EFFECT OF EVAPORATIVE COOLING ON SYSTEMIC AND MAMMARY INFLAMMATORY RESPONSES OF LACTATING DAIRY COWS DURING SUMMER

by

YUN-CHU CHEN

(Under the Direction of Sha Tao)

ABSTRACT

Heat stress may negatively influence the inflammatory responses both systemically and locally in the mammary gland. The objective of the experiment was to evaluate the effect of heat stress on performance and the systemic and mammary inflammatory response with or without mammary inflammation induced by lipopolysaccharide in lactating Holstein cows. Heat stress impaired cow's performance but did not change the systemic and mammary inflammation in healthy cows. However, following mammary endotoxin challenge, heat stress upregulated the mammary inflammation responses but downregulated the systemic inflammation.

INDEX WORDS: Heat stress, Mammary gland, Lactating cow, Lactation, Inflammation

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DEDICATION

To my beloved parents, Wen-Ling Lee and Chien-Chia Chen. Thank you all for your love and support anytime. You are always my great strength.

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CHAPTER 1

INTRODUCTION

Heat stress is defined as the external forces, including environmental temperature, relative humidity, solar radiation and wind speed, which increase the body temperature of dairy cattle beyond normal range. Heat stress causes significant economic losses in the livestock industry. Across the United States, St-Pierre et al. (2003) estimated $897 to $1500 million loss per year in the dairy industry due to heat stress. In a more recent analyses, Key and Sneeringer (2014) estimated that the reduced milk production due to heat stress in lactating dairy cows was equal to a $1.2 billion reduction in income in the US dairy sector, and Ferreira et al. (2016) estimated the loss due to reduced milk yield by prepartum heat stress is estimated to be approximately $800 million. Under heat stress condition, dairy cows have increased respiration rate and body temperature, and altered hormonal, metabolic, immunological and cellular responses. Common effects of heat stress on dairy cattle include but are not limited to reduced nutrient intake and milk yield, and impaired reproduction and animal health. Implementation of heat stress abatement is critical to maintain performance, health and overall welfare of dairy cows during summer.

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CHAPTER 2

LITERATURE REVIEW

1. Heat stress impacts on dairy cows

1.1.Feed intake

Reduced feed intake is a hallmark response to heat stress effect in lactating dairy cows and partially explain the decreased milk yield. Bouraoui et al. (2002) reported a 9.6% (1.73 kg/d) decrease in dry matter intake (**DMI**) but a 21% decrease in milk yield in lactating dairy cows when temperature-humidity index (**THI**) increased from 68 to 78. Milk yield and total digestible nutrient (**TDN**) of dairy cattle decreased 1.8 and 1.4 kg/d, respectively, when rectal temperature increased each 0.55 ℃ (West, 2003). Baumgard and Rhoads (2012) suggested that the decrease in milk synthesis by heat stress can be explained by both reduced DMI and mechanisms unrelated to intake. Indeed, Rhoads et al. (2009) and Wheelock et al. (2010) reported that the reduced DMI only account for 35 to 50% of the decrease in milk yield by heat stress.

1.2.Milk yield

Milk synthesis is sensitive to changes in ambient environment. When dairy cows are exposed to heat stress, the milk yield decreases. In the dairy industry, THI is widely used to evaluate the heat stress environment. It is a combined measure of relative humidity and ambient temperature but does not incorporate solar radiation and wind speed. External

or condition of heat define as heat stress which causes animals get strain in their body. Depending on strain evaluated, the THI threshold for heat stress varies (Tao et al., 2020). Regarding milk yield, past research reported that milk yield of lactating dairy cows starts to decrease when THI exceeds 72 (Armstrong, 1994). However, in modern high producing dairy cows (produce more than 35 kg/d), Zimbelman et al. (2009) reported that milk yield began to decrease when daily average THI was equal to 68. The milk yield response of a cow to heat stress is influenced by various factors.

1.2.1. Parity.

Parity has a strong effect on the animal's responses to heat stress. Milk yield is positively correlated with the cow's metabolic heat production. Because of the lower milk yield, primiparous cows generate less metabolic heat compared with multiparous cows. Therefore, primiparous cows were less susceptible to heat stress compare with multiparous dairy cows (Tao et al., 2020).

1.2.2. Stage of lactation.

Compared with dry cows, lactating dairy cows maintained greater body temperature when exposed to the same level of heat stress, suggesting greater susceptibility to heat stress (Araki et al., 1984). During lactation, higher milk yield is associated with greater metabolic heat production and results in greater susceptibility to heat stress. Compared with early- and late-lactation cows, mid-lactation cows have the highest milk yield and greatest metabolic heat production. Maust et al. (1972) separated dairy cows into three group: early lactation (<100 days in milk), mid-lactation (100-180 days in milk), and late lactation (180-260 days in milk). Under heat stress condition, all cows had increased body temperature, and the stage of lactation was not correlated with

body temperature response to heat stress. However, the decline in milk yield was greatest for cows in mid-lactation, the lowest for cows in early-lactation and intermediate for cows in late-lactation. Moreover, Tao et al. (2018) reported that deprivation of evaporative cooling during summer resulted in the greatest milk loss in mid lactation cows relative to early- and late-lactation cows (13.6, 9.3, and 9.3 kg/d, respectively) compared with those with evaporative cooling.

1.3.Milk composition

1.3.1. Protein.

A seasonal effect in on milk composition has been observed in the commercial dairies. Milk protein percentage in summer was 9.9% lower than spring, but milk collected in the winter has significant higher protein percentage than milk collected in both spring and summer (Bernabucci et al., 2002; Bernabucci et al., 2015). Gorniak (2015) reported that milk protein percentage decreases when THI exceeds 60. In contrast, Summer et al. (2019) reported that milk protein content declines when THI was greater than 75. In a controlled study, both milk protein percentage and yield were decrease by heat stress (Grummer, 1991). Similarly, Cowley et al. (2015) reported lower milk true protein concentration when dairy cows were under heat stress (THI $\geq$ 78), milk true protein concentration was lower than cows under thermoneutral condition where THI < 70.

The main types of milk protein include caseins and whey proteins. Approximately 77% of total milk protein are comprised by caseins (Summer et al., 2019). Cowley et al. (2015) reported that when dairy cattle were exposed to heat stress, milk casein concentration decreased. Milk casein is generally composed of 5 fractions, namely α_{s1},

α_{s2}, β, κ, and γ. Milk percentages of α_{s1}, β, and κ casein were lower during summer than winter, with spring intermediate. In contrast, concentrations of milk γ-casein were higher in the summer compared with spring and winter (Bernabucci et al., 2015). However, Cowley et al. (2015) reported that heat stress significantly lowered milk concentrations of both α_{s1} and α_{s2} casein without impacting on milk β and κ casein concentrations compared with thermoneutrality. The discrepancies between studies may attribute to experimental design (observational vs. controlled studies), diets used, and the manipulation of intake (ad libitum vs. pair-fed thermoneutral cows).

