CHAPTER I

INTRODUCTION

1.1 Introduction

The impact of heat stress (**HS**) on poultry is a major cause of economic losses in the poultry industry (Shehata et al., 2020). All homeothermic animals have a thermoneutral zone, and when the temperatures exceed this zone, animals cannot maintain a stable body temperature, resulting in HS. HS can be categorized as either acute (sudden, short-duration exposure to extremely high ambient temperatures and humidity) or chronic (extended period of elevated ambient temperatures with increased humidity) (Loyau et al., 2015). Both chronic and acute HS can compromise intestinal function through multiple mechanisms: reducing blood flow, increasing intestinal permeability, impairing intestinal barrier integrity, and causing microbiota dysbiosis, all of which affect nutrient digestion and absorption (Zhang et al., 2015; Gupta et al., 2017; Rostagno, 2020). In addition, HS triggers various pathophysiological changes, including immune dysregulation, overproduction of reactive oxygen species (ROS), decreased energy metabolism, and cell membrane lipid peroxidation. These changes ultimately increase susceptibility to pathogens and impair overall health (Liu et al., 2016; Shi et al., 2019). To combat the deleterious effects of HS in chickens, various genetic, management, and nutritional strategies have been implemented (Saeed et al., 2019). While genetic and management approaches may involve increased costs, technical complexity, and reduced genetic diversity, nutritional interventions offer a more feasible solution. These interventions, particularly the use of phytogenic compounds, vitamins (C and E), and minerals like selenium (Se), are gaining popularity as remedies for heat-stressed poultry (Kumar et al., 2021). Therefore, understanding the intrinsic physiological mechanisms of HS-induced intestinal damage in poultry and investigating the anti-HS effects of dietary phytochemicals and synthetic antioxidants, along with their underlying mechanisms, represents a critical area of research for improving intestinal barrier function and overall poultry health.

Similar to other animals, indigenous chickens demonstrate better adaptation to high temperatures compared to commercial high-performance broilers (Malila et al., 2024). Previous studies comparing the HS responses between slow-growing and fast-growing chickens have suggested that increased growth rates negatively correlate with birds' tolerance (Soleimani et al., 2011). Two breeder strains, heat-adapted (Leung Hang Kaeo breeder line) and heat-sensitive (Suranaree University of Technology (SUT) breeder line), are preserved by the avian research center of SUT in Thailand. These strains are used to produce crossbred meat chicken (Korat chicken) for the local niche market. Under elevated temperatures, the SUT breeder line exhibits significant signs of heat stress susceptibility and reduced productive performance, while the local breed shows minimal adverse effects. The investigation of gene biomarkers through transcriptomic analysis holds promise for optimizing feed modulation with antioxidant bioactive substances. These approaches could enhance HS adaptation while promoting gut health and increasing both efficiency and genetic gain (Gvozdanović et al., 2023).

The gut is the primary organ affected by HS (Chauhan et al., 2021). HS reduces blood flow to the gut, causing damage to the epithelial tissue and generating free radicals that impair nutrient digestion and absorption. The jejunum, which plays a critical role in nutrient digestion and absorption, is particularly sensitive to HS (Song et al., 2013), and is commonly used as a model in intestinal studies (Abdelli et al., 2021; Wang et al., 2021). Under HS conditions, birds activate defense mechanisms such as heat-shock proteins (HSPs) to alleviate or reduce the negative effects (Belhadj Slimen et al., 2016; Emami et al., 2021). The expression of *HSP70* and *HSP90* is upregulated to protect and repair cells (Varasteh et al., 2015). HSPs also play a regulatory role during cellular stress by inhibiting inflammatory cytokine production (Ferat-Osorio et al., 2014) and modulating tight junction proteins (Dokladny et al., 2008), thereby mitigating the detrimental effects of HS on gut health. Beyond molecular-level responses, various nutritional strategies are being tested, with dietary intervention emerging as a particularly cost-effective approach (Greene et al., 2021). Antioxidant supplementation combining vitamins E and C, minerals (Se, manganese, and zinc), and phytogenic bioactive compounds has demonstrated synergistic efficacy in enhancing antioxidant activity, reducing oxidative stress, strengthening immune function, and gut dysbiosis regulation (Ghazi Harsini et al., 2012; Kumbhar et al., 2018), while mitigating HS and lipid peroxidation in poultry (Leskovec et al., 2019). In addition, L-carnitine, a potent antioxidant, plays a crucial role in scavenging free radicals and protecting tissues from ROS-induced oxidative damage (Agarwal et al., 2018). Phytogenic compounds, rich in bioactive chemicals like polyphenols and flavonoids, can improve antioxidant capacity, and immunity, enhance gut microbiota and health, and reduce oxidative/inflammatory pathways (Yang et al., 2021; Reith et al., 2022). These properties contribute to increased resistance to external stress, garnering significant attention in recent research (Mahasneh et al., 2024). Notable herbs of interest include *Camellia sinensis* (green tea), which contains major antioxidant catechins (particularly epigallocatechin gallate), *Syzygium aromatum* (clove), which is rich in eugenol, and *Persicaria odorata* (Vietnamese coriander), which contains gallic acid and quercetin. These herbs have shown potential as feed additives to mitigate HS, providing several beneficial functionalities (Erener et al., 2011; Hosseinzadeh et al., 2014; El-Maati et al., 2016; Aziz-Aliabadi et al., 2023; Saracila et al., 2023). However, the role of synthetic and phytogenic antioxidants in gut health and production, along with their underlying mechanisms in heat-stressed breeder hens, remains to be fully explored.

