Effects of Heat Stress on Milk Protein Content and Composition and the Mechanism of Action: Postprint

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Abstract

Heat stress not only affects dairy cow health, but also predisposes to the occurrence of “heat stress-induced milk protein reduction syndrome”, thereby affecting the nutritional quality of milk. This article reviews recent research advances on the effects of heat stress on milk protein content and the fractions of casein and whey protein, discusses the pathogenic mechanism of heat stress-induced “heat stress-induced milk protein reduction syndrome” , aiming to provide reference for alleviating heat stress in dairy cows and improving milk quality.

Full Text

Preamble

Effects of Heat Stress on Milk Protein Content and Components in Cow’ s Milk and Its Mechanisms

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Abstract: Heat stress not only affects dairy cow health but also leads to the occurrence of “heat-stressed milk protein decrease syndrome,” thereby compromising milk nutritional quality. This paper reviews recent research advances on the effects of heat stress on milk protein content and the casein and whey protein components in cow’ s milk, and explores the mechanisms underlying heat stress-induced "heat-stressed milk protein decrease syndrome," with the aim of providing references for alleviating heat stress in dairy cows and improving milk quality.

Keywords: heat stress; dairy cow; heat-stressed milk protein decrease syndrome; mechanism

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1 Effects of Heat Stress on Milk Protein Content and Components

summarizes the potential biological functions and content changes of various milk protein components under heat stress conditions. During heat stress, milk protein components such as casein and whey protein undergo alterations [8], leading to decreased contents of casein, -lactoglobulin, and immunoglobulin, while -lactalbumin, lactoferrin contents, and lysozyme activity increase. These changes not only impair immune regulation in dairy cows but also affect the biological functions of milk proteins, including anti-inflammatory, antimicrobial, and antioxidant activities, consequently influencing milk nutritional quality [9] and constraining dairy industry development.

Table 1 Effects of heat stress on milk protein biological function and content in cow' s milk

Milk protein components	Biological function	Effects of heat stress on content
Casein (CN)	Source of amino acids, calcium, and phosphorus; immunomodulatory bioactivity; anti-inflammatory	[10-12]
-lactalbumin	Promotes lactose synthesis; calcium carrier; immunomodulation; potential anticancer activity	[11,13]
-lactoglobulin	Vitamin carrier; potential antioxidant; facilitates absorption of vitamin A and retinoic acid	

Milk protein components	Biological function	Effects of heat stress on content
Immunoglobulin	Natural antibodies; maintains and promotes immune function; reduces plasma cholesterol	
Lactoferrin (Lf)	Antimicrobial; antioxidant; anticancer; anti-inflammatory; immunomodulatory	
Lysozyme (LZM)	Anti-infective factor	

1.1 Effects of Heat Stress on Milk Protein Content in Cow's Milk

Numerous international studies have investigated the effects of heat stress on milk protein content. Beede et al. [16] reported that milk protein synthesis in dairy cows gradually decreases with increasing ambient temperature, with high temperature showing negative correlations with milk fat percentage and milk non-fat solids content (correlation coefficients of -0.23 and -0.61, respectively). Ravagnolo et al. [17] proposed that during heat stress, each unit increase in temperature-humidity index (THI) results in decreases of 12 g and 9 g in milk fat and milk protein synthesis, respectively. Barash et al. [18] reported a negative correlation between high temperature and milk protein synthesis in Holstein cows, with protein synthesis decreasing by 0.01 kg per 1 °C temperature increase.

Chinese researchers have also conducted extensive studies on this topic. Xue et al. [19] found that when THI exceeds 72, milk quality declines, with milk fat and protein percentages in summer being lower than in spring, autumn, and winter, though the differences were not significant. Wang et al. [20] demonstrated that high temperature reduces milk fat, protein, lactose, and non-fat solids contents; when temperature increased from 18 °C to 30 °C, milk yield decreased by 15%, net energy utilization for milk production dropped by 35%, and milk fat, non-fat solids, and protein contents decreased by 39.7%, 18.9%, and 16.9%, respectively. Li et al. [21] reported that heat stress significantly reduces milk fat and protein percentages, with milk fat percentage showing substantial seasonal variation and reaching its lowest level in summer. Cheng et al. [5] found that moderate heat stress significantly reduced feed intake, milk yield, fat-corrected milk yield, energy-corrected milk yield, milk fat percentage, milk protein content, and total milk solids content, while significantly increasing milk urea nitrogen content.