1.3.2. Fat.

Heat stress has been reported to decrease both milk fat percentage and yield in lactating dairy cows (Bouraoui et al., 2002; Vesna Gantner and Drago Solić, 2011; Gorniak, 2015). However, Grummer (1991) reported no difference in milk fat percentage for cows exposed to heat stress or thermal neutral conditions. Milk lipid composition, such as triacylglycerol (TAG) and polar lipid composition were significantly altered by heat stress. During summer or heat-stressed conditions, concentrations of TAG that containing short-chain fatty acids (C4-C10) and medium-chain fatty acids (C12-C16) decreased, while those with long chain fatty acids (≥ C18) especially C18:0, C18:1 and C18:2 significantly increased (Liu et al., 2017).

1.3.3. Lactose.

The reported impact of heat stress on milk lactose percentage are not consistent. Joksimovic-Todorovic et al. (2011) reported that the percentage of milk lactose percentage varied between milk collected during spring and summer (4.45 ± 0.54% and 4.03 ± 0.24% respectively). In contrast, milk lactose concentration and yield were not

different between heat-stressed cows and pair-fed thermoneutral cows (Gao et al., 2017). Cowley et al. (2015) also reported that heat stress did not affect lactose concentrations; however, Nardone et al. (1997) demonstrated that high air temperature in late pregnancy and the early postpartum period reduced colostrum lactose percentage in primiparous cows.

1.4.Mammary health

1.4.1. Somatic cell count (SCC).

The main components of somatic cell in milk are mammary epithelial cells, macrophages, and polymorphonuclear cells. Without infection, SCC are low in milk but increases with the age and declines with increased milk yield and days in milk (Schukken et al., 2003). During mammary infection or inflammation, bacteria enter the mammary gland through teat canal and subsequently are identified by local macrophages and mammary epithelial cells. These cells release proinflammatory cytokines and chemokines that attract additional immune cells, such as neutrophils, to migrate into the mammary gland, elevating SCC (Alhussien and Dang, 2018). Neutrophils comprise the majority of somatic cells in milk during an infection. Thus, SCC reflect the inflammatory response of the mammary gland. Because of the genetic selection, the milk yield of dairy cattle has increases which also resulted in an increased the risk of mammary infection. In the USA, the legal limit of milk SCC is 750×10^3 cells/mL, but most of producers maintain milk SCC below 400×10^3 cells/mL which meet European Union standards (Ruegg, 2017; Alhussien and Dang, 2018). During summer, the environment characterized by high ambient temperature and humidity support bacteria growth and survival, leading to a

higher risk of mammary infection (Tao et al., 2018). Olde Riekerink et al. (2007) reported highest bulk tank SCC in August and September within a year.

1.4.2. Mastitis.

Mastitis is the most common and detrimental disease in dairy cows. It is caused by mammary inflammation due to bacteria invasion. It leads to enormous economic loss of the dairy industry. Mastitis can be as either categorized clinical or subclinical mastitis. Clinical mastitis is visible and usually recognized by abnormal milk and udder swelling and redness. Subclinical mastitis does not change the appearance of milk or the udder appearance and is invisible to visual inspection. Subclinical mastitis defined as milk containing exceeding 200×10^3 cells/mL (Dohoo and Leslie, 1991). The incidence of clinical mastitis is highest in summer compared with other seasons. That is partly because the summer condition promotes pathogen survival and proliferation relative to dry and cold weather in winter. Additionally, heat stress impairs the immune function of a cow which also contribute to increased milk SCC and clinical mastitis incidence (Tao et al., 2018).

2. The responses of dairy cows to heat stress

When first exposed to heat stress, the first noticeable effect is the reduced DMI, which partially results in the decreased body weight (Shwartz et al., 2009). Reduced energy intake may also cause negative energy balance of lactating cows (Wheelock et al., 2010). However, the reduced DMI only explains 35-50% of the reduction in milk yield (Shwartz et al., 2009; Rhoads et al., 2010). The other 50 to 65% is due to, but are not limited to,

changes in nutrient absorption, increased maintenance requirement and altered endocrine status (Bernabucci et al., 2010).

2.1.Metabolic responses

2.1.1. Metabolic rates.

The thermoneutral zone of lactating dairy cows ranges from 5-25 °C (Kadzere et al., 2002). When the ambient temperature exceeds the upper critical temperature, heat will accumulate in the cow's body. This results in the spending more energy to dissipate heat in an attempt to maintain normal body temperature. As a result, the energy available for production decreases (Collier et al., 1982). The metabolic rate of the dairy cow is associated with feed intake and milk production, which are affected by heat stress. Appropriately 25-30% of the heat increment is derived from the digestive tract and half of heat production is derived nutrient metabolism in tissues (Webster et al., 1975). Therefore, the reduced milk yield and intake may reduce the metabolic heat production of dairy cows. Indeed, Yousef and Johnson (1966) reported that prolonged exposure to heat stress decreases cow oxygen consumption reducing metabolic heat production.

2.1.2. Lipid metabolism.

Heat stress exerts different effects on lipid metabolism at different stages of dairy cattle's life cycle. Monteiro et al. (2016) performed an adrenaline challenge in calves born to prepartum cooled or heat-stressed cows at 8, 29 and 57 days of age, and found that glucose or non-esterified fatty acid (NEFA) responses were not affected by prepartum heat stress. These data suggest that maternal heat stress during late gestation does not affect adrenaline-mediated fat mobilization and hepatic glycogenolysis of the pre-weaned calves. In 7 months-old Holstein bull calves, Yazdi et al. (2016) reported that heat stress increases

circulating NEFA concentrations. However, because both heat-stressed and pair-fed thermoneutral calves had similar increases in circulating NEFA, the increased adipose tissue mobilization by heat stress in post-weaned calves was mainly due to the reduced feed intake. In contrast, although a dramatic reduction in DMI, circulating NEFA concentration remain unchanged when lactating dairy cows were exposed to heat stress, suggesting inhibited adipose tissue mobilization (Wheelock et al., 2010).

In addition to adipose tissue mobilization, heat stress alters fatty acid composition in animal tissues or cells. Tian et al. (2015) reported that heat stress altered lipid metabolism because the concentration of choline and lysophosphatidylcholine in blood were change. These metabolites are regulated by phospholipases A1, A2 and D which were significantly increased by heat stress. Acute heat stress affects fat metabolism in isolated bovine adipocytes. The response of bovine primary adipocytes to lipolytic signals increase in vitro under heat stress condition (Faylon et al., 2015). The plasma concentration of fatty acid also affected by heat stress, because plasma linoleic acid, oleic acid and arachidonic acid concentrations were significantly increased by heat stress in lactating cows (Tian et al., 2015).

2.1.3. Carbohydrate and insulin metabolism.

Certain cell types or tissue, such as erythrocytes and brain, exclusively rely on glucose as an energy substrate (Aschenbach et al., 2010). In non-ruminants, glucose is directly absorbed from intestine (Aschenbach et al., 2010). However, ruminants absorb less than 10% of their glucose supply through the digestive tract (Young, 1977). The main energy sources used by ruminants are volatile fatty acids (VFA) that formed by microbial fermentation of dietary carbohydrates in the rumen (De Koster and Opsomer, 2013). The

primary VFA include acetate, butyrate and propionate. Propionate is the primary gluconeogenic precursors (De Koster and Opsomer, 2013). Circulating glucose in ruminants is mainly produced through hepatic and renal gluconeogenesis (De Koster and Opsomer, 2013). Circulating glucose concentrations are reduced in heat-stressed lactating dairy cows partially due to reduced DMI and associated hepatic gluconeogenesis (Marins et al., 2020).

