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Review Article

Rumen–mammary gland axis and bacterial extracellular vesicles: Exploring a new perspective on heat stress in dairy cows

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ABSTRACT

Heat stress poses a significant threat to the global livestock industry, particularly impacting dairy cows due to their higher metabolic heat production and increased susceptibility. The rumen microbiota plays a crucial role in regulating heat stress in dairy cows. Moreover, the rumen-mammary gland axis has been recently unveiled, indicating that rumen bacteria and their metabolites can influence mammary gland health and function. Extracellular vesicles, cell-derived vesicles, are known to carry various biomolecules and mediate intercellular communication and immune modulation. This review proposes the hypothesis that heat stress poses a threat to dairy cows via the rumen-mammary gland axis by regulating rumen microbiota and their secreted extracellular vesicles. It summarizes existing knowledge on bacterial extracellular vesicles and the rumen-mammary gland axis, suggesting that targeting the rumen microbiota and their extracellular vesicles, while enhancing mammary gland health through this axis, could be a promising strategy for preventing and alleviating heat stress in dairy cows. The aim of this review is to offer new insights and guide future research and development efforts concerning heat stress in dairy cows, thereby contributing to a deeper understanding of its pathogenesis and potential interventions.

1. Introduction

Heat stress is an organism's nonspecific physiological response to thermal environmental stimuli (Stott, 1981). It often occurs when the body's internal heat production or external heat absorption surpasses the body's ability to dissipate heat, leading to an imbalance in internal heat regulation (Ma et al., 2020a; Wang et al., 2021a). Heat stress poses a persistent and severe challenge to the global livestock industry, detrimentally impacting animal productivity, the quality of meat, eggs, and milk, and contributing to increased morbidity, mortality, and substantial economic losses (Liang et al., 2017). Among livestock, dairy cows face heightened vulnerability to heat stress due to their metabolic heat production during rumen fermentation and milk synthesis (Koch et al., 2019; Wolfenson and Roth, 2019; Yang et al., 2022). According to Armstrong (1994), when the temperature-humidity index (THI) falls between 72.0 and 79.0, it indicates mild heat stress, while a THI of 79.0 suggests high heat stress, and a THI surpassing 88.0 signifies severe heat stress. However, recent research has reported that dairy cows may suffer from heat stress when the THI is greater than 68.0 (Burgos-Zimbelman and Collier, 2011). Studies have demonstrated that heat stress significantly reduces milk yield and quality, triggers a systemic inflammatory response, lowers immune function, induces oxidative stress, and contributes to metabolic disorders and cellular damage in dairy cows (Gujar et al., 2023; Lemal et al., 2023). Additionally, the escalating global temperatures exacerbate the threat of heat stress, posing a severe challenge to the sustainable development of the entire livestock industry (Gujar et al., 2023).

The composition of the rumen microbiota plays a pivotal role in heat stress among dairy cows (Kim et al., 2022). Recent studies have proposed the concept of the intestinal/rumen-mammary gland axis, suggesting that metabolites from rumen bacteria migrate to the mammary gland through endogenous pathways, such as blood

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or lymph circulation, thereby regulating mammary gland health and function (Hu et al., 2024). Extracellular vesicles are membrane-bound structures released or shed by cells (Wei et al., 2021), carrying nuclei acids, lipids, and proteins that facilitate intercellular communication, immune regulation, and the pathological progression of various diseases (Aheget et al., 2020; Tkach and Théry, 2016). Considering the role of extracellular vesicles in bacteria-host communication, this review proposes the hypothesis that heat stress poses a threat to dairy cows via the rumen-mammary gland axis by regulating rumen microbiota and their secreted extracellular vesicles. This article aims to summarize recent advancements in understanding bacterial extracellular vesicles (BEVs) and the rumen-mammary gland axis, proposing strategies targeting the rumen microbiota and their extracellular vesicles to enhance mammary gland health through the rumen-mammary gland axis.

