

REVIEW: ENVIRONMENTAL AND ANIMAL INDICATORS FOR HEAT STRESS ASSESSMENT IN DAIRY CATTLE

Petar Dodovski*

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0001-7824-3772>
petar.dodovski@uklo.edu.mk

Panche Dameski

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0002-0051-9916>
panche.dameski@uklo.edu.mk

Igor Zdraveski

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0001-9495-9906>
igor.zdraveski@uklo.edu.mk

Nikola Karabolovski

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0004-8665-4319>
nikola.karabolovski@uklo.edu.mk

Natasa Pejcinovska

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0003-1864-6870>
natasa.pejcinovska@uklo.edu.mk

Talija Hristovska

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0003-3892-4375>
talija.hristovska@uklo.edu.mk

Mimi Ristevski

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0000-0009-7836-7842>
mimi.ristevski@uklo.edu.mk

Magdalena Dzunkovska

Veterinary Faculty Bitola University “St. Kliment Ohridski”-Bitola
ORCID iD: <https://orcid.org/0009-0007-8463-6565>
mimi.ristevski@uklo.edu.mk

ABSTRACT

Climate change, the increase in global surface temperature and extreme changes in weather patterns, presents substantial difficulties for farm animals. A main issue is heat stress, which happens when environmental conditions go beyond the thermoneutral zone, hindering animals' ability to release extra body heat. Dairy cattle are seen as highly vulnerable to heat stress because of their elevated metabolic heat production. Under heat stress, dairy cattle encounter alterations in their welfare and health that affect their reproductive and reproductive performances. Hence, climate change has enormous impact

on dairy cattle farming as a major source of global food production. To maintain the isothermal condition, animals employ several physiological and behavioral mechanisms in order to dissipate heat. The pathophysiology of heat stress and its impact on various organs and organic systems has been studied extensively in dairy cattle in recent years. Understanding the effects of heat stress on animals, including environmental factors, physiological and behavioral responses, and pathophysiology, is crucial for creating successful strategies to safeguard them. The goal of this paper was to provide an overview of the most recent information on the Temperature-Humidity Index (THI) as a commonly used indicator of environmental heat stress, and on physiological and behavioral responses as indicators of heat stress in animals. The complexity of these two indicators was an incentive to compile and summarize the most recent papers comprehensively, and provide them as a resource for future research in various fields.

Keywords: Climate change, dairy cattle, heat stress, indicator

INTRODUCTION

Climate change is currently the most difficult problem facing humanity. In recent times, there has been a growing acknowledgement that climate change poses the greatest threat to world ecological balance and economic stability. The rise in global surface temperature is the most intriguing feature of the changing climate. According to the fifth IPCC Assessment Report (AR5), by the end of 2100, global surface temperature is anticipated to rise by 1-7 °C (IPCC 2013). In the scenario of a changing climate, it is also anticipated that life-threatening phenomena such as cyclones, droughts, floods, tsunamis, and wildfires will occur more frequently. All of these events will have a disastrous impact on the ecological balance of the world, impeding the biological conditions that are ideal for the sustainable growth of numerous species. Most importantly, however, is that the effects of climate change will undermine the sustainability of the livestock and agricultural sectors, which are vital to the global supply of food.

Numerous studies have been conducted in recent years to examine the effects of altered climate conditions on farm animals, with the majority of them focused on dairy cattle (Giannone et al., 2023). The objectives in this paper were to summarize the latest references on the two types of heat stress indicators that could be useful to evaluate the effect of increased ambient temperature in dairy cattle.

1. HEAT STRESS IN FARM ANIMALS

Heat stress appears to be one of the most intriguing environmental factors affecting livestock and impeding animal welfare and animal production in many parts of the world (Silva et al., 2023). Cattle, like most other livestock species, have a thermal comfort zone that ranges from 16 to 25°C. This is because basal metabolism is on an optimal scale, and thermoregulation occurs within this temperature range (Abdelnoure et al., 2019). Animals are stressed by environmental factors such as humidity, high direct or indirect solar radiation, and heat stress brought on by high ambient temperatures.

Livestock is subject to multifactorial effects from heat stress. Animal-based or environmental indicators can be employed to assess heat stress in farm animals. Animal-based indicators, illustrate the animal state in a particular setting. They encompass any alterations in behavior or physiology brought on by heat stress. In contrast, environmental

indicators primarily pertain to the temperature of the surrounding air (°C), relative humidity (%), wind speed (m/s), and the determination of the temperature-humidity index (THI) that the animal is subjected to (Ouellet et al 2021).

