CHAPTER II

LITERATURE REVIEW

2.1 The impact of heat stress on the gut health of chickens

It is well-established that an increase in the energy requirements for maintenance leads to heat stress (HS) in poultry (Johnson et al., 2018). As a metabolically active organ, intestinal tissue, particularly the jejunum, is highly susceptible to the effects of HS (Calik et al., 2022). High temperatures can induce oxidative stress and cause a cellular imbalance between the production of reactive oxygen species (ROS) and antioxidant protective systems, thus further stimulating ROS production, which is responsible for various types of oxidative damage, such as lipids and protein oxidation, and ultimately contributes to tissue damage (Hidayat et al., 2023). Excessive production of ROS can compromise intestinal barrier function, induce aberrant immune responses, and increase intestinal permeability (Varasteh et al., 2015). These alterations facilitate the translocation of antigens, toxins, and pathogens across the tight junction (TJ) barrier, subsequently activating the immune system through Toll-like receptor (TLR) signaling, cytokines, and heat shock proteins (HSPs) (Wallin et al., 2002; Turner, 2009). This cascade of events ultimately leads to modifications in the intestinal mucosal microstructure, triggering inflammation and tissue damage. HS can significantly impair microbiota and metabolites in the intestinal of poultry, disrupt the dynamic balance between beneficial and pathogenic bacteria, and result in dysbiosis of the intestinal flora (Wang et al., 2022). In addition, HS impairs nutrient transport and digestion by decreasing enzymatic activity in the digesta, reducing the absorptive surface area, and modulating the expression of related nutrient transporters, proteins, and genes (Patra & Kar, 2021). It seems that many physiological alterations in immunity, barrier function, and nutrient transport within the intestines appear to result from heat stress-induced imbalances in the gut microbiome, which may subsequently provoke a systemic response, and thus impair intestinal health.

2.2 Heat stress and antioxidant defense system

Under HS, the oxidative and antioxidant systems in poultry lose their dynamic balance, leading to the overproduction of ROS. This imbalance results in inflammatory neutrophil infiltration and increased protease secretion (Habashy et al., 2017). Active oxygen refers to a group of free radicals that exist independently and contain unpaired electrons. These include superoxide anion ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) (Goncalves et al., 2020). The production of ROS occurs in specific parts of the mitochondrial electron transport chain, mainly in complexes I and III (Quijano et al., 2016). The main way of generating $O_2^{\bullet -}$ in the electron transport chain is auto radiation caused by the reaction between the reduced flavin protein and oxygen, under the condition of HS, the related enzymes in the complex undergo thermal denaturation, and the auto radiation rate of flavin increases in a temperature-dependent manner, increasing ROS generation (Messner & Imlay, 1999). In addition, high temperature down-regulated the expression of uncoupling proteins in the inner membrane of poultry mitochondria, hampering mild uncoupling in heat-stressed birds, which is a major contributor to the overproduction of ROS (Kikusato & Toyomizu, 2013). Under HS conditions, the overproduction of transitional metal ions will also increase the production of the Fenton reaction [$H_2O_2 + Fe^{2+} \longrightarrow OH^{\bullet +}\ (OH)^{-} + Fe^{3+}$], leading to increased ROS production. There are mainly antioxidant enzyme systems in animals, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). SOD can convert high-activity $O_2^{\bullet -}$ to low-activity H_2O_2; CAT can reduce H_2O_2 to H_2O (Nagami et al., 2005). GPx, with selenocysteine as its active center, reduces H_2O_2 and various organic hydroperoxides (Liochev & Fridovich, 1999) (Figure 2.1).

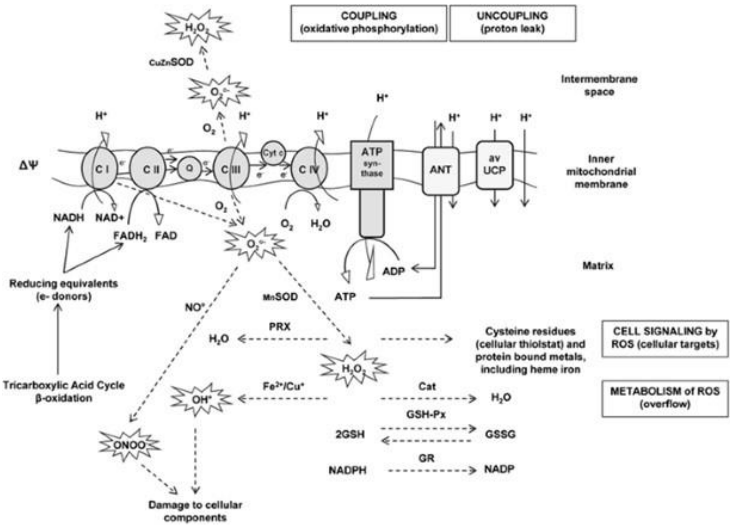

Figure 2.1 Mitochondrial energy transduction and pathophysiology of oxidative stress upon heat stress (Akbarian et al., 2016).

2.3 Heat stress on antioxidant status, barrier integrity, morphology, immunity, and production performance of chickens

HS has emerged as an important issue in the poultry production industry (Abdelnour et al., 2019). HS can be categorized into acute and chronic HS, depending on its duration and severity (Saeed et al., 2019). HS can adversely affect production performance, reproductive performance, gut health, economic traits, and poultry welfare (Tajima et al., 2007; Lara & Rostagno, 2013). In addition, HS induces a variety of negative effects on intestinal morphology, immunity, barrier integrity, digestive enzyme secretion, antioxidant status, and HSP expression in chickens, as summarized in Table 2.1.

Table 2.1 Effects of heat stress on antioxidant status, barrier integrity, morphology, immunity, and production performance in the gut of chickens.

Stains	**TN vs. HS conditions with RH**	**Results**	**Reference**
Broiler	TN (21°C) vs. HS (32°C) with 64% RH	HS decreased BW, ADG and increased FCR HS decreased villus length, villus surface area, and epithelium cell area.	(Al-Fataftah & Abdelqader, 2014)
Broilers	TN (20°C) vs. HS (30°C) with 70% RH	HS decreased BW, ADG and increased FCR Increased lipopolysaccharide, corticosterone, TNF-α, and IL2 in blood.	(Alhenaky et al., 2017)
Broilers	TN (23°C) vs. HS (30°C) with 67% RH	HS decreased crypt depth but did not affect villus height.	(Burkholder et al., 2008)
Wenchang chickens	TN (25.7°C, 88% RH) vs. HS (40.5°C, 52.4% RH)	HS reduced in villus length, mucosa thickness, intestinal wall thickness, and crypt depth in all three segments. Mucosal epithelia were detached with ruptured.	(Patra & Kar, 2021)
Broilers	TN (22°C) vs. HS (32°C) with (50 ± 10%) RH	HS decreased villus height the VH-to-crypt depth (CD) ratio (VCR), and antioxidant enzymes activity, while MDA content increased. HS reduced *Parabacteroides*, *Saccharimonas*, *Romboutsia*, and *Weissella* abundance.	(Liu et al., 2020)

Table 2.1 (continued).

