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Invited review: From heat stress to disease—Immune response and candidate genes involved in cattle thermotolerance

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ABSTRACT

Heat stress implies unfavorable effects on primary and functional traits in dairy cattle and, in consequence, on the profitability of the whole production system. The increasing number of days with extreme hot temperatures suggests that it is imperative to detect the heat stress status of animals based on adequate measures. However, confirming the heat stress status of an individual is still challenging, and, in consequence, the identification of novel heat stress biomarkers, including molecular biomarkers, remains a very relevant issue. Currently, it is known that heat stress seems to have unfavorable effects on immune system mechanisms, but this information is of limited use in the context of heat stress phenotyping. In addition, there is a lack of knowledge addressing the molecular mechanisms linking the relevant genes to the observed phenotype. In this review, we explored the potential molecular mechanisms explaining how heat stress affects the immune system and, therefore, increases the occurrence of immune-related diseases in cattle. In this regard, 2 relatively opposite hypotheses are under focus: the immunosuppressive action of cortisol, and the proinflammatory effect of heat stress. In both hypotheses, the modulation of the immune response during heat stress is highlighted. Moreover, it is possible to link candidate genes to these potential mechanisms. In this context, immune markers are very valuable indicators for the detection of heat stress in dairy cattle, broadening the portfolio of potential biomarkers for heat stress.
Key words: heat stress, immune markers, cortisol, molecular mechanisms, GWAS

INTRODUCTION

Heat stress (**HS**) appears when heat gain is greater than heat loss, inducing a loss of homeostasis and an increase in body temperature (Dikmen and Hansen, 2009; Herbut et al., 2018; Most and Yates, 2021). In causality, these changes trigger various animal responses, including behavioral, physiological, neuroendocrine, and molecular changes (Collier and Gebremedhin, 2015; Mishra, 2021). Heat stress especially impairs cows with high genetic merit for production traits (Nguyen et al., 2016). This may be linked to the fact that individuals who produce milk in large quantities have also a high metabolic heat production. Thus, Kadzere et al. (2002) indicated an air temperature of 25 to 26°C for HS in dairy cows. The temperature-humidity index (**THI**) combines both important environmental descriptors air temperature and humidity and is commonly used to define a threshold to determine the onset of HS (Halli et al., 2021). However, the environmental descriptor THI does not consider interindividual variability, indicating the need for additional HS markers to confirm the HS status of a given animal. Immune markers seem to be promising indicators in this regard. Indeed, HS implies unfavorable effects on the immune system, inducing a greater susceptibility to infectious diseases such as mastitis and metritis in cattle (Lacetera, 2018; Dahl et al., 2020). Also genetically, a higher level of somatic cells, indicating impaired udder health, has been associated with increased susceptibility to HS (Hammami et al., 2015). Moreover, animals classified as "high immune responders" (based on estimated breeding values for antibody and cell-mediated immune response) showed improved tolerance to HS compared with the responses of the control group [e.g., higher expression of heat shock proteins (**HSP**) and greater cell proliferation; Cartwright et al., 2021], highlighting the influence of thermotolerance on immune components. Currently, this knowledge is not considered when developing HS indicators, and the molecular mechanisms linking HS to immune-related diseases are rarely discussed. However, deep phenotyping strategies based on a better understanding of how HS affects immune response will contribute to the identification and use of novel potential HS biomarkers.

Consequently, this review focuses on the effects of HS on immune responses, specifically describing the

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mechanisms associating body temperatures, cortisol secretion, and the different immune response mechanisms observed in cattle. Additionally, we study the potential candidate genes (i.e., genes that have been detected based on GWAS in HS experiments) in the context of impaired immunity. Finally, we suggest some potential immune-related biomarkers for HS detection and for the discrimination of thermotolerant and thermosensitive animals.

LINKS BETWEEN HEAT STRESS, CORTISOL, AND IMPAIRED IMMUNITY

From Heat Stress to Cortisol Secretion

When reporting studies about the link between cortisol secretion and HS, it is important to note that cortisol levels are potentially evolving during the day. Glucocorticoid secretion follows a circadian rhythm, and, thus, cortisol secretion is variable depending on the period of the day. In mammals, cortisol levels increase in the morning and decrease in the evening (Chung et al., 2011). This pattern has been detected in lactating cows with a maximal peripheral blood cortisol level at around 0530 h and a minimal level at around 1800 h (Lefcourt et al., 1993). On top of this rhythm, cortisol secretion is triggered under stress, implying increased levels also due to HS. Specifically, during HS, the amount of cortisol reaches a plateau, and, afterward, cortisol levels decline with prolonged HS (Mishra, 2021). In this first section of this review, we address the assumption that the amount and duration of cortisol secretion during an HS event are sufficient to trigger the described responses. However, this hypothesis will be challenged in the following sections.

Cortisol secretion is accompanied by secretion of catecholamine, among other hormones. Indeed, HS also triggers the sympathetic-adreno-medullar axis (Mishra, 2021). Carroll and Forsberg (2007) reported that catecholamines stimulate cortisol secretions and act directly on immune cells functions. However, their action on the immune system (anti- or proinflammatory effect) is controversial in humans and in cattle (Bagath et al., 2019; Kruk et al., 2020). Similarly, estrogens and thyroid hormone secretions were reduced during HS (Mishra, 2021). These hormones are known to be able to influence immune function (Singh et al., 2008; Bagath et al., 2019).

Hypothalamic-Pituitary-Adrenal Axis

During HS, one of the axes triggered is the hypothalamic-pituitary-adrenal axis, which stimulates cortisol secretion in the bloodstream (Bagath et al., 2019; Mishra, 2021). Indeed, during HS, the paraventricular nucleus of the hypothalamus produces corticotropin-releasing hormone (Mishra, 2021). This hormone binds to the corticotropin-releasing hormone receptors expressed on the surface of anterior pituitary cells, triggering exocytosis of the adrenocorticotropic hormone (**ACTH**) stored in secretory vesicles (Stevens and White, 2010). The ACTH enters the adrenal gland through the bloodstream and causes the activation of the steroidogenic acute regulatory protein. This protein allows transport of cholesterol to the mitochondrial matrix to achieve the first rate-limiting step of cortisol synthesis (Gill, 1972; Arlt and Stewart, 2005; Galac et al., 2010; Feher, 2012).

Once released into the bloodstream, cortisol is able to passively cross the plasma membrane of target cells. A cortisol receptor, the glucocorticoid receptor (**GR**), is located in the cytoplasm, where it is sequestered by several proteins including HSP90. The binding of cortisol to the GR causes its dissociation from this protein complex and its translocation to the nucleus. The GR can then bind to the glucocorticoid response element regions of the promoter of the target genes. The GR-cortisol complex is also able to modulate the function of other transcription factors by interacting directly with them (Nicolaides et al., 2020).

In cattle, increases of blood cortisol and of milk cortisol have been identified during HS (Chen et al., 2018). Rees et al. (2016) detected an increase of fecal 11,17-dioxoandrostane from 8 to 16 h after the beginning of HS exposure. Indeed, cortisol degradation in the liver generates metabolites in the bile. In the ongoing process, the microbiota of the gut is able to convert these metabolites into 11,17-dioxoandrostane (Möstl et al., 2002).

From Cortisol Secretion to Impaired Immune Response

Among the target cells of cortisol are the immune cells. This section focuses on the effects of HS on different immune cell functions. Afterward, we will extend the aspects of HS and cortisol secretion, possibly inducing immunosuppression in cattle.

Neutrophils. Neutrophils are among the first-line defenders against pathogens. They act directly at the site of infection by various mechanisms such as phagocytosis and production of the neutrophil extracellular trap (Ohms et al., 2020). To reach the site of infection, neutrophils adhere to and pass through blood vessels. In the process of adherence to endothelial cells, neutrophils use a series of surface proteins (Ley, 2002). The neutrophils then travel to the site of infection by chemotaxis (Widdison and Coffey, 2011; Petri and Sanz,

2018). Generally, the binding of these molecules with their receptors leads to the activation of several pathways, including the mitogen-activated protein kinase (**MAPK**) pathways (Petri and Sanz, 2018). Once in proximity to pathogens, neutrophils are activated via their toll-like receptors (**TLR**), which trigger a signaling cascade dependent on the MAPK/ERK pathway. This activation allows neutrophils to carry out their antimicrobial activity (Ronchetti et al., 2018) and is characterized by the production of myeloperoxidase (**MPO**; Lau et al., 2005).

In lactating cattle, it has been shown that neutrophils exhibit decreased phagocytic activity during hot and humid seasons (Dahl et al., 2020; Tejaswi et al., 2020). Some in vitro studies have also showed a decrease in the number of neutrophils during HS, possibly following an increase in apoptosis (Lecchi et al., 2016; Catozzi et al., 2020). However, other studies have indicated an increase in neutrophil count during the summer (Bagath et al., 2019). An increase in the expression of adhesion proteins such as CD11b and CD44 has also been found in Indian native cattle commonly called zebu (i.e., *Bos indicus*) breeds, indicating an increase of their adhesive ability (Alhussien and Dang, 2018). Regarding these studies in cattle, HS unfavorably affected the activity of neutrophils. However, no consensus exists regarding how heat affects the neutrophil count. These differences could be due to the breed. In this regard, we could hypothesize that an increase of neutrophils with increasing HS appears in zebu breeds. Similarly, the increase of adhesion molecules expression appearing in zebu breeds, which are adapted to hot and humid climates, might mitigate the negative effects of HS on immunity.

The hypothalamic-pituitary-adrenal axis appears to be involved in linking HS to a functional change in neutrophils. Indeed, Salak-Johnson and McGlone (2007) showed that the injection of ACTH in a Japanese cattle breed impairs neutrophil functions. Moreover, the glucocorticoid-induced leucine zipper (**GILZ**) protein, whose expression is induced by glucocorticoids, can inhibit the MAPK pathway and, thus, block the activation of neutrophils (Ronchetti et al., 2018; Ricci et al., 2019). Regarding neutrophil count, cortisol might have a proapoptotic effect on neutrophils (Lecchi et al., 2016; Catozzi et al., 2020), but glucocorticoids also stimulate the mobilization of neutrophils from the bone marrow. This effect of cortisol on neutrophil mobilization appears to depend on the expression of L-selectin. This protein is known as a factor in the retention of neutrophils in the bone marrow (Furze and Rankin, 2008). In cattle, Burton et al. (1995) showed that neutrophils generated after the injection of cortisol downregulate L-selectin. This effect might be due to the upregulation of annexin A1, a protein whose expression is induced by glucocorticoids and which leads to a reduction in the expression of L-selectin (Feher, 2012; Ronchetti et al., 2018). Hence, an increase in cortisol concentration implies an increased number of neutrophils via their mobilization from the bone marrow. However, glucocorticoids could stimulate a decline in the number of neutrophils via apoptosis. Concerning adhesion, Burton et al. (1995) observed a downregulation of CD18 following the exposition of bovine cells to cortisol. CD18 together with CD11b form the Mac-1 adhesion molecule, which is downregulated in the presence of glucocorticoids. In this way, these results contrast with upregulate CD11b in zebu breeds (Alhussien and Dang, 2018). However, the downregulation of L-selectin in the presence of cortisol might contribute to impaired adhesion (Ronchetti et al., 2018). Identified differences in this regard might be attributable to the breed effect. Finally, glucocorticoids inhibit the production of proinflammatory cytokines such as tumor necrosis factor-α (**TNF-α**) in neutrophils (Hirsch et al., 2012). Considering all these different studies, cortisol regulates the neutrophil count, probably because of a balance between bone marrow recruitment and apoptosis. It is probably dependent on the produced cortisol level, whereas a higher concentration could promote apoptosis. Indeed, zebu breeds seem to have higher neutrophil counts, and zebu crossbred cattle produce less cortisol than purebred non-zebu cattle (Tejaswi et al., 2020). In all cases, cortisol was able to reduce the neutrophil activity and could be the cause for the activity decline observed during HS.

