Heat Stress: Can Animals be Stressed but still be Healthy?

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Abstract
In this era of rapid climate change, heat stress has emerged as a major problem for sheep farming. The quantity and quality of sheep production, as well as the welfare, are all shown to be negatively impacted by heat stress. Reduced cellular immune function in sheep exposed to high temperatures increases susceptibility to various diseases, although the underlying mechanisms are unclear. The imbalance between oxidants and antioxidants, known as oxidative stress, negatively impacts animal health due to heat stress-induced impairments in immune responses, increased production of reactive oxygen species, and/or a lack of antioxidants. Heat stress in livestock has negative effects on both the cell-mediated and humoral immune responses. However, the negative effects of heat stress on immune response in sheep can vary by breed. This review discusses how heat stress affects sheep's immunity. An overview of some molecular markers related to immunity, such as interleukin (ILs), Toll-like receptors (TLRs), and also the heat stress protein (HSPs) in sheep, is also discussed. Finally, recent research on the effects of antioxidant supplementation and other promising nutritional strategies for reducing heat stress and improving animal health is presented.

Keywords: animal health, antioxidant, heat stress, immunity, sheep.

1. Introduction

Heat stress (HS) is one of the main problems faced by livestock amid the recent increasing intensity of global climate change. According to National Oceanic and Atmospheric Administration National Centers for Environmental Information [1], at 0.86 °C above the 20th-century average of 13.9 °C, 2022 was the sixth warmest year on record since 1880. This resulted in record-breaking high temperatures across nearly every continent and a variety of extreme weather events (e.g., extreme heat waves, drought, severe wildfires, and tropical storms) across many countries.

In a normal condition, livestock could achieve minimal physiological costs and maximum productivity by maintaining a constant body temperature within a specific environmental temperature range [2]. However, in HS conditions, the livestock's body temperature rises, and they are unable to dissipate enough heat to maintain thermal equilibrium, leading to decreased productivity, reduced reproductive performance, weakened immunity, and increased mortality in some cases [3], as illustrated in Figure 1.

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In terms of animal health, the increased risk of disease occurs due to weakened immunity, as during HS, the animal elicits several thermoregulatory activities, including behavioural, physiological, neuroendocrine, and cellular responses to maintain homeostasis and survival. During these, immune responses in the animal tend to be suppressed [4,5] (Figure 2). According to [6], the dynamic relationship between the body's stress response and the immune system is referred to as stress-immune interactions. Intricately linked and influencing each other in a number of ways, the interplay between these two systems is complex and still not fully understood.