Despite a less important role in the ruminant, the intestinal glucose absorption may be altered by heat stress. Glucose transporters such as glucose transporters (GLUT) and sodium-glucose co-transporters (SGLT) regulate glucose absorption in intestines. In heat-stressed chicks, intestinal expression of SGLT-1 increases, enhancing glucose absorption (Garriga et al., 2006). In contrast, Habashy et al. (2017) reported that the gene expression of SGLT-5 was up-regulated and SGLT-1 was down-regulated in the ileum of heat-stressed broilers. In pigs, acute heat stress did not affect intestinal SGLT-1 expression but increased GLUT-2 protein expression compared with pigs under thermal neutrality (Pearce et al., 2013b). The GLUT-1 is the major glucose transporter in bovine mammary gland (Zhao et al., 1993). High producing dairy cows were expected with higher mammary gene expression of glucose transporter to support high milk lactose production (Lin et al., 2016). However, Marins et al. (2020) reported that the mammary expression of *SLC2A1* was not different between cooled and non-cooled lactating dairy cows during summer, suggesting that heat stress did not affect GLUT-1 gene expression in the lactating bovine mammary gland.

Blood glucose concentration is tightly regulated by insulin to maintain is one of the major hormones that regulate glucose homeostasis. Insulin is hypoglycemic. Increased

circulating insulin inhibits hepatic gluconeogenesis, increases glycogen synthesis and shunts glucose into muscle and adipose tissue through GLUT-4, decreasing blood glucose concentration. The regulatory functions of insulin in ruminants are similar to monogastric species (Sasaki, 2002). However, the response of tissue to insulin is weaker in ruminants than monogastric animals (Brockman, 1978).

Decreased DMI resulting from by heat stress is accompanied with increased or unaltered blood insulin concentration (Wheelock et al., 2010; Baumgard et al., 2011). When the circulating insulin concentration was calculated as the percentage of DMI, blood insulin concentration was elevated in heat-stressed mid-lactation dairy cows (Min et al., 2015). These data suggest that heat stress increases insulin secretion without regard to feed intake. Despite the reduced DMI, heat-stressed mid-lactation cows experience reduced mobilization of adipose tissue and increased glucose utilization by tissues rather than mammary gland (Wheelock et al., 2010). Increased insulin may partly explain these effects. In contrast, during the dry period, non-cooled heat-stressed cows have similar plasma insulin and glucose concentrations in the blood and insulin sensitivity on peripheral tissues compared with cooled cows (Tao et al., 2012). During the neonatal period, calves born to heat-stressed dry cows maintain similar circulating insulin and glucose concentrations but have greater glucose utilization by insulin independent tissues compared to calves born from cooled dry cows (Monteiro et al., 2016).The different results regarding glucose and insulin responses obtained from the dry, early- and mid-lactation cow suggest different metabolic responses to heat stress, and may result from different energy status of cows at different stage of lactation cycle.

2.2.Endocrine responses

2.2.1. Leptin.

Heat stress increased blood leptin and adiponectin concentrations in ruminants (Min et al., 2015; Al-Dawood, 2017). In mice, exposure to chronic heat stress also upregulates the leptin and adiponectin signaling pathways in adipose tissue, muscle and liver (Patrizia et al., 2012). The blood leptin and adiponectin increased in goat during heat stress (Al-Dawood, 2017). However, during the summer, cows provided with 8 cooling session each day had 1.4 times higher circulating leptin concentration compare with those that received 5 cooling session per day (Kleinjan-Elazary et al., 2020). The effects of altered leptin by heat stress are not clear, but may mediate body weight losses of the cows. Supplying human leptin during early lactation caused modest body weight loss and increased concentrations of T3 and T4 in dairy cows (Richard et al., 2016). Leptin and adiponectin also activate proinflammatory responses in placenta and adipose tissue (Lappas et al., 2005). In chicks, supplemental of leptin down-regulated the gene expression of heat shock protein (HSP) 70 (Figueiredo et al., 2007). In dairy cows, Min et al. (2015) reported that heat stress increases both serum leptin and HSP70 concentrations.

2.2.2. Cortisol.

The increases in salivary and blood cortisol concentrations are usually used as markers of stress in animals (Hellhammer et al., 2009; Caroprese et al., 2010). During systemic inflammation induced by intravenous injection of a LPS bolus, circulating cortisol concentration was also increased in ruminants (Herman et al., 2010; Marins et al., 2021, in press). In broilers, serum cortisol concentrations significantly increase during heat stress (Sohail et al., 2010). Upon exposure to a stressor, hypothalamus-pituitary-adrenal (HPA)

axis activated and corticotropin-releasing hormone released stimulating the secretion of adrenocorticotropic hormone (ACTH) from pituitary gland. The ACTH in turn triggers cortisol secretion from the adrenal gland. In dairy cattle, acute exposure to heat stress is reported to increase circulating cortisol concentration. In contrast, long-term exposure of heat stress either does not change or decrease blood cortisol concentrations (Christison and Johnson, 1972; Alvarez and Johnson, 1973; Hall et al., 2018). These datas suggest that chronic exposure to heat stress may inhibit cortisol secrection in dairy cattle. Increased circulating cortisol concentrations influence immune function and other stress responses of the animal. For instance, increased plasma cortisol concentrations down-regulates L-selectin expression on neutrophils of stressed animals. This further causes impaired neutrophil extravasation into infected tissue (Das et al., 2016). Increased circulating cortisol may also affect tissue HSP70 expression (Basu et al., 2001). Sharma et al. (2013) reported that increased serum cortisol concentration upregulates the gene expression of HSP from peripheral blood mononuclear (PBMC) in heat stress goats.

The changes in circulating glucocorticoids in response to heat stress may also affect the inflammatory responses by modulating the ratio of T-helper cell 1 (Th1) and T-helper cell 2 (Th2) (Bagath et al., 2019). T-helper cell 1 produces interferon-gamma (IFN-γ) and tumor necrosis factor (TNF)-β, which actives macrophages. T-helper cell 2 produces interleukin (IL)-10 that inhibits macrophages (Romagnani, 1999). Glucocorticoids are reported to upregulate proinflammatory cytokines such as IL-1, TNF-α and IFN-γ and downregulate anti-inflammatory cytokines such as IL-10 and IL-4 (Webster et al., 2002).

2.2.3. Thyroid hormone.

Thyroid hormones are important in growth regulation and maintaining basal metabolic rate. Magdub et al. (1982) reported lower plasma concentrations of triiodothyronine (T3) and thyroxine (T4) in heat-stressed lactating cows. In heat-stressed goats, circulating T4 was decreased but T3 concentrations remained unchanged (Sharma et al., 2013). Similarly, blood T3 concentrations in buffaloes were lower during summer compared with winter (Habeeb et al., 2000). Reduced in thyroid hormone secretion might be an adaptive mechanism in heat-stressed animals to decrease metabolic heat production (Marai and Haeeb, 2010; Bagath et al., 2019).

