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Gene Expression Profiles in Heat-Stressed Dairy Cattle

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Abstract Heat stress is a significant challenge in dairy cattle, impacting their physiological well-being, productivity, and overall health. Understanding the gene expression profiles in heat-stressed dairy cattle is crucial for developing effective management and breeding strategies. This study investigates the changes in gene expression in response to heat stress, focusing on the molecular mechanisms and key genes involved, and explores the physiological, behavioral, cellular, and molecular responses of dairy cattle to heat stress, with particular attention to heat shock proteins (HSPs) and their roles in maintaining cellular homeostasis. Through techniques such as RNA sequencing, quantitative PCR, and bioinformatics analysis, this study provides a comprehensive overview of the gene expression changes under heat stress conditions, highlighting the impact on metabolic pathways and immune responses. A case study is presented to illustrate the practical implications of these findings for dairy production, emphasizing the potential for genetic selection for heat tolerance and the importance of appropriate nutrition and management practices. This study concludes with recommendations for future research, addressing unresolved questions and exploring emerging technologies that could enhance our understanding of gene expression in heat-stressed dairy cattle.

Keywords Heat stress; Gene expression; Dairy cattle; Heat shock proteins; Transcriptomics

1 Introduction

Precision Livestock Farming (PLF) refers to the use of advanced technologies and data-driven approaches to monitor and manage livestock production at an individual animal level. Since its inception in the early 2000s, PLF has evolved significantly, integrating various technological advancements such as sensors, the Internet of Things (IoT), and data analytics to enhance the efficiency and sustainability of livestock farming (Halachmi and Guarino, 2016; Morrone et al., 2022). The primary goal of PLF is to optimize animal health, welfare, and productivity by providing real-time monitoring and control systems that allow farmers to make informed decisions (Vranken and Berckmans, 2017; Norton et al., 2019).

The importance of PLF in modern agriculture cannot be overstated (Lovarelli et al., 2020). As global demand for animal products continues to rise, there is an increasing need for sustainable and efficient farming practices (Tzanidakis et al., 2023). PLF addresses this need by enabling precise management of livestock, which can lead to improved animal welfare, reduced environmental impact, and enhanced economic viability of farming operations (Werkheiser, 2018; Monteiro et al., 2021). By continuously monitoring individual animals, PLF technologies help in early detection of diseases, optimizing feeding strategies, and ensuring better reproductive management, thereby contributing to the overall sustainability of livestock farming (Vaintrub et al., 2020; Tzanidakis et al., 2021).

This study provides a comprehensive overview of the advancements in Precision Livestock Farming (PLF) technology and its applications across diverse livestock production systems, exploring the current state of PLF, including the latest technological innovations and their practical applications in various agricultural environments, reviewing the benefits and challenges associated with PLF adoption, with a focus on its impact on animal welfare, farm productivity, and environmental sustainability, aiming to highlight the potential of PLF to transform modern livestock farming and identify areas for future research and development.

2 Mechanisms of Heat Stress in Dairy Cattle

2.1 Physiological impact of heat stress

Heat stress in dairy cattle leads to significant physiological changes that adversely affect their productivity and overall health. One of the primary impacts is a reduction in feed intake, which subsequently leads to decreased milk production. This reduction in feed intake is a direct response to the increased body temperature and the need to minimize metabolic heat production. Additionally, heat stress induces various physiological stress responses, including alterations in blood flow, increased respiration rate, and elevated heart rate, all aimed at dissipating excess body heat (Garner et al., 2020). Chronic heat stress can also lead to changes in the expression of genes related to inflammation, lipid metabolism, and the cardiovascular system, further exacerbating the physiological strain on the animals (Liu et al., 2020).