2. Rumen microbiota—the key target for regulating heat stress in dairy cows

The gastrointestinal microbiota, boasting approximately 150 times more genes than the human genome, constitutes a pivotal "organ" within the human body (Zimmermann et al., 2019). This microbial ecosystem significantly influences the host, playing critical roles in immune, metabolic, and intestinal homeostasis processes (Qi-Xiang et al., 2022). Uniquely present in ruminants, the rumen serves as a digestive organ colonized by diverse microbial communities crucial for aiding dairy cows in feed digestion, nutrient provisions, influencing their endocrine, immune, metabolic, and mammary gland functions through metabolic products (Hu et al., 2022; Park et al., 2022). Diet and environmental conditions profoundly impact the rumen microbiota of dairy cows (Wang et al., 2021b). Heat stress disrupts the rumen microbial community, reducing feed intake, and affecting the host's metabolism, physiology, immune system, and behavior (Wang et al., 2020). Under heat stress (THI = 87.2 for daytime and 81.8 for nighttime) conditions, the relative abundance of rumen *Bacteroidetes* increases while *Firmicutes* decreases, altering the *Firmicutes*-to-*Bacteroidetes* ratio in lactating dairy cows (Park et al., 2022). Zhao et al. (2019) reported that the negative effects of heat stress on milk production were related to the changes in ruminal bacterial composition and metabolites. Their findings revealed that heat stress decreased the dry matter intake (21.0 kg/d vs. 25.0 kg/d) and milk production (32.0 kg/d vs. 40.0 kg/d) of dairy cows (THI: 87.0 vs. 71.0). Simultaneously, heat stress increased the relative abundance of lactate-producing bacteria (e.g., *Streptococcus* and *unclassified Enterobacteriaceae*) and decreased that of acetate-producing bacteria (e.g., *Acetobacter*). Feng et al. (2023) reported that heat stress (THI = 81.1) increased the abundance of ruminal pathogens (e.g., *Treponema_2*, *Sphingobacterium*, and *Streptococcus*) in Holstein dairy cows across different growth stages, with varying impacts on the ruminal microbial community structure among heifers (highest effect in growing heifers and lowest effect in lactating cows)). Kim et al. (2020) also suggested that the impact of heat stress (THI = 87.5) on rumen bacterial taxa exhibited a higher sensitivity in Jersey cows compared to Holstein cows, demonstrating breed-related effects on rumen bacterial taxa and functional gene abundance.

Given the key role of rumen microbiota in ruminants, it remains a central focus in ruminant nutrition research. Studies reveal that *Saccharomyces cerevisiae* cultures improve antioxidant capacity and immunity in mid-lactation dairy cows and influence the health status of heat-stressed dairy cows by regulating the composition, function, and metabolites of rumen microbiota, thereby improving the rumen cellulolytic capacity (Du et al., 2022a, 2022b). Additionally, active dry yeast supplementation in mid-to-late lactation impacts the rumen microbial composition and fermentation of dairy cows (Uyeno et al., 2017). Moreover, low doses of betaine have been reported to alleviate the effects of heat stress on the archaeal community in vitro (Mahmood et al., 2020). Similarly, Wang et al. (2021c) reported that the increase in dietary cation–anion difference under heat stress conditions did not affect the α diversity and structure of the rumen microbiota of dairy cows; however, it did increase the relative abundance of *Fibrobactere*. Honeycomb flavonoid supplementation augments proliferative microbial and fiber-degrading bacteria abundance, thereby enhancing fiber digestibility, energy supply, and overall production performance of dairy cows under heat stress conditions (Liu et al., 2022). Numerous studies have delved into heat stress effects on the rumen microbiota of dairy cows and the regulatory impacts of different additives. However, understanding the mechanisms through which the rumen microbiota influence rumen fermentation, epithelial barrier function, and mammary gland function under heat stress necessitates further investigation.

3. Rumen-mammary gland axis—a breakthrough for understanding the occurrence of heat stress

Microbe-host interactions denote the reciprocal influence between microbes and host cells or tissues, pivotal for maintaining host health and defending against diseases. Recent advancements in high-throughput technologies such as molecular biology, metabolomics, and transcriptomics have unveiled intricate microbe-host interactions, particularly emphasizing connections between the intestinal microbiota and multiple organ systems such as the gut-liver and gut–brain axis (Lu and Stappenbeck, 2022). The microbiota's role in regulating diseases across distant organs is increasingly recognized (de Vos et al., 2022). Studies indicate that dysbiosis in cow intestinal or rumen microbiota can facilitate the translocation of harmful factors such as lipopolysaccharide (LPS) and pathogenic bacteria from the intestine to the mammary gland, fostering mastitis development (Zhao et al., 2022a, 2022b). Numerous investigations highlight the relationship between the intestinal microbiota, its metabolites, and the integrity of the blood-milk barrier. Fecal microbiota transplantation from mastitis cows into mice confirmed the damaging effect on the blood-milk barrier (Zhao et al., 2022a). Additionally, LPS diminishes the expression of tight junction proteins in mammary epithelial cells, impairing the integrity of the blood-milk barrier (Guo et al., 2019; Zhao et al., 2021). Notably, volatile fatty acids like butyrate and propionate can repair the blood-milk barrier damage in mastitis mouse models, thereby alleviating mammary gland inflammation (Hu et al., 2020), underscoring the intimate connection between gastrointestinal microbiota and the mammary gland in dairy cows.