2.2.4. Prolactin.

Prolactin (PRL) is a peptide hormone released from pituitary gland. It is associated with more than 300 functions in the animals such as cell proliferation, differentiation and survival (Yu-Lee, 2002). It also plays a critical role in lactogenesis and galactopoietics in dairy cows (Lacasse et al., 2016). The concentration of plasma prolactin increases in heat-stressed dry dairy cows (Tao et al., 2018). Interestingly, the reduced DMI alone also decreases blood prolactin concentrations in dairy cows (Bernabucci et al., 2010). However, Ronchi et al. (2001) reported that feed restriction in dairy heifers did not affect circulating PRL concentrations under elevated air temperature. Interestingly, the elevated PRL concentration in response to the blood by heat stress results in reduced gene expression of PRL receptor in circulating lymphocytes (Amaral et al., 2010; do Amaral et al., 2011). This leads to downregulate PRL signaling negatively affecting immune function of heat-stressed dairy cows (Amaral et al., 2010). In monogastric animals, circulating PRL is positively associated with milk yield. In dairy cattle, PRL is also suggested to be responsible for

maintaining milk synthesis (Lacasse et al., 2016). However, if altered PRL secretion by heat stress could affects mammary gland development, thereby altering milk synthesis is not clear. In contrast, altered circulating PRL and associated PRL signaling in the mammary gland by heat stress are suggested to affect mammary involution by impeding the mammary apoptosis during the early dry period (Accorsi et al., 2002; Tao et al., 2011). This negatively affects the following mammary growth and milk yield of cows in the following lactation (Tao et al., 2011).

2.2.5. Growth hormone.

Environmental stressors disrupt homeostasis, altering physical behavior and physiology to adapt the changing environment. The hypothalamic-pituitary-adrenal (HPA) axis is responsible for regulation of the neuro-endocrine responses to heat stress (Bernabucci et al., 2010; Afsal et al., 2018). Stress might stimulate hypothalamus to release corticotropin-release hormone (CRH) which can stimulate adrenocorticotropic hormone (ACTH) secretion from pituitary that and then induces glucocorticoid synthesis (Backström and Winberg, 2013). Corticotropin-release hormone has also been shown to stimulate the hypothalamus to secrete of somatostatin (Riedel et al., 1998). Increase somatostatin release is one possible reason why the reduced blood concentrations of growth hormone and thyroid hormones were observed in animals under heat stress (Bernabucci et al., 2010). McGuire et al. (1990) reported that plasma somatotropin concentration tended to decrease during heat stress. However, Rhoads et al. (2009) reported that plasma growth hormone concentrations were not different between thermoneutral and heat-stressed lactating dairy cows. Injection of bovine somatotropin (bST) to lactating dairy cows increases heat production and elevated the cow's rectal temperature (Mohammed and

Johnson, 1985; Elvinger et al., 1992). Administration of bST remains effective for increasing milk yield during heat stress (Mohammed and Johnson, 1985; Elvinger et al., 1992).

2.2.6. Reproductive hormones.

Hormones that regulate ovarian activity are gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol and progesterone. Heat stress reduced the length and intensity of estrus by affecting the secretion of these reproductive hormones (Das et al., 2016). Although reports with regarding to the effect of heat stress on the release of LH secretion were not consistent, the majority of research suggest that circulating LH decreases for animals experiencing heat stress environment (Rensis and Scaramuzzi, 2003). Further, Breen et al. (2005) reported that infusing cortisol in ewes suppressed the intensity of LH pulses by 35%. Low LH concentrations may impede follicle development, which in turn reduces estradiol secretion.

Heat stress also decreases the plasma concentrations of inhibin that elevates FSH secretion in dairy cattle (Roth et al., 2000). However, Vanselow et al. (2016) reported increased expression of follistatin is also increased which might be related to the reduced in synthesis of FSH and LH in the pituitary gland. Although inconsistent, these results may suggest that heat stress affects follicular dynamics impairing reproductive efficiency.

Circulating estradiol concentrations decreased for animals under heat stress. In heat-stressed laying hens, blood estradiol concentrations were significantly reduced (Rozenboim et al., 2007). Similarly, serum estradiol concentrations in dairy cows decreased under heat stress conditions (Wilson et al., 1998). Estradiol release from bovine follicles was lower when in vitro culture temperature was 41 °C compared with 37 or 39

°C (Bridges et al., 2005). Plasma progesterone concentration is related to oocyte maturation and embryo development (Wolfenson et al., 2000; Das et al., 2016). However, the effect of heat stress on circulating progesterone is inconsistently. When exposed to heat stress, circulating progesterone concentrations increased (Bridges et al., 2005), decreased (Wolfenson et al., 2000) or unchanged (Vanselow et al., 2016) in dairy cattle. These discrepancies may result from different degrees of heat stress or different levels of DMI of cows (Rensis and Scaramuzzi, 2003).

2.3.Immune responses to heat stress

Heat stress affects the immune function of dairy cattle in different stages of her life cycle. It is reported that heat stress affects hematological profiles of the dairy cow. In transition dairy cows, circulating white blood cells (WBC) and neutrophils concentrations were reduced under heat stress (Zachut et al., 2020). Similarly, Thompson et al. (2014) reported that prepartum heat-stressed cows had lower circulating WBC and neutrophils concentrations in early lactation compared with prepartum cooled cows. In contrast, blood neutrophil concentrations in lactating buffalo were higher during the increased in hot season compared with temperate climate in lactating buffalo (Yadav et al., 2016). The discrepancies with regard to the different impact of heat stress on hematological profiles may be explained by different species, stage of lactation, and various experimental models utilized in different experiment (Bagath et al., 2019).

2.3.1. Heat stress impact on innate immunity.

The immune system includes both innate and adaptive arms. Innate immunity provides a non-specific and rapid protection to the animal in the face of infection. Heat stress was reported to suppress innate immune function of dairy cows (Becker et al., 2020).

Neutrophils are the most abundant innate immune cells in the circulation and the first line defender from infection. Neutrophils engulf bacteria through a mechanism called phagocytosis, and kill the pathogen via oxidative burst (Amulic et al., 2012). Under in vitro condition, neutrophils isolated from thermo-neutral cows displayed reduced phagocytosis and oxidative burst when incubated under elevated temperature (Lecchi et al., 2016). During the dry period, neutrophil phagocytosis and oxidative burst were higher in cooling cows compared with heat stress cows (Dahl et al., 2020). But after parturition, both phagocytosis and oxidative burst were lower in prepartum heat stress cows than those cooled before calving (do Amaral et al., 2011). Heat-stressed heifers had similar neutrophil phagocytosis and oxidative burst compared with thermoneutral heifers (Dahl et al., 2020). Overall, these data suggest that heat stress may have negative effects on neutrophil phagocytosis and oxidative burst in mature cows (Amaral et al., 2010; Contreras-Jodar et al., 2018) but have little effect on neutrophil function of dairy heifers (Dahl et al., 2020).

2.3.2. Heat stress impact on adaptive immunity.

Adaptive immunity can be separated into cell-mediated and humoral immunity. T-lymphocytes mediate cell-mediated immunity in response to antigen-specific signals. Upon stimulation, T-helper cells proliferate and release cytokines, and cytotoxic T cells are the effector cells of the cell-mediated immune responses (Pearce et al., 2013a; Bagath et al., 2019). Heat stress effects on cell-mediated immunity are inconsistently reported. Lacetera et al. (2005) reported that DNA synthesis of PBMC was lower or unchanged in cows calving in the summer compared with spring. In vitro, Elvinger et al. (1991) indicated that the in vitro proliferation of lymphocytes isolated from cows maintained in thermal neutral conditions decreased when cells were cultured at 42℃ compared with 37℃. Blood

mononuclear cells isolated from non-cooled, heat-stressed mid-lactation cows had similar lipopolysaccharide stimulated proliferative response ex vivo compared with those from cooled cows (Marins et al., 2020). In ewes, exposure to solar radiation decreases lymphocyte proliferation ex vivo (Sevi and Caroprese, 2012). Different experimental conditions (e.g., in vivo vs. in vitro), distinct breed or species of animals, various duration and level of heat exposure may partially explain the inconsistent results (Lacetera et al., 2006).