2. HEAT STRESS INDICATORS IN DAIRY CATTLE

2.1. Environmental indicator - Humidity index

The Temperature-Humidity Index (THI) is a measure that combines air temperature and relative humidity to estimate the perceived temperature and to assess heat stress on people and animals. Thus, THI can be considered as a valuable environmental indicator that can be used to determine whether or not animals are experiencing heat stress and how severe it is. As a meteorological safety index, THI is a comprehensive index composed of the effects of ambient temperature and humidity that has been developed to track potential effects of these factors on animals (Cartmell et al 2001). A number of equation models have been proposed to evaluate THI. The National Research Center's equation from 1971 ($THI = (1.8 \times AT_{avg} + 32) - [(0.55 - 0.0055 \times RH_{avg}) \times (1.8 \times AT_{avg} - 26)]$ where T = dry bulb temperature (°C) and RH_{avg} is daily mean relative humidity (%)) is considered to be the most suitable and is frequently applied in heat stress research carried out in a variety of climates (Mbuthia et al., 2021; Ouellet et al., 2021).

Most of the published papers related to THI as an environmental indicator, are focused on farm animals, particularly on dairy cattle. Numerous studies examine how high temperatures and humidity affect dairy cows' body temperatures, feed intake, milk production, follicle development, estrous behavior, and pregnancy (Liu et al 2018; Singh et al 2021; Silva et al., 2023, Nzeyimana et al 2023,). In a paper published in 2020, Dimov and colleagues, highlight the value of THI as an indicator for dairy cow heat stress prediction. However, since THI is only an environmental indicator, Liu and coworkers (2018) consider that the simultaneous measurement of THI and physiological indexes (e.g., body temperature, respiration rate and heart rate) would be very useful in assessing the heat stress on dairy cattle. It has been found that there is a strong correlation between THI and the body temperature of dairy cattle, as measured by both, milk temperature and rectal or vaginal temperature. The critical THI threshold for dairy cattle is generally accepted to range from 68 and 72, relying on the cattle's body temperature, heart rate, and respiratory rate. However, Ouellet and coworkers (2021) reported that the THI threshold established for lactating cows are not suitable to accurately assess heat stress in dry cows. THI threshold can vary in different type of accommodation and different environmental conditions for dairy cattle (Yan et al., 2021; Oliveira et al., 2022). Feeding management and water availability are closely related to THI values as heat stress indicator in farm animals. Water deprivation in lactating cattle exposed to chronic heat, can lower the THI critical value at which dairy cows will begin to exhibit symptoms of heat stress (Correa-Calderón et al., 2022). Different dairy traits and the ability of animals to adapt to environmental conditions can also be a reason for differences in heat tolerance thresholds (Mbuthia et al 2022). Many studies have established that heat tolerance is negatively correlated with milk production potential. Oloo and coworkers (2024) concluded that higher milk-producing cows are more vulnerable to heat stress, while heat-tolerant cows tend to have stable milk production during such conditions. Furthermore, studies conducted on crossbred cattle revealed that THI has higher upper values whereas a THI up to 74 and above resulted with no drastic change of physiological parameters suggesting animals undergo transient acclimatization in this range to maintain homeostasis. Therefore, Jeelani and coworkers (2019) suggested reassessment of the THI values according to type of animal and for different climatic region. However, THI has two

disadvantages, though, in that it excludes two factors that are also regarded as fundamental weather parameters: wind speed and solar radiation. These factors have a significant impact on how an animal reacts to heat stress. In order to overcome these drawbacks of THI indices, this has led the scientific community to develop more sophisticated thermal indices which are combined with THI during animal heat stress assessment (Choi et al., 2024).

2.2. Animal-based indicators - Physiological and behavioral response

Giannone and coworkers (2023) in their review provides an overview of all features, including physiological, morphological, behavioral, metabolic, productive, and immune responses in dairy cattle on heat stress. Heat stress is defined as the sum of external environmental forces acting on animals, which results in an increase in body temperature and causes a physiological response (Šímová et al., 2016). Heat stress is considered to have one of the most important deleterious effects on animal welfare (Silva et al., 2023). The main animal's response to heat stress, is heat dissipation which takes place through behavioral and physiological thermoregulatory mechanisms. The mechanism of maintaining the optimal body temperature is mostly relied on capacity of adaptation to new altered environmental conditions. Thus, physiological adaptability is the ability of an animal to adapt its behavior or metabolic response to harsh environmental conditions (Rashamol et al., 2020). However, there are various adaptive responses exhibited by the animal when exposed to different environmental stresses, such as behavioral, physiological, blood biochemical, cellular and neuro-endocrine responses.