Macrophages and Monocytes. Macrophages exhibit a wide variety of phenotypes in vivo. This panel of phenotypes consists of 2 extremes, M1 and M2 macrophages. The M1 macrophages are associated with the initiation of inflammation and eradication of pathogens, and M2 macrophages are associated with the inhibition of inflammation and prevention of damage (Maciuszek et al., 2020). The polarization of macrophages in M1 is initiated, among other sources, by LPS of bacterial origins. In this regard, LPS bind on the surface of macrophages and activate the TLR4 to trigger a signaling cascade dependent on p50/p65 NFκB. This cascade induces the transcription of genes encoding proteins involved in the antimicrobial activity of macrophages such as the inducible nitric oxide synthase and cytokines such as TNF-α. TNF-α also triggers a signaling cascade dependent on c-Jun N-terminal kinase (**JNK**) by binding to the TNFR1 receptor that activates proinflammatory genes similarly to p50/p65 NFκB. Recognition of LPS also leads to the production of interferon-β (**IFN-β**), which binds to the surface of macrophages and triggers the phosphorylation of (JAK)-signal transducers and activators of transcription 1 (**STAT1**) and STAT2. Conversely, the binding of

IL-4 and IL-13 on their receptors triggers polarization in M2 macrophages. This results in phosphorylation of STAT6, which induces the transcription of genes associated with the M2 phenotype, such as *ARG1* (Tugal et al., 2013). However, in ruminants, it is *ARG2* transcription that is activated rather than *ARG1* (Young et al., 2018).

In cattle, Catozzi et al. (2020) showed in vitro that monocytes and macrophages subjected to HS overexpress proapoptotic markers such as caspase 3 (**CASP3**), B-cell lymphoma 2 (**BCL-2**) and BCL-2 antagonist/killer (**BAK**). Similar observations have been made based on peripheral blood mononuclear cells (**PBMC**; Lacetera et al., 2006; Catozzi et al., 2020) and neutrophils (Lecchi et al., 2016; Catozzi et al., 2020), indicating that disease susceptibility development during HS is due to decreased viability of immune cells. However, the in vitro study has also shown that HS induces upregulation of *STAT6* and downregulation of *STAT1* and *STAT2*. Such an expression profile is associated with an M2 phenotype (Catozzi et al., 2020). In this way, HS seems to reduce the number of macrophages but also affects their polarization.

Even if these experiments have been performed in vitro, cortisol is an inhibitor of inflammation and could accentuate the above-mentioned effects. Indeed, at the macrophage level, genes involved in M1 polarization such as *STAT1* are downregulated in the presence of cortisol in vitro. Billing et al. (2011) showed that cortisol impairs antigen presentation by the downregulation of chaperones, including HSP90A. In general, after binding to its receptor, cortisol is capable of sequestering the p65 subunit of NFκB and inducing the expression of IκB, an inactivator of NFκB (Feher, 2012; Nicolaides et al., 2020). *GILZ*, whose expression is controlled by the GR, is also capable of inhibiting NFκB in macrophages (Cannarile et al., 2019). In addition, cortisol is able to induce the expression of proapoptotic genes and to repress antiapoptotic genes encoding proteins of the BCL-2 family (Schmidt et al., 2004). However, apoptosis induced by glucocorticoids such as cortisol mainly affects T and B lymphocytes, but macrophages are quite resistant (Diaz-Jimenez et al., 2021). Hence, during HS, cortisol could be involved in macrophages M2 polarization, but it is probably not the only cause for macrophage apoptosis.

Cytotoxic T-Cells. Activation of cytotoxic T-cells occurs through binding of the T-cell receptor (**TCR**) to MHC class I expressed on the surface of most nucleated cells. These cells present a specific peptide as well as a specific costimulatory signal (Uzhachenko and Shanker, 2019). The interaction of TCR with MHC activates tyrosine kinases such as FYN proto-oncogene (**FYN**) and the lymphocyte-specific protein tyrosine kinase (**LCK**), which triggers the activation of a series of signaling pathways. Among these are the MAPK and NFκB pathways (Krawczyk and Penninger, 2014).

In cattle, several studies have been performed to assess the effect of HS on lymphocyte proliferation. However, the results vary widely from study to study. Most studies indicated a declining number of lymphocytes during HS, but others indicated an increase or stagnation (Elvinger et al., 1991; Lacetera et al., 2006; Pandey et al., 2017; Bagath et al., 2019). However, the increases in number of lymphocytes or stagnations were reported for breeds located in South Asia. More recently, Tejaswi et al. (2020) identified a decrease in lymphocyte proliferation during the summer in crossbreeds but not in native breeds from Pakistan. The same authors also noticed differences in cortisol levels between crossbreeds and native breeds. Once again, HS responses differed between breeds, probably due to the lower cortisol secretions during HS in breeds more adapted to heat.

Glucocorticoids modulate T-cell activation by interacting directly with the NFκB transcription factors, as well as by reducing the activity of FYN and LCK. This effect is intensified by GILZ, which is also able to inhibit NFκB. Moreover, GILZ impairs MAPK pathways (Cannarile et al., 2019). In this way, glucocorticoids affect the proliferation of T-cells (Löwenberg et al., 2007; Cain and Cidlowski, 2017). However, some studies on PBMC that focus principally on T lymphocytes were performed in vitro, and HS was simulated by incubating the cells at a higher temperature (Elvinger et al., 1991; Lacetera et al., 2006). In such designs, the effect of cortisol on immune cells was not considered. However, it is known that increasing incubation temperature leads to increased synthesis of HSP (Guerriero and Raynes, 1990). In addition, the expression of HSP such as *HSP70* and *HSP90* (as shown in PBMC) was increased in heat-stressed calves (Kim et al., 2020a). These proteins are chaperones, which have a protective role against cellular stresses mainly by maintaining protein folding. The expression of HSP is induced during stress via the heat shock transcription factor 1 (**HSF1**). Glucocorticoids can modulate HSP expression by binding, for instance, HSP70 and HSP90 to their receptors (Collier et al., 2008). In T-cells, the effect of HSP70 activation is controversial due to the potential use of contaminated recombinant proteins, but it appears that HSP70 negatively affects the responses of T-cells to various stimuli (Stocki et al., 2012). Recently, Gregg (2021) showed HSP70 interference with NFκB-dependent signaling. However, PBMC from cattle classified as high immune responders present greater concentrations of HSP and greater cell proliferation under HS compared with the control group (Cartwright et al., 2021). In summary,

HS seems to reduce T-cell proliferation and activity. The HSP may be involved in this reduction, but their effect on T-cells is not yet fully resolved. Due to their chaperone function, HSP also promote cell survival, which could explain the increased expressions in high immune responders.

Helper T-Cells. Activation of helper T-cells is similar to that of cytotoxic T-cells except that the TCR interacts with the MHC class II expressed on the surface of antigen-presenting cells. Additionally, a costimulatory signal is required (Kwek et al., 2012; Chen and Flies, 2013). Helper T-cells are divided into a variety of subtypes based on the types of cytokines produced. The best known are Th1 and Th2, characterized by the production of IFN-γ, TNF-α, IL-12, and of IL-4, IL-5, IL-13, IL-10, respectively. The Th1 type is essential to combat intracellular pathogens, whereas Th2 acts during extracellular infections, for example, by helminths (Li et al., 2019). The Th1 response is associated with a proinflammatory response, whereas Th2 produce anti-inflammatory cytokines (Toscano et al., 2006). In this way, the Th1/Th2 balance allows regulation of the immune response to eliminate the parasites, as well as preventing overly long and overly strong responses, which can cause damage (Infante-Duarte and Kamradt, 1999). The polarization of the helper T-cells depends on the cytokines released by the antigen-presenting cells. IL-12 and IFN-γ promote Th1 polarization, whereas IL-4 promotes Th2 polarization (Walsh and Mills, 2013). Indeed, the IL-12 binding to its receptor induces the phosphorylation of STAT4, which dimerizes and activates the transcription of genes encoding proinflammatory cytokines such as IFN-γ (Hamza et al., 2010). In the same way, IFN-γ binding to its receptor activates STAT1, which promotes IFN-γ expression (Abbas et al., 2018). Conversely, IL-4 triggers the phosphorylation of STAT6, which activates genes encoding anti-inflammatory cytokines (Vatrella et al., 2014).

For cattle kept under HS, the Th1/Th2 ratio was reduced by downregulating the Th1 response (Lacetera et al., 2005; Welsh et al., 2005; Inbaraj et al., 2016; Bagath et al., 2019).

In addition to the unfavorable effects of cortisol on T-cell activation described above, glucocorticoids promote the Th2 response. Glucocorticoids downregulate the expression of proinflammatory cytokines as well as the IL-12 receptor and upregulate the expression of anti-inflammatory cytokines (Cain and Cidlowski, 2017). In this way, Th2 polarization observed during HS could be directly due to cortisol secretion.

Dendritic Cells. These cells can be activated through pathogen recognition receptors expressed on their surface, through antigen uptake or by other cells. Once stimulated, dendritic cells migrate to the lymph nodes, where they activate and influence the polarization of T helper cells. The activation of dendritic cells occurs through binding of LPS on TLR4, which induces the expression of proinflammatory cytokines (e.g., IL-12p70 via NFκB) and promotes the Th1 polarization of T helper cells. Conversely, activation of TLR2 inhibits IL-12p70 production and stimulates IL-4 production, which, in turn, facilitates Th2 polarization (Walsh and Mills, 2013).

In cattle, a few studies have shown the role of HSP70 in the processing of antigens as well as its ability to activate TLR4 in dendritic cells when it is present in the extracellular environment. In this way, HS would boost innate immunity through dendritic cells (Archana et al., 2017; Bagath et al., 2019). Concerning the abundance of dendritic cells during HS, no significant difference has been observed in cattle (Joo et al., 2021). Thus, despite their high importance in the immune response mechanisms, studies addressing the effects of HS on dendritic cells in cattle are lacking.

However, glucocorticoids promote apoptosis of dendritic cells (Shodell et al., 2003; Cain and Cidlowski, 2017). Glucocorticoids especially act on the downregulation of MHC class II, the molecules expressed by antigen-presenting cells such as dendritic cells and involved in the costimulatory signal. Glucocorticoids also promote the expression of anti-inflammatory cytokines (Cain and Cidlowski, 2017). Thus, further studies on dendritic cells, especially in cattle, are imperative.

B-Cells. The activation of B-cells requires the binding of an antigen to the B-cell receptor and costimulation by a helper T-cell to produce high-affinity antibodies, including IgG (Abbas et al., 2018). This costimulation is mediated by the binding of the TCR of the T-cell to MHC class II of the B-cell. The T helper also secretes cytokines to activate the B-cells (Kwek et al., 2012). In more detail, when the B-cell receptor binds an antigen, it allows the recruitment of Lck/Yes novel tyrosine kinase and of spleen-associated tyrosine kinase. In causality, among others, the activation of MAPK and NFκB is induced (Haselager et al., 2020).

In lactating cows, HS probably causes a decrease in the amount of circulating IgG produced by B-cells. However, in most cattle studies, IgG of lactating cows has been measured in colostrum instead of using blood. Numerous studies have focused on IgG transfer from mother to calf. Indeed, HS affects milk IgG content, and has generally been found to hamper calves' IgG absorption (Monteiro et al., 2014; Dahl et al., 2020; Davidson et al., 2021). However, studies addressing IgG content in colostrum during HS provide very divergent results. Some studies indicate an increase in IgG concentrations during HS, but others show a decrease or no differences in IgG due to HS (Bagath et al., 2019;

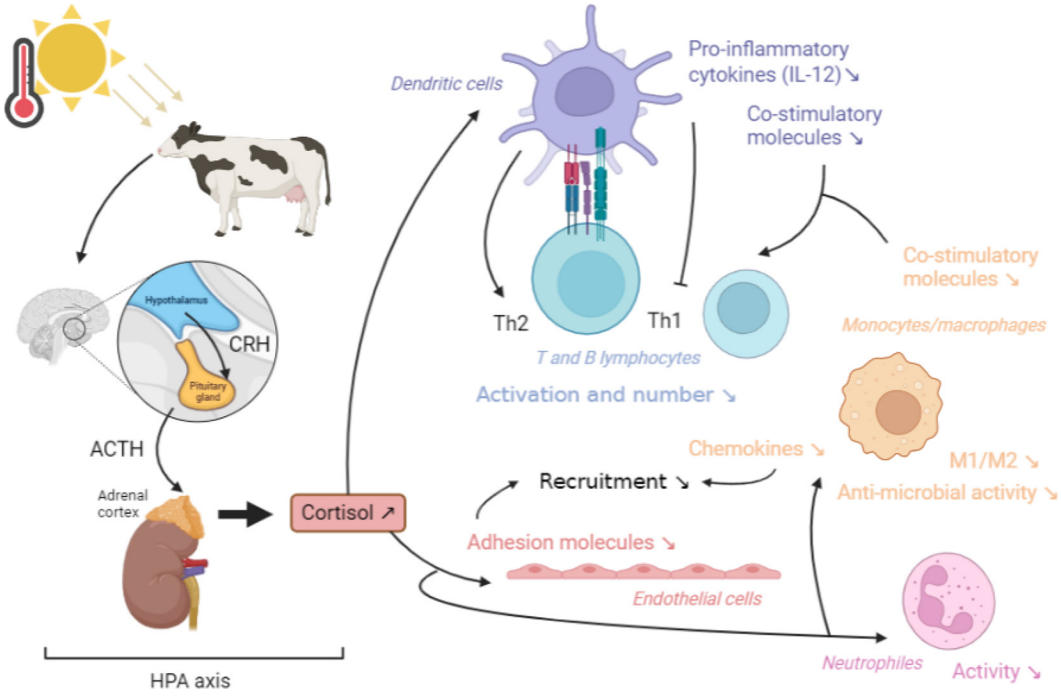

Figure 1. Possible mechanisms linking heat stress, cortisol secretion, and immunosuppression. CRH = corticotropin-releasing hormone; ACTH = adrenocorticotropic hormone; HPA = hypothalamic-pituitary-adrenal; IL-12 = interleukin 12; Th1, Th2 = helper T-cells of type 1 and type 2; M1, M2 = macrophage phenotypes M1 and M2.

