

Dietary Strategies Protecting Heat-stressed Farm Animals Against Increased Susceptibility to Inflammatory Diseases

Global warming and the growing consumer demand for animal-derived food and meat, particularly in newly industrialising countries with hot climates, raise public concerns on animal welfare, long-term sustainable livestock production and food and consumer safety (see Figure 1). In these areas, heat stress is one major reason for economic losses in livestock production. Besides lowering the quality of forage and feedstuffs, a hot and humid climate reduces reproduction performance and productivity of livestock animals. Moreover, heat stress affects general health and the susceptibility of farm animals to infectious diseases. In addition, new pathogens or vectors may be introduced into areas with changing climate.

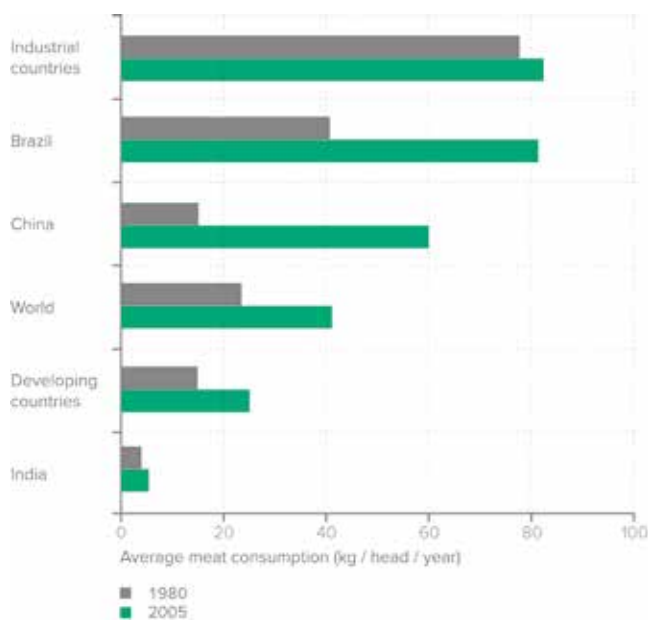


Figure 1. Development of average meat consumption within 15 years depending on different regional areas (modified after FAO).



Under heat stress, animals alter their behaviour and physiology in order to dispose of heat and to decrease body temperature (see Figure 2). They spend less time feeding and moving, while spending more time on resting, drinking and panting. Obvious signs of heat stress in livestock include the decrease in feed intake and daily gain, the deterioration of product quality (meat, eggs and milk), and the increase in morbidity and mortality.

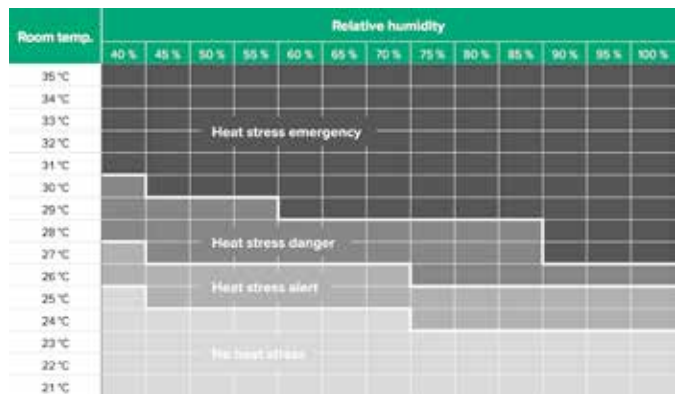


Figure 2. Heatstress index for growing-finishing pigs. Source: Iowa State University

To address and counteract these challenges, a detailed knowledge of the physiological mechanisms of heat stress in livestock species is required. Consequently, the purpose of this article is to provide a brief overview on heat stress-induced physiological and molecular changes in livestock. Figure 3 summarises direct and indirect heat stress-induced consequences for livestock.

