

# Physiological and Intestinal Biomarkers as Indicators of Heat Stress in Small Ruminants

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**Abstract** Small ruminants (sheep and goats) play a major role in the economy of million people, and have provided meat, milk, skin, wool and fiber for centuries. Stress is the body's mechanism for responding to a challenge or a problem. In response to a stressor, an organism undergoes physiological or biological stress. Animals are exposed to many kinds of stressors, including heat stress (HS), nutritional stress, chemical stress, and psychological stress. Physiologically heat stress responses can be estimated by changes in intrarectal temperature (°C), respiratory rate (breaths/min), and heat tolerance index. To assess the impact of thermal stress on overall body functions in small livestock, it is necessary to include indicators that effectively demonstrate the extent to which vital body systems are affected. The inclusion of hormonal change indicators is one of the important aspects in assessing heat stress on animals; however, indicators of gastrointestinal function have not received significant attention in the research field. Accordingly, the research aims to shed some light on the important indicators related to the physiological response of small livestock under thermal stress conditions.

**Keywords** — heat stress, heat tolerance index (HTI), intestinal permeability, intestinal markers.

## INTRODUCTION

Climate changes are presenting important problems to the farm animals industry. When temperatures climb beyond the 25°C threshold, animals' thermoregulatory systems are put to the test (1), which interferes with their capacity to maintain homeothermy (2). When an animal can no longer transfer excess heat in order to maintain body thermal equilibrium, it is said to be suffering from heat stress (HS) (3). Air temperature and humidity are two of the many climatic factors that meteorological stations frequently measure and may be used to assess the state of HS. When measuring the HS condition, one of the most popular measures is the temperature-humidity index (THI). In order to account for the varying sensitivity among species, the temperature and humidity of the air are combined into a single number (4,5). Reduced growth, production, reproduction, milk quality and quantity, and natural immunity are all impacted by heat stress, which leaves animals more susceptible to health problems and even death. As a result, heat stress causes substantial economic damage,

which highlights the need to evaluate the safety of animals objectively. The agriculture industry is being threatened by the rising demand for animal products and the frequently hot weather (6). Animals' reactions to stress are influenced by a wide range of interrelated variables, including the type of stressor (physiological, psychological, and physical), its duration (chronic and acute), heredity, age, and social standing. However, one of the main causes of stress in animals is exposing them to adverse environmental factors. Physiological, metabolic, and behavioral changes induced by heat stress (HS) have a detrimental impact on the performance and general health of sheep. High ambient temperatures cause animals to consume less dry food, which lowers the proportion of fiber they consume. This, in turn, influences the rate of fatty acid synthesis (7).

Accurate and non-invasive HS measurement is just as crucial as developing appropriate ways to mitigate the effects of HS in sheep. Rectal temperature (RT) has historically been used to evaluate the effects of hot weather on sheep and is regarded as an indicator of thermal balance (8). Heat stress (HS), also known as excessive heat load, is the term used to characterize a condition in which an animal's thermoregulatory systems are unable to adequately control its body temperature within the typical range. HS significantly impairs the productivity and well-being of agricultural animals, including sheep raised in tropical and subtropical climates (9,10). Increased body temperature and respiration rate are the main physiological reactions to Heat stress in ruminants. Sheep typically have body temperatures and respiration rates between 38.1-39.9 °C and 12-30 beats per minute, though these can vary depending on a number of factors, including activity level, breed, age, and diet (11,12). Long-term heat stress frequently results in increased morbidity and mortality (13), weakened immunological function (14,15,16), and reduced productivity (17,18). It is especially challenging to conduct comprehensive screening for HS symptoms in agricultural settings, particularly in grazing systems with high animal populations. The degree of HS in an animal is usually determined by its genetic susceptibility to stressful situations, which might differ depending on the breed, species, age, and physiological stage (19,9). Therefore, techniques to measure and track physiological reactions like body temperature in real-time may provide further information on the early detection of HS in sheep, regardless of the information already

available. This method uses the body's equivalent heat radiation expelled to determine the surface temperature (20,21).