Dahl et al., 2020). Conversely, in blood, HS is associated with decreased IgG content (Dahl et al., 2020).

In humans, the effect of glucocorticoids on the production of immunoglobulins has not been fully described. In general, glucocorticoids seem to cause a decrease in overall Ig concentrations (Cain and Cidlowski, 2017). In addition, cortisol has been found to induce apoptosis in mouse B-cells, mainly in immature B-cells (Gruver-Yates et al., 2014). Furthermore, glucocorticoids affect the activity of transcription factors involved in the activation of B-cells such as NFκB, as well as of T-cells (Cain and Cidlowski, 2017). Thus, the decreased IgG content in the blood of heat-stressed cows might be due to the detrimental effects on the number and activity of B-cells.

Global Effect on Immune Cells. In addition to the cortisol effects observed individually on the different cell types described above, cortisol affects their interactions. Indeed, cortisol has been found to hamper the expression of proinflammatory cytokines such as IL-12 by dendritic cells, and indirectly promotes the Th2 polarization of helper T-cells (Walsh and Mills, 2013; Cain and Cidlowski, 2017). The impaired production of proinflammatory cytokines as well as the lower expression of molecules involved in the costimulatory signal by dendritic cells and macrophages reduce the activation and proliferation of T- and B-lymphocytes (Cannarile et al., 2019). In addition to affecting the phenotype of macrophages, glucocorticoids reduce their production of chemokines and thus limit the recruitment of immune cells to the site of infection. Likewise, cortisol reduces the expression of adhesion molecules by endothelial cells (Cain and Cidlowski, 2017; Figure 1).

Based on the results described above, the complex formed by cortisol and its receptor reduces the ability of immune cells to react to infection, which is associated with a weaker immune response against pathogens. In addition, cortisol could affect the number of immune cells through its proapoptotic effect and by reducing lymphocyte proliferation. Cortisol also limits the recruitment of immune cells to the site of infection. However, once again, even if a strong effect of cortisol on immune cells is detected, the amount of cortisol secreted during HS should be sufficient to trigger these responses. This seems to be dependent on the animal breed (Tejaswi et al., 2020), and, thus, genetics are probably one of the key elements for differentiated tolerance to HS on an animal level.

LINK BETWEEN HEAT STRESS AND INFLAMMATION

In contrast to the previous section, several studies focusing on transcriptome modification during HS have highlighted genes involved in inflammation and

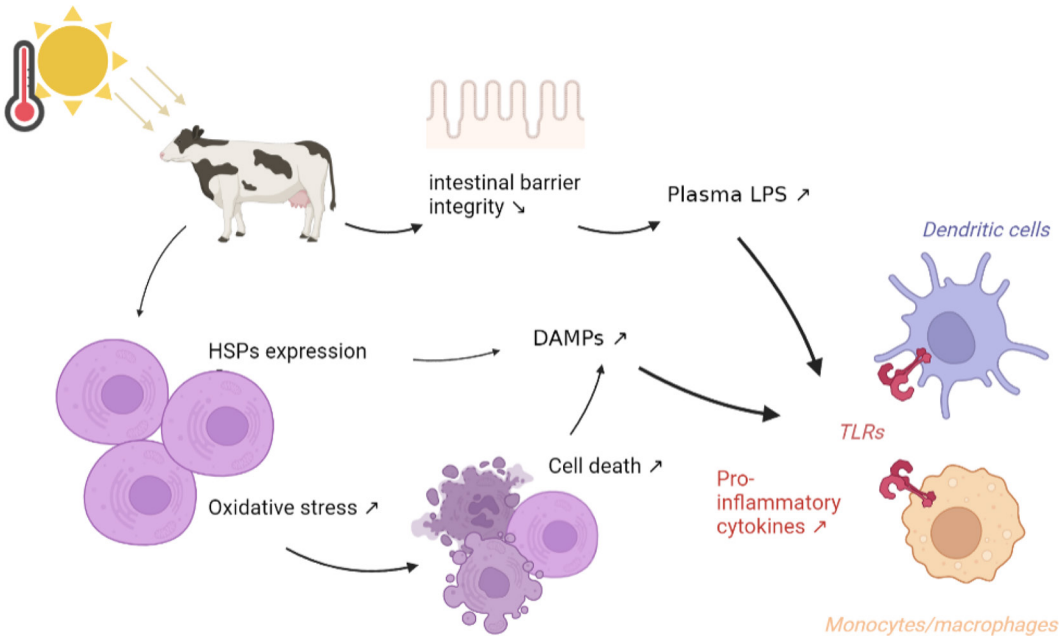

Figure 2. Possible mechanisms linking heat stress and proinflammatory cytokine production. HSP = heat shock protein; DAMP = damage-associated molecular pattern; TLR = toll-like receptor.

indicated an increase in inflammation in cattle during HS (Gao et al., 2019; Garner et al., 2020; Ahlawat et al., 2021). Accordingly, Chen et al. (2018) observed an increase in proinflammatory cytokines (IL-6, IL-1β, IFN-γ, TNF-α) under HS. The proinflammatory effect of HS could be associated with low levels of cortisol following HS. As previously mentioned, cortisol levels vary widely with the time of exposure to HS. In addition, cortisol production varies between animals. Hence, in some cases, cortisol levels are probably not sufficient to generate the consequences described above. In this regard—that is, in the absence of a sufficient anti-inflammatory effect of cortisol—inflammation could appear in affected animals (Min et al., 2016; Most and Yates, 2021). Moreover, in humans, Cruz-Topete and Cidlowski (2015) showed that glucocorticoids were also able to trigger inflammation, depending on the time of exposure and the basal state of the immune system.

This increase in inflammation during HS might be due to the ability of HSP to generate a proinflammatory response in cells expressing TLR4 and CD14. Indeed, as already mentioned, HSP70 can activate the TLR4 of dendritic cells (Archana et al., 2017; Bagath et al., 2019) and a variety of HSP, including HSP70, which are upregulated during HS (Mishra, 2021). Generally, HSP act intracellularly. However, HSP appear extracellular after release by injured or dying cells, then acting as damage-associated molecular patterns to activate surface TLR4 (Abbas et al., 2018). Heat stress induces oxidative cell damage by promoting the production of free radicals and induces cell death (Belhadj Slimen et al., 2016). Consequently, a release of HSP as damage-associated molecular patterns is expected. In addition, Johnson and Fleshner (2006) showed that several cell types are able to release HSP72 following a stressor. This increased release of HSP during HS has been observed in several species, including cattle (Collier et al., 2008).

In several species, including cattle, HS has been found to affect the integrity of the intestinal barrier, which allows the passage of LPS to the circulatory system and, thus, stimulates the production of proinflammatory cytokines (Lian et al., 2020; Patra and Kar, 2021; Fontoura et al., 2022). This condition is called "leaky gut." In cattle, HS alters the tight junctions between enterocytes of the jejunum, causing leaky gut and allowing passage of LPS for the activation of TLR (Mani et al., 2012). These findings indicate that HS affects the integrity of the intestinal barrier in cattle and, thus, promotes the production of proinflammatory cytokines (Koch et al., 2019).

In this context, we expect the emergence of inflammation in cattle with too low a production of cortisol to trigger an anti-inflammatory response following HS induction. The inflammatory state might be due to the increased release of HSP and the HS-induced leaky gut, enabling the passage of bacteria across the gut epithelium but not, at least initially, following an infection (Figure 2).

LINKS BETWEEN REDUCED IMMUNITY, INFLAMMATION, AND DISEASE OCCURENCE

As addressed above, a well-known example of an immune-related disease with increasing incidences during HS is mastitis (Lacetera, 2018; Dahl et al., 2020). Mastitis is a mammary gland inflammation generally caused by bacterial infection (Cheng and Han, 2020). Consequently, the impaired immunity induced by cortisol during HS could facilitate the development and duration of mastitis and other bacterial infections (Calcagni and Elenkov, 2006; Bronzo et al., 2020; Cheng and Han, 2020).

Conversely, studies investigating associations between inflammation and occurrences of infectious diseases in heat-stressed cattle are lacking. In humans, Hunter (2012) showed that with greater inflammation the symptoms of a disease can also be more pronounced. Hence, HS-induced inflammations could cause more severe mastitis symptoms, implying a shift from subclinical to more clinical cases. This hypothesis relies on the stacking effect. Indeed, a quick succession of stressors will prevent the return to the initial state (Edwards et al., 2019). In this way, inflammation following HS could facilitate the onset of inflammatory diseases, such as clinical mastitis.

By linking these 2 aspects, we expect that a thermotolerant animal produces low levels of cortisol and thus avoids its immunosuppressive action. Simultaneously, a thermotolerant animal expresses high levels of HSP, which limits cell stress and the initiation of inflammation.

In addition to the immune competence and the inflammation level of the affected animal, further factors promote infectious diseases during HS. Bronzo et al. (2020) highlighted the involvement of commensal microbiota with regard to susceptibility to mastitis and to other infectious diseases. Heat stress modifies the microbiome composition of cattle, implying increased susceptibility to infectious diseases (Czech et al., 2022; Kim et al., 2022). Hamel et al. (2021) showed that HS contributed to the shedding of some pathogens (yeasts and *Streptococcus uberis*) by intramammary-infected quarters. The observed mechanism could be due to defective immune function, as well as to a higher milk temperature, both promoting pathogen proliferation (Hamel et al., 2021). As shown by Salama et al. (2019), high temperatures also increase the membrane permeability of bovine mammary epithelial cells, with effects on impaired udder health. In the same way, Almeida et al. (2018) and Rakib et al. (2020) identified stronger pathogen adherence and internalization in heat-stressed compared with non-heat-stressed bovine mammary epithelial cells. Concerning metritis, Menta et al. (2022) indicated an increased incidence for retained placenta due to HS, causing an increased risk for metritis. Molinari et al. (2022) found that the increased occurrence of metritis during HS was not associated with a higher bacterial load. Hence, the increased occurrence of metritis is probably due to lower host resistance during the hot season, rather than to greater proliferation of pathogens.

In conclusion, the detrimental effect of HS on the immune system seems to be the main cause of the increased occurrence of infectious diseases. However, some other causes in this regard exist, such as induction through the specificities of the infected organs. For example, in the case of metritis, greater occurrence of membrane retention has been associated with high cortisol and progesterone levels and with low estrogen levels (Amin et al., 2013). The effects of HS on the levels of these molecules (Mishra, 2021) could thus explain the increased occurrence of metritis.

GWAS STUDIES ON HEAT STRESS AND THERMOTOLERANCE

Several immediate and time-lagged studies have relied on results from GWAS used for the ongoing annotation of potential candidate genes involved in cattle response to HS (Table 1; Hayes et al., 2009; Dikmen et al., 2013; Howard et al., 2014; Macciotta et al., 2017; Otto et al., 2019; Sigdel et al., 2019, 2020; Cheruiyot et al., 2021; Halli et al., 2021; Luo et al., 2021, 2022; Bohlouli et al., 2022; Sölzer et al., 2022). Some of these studies focused on physiological parameters affected by HS such as body temperature, whereas others analyzed production trait alterations by HS. In both cases, several genes identified may regulate immune system mechanisms and cortisol production, suggesting the importance of these traits as indicators for thermotolerance. In this section, we explore the potential underlying genetic mechanisms associating some of these genes with thermotolerance and immunity. The studied genes have been chosen according to their link with the previously described mechanisms and their apparent importance in immune response. However, further genes might be involved in the respective mechanisms, indicating the necessity of additional research.

