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Heat Stress in Chickens: Implications for Gut Health and Dietary Interventions with Phytobiotics

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INTRODUCTION

Heat stress (HS) occurs when an animal generates more heat than it can effectively release into its surrounding environment. The discrepancy can arise from fluctuations in a blend of environmental factors (such as sunlight, thermal irradiation, air temperature, humidity, and movement) and attributes of the animal (including species, gender, and metabolic rate) (Akbarian *et al*., 2016). Increased environmental temperatures have notable adverse impacts on productivity, feed consumption, growth performance, meat production, welfare, and mortality rates in contemporary broiler chickens. Different managerial and nutritional approaches have been suggested to alleviate the adverse impacts of HS in chickens, with indications of potential effectiveness in plant-based additives. The term phytogenic feed additives (PFA) refers to a diverse array of bioactive substances derived from plants, such as herbs, spices, extracts, and essential oils, which are included in animal diets (Windisch *et al*., 2008). Adding phytobiotics to the diet positively impacts gastrointestinal health and function, enhances nutrient digestibility, influences gut microbiota, boosts the immune system, improves oxidative status, and enhances the growth performance of both broilers and laying hens (Abdelli *et al*., 2021).

Heat stress: Effects on physiology of chicken

Elevated environmental temperatures can surpass the thermoregulatory mechanisms, leading to a mismatch between the metabolic heat produced by chickens and their ability to effectively release body heat into the environment. This modification leads to an abnormal elevation in body temperature, known as hyperthermia, and initiates heat stress (HS) (Rostagno,

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2020). Aside from its potential lethality, heat stress exerts a wide-ranging impact on the behavior, physiology, gut health, welfare and productive performance of chickens (Brugaletta *et al*., 2022). Fast-growing and highly efficient broiler chickens exhibit lower thermotolerance and increased susceptibility to heat stress (HS) compared to slow-growing lines (Tallentire *et al*., 2018). This is attributed to their exceptionally high metabolic rates and underdeveloped cardiovascular and respiratory systems (Xu *et al*., 2018). The physiological reaction to HS is more pronounced, and heat tolerance is diminished in heavier broilers as opposed to lighter birds of the same line and age (Gogoi *et al*., 2021).

Effect of heat stress on gut health

The intestinal tract shows heightened responsiveness to various forms of stress, including heat stress (Slawinska *et al*., 2019). The efficient functioning of the intestinal tract is pivotal in poultry production, given its significant implications for the overall health and performance of the birds (Kadykalo *et al*., 2018). Continuous and bidirectional communication occurs between the brain and digestive systems through complex pathways involving the enteric nervous system (ENS), the autonomic nervous system (ANS), the hypothalamus-pituitary axis (HPA), and the central nervous system (CNS). This interconnected network is recognized as the brain-gut axis (Rostagno, 2020). Signals originating from the brain have the capacity to alter the motor, sensory, secretory, and immune functions of the intestinal tract. Conversely, visceral messages from the intestinal tract can impact brain functions through a reciprocal top-down and bottom-up relationship (Cryan *et al*., 2019). Situated within the entire intestinal tract wall, the enteric nervous system (ENS) is an integrative network comprising millions of neurons. It regulates microcirculation, motility, and all exocrine and endocrine secretions (Costa *et al*., 2000). A comprehensive approach to addressing gut health involves considering the major elements that synergistically influence it, specifically the GI epithelium, the GI immune system, and the GI microbiota (Kogut *et al*., 2017).

Heat stress and gastrointestinal epithelium

The gastrointestinal (GI) epithelium, organized as a single-cell layer, actively participates in the coordinated gut immune system. It establishes a protective barrier strengthened by tight junction (TJ) proteins, produces mucus and antimicrobial/host defense peptides (AMP/HDP), and expresses pattern recognition receptors (PRR) that coordinate the immune response within the intestine (Brugaletta *et al*., 2022). The initial protective barrier for the epithelium against various

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luminal threats, such as mechanical forces during digestion, enzymes, and gut bacteria, is provided by the intestinal mucus layer (Duangnumsawang *et al*., 2021).