2.2 Behavioral responses to heat stress

Behavioral adaptations are crucial for dairy cattle to cope with heat stress. Cows often seek shade and increase their water intake to cool down. They may also reduce their physical activity to lower metabolic heat production. These behavioral changes are essential for maintaining homeostasis but can lead to reduced feed intake and, consequently, lower milk yield (Choi et al., 2021). The behavioral responses are often accompanied by physiological changes, such as increased sweating and panting, which help in thermoregulation but can also lead to dehydration and electrolyte imbalances.

2.3 Cellular and molecular responses

At the cellular and molecular levels, heat stress triggers a complex network of responses aimed at protecting cellular integrity and function. One of the key responses is the upregulation of heat shock proteins (HSPs), which play a critical role in protein folding and protection against thermal damage (Sengar et al., 2017). Genes involved in oxidative stress, apoptosis, and glucose metabolism are also differentially expressed under heat stress conditions, indicating a broad cellular response to mitigate damage and maintain cellular function (Fang et al., 2021). Additionally, miRNAs have been identified as important regulators of the heat stress response, targeting genes involved in stress signaling pathways and protein synthesis. For instance, miRNAs such as bta-miR-423-5p and bta-miR-2898 have been shown to regulate the expression of heat shock proteins and other stress-related genes, highlighting their role in the post-transcriptional regulation of the heat stress response (Deb and Sengar, 2020; Liu et al., 2020).

3 Gene Expression Changes in Heat-Stressed Dairy Cattle

3.1 Key genes affected by heat stress

Heat stress significantly impacts the expression of various genes in dairy cattle. Notably, genes encoding heat shock proteins (*HSPs*) such as *HSP70, HSP90*, and *HSP27* are upregulated in response to elevated temperatures. For instance, the expression of *HSP70* and *HSP90* genes was significantly higher in Hanwoo calves exposed to high temperature-humidity indices (THIs) (Kim et al., 2020). Similarly, in Sahiwal and Tharparkar breeds of zebu cattle, HSP70 family genes (*HSPA1A, HSPA1B, and HSPA8*) showed maximal induction during summer (Kumar et al., 2015). These findings suggest that HSP genes are crucial markers for assessing heat stress in dairy cattle.

3.2 Heat shock proteins (HSPs) and their roles

Heat shock proteins (*HSPs*) play a vital role in protecting cells from heat-induced damage. HSP70, one of the most studied HSPs, is highly sensitive to heat stress and is primarily responsible for cellular protection. In bovine mammary epithelial cells, *HSP70* expression peaked at 14 times the control level after 1 hour of heat exposure (Hu et al., 2016). HSP90 also plays a significant role, with its expression being higher in Sahiwal cattle compared to Frieswal cattle under both in vitro and environmental heat stress conditions (Deb et al., 2014). These proteins help maintain cellular homeostasis by preventing protein aggregation and assisting in protein refolding.

3.3 Impact on metabolic pathways

Heat stress affects various metabolic pathways in dairy cattle, leading to altered nutrient utilization and metabolic disorders. For example, heat-stressed cows exhibit decreased ruminal pH and acetate concentration, along with increased ruminal lactate levels (Kim et al., 2022). This shift in rumen fermentation is associated with changes in the abundance of specific microbial populations, such as an increase in lactate-producing bacteria and a decrease in acetate-producing bacteria. Additionally, key metabolic markers like isoleucine, methionine, and tryptophan are reduced under heat stress conditions, impacting feed consumption and milk production efficiency.

3.4 Influence on immune response

Heat stress also influences the immune response in dairy cattle. *HSPs*, particularly *HSP70* and *HSP90*, are involved in modulating immune functions (Guzmán et al., 2023). In channel catfish, HSP90 genes showed pathogen-specific expression patterns, indicating their role in immune responses (Xie et al., 2015). Similarly, in dairy cattle, the expression of immune-related genes such as bovine lymphocyte antigen and histocompatibility complex class II (*DRB3*) is downregulated during heat stress, while *HSP70* and *HSP90* are upregulated. These changes suggest that HSPs not only protect against thermal stress but also play a role in enhancing immune resilience.