Genes Involved in Inflammation and Immunity

HSF1. *HSF1* is well known to be involved in response to HS and has been highlighted as a candidate gene in 2 HS GWAS (Macciotta et al., 2017; Sigdel et al., 2019). Indeed, during cellular stress, HSF1 is activated by phosphorylation and promotes the expression of HSP by binding to heat shock elements (Jee, 2016).

Table 1. GWAS studies on cattle response to heat stress

Study	Type of heat stress	Trait(s)	Threshold[1]	Candidate genes/enriched transcription factors
Bohlouli et al., 2022	Immediate	Milk fatty acids (C18:0, PUFA, SFA, UFA)	10 THI classes	*AMFR*, *ADGRB1DENND3*, *DUSP16*, *EFR3A*, *EMP1*, *ENSBTAG00000003838*, *EPS8*, *MGP*, *PIK3C2G*, *STYK1*, *TMEM71*, *GSG1*, *SMARCE1*, *CCDC57*, *FASN*, *ENSBTAG00000048091*, *PAEP*, *EPPK1*
Cheruiyot et al., 2021	Immediate	Milk yield, fat yield, protein yield	THI ≥60	>50 genes
Dikmen et al., 2013	Immediate	Rectal temperature	THI ≥78.2	*U1*, *NCAD*, *SNORA19*, *RFWD2*, *SCARNA3*, *CEP170*, *PLD5*, *SLCO1C1*, *PDE3A*, *KBTBD2*, *LSM5*, *GOT1*
Halli et al., 2021	Time-lagged	Milk yield, fat percentage, fat yield, protein percentage, protein yield, SCC	THI ≥60	>50 genes
Hayes et al., 2009	Immediate	Milk yield	THI ≥60	>50 genes
Howard et al., 2014	Immediate	Body temperature	All animals in the same conditions, 5 d of recording during summer	*TUBB2A*, *TUBB2B*, *STAC*, *WRNIP1*, *MLH1*, *RIPK1*, *SMC6*, *GEN1*, *SERPINB9*, *KCNS3*, *SLC22A23*, *TRPC4*
Luo et al., 2021	Immediate	Rectal temperature	DBT >25°C	*SPAG17*, *FAM107B*, *TSNARE1*, *RALYL*, *PHRF1*
Luo et al., 2022	Immediate	Rectal temperature, respiration rate score, drooling score	THI ≥70.5	*PMAIP1*, *SBK1*, *TMEM33*, *GATB*, *CHORDC1*, *RTN4IP1*, *BTBD7*
Macciotta et al., 2017	Immediate	Milk yield, fat percentage, protein percentage, SCC	11 THI classes (from 50 to 52 to >79)	*BTRC*, *FGF8*, *MGEA5*, *KCNIP2*, *HPS6*, *CCSER1*, *DGAT1*, *HSF1*, *ARHGAP39*, *RPL8*, *MAPK 15*, *ZNF34*, *MCAT*, *SAMM50*, *TSPO*
Otto et al., 2019	Immediate	Change in rectal temperature	All animals placed 6 h in heat chambers at 42°C and 60% relative humidity	>50 genes/*ARNT*, *NFκB*, *STAT3*
Sigdel et al., 2019	Immediate	Milk yield, fat yield, protein yield	THI >68	*CDKN1B*, *DUSP16*, *HSF1*, *EEF1D*, *VPS28*, *TONSL*, *PEX16*, *MAPK8IP1*, *CREB3L1*, *CRY2*
Sigdel et al., 2020	Immediate	Outcome of the insemination	THI >68	*EXD2*, *SLC10A1*, *ADAM20*, *FSD2*, *AP3B2*, *EPAS1*, *TAOK3*, *NOS1*, *BRWD1*
Sölzer et al., 2022	Immediate	Claw disorder frequency (dermatitis digitalis, interdigital hyperplasia, sole ulcer)	THI >68	*FSIP2*, *CLCN1*, *ADGRV1*, *DOP1A*, *THBD*, *RHOBTB1*, *ENSBTAG00000052131*, *OR6C8*, *ENSBTAG00000050222*, *ASTN2*, *TRH*, *ERCC4*

[1]THI = temperature-humidity index; DBT = dry bulb temperature.

A missense mutation was detected in zebu cattle (*Bos indicus*) adapted to subtropical conditions, as well as in *Bos taurus* from the south of China under HS conditions, suggesting that this mutation is associated with heat tolerance (Rong et al., 2019). As previously discussed, HSP can modulate the immune response, and, thus, mutations in *HSF1* could affect immune response under HS conditions.

NFKB1. In the GWAS by Otto et al. (2019), NFκB was highlighted as an enriched transcription factor. These findings were based on the analysis of the regulatory sequences of candidate genes for rectal temperature variation during HS in cattle. As already described, NFκB is involved in the activation of numerous immune cells and promotes inflammation. This is a first example for the importance of genes involved in immunity during HS. On this basis, it is expected that a difference in NFκB activity will affect the HS response of an individual.

STAT3. Otto et al. (2019) also identified STAT3 as an enriched transcription factor. As stated for STAT4, which is associated with the Th1 response, and STAT6, which is linked with the Th2 response, STAT3 activation is associated with the Th17 response in helper T-cells. This type of response is triggered by IL-6 and implies the production of cytokines such as IL-17 by the Th17 lymphocyte. The Th17 response plays a role in the defense against extracellular pathogens by promoting, among other effects, granulocyte recruitment (Chaudhry et al., 2009; Abbas et al., 2018). In this way, STAT3 modulates the Th balance and thus influences the response against pathogens and affects the immune response during HS.

MAPK8IP1. Sigdel et al. (2019) identified *MAPK8IP1* as a candidate gene for the decline of milk production under HS. *MAPK8IP1* encodes the IB1 protein, which maintains JNK in the cytoplasm and prevents c-Jun activation. *MAPK8IP1* affects the immune response by modulating leukocyte activation. However, IB1 is expressed in β-pancreatic cells and prevents the inhibitory action of JNK on the expression of the gene coding for insulin. Therefore, not surprisingly, a mutation in *MAPK8IP1* was also segregated with diabetes in some human families (Waeber et al., 2000). Consequently, *MAPK8IP1* could affect HS tolerance by affecting insulin production. Indeed, as reviewed by König and May (2019) and Mishra (2021), HS is associated with a modification of plasma insulin of plasma glucose, both affecting energy balance.

DUSP16. *DUSP16* is a second candidate gene that has been identified by 2 GWAS (Sigdel et al., 2019; Bohlouli et al., 2022). The protein DUSP16 encoded by this gene, like other dual-specificity phosphatases, deactivates MAPK actors by dephosphorylation (Thiriet, 2013). More precisely, DUSP16, also named MKP7, is able to reduce JNK activation (Willoughby et al., 2003). Due to its expression in leukocytes, *DUSP16* could therefore affect the number and activation of leukocytes. Moreover, in DUSP16-deficient mice, greater production of IL-12 was also observed after stimulation with LPS (Niedzielska et al., 2014). In this way, variations in DUSP16 activity could affect the level of immune system activation in cattle.

ADGRB1. Bohlouli et al. (2022) highlighted *ADGRB1* as a candidate gene for a reduction in milk content of saturated fatty acid during HS. This gene encodes the BAI1 protein, one of whose functions is to recognize gram-negative bacteria. Once activated, the BAI1 receptor will trigger a rearrangement of the cytoskeleton, which promotes phagocytosis as well as the activity of NADPH oxidase via the Rac protein. In this way, the bactericidal activity of macrophages is increased. In addition, Billings et al. (2016) identified stronger susceptibility to bacterial infections in BAI1-deficient mice. Hence, it is expected that this protein modulates resistance to bacteria also in cattle, implying improved resistance to infectious diseases during HS. However, more recent studies in mice and humans suggest that this protein is not expressed in monocytes and macrophages (Hsiao et al., 2019).

EPS8. Bohlouli et al. (2022) detected *EPS8* as a potential candidate gene. The EPS8 protein is involved in actin remodeling. It has been shown that this protein is expressed by dendritic cells and is essential for their migration by allowing the formation and maintenance of cellular protuberances (Frittoli et al., 2011). In addition, EPS8 also promotes the phagocytosis activity of LPS-stimulated macrophages (Chen et al., 2012). Again, a polymorphism of this gene could therefore modify the ability of cattle to respond to an infection and to HS.

Genes Involved in Cortisol Production

CRY2. Sigdel et al. (2019) identified *CRY2* as a candidate gene for HS. They also mentioned its role in thermotolerance. Indeed, *CRY2* knockdown in plants is associated with increased sensitivity to temperature (Sanchez-Bermejo et al., 2015; Sigdel et al., 2019). Torres-Farfan et al. (2009) showed that a knockdown of *CRY2* in the monkey adrenal gland implied an inhibition of cortisol production during ACTH stimulation. This suggests that a polymorphism in this gene has a strong effect on the cortisol secretion levels under HS, as well as influencing the immune response.

TSPO. Macciotta et al. (2017) proposed *TSPO* as a candidate gene associated with the slope of protein percentage during HS in dairy cattle. This gene en-

codes a protein involved in cholesterol transportation. This protein even seems to work with the steroidogenic acute regulatory protein to transport cholesterol to the mitochondrial matrix to achieve the first rate-limiting step of cortisol synthesis (Selvaraj et al., 2015). Moreover, in *TSPO* knockout mice and in humans with a specific polymorphism in this gene, Owen et al. (2017) found impaired cortisol secretion following ACTH stimulation. Based on these physiological causalities, polymorphisms linked to *TSPO* in cattle might affect cortisol production and, thus, the response to HS.

POTENTIAL IMMUNE-RELATED BIOMARKERS FOR HEAT STRESS AND THERMOTOLERANCE

Consideration of candidate genes based on GWAS to select animals presenting favorable genotypes is an approach to improve thermotolerance. However, this approach does not consider environmental interactions. Thus, a possibility to identify novel biomarkers of HS is to focus on differences in gene expressions. Hence, simultaneously considering the pathways discussed previously, the candidate genes identified on the basis of GWAS and gene expression studies will contribute to the detection of potential immune-related biomarkers for HS in cattle (Table 2). Moreover, the identified biomarkers potentially could be used to discriminate thermotolerant and thermosensitive animals.

Genes Highlighted on the Basis of GWAS

HSF1 and HSP. The genes coding for HSP and their regulator HSF1 are upregulated during HS in several species and tissues (Archana et al., 2017). Hence, they are proper indicators for HS.

Concerning the possible difference between thermotolerant and thermosensitive cattle, Li et al. (2011) showed that *HSF1* polymorphism affects thermotolerance. In this regard, the homozygotes TT at locus *G4693T* were more thermotolerant than heterozygotes and homozygotes GG. Moreover, this polymorphism affects the expression of *HSF1*. Indeed, the homozygotes TT also overexpressed *HSF1* compared with the other genotypes. In this way, the expressions of the *HSF1* and *HSP* genes seem to be potentially potent biomarkers to discriminate thermotolerant and thermosensitive animals.

NFKB1, STAT3, MAPK8IP1, DUSP16, ADGRB1, EPSO, CRY2, and TSPO. The candidate genes highlighted by the previously mentioned GWAS are potential biomarkers for HS. Due to their important functions for thermotolerance, HS might affect the respective gene expressions. In vitro studies have observed a downregulation of *MAPK8IP1* (Khan et al., 2020), an upregulation of *DUSP16*, and an upregulation of *TSPO* (Kapila et al., 2016). Such results are expected, because, as already mentioned, TSPO can affect cortisol synthesis. Conversely, *MAPK8IP1* seems to promote insulin synthesis, possibly due to increased insulin production during HS, implying an expected upregulation of this gene. However, this study was performed on granulosa cells, indicating that *MAPK8IP1* additionally influences follicular growth (Fayad et al., 2007). Hence, differences in expression pattern could depend on the cell type studied. A transcriptomic study (Martínez et al., 2021) showed an upregulation of *STAT3*, and a further study (Srikanth et al., 2017) with focus on the hepatic response to HS showed an upregulation of *NFKB1*. These results suggest an activation of the immune system during HS. Concerning the other genes, currently no studies showed an up- or downregulation in cattle during HS.