### **HEAT STRESS RESPONSE**

Anthropogenic climate change is expected to increase the risk of high heat stress (HS) in livestock in the future, particularly in the tropical zone (22). One of the main things influencing animals on farms is HS (23). Animals undergo stress in all types of production systems, this response can be at several levels, including behavioural, physiological, cellular, or molecular. Understanding how response can be influenced by a number of intricate and overlapping elements is essential to understanding how stress impacts an organism. Acute HS may lead to chronic stress, but does not cause it directly, which happens when the ambient temperature rises quickly and stays that way for days or weeks. When there is a short period of exposure to heat (such in the middle of the day) and comfortable temperatures for the remainder of the day, this can then be either constant (continuous) or cyclic.

To keep homeostasis, the stress reactions can lead to metabolic alterations. These consist of the mobilization of water and energy, the activation of the immune system, and decreased activity of the reproductive and digestive systems. Production and reproduction may be altered when the immune system changes, which might lead to lower food intake and disease susceptibility (24). The reaction is not a straight line. Research has demonstrated that even minor exposure to stresses can trigger an adaptive response that improves the organism's defenses (25). This, in turn, is accompanied with an enhancement in efficiency. Financial success thus depends on an understanding of the connection between HS and production/reproduction. Heat stress can suppress the immune system, limit milk production in dairy cows, and induce reproductive disorders like lower birth weights and lower semen quality (26,27,28,29). Animals are more susceptible to infection in the days and weeks that follow heat stress because it reduces their natural immunity (30). In heat stress there was revealed a decrease in immune response with elevated in Heterophil/lymphocyte ratio (31).

### **Physiological response to heat stress**

Reproductive success is impacted by physiological, behavioral, and metabolic responses to increased heat that the animal body perceives. Sheep that are exposed to high ambient temperatures initiate a number of physiological compensatory processes, such as elevated heart rate, respiration rate, and rectal temperature, that enable the body to adjust to excessive environmental circumstances (32,33). Evaporative heat loss processes, such as thermal polypnea, are initiated when the skin and coat are no longer adequate to stop heat accumulation.

### **Rectal Temperature**

In animals, body temperature is a good indicator of heat tolerance because it is the result of all heat gain and loss processes in the body. The maintenance of body temperature is controlled by neurons in a system known as negative feedback (34). Rectal temperature is a significant body

temperature indicator that can be utilized to evaluate the intensity of heat stress. Animal performance can be negatively impacted by a rectal temperature increase of no more than 1°C. Under heat stress, small ruminants' rectal temperatures were observed to be greater (33,35). According to some theories, this is a natural way for them to keep their body temperature from rising during heat stress and help them from dying from heat stroke. Its variance, both above and below average, indicates how well the animal tolerates environmental stressors. But according to (36) and (37), sheep's rectal temperatures ranged from 38.3 to 39.9°C in case of heat stress. Death may result from the degenerative conditions of animal cells if the rectal temperature rises above 41.7°C (33). The rectal temperature of sheep exposed to heat was greater, ranging from 39.5 to 39.8°C (38,39). Increased body temperature of the core, decreased immunity, decreased voluntary feed intake, impaired endocrine and reproductive processes, decreased energy availability for cells, altered blood pH and electrolyte balance, altered digestibility and metabolism of different nutrients, disruption of intestinal epithelium structure and function, altered normal and protective microbiota, and elevated levels of cortisol and corticosterone in the blood are some of the physiological changes associated with Heat stress (40). According to (41), two of an animal's automatic major reactions to heat stress are sweating and panting. Due to the wool coat, respiratory evaporation is far more significant in wool sheep than sweating. (32) state that sheep can release heat from their bodies through insensible perspiration, which is the result of water diffusing through their skin.