4 Techniques for Analyzing Gene Expression in Heat-Stressed Cattle

4.1 RNA sequencing and transcriptomics

RNA sequencing (RNA-seq) is a powerful technique used to analyze the complete transcriptome of an organism, providing insights into gene expression changes under various conditions, including heat stress. In dairy cattle, RNA-seq has been employed to identify differentially expressed (*DE*) genes and non-coding RNAs, such as microRNAs (miRNAs) and circular RNAs (circRNAs), in response to heat stress. For instance, a study on the hypothalamic-pituitary-mammary gland axis of dairy cows under heat stress identified numerous DE circRNAs, miRNAs, and mRNAs, highlighting the MAPK signaling pathway as a key player in the heat stress response (Zeng et al., 2023). Another study focused on the differential expression of miRNAs in buffalo heifers under heat stress, revealing significant changes in the expression of miRNAs and their target genes, which are involved in heat tolerance mechanisms (Yadav et al., 2021).

4.2 Quantitative PCR (qPCR)

Quantitative PCR (qPCR) is a widely used method for quantifying gene expression due to its specificity, sensitivity, and reproducibility. It is particularly useful for validating RNA-seq results and for studying the expression of specific genes under heat stress conditions. For example, qPCR was used to validate the expression of DE miRNAs identified through RNA sequencing in dairy cows, confirming the downregulation of several miRNAs post-calving (Figure 1) (Webb et al., 2020). Additionally, qPCR has been employed to study the expression of heat shock proteins (HSPs) in different cattle breeds, revealing higher expression levels of Hsp90 in the Sahiwal breed compared to the Frieswal breed under heat stress, which may contribute to better heat tolerance in Sahiwal cattle (Deb et al., 2014).

The research of Webb et al. (2020) illustrates the relative expression of different microRNAs (miRNAs) in dairy cows with high and normal body conditions at various time points relative to calving. It shows distinct patterns of miRNA expression, with significant differences between time points within each group and between the groups at specific time points. These variations suggest that miRNA levels are dynamically regulated in response to physiological changes around parturition, potentially influencing the health and metabolic status of the cows during this critical period. The trends indicate potential biomarkers for monitoring dairy cow health in relation to body condition and calving.

4.3 Bioinformatics tools for data analysis

Bioinformatics tools are essential for analyzing the large datasets generated by RNA-seq and qPCR. These tools facilitate the identification of DE genes, pathway enrichment analysis, and the construction of gene regulatory networks. For instance, bioinformatics analysis was used to identify putative target genes and enriched biological pathways for DE miRNAs in dairy cows, revealing associations with cell cycle, insulin signaling, and lipid metabolism (Webb et al., 2020). Similarly, competitive endogenous RNA (ceRNA) networks were established to understand the molecular basis of heat stress response and lactation regulation in dairy cows. Tools such as geNorm and NormFinder are also used to evaluate the stability of reference genes for qPCR, ensuring accurate normalization of gene expression data (Lozano-Villegas et al., 2021).