Heat stress leads to decreased milk fat percentage, milk protein percentage, and milk non-fat solids content. Milk fat and protein synthesis are particularly sensitive to heat stress; dairy cows first reduce milk protein synthesis during heat stress, accompanied by a significant increase in milk urea nitrogen content. This alteration in nitrogen metabolism pathways results in what is termed "heat-stressed milk protein decrease syndrome."

1.2 Effects of Heat Stress on Casein in Cow' s Milk

Casein (CN) constitutes over 80% of milk protein and primarily includes S-casein, -casein, -casein, and -casein. In milk, S-casein is the main component, accounting for 45%-55% of total casein, comprising S1-casein and S2-casein. -casein is the second most abundant component, with content comparable to S1-casein, representing 35% and 38% of casein, respectively. -casein is a hydrolytic fragment of -casein, existing in three forms (1-, 2-, and 3-casein) based on the starting amino acid residue of the fragment. -casein appears as polymers linked by intermolecular disulfide bonds in a mixture form, distributed throughout the casein micelle and serving to stabilize the micelle structure. Chatterton et al. [22] reported that casein possesses important anti-inflammatory effects and contributes to intestinal health in young animals.

Heat stress affects casein component composition in milk. Moore et al. [10] found that milk from cows experiencing heat stress during the 60 days prepartum showed decreased protein and casein contents. Bernabucci et al. [13] measured -casein, -casein, and -casein contents in spring and summer milk, finding that total casein content decreased by 5.5% in summer compared to spring, with highly significant differences in -casein and -casein contents between seasons, while -casein content was not affected by season. Cowley et al. [12] measured milk protein, casein content, and urea nitrogen concentration, finding that heat stress reduced milk protein and casein contents while increasing milk urea nitrogen concentration. To further investigate casein component changes during heat stress-induced protein reduction, they measured specific casein components and found that heat stress increased total casein and S1-casein contents while decreasing S2-casein content, suggesting that the reduction in casein content was primarily caused by decreased S2-casein. Bernabucci et al. [11] used SDS-PAGE to examine casein component contents in 25 cows at the same lactation stage during winter, spring, and summer, finding that S-casein, -casein, and -casein contents decreased in summer milk, while -casein content was 50% higher than in winter and 59% higher than in spring. Except for -casein, all other casein components decreased in summer, leading to the conclusion that reduced total casein content likely resulted from decreased S-casein and -casein contents.

Although heat stress increased S1-casein content, -casein content significantly decreased under heat stress conditions. Heat stress reduces -casein and -casein contents, leading to decreased total casein content and the manifestation of "heat-stressed milk protein decrease syndrome." Casein serves as the primary source of various bioactive peptides and supplies amino acids, calcium, and phosphorus, with high digestibility and the ability to form curds in the stomach to promote digestion [23]. However, under heat stress conditions, “heat-stressed milk protein decrease syndrome” reduces casein content and diminishes its anti-inflammatory effects [24], potentially compromising milk quality.