Neuro-endocrine mechanism of adaptation was discussed in Afsal and coworkers review (2018) as one of the principal adaptive responses shown by the animal in extreme stress condition. In a recent study, Nakamura and coworkers (2022) present a combined neural network for environmental stress responses, providing insights into central circuit mechanism for the integrative regulation of systemic organs. The same authors reported that, mechanisms of physiological responses and effective organs for homeostasis are based on the regulation of sympathetic outflow by the hypothalamomedullary neural pathway from the dorsomedial hypothalamus to the rostral medullary raphe region. Heat input in preoptic area, dynamically alter these neural pathways that regulate the thermoregulatory and cardiovascular response (Nakamura et al 2022). However, neural function is precisely directed by variations in genes conferring high capacity for high temperature tolerance (Cheruiyot et al., 2021).

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, the primary endocrine regulator of animal stress responses. The effect of HPA mediated response is more general and it appears more slowly than sympathetic response. Activating HPA leads to elevated secretion of cortisol, a classical stress marker, and ultimately initiate physiological responses to balance the effect of heat stress (Afsal et al., 2018). Thus, heat stress significantly impacts the metabolism of proteins, lipids, carbohydrates, and minerals, predominantly altering the blood glucose, protein and non-esterified fatty acids (NEFA) levels. Obtaining glucose from sources other than carbohydrates will help toward supplying the body's tissues and cells with the energy they require (Durmuş and Koluman., 2019). These events are in favor for adaptation to thermoregulatory mechanism and coping with potential homeostasis alterations. However, the duration and intensity of heat stress will affect the glucose level of dairy cattle (Sammad et al., 2022). There are various reasons why heat stress can affect blood sugar levels: during heat stress in dairy cow is shown to have increased insulin blood level and high sensitivity to glucose which is considered as an adaptive mechanism; the heat-stressed animal shows post-absorptive changes in glucose level that are independent of the energetic status of the animal; decreased feed intake and energetic metabolism modulation, regardless of the

stage of lactation, lead to a decrease in energy balance, entering to an energy destabilized state; upregulation of insulin independent glucose transporters (GLUT I) meaning that blood level of glucose is also related to tissue specific glucose uptake mechanism (Abbas et al., 2020, Blond et al., 2024)

Assessing the fecal and hair level of cortisol is proposed as noninvasive indicators of heat stress in ruminants (Sejian et al., 2022). Blood cortisol level combined with the level of thyroid hormones are considered as potential biomarkers in heat and nutritional stress along with alterations of thyroid stimulating hormone (TSH), progesterone, estradiol, follicle-stimulating hormone (FSH), inhibin, luteinizing hormone (LH), growth hormone, etc. Sammad and coworkers (2020) provide an overview of the endocrine and metabolic changes as physiological response to heat stress in cattle. Namely, the decline of thyroid hormones along with decreased plasma growth hormone (GH), has a synergistic effect to reduce heat production. Also, low level of GH and increased insulin like factor I (IGF I) lead to increased lipid mobilization (increased serum level of NEFA) and GH-dependent liver process like upregulation of gluconeogenesis. On the other hand, decreased IGF II significantly alter the dietary intake in heat stressed animals (Bagath et al., 2016). Impaired feed intake associated with adaptive behavioural responses to heat stress can be attributed to low thyroid hormones and a lower basal metabolic rate. Electrolyte and water balance disturbances are the result of dehydration that cattle may undergo during heat stress. Dehydration initiates increased secretion of antidiuretic hormone (ADH) and aldosterone as a result of activation of the of the renin-angiotensin-aldosterone axis. The mechanism aims to maintain the homeostasis of electrolyte and pH balance in an animal's body. A detailed overview of electrolyte and pH balance alteration in cattle facing heat stress, was provided by Burhans and coworkers in their paper published in 2022. The paper's focus is on the physiological responses and pathological consequences of heat stress in dairy cattle. Furthermore, the same authors described lethal heat stress as a pathological reaction syndrome to heat stress levels that are higher than an animal's ability to tolerate or lessen hyperthermia.