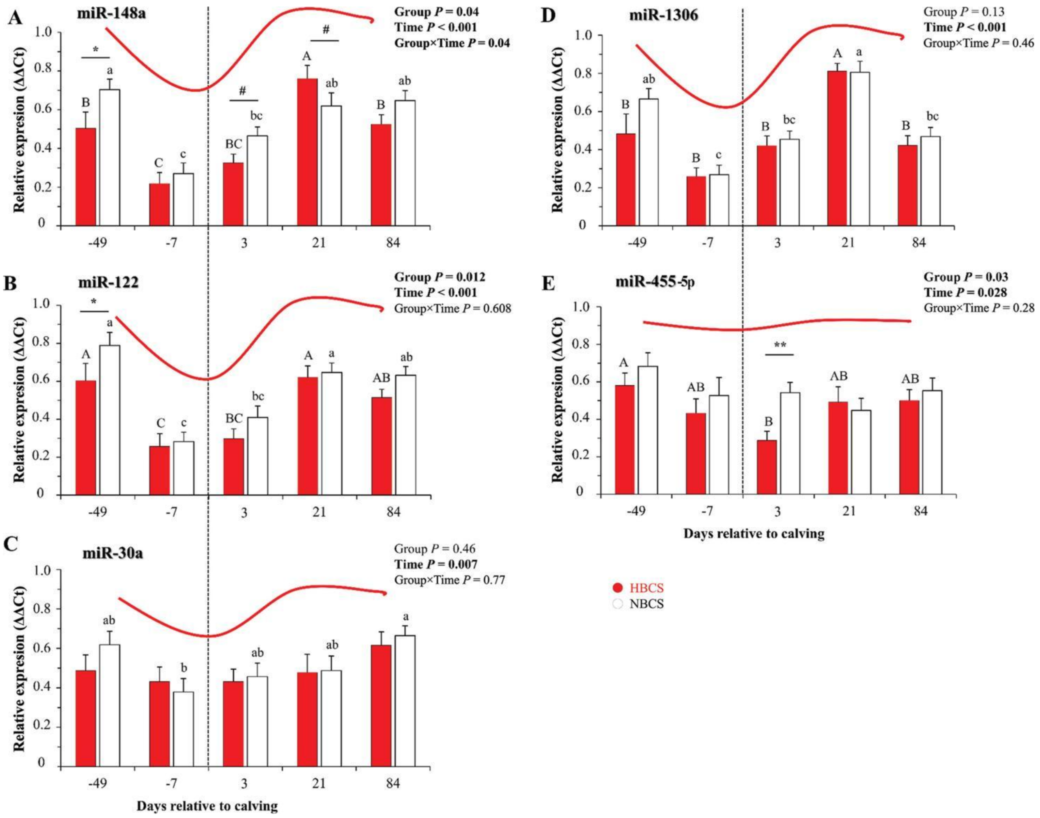

Figure 1 Relative abundance of serum microRNA (miRNA) as assessed by quantitative PCR in high (HBCS; n=15) and normal body condition (NBCS; n=15) dairy cows on d-49, -7, 3, 21 and 84 relative to parturition (Adopted from Webb et al., 2020)

Image caption: Data are given as means±SEM. Different uppercase letters (A-C) illustrate differences ($P<0.05$) between time points within HBCS; Different lowercase letters (a-c) illustrate differences ($P<0.05$) between time points within NBCS. Differences between HBCS and NBCS within 1 time point are labeled with * ($0.01<P<0.05$) or ** ($P<0.01$). Trends ($0.05<P<0.1$) are labeled with hashtags (#); The dashed line indicates calving (Adopted from Webb et al., 2020)

4.4 Comparative approaches: normal vs. heat-stressed conditions

Comparative approaches involve analyzing gene expression profiles in cattle under normal and heat-stressed conditions to identify stress-responsive genes and pathways. Studies have shown significant differences in the expression of miRNAs and their target genes between heat-stressed and non-heat-stressed cattle. For example, miRNA profiling in Holstein cows under summer heat stress revealed differential expression of miRNAs associated with progesterone biosynthesis and immune responses, suggesting that pregnant cows are more susceptible to heat stress (Lee et al., 2020). Another study on buffalo heifers demonstrated significant upregulation of heat shock proteins and miRNAs involved in heat tolerance under heat stress conditions (Yadav et al., 2021). These comparative analyses provide valuable insights into the molecular mechanisms underlying heat stress response in dairy cattle (Sigdel et al., 2020).

5 Case Study: Gene Expression Profiles in Heat-Stressed Dairy Cattle

5.1 Overview of the case study

This case study investigates the gene expression profiles in dairy cattle subjected to heat stress, a condition that significantly impacts their productivity and overall health. The study focuses on understanding the molecular mechanisms underlying the heat stress response by examining various tissues and biological markers in dairy cows.