As shown for *HSF1*, significantly associated SNPs from GWAS could affect the expression of annotated potential candidate genes. Different expression levels might contribute to discriminate between thermotolerant and thermosensitive animals.

Other Genes Associated With the Immune Response and Cortisol Secretion

IL1B, IL10, TNFA, TNFR, NOS2, and MPO. Modifications of the expression or the activity of NFκB, an important regulator of inflammation, might be associated with modifications of its target gene expression. Among them are *NOS2* encoding inducible nitric oxide synthase and the genes coding for the proinflammatory cytokines IL-1β and TNF-α. *IL1B* and *NOS2* seem to be upregulated during HS, whereas *TNFA* and its receptor (*TNFR1)* seem to be downregulated (Table 2). Moreover, expression of *IL10*, an anti-inflammatory cytokine, seems also to be upregulated during HS. As already mentioned, at the protein level, it is not clear whether HS promotes a proinflammatory or an anti-inflammatory environment. Similarly, *NOS2*, which is associated with the polarization of macrophages, has been found to be upregulated (Bharati et al., 2017). In contrast, *MPO*, associated with the activation of neutrophils, has been found to be downregulated during HS (Park et al., 2021).

As suggested by Cartwright et al. (2021), thermotolerant animals could conserve a better immune response during HS. In this context, different patterns of cytokine and cell activation marker expressions can be expected between thermotolerant and thermosensitive animals.

GILZ, NFKBIA, ANXA1, DUSP1, and TPP. Target genes of the GR are potential biomarkers for

Table 2. Gene expression studies for genes associated with immunity and heat stress in cattle[1]

Gene	Article(s) showing variation of expression during heat stress	Intensity of HS	Duration of heat stress	Animal	Tissue	Direction of variation
BAK	Somal et al., 2015	THI 79.9	Not determined	Sahiwal cows	PBMC	Upregulation
BCL2	Somal et al., 2015	THI 79.9	Not determined	Sahiwal cows	PBMC	Upregulation
CASP-3	Somal et al., 2015	THI 79.9	Not determined	Sahiwal cows	PBMC	Upregulation
DUSP16	Kapila et al., 2016	42°C in vitro	1 h in vitro	Riverine buffalo	Mammary epithelial cells	Upregulation
HSF1	Li et al., 2011	25–35°C and 45–65% humidity	Not determined	Holstein cows	Liver	Upregulation
HSP	Archana et al., 2017	Various	Various	Various	Various	Upregulation
IL10	Park et al., 2021	THI 79.13	Not determined	Dairy cows	Immune cells	Upregulation
IL1B	Park et al., 2021; Thompson et al., 2014	THI 79.13; absence of cooling	Not determined	Dairy cows	Immune cells; neutrophils	No change; upregulation
NOS2	Bharati et al., 2017	42°C	6 h/d during 23 d	Tharparkar cattle	PBMC	Upregulation
MAPK8IP1	Khan et al., 2020	41°C in vitro	2 h in vitro	Dairy cattle	Granulosa cells	Downregulation
MPO	Park et al., 2021	THI 79.13	Not determined	Holstein cows	Neutrophils	Downregulation
NFKB1	Martínez et al., 2021	Not determined	Not determined	Holstein cows	Liver	Upregulation
NFKBIA	Kim et al., 2020b	THI 87.5	Not determined	Jersey cows	PBMC	Downregulation
STAT3	Srikanth et al., 2017	THI from 90 to 93	9 h	Holstein calves	Whole blood	Upregulation
TNFA	Lendez et al., 2021; Park et al., 2021	Summer (between 5.1 and 32.5°C); THI 79.13	Not determined	Dairy cows	Immune cells; PBMC	Downregulation; no change
TNRF1	Lendez et al., 2021	Summer (between 5.1 and 32.5°C)	Not determined	Dairy cows	PBMC	Downregulation
TSPO	Kapila et al., 2016	42°C in vitro	1 h in vitro	Riverine buffalo	Mammary epithelial cells	Upregulation

[1]HS = heat stress; THI = temperature-humidity index; PBMC = peripheral blood mononuclear cells.

HS, because HS is associated with cortisol secretion. *GILZ*, *NFKBIA*, *ANXA1*, *DUSP1*, and *TPP* are some example genes in this regard (Xavier et al., 2016). In addition, studying their gene expressions could help to determine whether cortisol levels during HS are sufficient to trigger anti-inflammatory effects. Of course, these levels can depend on the intensity and duration of HS, and may vary from one individual to another. Currently, very few studies have focused on the expression of these genes during HS. Kim et al. (2020b) highlighted *NFKBIA* as one of the genes downregulated during HS. This suggests that the cortisol level was not able to promote the expression of the inhibitor of NFκB, which promotes inflammation.

Tejaswi et al. (2020) showed that crossbred cattle present a higher level of cortisol than purebred zebu cattle. Because zebus are more resistant to HS than crossbreds, we can hypothesize that thermotolerant animals in general present lower cortisol levels than thermosensitive. In this case, expressions of genes controlled by glucocorticoids are lower in thermotolerant than in thermosensitive animals.

Genes Associated With Apoptosis: BAK, CASP3, and BCL2

As already discussed, HS triggers oxidative stress, and glucocorticoids can promote cell death. In such a context, apoptosis markers are potential biomarkers for HS. Somal et al. (2015) obtained an increase in the expression of *BAK* and *CASP3*, as well as of *BCL2*. BAK and CASP3 are proteins associated with apoptosis, whereas *BCL2* is an antiapoptotic protein. In this case, the upregulation of *BAK* was stronger than that of *BCL2*, suggesting an increase of apoptosis.

We expect that thermotolerant animals are able to limit cell death during HS. This could be evidenced by a lower expression level of proapoptotic genes such as *BAK* and *CASP3* or by a greater expression of antiapoptotic genes such as *BCL2*, or both, by thermotolerant compared with thermosensitive animals.

CONCLUSIONS

Heat stress modulates the immune system of affected animals, which may directly influence health status. As illustrated in this review, all mechanisms associating HS with the greater occurrence of immune-related diseases have not been fully explained. Indeed, HS leads, among other effects, to the production of cortisol, which is well known for its immunosuppressive effect. However, several studies have also shown an increase in proinflammatory cytokines under HS conditions. The opposite effects might be due to interindividual variability, including genetic differences, as well as to the duration and intensity of HS. The production of cortisol depends on these parameters. In all cases, immune parameters are modulated by HS. Additionally, the importance of the link between immune response and thermotolerance is highlighted by GWAS, and corresponding annotated potential candidate genes are associated with immune response and cortisol production. Therefore, it is suggested to use immune parameters as biomarkers for the detection of HS. In addition, the expression levels of genes highlighted in this review could be used as biomarkers. Moreover, polymorphisms present in certain genes associated with the immune system could potentially be used in the context of the prediction of an individual being tolerant to HS and considered in genetic evaluations aiming at improved thermotolerance.

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REFERENCES

Abbas, A. K., A. H. Lichtman, S. Pillai, D. L. Baker, and A. Baker. 2018. Cellular and Molecular Immunology. 9th ed. Elsevier.

Ahlawat, S., R. Arora, U. Sharma, A. Sharma, Y. Girdhar, R. Sharma, A. Kumar, and R. K. Vijh. 2021. Comparative gene expression profiling of milk somatic cells of Sahiwal cattle and Murrah buffaloes. Gene 764:145101. https://doi.org/10.1016/j.gene.2020.145101.

Alhussien, M. N., and A. K. Dang. 2018. Impact of different seasons on the milk somatic and differential cell counts, milk cortisol and neutrophils functionality of three Indian native breeds of cattle. J. Therm. Biol. 78:27–35. https://doi.org/10.1016/j.jtherbio.2018.08.020.

Almeida, R. A., O. Kerro-Dego, and A. G. Rius. 2018. Effect of heat stress on the interaction of *Streptococcus uberis* with bovine mam-

mary epithelial cells. J. Dairy Res. 85:53–56. https://doi.org/10.1017/S0022029917000875.

Amin, R., G. R. Bhat, A. Ahmad, P. Swain, and G. Arunakumari. 2013. Understanding patho-physiology of retained placenta and its management in cattle: A review. Vet. Med. Sci. 1:1–9.

Archana, P. R., J. Aleena, P. Pragna, M. K. Vidya, A. P. A. Niyas, M. Bagath, G. Krishnan, A. Manimaran, V. Beena, E. K. Kurien, V. Sejian, and R. Bhatta. 2017. Role of heat shock proteins in livestock adaptation to heat stress. J. Dairy Vet. Anim. Res. 5:00127. https://doi.org/10.15406/jdvar.2017.05.00127.

Arlt, W., and P. M. Stewart. 2005. Adrenal corticosteroid biosynthesis, metabolism, and action. Endocrinol. Metab. Clin. North Am. 34:293–313. https://doi.org/10.1016/j.ecl.2005.01.002.

Bagath, M., G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, and V. Sejian. 2019. The impact of heat stress on the immune system in dairy cattle: A review. Res. Vet. Sci. 126:94–102. https://doi.org/10.1016/j.rvsc.2019.08.011.

Belhadj Slimen, I., T. Najar, A. Ghram, and M. Abdrrabba. 2016. Heat stress effects on livestock: Molecular, cellular and metabolic aspects, a review. J. Anim. Physiol. Anim. Nutr. (Berl.) 100:401–412. https://doi.org/10.1111/jpn.12379.

Bharati, J., S. S. Dangi, S. Bag, V. P. Maurya, G. Singh, P. Kumar, and M. Sarkar. 2017. Expression dynamics of HSP90 and nitric oxide synthase (NOS) isoforms during heat stress acclimation in Tharparkar cattle. Int. J. Biometeorol. 61:1461–1469. https://doi.org/10.1007/s00484-017-1323-3.

Billing, A. M., F. Fack, J. D. Turner, and C. P. Muller. 2011. Cortisol is a potent modulator of lipopolysaccharide-induced interferon signaling in macrophages. Innate Immun. 17:302–320. https://doi.org/10.1177/1753425910369269.

Billings, E. A., C. S. Lee, K. A. Owen, R. S. D'Souza, K. S. Ravichandran, and J. E. Casanova. 2016. The adhesion GPCR BAI1 mediates macrophage ROS production and microbicidal activity against Gram-negative bacteria. Sci. Signal. 9. https://doi.org/10.1126/scisignal.aac6250.

Bohlouli, M., K. Halli, T. Yin, N. Gengler, and S. König. 2022. Genome-wide associations for heat stress response suggest potential candidate genes underlying milk fatty acid composition in dairy cattle. J. Dairy Sci. 105:3323–3340. https://doi.org/10.3168/jds.2021-21152.

Bronzo, V., V. Lopreiato, F. Riva, M. Amadori, G. Curone, M. F. Addis, P. Cremonesi, P. Moroni, E. Trevisi, and B. Castiglioni. 2020. The role of innate immune response and microbiome in resilience of dairy cattle to disease: The mastitis model. Animals (Basel) 10:1397. https://doi.org/10.3390/ani10081397.

Burton, J. L., M. E. Kehrli Jr., S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: Effects of cortisol and dexamethasone. J. Leukoc. Biol. 57:317–325. https://doi.org/10.1002/jlb.57.2.317.

Cain, D. W., and J. A. Cidlowski. 2017. Immune regulation by glucocorticoids. Nat. Rev. Immunol. 17:233–247. https://doi.org/10.1038/nri.2017.1.

Cannarile, L., D. V. Delfino, S. Adorisio, C. Riccardi, and E. Ayroldi. 2019. Implicating the role of GILZ in glucocorticoid modulation of T-cell activation. Front. Immunol. 10:1823. https://doi.org/10.3389/fimmu.2019.01823.

Carroll, J. A., and N. E. Forsberg. 2007. Influence of stress and nutrition on cattle immunity. Vet. Clin. North Am. Food Anim. Pract. 23:105–149. https://doi.org/10.1016/j.cvfa.2007.01.003.

Cartwright, S. L., M. McKechnie, J. Schmied, A. M. Livernois, and B. A. Mallard. 2021. Effect of in vitro heat stress challenge on the function of blood mononuclear cells from dairy cattle ranked as high, average and low immune responders. BMC Vet. Res. 17:233. https://doi.org/10.1186/s12917-021-02940-8.