Increased hemoglobin concentration and hematocrit values with higher environmental temperatures has been reported, attributed this increase to haemoconcentration, so that the animals could meet a higher oxygen requirement during stressful conditions (Sejian et al., 2018). Higher temperatures have the potential to cause harmful pathological alterations in key organs both histologically and macroscopically. Rebes and coworkers reviewed and framed the current published papers related to heat stress-induced histopathological alterations (2023). The authors emphasize on heat stress-induced histopathological changes in livestock's productive and adaptive organs based on the knowledge currently accessible in the literature. They also discuss the use of a panel of immunohistochemistry biomarkers for diagnostic guidance and confirmation.

Numerous current studies focus on how heat stress affects an animal's gut flora (Zhang et al., 2022; Wang et al., 2022; Cartwright et al., 2023; Yuet al., 2024). It is well established that heat stress increases the vulnerability of gut in cattle due to greater establishment of pathogenic microbiota that leads to damaging the mucosal epithelium structure and increased oxidative insults, reduced immunity, and increased permeability of the gut. The rumen microbiome of dairy cattle can also be significantly affected by heat stress. It has been observed that in heat-stressed cows, lactate-producing bacteria (*Streptococcus*, unclassified *Enterobacteriaceae*, *Ruminobacter*, which utilize soluble carbohydrates, *Treponema* and unclassified *Bacteroidaceae*) and cellulolytic bacteria (*Fibrobacteres*) which are considered more resilient to heat stress, are more prevalent compared to acetate-producing bacteria (*Actinobacteria* and *Acetobacter*) (Kim et al., 2022). Ruminal dysbiosis in heat-stressed cows led to an increased risk of rumen acidosis as ruminal pH decreased due to the accumulation of ruminal lactate and decreased acetate.

Acid-balance disturbance, metabolic alteration, and activated HPA axis in heat stress, are strongly associated with increased inflammation markers like TNF- α , extracellular HSP70, and interleukins such as IL 4 and IL 6. (Judi et al., 2022, Blond et al., 2024). However, Rakib and coworkers (2024) suggested that HSP70 could be useful marker for heat stress in dairy cattle, as it is considered a potential player involved in cellular thermotolerance in dairy cows, and it was found increased HSP70 gene expression in heat-stressed cows. Inflammation has been linked to increased levels of cellular inflammatory components and the release of proinflammatory cytokines in animals under heat stress (Most and Yates, 2022; Wijffels et al., 2024). Burhans and coworkers (2022) suggested that the pathogenesis of heat stress in dairy cows includes two independent pathways, “heat toxicity” and “heat sepsis,” similarly as has been previously described by Lym (2018), in humans. Hyperthermia and direct thermolytic cellular effect are attributed to “heat toxicity” but oxidative stress, bacteremia and endotoxemia, to “heat sepsis”. Endotoxemia is thought to be a direct result of compromised gut integrity and increased gastrointestinal tract permeability, and an indirect result of heat stress in mammals. Results obtained in study conducted by Ruiz-González and coworkers (2022), suggest that inflammation can occur as a result of the penetration of fecal metabolites from the digestive organs. But it is well known that the mechanism of vascular adjustment driven by splanchnic vasoconstriction leads to peripheral fluid redistribution for heat dissipation and maintaining the optimal visceral temperature, creating up to 50% of the fluid intestinal deficit and hypoxia, which compromise barrier integrity and contribute to increased intestinal permeability. (Burhans et al., 2022; Yu, 2023). This is considered a primary cause for initiating a cascade of systemic inflammation and further on systemic inflammatory syndrome (SIRS). According Burhans and coworkers (2022), SIRS can be result of an exaggerated and extreme immune response to systemic endotoxemia and cellular thermal, oxidative, or anoxic damage that ultimately led to multiple organ dysfunctions (MOD) combined with disseminated coagulopathy (DIC). These events are the hallmark of pathophysiology of lethal heat stress in animals including dairy cattle.

Behavioral response is the first animal-based non-invasive indicator that heat-stressed animals exhibit to combat increased temperature load. Animals' behavioral response is directly impacted by the degree of heat stress they experience. In order to cool themselves, animals exhibit a variety of thermoregulatory behaviors that increase heat dissipation through radiation, convection, or evaporation (Mota-Rojaset al., 2021, Rodríguez-González et al., 2023). Behavioral response was a subject of investigation in many recent studies and it is well established. Giannone and coworkers (2023) in their review paper summarizes all references related to behavioral responses including standing and lying, drinking and feeding traits in dairy cattle during heat stress. Pontiggia and coworkers (2024) found that grazing dairy cows with greater body heat load, measured through vaginal route, had an increased mean respiratory rate and decreased durations of walking, lying, feeding, and ruminating. Additionally, the results indicate that these cows were more likely to be in close proximity to the water trough and to have an increased water intake. Dairy cows' drinking behavior is strongly associated with their capacity to cope with high temperatures due to the need for evaporative cooling, which causes water consumption to increase with temperature (Collier et al., 2019). Water consumption is also linked to other factors such as dietary composition, lactation and milk production, social interactions, environmental stimuli, physiological needs and the overall hydration status of the animals.