5.2 Study design

The study employed a multi-faceted approach to analyze gene expression in dairy cattle under heat stress. Different research methodologies were utilized, including RNA sequencing, genome-wide association studies (GWAS), and proteomics. Samples were collected from various tissues such as the hypothalamic-pituitary-mammary gland axis (Figure

2), peripheral white blood cells, and milk somatic cells. Additionally, physiological parameters like rectal temperature and milk yield were measured to correlate with gene expression data (Zeng et al., 2023).

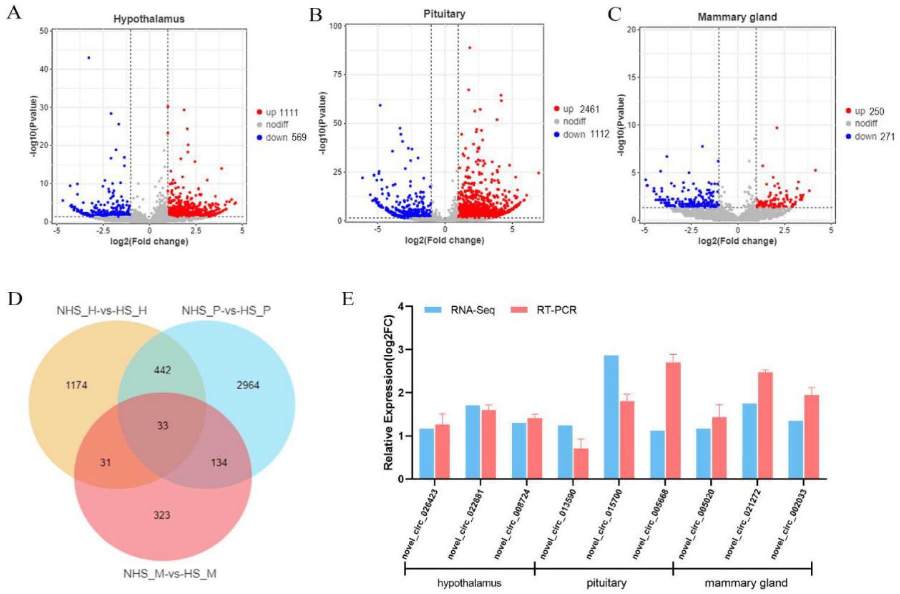

Figure 2 (A) Volcano plot of DEcircRNAs in hypothalamus; (B) Volcano plot of DEcircRNAs pituitary; (C) Volcano plot of DEcircRNAs mammary gland. Red dots represent up-regulated circRNAs and blue dots represent down-regulated circRNAs; (D) Venn diagram of DEcircRNAs in the HPM axis-related tissues between NHS and HS groups; (E) Comparison of the circRNAs expression levels determined by RNA-seq and RT-qPCR (Adopted from Zeng et al., 2023)

The research of Zeng et al. (2023) presents a comparative analysis of differentially expressed circular RNAs (circRNAs) in the hypothalamus, pituitary, and mammary gland under varying conditions. The volcano plots indicate significant upregulation and downregulation of circRNAs in these tissues, with more pronounced changes in the pituitary. The Venn diagram illustrates the overlap of these differentially expressed circRNAs across tissues, highlighting shared and unique circRNAs between groups. The bar graph compares expression levels of select circRNAs, confirming RNA-seq findings with RT-qPCR, showing consistent trends across both methods. This suggests potential regulatory roles of circRNAs in these tissues under stress conditions.