Stains	**TN vs. HS conditions with RH**	**Results**	**Reference**
Cobb chicks	TN (24°C) vs. HS (33°C) with (50 ± 5%) RH	HS decreased the final BW, downregulated the expression of *GPX3*, *IL4*, and *CLDN2,* and decreased *Ruminococcus*, *Ocillospira*, and *Lactobacillus* abundance.	(Chaudhary et al., 2023)
Indigenous broilers	TN (21°C) vs. HS (32°C) with (55–70%) RH	HS decreased the BWG. HS had lower T-AOC, GSH-Px and SOD in serum and jejunum. HS downregulated *Occludin, Claudin-1, Claudin-4, ZO-1, Mucin-2* in the jejunum.	(Liu et al., 2022)
Broilers	TN (21°C) vs. HS (31°C) with (70%) RH	HS increased *Proteobacteria* abundance and decreased *Firmicutes* abundance.	(Liu et al., 2023)
Broilers	TN (24-26°C) vs. HS (334-38°C)	HS increased concentrations of HSP70 and cortisol. HS increased *Firmicutes*, *Tenericutes*, and *Proteobacteria* and decreased *Bacteroidetes* and *Cyanobacteria* abundance.	(Shi et al., 2019)
Broilers	TN (20°C) vs. HS (32°C)	HS decreased ADG, ADFI, and FCR. HS reduced villi height and VCR in the jejunum and ileum. HS reduced mRNA levels of jejunal *MUC2* and *OCLN*, and ileal *MUC2*, *ZO1*, *OCLN*, and *CLDN3.*	(Gu et al., 2012)

Table 2.1 (continued).

Stains	**TN vs. HS conditions with RH**	**Results**	**Reference**
Broilers	TN (20°C) vs. HS (27.8°C) with 53.0% RH	HS decreased BW, ADG, ADFI, and feed efficiency. HS did not affect jejunal gene expressions of *OCLN*, *ZO1*, *CLDN1*, and *JAM2*.	(Koch et al., 2019)
Broilers	TN (23°C) vs. cyclic HS (28°C–35°C–28°C)	HS decreased ADFI, ADG, and FCR. HS reduced villus height and VCR in the jejunum. HS increased serum TNFα, IL6, and IL1β levels, but decreased anti-inflammatory cytokine IL10 levels.	(Patra, 2018)
Broilers	TN (26°C) vs. HS (34°C)	HS decreased FI and BW. HS decreased gene expressions of *CLDN3* and *OCLN* but not *CLDN1*. HS increased gene expressions of *HSPA1A*, *HSPD1*, and *HSPB1*.	(Jimoh et al., 2018)
Broilers	TN (22°C) vs. HS (38°C)	HS increased *HSF-3*, *HSP70*, *HSP90*, *CLDN*, *CLDN5*, *ZO1*, *TLR-4*, *IL6*, *IL8*, and *HSP70* mRNA expression in jejunum.	(Varasteh et al., 2015)
Layers	TN (21°C, RH 62%) vs. HS (35°C, 64% RH)	HS decreased duodenal, jejunal, and ileal villus height, crypt depth, and absorptive epithelial cell area.	(Abdelqader et al., 2017)

Table 2.1 (continued).

Stains	TN vs. HS conditions with RH	Results	Reference
White Leghorn hens	TN (20°C–22°C, 50%–60% RH) vs. HS (30°C–33°C, 70%–80% RH)	HS decreased egg weight, eggshell thickness, percentage, and density. HS decreased calcium-binding protein (calbindin) in the ileum, cecum, and colon.	(Shakeri et al., 2019)
Commercial laying hens	TN (26°C) vs. HS (33°C), with 60%–70%	HS reduced egg production rate, feed intake, and egg weight while increasing the feed-to-egg ratio, broken egg ratio, and mortality—down-regulated expression levels of *OCLN*, *ZO1*, and *JAM-A* in ileum and cecum.	(Tajima et al., 2007)

TN, thermoneutral; HS, heat stress; RH, relative humidity; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio; TNF-α, tumor necrosis factor α; IL, interleukin; VCR, villus height to crypt depth ratio; ADFI, average daily feed intake; MDA, malondialdehyde; SOD, superoxide dismutase; CLDN, claudins; MUC, mucin; OCLN, occludin; ZO1, zonula occludens 1; HSP, heat shock protein; SGLT1, sodium-dependent glucose cotransporter 1; GPx, glutathione peroxidase; T-AOC, total antioxidant capacity; HSPA1A, heat shock protein family A (HSP70) member 1A; HSPD1, heat shock protein family D (HSP60) member 1, HSPB1, heat shock protein family B (small) member 1; HSF, heat shock factor; GLUT, facilitative glucose transporter; VH, villus height.

2.3.1 Heat stress on oxidative stress in the gut

HS typically induces oxidative stress. SOD, CAT, and GPx represent the primary components of the antioxidant defense system, playing a critical role in maintaining oxidative stability within the host. They work synergistically to eliminate superoxide anions and hydrogen peroxide within cells (Gu et al., 2012). HS resulted in a significant decrease in various oxidative stress markers, such as GPx, SOD, and CAT, total antioxidant capacity, and nuclear factor erythroid 2-related factor 2 (Nrf2), while increasing Kelch-like ECH-associated protein 1 (Keap1) transcripts and malondialdehyde (MDA) levels in broilers (Hidayat et al., 2023; Algothmi et al., 2024). Previous studies have found that chronic HS (20°C vs. 32°C–33°C 8 h/day) leads to increased MDA content in the jejunal mucosa and decreased SOD levels in the ileal mucosa of chickens (Gu et al., 2012). Similarly, after 2 weeks of cyclic HS (22°C, 24 h/d vs. 32°C, 10 h/d), chickens showed increased MDA content and decreased GPx levels in the ileum and jejunum (Roushdy et al., 2018). The Nrf2-mediated antioxidant response pathway plays a crucial role in maintaining cellular redox balance by promoting the transcription of various cytoprotective genes (Liu et al., 2016). Moreover, Du et al. (2022) reported that HS resulted in reduced SOD levels in jejunal mucosa and increased MDA content in the serum, liver, and intestinal of heat-stressed broilers. In conclusion, HS causes redox dysfunction and hemostasis imbalance by decreasing the activities of SOD, GPx, and CAT, as well as the total antioxidant capacity in jejunal and ileal tissues and serum, while simultaneously elevating the levels of oxidative markers such as MDA and H_2O_2 levels.