Impairment of the integrity of the intestinal barrier, also known as intestinal barrier dysfunction, results in heightened intestinal permeability. This condition is characterized by the unmediated diffusion of typically restricted large molecules (with a molecular weight exceeding 150 Da) from the intestinal lumen into the circulatory system (Lambert, 2009). Elevated temperatures can interfere with these junctions, leading to unfavorable outcomes by permitting the leakage of toxins and infectious agents (Hoover, 2020).

During heat stress (HS), the cardiovascular system initiates an evolutionarily conserved adaptation, directing a substantial volume of blood from the splanchnic tissues to peripheral areas of the body. This process aims to optimize the dissipation of sensible heat (Lambert, 2009). This negatively impacts the gastrointestinal tract (GIT) as the changes in blood pressure are predominantly offset by sympathetic-induced vasoconstriction of visceral vessels. The ensuing hypoperfusion leads to a diminished delivery of nutrients and oxygen to the GIT, thereby causing harmful effects on the intestinal mucosa (Lambert, 2009; Rostagno, 2020). Diminished blood supply and reduced feed intake contribute to a decrease in oxygen and nutrient availability, leading to morphological alterations and mucosal damage. This is a consequence of oxidative stress and inflammation. Additionally, heightened serum levels of corticosterone and catecholamines (such as epinephrine and norepinephrine) induced by heat stress also impact tight junctions and the immune system. Indeed, numerous investigations (Quinteiro-Filho *et al*., 2010, 2012a,b, 2017) have illustrated the impact of heat stress on the integrity of the intestinal barrier. This results in heightened permeability of the intestine and localized inflammation in poultry. The inflammation is characterized by an augmented presence of lympho-plasmacytic infiltrate throughout the small intestine, encompassing the duodenum, jejunum, and ileum. The disruption caused by heat stress affected the integrity of the intestinal barrier, leading to an elevation in intestinal permeability to endotoxin and the translocation of intestinal pathogens like *Salmonella spp*. (Alhenaky *et al*., 2017).

Heat stress and immune system

The immune system in poultry includes the gut-associated lymphoid tissue (GALT), comprising lymphoid cells situated in the epithelial lining (intraepithelial lymphocytes) and the lamina propria. Additionally, specialized lymphoid structures such as Peyer's patches, Meckel's

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diverticulum, cecal tonsils, and the bursa of Fabricius play a crucial role in this immune system component (Casteleyn *et al*., 2010; Taha-Abdelaziz *et al*., 2018).

Enteric neurons and intestinal immune cells employ shared regulatory mechanisms and can collaborate in responding to challenges. Dysregulation of the immune response can occur due to various stressors that impact the interplay between these systems. Consequently, this dysregulation increases susceptibility to pathogens, affects the severity of infections and pathologies, and may even have a negative impact on responses to vaccines (Rostagno, 2020).

An immunosuppressive effect of heat stress is observed in broilers and laying hens, as indicated by various measurements. These include a decrease in the number of intraepithelial lymphocytes and IgA-secreting cells along the intestinal tract, a diminished antibody response, and a reduction in the phagocytic activity of macrophages (Lara and Rostagno, 2013; Wu *et al*., 2021). Broilers and laying hens exposed to heat stress have been noted to exhibit reduced relative weights in their thymus, bursa, spleen, and liver (Lara and Rostagno, 2013; He *et al*., 2020). Numerous studies have shown that heat stress can modify the levels of circulating cells, resulting in an elevated heterophil:lymphocyte (H:L) ratio. This shift is attributed to a decrease in the count of circulating lymphocytes and an increase in the number of heterophils, which is influenced by higher concentrations of glucocorticoids, especially corticosterone, released due to the activation of the HPA axis (Altan *et al*., 2003; Mashaly *et al*., 2004).