Catozzi, C., G. Ávila, V. Zamarian, D. Pravettoni, G. Sala, F. Ceciliani, N. Lacetera, and C. Lecchi. 2020. In vitro effect of heat stress on bovine monocytes lifespan and polarization. Immunobiology 225:151888. https://doi.org/10.1016/j.imbio.2019.11.023.

Chaudhry, A., D. Rudra, P. Treuting, R. M. Samstein, Y. Liang, A. Kas, and A. Y. Rudensky. 2009. CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science 326:986–991. https://doi.org/10.1126/science.1172702.

Chen, L., and D. B. Flies. 2013. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat. Rev. Immunol. 13:227–242. https://doi.org/10.1038/nri3405.

Chen, S., J. Wang, D. Peng, G. Li, J. Chen, and X. Gu. 2018. Exposure to heat-stress environment affects the physiology, circulation levels of cytokines, and microbiome in dairy cows. Sci. Rep. 8:14606. https://doi.org/10.1038/s41598-018-32886-1.

Chen, Y.-J., M.-Y. Hsieh, M. Y. Chang, H.-C. Chen, M.-S. Jan, M.-C. Maa, and T.-H. Leu. 2012. Eps8 protein facilitates phagocytosis by increasing TLR4-MyD88 protein interaction in lipopolysaccharide-stimulated macrophages. J. Biol. Chem. 287:18806–18819. https://doi.org/10.1074/jbc.M112.340935.

Cheng, W. N., and S. G. Han. 2020. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. Asian-Australas. J. Anim. Sci. 33:1699–1713. https://doi.org/10.5713/ajas.20.0156.

Cheruiyot, E. K., M. Haile-Mariam, B. G. Cocks, I. M. MacLeod, R. Xiang, and J. E. Pryce. 2021. New loci and neuronal pathways for resilience to heat stress in cattle. Sci. Rep. 11:16619. https://doi.org/10.1038/s41598-021-95816-8.

Chung, S., G. H. Son, and K. Kim. 2011. Circadian rhythm of adrenal glucocorticoid: Its regulation and clinical implications. Biochim. Biophys. Acta. 1812:581–591. https://doi.org/10.1016/j.bbadis.2011.02.003.

Collier, R. J., J. L. Collier, R. P. Rhoads, and L. H. Baumgard. 2008. Invited review: Genes involved in the bovine heat stress response. J. Dairy Sci. 91:445–454. https://doi.org/10.3168/jds.2007-0540.

Collier, R. J., and K. G. Gebremedhin. 2015. Thermal biology of domestic animals. Annu. Rev. Anim. Biosci. 3:513–532. https://doi.org/10.1146/annurev-animal-022114-110659.

Contreras, G. A., and L. M. Sordillo. 2011. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comp. Immunol. Microbiol. Infect. Dis. 34:281–289. https://doi.org/10.1016/j.cimid.2011.01.004.

Cruz-Topete, D., and J. A. Cidlowski. 2015. One hormone, two actions: Anti- and pro-inflammatory effects of glucocorticoids. Neuroimmunomodulation 22:20–32. https://doi.org/10.1159/000362724.

Czech, B., J. Szyda, K. Wang, H. Luo, and Y. Wang. 2022. Fecal microbiota and their association with heat stress in *Bos taurus*. BMC Microbiol. 22:171. https://doi.org/10.1186/s12866-022-02576-0.

Dahl, G. E., S. Tao, and J. Laporta. 2020. Heat stress impacts immune status in cows across the life cycle. Front. Vet. Sci. 7:116. https://doi.org/10.3389/fvets.2020.00116.

Davidson, B. D., B. Dado-Senn, V. Ouellet, G. E. Dahl, and J. Laporta. 2021. Effect of late-gestation heat stress in nulliparous heifers on postnatal growth, passive transfer of immunoglobulin G, and thermoregulation of their calves. JDS Commun. 2:165–169. https://doi.org/10.3168/jdsc.2020-0069.

Diaz-Jimenez, D., J. P. Kolb, and J. A. Cidlowski. 2021. Glucocorticoids as regulators of macrophage-mediated tissue homeostasis. Front. Immunol. 12:669891. https://doi.org/10.3389/fimmu.2021.669891.

Dikmen, S., J. B. Cole, D. J. Null, and P. J. Hansen. 2013. Genome-wide association mapping for identification of quantitative trait loci for rectal temperature during heat stress in Holstein cattle. PLoS One 8:e69202. https://doi.org/10.1371/journal.pone.0069202.

Dikmen, S., and P. J. Hansen. 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J. Dairy Sci. 92:109–116. https://doi.org/10.3168/jds.2008-1370.

Edwards, P. T., B. P. Smith, M. L. McArthur, and S. J. Hazel. 2019. Fearful Fido: Investigating dog experience in the veterinary context in an effort to reduce distress. Appl. Anim. Behav. Sci. 213:14–25. https://doi.org/10.1016/j.applanim.2019.02.009.

Elvinger, F., P. J. Hansen, and R. P. Natzke. 1991. Modulation of function of bovine polymorphonuclear leukocytes and lymphocytes by high temperature in vitro and in vivo. Am. J. Vet. Res. 52:1692–1698.

Fayad, T., R. Lefebvre, J. Nimpf, D. W. Silversides, and J. G. Lussier. 2007. Low-density lipoprotein receptor-related protein 8 (LRP8) is upregulated in granulosa cells of bovine dominant follicle: Molecular characterization and spatio-temporal expression studies. Biol. Reprod. 76:466–475. https://doi.org/10.1095/biolreprod.106.057216.

Feher, J. 2012. The adrenal cortex. Pages 810–819 in Quantitative Human Physiology: An Introduction. Academic Press. https://doi.org/10.1016/B978-0-12-382163-8.00088-8.

Fontoura, A. B. P., A. Javaid, V. Sáinz de la Maza-Escolà, N. S. Salandy, S. L. Fubini, E. Grilli, and J. W. McFadden. 2022. Heat stress develops with increased total-tract gut permeability, and dietary organic acid and pure botanical supplementation partly restores lactation performance in Holstein dairy cows. J. Dairy Sci. 105:7842–7860. https://doi.org/10.3168/jds.2022-21820.

Frittoli, E., G. Matteoli, A. Palamidessi, E. Mazzini, L. Maddaluno, A. Disanza, C. Yang, T. Svitkina, M. Rescigno, and G. Scita. 2011. The signaling adaptor Eps8 is an essential actin capping protein for dendritic cell migration. Immunity 35:388–399. https://doi.org/10.1016/j.immuni.2011.07.007.

Furze, R. C., and S. M. Rankin. 2008. Neutrophil mobilization and clearance in the bone marrow. Immunology 125:281–288. https://doi.org/10.1111/j.1365-2567.2008.02950.x.

Galac, S., M. M. J. Kool, E. C. Naan, S. Daminet, J. A. Mol, and H. S. Kooistra. 2010. Expression of the ACTH receptor, steroidogenic acute regulatory protein, and steroidogenic enzymes in canine cortisol-secreting adrenocortical tumors. Domest. Anim. Endocrinol. 39:259–267. https://doi.org/10.1016/j.domaniend.2010.07.001.

Gao, S. T., L. Ma, Z. Zhou, Z. K. Zhou, L. H. Baumgard, D. Jiang, M. Bionaz, and D. P. Bu. 2019. Heat stress negatively affects the transcriptome related to overall metabolism and milk protein synthesis in mammary tissue of midlactating dairy cows. Physiol. Genomics 51:400–409. https://doi.org/10.1152/physiolgenomics.00039.2019.

Garner, J. B., A. J. Chamberlain, C. Vander Jagt, T. T. T. Nguyen, B. A. Mason, L. C. Marett, B. J. Leury, W. J. Wales, and B. J. Hayes. 2020. Gene expression of the heat stress response in bovine peripheral white blood cells and milk somatic cells in vivo. Sci. Rep. 10:19181. https://doi.org/10.1038/s41598-020-75438-2.

Gill, G. N. 1972. Mechanism of ACTH action. Metabolism 21:571–588. https://doi.org/10.1016/0026-0495(72)90101-1.

Gregg, R. K. 2021. Implications of microgravity-induced cell signaling alterations upon cancer cell growth, invasiveness, metastatic potential, and control by host immunity. Int. Rev. Cell Mol. Biol. 361:107–164. https://doi.org/10.1016/bs.ircmb.2021.01.004.

Guerriero, V., and D. A. Raynes. 1990. Synthesis of heat stress proteins in lymphocytes from livestock. J. Anim. Sci. 68:2779. https://doi.org/10.2527/1990.6892779x.

Halli, K., S. F. Vanvanhossou, M. Bohlouli, S. König, and T. Yin. 2021. Identification of candidate genes on the basis of SNP by time-lagged heat stress interactions for milk production traits in German Holstein cattle. PLoS One 16:e0258216. https://doi.org/10.1371/journal.pone.0258216.

Hamel, J., Y. Zhang, N. Wente, and V. Krömker. 2021. Heat stress and cow factors affect bacteria shedding pattern from naturally infected mammary gland quarters in dairy cattle. J. Dairy Sci. 104:786–794. https://doi.org/10.3168/jds.2020-19091.

Hammami, H., J. Vandenplas, M.-L. Vanrobays, B. Rekik, C. Bastin, and N. Gengler. 2015. Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. J. Dairy Sci. 98:4956–4968. https://doi.org/10.3168/jds.2014-9148.

Hamza, T., J. B. Barnett, and B. Li. 2010. Interleukin 12 a key immunoregulatory cytokine in infection applications. Int. J. Mol. Sci. 11:789–806. https://doi.org/10.3390/ijms11030789.

Haselager, M. V., A. P. Kater, and E. Eldering. 2020. Proliferative signals in chronic lymphocytic leukemia; What are we missing? Front. Oncol. 10:592205. https://doi.org/10.3389/fonc.2020.592205.

Hayes, B. J., P. J. Bowman, A. J. Chamberlain, K. Savin, C. P. van Tassell, T. S. Sonstegard, and M. E. Goddard. 2009. A validated genome wide association study to breed cattle adapted to an environment altered by climate change. PLoS One 4:e6676. https://doi.org/10.1371/journal.pone.0006676.

Herbut, P., S. Angrecka, and J. Walczak. 2018. Environmental parameters to assessing of heat stress in dairy cattle—A review. Int. J. Biometeorol. 62:2089–2097. https://doi.org/10.1007/s00484-018-1629-9.

Hirsch, G., A. Lavoie-Lamoureux, G. Beauchamp, and J.-P. Lavoie. 2012. Neutrophils are not less sensitive than other blood leukocytes to the genomic effects of glucocorticoids. PLoS One 7:e44606. https://doi.org/10.1371/journal.pone.0044606.

Howard, J. T., S. D. Kachman, W. M. Snelling, E. J. Pollak, D. C. Ciobanu, L. A. Kuehn, and M. L. Spangler. 2014. Beef cattle body temperature during climatic stress: A genome-wide association study. Int. J. Biometeorol. 58:1665–1672. https://doi.org/10.1007/s00484-013-0773-5.

Hsiao, C. C., M. van der Poel, T. J. van Ham, and J. Hamann. 2019. Macrophages do not express the phagocytic receptor BAI1/ADGRB1. Front. Immunol. 10:962. https://doi.org/10.3389/fimmu.2019.00962.

Hunter, P. 2012. The inflammation theory of disease: The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. EMBO Rep. 13:968–970. https://doi.org/10.1038/embor.2012.142.

Inbaraj, S., V. Sejian, M. Bagath, and R. Bhatta. 2016. Impact of heat stress on immune responses of livestock: A review. Pertanika, J. Trop. Agric. Sci. 39:459–482.

Infante-Duarte, C., and T. Kamradt. 1999. Th1/Th2 balance in infection. Springer Seminars in Immunopathology 21:317–338. https://doi.org/10.1007/BF00812260.

Jee, H. 2016. Size dependent classification of heat shock proteins: A mini-review. J. Exerc. Rehabil. 12:255–259. https://doi.org/10.12965/jer.1632642.321.

Johnson, J. D., and M. Fleshner. 2006. Releasing signals, secretory pathways, and immune function of endogenous extracellular heat shock protein 72. J. Leukoc. Biol. 79:425–434. https://doi.org/10.1189/jlb.0905523.

Joo, S. S., S. J. Lee, D. S. Park, D. H. Kim, B.-H. Gu, Y. J. Park, C. Y. Rim, M. Kim, and E. T. Kim. 2021. Changes in blood metabolites and immune cells in Holstein and Jersey dairy cows by heat stress. Animals (Basel) 11:974. https://doi.org/10.3390/ani11040974.

