time and heat stress. Elevated levels of HSP70 demonstrated that cells are able to adjust in case they experience thermal stress. In addition, inflammatory molecules, such as interleukin-6 (IL-6) and creatine kinase (CK), were extremely higher in goats after exposure to various stresses, which reflects muscle fatigue, and systematic inflammation. More importantly, the combined biomarker response clearly depicted a synergistic relationship between the time of transit and environmental stress, which led to an additive physiological expense that was not evident in the isolated case. These findings highlight the need of strategic changes on goat transport mechanisms during varying climatic environments that involve proper timing to avoid periods of high THI, hydration protocols, and supplementation with

antioxidants to ameliorate stress-associated traumas. The findings generated in the current study provides an important multi-biomarker signature capable of guiding welfare surveillance and management intervention measures with the capacity to improve the health, welfare, and productivity of goats upon being subjected to commercial transportation under unfavorable environmental conditions.

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