2.3.2 Heat stress on immunity in the gut

HS can induce the release of proinflammatory cytokines and mediators (such as nuclear factor **K**B [NF-kB], Toll-like receptor [TLR], and interleukin [**IL**]) in broilers, leading to decreased growth rate and weakened immune system in most broilers. In general, pro-inflammatory mediators contribute to inflammatory damage, while anti-inflammatory mediators help alleviate inflammation and facilitate the healing process in response to environmental triggers (Bamias et al., 2014). Studies have shown that HS (38°C - 39°C, 8 hours per day, for 5 consecutive days) resulted in upregulating of *TLR4*, *IL6*, and *IL8* levels in the jejunum of chickens (Varasteh et al., 2015). The transcription factor NF-**K**B plays a crucial role in the regulation of genes activated by inflammatory cytokines, pathogens, and oxidative stress (Sanz Fernandez et al., 2014). HS leads to increased levels of proinflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor-alpha [TNF-α]) by affecting the redox-sensitive downstream pathways of NF-κB (Fang et al., 2023). In addition, IL-10 is a key anti-inflammatory mediator that plays a pivotal role in regulating the inflammatory response. Several studies have highlighted IL-10 as one of the most important cytokines involved in various pathophysiological conditions, where it inhibits the production of pro-inflammatory mediators (Hidayat et al., 2023). TNF-α is a proinflammatory mediator that is widely used in animal models (Abdelnour et al., 2019). In addition, TNF-α is also an important mediator of early liver injury (Hoek & Pastorino, 2002). However, it is well known that HS is an important environmental factor leading to liver injury. Therefore, elevated TNF-α levels in the liver or serum may lead to liver dysfunction (Li et al., 2023). Notably, IL-6 exhibits both pro- and anti-inflammatory properties, influencing both metabolic and inflammatory pathways (Su et al., 2013). Additionally, IL-6 impacts the tight junctions in the gastrointestinal tract, while TNF-α is known to increase intestinal permeability (Su et al., 2013). Furthermore, HS can trigger inflammation through the release of inflammatory markers, including IL-2, TNF-α, and IL-4 (He et al., 2019). TLRs, particularly TLR4, serve as key biosensors for stress. TLR4 activation can stimulate NF-κB, a central nuclear transcription factor in inflammatory and immune responses, influencing the expression of various inflammatory markers such as IL-6, TNF-α, and IL-1β (He et al., 2019). Previous studies have shown that HS increases the expression of both NF-κB and TLR4 (Cheng et al., 2019). Therefore, the activation of inflammatory signaling pathways may serve as a key factor in the disruption of innate immunity and the initiation of the inflammatory response during HS.

2.3.3 Heat stress on barrier integrity in the gut

HS-induced hypoxia impairs the balance of antioxidants and immune responses, resulting in epithelial damage and compromised barrier integrity (Lian et al., 2020). The preservation of intestinal mucosal integrity is crucial for optimal nutrient absorption and preventing the paracellular passage of harmful antigens. Tight junction (TJ) proteins, which seal the spaces between adjacent epithelial cells, are particularly vulnerable to the effects of heat stress (Varasteh et al., 2015). The junctional transmembrane proteins, occludins (OCLN), claudins (CLDN), junctional adhesion molecules (JAMs), and tricellular interact with the intracellular scaffolding protein zona occludens (ZO), which is anchored to the actin cytoskeleton (Lee, 2015). However, oxidative damage caused by heat stress impairs digestion and absorption of nutrients in poultry (Mishra & Jha, 2019). Previous research has shown that HS significantly increases the expression of E-cadherin, claudin-1, claudin-5, and ZO-1 proteins in the small intestine of broilers. Furthermore, HS-induced upregulation of HSF-1 enhances occludin expression by binding to its promoter, thereby promoting its involvement in the junctional complex (Dokladny et al., 2008). Consequently, the elevated levels of HSPs may be linked to the increased mRNA levels of TJ proteins under HS conditions (Lian et al., 2020). In addition, HS compromises the gut barrier integrity, leading to increased intestinal permeability, which can trigger chronic systemic inflammation and reduce disease resistance in broilers (Zhang et al., 2022; Saracila et al., 2023). Also, studies have shown that HS can reduce the number of goblet cells in the intestinal mucosa of chickens (Yu et al., 2010) and mucin 2 mRNA levels (Pearce et al., 2014). The above studies indicate that HS induces intestinal dysfunction by changing TJ proteins, leading to increased pathogen invasion, increased susceptibility to the mucosa, and reduced nutrient absorption, thus causing growth retardation in broilers.

2.3.4 Heat stress on heat shock protein in the gut

HSPs are well-known stress response proteins and molecular chaperones. They safeguard cells by aiding in protein folding, repair, localization, and degradation. HSPs facilitate the production of proteins involved in highly conserved cellular response mechanisms under stress conditions (Zilaee et al., 2014). The most prevalent HSPs induced by HS are *HSP70* and *HSP90*, which play crucial roles in cell protection. Among HSPs, the *HSP70* family is the most conserved and abundant protein in organisms (Milarski & Morimoto, 1989). Under HS conditions, *HSP70* is activated to eliminate denatured or misfolded proteins within cells, thereby enhancing cell viability and increasing resistance to heat stress (Bhat et al., 2016). *HSP70* has been detected in the pectoral muscle, liver, heart, and lungs, with its concentration in the brain being 2 to 5 times higher than in other embryonic tissues. This suggests that the elevated expression of *HSP70* in various embryonic tissues serves as an adaptive mechanism to mitigate stress conditions (Leandro et al., 2004). HSP expression serves as a potent antioxidant defense mechanism in the chicken intestine (Surai et al., 2019). However, under HS conditions, the overexpression of *HSP70* did not influence intestinal morphology. Notably, a significant correlation was observed between *HSP70* expression and digestive enzyme activity in hens (Hao et al., 2012). In addition, Studies have found that inducing *HSP70* expression can protect the intestinal mucosa from HS damage by increasing the activity of antioxidant enzymes, preventing lipid peroxidation, and enhancing the antioxidant capacity of broilers (Gu et al., 2012). The *HSP90* family, a crucial group of chaperones downstream of *HSP70*, aids in the final structural maturation and conformational changes of proteins, thereby preserving homeostasis and cellular integrity under heat stress conditions (Dangi et al., 2014). It has been reported that HS increased HSP90 levels, which helps correct the folding, stability, and function of other proteins (Hong et al., 2013). In addition, HSP90 is involved in various cellular processes such as cell survival, cell cycle regulation, and other signal transduction pathways (Jackson, 2012). Calik et al. (2022) found that HS significantly upregulated *HSP70*, *HSP90* levels in the jejunum of broilers. To sum up, the findings of the above studies, the enhanced expression of *HSP70* and *HSP90* is one of the important defense responses to avoid or cope with the adverse changes in protein function and structure caused by different stresses, effectively inhibiting lipid peroxidation, improving antioxidant capacity, and playing an important role in protecting the integrity of the intestinal mucosa from HS damage, thereby contributing to cell function under stress conditions. However, more research is needed to better understand the molecular mechanisms of *HSP70* and *HSP90* regulation in avian organisms.