Transcriptome sequencing (RNA-seq) serves as a powerful tool for analyzing molecular mechanisms and revealing intrinsic cellular biological regulatory mechanisms underlying various physiological conditions in animals (Wang et al., 2019). This technology has been successfully employed across species, including poultry, cattle, and pigs, to identify genes that play key roles in responses to high ambient temperatures (Coble et al., 2014; Srikanth et al., 2017). Previous RNA-seq studies have revealed differential expression of genes in the jejunal mucosa of heat-stressed animals. In broilers subjected to chronic HS, differentially expressed genes (DEGs) were associated with immune response, glutathione metabolism, defense mechanisms, and detoxification of xenobiotics (Kim et al., 2022). Similarly, in breeder chickens under acute HS, RNA-seq analysis demonstrated DEGs involved in steroid biosynthesis, steroid hormone biosynthesis, protein processing in endoplasmic reticulum, the peroxisome proliferator-activated receptor signaling pathway, and the adipocytokine signaling pathway (Zhu et al., 2025). While these studies have provided valuable insights into the gene expression profiles of poultry under HS, there remains a critical need to investigate the long-term effects of HS on gut health at the molecular level. Such research could reveal novel mechanisms and potential therapeutic targets for improving heat tolerance in poultry.

Therefore, this study pursued a comprehensive investigation with two interconnected objectives. The first objective was to conduct transcriptomic analysis in the jejunal mucosal tissue of breeder hens exposed to HS, aiming to identify relevant gene markers by comparing heat-adapted and heat-sensitive breeds and track progressive changes in selected candidate genes among heat-sensitive breeder hens under HS conditions. The second objective was to evaluate the efficacy of two antioxidant sources in breeder hens' diets under HS conditions: synthetic antioxidants (a combination of vitamin E, vitamin C, Se, and L-carnitine) and phytogenic antioxidants (a combination of clove, green tea pomace, and Vietnamese coriander). The evaluation encompassed antioxidants genes (SOD, GSH-Px), HSPs (HSP70, HSP90), immune-related genes (IL-10, IL-6, TNF-α, NF-κB and TLR4), and TJ protein genes (ZO-1, CLND1), as well as parameters such as short-chain fatty acids (SCFAs) concentration, ammonia production, and microbiota composition.

1.2 Research objectives

The objectives of this study were:

1.2.1 To identify heat tolerance mechanisms in breeder hens by comparing jejunal transcriptome profiles between heat-adapted and heat-sensitive hens, and tracking expression changes of selected candidate genes in heat-sensitive hens supplemented with dietary antioxidants under HS conditions.

1.2.2 To evaluate the efficacy of dietary supplementation, either with synthetic or phytogenic antioxidant mixtures, on alleviating the deleterious impact of HS in heat-sensitive breeder hens by measuring gut health parameters, including gene expression related to intestinal function, antioxidant capacity, short-chain fatty acid (SCFAs) and ammonia concentrations, and microbial populations.

1.3 Research hypotheses

1.3.1 Heat-adapted and heat-sensitive breeder hens exhibit distinct jejunal transcriptome profiles under HS conditions, with differentially expressed genes and biological pathways that can explain the variation in heat tolerance. Dietary antioxidant supplementation in heat-sensitive breeder hens under HS can normalize the expression of selected candidate genes.

1.3.2 Dietary antioxidant supplementation with synthetic or phytogenic antioxidant mixtures in heat-sensitive breeder hens under HS can improve expression of the genes related to gut barrier integrity (ZO-1, CLDN1), anti-inflammatory cytokine (IL-10) and antioxidant capacity (SOD, GSH-Px), reduce pro-inflammatory cytokines (IL-6, TNF-α, NF-κB and TLR4) and HSPs (HSP70, HSP90) expression, increase cecal SCFAs content, reduce ammonia production, and modify microbial population.

1.4 Scope of the study

In this study, two 28-week-old broiler breeder strains (heat-adapted [Leung Hang Kaeo breeder line] and heat-sensitive [Suranaree University of Technology (SUT)] breeder line) were used to acquire a deeper understanding of their transcriptomic responses to HS. In addition, this study aims to leverage these findings to develop innovative strategies for mitigating HS impacts by evaluating the effects of dietary synthetic antioxidants (a combination of vitamin E, vitamin C, Se, and L-carnitine) and phytogenic antioxidants (a combination of clove, green tea pomace, and Vietnamese coriander) supplementation in heat-sensitive breeders under HS conditions. This study was assessed various gut health parameters, including the expression of antioxidant enzymes (SOD and GSH-Px), HSPs (HSP70 and HSP90), immune-related genes (IL-10, IL-6, TNF-α, NF-κB and TLR4), TJ protein genes (ZO-1, CLDN1), cecal short-chain fatty acid (SCFAs) concentration, ammonia production, and the composition of the cecal microbiota. By integrating transcriptomic analysis with gut health evaluations, this study seeks to identify molecular and physiological mechanisms underlying heat resilience and propose targeted nutritional interventions to enhance poultry health and performance under HS conditions.

1.5 Expected benefits

1.5.1 The knowledge obtained from transcriptomic profiling of two breeding strains with different tolerance to HS can provide a deeper understanding of the molecular responses and biological pathways involved in heat adaptation, which is crucial for developing targeted feed additive interventions to cope with HS in poultry production systems.

1.5.2 Dietary supplementation with either synthetic or phytogenic antioxidants can be applied to breeder hen diets and can be further studied for potential applications in other animal production systems facing HS challenges.

1.5.3 The marker genes identified in this study can serve as monitoring tools to evaluate the efficacy of feed additives designed to mitigate HS effects in poultry and potentially other livestock species. While the primary focus of this study is on dietary supplementation effects, the identified candidate genes may also provide preliminary insights for future genetic selection research.

1.6 References

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