Humoral immunity is mediated by B-lymphocytes that produce antibodies against antigens. In dairy cows, exposure to heat stress during the dry period lowers IgG production against ovalbumin challenge during the dry and early lactating periods (do Amaral et al., 2011; Gomes et al., 2014). In early lactation, deprivation of evaporative cooling reduces circulating immunoglobulins (IgA, IgG, IgM) concentrations of dairy cows in summer (Safa et al., 2019). However, IgM secretion by PBMC in vitro from cows that calved in the spring was lower than those that calved in the summer. The differences between studies are possibly due to different experimental design (Lacetera et al., 2005).

2.3.3. Inflammatory Responses.

Heat stress alters plasma concentration and tissue gene expression of inflammatory cytokines in various species including humans, chickens, and dairy cows with inconsistent results (Starkie et al., 2005; Ohtsu et al., 2015; Chen et al., 2018). Koch et al. (2019) reported that the gene expression of TNF-α, IL-6, IL-10, C-X-C motif chemokine ligand 5 (CXCL5) from jejunal mucosa were not different between heat-stressed and pair-fed thermal neutral cows. However, Safa et al. (2019) reported that plasma concentrations of TNF-α were reduced by deprivation of evaporative cooling in early lactation cows (THI =

83.4). In contrast, Zhang et al. (2014) reported that circulating concentrations of both TNF-α and IL-10 increased in cows exposure to high THI (THI = 80.3) compared with moderate THI (THI = 73.9) and low THI (THI = 56.4). Additionally, heat stress during the dry period upregulates the gene expression of TNF-α in circulating blood mononuclear cells during the transition period (Tao et al., 2013). Chen et al. (2018) also reported increased IL-1β, IL-6, IFN-γ, and TNF-α concentrations in plasma of cows under high THI environment (THI = 80.5) compared with those under lower THI environment (THI = 66). Similarly, Min et al. (2016) reported that circulating TNF-α and IL-6 concentrations was increased in dairy cows exposed to chronic moderate heat stress; however, plasma IL-1β concentration was not affected. These results provide evidence that heat stress may elicit acute-phase responses in dairy cattle (Tao and Dahl, 2013). A summarization of the result of heat stress impact on inflammatory cytokine production is presented in Table 2.1.

2.4.Heat shock responses

Heat shock proteins are found in all cells of plants and animals. They protect cells against harmful conditions such as hyperthermia, ultraviolet irradiation and inflammation (Elmore, 2007). As molecular chaperones HSP ensure correct protein folding. Heat shock transcription factors are the first responders to stress. They bind to the heat shock element and stimulate gene expression of HSP when cells are exposed to high ambient temperature. Heat shock proteins are categorized and named according to their molecular weight (e.g., the 70 kDa HSP70 family). Both HSP70 and HSP90 play important roles in the development of thermo-tolerance and protect cells are from heat stress. The HSP70 is a well-studied HSP and found in all organisms from archaebacteria to mammals. When an animal's body temperature increases, HSP70 synthesis increases becoming the most

abundant protein in cells (Daugaard et al., 2007). Therefore, HSP70 is considered as the ideal biological marker for heat stress (Beere, 2004; Collier et al., 2008; Archana et al., 2017; Bagath et al., 2019). Interestingly, extracellular HSP70 was reported to act as damage-associated molecular patterns which interacts with toll-like receptor 4 (TLR4) and triggers innate immunity inducing inflammatory responses (Calderwood et al., 2016). Heat shock proteins also inhibit caspase activation promoting cell survival. Beere (2004) reported that HSP70 prevent the formation of apoptosome by inhibiting cytochrome *c* combining with Apaf-1.

Heat stress alters the gene and protein expression of HSP in dairy cattle. Collier et al. (2006) reported that, under in vitro conditions, gene expression of HSP70 of bovine mammary epithelial cells (BMEC) increases immediately and achieves peak expression at 4h after exposure to elevated culture temperatures. However, after 8 h of exposure, the gene expression of HSP70 declined to the basal concentrations level suggesting that cells lose thermotolerance. In contrast, under *in vivo* conditions, Orellana Rivas et al. (2021) reported that the gene expression of HSP70 of the lactating mammary gland was upregulated by both acute and chronic heat stress. Han et al. (2015) reported that heat stress increased the gene expression of HSP70 in mammary epithelial cells. Compared with spring season (THI = 52.1 - 65.2), lactating cows in the summer (THI = 72.5 - 86.9) have greater circulating HSP70 concentrations and gene expression of HSP70 and HSP901B were upregulated in the mammary gland (Yue et al., 2020). Further, Orellana Rivas et al. (2021) reported that prolonged, but not acute, exposure to heat stress is associated with increased circulating HSP70.

3. The impact of heat stress on mammary gland development

The mammary gland is one of the places to produce heat in cows' body (Gebremedhin and Wu, 2016). Thus, it is not surprised that milk synthesis is impaired by heat stress.

3.1.Nutrient uptake

Heat stress reduces DMI and alters systemic metabolism, decreasing circulating concentrations of glucose, BHB, and triglycerides (Marins et al., 2020). These reduction in circulating nutrient concentrations may partially reduce mammary nutrient uptake. Mammary nutrient uptake also depends on mammary blood flow (Prosser et al., 1996) which is reduced for cows under heat stress compared with those maintained in thermoneutrality (Lough et al., 1990). The decrease in blood flow was mainly due to the reduced DMI rather than directly exposure in high ambient temperature. However, although the level of nutrients changed during heat stress, the expression of nutrient transporters of the mammary gland are not affected by heat stress (Tao et al., 2018).

3.2.Mammary Apoptosis

3.2.1. Apoptosis.

Apoptosis, or programmed cell death, is a normal but critical physiological process to maintain tissue homeostasis. It also occurs when cells or tissues are injured. Necrosis is the other type of cell death. Necrotic cells release their cellular contents into the surrounding environment leading to tissue inflammation. In contrast, apoptosis does not induce inflammation or damage to adjacent cells or tissue. Apoptosis is triggered by a variety of stimuli or conditions and this cellular process is executed by cysteine aspartic proteases or caspases. Caspases are categorized into initiator caspases (caspases 2, 8, 9,

and 10), executioner caspases (caspases 3, 6, and 7) and inflammatory caspases (caspases 1, 4, 5, 11, 12, and 13). The function of initiator caspases is to start the apoptosis signaling and executioner caspases can lead to apoptosis. For inflammatory caspases, they are involved in the inflammatory cytokine signaling but do not function in the process of apoptosis. Upon induction by upstream signals, initiator caspases form dimers that cleave executioner caspases for proteolytic dismantling of the cells (Beere, 2004; Sakamaki and Satou, 2009; McIlwain et al., 2013).