2.3.5 Heat stress on microbiota in the gut

The gut microbiota plays a crucial role in providing nutrients from the diet and regulating both the digestive and immune systems; therefore, maintaining a healthy gastrointestinal microbiome is essential for animals (Obianwuna et al., 2024). Studies have shown that heat stress affects the structure of the intestinal flora, which may be a key factor in the health of host animals. The distribution of intestinal flora can also affect the host's stress response (Chen et al., 2021). HS altered the profile of the cecal microbiome, with increased abundances of *Firmicutes* and *Tyzzerella*, and decreased abundances of *Bacteroidetes* and the genera *Bacteroides*, *Parabacteroides*, and *Romboutsia* (Liu et al., 2020). Wang et al. (2018) reported that HS (31 ± 1 °C) significantly increased the α diversity (observed species, PD of the whole tree, and Chao 1) of the ileal microbiota of 42-day-old broilers. In addition, HS induces oxidative stress and produces excessive ROS, leading to an imbalance between endogenous antioxidant defense mechanisms. Excessive ROS production can lead to the invasion of facultative anaerobic bacteria and cause membrane permeability damage (Tomasello et al., 2016). Fang et al. (2023) demonstrated that HS led to a reduction in the relative abundance of *Firmicutes*, while the abundance of *Bacteroidetes* also decreased. These findings indicate that heat stress disrupts the homeostatic balance of the intestinal microbiota. Dysbiosis refers to an imbalance in the intestinal microbiota, characterized by an overgrowth of harmful microorganisms or a reduction in beneficial bacteria, which disrupts the delicate equilibrium between the host and its gastrointestinal microbiota (Walker, 2017; Ducatelle et al., 2018). It is commonly linked to nutrient maldigestion, compromised intestinal barrier function, and gastrointestinal inflammation (Chen et al., 2015). Despite the availability of advanced analytical methods for studying the gastrointestinal microbiota (Borda-Molina et al., 2018), the alterations in the structure, composition, and function of the microbiota in heat-stressed chickens are not yet fully understood (He et al., 2021; Liu et al., 2022).

2.4 Effect of dietary vitamin C, vitamin E, selenium, L-carnitine, and their combined supplementation in poultry under heat stress conditions

With the advancement of modern farming, the requirements for equipment and management technology are getting higher and higher, such as chicken houses, ventilation, cooling systems, and management technology have been greatly improved, which has a positive effect on poultry HS management. However, the high cost of this method and the rapid update rate of equipment make this technology not always feasible. Therefore, it is necessary to start from a nutritional strategy and consider inexpensive and nutritious antioxidant additives to alleviate HS in poultry. Therefore, during the HS, it is urgent to find efficient and feasible methods to enhance the technical effect, to alleviate or reduce the heat stress of poultry. At present, most studies have shown that different nutritional strategies can alleviate the negative effects of HS, thereby improving the breeding efficiency and production performance of poultry in high-temperature environments, such as supplementing the diet with plant-derived antioxidants (polyphenols, EGCG) minerals (selenium, zinc), vitamins (vitamin C, vitamin E), electrolytes, phytobiotics, probiotics, fats and amino acids can effectively relieve HS (Kumar et al., 2021; Calik et al., 2022).

2.4.1 Vitamin C

Vitamin C, also known as L-ascorbic acid, is a water-soluble antioxidant compound that is usually synthesized in the liver and kidneys of poultry, but environmental stressors can affect its synthesis and utilization, supplementing vitamin C in poultry diets can help enhance immunity, which can scavenge ROS to prevent cellular damage caused by oxidative stress. Vitamin C may act as a co-antioxidant with other antioxidants by synergistic effects, to relieve HS. The antioxidant activity of vitamin E increased in the presence of vitamin C through reducing tocopheroxy radicals back to their active form of vitamin E (Calik et al., 2022). A number of studies have found that vitamin C supplementation of 150-500 mg/kg in broiler diets under high temperature conditions can effectively improve various production factors and growth performance, and reduce the expression of *HSP70* (Farooqi et al., 2005; Attia et al., 2011; Kumar et al., 2017). Under HS conditions, the poultry's own endogenous synthesis of vitamin C is hindered, and the demand for vitamin C will increase at this time (Ghazi Harsini et al., 2012). Furthermore, the authors found that vitamin C supplementation in the diets of heat-stressed layer hens improved laying performance and egg quality, significantly increased the length, width and diameter of intestinal crypts, and protected intestinal epithelial cells from HS caused oxidative damage (Ajakaiye et al., 2011). This indicates that higher concentrations of vitamin C in broiler diets can effectively combat HS.

2.4.2 Vitamin E

Vitamin E contains four tocopherols (α-、β-、γ- and δ-), of which α-tocopherol is biologically active and able to meet the needs of animals. Vitamin E is a group of compounds containing both tocopherols and tocotrienols, which are the first line of defense against lipid peroxidation caused by HS in poultry, it is a biological antioxidant and free radical scavenger (Ajakaiye et al., 2011). Dietary supplementation with vitamin E at various levels (150–500 mg/kg) improved poultry performance (Ghazi Harsini et al., 2012), and reduced the negative effects of HS (Maini et al., 2007). Additionally, vitamin E increased the secretion of growth hormone, which may be responsible for improved growth performance (Khan et al., 2013). Habibian et al. (2014) reported that vitamin E supplementation increased the production of antibodies against different diseases in heat-stressed poultry, which improved their immunity. The literature showed that supplementation with vitamin E at an average concentration of 250 mg/kg is a viable protective measure to reduce HS symptoms and the best possible broiler performance (Maini et al., 2007). Supplementation with 200 mg/kg vitamin E to grain could significantly increase the activity of SOD, CAT and GSH, and reduce the concentration of MDA in blood and liver of heat-stressed broilers (Calik et al., 2022). Vitamin E supplementation had positive effects on laying hens' egg production and egg quality (Ajakaiye et al., 2011). Sahin et al. (2006) found that supplementing 250mg/kg of vitamins in heat-stressed quail diets significantly improved growth performance, increased blood vitamins E and A concentrations, and decreased MDA concentrations.

2.4.3 Selenium

In recent years, the role of selenium (Se) in poultry nutrition has been studied more and more, especially the supplementation of selenium to reduce or alleviate the HS of poultry, because the main form of glutathione peroxidase is selenium-dependent. (Calik et al., 2022). In addition, Se can act as a cofactor of glutathione peroxidase and superoxide dismutase, play a major role in the antioxidant system, and can scavenge free radicals to protect poultry from HS damage (Shakeri et al., 2020). When dietary Se is deficient, poultry exhibit poor appetite and inefficient utilization, which negatively affects growth performance (Wasti et al., 2020). Studies have shown that supplementation of Se can increase the feed intake and body weight of heat-stressed broilers and reduce the feed conversion ratio (Niu et al., 2009; Ghazi Harsini et al., 2012). In addition, supplementation with 0.2-1 mg/kg Se in poultry diets can improve heat stress-induced antioxidant status (Khoi et al., 2021). Supplementation with Se (0.3-0.5 mg/kg) in heat-stressed broiler diets increased the activities of SOD and GPx and decreased MDA in serum (Xu et al., 2014). Sahin et al. (2008) found that supplementation with 0.3 mg/kg Se in heat-stressed quail diets improved growth performance and decreased serum and liver MDA concentrations. A study showed that supplementation with 0.3 mg/kg Se in diets of broiler chickens under HS increased the levels of TNF-α, IL-4, TNF-γ and IL-2 (Habibian et al., 2015). Moreover, nicotinamide adenine dinucleotide phosphate (NADPH) levels were elevated due to Se supplementation, which further promotes glutathione reductase activation, resulting in increased GSH-Px production (Zhao et al., 2018).