### **Respiratory rate**

Respiration rate (breaths/min) is a practical and reliable measure of heat load and an indicator of HS (42,43). It is measured by counting the number of flank movements per minute from a distance of 4–5 m without causing any disruption the animals (44) or from a non-obstructive distance (45). The respiration rate (breaths/min) is often variable and indirectly related to the animal's activities (metabolism and muscle activity) (46) and environmental conditions (47). According to (48), sheep have a standard reference respiration rate of 16–30 breaths per minute. Therefore, the most accessible and simple method for assessing the effects of HS on animals under extreme conditions seems to be measuring respiration rate, determining whether an animal is panting, and classifying the severity of HS utilizing panting rate (breaths/min) (low: 40–60, medium: 60–80, high: 80–120, and severe HS: >200) (47). According to (47) openmouthed panting and a respiratory rate of about 300 breaths per minute are signs of severe HS. Sheep have been observed to have an elevated respiratory rate (74 breaths/min) after HS (49). Numerous studies demonstrate that differences in respiratory rate responses to heat stress within and across breeds can be used to identify phenotypes that are more vulnerable to the effects of heat stress (32). The elevated respiration rate most likely reflects an attempt by the animals to boost their heat expulsion through higher respiratory elimination in order to maintain a normal body temperature (50).

### Heat tolerance index HTI

A heat tolerance equation calculated using Rectal temperature (RT) and respiratory rate (RR) has been identified by Benezra (51). The Benezra index is the only one of the heat tolerance indicators that takes RR as factor. Animals with lower RR and RT are thought to be better acclimated, according to the direct correlation among this index and physiological measures like RR and RT (52). Benezra test (51) was used to determine the heat tolerance index; the lower the number, the more heat tolerant the animal was thought:

$$HTI = (RT / RR) + (39.1 / 27)$$

Where: HTI = heat tolerance index; RT = Rectal Temperature in °C; RR = Respiratory Rate in breaths per minute; 39.1 = normal RT of sheep (°C); and 27 = normal respiration rate of sheep (breaths/minute).

**Table 1.** THI value which outcome from Rectal Temperature and Respiratory Rate has an Influence on HTI

| Refrence                           | Rectal temperatue (°C) | Respirator y rate (breath/mi n) | THI value | influence on HTI                               |
|------------------------------------|------------------------|---------------------------------|-----------|--|
| 53- Khan <i>et al.</i> , 2020      | 39.1                   | 82                              | 80        | Slightly decrease in heat tolerance            |
| 54- naga <i>et al.</i> , 2021      | 39.3                   | 88                              | 82        | Moderate decline in heat tolerance             |
| 55- Ibrahim <i>et al.</i> , 2023   | 39.6                   | 92                              | 84        | Significant decrease in heat tolerance         |
| 56- Yuan <i>et al.</i> , 2025      | 39.8                   | 95                              | 85        | A marked decline in heat tolerance             |
| 57- Arero & Ozmen, 2025            | 39.3                   | 77                              | 84        | Decline heat tolerance with increase RR and RT |
| 58- Rodrigues <i>et al.</i> , 2024 | 40.0                   | 90                              | 79        | Significant Decrease in HT with high RR        |

### Neuroendocrine responses

Animals undergo a number of neuroendocrine reactions in response to stress, including the production of tropic hormones and the activation of several hormonal axis, which mediate the adaptive and behavioral responses in animals. Decreases in anabolic and catabolic hormones, respectively, are the primary indicators of changes in the hormonal profile (59). These include follicle-stimulating (FSH) and somatotrophic or growth hormone (GH), prolactin (PRL), luteinizing (LH) hormones, thyroid stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH), and others. Although reproductive hormones, particularly in females, are more challenging to interpret, particularly because of differences in the estrus cycle, plasma levels of these hormones may be possible markers of physiological changes in the bodies of heat-exposed animals (60). Regardless of reduced feed intake, heat stress also lowers metabolic rates and modifies post-absorptive metabolism. This suggests that the metabolic processes of heat-stressed animals does not

prioritize growth, productivity, reproduction, or health (61). An increase in ambient temperature triggers the production of stress hormones, which mobilize energy mainly for the maintenance of brain and muscle activities. In reaction to heat load, the body changes post-absorptive energy, produces oxidative stress, damages the liver, lipid and protein metabolism, compromises the immune system, and reduces reproductive capacity (59). These change the distribution of nutrients and could prevent animals that suffering from heat stress from consuming glucose-sparing. The activity of the hypothalamic-hypophyseal axis also gets affected by heat, which may decrease thyroid hormone secretion and lower the animal's normal metabolic rate (32,62). The thyroid hormones thyroxine (T4) and triiodothyronine (T3) raise the metabolic activity in numerous tissues, which speeds up the heat generation of body cells and consumption of oxygen. When the air temperature rises significantly, animals under thermal stress may produce a lesser amount of these hormones (63,64,65,18). As a result, thyroid hormones have an influence on thermogenesis, and changes in their production may serve as a physiological indicator of environmental adaptation (66).