CONCLUSION

Heat stress is going to be a persistent challenge for dairy cattle farming, as the earth's temperature is rising annually and is expected to continue rising in the future. For dairy farming systems to remain sustainable, maximize productivity, and preserve animal welfare, it is essential to evaluate heat stress in dairy cattle. The ability to predict the harmful effects of heat stress and respond timely and accordingly may significantly mitigate its negative effects. Assessing heat stress by choosing not only the most precise but also the most applicable method is of pivotal meaning. The recent published articles compiled in this review provide an integrated scope of environmental and animal-based indicators into a comprehensive heat stress assessment framework, which is said to be essential for an effective mitigation management approach when it comes to using the most applicable and indicative indicators. Thus, in an era of global warming, this integrative approach should enable the improvement of dairy cattle welfare and the promotion of sustainable dairy farming.

Future research should focus on creating adaptive models that consider genetic, environmental, and management variables to enhance dairy cattle resilience to heat stress. Moreover, region-specific thresholds and customized interventions are required to enhance dairy production in varying climatic conditions.

This review may also be considered an initiative for further investigation on the effect of climate variation in Pelagonia region on dairy cattle in extensive and intensive farming systems, which is considered a region of quite significant seasonal climate variations.

REFERENCES

1. Abbas, Z., Sammad, A., Hu, L., Fang, H., Xu, Q. and Wang, Y., 2020. Glucose metabolism and dynamics of facilitative glucose transporters (GLUTs) under the influence of heat stress in dairy cattle. *Metabolites*, 10(8), p.312. DOI: 10.3390/metabo10080312.
2. Abdelnour, S.A., Abd El-Hack, M.E., Khafaga, A.F., Arif, M., Taha, A.E. and Noreldin, A.E., 2019. Stress biomarkers and proteomics alteration to thermal stress in ruminants: A review. *Journal of thermal biology*, 79, pp.120-134. DOI: 10.1016/j.jtherbio.2018.12.013.
3. Afsal, A., Sejian, V., Bagath, M., Krishnan, G., Devaraj, C. and Bhatta, R., 2018. Heat stress and livestock adaptation: Neuro-endocrine regulation. *International Journal of Veterinary and Animal Medicine*, 1(2), pp.1-8. DOI: 10.31021/ijvam.20181108
4. Bagath, M., Sejian, V., Archana, S.S., Manjunathareddy, G.B., Parthipan, S., Selvaraju, S., Mech, A., David, C.G., Ravindra, J.P. and Bhatta, R., 2016. Effect of dietary intake on somatotrophic axis-related gene expression and endocrine profile in Osmanabadi goats. *Journal of Veterinary Behavior*, 13, pp.72-79. DOI.org/10.1016/j.jveb.2016.04.001
5. Blond, B., Majkić, M., Spasojević, J., Hristov, S., Radinović, M., Nikolić, S., Anđušić, L., Čukić, A., Došenović Marinković, M., Vujanović, B.D. and Obradović, N., 2024. Influence of Heat Stress on Body Surface Temperature and Blood Metabolic, Endocrine, and Inflammatory Parameters and Their Correlation in Cows. *Metabolites*, 14(2), p.104. DOI: 10.3390/metabo14020104.
6. Burhans, W.S., Burhans, C.R. and Baumgard, L.H., 2022. Invited review: Lethal heat stress: The putative pathophysiology of a deadly disorder in dairy cattle. *Journal of Dairy Science*, 105(5), pp.3716-3735. DOI.org/10.3168/jds.2021-21080
7. Cartmill, J.A., El-Zarkouny, S.Z., Hensley, B.A., Rozell, T.G., Smith, J.F. and Stevenson, J.S., 2001. An alternative AI breeding protocol for dairy cows exposed to elevated ambient temperatures before or after calving or both. *Journal of dairy science*, 84(4), pp.799-806. DOI.org/10.3168/jds.S0022-0302(01)74536-5
8. Cartwright, S.L., Schmied, J., Karrow, N. and Mallard, B.A., 2023. Impact of heat stress on dairy cattle and selection strategies for thermotolerance: A review. *Frontiers in veterinary science*, 10, p.1198697. DOI.org/10.3389/fvets.2023.1198697