2.4.4 L-carnitine

L-carnitine is a water-soluble product found in animals, plants, and microorganisms. It is synthesized from two important amino acids, lysine and methionine. In essence, L-carnitine plays an intermediary role in metabolism, promotes cell energy metabolism, regulates the concentration of coenzyme A in cytoplasm and mitochondria, and plays an important role in glycolipid metabolism. In addition, L-carnitine reduces liver toxicity and enhances antioxidant capacity (Abu-El-Zahab et al., 2019). Dietary supplementation with 100-160 mg/kg L-carnitine can improve heat-stressed broiler feed intake and feed conversion ratio, and reduce serum triglyceride and cholesterol levels (Kuter & Onol, 2021; Qiao et al., 2021). In addition, Supplementation with 100 mg/kg L-carnitine reduced MDA in heart tissue of broilers raised under low temperature environment, and increased SOD and GSH-Px activities (Wang et al., 2013). In addition, supplementation with 50 mg/kg L-carnitine in the diet increased sheep red blood cell (SRBC) antibody titers in broiler chickens under HS (Rehman et al., 2017). Moreover, Yousefi et al. (2023) showed that dietary supplementation with 0.5g/kg L-carnitine increased average daily gain, reduced the concentration of MDA in serum and the depth of jejunal crypts in heat-stressed broilers.

2.4.5 The combination of selenium with vitamin E, or vitamin C

High temperature will affect the absorption of vitamins A, C, and E, and reduce the concentration of iron, zinc, and Se in tissues and blood; therefore, vitamins and trace elements should be supplemented in the diet at this time to maintain the normal requirements of the poultry body. However, based on the available literature, there is currently a limitation on the simultaneous supplementation of vitamins E, C, Se, and carnitine to alleviate HS in poultry. However, the combination of selenium with vitamin E, or with vitamin C, has been shown to have a positive facilitative effect in reducing the negative effects of heat stress in poultry. Studies have found that supplementing selenium and vitamin E (0.5mg/kg+250mg/kg) or (0.5mg/kg+150mg/kg) in heat-stressed broiler diets improved antibody responses to some diseases and improved performance production (FI, weight gain, and FCR) and antioxidant capacity (Ghazi Harsini et al., 2012; Habibian et al., 2014). In addition, Ajakaiye et al. (2011) reported that supplementation with 150 mg Vit C + 150 mg Vit E in heat-stressed layer diets significantly improved laying performance and egg quality. Supplementation with vitamin C (200 mg/kg diet) and vitamin E (100 mg/kg diet) to broiler chickens diets increased total antioxidant capacity, SOD, and GPx enzyme activity under HS (Hosseini-Mansoub et al., 2010). Dietary supplementation with 15000IU Vit A + 30 mg Se increased feed intake and body weight and reduced feed conversion ratio and lipid peroxidation in heat-stressed broilers (Kucuk et al., 2003). In addition, Calik et al. (2022) found that supplantation with combination vitamins E (250 mg/kg) and Se (1 mg/kg) significantly downregulated the mRNA levels of HSPs in liver and jejunal tissues of the HS-challenged birds both on d 28 and d 35, while mRNA abundance of TLR2, TNFα, IFNγ, IL-1β, IL-10, and iNOS in the liver was significantly downregulated in birds fed the vitamin E, Se diet on d 35. Moreover, *Lachnospiraceae FE2018* and *Ruminococcaceae NK4A214* groups were enriched in the vitamin E, Se birds on day 35. The combination of vitamins E, C, and Se effectively reduces the impact of heat stress on poultry, and the effect is better when these additives are used alone.

2.5 Mechanism of action of phytogenic compounds to mitigate oxidative stress and heat stress

Phytogenic compounds can act in several ways to scavenge ROS. For example, Sandoval-Acuña et al. (2014) defined two kinds of ROS-scavenging activities: direct activities to scavenge ROS or indirect activities by inducing the synthesis of ROS-removing enzymes (e. g, SOD, CAT). Figure 2.2 schematically shows these activities. Polyphenols also have the ability to directly chelate transition metal ions, especially Fe^{2+} and Cu^{2+}, which can generate highly reactive oxygen radicals (Karamać, 2009).

It is generally known that HS can induce free radicals in the animal body, leading to oxidative stress and reduced production performance (Chauhan et al., 2021). The antioxidant properties of phytogenic additives can be attributed to their chemical structure, including the presence of hydroxyl groups attached to the benzene ring, which are good hydrogen donors (Saracila et al., 2021). In the case of polyphenols and flavonoids, it was reported that the B ring hydroxyl structure has a major role in the activity of free radical scavenging (Salehi et al., 2020). Polyphenols participate in the elimination of numerous ROS and RNS, such as hydroxyl radicals, peroxyl radicals, hypochlorous acids, superoxide anions, and proximities (Halliwell, 2006) by transferring the H atom from the

OH group (polyphenols) or a single electron to the free radical or a transition metal ion (Sandoval-Acuña et al., 2014; Papuc et al., 2017) as shown in Figure 2.3.

Phytogenic additives are rich in catechins, polyphenols, flavonoids and volatile oils, gallic acid, tannins, and flavonoids; this has been shown to alleviate the negative effects of HS (Chauhan et al., 2021). Most bioactive polyphenol compounds have been assessed partly through biological properties and bioavailability, especially strong radical scavenging activities for their antioxidant ability which is dependent on the quantity and quality of polyphenol compounds in each plant species that act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin (Lee et al., 2016). Polyphenols elevate the expression of stress response proteins such as heat shock proteins and antioxidant enzymes, which can suppress ROS and interfere with many players of HS responses (Yin et al., 2021). Phytogenic compounds enhance the expression of antioxidant enzymes mainly by activating nuclear factor erythroid 2-related factor 2 (Nrf2) (Saracila et al., 2023). Normally, Nrf2 is combined with kelch-like epichlorohydrin-related protein 1 (Keap1) in the cytoplasm, where it is inactive at this time; however, after cells are treated with phytogenic additives, Nrf2 will separate from Keap1 and translocate to the nucleus, it plays a role in up-regulating the gene expression of antioxidant defense enzyme 1 (NQO1) and heme oxygenase 1 (HO1), thereby enhancing the expression of antioxidant enzymes, such as SOD, CAT, GSH-Px, GR, and GST, etc (Kurutas, 2015) (Figure 2.2 and 2.3). Firstly, SOD can scavenge $O_2^{\bullet-}$ free radicals, and secondly, in the presence of CAT, H_2O_2 can rapidly decompose to produce active oxygen such as $O_2^{\bullet-}$ and $HO^{\bullet}$, which scavenging free radicals; thirdly, glutathione peroxidase (the sulfhydryl group (-SH) in GSH-Px) provides reduced hydrogen, gives free radicals a paired electron, and makes free radicals lose their strong oxidative and erosive properties (Calik et al., 2022). Glutathione itself becomes the oxidation state of this disulfide bond, and then, through the reduced hydrogen, is converted into reduced glutathione, thus continuously circulating and continuing to exert its antioxidant effect (Georgiou-Siafis & Tsiftsoglou, 2023). On the other hand, due to the special molecular structure of the main active species, Fe^{2+} can be released by increasing the Fenton reaction to generate more $OH^{\bullet}$ and H_2O_2 (Zhou et al., 2021). In addition, since the main active substances of phytogenic are polyphenols, polyphenols (Phen-OH) react with free radicals ($R^{\bullet}$) to generate active oxygen, including superoxide ions ($O_2^{\bullet-}$) and hydroxyl radicals ($OH^{\bullet -}$), thus scavenging free radicals (Guo et al., 1999; Michalak, 2006) (Figure 2.3). HSP (HSP70) reduces the release of oxygen free radicals and increases the activity of SOD, thereby scavenging free radicals (Figure 2.2).