Heat stress and gastrointestinal microbiota

Numerous factors, such as genetics, diet, diseases, medications, and various environmental elements, collectively influence the composition of intestinal microbiomes. Factors related to the environment, such as crowding and heat stress, can impact the composition of intestinal flora and the growth of rats and chicks. The absence of gut microbiota intensifies neuroendocrine and behavioral responses induced by acute stress. Consequently, gut microbiota play crucial roles in various aspects of physical health, encompassing growth performance, immunity, metabolism, and brain behavior (Shi *et al*., 2019). In chickens, the gut microbiome undergoes rapid development following hatching. Initially, the dominant members are *Enterobacteriaceae* from the *Proteobacteria* phylum, which swiftly transition to *Clostridiales* within the *Firmicutes* phylum within the first week post-hatching. As broilers and laying hens progress through their growth period, the major constituents of the gut microbiota include the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. However, the microbial composition varies among different sections of the gut (duodenum, jejunum, ileum, cecum, and colon), as well as with age and feeding patterns (He *et al*., 2021).

Studies conducted in poultry, focusing on both broilers and layers, have revealed notable impacts of heat stress on the composition and structure of the intestinal microbiota. Specific changes reported encompass reduced levels of *Lactobacillus* and *Bifidobacterium*, along with elevated levels of *Clostridium* and total coliforms (Song *et al*., 2014). Notably, these studies propose varied effects along the intestinal tract, highlighting the greater sensitivity of the small intestine in contrast to the ceca (Rostagno, 2020). Shi *et al*. (2019) conducted a study on broiler chickens, revealing that at the phylum level, the heat-stressed group exhibited an elevation in the abundance of Firmicutes, Tenericutes, and Proteobacteria, while Bacteroidetes and Cyanobacteria were diminished compared to the control group.

Enhancing Heat Stress Resilience through Phytobiotics

Phytobiotics, commonly known as phytochemicals or phytogenics, encompass a diverse range of bioactive compounds derived from plants. To date, over 5,000 distinct dietary phytobiotics have been recognized in fruits, vegetables, whole grains, legumes, nuts, herbs, and essential oils (Kikusato, 2021). Under heat stress (HS), oxidative stress occurs, prompting cellular alterations aimed at neutralizing the detrimental impact of reactive oxygen species (ROS). Alterations in antioxidant enzyme activity are intricately linked to elevated temperatures. Broiler livers exposed to high temperatures exhibited increased levels of superoxide dismutase (SOD) and catalase (CAT) activity, as well as elevated malondialdehyde (MDA) levels and total antioxidant capacity (T-AOC) (Zeng *et al*., 2014). In general, the activity of antioxidant enzymes such as CAT, GSH-Px, and SOD is significantly elevated to safeguard cells against excessive superoxide formation (Akbarian *et al*., 2016). The positive attributes of phytogenic substances stem from their bioactive components, exemplified by curcumin, thymol, cineole, linalool, allicin, capsaicin, and carvacrol (Oke, 2018). Additionally, the phenolic compounds found in plant products, recognized as primary antioxidants, render them bioactive substances beneficial for health and growth (Tuorkey, 2015).

Phytobiotics commonly utilised in chicken

Ginger (*Zingiber officinale* Roscoe) is globally renowned as one of the most popular spices, boasting a spectrum of health benefits including antioxidant, hepatoprotective, antimicrobial, analgesic, radio-protective, and anti-inflammatory properties. Among its numerous bioactive

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components, the gingerols stand out as pivotal agents, comprising a group of phenolic compounds such as 6-, 8-, and 10-gingerol. Notably, 6-gingerol emerges as the primary constituent responsible for a multitude of pharmacological effects attributed to ginger (Alsherbiny *et al*., 2019). The group administered with 150 mg/kg of ginger essential oil exhibited an elevation in total superoxide dismutase (TSOD) activity in the liver compared to the control group. Moreover, both the ginger powder and essential oil groups showed decreased concentrations of malondialdehyde (MDA) in the liver compared to the control. Additionally, all dietary groups demonstrated increased total antioxidant capacity (TAC) and decreased MDA concentration in serum compared to the control group (Habibi *et al*., 2014). Similarly, supplementation with ginger extract led to an increase in total antioxidant capacity and a decrease in malondialdehyde content in both the liver and breast muscle. Furthermore, it elevated glutathione peroxidase activity in both serum and breast muscle (Wen *et al*., 2020). Supplementing with ginger enhanced the antioxidant status of both broiler (Safiullah *et al.*, 2019) and layer chickens (Ibtisham *et al*., 2019).