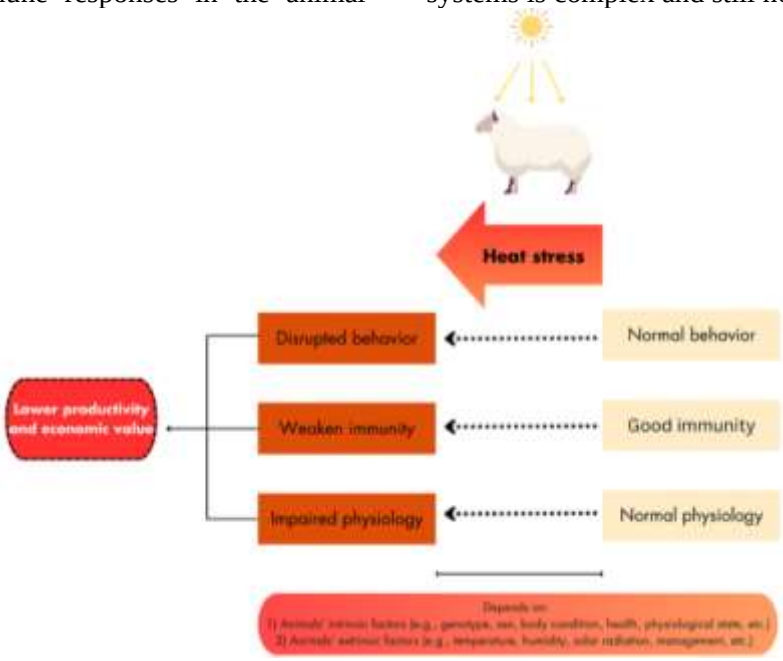

Figure 1. Heat stress deleterious impact to livestock through various ways

There are two parts of the immune system, the innate immune system, and the adaptive immune system. While pathogen exposure is necessary for the development of an adaptive immune response, the innate immune system can function autonomously [7]. Both of the immune systems are disrupted during HS. As the first line of defence against external assaults, HS can compromise innate immunity. Adaptive immunity is the process through which cells such as T- and B-lymphocytes, antigen-presenting cells, and natural killer cells produce specific antibodies against antigens or foreign proteins. During the HS, the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axes are activated to control how the body reacts to stressors and, as a result, alter immunological responses [8].

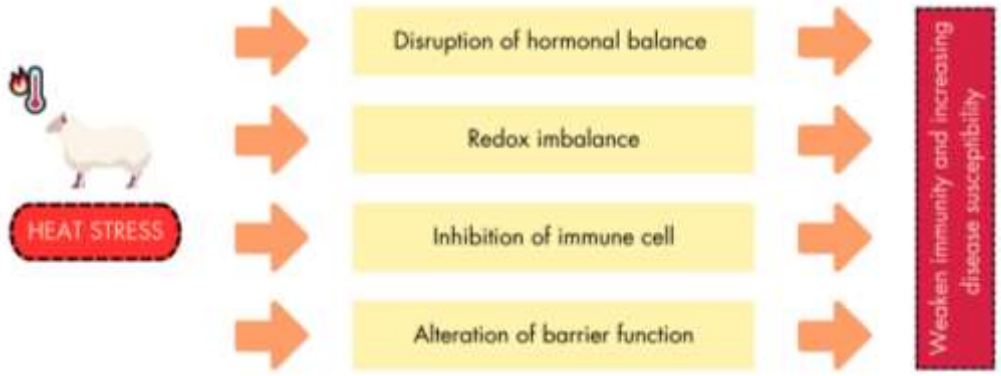

Figure 2. Heat stress weaken immunity and increases disease susceptibility

Detection of stressors (heat) triggers the activation of the HPA axis that would involve a rapid of hormonal secretions that lead to the net increase in glucocorticoid levels in the body. In acute stress, glucocorticoid levels rise with the purpose of preserving homeostasis by acting on vital systems [9]. Once the stressor diminishes, glucocorticoid levels quickly taper off and return to normal through a negative feedback mechanism [10]. In contrast, during chronic stress, in the continuous presence of stressors, the HPA sustains the release of glucocorticoid that maintains its high levels in circulation. This is also exacerbated due to the impairment of the negative feedback mechanism [11].
In this article, the relationship between HS and immunity in livestock is summarized. As supporting evidence, a selection of current studies on sheep is presented. In the end, a HS mitigation strategy through nutritional supplementation to strengthen livestock's immunity is summarized.

2. Discussion

Stress hormone

HS induces endocrine changes to accommodate the release of internal heat. Major changes were observed in prolactin and cortisol, which are stress hormones.
An increase in circulating prolactin may influence heat-suppressing and heat-generating mechanisms that are adaptive for maintaining homeothermic body condition through water conservation, increasing sweating rate, reducing renal fluid, electrolyte excretion, and induction of heat shock protein (HSP) [12,13]. Disruptions in prolactin signalling are also observed and have been linked to impairments in both innate and adaptive immunity [14].
Heat acclimation is also known to increase circulating cortisol by the adrenal gland and decrease endogenous levels of thyroid hormones, both in an effort to decrease endogenous heat production [15]. Cortisol regulates the immune system by targeting genes involved in the production of cytokines, chemokines, inflammatory proteins and their receptors, and cell adhesion. While the secretion of cortisol during times of acute stress stimulates the immune system, it has been linked to immune suppression during times of chronic stress. HS impairs the cellular immune response by increasing cortisol concentrations, which bind to DNA and inhibit the expression of genes involved in T-cell activation and cytokine production, resulting in an immune system inhibitory effect associated with repression of immune cells' anti-inflammatory action [16,17].
Several studies in sheep have proven that heat stress induced prolactin and cortisol production. For instance, exposure to HS in Dorper lambs elevated the plasma prolactin concentration [13] and serum cortisol level in Iranian fat-tailed sheep, which was found to increase under both cold and HS conditions [18], with the increase being higher under the latter. Similar result was found in Merino sheep, but after long exposure to HS, the serum cortisol level gradually decreased as an act of adaptation [19].