Gao et al. (2017) suggested that the reduction in blood supply of milk protein precursors and the decreased synthesis of rumen microbial proteins (MCP) might be the reasons for the decline in milk protein (4.1%) during heat stress conditions (THI = 84.5). Studies have shown that the interaction between rumen microbes and their metabolites with host metabolism contributes to increased milk protein yield, with the high abundance of *Prevotella* in the rumen meeting the host's demand for utilizing MCP for milk protein biosynthesis (Xue et al., 2020). Zhang et al. (2023) investigated the role of host-microbe interactions in regulating milk protein synthesis, finding that *Prevotella*- and *Ruminococcus*-dominated enterotypes could regulate milk protein synthesis by influencing rumen L-tyrosine and L-tryptophan. Cows with high milk protein and fat concentrations harbor a variety of *Prevotella* species in the rumen associated with total volatile fatty acid, acetate, and amino acid synthesis, meeting the host's needs for energy, fat, and MCP synthesis, which can be utilized to enhance the

biosynthesis of milk fat and protein (Wu et al., 2021). The gut/rumen-mammary gland axis, a recent concept, delineates the interplay the between gut/rumen microbiota and the mammary gland. Imbalances in gut/rumen microbiota compromise the intestinal mucosal barrier, enabling bacteria and their metabolites to reach the mammary gland through endogenous pathways (such as blood or lymph circulation), influencing mammary gland health and function (Hu et al., 2024). Therefore, the gut/rumen-mammary gland axis significantly contributes to the occurrence and development of mammary gland diseases. Sialic acid aggravates intestinal dysbiosis and mastitis through the microbiota-gut-mammary gland axis, regulated by symbiotic bacteria (Zhao et al., 2023), suggesting potential applications in modulating mammary gland function. Considering the alteration in rumen microbes under heat stress conditions and their pivotal role in the nutritional regulation of heat-stressed dairy cows, it can be speculated that the rumen-mammary gland axis serves as a primary pathway for heat stress to influence rumen microbes and mammary gland function. Despite limited reports on the rumen-mammary gland axis in heat-stressed dairy cows, its potential significance warrants further exploration.

4. Extracellular vesicles: deciphering the code of communication between bacteria and host cells

Microorganisms predominantly inhabit the gastrointestinal tract and their entry into the systemic circulation is restricted by the gastrointestinal barrier. Thus, the extensive impact of microbes on host physiology, explained solely through direct interaction, remains challenging. Current research into microbe-host interactions emphasizes the direct transfer of bioactive molecules, such as enzymes, metabolites, and toxins, to host cells through various secretion systems (Chanson et al., 2021; Ma et al., 2020b). Recent studies increasingly recognize extracellular vesicles as crucial mediators of communication between the intestinal microbiota and the host (Díaz-Garrido et al., 2021a). These extracellular vesicles play key roles in regulating metabolism, immune responses, oxidative stress, and other aspects (Chan et al., 2019; Fontana et al., 2022; Liu et al., 2019). Both Gram-negative and Gram-positive bacteria release diverse BEVs through distinct pathways (Wei et al., 2022). For instance, BEVs secreted by gastrointestinal bacteria (symbionts, probiotics, and pathogens) spread throughout the body via the blood or lymph nodes, interacting with host cells (Díaz-Garrido et al., 2021a; Liu et al., 2021b; Nishiyama et al., 2020). Notably, BEVs, containing toxins, quorum sensing molecules, and nucleic acids, regulate the gastrointestinal microenvironment and host health (Liang et al., 2022), influencing microbiota stability, promoting bacterial colonization and proliferation, modulating host metabolism, inflammation, immune response, and accelerating disease development (Díaz-Garrido et al., 2021b; Wang et al., 2023).