5.3 Key findings

The studies revealed several key findings. Differentially expressed genes (*DEGs*) were identified in various tissues, with significant changes observed in the expression of circRNAs, miRNAs, and mRNAs. For instance, the MAPK signaling pathway was highlighted as a crucial pathway in the heat stress response. Genes related to cellular stress response, apoptosis, oxidative stress, and glucose metabolism were commonly differentially expressed in both peripheral white blood cells and milk somatic cells (Figure 3) (Garner et al., 2020). Additionally, specific miRNAs were found to target progesterone biosynthesis and corpus luteum-related genes, indicating that pregnant cows are more susceptible to heat stress (Lee et al., 2020). Proteomics analysis showed alterations in the complement and coagulation cascades, suggesting impaired immune function in heat-stressed cows (Min et al., 2016).

The research of Fang et al. (2021) illustrates the cellular responses to cold and heat stress in peripheral blood mononuclear cells. Under cold stress, cells experience hypoxic stress, leading to decreased metabolic rates and reduced protein synthesis. This response is aimed at conserving energy and preventing apoptosis by regulating gene expression through the endoplasmic reticulum and mitochondria. In contrast, heat stress triggers oxidative damage and mitochondrial dysfunction, leading to protein misfolding. Heat shock proteins (HSPs) play a crucial role in stabilizing these denatured proteins, preventing further cellular damage, and aiding in protein refolding, thereby protecting cells from heat-induced apoptosis and necrosis.

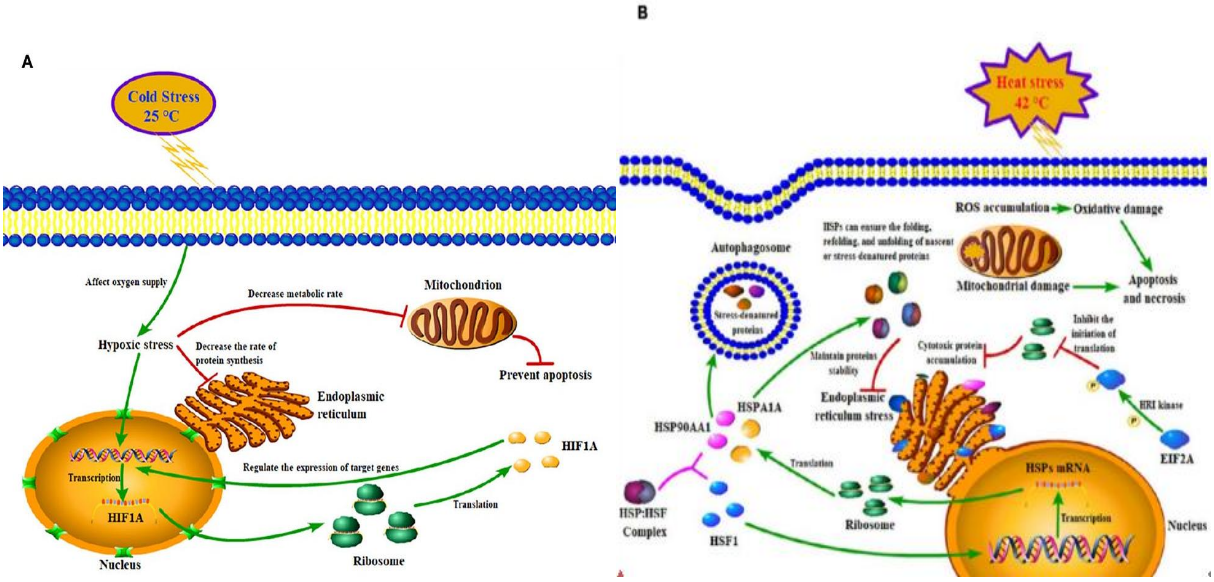

Figure 3 Models of the mechanisms of key genes in cold (A) and heat (B) stress responses of peripheral blood mononuclear cell (Adopted from Fang et al., 2021)

5.4 Implications for dairy production

The findings have significant implications for dairy production. Understanding the genetic and molecular basis of heat stress response can aid in developing strategies to mitigate its adverse effects. For example, identifying candidate genes for heat tolerance can inform selective breeding programs aimed at enhancing thermotolerance in dairy cattle (Otto et al., 2019; Luo et al., 2021). Additionally, dietary interventions, such as the supplementation of rumen-protected tryptophan, have been shown to improve feed intake, milk yield, and overall stress resilience in heat-stressed cows (Choi et al., 2021). These insights can help optimize management practices and improve the welfare and productivity of dairy cattle in hot climates.