Apoptosis can be activated by either extrinsic or intrinsic pathways. The extrinsic pathway is mediated by extracellular signals or ligands that trigger apoptosis by binding with death receptors on the cell surface. Common death receptors include tumor necrosis factor (TNF) receptor-1 (TNFR1) and CD95 (also called FAS). Both belong to the TNF receptor superfamily (McIlwain et al., 2013). The FAS contain FAS-associated death domain (FADD) and TNFR1 contain TNFR-associated death domain (TRADD). Both death domains recruit adaptors (Beere, 2004) that transmit a cell death signal from the surface to inside of the cell (Elmore, 2007). Specifically, TRADD recruit FADD that which leads to recruitment, dimerization, and activation of pro-caspase 8 (Beere, 2004; McIlwain et al., 2013).

The intrinsic pathway is initiated by either positive or negative factors. The stimuli of positive factors include toxins, hyperthermia and free radicals whereas negative factors include growth factors, hormone and cytokines. These stimuli initiate apoptosis through the opening of the mitochondrial permeability transition pore that releases mitochondria contents into the cytosol, such as cytochrome *c* (Elmore, 2007). In cytosol, cytochrome *c*

binds with the adaptor protein apoptotic protease-activating factor-1 (APAF1), which recruit pro-caspase-9 to form apoptosome (Beere, 2004).

3.2.2. Apoptosis and heat shock protein.

Heat shock proteins responses to prevent the cell from programed death or damage so it can survive (Beere, 2004). In the intrinsic pathway, HSP prevent pro-apoptotic factors such as cytochrome c from being released from the mitochondria. Bid is one of pro-apoptotic proteins that regulates the cytochrome *c* release from the mitochondria. Both HSP27 and HSP70 are reported to inhibit apoptosis by preventing the activation and translocation of Bid (Beere, 2004). In mouse embryo fibroblasts, increased expression of HSP70 significantly reduced apoptosis induced by TNF-α (Buzzard et al., 1998), suggesting that HSP70 block extrinsic pathway-mediated apoptosis.

3.2.3. Heat stress effects on apoptosis.

Hyperthermia leads to a dysfunctional mammary gland and induces apoptosis and autophagy. Collier et al. (2006) reported that BMEC had increased apoptosis after exposure to elevated culture temperature. The BMEC incubated at 42 ℃ have increased cytochrome *c* content in the cytoplasm suggesting that the heat stress induced apoptosis is through the intrinsic pathway (Chen et al., 2020). Similarly, Du et al. (2008) reported that exposure of BMEC to 40 ℃ decreased the ratio of BCL2/BAX gene expression at 3 h following exposure and increased apoptosis rate at 6 h after heat exposure. In vivo, Orellana Rivas et al. (2021) reported that mammary gene expression of both BCL2 and BAX were higher in non-cooled cows compared with cooled cows, but the ratio of BCL2/BAX was similar between treatments. These results suggested a balance between pro- and anti-apoptotic factors in heat-stressed mammary glands that may prevent apoptosis mediated by intrinsic

pathway. However, mammary gene expression of FAS in non-cooled cows was significantly higher than for cooled cows immediately after initiation of treatment (deprivation of cooling), suggesting upregulated mammary apoptosis during acute heat stress.

3.3.Mammary cell proliferation

Mammary growth is affected by factors such as diet, photoperiod, and heat stress. Wall et al. (2005) reported that dry cows maintained under short-day photoperiod increased mammary cell proliferation before calving resulting in greater milk production in next lactation compared with long-day photoperiod. Nutrition affects mammary cells proliferation as well. Compare with high energy density diets, feeding low-energy density diet lowered the proliferation rate of mammary epithelial cells in early lactation cows (Nørgaard et al., 2005; Dessauge et al., 2011).

The impact of heat stress on mammary cell proliferation has been studied. Adin et al. (2009) reported that the mammary gland of dry cows provided evaporative cooling had higher gene expression of the enzyme associated with fatty acid metabolism compared with dry cows without cooling. These authors suggested that these upregulated gene expressions may reflect higher mammary cell proliferation (Adin et al., 2009). Tao et al. (2011) reported that dry cows without evaporative cooling had lower mammary cell proliferation 20 d before calving compared with prepartum cooled dry cows. The decreased mammary growth resulted in reduced milk yield in the subsequent lactation (Tao et al., 2011). These changes might partially explain the lower milk yield reported with prepartum heat stress. Orellana Rivas et al. (2021) collected mammary biopsy from cooled and non-cooled lactating cows at 7 and 56 d after deprivation of evaporative cooling to assess mammary

growth. Compared with cooled cows, the mammary gland of non-cooled cows had increased mammary cell proliferation at d 56 but not d 7 after cooling deprivation. The results potentially suggests that the mammary gland tended to growth in order to compensate for the reduced milk synthesis when exposed to long-term heat stress. However, the cellular mechanisms are not clear and further research is needed.

4. Conclusions

Heat stress increases the heat load carried by dairy cows, resulting in reduced DMI, lower milk yield, impaired reproduction and weaken health during the summer. These significantly reduce the profitability of the dairy farm. Reduce feed intake and energy balance lead to metabolic change, which are also influenced by increased hormone concentrations such as insulin and cortisol by heat stress. The immune system including innate and adaptive immunity are impacted by heat stress. Studies also indicated that the gene expression or circulating concentration of inflammatory cytokines are changed by heat stress. In dry cows, the reduction in milk yield in the next lactation by heat stress is partially due to the impaired mammary growth before calving. In contrast, during lactation, the effect of heat stress on mammary development is different from the dry period. Additionally, acute or chronic heat stress seem to have distinct impacts on mammary cell turnover. However, if the altered mammary gland development by heat stress explain part of the reduction in milk yield is still unclear. Additionally, research is needed.

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Table2.1. Summary of heat stress impact on changes in cytokine production in cells, tissues or circulation of dairy cows

Period	Sample	Effect on cytokines		Reference
Transition	PBMC[1]	TNF[2]	Increase	Tao et al. (2013)
Postpartum	PBMC	IL-8[3]	Increase	
Dry	Blood neutrophil	IL-1β, TNF-α, IL-8, IL-6	No difference	Thompson et al. (2014)
		IL-10	Increase	
Lactation	Blood	TNF-α, IL-10	Increase	Zhang et al. (2014)
Lactation	Blood	TNF-α, IL-6	Increase	Min et al. (2016)
		IL-1β	No difference	
Lactation	Blood	IL-1β, TNF-α, IL-6, IFN-γ[4]	Increase	Chen et al. (2018)
Lactation	Intestine mucosa	TNF-α, IL-6, IL-10, CXCL5[5]	No difference	Koch et al. (2019)
Postpartum	Blood	TNF-α, IL-1α, IL-2	Low	Safa et al. (2019)
Dry	PBMC	TNF-α	Low	Amaral et al. (2010)
		IL-4, IL-6, IFN-γ	No difference	

[1]Peripheral blood monocular cell
[2]Tumor necrosis factor
[3]Interleukin
[4]Interferon-gamma
[5]C-X-C motif chemokine ligand 5

CHAPTER 3

EFFECT OF EVAPORATIVE COOLING ON SYSTEMIC AND MAMMARY INFLAMMATORY RESPONSES OF LACTATING DAIRY COWS DURING SUMMER[1]

[1]Y.-C. Chen, R. M. Orellana Rivas, T. N. Marins, V. Lacerda, Z. Wang, M. Garrick, H. Liu, J. K. Bernard, P. Melendez and S. Tao. To be submitted to *Journal of Dairy Science*