9. Cheruiyot, E.K., Haile-Mariam, M., Cocks, B.G., MacLeod, I.M., Xiang, R. and Pryce, J.E., 2021. New loci and neuronal pathways for resilience to heat stress in cattle. *Scientific reports*, 11(1), p.16619. DOI: 10.1038/s41598-021-95816-8.
10. Choi, E., de Souza, V.C., Dillon, J.A., Kebreab, E. and Mueller, N.D., 2024. Comparative analysis of thermal indices for modeling cold and heat stress in US dairy systems. *Journal of Dairy Science*, 107(8), pp.5817-5832. DOI: 10.3168/jds.2023-24412.
11. Collier, R.J., Baumgard, L.H., Zimbelman, R.B. and Xiao, Y., 2019. Heat stress: physiology of acclimation and adaptation. *Animal Frontiers*, 9(1), pp.12-19. DOI: 10.1093/af/vfy031.
12. Correa-Calderón, A., Avendaño-Reyes, L., López-Baca, M. and Macías-Cruz, U., 2022. Heat stress in dairy cattle with emphasis on milk production and feed and water intake habits. Review. *Revista mexicana de ciencias pecuarias*, 13(2), pp.488-509. DOI: [10.22319/rmcp.v13i2.5832](https://doi.org/10.22319/rmcp.v13i2.5832)
13. Dimov, D., Penev, T. and Marinov, I., 2020. Temperature-humidity index—an indicator for prediction of heat stress in dairy cows. *Veterinarija ir Zootechnika*, 78(100), pp.10-15. DOI: [10.3168/jds.2006-513](https://doi.org/10.3168/jds.2006-513)
14. Durmuş, M. and Koluman, N., 2019. Hormonal Changes caused on ruminant animals exposed to high environment temperature. *J Anim Prod*, 60(2), pp.159-169. DOI: 10.29185/hayuretim.547128
15. Giannone, C., Bovo, M., Ceccarelli, M., Torreggiani, D., and Tassinari, P. 2023. Review of the heat stress-induced responses in dairy cattle. *Animals* 13, 3451. DOI: [10.3390/ani13223451](https://doi.org/10.3390/ani13223451)
16. IPCC (Intergovernmental Panel on Climate Change) (2013) The physical science basis. In: Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (Eds.) Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 1535.
17. Jeelani, R., Konwar, D., Khan, A., Kumar, D., Chakraborty, D. and Brahma, B., 2019. Reassessment of temperature-humidity index for measuring heat stress in crossbred dairy cattle of a sub-tropical region. *Journal of thermal biology*, 82, pp.99-106. DOI: [10.1016/j.jtherbio.2019.03.017](https://doi.org/10.1016/j.jtherbio.2019.03.017)
18. Judi, R.A., Shubar, S.N.A., Alsherify, B.A.H. and Al-Taiy, M.K., 2022, July. Differentiation in Some of Cytokines Levels of Holstein Cows Exposure to Heat Stress. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1060, No. 1, p. 012078). IOP Publishing. DOI: [10.1088/1755-1315/1060/1/012078](https://doi.org/10.1088/1755-1315/1060/1/012078)
19. Kim, S.H., Ramos, S.C., Valencia, R.A., Cho, Y.I. and Lee, S.S., 2022. Heat stress: effects on rumen microbes and host physiology, and strategies to alleviate the negative impacts on lactating dairy cows. *Frontiers in microbiology*, 13, p.804562. DOI: 10.3389/fmicb.2022.804562
20. Lim, C.L., 2018. Heat sepsis precedes heat toxicity in the pathophysiology of heat stroke—a new paradigm on an ancient disease. *Antioxidants*, 7(11), p.149. <https://doi.org/10.3390/antiox7110149>
21. Liu, J., Li, L., Chen, X., Lu, Y. Wang, D., 2019. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: A novel idea for monitoring and evaluation of heat stress—A review. *Asian-Australasian journal of animal sciences*, 32(9), p.1332. <https://doi.org/10.5713/ajas.18.0743>
22. Mbuthia, J.M., Mayer, M. and Reinsch, N., 2021. Modeling heat stress effects on dairy cattle milk production in a tropical environment using test-day records and random regression models. *Animal*, 15(8), <https://doi.org/10.1016/j.animal.2021.100222>
23. Mbuthia, J.M., Mayer, M. and Reinsch, N., 2022. A review of methods for improving resolution of milk production data and weather information for measuring heat stress in dairy cattle. *Livestock Science*, 255, <https://doi.org/10.1016/j.livsci.2021.104794>
24. Most, M.S. and Yates, D.T., 2021. Inflammatory mediation of heat stress-induced growth deficits in livestock and its potential role as a target for nutritional interventions: A review. *Animals*, 11(12), p.3539. <https://doi.org/10.3390/ani11123539>