1.3 Effects of Heat Stress on Whey Protein in Cow’ s Milk

Whey proteins are those that remain dispersed in whey at pH 4.6. In addition to the predominant casein fraction, cow’ s milk contains certain amounts of whey protein, which accounts for 18%-25% of total milk protein and includes various bioactive proteins such as -lactalbumin (-La), -lactoglobulin (-Lg), immunoglobulins (Ig), lactoferrin (Lf), and lysozyme (LZM). -La functions as a coenzyme in lactose biosynthesis in mammary glands, controlling lactose content [25-26] and serving as a key protein for lactose synthesis and secretion. Research has shown that -La in milk can inhibit cyclooxygenase-2 activity, thereby exerting anti-inflammatory effects [27]. -Lg belongs to the lipid transport protein family, capable of binding fatty acids, vitamins [28], and polyphenols, facilitating the absorption of vitamin A and retinoic acid. Immunoglobulins (including IgG, IgA, and IgM) are natural antibody components in milk and plasma, important for maintaining and promoting immune function [29] and capable of reducing plasma cholesterol content to lower blood pressure [30-31]. Lf possesses antimicrobial, antioxidant, anticancer, anti-inflammatory, and immunomodulatory functions. LZM inhibits both Gram-positive and Gram-negative bacteria, and through synergistic action with Lf, can disrupt the outer membrane of Gram-negative bacteria.

Conesa et al. [32] measured IgG content in milk from various regions of Spain, finding seasonal variations in IgG content within the same region, with summer milk showing lower IgG content than spring milk. Ravagnolo et al. [17] found that when THI exceeds 72, the proportions of IgA and IgG in milk decrease. Brodziak et al. [15] reported that milk from cows calving in spring and summer had significantly higher Lf content than that from cows calving in autumn and winter, and that Lf content was significantly higher in milk from free-grazing cows than from restricted-fed cows. Additionally, Brodziak et al. [15] measured LZM activity in spring-summer and autumn-winter seasons, finding higher LZM activity in milk from spring-summer calving cows. Yang et al. [33] investigated seasonal variations in IgA, IgM, and Lf contents in milk from different dairy farms, finding that IgA content was not significantly affected by season, but IgM content showed highly significant differences between spring and summer in 5 out of 6 farms, and Lf content showed highly significant differences in 3 out of 6 farms.

These results indicate that changes in IgG, IgA, IgM, and Lf contents in milk may be influenced by various factors including temperature and geographical location. Bernabucci et al. [13] measured -La and -Ig contents in spring and summer milk, finding no significant differences and concluding that neither was significantly affected by season. However, subsequent research by Bernabucci et al. [11] revealed that -La content was highest in summer and lowest in winter, while -Ig content was lower in summer than in winter, indicating that different seasons have different effects on -La and -Ig contents.

Heat stress affects whey protein component composition in milk, increasing -La content. When dairy cows experience heat stress, IgG, IgM, and IgA contents in whey protein decrease, impairing functions such as bacterial metabolism inhibition, bacterial aggregation, and enhanced phagocytic activity. The increased LZM activity and Lf content under heat stress conditions [34] may result from elevated somatic cell counts in milk, as LZM activity increases with somatic cell count and can act synergistically with Lf as a bactericidal agent.

2.1 Regulation of Milk Protein Synthesis in Heat-Stressed Dairy Cows by the AMPK/TSC/mTOR Signaling Pathway

AMP-activated protein kinase (AMPK) is a cellular energy regulator [35] that can be activated by various stimuli and participates in many different types of stress responses [36]. Frederich et al. [37] investigated the effects of different temperature stresses on AMPK activity in rock crabs, finding that AMPK activity remained stable at 12-18 °C but increased 9.1-fold when ambient temperature rose to 18-30 °C, demonstrating that heat stress activates AMPK signaling molecules. Furthermore, Li et al. [38] studied changes in AMPK activity in heat-stressed dairy cows, using bovine-specific enzyme-linked immunosorbent assay kits for rapid detection of AMPK activity in cow blood, and confirmed that heat stress activates AMPK signaling molecules in dairy cows. Although research on the pathways through which AMPK signaling molecules enter the bloodstream remains limited, numerous studies have shown that AMPK signaling molecules regulate milk protein synthesis by acting on protein translation processes through the AMPK/TSC/mTOR signaling pathway [39-41].

Mammalian target of rapamycin (mTOR) is an important downstream target of the AMPK signaling pathway, existing as two protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Burgos et al. [42] demonstrated that energy deficiency in bovine mammary epithelial cells activates AMPK and reduces protein synthesis by inhibiting the mTORC1 signaling pathway. Therefore, heat stress activates AMPK, which inhibits the mTOR signaling pathway, reduces milk protein synthesis in mammary epithelial cells, and leads to "heat-stressed milk protein decrease syndrome" in dairy cows.