Abstract

To examine the effect of evaporative cooling on systemic and mammary inflammation of lactating dairy cows during the summer, 30 multiparous Holstein cows (parity = 2.4, DIM = 156 d) were randomly assigned to one of 2 treatments: cooling (**CL**) with fans and misters or not (**NC**). The experiment was divided into a 14 d baseline and a following environmental challenge (36 d). Temperature-humidity index averaged 78.4 during the 36 d experiment. Milk yield and DMI were recorded daily. Blood and milk samples were collected from a subset of cows (n = 9/ per treatment) on d -3, 1, 3, 7, 14, and 28 of the experiment to measure cortisol, interleukin-10 (**IL-10**), tumor necrosis factor-α (**TNF-α**), haptoglobin (**HP**), and lipopolysaccharide binding protein (**LBP**). Mammary biopsies were collected from another subset of cows (n = 6/ per treatment) on d -9, 2, 10, and 36 to analyze gene expression of cytokines and acute phase proteins. Fourteen cows received a bolus of lipopolysaccharides (**LPS**) in left rear quarter on d 30 of the experiment. Blood and milk samples from the LPS infused quarter were collected at -4, 0, 3, 6, 12, 24, 48, 96 and 144 h relative to infusion to analyze inflammatory products. Deprivation of cooling decreased milk yield ($P < 0.01$) and DMI ($P < 0.01$). Compared with CL, plasma cortisol concentration of NC cows was higher on d 1 but lower on d 28 of the experiment (cooling [C] × time [T]: $P < 0.01$). Deprivation of cooling tended to reduce serum IL-10 concentrations on d 14 of the experiment (C × T: $P = 0.02$) but did not ($P \geq 0.50$) affect circulating TNF-α, HP, or LBP. Compared with CL, NC cows tended to have higher ($P = 0.08$) milk IL-10 concentrations but did not affect TNF-α ($P \geq 0.17$) or HP ($P > 0.7$). No differences ($P \geq 0.70$) were observed in mammary tissue gene expression of TNF-α, IL-10 and HP. Milk yield declined after LPS infusion but not affected by treatments. NC cows

had similar TNF-α but had lower IL-10 ($P < 0.01$) and tended to have lower ($P = 0.07$) HP concentrations than CL. Milk IL-10 and TNF-α concentrations were higher ($P < 0.01$) 3 h after LPS infusion (C × T: $P \leq 0.15$) for NC compared with CL. In conclusion, deprivation of evaporative cooling had minimal effects on lactating cows' basal inflammatory status, but upregulated mammary inflammatory responses after mammary inflammation induced by LPS.

Key words: Lactation, inflammation, heat stress

Introduction

Heat stress is defined as the external forces that increase the cow's body temperature. It not only impairs production (Rachid et al., 2002; Wheelock et al., 2010; Gao et al., 2017), but also negatively affects the health of dairy cows. The reduced milk yield due to heat stress represents a $1.2 billion loss per year in the dairy industry (Key et al., 2014). The lower milk yields due to heat stressed lactating dairy cow is partly explained by the accompanying decrease in dry matter intake (**DMI**) (Rhoads et al., 2009), but the contribution of other factors influencing milk synthesis during heat stress are still not clear. Tao et al. (2011) and Orellana Rivas et al. (2021) reported that deprivation of evaporative cooling impacts mammary cell turnover during both dry and lactating periods. This suggests altered mammary gland development and provides a possible reason for the reduced milk and components synthesis by heat stress. Additionally, there is a seasonal effect on mammary health. During the hot summer months, there are often periods of elevated bulk milk somatic cell counts (**SCC**) and a concomitant increase in observed

clinical cases of environmental mastitis (Hogan et al., 1989; Olde Riekerink et al., 2007). This suggest that heat stress negatively affects mammary health.

Heat stress has been reported to impact the inflammatory responses of animals with inconsistent results (Starkie et al., 2005; Ohtsu et al., 2015; Chen et al., 2018). In early lactation dairy cows, plasma concentrations of tumor necrosis factor-α (**TNF-α**) were reduced by deprivation of evaporative cooling (THI = 83.4) (Safa et al., 2019). Zhang et al. (2014) reported that circulating TNF-α and interleukin-10 (**IL-10**) were increased in dairy cows housed high temperature-humidity index (**THI**) period (THI = 80.3) compared with moderate and low THI periods (THI = 73.9 and 56.4). Similarly, Min et al. (2016) reported that circulating TNF-α and interleukin-6 (**IL-6**) concentrations were increased in dairy cows exposed to chronic moderate heat stress compared with no heat stress cows. The inconsistent inflammatory responses to heat stress may depend on different stage of life cycle, the part of sample collection, and experiment model. Synthesis and release of acute phase proteins (**APP**) follow acute inflammation (Horadagoda et al., 1999; Eckersall et al., 2001) or exposure to stress. Increased serum concentrations of haptoglobin (**HP**) and lipopolysaccharide bounding protein (**LBP)** had been observed in finishing pig under heat stress (Cui et al., 2019). In lactating dairy cows, hyperthermia induced by an electric blanket increases the circulating LBP and serum amyloid A (**SAA**) concentrations (Al-Qaisi et al., 2020). Additionally, dairy cows under complex stress (combine multiple stressors: transportation during spring/summer, social isolation and tie stall housing) had also been reported to increase blood SAA and haptoglobin concentrations (Lomborg et al., 2008).

It is important to note that the impact of stress, including heat stress, on mammary inflammatory responses has not been widely studied. Additionally, if heat stress affects responses to mammary infection is seldomly researched. Intramammary LPS infusion is a common experimental model to study mastitis (Rainard and Riollet, 2006). Salama et al. (2020) reported the alteration of milk protein and lactose when dairy goats were infused with lipopolysaccharide (**LPS**) under heat stress conditions. Marins et al. (2019) reported that lactating dairy cows deprived of evaporative cooling during summer had a greater reduction in circulating neutrophils and lymphocytes and had greater increase in plasma lactose concentrations following intramammary LPS infusion compared with cooled cows. These data may suggest that heat stress enhances immune cell migration into the mammary gland following mammary inflammation induced by LPS, indicating stronger mammary inflammatory responses. However, to the best of our knowledge, research on the systemic and mammary inflammatory response to intramammary LPS infusion of heat-stressed lactating dairy cows is lacking. Therefore, we hypothesized that heat stress upregulates systemic and mammary inflammation following mammary inflammation induced by LPS in lactating dairy cows. The objective of this experiment was to evaluate the effects of deprivation of evaporative cooling on the performance and systemic and mammary inflammation of lactating dairy cows with and without an intramammary LPS infusion.

Materials and Methods

Animals and experimental design

The experiment was conducted at the Dairy Research Center on the University of Georgia Tifton campus from June to August 2019. Experimental procedures and animal

handling were approved by the University of Georgia Institutional Animal Care and Use Committee before initiation of the experiment.