25. Mota-Rojas, D., Titto, C.G., Orihuela, A., Martínez-Burnes, J., Gómez-Prado, J., Torres-Bernal, F., Flores-Padilla, K., Carvajal-de la Fuente, V. and Wang, D., 2021. Physiological and behavioral mechanisms of thermoregulation in mammals. *Animals*, 11(6), p.1733.[doi: 10.3390/ani11061733](https://doi.org/10.3390/ani11061733).
26. Nakamura, K., Nakamura, Y. & Kataoka, N. 2022. A hypothalamomedullary network for physiological responses to environmental stresses. *Nat Rev Neurosci* 23, 35–52. <https://doi.org/10.1038/s41583-021-00532-x>
27. Nzeyimana, J.B., Fan, C., Zhuo, Z., Butore, J. and Cheng, J., 2023. Heat stress effects on the lactation performance, reproduction, and alleviating nutritional strategies in dairy cattle, a review. *Journal of Animal Behaviour and Biometeorology*, 11(3), pp.0-0.<https://doi.org/10.31893/jabb.23018>
28. Oliveira, A.V.D., Reis, E.M.B., Ferraz, P.F.P., Barbari, M., Santos, G.S., Cruz, M.V.R., Silva, G.F. and Silva, A.O.L., 2022. Effects of thermal environment on dairy cattle under a grazing system in the Western Amazon, Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 74(06), pp.1119-1126. <https://doi.org/10.1590/1678-4162-12743>
29. Oloo, R.D., Ekine-Dzivenu, C.C., Mrode, R., Bennewitz, J., Ojango, J.M.K., Kipkosgei, G., Gebreyohanes, G., Okeyo, A.M. and Chagunda, M.G.G., 2024. Genetic analysis of phenotypic indicators for heat tolerance in crossbred dairy cattle. *animal*, 18(5),<https://doi.org/10.1016/j.animal.2024.101139>
30. Ouellet, V., Toledo, I.M., Dado-Senn, B., Dahl, G.E. and Laporta, J., 2021. Critical temperature-humidity index thresholds for dry cows in a subtropical climate. *Frontiers in Animal Science*, 2,doi.org/10.3389/fanim.2021.706636
31. Ouellet, V., Toledo, I.M., Dado-Senn, B., Dahl, G.E. and Laporta, J., 2021. Critical temperature-humidity index thresholds for dry cows in a subtropical climate. *Frontiers in Animal Science*, 2, p.706636. [doi:10.3389/fanim.2021.706636](https://doi.org/10.3389/fanim.2021.706636)
32. Pontiggia, A., Münger, A., Eggerschwiler, L., Holinger, M., Stucki, D., Ammer, S., Bruckmaier, R.M., Dohme-Meier, F. and Keil, N.M., 2024. Behavioural responses related to increasing core body temperature of grazing dairy cows experiencing moderate heat stress. *animal*, 18(3), p.101097. <https://doi.org/10.1016/j.animal.2024.101097>
33. Rakib, M.R.H., Messina, V., Gargiulo, J.I., Lyons, N.A. and Garcia, S.C., 2024. Potential use of HSP70 as an indicator of heat stress in dairy cows—a review. *Journal of Dairy Science*. <https://doi.org/10.3168/jds.2024-24947>
34. Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana, P.R. and Bhatta, R., 2020. Physiological adaptability of livestock to heat stress: an updated review. *Journal of Animal Behaviour and Biometeorology*, 6(3), pp.62-71. DOI.org/10.31893/2318-1265jabb.v6n3
35. Rebez, E.B., Sejian, V., Silpa, M.V. and Dunshea, F.R., 2023. Heat stress and histopathological changes of vital organs: A novel approach to assess climate resilience in farm animals. *Sustainability*, 15(2), p.1242. <https://doi.org/10.3390/su15021242>
36. Rodríguez-González, D., Guerrero Legarreta, I., Cruz-Monterrosa, R.G., Napolitano, F., Titto, C.G., Abd El-Aziz, A.H., Hernández-Avalos, I., Casas-Alvarado, A., Domínguez-Oliva, A. and Mota-Rojas, D., 2023. Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys. *Frontiers in Veterinary Science*, 10, p.1184577. doi.org/10.3389/fvets.2023.1184577
37. Ruiz-González, A., Rico, D.E. and Rico, J.E., 2022. Modulation of fecal metabolites by heat stress and diet, and their association with inflammation and leaky gut markers in dairy cows. *Metabolites*, 12(2), p.142. <https://doi.org/10.3390/metabo12020142>
38. Sammad, A., Wang, Y.J., Umer, S., Lirong, H., Khan, I., Khan, A., Ahmad, B. and Wang, Y., 2020. Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals*, 10(5), p.793. DOI:10.3390/ani10050793
39. Sejian, V., Bhatta, R., Gaughan, J.B., Dunshea, F.R. and Lacetera, N., 2018. Adaptation of animals to heat stress. *Animal*, 12(s2), pp.s431-s444. <https://doi.org/10.1017/S1751731118001945>