Antibody production

HS has been linked to a decrease in both primary and secondary lymphoid organs, circulating leukocyte profiles, T cells in the blood, and antibody response to sheep’s red blood cells [20]. A decrease in antibody synthesis can be linked to cytokine release during HS, which stimulates the hypothalamus to release corticotrophin releasing hormone, resulting in an increase in corticosterone secretion with an intermediate generation of adrenocorticotropic hormone. Corticosterone inhibits antibody synthesis, resulting in immune system dysfunction [21]. Corticosteroids, which can be produced in response to stress, have been shown to bind to DNA and block the expression of genes involved in T-cell activation and cytokine production. Corticosteroid anti-inflammatory characteristics reduce phagocytic cell activity, which changes lymphocyte function [22].
A study in heat-stressed lamb showed that IgG level was significantly reduced and IgA level was significantly increased. IgG is the most important immunoglobulin, promoting immune cells to swallow pathogens and neutralize bacterial toxins, whereas IgA has an evident protective surface and defence function against invading pathogens despite having a low amount in serum [23]. In ewes, a reduced cellular immunological response after an intradermal injection of mitogens, as well as higher cortisol levels, was observed [22]. Another evidence is that the concentration of neutrophils, coliforms, and staphylococci in ewe milk were all found to be higher after being exposed to solar radiation under elevated ambient temperature [24].

Barrier function

Wound healing and tissue repair are also highlighted characteristics of the humoral immune response. With their anti-inflammatory properties, inflammation is withdrawn from cells, which would result in the creation of a conducive environment for the factors of healing and repair of tissues [25]. Interleukin 10 (IL10) and Interleukin 4 (IL4) cause the shifting of macrophages phenotypes that promote proper healing of tissues [26,25]. IL10 has also demonstrated putative roles in repairing and preserving homeostasis during stress situations. [27] reported its role in activating heat shock protein (HSP) 90, which is a HSPs that act as a critical molecular chaperone to protect cells from damage and has involvement in enhancing antigen presentation. Its action in antagonizing fever mediators suggests its benefit in reducing heat load during heat stress [28].

When animals are exposed to high ambient temperatures, their blood flows rapidly to get rid of heat. This causes blood flow, oxygen, and nutrient intake to splanchnic tissues to decrease. Metabolic changes and gut problems cause oxidative stress, the movement of lumen contents, and the release of proinflammatory mediators. This starts a system-wide inflammatory reaction [8].

The suppression of general inflammatory mechanisms of innate and cell-mediated immunity leaves the body vulnerable to general infectious challenges. Particularly, immune defence against viruses is also significantly diminished. On the other hand, the overall result of modulation by the mentioned cytokines interaction creates a protective action against overwhelming and overshooting of immune defences that can prove detrimental to the overall health of the animal and the normal functioning and survival of its tissues in the presence of exogenous stressors [29,30], as evidenced by a study in the intestinal barrier which was compromised by direct HS in a lactating bovine model, allowing microbes and toxic compounds to enter the system and set off immune, cytokine, lipopolysaccharide (LPS), and phagocytic-related pathways [31].

Oxidative stress

Living organisms produce reactive oxygen species (ROS) as a by-product of normal cellular metabolism. They function in physiological cell processes at low to moderate concentrations, but at high concentrations, they cause adverse changes to cell components. Redox imbalance, caused by either the depletion of antioxidants or the accumulation of ROS, is known as oxidative stress. When cells experience oxidative stress, they attempt to counteract the oxidant effects and restore redox balance by activating or silencing genes that encode defensive enzymes and damaging the DNA, lipid, and proteins [32,33].