**Table 2.** Overview of pathways affected by heat stress in livestock species

| Reference                          | Animal Type | Target organ                 | Effects of heat stress  |
|------------------------------------|-------------|------------------------------|---|
| 67-Wang <i>et al.</i> , 2024       | Goat        | systemic circulation         | ↑ Respiration rate, ↑ rectal temperature, ↑ cortisol, ↑ total protein, ↑ urea, ↓ glucose, ↓ insulin |
| 68-Rebez <i>et al.</i> , 2023      | Goat        | Liver                        | Cellular degeneration, lipid accumulation in hepatocytes, reduced tissue oxygenation                |
| 68-Rebez <i>et al.</i> , 2023      | Goat        | Thyroid Gland                | ↓ Thyroid activity, inactive follicles, atrophied cells, ↓ T3/T4 hormone secretion                  |
| 57-Arero & Ozmen, 2025             | Sheep       | Reproductive System (Male)   | ↓ Sperm quality and motility, reduced libido, disruption in HPA axis                                |
| 57-Arero & Ozmen, 2025             | Sheep       | Reproductive System (Female) | ↓ Fertility, embryonic loss, fetal growth retardation, low birth weight or stillbirths              |
| 69-Tüfekci & Sejian, 2023          | Sheep       | Adrenal Glands               | ↑ Cortisol release → increased metabolic stress and immune suppression                              |
| 61-Belhadj <i>et al.</i> , 2016    | Goat/ Sheep | Gut/ Digestive System        | ↓ Blood flow → intestinal permeability ("leaky gut"), impaired nutrient absorption, dysbiosis       |
| 70-Mehrani <i>et al.</i> , 2023    | Sheep       | Muscle / Meat Quality        | ↓ Glycogen → DFD (Dark, Firm, Dry) meat, ↑ pH, poor storage quality                                 |
| 71-Bhateshwar <i>et al.</i> , 2023 | Sheep       | Brain / CNS (Hypothalamus)   | Disrupted appetite center → ↓ feed intake, altered central thermoregulation                         |
| 72-Kadim <i>et al.</i> , 2014      | Goat        | Muscle / Meat Quality        | ↓ Glycogen → DFD (Dark, Firm, Dry) meat, ↑ pH, poor storage quality                                 |

### Intestinal Biomolecules markers

The gut, one of the greatest digestive organs in the body, serves as the first line of defense against the body's

nonspecific immunity and is also the biggest center for bacteria and endotoxins to be accumulated." According to (73), a healthy and full intestinal mucosal barrier can keep bacteria and metabolites within a certain range in the gut while they are digesting and absorbing different nutrients. This avoids them from invading the blood circulation system and causing infections. A mechanical barrier, a chemical barrier, an immunological barrier, and a biological barrier are the four successive divisions of the intestinal cavity to the mucosal layer based on the components and functions of the animal body's intestinal mucosal barrier (74). Animal immune systems and organs suffer damage by heat stress. In addition, heat stress affects the intestines and causes inflammation in the body, which results in low body weight, appetite loss, and liver conditions. Ischemia occurs in the small intestine, and intestinal barrier permeability rises. moreover, stress-induced cellular and organ damage is significantly influenced by the processes of oxidative stress and mitogen-activated protein kinase (MAPK) pathway. Heat stress enhanced MAPK activity in the small intestine. According to (75), heat stress triggered MAPK signaling pathways, increased oxidative stress, and severely damaged the small intestine. The gastrointestinal system keeps the internal environment from risky items by acting as a physical barrier against poisonous compounds. The process of protein harmonization in cell-to-cell connections, such tight junctions, may make it easy (76). On the intestinal epithelium, exposure as little as 4–6 hours might have serious implications (77). Furthermore, intestinal wall thermal stress may produce inflammation and sepsis (78), as well as disruption to the gut barrier and a rise in permeability of tight junctions (79). Heat stress causes heat stress transcription factors to become more active, which in turn causes the transcription of the HSP70 gene to develop quickly. This raises the amount of HSP70 protein in cells and the degree of HSP70 expression (80,81). HSP70 is produced in brain tissues, liver, lung, heart, leukocytes, and other tissues when the environmental temperature is high (82,83). The gut is altered by HS in a number of ways, such as increased permeability to toxins and pathogens, decreased immunity, higher oxidative injuries, and damage to the mucosal epithelial microstructures. When the integrity of the intestinal barrier is compromised, harmful bacteria can infiltrate the body and move antigens into the bloodstream, which can ultimately result in immunological reactions and systemic inflammations. Furthermore, decreased enzymatic activity in the digesta, decreased surface areas for absorption, damage to the mucosal structure, and changed expressions of the proteins that transport nutrient materials and genes all these can compromise the gut's ability to digest nutrients. In farm animals and poultry, the systemic hormonal changes caused by HS, in addition to changes in immunological and inflammatory responses, frequently result in decreased feed intake and the production efficiency (84). In addition to the physiological reactions, enzymatic reactions, energy metabolism, disruptions in water, protein, and mineral balance, blood hormone, metabolite release and heat stress adversely impacts the oxidative condition of sheep (32,33). One environmental component implicated in promoting the