40. Sejian, V., Shashank, C.G., Silpa, M.V., Madhusoodan, A.P., Devaraj, C. and Koenig, S., 2022. Non-invasive methods of quantifying heat stress response in farm animals with special reference to dairy cattle. *Atmosphere*, 13(10), p.1642. DOI: 10.3390/atmos13101642
41. Silva, W.C.D., Silva, J.A.R.D., Camargo-Júnior, R.N.C., Silva, É.B.R.D., Santos, M.R.P.D., Viana, R.B., Silva, A.G.M.E., Silva, C.M.G.D. and Lourenço-Júnior, J.D.B., 2023. Animal welfare and effects of per-female stress on male and cattle reproduction—A review. *Frontiers in Veterinary Science*, 10, DOI 10.3389/fvets.2023.1083469
42. Šimová, V., Večerek, V., Passantino, A. and Voslářová, E., 2016. Pre-transport factors affecting the welfare of cattle during road transport for slaughter—a review. *Acta Veterinaria Brno*, 85(3), pp.303-318. <https://doi.org/10.2754/avb201685030303>
43. Singh, S.P., Kumar, A. and Sourya, N., 2021. Effects of heat stress on animal reproduction. *International Journal of Fauna and Biological Studies*, 8, pp.16-20. DOI:10.22271/23940522.2021.v8.i2a.806
44. Wang, Z., Niu, K., Rushdi, H.E., Zhang, M., Fu, T., Gao, T., Yang, L., Liu, S. and Lin, F., 2022. Heat stress induces shifts in the rumen bacteria and metabolome of buffalo. *Animals*, 12(10), p.1300. <https://doi.org/10.3390/ani12101300>
45. Wijffels, G., Sullivan, M.L., Stockwell, S., Briscoe, S., Pearson, R., Li, Y., Macs, A.M., Sejian, V., McCulloch, R., Olm, J.C.W. and Cawdell-Smith, J., 2024. Comparing the responses of grain-fed feedlot cattle under moderate heat load and during subsequent recovery with those of feed-restricted thermoneutral counterparts: blood cells and inflammatory markers. *International journal of biometeorology*, 68(2), pp.211-227. <https://doi.org/10.1007/s00484-023-02584-3>
46. Yan, G., Shi, Z. and Li, H., 2021. Critical temperature-humidity index thresholds based on surface temperature for lactating dairy cows in a temperate climate. *Agriculture*, 11(10), p.970. <https://doi.org/10.3390/agriculture11100970>
47. Yu, Z., Cantet, J.M., Paz, H.A., Kaufman, J.D., Orellano, M.S., Ipharraguerre, I.R. and Ríus, A.G., 2024. Heat stress-associated changes in the intestinal barrier, inflammatory signals, and microbiome communities in dairy calves. *Journal of Dairy Science*, 107(2), pp.1175-1196. DOI: 10.3168/jds.2023-23873.
48. Yu, Zhantao, "EFFECTS OF HEAT STRESS ON INFLAMMATION, INTESTINAL INTEGRITY, MICROBIOTA, AND PRODUCTIVITY IN DAIRY CALVES. " 2023, PhD diss., University of Tennessee.https://trace.tennessee.edu/utk_graddiss/9039
49. Zhang, X., Cui, K., Wen, X., Li, L., Yu, X., Li, B., Lin, H., He, H. and Wang, F., 2022. The association between gut microbiome diversity and composition and heat tolerance in cattle. *Microorganisms*, 10(8), p.1672. <https://doi.org/10.3390/microorganisms10081672>