Bittel et al. (2021) first demonstrated that mammalian intestinal microbiota package biomolecules in small vesicles, which are delivered to other organs via the blood, even crossing the blood–brain barrier and impacting brain neurons. Bacterial extracellular vesicles derived from *Akkermansia muciniphila* promote the proliferation of beneficial bacteria, regulate mucosal immunoglobulin A response, and maintain the intestinal physical and chemical barrier via selective membrane fusion (Wang et al., 2023). Furthermore, Liu et al. (2021a) also found that extracellular vesicles secreted by the intestinal microbiota and *A. muciniphila* can enter and accumulate in the bone tissue, exerting a bone-protective effect. Similarly, BEVs derived from *Fusobacterium nucleatum* can induce epithelial barrier damage, increase intestinal permeability, and activate RIPK1-mediated apoptosis (Liu et al., 2021b). Additionally, *F. nucleatum* infection can alter the exosomes secreted by intestinal epithelial cells, promoting cell senescence and colonic inflammation through miR-129-2-3p (Guo et al., 2021). Bacterial extracellular vesicles containing microbial DNA can cross the damaged intestinal barrier of patients with obesity, aggravating tissue inflammation and metabolic disorders (Luo et al., 2021). Recently, researchers have detected BEVs in the amniotic fluid of healthy pregnant women, similar to the extracellular vesicles found in the intestinal microbiota. Increasing evidence suggests that BEVs can cross the intestinal barrier and reach the amniotic space (Kaisanlahti et al., 2023). Additionally, *Bacteroides thetaiotaomicron* can regulate the production of BEVs, regulating the host's utilization of polysaccharides (Sartorio et al., 2023). Although BEVs have been proven to be key mediators of microbe-host interactions in human and mouse models, studies on ruminants are limited.

Studies have suggested that exosomes play critical roles in regulating animals' heat stress response. For instance, heat stress increases exosome production in sea cucumbers, altering microRNA (miRNA) expression profiles within these exosomes (Huo et al., 2023). Ross et al. (2017) showed that exosomes can mediate molecular communication between different tissues and organs, potentially affecting reproduction. For example, under heat stress conditions, extracellular vesicles from granulosa cells reduce reactive oxygen species accumulation, improve mitochondrial function, and inhibit stress-related gene expression to alleviate the adverse effects of heat stress on bovine follicular cells and embryos (Menjivar et al., 2023). Additionally, the concentration of exosomes in oviduct fluid may relate to heat stress -induced (THI = 78.4) fertility changes in dairy cows (Stamperna et al., 2022). Moreover, the miRNA expression profile of bovine follicular fluid extracellular vesicles also changes with seasons, and the differentially expressed miRNAs (DE miRNAs) could serve as good indicators of mediating the heat stress response (Gad et al., 2023). Heat stress also induces an increase in the release of hepatic exosomes in mice, which changes the protein expression profile, and activates the nucleotide-binding and oligomerization domain -like receptor signaling pathway through exosomes to promote liver injury and inflammation (Li et al., 2019). These findings suggest that exosomes' production and composition could serve as potential heat stress biomarkers.

Exosomes also play a key role in the growth of bovine mammary gland tissue and milk synthesis. A comparison of the milk extracellular vesicles miRNA profiles of heat-stressed (THI = 79.6) and normal (THI = 53.8) Holstein cows revealed that the DE miRNAs play a role in cell apoptosis, autophagy, and the p38 MAPK signaling pathway, suggesting that milk extracellular vesicles miRNA are important regulators in the damage response of heat-stressed dairy cows (Wang et al., 2022a). Additionally, exosomes are essential for maintaining the normal physiological function of bovine mammary epithelial cells (BMECs) and regulating milk fat and protein synthesis. According to Wang et al. (2022b), serum-derived exosome depletion aggravates the damage of BMECs induced by heat stress, exacerbates apoptosis and oxidative stress, and inhibits milk fat and protein synthesis. Moreover, supplementing inulin in the diet changes the serum extracellular vesicles miRNA expression profile of mastitis cows, indicating that DE miRNAs are involved in lipid oxidation and metabolism, immunity, and inflammation (Yu et al., 2023).

Therefore, extracellular vesicles may represent a novel molecular mechanism regulating heat stress in dairy cows. Exploring the signal transduction and effects of extracellular vesicles within the microbe-rumen-mammary gland axis could elucidate heat stress mechanisms and offer novel prevention and treatment strategies.