2.2 Involvement of Heat Shock Proteins (HSPs) in Heat Stress Response

Heat shock proteins (HSPs) are highly conserved proteins primarily classified into several families based on molecular weight, including HSP90, HSP70, and HSP29. The transcriptional regulation of HSPs mainly depends on heat shock transcription factors (HSF), and heat stress induces the expression of both HSPs and HSF. Dairy cows protect themselves and adapt to heat stress environments by activating HSF and increasing HSP expression [43]. Under heat stress conditions, protein synthesis and expression decrease in the body, which compensates by increasing HSP synthesis to repair damage caused by high temperature and enhance heat stress resistance. HSP70 is the most important member of the HSPs family, closely related to ambient temperature and humidity changes, and plays a major role in biological heat tolerance. Hu et al. [44] used in vitro cultured bovine mammary epithelial cells as a model to investigate the effects of heat stress on mRNA expression levels of HSPs and milk protein synthesis-related genes, finding upregulated expression of HSP27 and HSP70, indicating that heat stress increases HSP synthesis while decreasing milk protein synthesis. “Heat-stressed milk protein decrease syndrome” occurs because when animals are exposed to heat stress, dairy cows significantly increase HSP70 mRNA expression and HSP70 synthesis to protect against irreversible heat-induced damage, resulting in increased total HSP synthesis and suppressed synthesis of normal proteins [43,45], thereby reducing milk protein content.

2.3 Effects of Heat Stress on Synthetic Capacity of Mammary Epithelial Cells

Numerous studies have shown that heat stress reduces cell viability and induces apoptosis [46]. Zhou et al. [47] reported that after high-temperature treatment at 42 °C, bovine mammary epithelial cell growth arrested, with cell numbers significantly decreasing by day 2 of culture, demonstrating that heat stress inhibits growth and induces apoptosis in bovine mammary epithelial cells. Collier et al. [43] found that bovine mammary epithelial cells grew normally at 38 °C, but their growth was inhibited and cells entered an apoptotic state after treatment at 42 °C. Hu et al. [48] observed that heat stress at 42 °C in vitro upregulated expression of the B-cell lymphoma-2-associated X protein (BAX) gene (a pro-apoptotic gene) in mammary epithelial cells, while B-cell lymphoma-2 (Bcl-2) gene (an anti-apoptotic gene) expression was first upregulated then downregulated, indicating that heat stress induces apoptosis in bovine mammary epithelial cells. Additionally, Hu et al. [44] found that high-temperature culture of bovine mammary epithelial cells downregulated expression of casein synthesis genes (CSN2) and milk fat synthesis genes (BTN1A1), reducing total casein content. Gao et al. [49] investigated the relationship between milk protein changes during heat stress and milk protein synthesis-related signaling pathways and mammary cell apoptosis, concluding that heat stress reduces milk protein content and yield by inducing mammary cell apoptosis and decreasing the number of mammary cells available for milk protein synthesis. These results collectively demonstrate that bovine mammary epithelial cells respond to heat stress and that high temperature significantly affects the lactation function of mammary epithelial cells. Thus, heat stress not only inhibits mammary epithelial cell growth and induces apoptosis but also impairs lactation function, reducing the cells’ capacity to synthesize milk protein and ultimately leading to “heat-stressed milk protein decrease syndrome.”

Heat stress causes changes in milk composition, reduces milk protein content, and damages various functional proteins, thereby decreasing milk nutritional quality. Although heat stress affects all milk protein components to varying degrees, many aspects of these effects remain unresolved due to the complexity of the mechanisms and differences in experimental conditions, temperatures, and durations. Further improvements in research techniques are needed to investigate the causes of milk protein content reduction under heat stress, providing a theoretical basis for developing measures to alleviate heat stress in dairy cows and achieve the goals of increasing milk protein yield and improving milk quality.

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