5.5 Lessons learned

Several lessons were learned from this case study. First, a comprehensive approach that integrates various omics technologies is essential for a thorough understanding of the heat stress response in dairy cattle. Second, the identification of specific biomarkers, such as miRNAs and proteins, can provide valuable tools for monitoring and managing heat stress in dairy herds. Lastly, the genetic variability in heat tolerance among different breeds and crossbreeds underscores the importance of tailored breeding programs to enhance resilience to heat stress (Macciotta et al., 2017; Liu et al., 2020). These lessons highlight the need for continued research and innovation to address the challenges posed by heat stress in the dairy industry.

6 Implications for Dairy Management and Breeding

6.1 Strategies for mitigating heat stress

Heat stress in dairy cattle significantly impacts milk production, fertility, and overall animal welfare. Effective strategies to mitigate heat stress include improving housing conditions, such as providing shade, fans, and sprinklers to cool the animals. Additionally, adjusting feeding times to cooler parts of the day and ensuring access to cool, clean water can help reduce the effects of heat stress. Implementing these strategies can help maintain productivity and animal health during periods of high temperature and humidity (Garner et al., 2016; Habimana et al., 2023).

6.2 Potential for genetic selection for heat tolerance

Genetic selection for heat tolerance offers a promising long-term solution to mitigate the effects of heat stress in dairy cattle. Studies have shown that genomic selection using DNA markers can predict heat tolerance, allowing for the breeding of more resilient cattle. For instance, genomic estimated breeding values (GEBV) for heat tolerance have been developed, which can be used to select animals that maintain higher production levels under heat stress conditions (Nguyen et al., 2016). Additionally, specific genes, such as FAM107B and PHRF1, have been identified as influencing heat stress response, providing targets for genetic improvement (Luo et al., 2021). The integration of genomic information with environmental and physiological data can enhance the accuracy of selecting heat-tolerant animals (Sungkhapreecha et al., 2022).

6.3 Role of nutrition and management in supporting gene expression

Nutrition and management practices play a crucial role in supporting gene expression related to heat tolerance. Proper nutrition can help mitigate the negative effects of heat stress by ensuring that cattle receive adequate energy and nutrients to maintain production and health. For example, feeding strategies that include high-quality forages and balanced rations can support metabolic functions and reduce heat stress impacts (König and May, 2019). Additionally, management practices such as optimizing milking schedules and providing comfortable resting areas can enhance the expression of genes associated with heat tolerance, further supporting the overall well-being and productivity of dairy cattle (Carabaño et al., 2017; Nguyen et al., 2017). Integrating these practices with genetic selection can create a comprehensive approach to managing heat stress in dairy herds.

7 Future Directions and Research Gaps

7.1 Unresolved questions in gene expression studies

Despite significant advancements in understanding the genetic basis of heat stress response in dairy cattle, several unresolved questions remain. One key area is the precise genetic mechanisms that confer heat tolerance. While studies have identified candidate genes such as HSF1 and MCAT (Macciotta et al., 2017), and others like LIF, OSM, and TXNRD2 (Otto et al., 2019), the functional roles of these genes in heat stress response need further elucidation. Additionally, the genetic correlations between heat tolerance and other economically important traits, such as milk yield and fertility, are not fully understood. For instance, while some studies have shown unfavorable correlations between heat tolerance and production traits, others have identified potential favorable genetic correlations with fertility (Nguyen et al., 2016). This discrepancy highlights the need for more comprehensive studies to clarify these relationships.