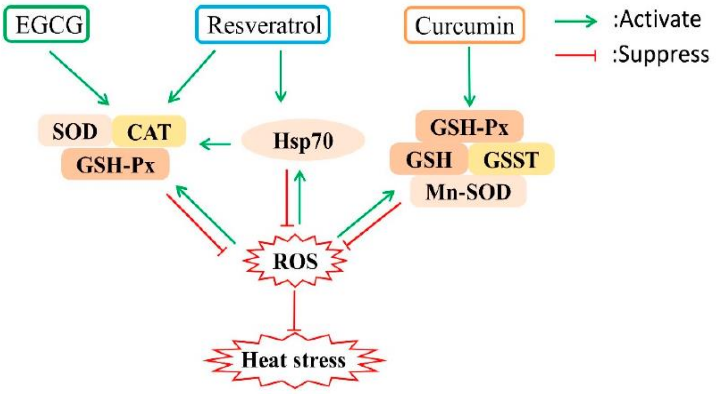

Figure 2.2 Potential mechanisms underlying the protective effect of polyphenols against heat stress (Saracila et al., 2021).

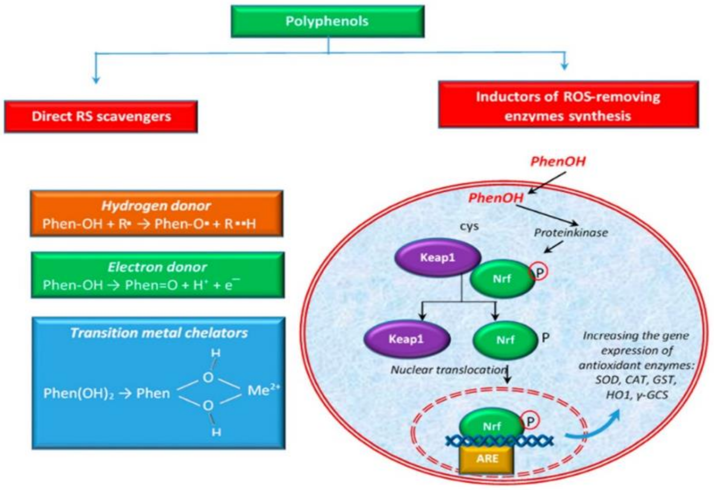

Figure 2.3 Role of polyphenols in scavenging reactive oxygen species and stimulating antioxidant enzymes (Saracila et al., 2021).

2.6 Effects of phytogenic supplementation to mitigate the negative effects of heat stress in broilers.

HS increased intestinal inflammation, oxidative stress, and reduced antioxidant and immune markers in broilers. Phytogenic antioxidants in the diet help alleviate stress in broilers. Under stress, phytogenic antioxidants contain bioactive components that can improve immunity, antioxidant capacity, enhance gut microbiota and health, and reduce oxidative/inflammatory pathways (Yang et al., 2021; Reith et al., 2022), making animals more resistant to external stress (Al-Garadi et al., 2023). Previous studies found that most phytogenic antioxidant substances are mostly supplemented in both broiler and laying hen diets under HS and improved the total antioxidant capacity (T-AOC), GSH, GSH-PX, SOD and CAT, while lipid peroxidation, MDA and nitric oxide (NO) reduced in blood and tissues (El-Maaty et al., 2014; Hosseini-Vashan et al., 2016; Ibtisham et al., 2019; Reis et al., 2019). Studies have shown that the supplementation with *Artemisia annua* (0.75-1.25 g/kg) significantly increased body weight, reduce oxidative stress biomarkers (MDA, corticosterone), and improved liver function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) and antioxidant capacity (Wan et al., 2017). Olive oil or its leaf extract enhanced the health and redox status balance of chickens by increasing plasma SOD levels and decreasing MDA content (Lee et al., 2016). In addition, Fatima et al. (2022) explored the effect of fennel seeds (20-25 g/kg) on improving growth, antioxidant status, carcass characteristics, and immune responses in broiler chickens under HS conditions. Likewise, Khalil et al. (2020) demonstrated that supplementation with thyme essential oil (150 or 200 mg/kg) in broilers' diets resulted in enhanced growth performance, improved immune function, and favorable alterations in blood metabolites. Moreover, it contributed to a reduction in stress-related biomarkers, including corticosterone and malondialdehyde (MDA), under HS conditions. The beneficial impact of rosemary on productivity and the health of broilers under stress conditions can be ascribed to its potential to augment antioxidant activity (Hosseinzadeh et al., 2023), regulate the composition of intestinal microbiota (Liu et al., 2022), enhance intestinal morphology, boost immune function (Rostami et al., 2018), and improve plasma biochemical parameters in broilers (Torki et al., 2018). Mirzaei et al. (2023) found that fennel nanoemulsion significantly improved the antioxidant capacity and immunity of stressed broilers. At the same time, it also led to a decrease in *Escherichia coli* levels and an increase in the levels of beneficial bacteria such as *Lactobacillus*. Song et al. (2017) showed that supplementation with 1 g of *Artemisia annua* to heat-stressed broilers (34 ± 1°C) reduced plasma diamine oxidase (DAO) activity, *HSP70* mRNA expression, and *TLR-4*, *IL-6*, *IL-1*β, and *IFN-*γ expression in intestinal tissues. In the intestinal mucosa, mature epithelial cells showed higher oxidase activity. Moreover, rosemary modulates intestinal microbiota (Liu et al., 2022), improves intestinal morphology, and enhances immune activity (Rostami et al., 2018), which may be attributed to its enhanced antioxidant activity (Hosseinzadeh et al., 2023). Phytogenics have demonstrated potential in mitigating the negative effects of HS in chickens by promoting gut health, preserving microbiota balance, attenuating inflammatory and oxidative stress pathways, enhancing immune function, boosting antioxidant capacity, and improving production performance. Nonetheless, additional research is required to explore the molecular mechanisms underlying phytogenics, as well as the interactions between their active components, gut microbiota, and the gut barrier. These studies may enhance broiler welfare and foster a more sustainable and efficient poultry industry. The supplementation of phytogenic substances to broilers to mitigate the negative effects of HS is summarized in Table 2.2.