Black cumin seeds contain drug-like compounds like thymohydroquinone, thymol, carvacrol, and dithymoquinone (Al-Saleh *et al*., 2006). Thymohydroquinone constitutes approximately 24% of the essential oil composition, exhibiting antioxidant, antihistaminic, antimicrobial, and anti-inflammatory properties (Arslan *et al*., 2005). Considerable research has explored the effects of incorporating dietary black cumin on poultry, with reported positive impacts on broilers' feed consumption, body weights, and growth performance (Abu-Dieyeh *et al*., 2008). Responding to the deman Black cumin seeds contain drug-like compounds like thymohydroquinone, thymol, carvacrol, and dithymoquinone (Al-Saleh *et al*., 2006). d for eggs and laying hens with enhanced antioxidant properties, black cumin has been included in poultry diets, resulting in reduced cholesterol and MDA concentrations in eggs and blood (Firdaus *et al*., 2019). Furthermore, Oke *et al*. (2021) demonstrated the favorable outcomes of in ovo injection of 6 mg of black cumin extract on blood antioxidant indices, hatchability, and posthatch performance of thermally stressed broiler embryos. Studies by various researchers have shown that black cumin seeds or extracts can mitigate oxidative stress, decrease serum MDA levels, and promote brain and spinal cord tissue health (Farah *et al*., 2005). Additionally, feeding Cobb broilers with 0.5% N. sativa along with 500 ppm vitamin C in a hot tropical environment resulted in decreased high-density lipoprotein (HDL) levels and improved H/L ratios (Ali *et al*., 2014). El-Shoukary *et al*. (2014) observed increased feed intake, daily body gain, dressing percentage, and reduced panting behavior, water consumption, and corticosterone levels when broiler diets were supplemented with 1% black seed under heat stress conditions.

Demethoxycurcumin, curcumin, and bisdemethoxycurcumin constitute the curcumoids found in turmeric. They possess antihepatotoxic, anticarcinogenic, antioxidative, hypocholesterolemic, and anti-inflammatory properties. These curcuminoids serve as pivotal antioxidative elements within turmeric (Cousins *et al*., 2007). Supplementing the diet with 100 mg curcumin/kg resulted in improved final body weight, reduced mitochondrial malondialdehyde concentration, and lowered reactive oxygen species production by activating glutathione peroxidase, superoxide dismutase, and glutathione S-transferase. Additionally, it enhanced the gene expression of peroxiredoxin-3 and thioredoxin-2 in broiler chickens under heat stress conditions (Zhang *et al*., 2018). In layers exposed to heat stress conditions, a diet enriched with curcumin led to notable enhancements in the activities of glutathione peroxidase (GSH-Px) and catalase (CAT), increasing by 49% and 31%, respectively (Liu *et al.*, 2020). Supplementing *Curcuma longa* improved the resilience of broiler chickens to heat stress, while a dosage of 8 g/kg enhanced nutrient absorption and juvenile growth by improving intestinal morphology in a hot and humid tropical environment (Kpomasse *et al*., 2023). Studies have demonstrated that administering phytobiotics containing lycopene, resveratrol, and epigallocatechin gallate also has a beneficial impact on mitigating heat stress in chickens (Wasti *et al.*, 2020). Adding Sweet wormwood (Artemisia annua), Hot red pepper (Capsicum frutescens), Moringa (Moringa oleifera), Thyme (Thymus vulgaris), Coriander (Coriandrum sativum) seeds, and Cinnamon (Cinnamomum zeylanicum) to the diet also demonstrates heat stress-alleviating effects (Oni *et al*., 2023).

CONCLUSION

As global temperatures continue to rise, heat stress poses a significant challenge to the poultry industry's growth. Various strategies have been implemented and evaluated to mitigate heat stress in poultry. Phytobiotic feed additives offer beneficial effects in reducing the impact of stress on birds by acting as a natural and potent source of antioxidants for avian species when faced with thermal challenges.

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