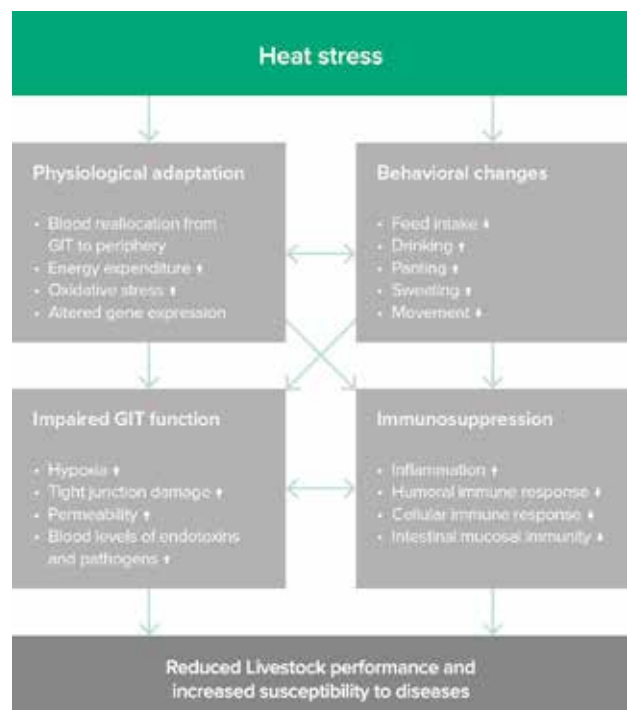


Figure 3. Heat stress reduces performance of livestock animals due to various direct and indirect negative influences.

Intestinal Morphology

The results of studies reporting on heat stress-induced changes in intestinal morphology are not consistent. Thus, contradictory information on the alteration of villus height and crypt depth, two key parameters for the intestinal capacity of nutrient absorption, exist. For instance, Song *et al.* (2014) found a reduction of villus height and an increase in crypt depth in the jejunum of Ross 308 broilers, exposed to cyclic heat stress. Other studies reported on a reduced ileal crypt depth, but discovered no changes in villus height of Ross 308 broilers kept at 30°C compared to their littermates kept at 23°C (Burkholder *et al.*, 2008). In heat-stressed pigs, Pearce *et al.* (2015) observed a villus height reduction in tendency, but found no changes in crypt depth. In conclusion, the differing and contradictory results regarding gut morphology may reflect differences in the applied heatstress regimes and/or species- and breed-specific differences.

Metabolic Changes

The initial cellular response to heat stress seems to consist of an increase in energy expenditure. For this purpose, mitochondrial activity is increased to produce sufficient amounts of ATP for essential cellular processes, like Na⁺/K⁺-ATPase activity. The up-regulation of mitochondrial capacity is linked to a higher production of reactive oxygen species, resulting in an increased level of endogenous oxidative stress (Akbarian *et al.*, 2016). As a consequence, heat-mediated oxidative stress produces alterations in cell signalling, affecting heat shock protein expression (HSP) as well as damage to the intestinal barrier (Lambert *et al.*, 2002) and hyperthermia-induced apoptotic cell death (Katschinski *et al.*, 2000). The up-regulated expression of HSPs is an endogenous mechanism conferring cells protection against stress. For instance, HSP70 has been shown to protect intestinal mucosa of heat-stressed broilers by improving antioxidant capacity and inhibiting lipid peroxidation (Gu *et al.*, 2012).

A study with rats has provided evidence for the increased energy consumption during hyperthermia. In this study, liver glycogen levels declined by about 20% after exposure to heat stress for two hours (Hall *et al.*, 2001). Whole-body hyperthermia induces re-distribution of blood from internal organs to the periphery to intensify heat loss from the body (Lambert *et al.*, 2002). Due to this shift, cellular hypoxia and metabolic stress are induced in the intestinal tract (Hall *et al.*, 2001). Since heat stress affects in particular the insulin signalling pathway, changes in whole-body energy metabolism are partially caused through changes in glucose uptake and metabolism. This hypothesis is supported by the results of several studies, showing an increased intestinal glucose uptake in heat-stressed animals (e.g. Pearce *et al.*, 2013; Fernandez *et al.*, 2015). Different other tissues might also show alterations in individual glucose uptake and utilisation. In this context, Fernandez *et al.* (2015) observed a higher insulin receptor substrate-1 abundance in skeletal muscle, but not in adipose tissue of pigs. The differential effects of heat stress in various tissues might ultimately induce changes in energy deposition and cause shifts in lean body mass of the animals. This phenomenon was described for post-natal body composition of dams exposed to heat stress during gestation. Piglets from sows exposed to heat stress (28°C to 34°C) during the first half of gestation showed a reduced longissimus dorsi cross-sectional area and an increased subcutaneous fat thickness and blood insulin concentrations, compared to piglets from sows kept under normal temperature conditions (18°C to 22°C) (Boddicker *et al.*, 2014). Interestingly, Fernandez *et al.* (2015) reported



on increased insulin receptor substrate-1 abundance in skeletal muscle but not in adipocytes, suggesting a higher energy uptake into the muscle but not fat tissue. In summary, these results suggest that further research is needed to evaluate the detailed mechanism as to how heat stress-induced metabolic changes in dams cause an increased fat deposition in piglets.