Oxidative stress can directly damage immune cells, resulting in impaired function and reduced ability to combat infections. It can also modulate the immune response by altering the production of cytokines and chemokines, which are crucial immune signalling molecules. For instance, elevated levels of ROS can cause an increase in pro-inflammatory cytokine production, which can contribute to chronic inflammation and autoimmune diseases [34].

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) functions were all found to be elevated in response to heat stress. (GPX). Antioxidant enzyme activities rise in reaction to elevated ROS levels, with the goal of keeping free radical concentrations at a steady state [35].

When the THI is above 72, [35] found that the SOD activity, oxidative stress index, ROS production, and protein oxidation of sheep are all substantially elevated, which may lead to cytotoxicity. Additionally, redox status changes caused by HS have been related to embryonic loss [36].

When there is a change in the steady-state concentration of ROS, the mitochondria are the first part of a cell to be damaged, as Mitochondria are the biggest producer of ROS. In fact, it has been shown that changing the steady-state level of ROS production causes the respiratory chain to stop working. As a result, as the flow of electrons decreases, the production of adenosine triphosphate (ATP) goes down, and the energy production in the cell is slowed down [37, 35].

Biological marker for heat stress and immunity

Biological markers can work as surrogate markers for cellular activation and are crucial in combating heat stress and maintaining immunity. Heat shock proteins (HSPs), Toll-like receptors (TLRs), and interleukins (ILs) are some of the biological

markers being investigated in relation to heat stress and immunity. Animals' superiority in heat adaptability and immunity characteristics can be determined by referring to these genes.

HSPs are biological markers that function as protein chaperones and increase in response to different stressors. They also play an important role in antigen processing and presentation. Increased HSP expression could be interpreted as an adaptation in antigen processing and presentation and an increased ability to activate the innate and acquired immune systems directly [38]. Under 43 °C ambient temperature, an increase in HSP70 concentration in blood was observed in Pelibuey and Suffolk sheep, which was synthesized abundantly to avoid cellular protein damage through the chaperonic activity ensuring the folding, unfolding, and refolding of stress-denatured proteins and may also be part of the acclimation mechanism [39].

Leukocyte-derived ILs are a class of cytokines—secreted proteins and signal molecules—that play a crucial role in immune system promotion and regulating the ratio of humoral to cell-mediated Th1 and Th2 reactivity [40]. A study in Barki sheep demonstrated that the temperature-sensitive genes IL-2, IL-6, and HSP 70 have seasonal impacts on their expression, which means it is related to thermotolerance [41]. Research in hyperthermic lambs revealed that the level of IL-2, a critical cytokine that primarily promotes T cell proliferation and differentiation, mediates cell immunity, and mediates inflammatory responses, is significantly reduced. This reduction could be attributed to a rise in cortisol levels in lambs exposed to HS, which could result in a shift from a Th1 immune-driven response to a Th2 response, suppressing IL-2 secretion and effect [23].

TLRs are predominantly expressed in monocytes, macrophages, dendritic cells, and B lymphocytes, which are antigen-presenting cells. In a unique, non-self-reactive manner, TLRs recognize microbial markers such as protein, carbohydrate, lipid, nucleic acid, and/or their combinations to initiate a complex signalling cascade that activates a wide variety of transcription factors and inflammatory cytokines. Each TLR is an immune system alert signal able to induce proinflammatory mediators in response to the activation of the innate immune system and initiate adaptive immune responses [40.42,43].

TLR2 expression increased during Short Term Heat Stress Acclimatation (STHSA) and returned to basal level during Long Term Heat Stress Acclimatation (LTHSA) and recovery phase in Thapakar cattle's peripheral blood mononuclear cells, whereas TLR4 expression increased during STHSA, LTHSA, and decreased during the recovery phase. This result suggested that TLR2 may play a role in the acute immune response during STHSA, whereas TLR4 may be essential for the long-term immune response necessary to mitigate the harmful effects of heat stress and confer thermo-tolerance [40], which is also supported by the observation in Black Bengal goats [44].