Kadzere, C. T., M. R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: A review. Livest. Prod. Sci. 77:59–91. https://doi.org/10.1016/S0301-6226(01)00330-X.

Kapila, N., A. Sharma, A. Kishore, M. Sodhi, P. K. Tripathi, A. K. Mohanty, and M. Mukesh. 2016. Impact of heat stress on cellular and transcriptional adaptation of mammary epithelial cells in riverine buffalo (*Bubalus bubalis*). PLoS One 11:e0157237. https://doi.org/10.1371/journal.pone.0157237.

Khan, A., J. Dou, Y. Wang, X. Jiang, M. Z. Khan, H. Luo, T. Usman, and H. Zhu. 2020. Evaluation of heat stress effects on cellular and transcriptional adaptation of bovine granulosa cells. J. Anim. Sci. Biotechnol. 11:25. https://doi.org/10.1186/s40104-019-0408-8.

Kim, W.-S., J. Ghassemi Nejad, S.-G. Roh, and H.-G. Lee. 2020a. Heat-shock proteins gene expression in peripheral blood mononuclear cells as an indicator of heat stress in beef calves. Animals (Basel) 10:895. https://doi.org/10.3390/ani10050895.

Kim, E. T., S. S. Joo, D. H. Kim, B.-H. Gu, D. S. Park, R. M. Atikur, J. K. Son, B. Y. Park, S. B. Kim, T.-Y. Hur, and M. Kim. 2020b. Common and differential dynamics of the function of peripheral blood mononuclear cells between Holstein and Jersey cows in heat-stress environment. Animals (Basel) 11:19. https://doi.org/10.3390/ani11010019.

Kim, S. H., S. C. Ramos, R. A. Valencia, Y. I. Cho, and S. S. Lee. 2022. Heat stress: Effects on rumen microbes and host physiology, and strategies to alleviate the negative impacts on lactating dairy cows. Front. Microbiol. 13:804562. https://doi.org/10.3389/fmicb.2022.804562.

Koch, F., U. Thom, E. Albrecht, R. Weikard, W. Nolte, B. Kuhla, and C. Kuehn. 2019. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine.

Proc. Natl. Acad. Sci. USA 116:10333–10338. https://doi.org/10.1073/pnas.1820130116.

König, S., and K. May. 2019. Invited review: Phenotyping strategies and quantitative-genetic background of resistance, tolerance and resilience associated traits in dairy cattle. Animal 13:897–908. https://doi.org/10.1017/S1751731118003208.

Krawczyk, C., and J. M. Penninger. 2001. Molecular motors involved in T cell receptor clusterings. J. Leukoc. Biol. 69:317–330. https://doi.org/10.1189/jlb.69.3.317.

Kruk, J., K. Kotarska, and B. H. Aboul-Enein. 2020. Physical exercise and catecholamines response: Benefits and health risk: Possible mechanisms. Free Radic. Res. 54:105–125. https://doi.org/10.1080/10715762.2020.1726343.

Kwek, S. S., E. Cha, and L. Fong. 2012. Unmasking the immune recognition of prostate cancer with CTLA4 blockade. Nat. Rev. Cancer 12:289–297. https://doi.org/10.1038/nrc3223.

Lacetera, N. 2018. Impact of climate change on animal health and welfare. Anim. Front. 9:26–31. https://doi.org/10.1093/af/vfy030.

Lacetera, N., U. Bernabucci, D. Scalia, L. Basiricò, P. Morera, and A. Nardone. 2006. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. J. Dairy Sci. 89:4606–4612. https://doi.org/10.3168/jds.S0022-0302(06)72510-3.

Lacetera, N., U. Bernabucci, D. Scalia, B. Ronchi, G. Kuzminsky, and A. Nardone. 2005. Lymphocyte functions in dairy cows in hot environment. Int. J. Biometeorol. 50:105–110. https://doi.org/10.1007/s00484-005-0273-3.

Lau, D., H. Mollnau, J. P. Eiserich, B. A. Freeman, A. Daiber, U. M. Gehling, J. Brümmer, V. Rudolph, T. Münzel, T. Heitzer, T. Meinertz, and S. Baldus. 2005. Myeloperoxidase mediates neutrophil activation by association with CD11b/CD18 integrins. Proc. Natl. Acad. Sci. USA 102:431–436. https://doi.org/10.1073/pnas.0405193102.

Lecchi, C., N. Rota, A. Vitali, F. Ceciliani, and N. Lacetera. 2016. In vitro assessment of the effects of temperature on phagocytosis, reactive oxygen species production and apoptosis in bovine polymorphonuclear cells. Vet. Immunol. Immunopathol. 182:89–94. https://doi.org/10.1016/j.vetimm.2016.10.007.

Lefcourt, A. M., J. Bitman, S. Kahl, and D. L. Wood. 1993. Circadian and ultradian rhythms of peripheral cortisol concentrations in lactating dairy cows. J. Dairy Sci. 76:2607–2612. https://doi.org/10.3168/jds.S0022-0302(93)77595-5.

Lendez, P. A., L. Martinez Cuesta, M. V. Nieto Farias, A. A. Vater, M. D. Ghezzi, D. Mota-Rojas, G. L. Dolcini, and M. C. Ceriani. 2021. Alterations in TNF-α and its receptors expression in cows undergoing heat stress. Vet. Immunol. Immunopathol. 235:110232. https://doi.org/10.1016/j.vetimm.2021.110232.

Ley, K. 2002. Integration of inflammatory signals by rolling neutrophils. Immunol. Rev. 186:8–18. https://doi.org/10.1034/j.1600-065X.2002.18602.x.

Li, Q., B. Wang, K. Mu, and J. Zhang. 2019. The pathogenesis of thyroid autoimmune diseases: New T lymphocytes—Cytokines circuits beyond the Th1–Th2 paradigm. J. Cell. Physiol. 234:2204–2216. https://doi.org/10.1002/jcp.27180.

Li, Q.-L., Z.-H. Ju, J.-M. Huang, J.-B. Li, R.-L. Li, M.-H. Hou, C.-F. Wang, and J.-F. Zhong. 2011. Two novel SNPs in *HSF1* gene are associated with thermal tolerance traits in Chinese Holstein cattle. DNA Cell Biol. 30:247–254. https://doi.org/10.1089/dna.2010.1133.

Lian, P., S. Braber, J. Garssen, H. J. Wichers, G. Folkerts, J. Fink-Gremmels, and S. Varasteh. 2020. Beyond heat stress: Intestinal integrity disruption and mechanism-based intervention strategies. Nutrients 12:734. https://doi.org/10.3390/nu12030734.

Löwenberg, M., A. P. Verhaar, G. R. van den Brink, and D. W. Hommes. 2007. Glucocorticoid signaling: A nongenomic mechanism for T-cell immunosuppression. Trends Mol. Med. 13:158–163. https://doi.org/10.1016/j.molmed.2007.02.001.

Luo, H., L. Hu, L. F. Brito, J. Dou, A. Sammad, Y. Chang, L. Ma, G. Guo, L. Liu, L. Zhai, Q. Xu, and Y. Wang. 2022. Weighted single-step GWAS and RNA sequencing reveals key candidate genes associated with physiological indicators of heat stress in Holstein cattle. J. Anim. Sci. Biotechnol. 13:108. https://doi.org/10.1186/s40104-022-00748-6.

Luo, H., X. Li, L. Hu, W. Xu, Q. Chu, A. Liu, G. Guo, L. Liu, L. F. Brito, and Y. Wang. 2021. Genomic analyses and biological validation of candidate genes for rectal temperature as an indicator of heat stress in Holstein cattle. J. Dairy Sci. 104:4441–4451. https://doi.org/10.3168/jds.2020-18725.

Macciotta, N. P. P., S. Biffani, U. Bernabucci, N. Lacetera, A. Vitali, P. Ajmone-Marsan, and A. Nardone. 2017. Derivation and genome-wide association study of a principal component-based measure of heat tolerance in dairy cattle. J. Dairy Sci. 100:4683–4697. https://doi.org/10.3168/jds.2016-12249.

Maciuszek, M., K. Klak, L. Rydz, B. M. L. Verburg-van Kemenade, and M. Chadzinska. 2020. Cortisol metabolism in carp macrophages: A role for macrophage-derived cortisol in M1/M2 polarization. Int. J. Mol. Sci. 21:8954. https://doi.org/10.3390/ijms21238954.

Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. Growth and Development Symposium: Endotoxin, inflammation, and intestinal function in livestock. J. Anim. Sci. 90:1452–1465. https://doi.org/10.2527/jas.2011-4627.

Martínez, R. S., R. A. Palladino, G. Banchero, R. Fernández-Martín, M. Nanni, N. Juliano, J. Iorio, and A. La Manna. 2021. Providing heat-stress abatement to late-lactation Holstein cows affects hormones, metabolite blood profiles, and hepatic gene expression but not productive responses. Appl. Anim. Sci. 37:490–503. https://doi.org/10.15232/aas.2020-02109.

Menta, P. R., V. S. Machado, J. M. Piñeiro, W. W. Thatcher, J. E. P. Santos, and A. Vieira-Neto. 2022. Heat stress during the transition period is associated with impaired production, reproduction, and survival in dairy cows. J. Dairy Sci. 105:4474–4489. https://doi.org/10.3168/jds.2021-21185.

Min, L., N. Zheng, S. Zhao, J. Cheng, Y. Yang, Y. Zhang, H. Yang, and J. Wang. 2016. Long-term heat stress induces the inflammatory response in dairy cows revealed by plasma proteome analysis. Biochem. Biophys. Res. Commun. 471:296–302. https://doi.org/10.1016/j.bbrc.2016.01.185.

Mishra, S. R. 2021. Behavioural, physiological, neuro-endocrine and molecular responses of cattle against heat stress: An updated review. Trop. Anim. Health Prod. 53:400. https://doi.org/10.1007/s11250-021-02790-4.

Monteiro, A. P. A., S. Tao, I. M. Thompson, and G. E. Dahl. 2014. Effect of heat stress during late gestation on immune function and growth performance of calves: Isolation of altered colostral and calf factors. J. Dairy Sci. 97:6426–6439. https://doi.org/10.3168/jds.2013-7891.

Most, M. S., and D. T. Yates. 2021. Inflammatory mediation of heat stress-induced growth deficits in livestock and its potential role as a target for nutritional interventions: A review. Animals (Basel) 11:3539. https://doi.org/10.3390/ani11123539.

Möstl, E., J. L. Maggs, G. Schrötter, U. Besenfelder, and R. Palme. 2002. Measurement of cortisol metabolites in faeces of ruminants. Vet. Res. Commun. 26:127–139. https://doi.org/10.1023/A:1014095618125.

Nguyen, T. T., P. J. Bowman, M. Haile-Mariam, J. E. Pryce, and B. J. Hayes. 2016. Genomic selection for tolerance to heat stress in Australian dairy cattle. J. Dairy Sci. 99:2849–2862. https://doi.org/10.3168/jds.2015-9685.

Nicolaides, N. C., G. Chrousos, and T. Kino. 2020. Glucocorticoid receptor. In Endotext. K. R. Feingold, B. Anawalt, M. R. Blackman, ed. MDText.com. Accessed Apr. 13, 2023. https://www.ncbi.nlm.nih.gov/books/NBK279171/.

Niedzielska, M., B. Bodendorfer, S. Münch, A. Eichner, M. Derigs, O. da Costa, A. Schweizer, F. Neff, L. Nitschke, T. Sparwasser, S. M. Keyse, and R. Lang. 2014. Gene trap mice reveal an essential function of dual specificity phosphatase Dusp16/MKP-7 in perinatal survival and regulation of toll-like receptor (TLR)-induced cytokine production. J. Biol. Chem. 289:2112–2126. https://doi.org/10.1074/jbc.M113.535245.

Ohms, M., S. Möller, and T. Laskay. 2020. An attempt to polarize human neutrophils toward N1 and N2 phenotypes in vitro. Front. Immunol. 11:532. https://doi.org/10.3389/fimmu.2020.00532.

Otto, P. I., S. E. F. Guimarães, L. L. Verardo, A. L. S. Azevedo, J. Vandenplas, C. A. Sevillano, D. B. D. Marques, M. Pires, C. de Freitas, R. S. Verneque, M. F. Martins, J. C. C. Panetto, W. A. Carvalho, D. O. R. Gobo, M. V. G. B. da Silva, and M. A. Machado. 2019. Genome-wide association studies for heat stress response in *Bos taurus* × *Bos indicus* crossbred cattle. J. Dairy Sci. 102:8148–8158. https://doi.org/10.3168/jds.2018-15305.