Intestinal Barrier Integrity

One explanation for an increased susceptibility to diseases of livestock animals under heat stress consists in the occurrence of an impaired intestinal barrier function. The intestinal barrier is the first-line defence against harmful microbial pathogens, their toxins and antigens from the intestinal lumen. The intestinal barrier is formed by a layer of epithelial cells, and the cellular interspaces are sealed by tight junctions, which are mainly composed of proteins of the claudin and the occludin family. Tight junctions act as selective barriers regulating the paracellular transport. Heat stress compromises the integrity of the tight junctions and thereby increases the permeability of the intestinal mucosa, disturbing its original function in absorbing nutrients and keeping pathogens at bay. Measurement of the transepithelial electrical resistance (TEER) is routinely used to determine this integrity, respectively the permeability of the intestinal barrier in freshly obtained intestinal samples or cell cultures. The lower the TEER, the higher the degree of impairment of the intestinal barrier integrity. It has been shown that heat stress reduces the TEER in Caco-2 monolayers (Xiao *et al.*, 2013), but also in pigs *in vivo* (Sanz Fernandez *et al.*, 2013). In the literature, numerous studies report on the gene- or protein-expression of tight junction proteins under heatstress conditions. For instance, Xiao *et al.* (2013) have detected in Caco-2 an increase in occludin mRNA and protein levels when culture temperature of the cells was raised from 37°C to 41°C. In contrast, increasing the temperature up to 43°C reduced Zonula occludens-1 mRNA- and protein-expression, whereas no changes in claudin-2 abundance could be observed (Xiao *et al.*, 2013). Exposition of broilers to heat stress at 33°C for 10 h resulted in a significant decrease of occludin and Zonula occludens-1 protein levels (Song *et al.*, 2014). In pigs, colonic occludin gene abundance has been found to be decreased under heatstress conditions (Pearce *et al.*, 2015). In contrast, claudin-3 and occluding protein expression increased in the ileum of growing pigs under heat stress (Pearce *et al.*, 2013). In summary, these studies demonstrate that temperature influences tight junction protein expression. Although in most of the studies, tight junction protein abundance was decreased by heat stress due to damage and loss of function, others have shown an upregulation of their gene expression. This phenomenon may be explained as a direct consequence of the heat stress: the upregulation of tight junction gene expression might serve as an intestinal barrier function to counter-regulate the loss of tight junction proteins induced by heat stress.