Table 2.2 Effects of phytogenic supplementation to mitigate the negative effects of heat stress in broilers.

Spices and Conditions	Phytogenic substances	Results	Bioactive Compounds and References
Broilers, TN (24°C, 50% RH), HS (36°C, 78% RH, 2 h/day) for 0 days	Basal diets (control) Basal diets (HS) PFA-C 250 ppm (encapsulated essential oils, dried herbs and spices, saponins, and anticaking agents) under HS PFA-C 400 ppm (encapsulated essential oils, dried herbs and spices, saponins, and anticaking agents) under HS	PFA 250 upregulated the expression of SOD1 and downregulated GPX-3, but was unchanged for PFA-C400. PFA 400 decreased TNFα levels. HSP and HSF were unaffected by PFA. Total antioxidant capacity, MDA content was increased by HS, but were unchanged for PFA. Expression of IL-18 is unaffected by PFA.	Phenolic compounds PFA-C exhibited a significantly higher antioxidant capacity. 8.9- and 15.5-fold increase for PFA-C250 and PFA-C400, respectively. (Greene et al., 2021).
Broilers, TN (21°C, 55% RH), HS (34°C, 55% RH, 4h/day) for 28 days	Basal diets (control) Basal diets (HS) 3% Dried tomato pomace DTP under HS 5% Dried tomato pomace DTP under HS	Enhanced GSP-PX and SOD Reduced MDA in plasma by 5% DTP	3 and 5% of DTP contains 420 and 708 mg lycopene/kg diet, respectively. (Hosseini-Vashan et al., 2015).

Table 2.2 (continued).

Spices and Conditions	Phytogenic substances	Results	Bioactive Compounds and References
Broilers, HS (32±2°C, 50±5% RH 24h/day) for 14 days	Basal diets (control) Basal diets (HS) 50 (R1) and 100 (R2) mg/kg of rosemary leaves extract (RLE) under HS 50 (O1) and 100 (O2) mg/kg of oregano leaves extract (OLE) under HS	Total lipids and LDL-cholesterol levels decreased in the R2 and O2 groups. Total antioxidant capacity increased in the R1 and R2 groups. The mRNA expression of HSP70 and HSP90A was downregulated in R1, R2, O1, and O2 groups.	Ferruginol, triterpenoid diterpenoid, sesquiterpene, eucalyptol, camphor, and monoterpenoids. (Madkour et al., 2024).
Broilers, TN (24°C, 55% RH), HS (35°C, 50-60% RH 8h/day) for 28 days	Basal diets (control) Basal diets (HS) Mixture of thymol and carvacrol (60, 100, and 200 mg/kg) under HS	The addition of this mixture improved the GPx and SOD, pancreatic enzymes (trypsin, lipase, and protease) and immune markers.	Thymol, carvacrol, resin, tannins, steroids, saponins, flavonoids, and alkaloids. (Mahasneh et al., 2024).
Broilers, TN (22°C 24 h/day), HS (33°C, 60-70% RH 10h/day) for 21 days	Basal diets (control) (TN) Basal diets (HS) Inclusion of 400 mg/kg resveratrol under HS	The HS+ resveratrol increased serum IgY, IgA, and IL-10 contents, while lowering splenic TLR4, TNF-α, IL-1β, and NF-κB mRNA levels.	Resveratrol, extracted from a variety of plants. (Li et al., 2023).

Table 2.2 (continued).

Spices and Conditions	Phytogenic substances	Results	Bioactive Compounds and References
Broilers, TN (22-28°C), HS (32-38°C, 60-80% RH)	Basal diets (control) (TN) Basal diets (HS) HS+1% Ginger powder HS+0.332% Herbal medicine HS+0.1% Ginger powder + 0.332 g/kg Herbal medicine	Improved SOD, NO, T-AOC, MDA, and GSH-PX in serum by supplemented groups Improved CAT in serum by HS + Chinese herbal medicine and ginger powder + Chinese herbal medicine	Ginger: gingerol, gingerdione, and shogaols; Chinese herbal medicine: vitamins, lipids, amino acids, proteins, trace elements. (Ibtisham et al., 2019).
Broilers, TN (22°C), HS (34°C, 50-60% RH 8h/ day) for 20 days	Basal diets (control) (TN) Basal diets (HS) HS+1 g/kg *Artemisia annua* (EA)	EA decreased the expression of HSP70, TLR4, IL-6, IL-1β, and INF-γ in the intestine, whereas it increased jejunal zonula occludens-1 and occluding.	*Artemisia annua* contains antioxidant compounds such as flavonoids and phenolics. (Song et al., 2017).
Broilers, TN (24°C), HS (34°C, 60% RH 4h/ day)	Basal diets (control) (TN) HS+ probiotics 2 g/kg (PRO) HS+ clove essential oil 300 mg/kg (CEO) HS + PRO and CEO (PC)	decrease in TNF-α in all supplemented groups compared with the control. SOD and GPx levels were elevated and the MDA level value decreased.	The probiotic mixture L*actobacillus Acidophilus* and *Bacillus Subtilis*. eugenol, gallic acid, catechin. (Elbaz et al., 2023).

Table 2.2 (continued).

Spices and Conditions	Phytogenic substances	Results	Bioactive Compounds and References
Broilers, TN (28°C), HS (35°C, 70% RH 12h/ day)	Basal diets (control) (TN) HS+Epigallocatechin-3-gallate (EGCG) 0 mg/kg HS+EGCG 300 mg/kg HS+EGCG 600 mg/kg	Supplemented with EGCG increased VH, VH/CD (V/C), and the activities of GSH-Px, SOD and CAT, and decresed the crypt depth and MDA content. Supplementation increases the gene expression of *Nrf2*, *Claudin-1*, and Mucin 2, and reduces the *NF-κB*.	Epigallocatechin-3-gallate of green tea. (Song et al., 2019).

Abbreviation: TN, thermoneutral condition; HS, heat stress condition; PFA, phytogenic feed additive; SOD1, superoxide dismutase 1; GPX-3, glutathione peroxidase-3; HSP, heat shock proteins; HSF, heat shock factors; MDA, malondialdehyde; IL-18, interleukin-18; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; NF-κB, nuclear Factor kappa B; NO, nitric oxide; T-AOC, total Antioxidant Capacity; CAT, catalase; INF-γ, interferon-γ; Nrf2, nuclear factor-erythroid 2-related factor 2.