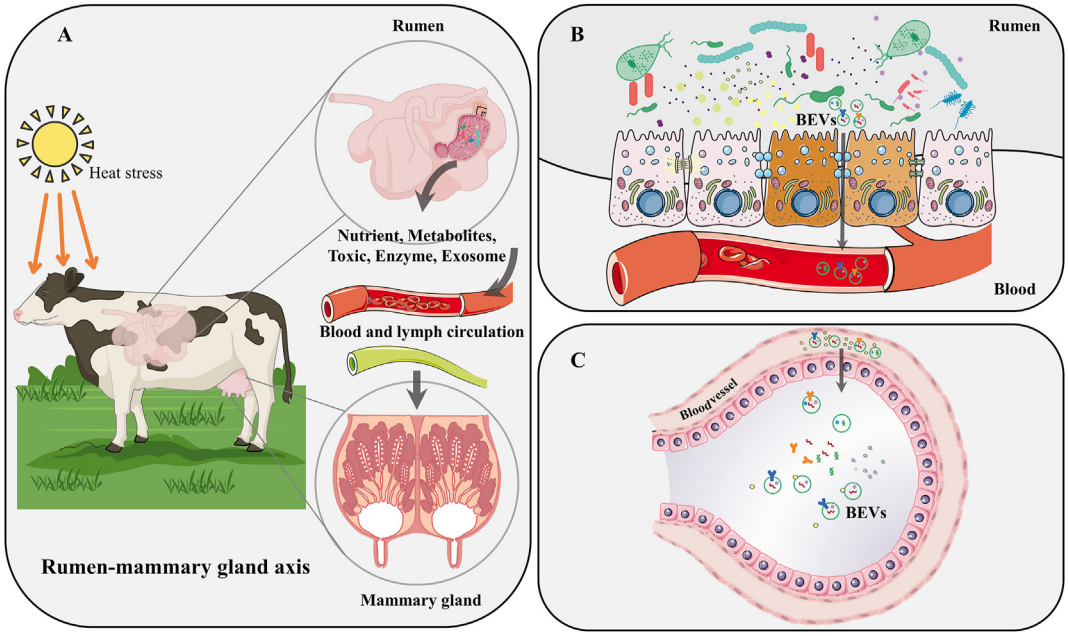

Fig. 1. The mechanism of rumen–mammary gland axis under heat stress in dairy cows. (A) Under heat stress conditions, nutrients, metabolites, toxins, enzymes, and exosomes produced by rumen microbes can enter the mammary gland through the blood and lymph circulation, and thereby affect mammary functions and health. (B) Rumen microbes can release bacterial extracellular vesicles (BEVs) to regulate the rumen barrier function and cross the rumen epithelial cells to enter the blood circulation and reach distant tissues. (C) Bacterial extracellular vesicles in the blood can regulate the permeability of the blood–milk barrier and cross the mammary epithelial cells to enter the milk.

5. Conclusion

This review comprehensively outlines the latest advancements in understanding extracellular vesicles and the rumen-mammary gland axis, elucidating their roles and mechanisms in regulating heat stress in dairy cows. Furthermore, it proposes a theoretical hypothesis that heat stress poses threats to dairy cows via the rumen-mammary gland axis by regulating rumen microbiota and their secreted extracellular vesicles (Fig. 1). The rumen microbiota and their BEVs serve as crucial targets and biomarkers of heat stress. Enhancing mammary gland health through the rumen-mammary gland axis represents viable strategies and research directions for the future prevention and treatment of heat stress in dairy cows. Further investigation is warranted to comprehend the mechanisms by which heat stress influence the rumen microbiota and their BEVs, as well as their impact on rumen fermentation, epithelial barrier function, and mammary gland function via the rumen-mammary gland axis. However, it should be noted that the current knowledge of the molecular cargo and biogenesis of BEVs in ruminants is limited. Moreover, data on the effects of BEVs on ruminants are scarce. Future investigations should employ multi-omics methods to uncover the molecular profiles and functions of BEVs, exploring the intricate interactions and feedback loops between BEVs and the host. Furthermore, novel strategies should be developed to modulate the production and composition of BEVs, aiming to improve the health and productivity of dairy cows under heat stress conditions.

Author contributions

Qi Huang: Investigation, Writing–Original draft preparation. **Yang Xiao**: Writing–Review & editing. **Peng Sun**: Supervision, Writing–Review & editing, Funding acquisition, Project administration, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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