Owen, D. R., J. Fan, E. Campioli, S. Venugopal, A. Midzak, E. Daly, A. Harlay, L. Issop, V. Libri, D. Kalogiannopoulou, E. Oliver, E. Gallego-Colon, A. Colasanti, L. Huson, E. A. Rabiner, P. Suppiah, C. Essagian, P. M. Matthews, and V. Papadopoulos. 2017. *TSPO* mutations in rats and a human polymorphism impair the rate of steroid synthesis. Biochem. J. 474:3985–3999. https://doi.org/10.1042/BCJ20170648.

Pandey, P., O. K. Hooda, and S. Kumar. 2017. Impact of heat stress and hypercapnia on physiological, hematological, and behavioral profile of Tharparkar and Karan Fries heifers. Vet. World 10:1149–1155. https://doi.org/10.14202/vetworld.2017.1149-1155.

Park, D. S., B.-H. Gu, Y. J. Park, S. S. Joo, S.-S. Lee, S.-H. Kim, E. T. Kim, D. H. Kim, S. S. Lee, S. J. Lee, B.-W. Kim, and M. Kim. 2021. Dynamic changes in blood immune cell composition and function in Holstein and Jersey steers in response to heat stress. Cell Stress Chaperones 26:705–720. https://doi.org/10.1007/s12192-021-01216-2.

Patra, A. K., and I. Kar. 2021. Heat stress on microbiota composition, barrier integrity, and nutrient transport in gut, production performance, and its amelioration in farm animals. J. Anim. Sci. Technol. 63:211–247. https://doi.org/10.5187/jast.2021.e48.

Petri, B., and M.-J. Sanz. 2018. Neutrophil chemotaxis. Cell Tissue Res. 371:425–436. https://doi.org/10.1007/s00441-017-2776-8.

Rakib, M. R. H., M. Zhou, S. Xu, Y. Liu, M. A. Khan, B. Han, and J. Gao. 2020. Effect of heat stress on udder health of dairy cows. J. Dairy Res. 87:315–321. https://doi.org/10.1017/S0022029920000886.

Rees, A., C. Fischer-Tenhagen, and W. Heuwieser. 2016. Effect of heat stress on concentrations of faecal cortisol metabolites in dairy cows. Reprod. Domest. Anim. 51:392–399. https://doi.org/10.1111/rda.12691.

Ricci, E., S. Ronchetti, E. Gabrielli, E. Pericolini, M. Gentili, E. Roselletti, A. Vecchiarelli, and C. Riccardi. 2019. GILZ restrains neutrophil activation by inhibiting the MAPK pathway. J. Leukoc. Biol. 105:187–194. https://doi.org/10.1002/JLB.3AB0718-255R.

Ronchetti, S., E. Ricci, G. Migliorati, M. Gentili, and C. Riccardi. 2018. How glucocorticoids affect the neutrophil life. Int. J. Mol. Sci. 19:4090. https://doi.org/10.3390/ijms19124090.

Rong, Y., M. Zeng, X. Guan, K. Qu, J. Liu, J. Zhang, H. Chen, B. Huang, and C. Lei. 2019. Association of *HSF1* genetic variation with heat tolerance in Chinese cattle. Animals (Basel) 9:1027. https://doi.org/10.3390/ani9121027.

Salak-Johnson, J. L., and J. J. McGlone. 2007. Making sense of apparently conflicting data: Stress and immunity in swine and cattle. J. Anim. Sci. 85(Suppl. 13):E81–E88. https://doi.org/10.2527/jas.2006-538.

Salama, A. A. K., M. Duque, L. Wang, K. Shahzad, M. Olivera, and J. J. Loor. 2019. Enhanced supply of methionine or arginine alters mechanistic target of rapamycin signaling proteins, messenger RNA, and microRNA abundance in heat-stressed bovine mammary epithelial cells in vitro. J. Dairy Sci. 102:2469–2480. https://doi.org/10.3168/jds.2018-15219.

Sanchez-Bermejo, E., W. Zhu, C. Tasset, H. Eimer, S. Sureshkumar, R. Singh, V. Sundaramoorthi, L. Colling, and S. Balasubramanian. 2015. Genetic architecture of natural variation in thermal responses of Arabidopsis. Plant Physiol. 169:647–659. https://doi.org/10.1104/pp.15.00942.

Schmidt, S., J. Rainer, C. Ploner, E. Presul, S. Riml, and R. Kofler. 2004. Glucocorticoid-induced apoptosis and glucocorticoid resistance: Molecular mechanisms and clinical relevance. Cell Death Differ. 11(Suppl. 1):S45–S55. https://doi.org/10.1038/sj.cdd.4401456.

Selvaraj, V., D. M. Stocco, and L. N. Tu. 2015. Minireview: Translocator protein (TSPO) and steroidogenesis: A reappraisal. Mol. Endocrinol. 29:490–501. https://doi.org/10.1210/me.2015-1033.

Shodell, M., K. Shah, and F. P. Siegal. 2003. Circulating human plasmacytoid dendritic cells are highly sensitive to corticosteroid administration. Lupus 12:222–230. https://doi.org/10.1191/0961203303lu362xx.

Sigdel, A., R. Abdollahi-Arpanahi, I. Aguilar, and F. Peñagaricano. 2019. Whole genome mapping reveals novel genes and pathways involved in milk production under heat stress in US Holstein cows. Front. Genet. 10:928. https://doi.org/10.3389/fgene.2019.00928.

Sigdel, A., L. Liu, R. Abdollahi-Arpanahi, I. Aguilar, and F. Peñagaricano. 2020. Genetic dissection of reproductive performance of dairy cows under heat stress. Anim. Genet. 51:511–520. https://doi.org/10.1111/age.12943.

Singh, J., R. D. Murray, G. Mshelia, and Z. Woldehiwet. 2008. The immune status of the bovine uterus during the peripartum period. Vet. J. 175:301–309. https://doi.org/10.1016/j.tvjl.2007.02.003.

Sölzer, N., K. May, T. Yin, and S. König. 2022. Genomic analyses of claw disorders in Holstein cows: Genetic parameters, trait associations, and genome-wide associations considering interactions of SNP and heat stress. J. Dairy Sci. 105:8218–8236. https://doi.org/10.3168/jds.2022-22087.

Somal, A., A. Aggarwal, and R. C. Upadhyay. 2015. Effect of thermal stress on expression profile of apoptosis related genes in peripheral blood mononuclear cells of transition Sahiwal cow. Iran. J. Vet. Res. 16:137–143.

Srikanth, K., A. Kwon, E. Lee, and H. Chung. 2017. Characterization of genes and pathways that respond to heat stress in Holstein calves through transcriptome analysis. Cell Stress Chaperones 22:29–42. https://doi.org/10.1007/s12192-016-0739-8.

Stevens, A., and A. White. 2010. ACTH: Cellular peptide hormone synthesis and secretory pathways. Pages 63–84 in Cellular Peptide Hormone Synthesis and Secretory Pathways.J. F. Rehfeld and J. R. Bundgaard, ed. Springer.

Stocki, P., X. N. Wang, and A. M. Dickinson. 2012. Inducible heat shock protein 70 reduces T cell responses and stimulatory capacity of monocyte-derived dendritic cells. J. Biol. Chem. 287:12387–12394. https://doi.org/10.1074/jbc.M111.307579.

Tejaswi, V., B. Balachander, H. A. Samad, M. Sarkar, V. P. Maurya, and G. Singh. 2020a. Assessment of heat stress induced alterations in polymorphonuclear (PMN) cell activity in native and crossbred cows. J. Appl. Anim. Res. 48:549–552. https://doi.org/10.1080/09712119.2020.1829629.

Tejaswi, V., B. Balamurugan, H. A. Samad, M. Sarkar, V. P. Maurya, and G. Singh. 2020b. Short communication: Differential endocrine and antioxidant responses to heat stress among native and crossbred cattle. J. Vet. Behav. 39:1–5. https://doi.org/10.1016/j.jveb.2020.06.001.

Thiriet, M. 2013. Mitogen-activated protein kinase module. Pages 311–378 in Intracellular Signaling Mediators in the Circulatory and Ventilatory Systems. Springer.

Thompson, I. M. T., S. Tao, A. P. A. Monteiro, K. C. Jeong, and G. E. Dahl. 2014. Effect of cooling during the dry period on immune response after *Streptococcus uberis* intramammary infection challenge of dairy cows. J. Dairy Sci. 97:7426–7436. https://doi.org/10.3168/jds.2013-7621.

Torres-Farfan, C., L. Abarzua-Catalan, F. J. Valenzuela, N. Mendez, H. G. Richter, G. J. Valenzuela, and M. Serón-Ferré. 2009. Cryptochrome 2 expression level is critical for adrenocorticotropin stimulation of cortisol production in the capuchin monkey adrenal. Endocrinology 150:2717–2722. https://doi.org/10.1210/en.2008-1683.

Toscano, M. A., A. G. Commodaro, J. M. Ilarregui, G. A. Bianco, A. Liberman, H. M. Serra, J. Hirabayashi, L. V. Rizzo, and G. A. Rabinovich. 2006. Galectin-1 suppresses autoimmune retinal disease by promoting concomitant Th2- and T regulatory-mediated anti-inflammatory responses. J. Immunol. 176:6323–6332. https://doi.org/10.4049/jimmunol.176.10.6323.

Tugal, D., X. Liao, and M. K. Jain. 2013. Transcriptional control of macrophage polarization. Arterioscler. Thromb. Vasc. Biol. 33:1135–1144. https://doi.org/10.1161/ATVBAHA.113.301453.

Uzhachenko, R. V., and A. Shanker. 2019. CD8+ T lymphocyte and NK cell network: Circuitry in the cytotoxic domain of immunity. Front. Immunol. 10:1906. https://doi.org/10.3389/fimmu.2019.01906.

Vatrella, A., I. Fabozzi, C. Calabrese, R. Maselli, and G. Pelaia. 2014. Dupilumab: A novel treatment for asthma. J. Asthma Allergy 7:123. https://doi.org/10.2147/JAA.S52387.

Waeber, G., J. Delplanque, C. Bonny, V. Mooser, M. Steinmann, C. Widmann, A. Maillard, J. Miklossy, C. Dina, and E. H. Hani. 2000. The gene *MAPK8IP1*, encoding islet-brain-1, is a candidate for type 2 diabetes. Nat. Genet. 24:291–295. https://doi.org/10.1038/73523.

Walsh, K. P., and K. H. G. Mills. 2013. Dendritic cells and other innate determinants of T helper cell polarisation. Trends Immunol. 34:521–530. https://doi.org/10.1016/j.it.2013.07.006.

Welsh, M. D., R. T. Cunningham, D. M. Corbett, R. M. Girvin, J. McNair, R. A. Skuce, D. G. Bryson, and J. M. Pollock. 2005. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology 114:101–111. https://doi.org/10.1111/j.1365-2567.2004.02003.x.

Widdison, S., and T. J. Coffey. 2011. Cattle and chemokines: Evidence for species-specific evolution of the bovine chemokine system. Anim. Genet. 42:341–353. https://doi.org/10.1111/j.1365-2052.2011.02200.x.

Willoughby, E. A., G. R. Perkins, M. K. Collins, and A. J. Whitmarsh. 2003. The JNK-interacting protein-1 scaffold protein targets MAPK phosphatase-7 to dephosphorylate JNK. J. Biol. Chem. 278:10731–10736. https://doi.org/10.1074/jbc.M207324200.

Xavier, A. M., A. K. O. Anunciato, T. R. Rosenstock, and I. Glezer. 2016. Gene expression control by glucocorticoid receptors during innate immune responses. Front. Endocrinol. (Lausanne) 7. https://doi.org/10.3389/fendo.2016.00031.

Young, R., S. J. Bush, L. Lefevre, M. E. B. McCulloch, Z. M. Lisowski, C. Muriuki, L. A. Waddell, K. A. Sauter, C. Pridans, E. L. Clark, and D. A. Hume. 2018. Species-specific transcriptional regulation of genes involved in nitric oxide production and arginine metabolism in macrophages. Immunohorizons 2:27–37. https://doi.org/10.4049/immunohorizons.1700073.

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