2.7 The application of the transcriptomic technique in animal research

The study of transcriptomes (RNA-seq)-the complete set of RNA molecules transcribed in an organism-provides insight into gene expression, regulation, and cellular processes. In recent years, RNA-seq (Nagalakshmi et al., 2008), a methodology for RNA profiling based on next-generation sequencing (NGS) (Shendure & Ji, 2008), is replacing microarrays for the study of gene expression. In animal research, transcriptomics technologies have facilitated the comprehensive analysis of gene activity under different conditions, such as disease states, environmental stressors, and genetic mutations (Qian et al., 2014). This has proven instrumental in understanding complex biological phenomena that are difficult to elucidate with traditional genomic approaches. Recent advancements in transcriptomics technologies, including RNA-Seq, microarray-based techniques, and single-cell sequencing, have significantly enhanced the scope and depth of animal research. Transcriptome sequencing has been used in several species, including poultry, cattle, and pigs, to identify genes that play key roles in responses to different conditions (Coble et al., 2014; Srikanth et al., 2017). RNA-seq has become the gold standard for transcriptomic analysis due to its ability to generate highly accurate and detailed gene expression profiles. Unlike microarrays, RNA-Seq does not rely on pre-existing knowledge of the genome and can identify novel transcripts, alternative splicing events, and rare RNA species. Studies utilizing RNA-Seq in animal models have provided critical insights into the molecular mechanisms underlying various physiological processes and diseases (Wang et al., 2009). Studies have used RNA-seq to investigate gene expression profiles in response to avian influenza (AI) and Newcastle disease (ND), providing insights into the molecular mechanisms of immunity in chickens (Wang et al., 2014). By profiling immune-related genes, researchers have been able to identify genetic markers associated with resistance to these diseases. In addition, RNA-seq analysis in poultry has also been used to study muscle development and fat deposition. For instance, the differential expression of genes involved in myogenesis and adipogenesis has been studied to identify potential targets for improving meat yield and quality in broilers (Malila et al., 2022). Transcriptomic studies in dairy cattle have provided insights into the molecular basis of lactation. RNA-Seq has been employed to investigate gene expression in mammary tissue during different stages of lactation, identifying key regulatory genes involved in milk synthesis and secretion (Seo et al., 2016). This information has led to a better understanding of factors affecting milk yield and quality. Previous studies using RNA-Seq have revealed that genes such as *FLNC*, *COL1A1*, *NRAP*, *SMYD1*, *TNNI3*, *CRYAB* and *PDLIM3* played vital roles in the muscle growth, and genes such as *CCDC71L*, *LPIN1*, *CPT1A*, *UCP3*, *NR4A3* and *PDK4* played dominant roles in the lipid metabolism in Shaziling pigs (Zheng et al., 2024). Moreover, RNA-seq is also used in the animal models field, animal models are indispensable in understanding the molecular mechanisms of diseases such as cancer, neurological disorders, and metabolic diseases. For instance, RNA-Seq has been used to investigate the gene expression alterations in mouse models of Alzheimer's disease, offering new targets for therapeutic intervention (Wan et al., 2020). Similarly, transcriptomics has been used to uncover genetic factors involved in obesity and type 2 diabetes in rodent models (Agueda-Oyarzabal et al., 2025). However, one of the major challenges in transcriptomics is the sheer complexity of the data. For example, integrating transcriptomic data with other omics technologies (proteomics, metabolomics) remains a significant hurdle in comprehensive systems biology studies (Jendoubi, 2021). Despite its advantages, RNA-Seq is still associated with several technical challenges, such as bias in transcript quantification, particularly for low-abundance transcripts. As transcriptomics technologies continue to evolve, several advancements hold promise for the future of animal research. These include improvements in single-cell sequencing, better integration of multi-omics data, and advancements in spatial transcriptomics that allow the mapping of gene expression within intact tissues (Kleino et al., 2022).

2.8 Transcriptome responses to heat stress for gene marker identification in poultry research

HS can induce a change in the physiology and metabolism of poultry. In response to HS, poultry activate various stress response mechanisms at the molecular level, including changes in gene expression, protein synthesis, and metabolic pathways. Transcriptomics, the study of the complete set of RNA transcripts, has provided valuable insights into these molecular responses. Transcriptome analysis based on RNA-seq can contribute to an improvement in the current understanding of the molecular and functional mechanisms of physiological changes in poultry exposed to HS conditions. Previous studies using RNA-seq have shown that HS leads to increased expression of genes related to various nutrient metabolism and HSPs in the liver of poultry (Xie et al., 2014). By sequencing the RNA content of cells under stress conditions, RNA-Seq allows for the identification of differentially expressed genes (DEGs), alternative splicing events, and the characterization of non-coding RNAs that contribute to stress responses (Wang et al., 2009). In poultry, RNA-Seq has been used to investigate the transcriptional changes in various tissues, including the liver, small intestine, muscle, and brain, under heat stress (Kim et al., 2022; Wu et al., 2024; Zhu et al., 2025). HS induces the upregulation of HSPs, which act as molecular chaperones to protect cellular proteins from denaturation. The transcriptomic analysis of broilers and breeder hens in jejunal mucosa under HS has highlighted key HSPs, including HSP40 (*DNAJA1*), HSP70 (*HSPA2* and *HSPA8*), HSP90 (*HSP90AA1*), and *HSP110*, as important markers of heat stress tolerance (Kim et al., 2022; Zhu et al., 2025). It has been reported that genes related to immune responses and glutathione metabolism in the small intestine are affected by HS. Kim et al. (2022) found that upregulated glutathione-S-transferases (*GSTA3* and *GSTA4*) and downregulated interleukin-1 beta (*IL1B*) and interleukin-1 receptor type 2 (*IL1R2*) in the jejunal mucosa of heat-stressed broilers, which suggests that HS conditions may impair mucosal integrity and functions and decrease the immune systems. Moreover, RNA-seq analysis between native and commercial chicken breeds to HS response found that the expression of *PT1A* and *ANGPTL4* genes in native chickens, and *HSP90B1* and *HSPA5* genes in commercial chickens could be potential candidate genes involved with HS. These genes exhibited enriched pathways related to metabolic activity and inflammatory reactions (Barreto Sánchez et al., 2022). Hosseinzadeh & Hasanpur (2023) found that under acute HS, the endoplasmic reticulum chaperone complex (*HSPA5*, *SDF2L1*) was inhibited, and *SSR1* and *SEC23B* genes were downregulated, suggesting that acute HS may lead to protein structure disruption, protein binding, protein transport, protein formation, and degradation of misfolded proteins. Furthermore, transcriptome analysis in the jejunal mucosa in breeder hens under thermoneutral (23°C) and HS (36°C for 6 h) indicated that the DEGs of *HSPA2*, *DNAJA4*, *HSP90AA1*, *PDK4*, *SLC10A2*, *PPARA*, and *CD36* were associated with steroid biosynthesis, steroid hormone biosynthesis, protein processing in endoplasmic reticulum, the peroxisome proliferator-activated receptor signaling pathway, and the adipocytokine signaling pathway, which contribute to a deeper understanding of the jejunal mucosal response in breeder hens to acute HS (Zhu et al., 2025). Therefore, transcriptome sequencing provides a powerful tool to analyze the molecular mechanism of poultry that are exposed to HS conditions, and RNA-seq analysis identified key pathways and candidate genes that can be used as indicators to monitor acute or chronic HS responses in poultry and may provide strategies for the development of heat-resistant strains.

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