Nutrition intervention to reduce heat stress

It is essential to employ effective nutritional strategies to reduce the effects of HS, such as increasing nutrient density, ration reformulation to account for the decreased dry matter intake, and mineral, vitamin, and antioxidant supplementation. HS can be mitigated by using exogenous antioxidants and salt supplementation in the diet [7].

Damage from HS can be mitigated in several ways. However, one of the most important is through antioxidant supplementation, both enzymatic (e.g., superoxide dismutase, glutathione peroxidase, and catalase) and nonenzymatic antioxidants (e.g., vitamins C, E, A, and glutathione, and pyruvate) [32]. Antioxidant defence mechanisms protect cells from cellular oxidants, while repair systems keep oxidatively damaged molecules from building up, potentially reducing the amount of oxidative damage caused by HS [45].

Micronutrients are frequently added to diets to promote livestock production and immune competence (Table 1), such as selenium (Se) and vitamin E. Se, as a component of GSH-Px and thioredoxin reductase, and vitamin E, as a key lipid peroxidation chain breaker, both play important roles in antioxidant activity regulation by balancing the body's pro- and antioxidant status. All three types of antioxidant defence systems in animal cells are involved in their beneficial effects: 1) radical formation prevention, 2) chain formation and propagation prevention, and 3) excision and repair of damaged molecules [46,47].

Table 1. Nutritional intervention in sheep that successfully reduce heat stress

Breed	Supplementation (per kg feed)	Reference
Malpura ewes	20 gr mineral and antioxidant mixture (164.0 mg zinc sulphate, 0.95 mg cobalt sulphate, 1.2 g chromium acetate, 0.1 mg selenium chloride and 40.0 mg vitamin E)	[48]
Merino × Poll Dorset crossbred ewes	100 IU vitamin E and 1.20 mg Selenium	[49]
Katahdin × Dorper ewe lambs	250 mg free ferulic acid	[50]
Crossbred lambs ((Merino × Border Leicester) × Dorset)	228 mg vitamin E and 1.16 mg Selenium	[38]
Katahdin × Pelilbuey and Dorper × Pelibuey crossbred ewes	10 mg of zilpaterol hydrochloride	[51]
Australian Merino rams	0.8 mg Selenium and 150 mg vitamin E	[47]
Barki rams	4% *Sargassum latifolium* algae (contain β-carotene, fucoxanthin, and tocopherol)	[52]
Merino x Poll crossbred sheep	0.4 and 0.8 µg nano chromium picolinate	[53]
Chios cross-bred ewes	0.515 g cornus extract with oregano and thyme essential oils	[54]
Afshari × Chaal lambs	2% phytogenic-rich herbal mixture (cinnamon, turmeric, rosemary, and clove buds)	[55]
Ujumqin lambs	5-10 g chestnut tannins	[56]
Iraqi ewes	40 mg vitamin C	[57]

4. Conclusions

The answer to the title of the review is definitely a 'no', animals that have been exposed to extreme temperatures cannot maintain their health indefinitely. Heat stress compromises immunity through a complex way, including disruption of hormonal and redox balance, inhibition of immune cells, and alteration of barrier function. Immunological indicators such as interleukins (ILs), toll-like receptors (TLRs), and heat shock proteins (HSPs) can be used to assess the heat stress and thermotolerance ability of an animal. Nutritional interventions in form of feed supplementation are an immediate solution for mitigating the effects of heat stress in addition to practical management and environmental interventions such as improving housing and ventilation.

Acknowledgements

This study was supported by the bilateral S&T cooperation programme, within the project "*Effect of heat stress in Pramenka types of sheep using DNA and RNA based methods*" from the National Development, Research and Innovation Fund (2021-1.2.4-TÉT-2021-00047). This study was also supported by the bilateral S&T cooperation programme, within the project "*Genetic characterization of native sheep in Carpathian basin and Morocco as a potential factor for climate change adaptation*" from the National Development, Research and Innovation Fund (2021-1.2.4-TÉT-2021-00014). Thanks to Stipendium Hungaricum Scholarship from Tempus Public Foundation for supporting PKA, RF, GW, and NK studies.

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