development of reactive oxygen species (ROS) is heat stress. The majority of research on ROS in sheep examines that antioxidants might reduce or modulate HS (11,85). Early embryonic death has been connected to redox status changes caused by HS (86). According to (40), heat stress raises the generation of ROS, SOD activity, oxidative stress index, and protein oxidation. Erythrocyte deformation brought on by respiratory oxidative stress increases the generation of ROS and facilitates their spread throughout the body (87).

### **Intestinal permeability markers**

A common method for identifying defects in the intestinal mucosal barrier is intestinal permeability, which is a measure of the integrity of the intestinal barrier. components of the intestinal barrier, beginning with tight junction proteins, mucosal epithelia, and the mucous layer. The intestinal barrier keeps host immune cells and gut bacteria apart and promotes gut homeostasis. The systemic circulation for bacteria and toxins such as endotoxins can be increased by a number of physiological conditions that affect intestinal permeability. Disruption or modification to the intestinal barrier layers is one of these causes (88,89). Circulating markers or by fecal markers can be used to measure intestinal permeability. Intestinal permeability is commonly utilized to evaluate intestinal mucosal damage in a number of gastrointestinal illnesses and serves as a diagnostic indicator of the integrity of the intestinal barrier (89,90). In addition to gastrointestinal problems, a variety of circumstances, such as aging (91), can result in higher permeability of the intestines, which has been linked to a number of medical conditions including diabetes, cardiovascular disease, and cancer (89). A few suggested biomarkers monitor the movement of substances across the intestinal barrier either directly or indirectly. Albumin can move from blood vessels into the interstitial space and subsequently into the gut lumen when the intestinal barrier is impaired. Consequently, fecal albumin, which is often employed in animal models, has been proposed as an indicator of permeability of the intestinal tract (92).

### **The Tight Junction**

Is a complex mechanism that keeps water, molecules, and ions moving. Occludin, tricellulin, claudin, and junctional adhesion molecule (JAM) are examples of transmembrane proteins. According to the majority of studies, signaling pathways such MAPK, PKC, PI3K, and protein phosphatases maintain the permeability of tight junction and tight junction protein expression/cytoskeleton (93). Tight junctions, which form the base of the linkage between epithelial cells, control the movement of substances between them and, as a result, influence the intestinal mucosal barrier's permeability. Tight junctions also contribute to the preservation of intestinal mucosal barrier function and facilitate the transfer of data between epithelial cells and the external and internal environments (94,95). Pathogens translocate as a result of tight connections being destroyed by prolonged exposure to high temperatures. Although the minute alterations in tight junction protein composition are being studied, these results



show that heat damages the intestinal wall and alters tight junction proteins (96).

### **Lipopolysaccharide binding protein (LBP)**

is a protein in acute phase that is generated by hepatocytes and circulates in the circulation. Furthermore, it is evaluated in plasma (97). LBP is regarded as an indicator to the permeability of intestinal and endotoxemia because it binds to bacterial lipopolysaccharides, which are partially derived via intestinal permeability (97). Zonulin, commonly known as the zonulin family peptides, is a group of structurally and functionally analogous proteins (98).