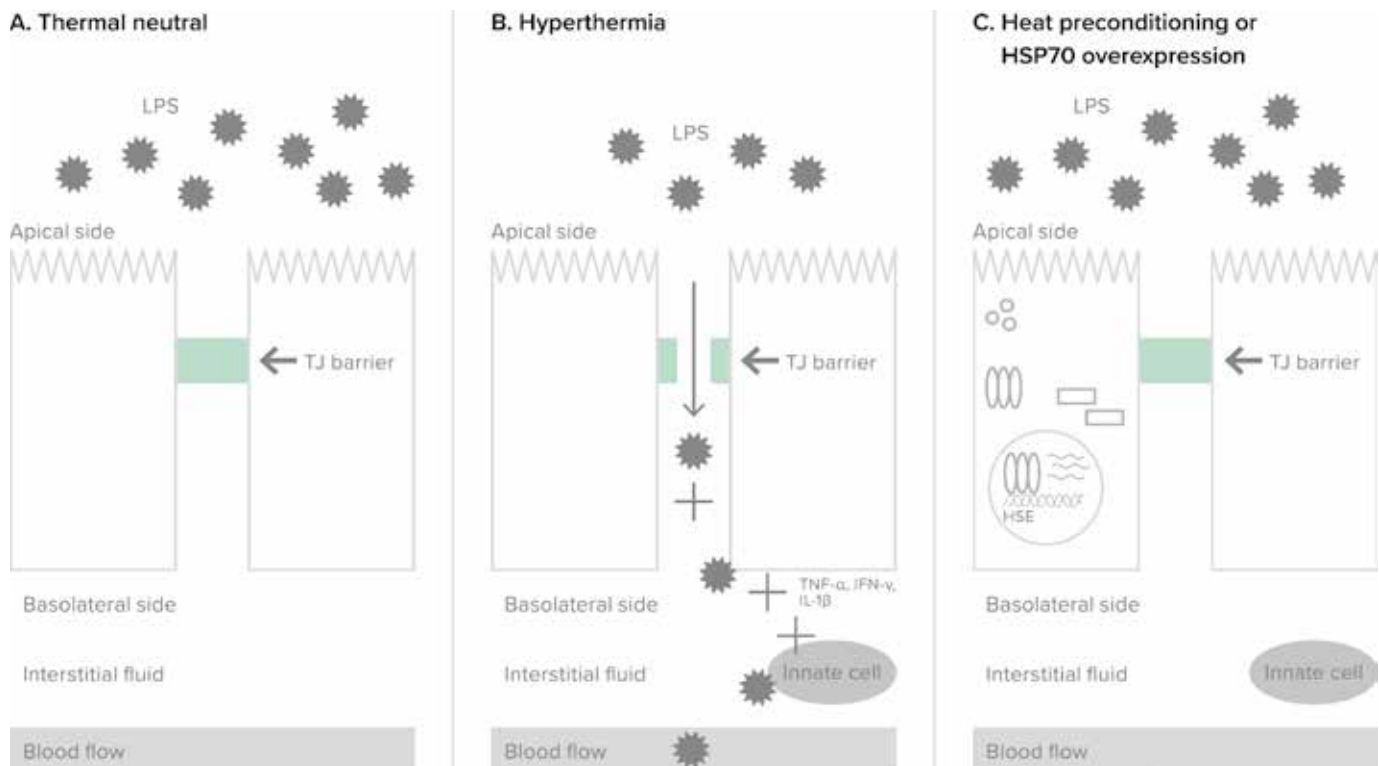


Figure 4. Temperature-induced reduction of tight junction protein abundance (Modified after Dokladny *et al.*, 2016). A) Intact tight junction barrier at thermoneutral ambient temperature. B) Heat stress-induced breakdown of tight junctions C) Upregulation of tight junction gene expression to counteract heat stress-afflicted damage of intestinal barrier. For details read Dokladny *et al.* (2016).

In conclusion, the results of all studies investigating the link between heat stress and intestinal barrier integrity unambiguously demonstrate the negative impact of heat stress on the intestinal barrier. The impairment of the intestinal barrier integrity results in an increased permeability of the gut to different molecules and pathogens. Consequently, increased concentrations of substances from the gut can be found in the blood of the animals. For example, blood endotoxin levels in heat-stressed pigs increased by 200 % when compared to pigs kept under normal temperature conditions (Pearce *et al.*, 2013). Comparing the effect of prolonged heat stress to short-term exposure have shown also remarkable differences in endotoxin blood levels. Gilts exposed to 35°C for seven days showed 260–390% increased blood endotoxin levels compared to gilts exposed to heat stress at 35°C only for 24 hours (Sanz Fernandez *et al.*, 2013). The increased endotoxin levels under heat stress exposure can cause a systemic inflammatory response, resulting in decreased growth performance and increased morbidity and mortality (Xu *et al.*, 2015; Dokladny *et al.*, 2008). Increased migration of bacteria from the gut to other parts of the body could be directly demonstrated by Quinteiro-Filho *et al.* (2012). In their study, broilers infected with *Salmonella enteritidis* showed a decreased performance and were linked to 3.9-times higher *Salmonella* counts in the spleen of heat-stressed animals (31°C), compared to control animals (21°C).