7.2 Emerging technologies for gene analysis

Emerging technologies offer promising avenues for advancing our understanding of gene expression in heat-stressed dairy cattle. High-throughput sequencing technologies, such as RNA-Seq, have already been employed to identify differentially expressed genes under heat stress conditions (Garner et al., 2020). These technologies can be further leveraged to perform more detailed transcriptomic analyses, including single-cell RNA sequencing, which could provide insights into cell-specific responses to heat stress. Additionally, genome-wide association studies (GWAS) combined with advanced bioinformatics tools can help identify novel genetic markers and pathways involved in heat tolerance (Sigdel et al., 2019; Bohlouli et al., 2022). The integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, could also provide a more holistic understanding of the biological processes underlying heat stress response.

7.3 Potential applications in dairy industry

The findings from gene expression studies have several potential applications in the dairy industry. One immediate application is the development of genetic selection programs aimed at improving heat tolerance in dairy cattle. Genomic selection for heat tolerance, as demonstrated by the development of genomic estimated breeding values (GEBV) for heat tolerance traits, can be integrated into breeding programs to produce more resilient cattle. Additionally, the identification of specific biomarkers, such as differentially expressed miRNAs (Lee et al., 2020) and heat shock proteins (Kumar et al., 2015), can be used for early detection and management of heat stress in dairy herds. These biomarkers can also be incorporated into precision farming technologies to monitor and mitigate the effects of heat stress in real-time. Furthermore, understanding the genetic basis of heat tolerance can inform the development of nutritional and management strategies tailored to enhance the resilience of dairy cattle to heat stress.

8 Concluding Remarks

Heat stress (HS) significantly impacts the physiological and molecular functions of dairy cattle, leading to reduced milk production and overall health. Various studies have identified differentially expressed genes, miRNAs, and proteins that play crucial roles in the heat stress response. For instance, the MAPK signaling pathway and competitive endogenous RNA (ceRNA) networks are key in regulating stress responses and lactation in heat-stressed cows. Genes associated with cellular stress response, apoptosis, and glucose metabolism were differentially expressed in peripheral white blood cells and milk somatic cells under heat stress. Genome-wide association studies have identified candidate genes such as *LIF, OSM,* and *TXNRD2* that are involved in heat stress response. Additionally, miRNAs like bta-miR-146b and bta-miR-20b have been linked to progesterone biosynthesis and immune responses in heat-stressed cows. Proteomic analyses have shown that heat stress leads to a decrease in complement system proteins, indicating impaired immune function.

To mitigate the adverse effects of heat stress on dairy cattle, it is recommended to implement genetic selection for heat tolerance traits, such as rectal temperature, which has been shown to be heritable and genetically variable. Dietary interventions, such as the supplementation of rumen-protected tryptophan, can improve feed intake, milk yield, and stress resilience. Further research should focus on validating the identified candidate genes and miRNAs through functional studies to better understand their roles in heat stress response. Additionally, exploring the integration of multi-omics approaches, including genomics, transcriptomics, and proteomics, will provide a comprehensive understanding of the molecular mechanisms underlying heat stress in dairy cattle. Investigating the role of specific pathways, such as the MAPK signaling pathway and the complement and coagulation cascades, could lead to the development of targeted interventions to enhance heat tolerance.

The findings from various studies underscore the complexity of the heat stress response in dairy cattle, involving a multitude of genetic, molecular, and physiological changes. By leveraging advanced genomic and proteomic technologies, researchers can identify key biomarkers and pathways that contribute to heat tolerance. Implementing these insights into breeding programs and management practices will be crucial for improving the resilience of dairy cattle to heat stress, thereby ensuring sustainable milk production in the face of global climate change. Continued interdisciplinary research efforts are essential to develop effective strategies to combat the challenges posed by heat stress in the dairy industry.

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Conflict of Interest Disclosure

Authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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