### **Zonulin**

According to (99), a protein that regulates intestinal permeability is elevated in a number of autoimmune disorders and plays a role in the etiology of autoimmune diabetes. Zonulin, commonly known as the zonulin family peptides, is a group of functionally and structurally equivalent proteins (98). Increased zonulin levels have been linked to elevated permeability in the intestinal tract (100,99), and zonulin is an acute-phase signaling protein that regulates intestinal permeability by weakening tight junctions (100). It can be detected in both blood and fecal samples. By controlling tight junctions, which are cell-cell junctions that allow dietary and microbiological antigens to enter the body through the paracellular route of intestinal absorption, it plays a crucial role in preserving the homeostasis of the intestinal mucosa. Changes in intestinal permeability can result in increased exposure to luminal antigens and a subsequent loss of immune tolerance, which can cause the onset and progression of a number of long-term inflammation-related conditions (101).

### **Intestinal Fatty Acids Binding protein (IFABP)**

A cytosolic protein called intestinal fatty acid binding protein (I-FABP; occasionally referred to as fatty acid binding protein 2) is released into the gut lumen when there is damage and is essential for the cellular absorption and utilization of fatty acids in enterocytes (102). Low levels of I-FABP are present in the circulation under normal conditions (103). Thus, circulating I-FABP has been proposed as an indicator of intestinal permeability and may represent migration from the gut into the circulation of blood (104). The intestinal Fatty acid binding proteins are a class of proteins that are thought to be an indication for intestinal mucosal activity since they are important in the intestinal mucosa's metabolism of long-chain fatty acids. According to (105), those proteins are low molecular weight proteins that are found intracellularly. Because of their tissue characteristics and intracytoplasmic location, they are released from intestinal cells into the bloodstream only in intestinal disease and serve as a particular indicator for enterocyte activity. An elevated IFABP plasma level suggested intestinal epithelium injury. They are utilized as therapeutic targets besides to their significance as a biomarker for the integrity of the intestinal mucosa (106). In addition to their involvement in splitting and intracellular receptor transportation, IFABPs have a crucial role in the transcellular membrane transport of hydrophobic compounds,

particularly fatty acids (107). Because FABP plays a part in intracellular transport and FA absorption. It was recently proposed by (108) that the variety of dietary fatty acids influences the regulation of gastrointestinal motility via FABP4.

### **Calprotectin**

is secreted as a component of the inflammatory response by neutrophils. Fecal calprotectin levels (88,109) have been suggested as an indirect indicator of intestinal permeability since intestinal inflammation is associated with both increased intestinal permeability and higher calprotectin production.

**Table 3.** changes of intestinal marker and it's effect on body

| Refrence                          | Animals type | Intestinal marker | Changes in intestinal markers   |
|-----------------------------------|--------------|-------------------|---|
| 110-Soares <i>et al.</i> , 2022   | Sheep        | zonulin           | ↑ increased permeability via tight-junction disassembly → promotes leaky gut and endotoxin leakage      |
| 111-Ural <i>et al.</i> , 2021     | Goat         | zonulin           | Heat stress elevates serum zonulin, disrupting barrier function and reducing nutrient absorption        |
| 112-Snipe <i>et al.</i> , 2018    | Sheep        | I-FABP            | ↑ expression → indicates enterocyte damage; heat causes epithelial injury and compromised absorption    |
| 113-Beiter <i>et al.</i> , 2025   | Goat         | LBP               | ↑ elevated post heat-exercise → reflects increased LPS translocation → systemic inflammation            |
| 88-Seethaler <i>et al.</i> , 2021 | Sheep        | LBP               | LBP correlates strongly with gut permeability; rise indicates leak of bacterial endotoxins              |
| 112-Snipe <i>et al.</i> , 2018    | Goat         | Calprotectin      | ↑ higher fecal calprotectin → marker of intestinal inflammation and disrupted barrier                   |
| 110-Soares <i>et al.</i> , 2022   | Sheep/Goat   | Calprotectin      | Chronic low-grade heat → sustained calprotectin → prolonged gut inflammation and nutrient malabsorption |

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