Intestinal Microbiota

Heat stress has been shown to affect the composition of the intestinal microbiota in several recent studies. In Holstein heifers, an increase of environmental temperature from 20°C to 33°C induced significant changes to the major phylogenetic groups in the rumen, accompanied by a reduction of short-chain fatty acid (SCFA) production (Tajima *et al.*, 2017). These changes could be related to the

reduced dry matter intake of the animals under heat stress. Changes in SCFA production due to heat stress have been also observed in pigs, although in this study, overall SCFA production was not reduced. However, the proportion of the different SCFAs was altered to a higher percentage of acetate and a lower abundance of propionate (Song *et al.*, 2011). A change in the proportion of produced SCFA might reflect both a shift in the metabolic activity of bacteria and/or a changed composition of the intestinal microbiota. It has been also shown that heat stress directly alters the intestinal microbial diversity. In this context, Burkholder *et al.* (2008) observed a reduced ileal microbial diversity in broilers kept at 30°C compared to broilers kept at 23°C. Another study with poultry showed the context between heat stress and changes in some bacterial groups in more detail. Cyclic heat stress decreased the counts of *Lactobacilli* and *Bifidobacteria* in the small intestine, whereas time counts of coliforms and *Clostridium* increased (Song *et al.*, 2014). This might reflect an unfavourable change, since numerous members of the lactobacilli- and bifidobacterial families are considered as advantageous for the host. Although most coliforms and clostridia are commensal members of the gut microbiota, some of them are associated with proteolytic fermentation, and contain a range of potential pathogenic members (e.g. *C. perfringens*, *Salmonella* or endotoxin producing *E. coli*). Consequently, increased counts of these bacterial groups might result in increased production of potentially harmful fermentation products and proliferation of potential pathogens.

Immune System

Heat stress leads to an overall immunosuppression in livestock, representing another possible mechanism explaining the increased susceptibility of animals to illnesses. In poultry, heat stress suppresses the innate and adaptive immunity. Broilers under heat stress have shown lower levels of total circulating antibodies, as well

as lower specific IgM and IgG levels against Newcastle disease virus and infectious bursal disease virus (Niu *et al.*, 2009; Akhavan-Salamat *et al.*, 2016; Sohail *et al.*, 2010). Exposure of chickens to a high temperature also decreases the percentage of CD4+ T cells and CD8+ T cells, which impairs the cellular immune response (Khajavi *et al.*, 2003; Trout and Mashaly, 1994). Moreover, heat stress has a negative impact on intestinal mucosal immunity. The intestinal mucosa is an important barrier against pathogen invasion. A mucus layer covers the luminal epithelium, and the lamina propria tissue below harbours a wide range of immune cells, including intraepithelial lymphocytes, regulatory T cells, secretory IgA cells and others. Under heatstress conditions, the integrity of the mucosal epithelial cells is compromised, allowing bacteria and other antigens to invade into the lamina propria. The massive invasion of antigens triggers inflammatory processes. The expression of IL-10 and IL-4 from the intestinal mucosa has been shown to decrease in heat-stressed animals, indicating an impairment of anti-inflammatory function and immune cell proliferation. Additionally, the expression of Toll-like receptors decreases, reflecting attenuation to resist bacterial invasion (Liu *et al.*, 2012). Also in swine, heat stress has been demonstrated to influence cellular immunity. Exposure to heat stress reduced the proliferation of porcine T cells in peripheral blood (Kelley, 1985). Jensen *et al.* (1983) found that phytohemagglutinin skin-test reaction was weakened in barrows under heat stress, which is a reliable indicator for assaying cellular immunity. Heat stress also has been shown to cause neutrophil infiltration in ileum and inflammation, which worsens the integrity of the intestinal barrier (Pearce *et al.*, 2013).

Conclusion

- Heat stress has multiple etiological factors causing physiological, behavioural and metabolic changes.
- Heat stress has substantial effects on the immune system, causing suppression of immunity and reduction of the ability to resist pathogen invasion. This results in a higher morbidity and mortality.
- Heat stress reduces the intestinal absorption surface (villi height), resulting in lower nutrient utilisation and higher substrate for fermentation.
- Heat stress severely alters signalling pathways, regulating major processes of glucose and lipid metabolism, resulting in unfavourable changes in the relation of protein and fat deposition.
- Heat stress triggers the proliferation of undesired bacteria, resulting in higher numbers of proteolytic bacteria and potential pathogenic bacteria.
- Heat stress decreases intestinal barrier integrity, resulting in the invasion of a higher number of antigens and pathogens into the bloodstream and the organism.
- Heat stress-induced changes in the organism represent a complex of the above-mentioned symptoms (=syndrome), resulting in a strong general impairment of animal performance and health.

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