Thirty multiparous mid-lactating Holstein cows (parity = 2.4, DIM = 156 d) were randomly assigned to 1 of 2 treatments, cooling (**CL**) or without cooling (**NC**). Cows were housed in the same free stall and managed in the same manner. The experiment was divided into a 14 d baseline period followed by a 36 d environmental challenge. During the baseline period, all cows received evaporative cooling from fans and misters attached to the front face of fans. Fans (0.9-m diameter) were placed on 6-m intervals above feed bunk and free stalls and provided a minimum of 9.5 km/h wind speed at the cow level. Fans were switched on when the air temperature exceeded 20°C and misters were activated when ambient relative humidity ≤ 85%. During the environmental challenge, cooling was provided continuously to CL cows but not to NC cows. The parity and DIM (163 vs. 149 d, SEM = 15 d, respectively, $P = 0.51$) were similar between CL and NC cows at the onset of the environmental challenge. The onset of environmental challenge was considered as the d 1 of the experiment.

Data and sample collection

Air temperature and relative humidity in the barn was monitored every 15 minutes throughout the experiment using the Hobo Pro Series Temp probes (Onset Computer Corp., Pocasset, MA). The THI was calculated based on THI = (1.8 × T + 32) – [(0.55 – 0.0055 × RH) × (1.8 × T – 26)], where T = air temperature (°C) and RH = relative humidity (%) (Council, 1971). Vaginal temperature was measured every 5 min for 4 consecutive d each week in all cows using an iButton (Mouser Electronics, Mansfield, TX) attached to a blank intravaginal implant. Respiration rate was measured for all cows by counting the flank

movement for one min 3 times each week (1330 h, Tuesday, Thursday and Saturday) during the experiment.

The same diet (Table 3.1) was fed to all cows as a total mixed ration (**TMR**) once daily (1600 h) throughout the experiment. Daily feed intake was recorded using the Calan Broadbent feeding system (American Calan Inc., Northwood, NH). Representative forage samples were collected daily and remaining ingredients and TMR were sampled three times each week. Samples were dried in a forced-air oven at 55°C for 48 h to measure DM. Amounts of each dietary ingredient were adjusted according to changes in DM of each ingredient. Ingredient samples were composited weekly and grounded to pass through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Chemical composition (ash [Method 942.05, AOAC, 2000], CP [Leco FP-528 Nitrogen Analyzer, St. Joseph, MO], sugar (DuBois et al., 1956), starch (Hall, 2009), ADF [Method 973.18, AOAC, 2000], ether extract [Method 920.39, AOAC, 2000], neutral detergent fiber treated using amylase and corrected for ash [aNDFom, (Van Soest et al., 1991)] were determined for each dietary ingredient to calculate the composition of the TMR nutrient composition (Table 3.1). Body weight (**BW**) was measured and body condition score (**BCS**) was assessed (Wildman et al., 1982) after the morning milking and before eating (0530 h) every week. Cows were milked twice daily times (0500 and 1700 h) every day, and yield recorded at each milking (Delpro, DeLaval, Kansas City, MO). Milk samples were collected from 2 consecutive milkings each week and stored at 4°C with bronopol-B-14 as a preservative until analyzed for milk components (fat, protein, lactose, SNF, MUN, and SCC) at the Dairy One Cooperative (Ithaca, NY).

On d -3, 1, 3, 7, 14, and 28 of the experiment, additional milk samples were collected from a subset cows (n = 9/treatment) in the afternoon milking. Samples were stored without preservatives and transported to the lab at room temperature within 20 min after collection. Samples were centrifuged at 1,700 × *g* for 15 min at 4°C to obtain skim milk that was stored at -80 °C for until analysis. Blood was drawn from the same subset of cows as the skim milk collection (n = 9/treatment) at d -3, 1, 3, 7, 14, and 28 (1300 h) of the experiment. Samples were collected from coccygeal vessels into additive-free and sodium-heparinized vacutainers and maintained at room temperature for 1 h or on ice before centrifugation at 1,700 × g for 30 min at 4°C to collect plasma and serum, respectively. Samples were stored in -20°C for analysis in the same batch.

Mammary biopsy collections

Mammary biopsies were collected from a subset of cows (n = 6/treatment) that were not involved in the blood and skim milk collection at d -9 (as baseline samples) 2, 10, and 36 of the experiment according to procedures described by Weng et al. (2018). Biopsies were only collected from the rear quarters. Tissues were collected from left rear quarter on d -9 and 10, and from right rear quarter on d 2 and 36 of the experiment. Before biopsy collection, cows were systemically sedated by intravenous injection of xylazine hydrochloride (20 μg/kg of BW; Phoenix Pharmaceuticals, St. Joseph, MO). The skin area to perform tissue collection was carefully shaved and sanitized three times by scrubbing with iodine and 70% ethanol. Three mL of lidocaine hydrochloride (Animal Rx Pharmacy, Atlanta, GA) was subcutaneously injected above the biopsy region for local anesthesia. A 3 cm incision was made to penetrate the skin and connective tissue. Mammary tissue was collected using a rotating stainless-steel cannula with retractable blade connected to a

cordless drill (Farr et al., 1996). Incisions were closed by 18-mm stainless steel Michel wound clips (GerMedUSA, Garden City Park, NY) and sprayed with aerosol bandage (Neogen Corp., Lexington, KY) to prevent infection. The tissue was rinsed with saline, fat trimmed, and placed in 3 mL of RNA*later*™ stabilization solution (Thermo Fisher Scientific, Waltham, MA), and then stored at -80°C until RNA extraction.

Intramammary LPS infusion

Intramammary LPS infusion (**i.m. LPS**) was performed on d 30 during the environmental challenge for a subset of cows (n = 7/treatment) that were not enrolled in the mammary biopsy collection. All cows enrolled in the intramammary LPS infusion were free of mastitis during the experiment and had SCC below 200,000 cells/mL in the d 28 milk sample. During and after i.m. LPS, all cows remained in their respective pens. Two h after morning milking (0700 h), the left rear quarter of each cow were infused with 10 µg of *Escherichia coli* O111:B4 LPS (Sigma-Aldrich, St. Louis, MO; Marins et al., 2019) dissolved in 5 mL pyrogen-free saline solution, and were massaged throughout to move the LPS into the gland cistern. The vaginal temperature was recorded every 5 min from 24 h before through 48 h after i.m. LPS. Blood was drawn from coccygeal vessels of all cows into additive-free and sodium-heparinized vacutainers (Becton Dickinson) at -4, 0, 3, 6, 12, 24, 48, 96, and 144 h relative to infusion to collect serum and plasma, respectively. Additional blood samples were collected into vacutainers containing K3 EDTA vacutainers (Becton Dickinson) at 0, 3, 6, 12, and 24 h relative to infusion for hematologic profile analyzed at the University of Georgia Veterinary Diagnostic Laboratory (Tifton, GA) using the ADVIA 2120i Hematology System (Siemens, Tarry town, NY). Individual milk samples from the left rear quarters were collected at -4, 0 (immediately before infusion), 3,

6, 12, 24, 48, 96, and 144 h relative to infusion to measure milk composition and to collect skim milk as described above. Milk samples were also collected from the right rear quarters for milk composition analysis at the same time as non-infused controls. Before collection, teats were dipped with an iodine-based solution (0.1%) and cleaned using paper towels. The first 10 stream of milk was discarded.

Sample analysis

Plasma or serum concentrations of cortisol (Cortisol Elisa kit, Arbor Assay), LBP (multispecies reactive ELISA kit, Cell Science, Inc., Newburyport, MA), TNF- α (Bovine TNF-α DIY ELISA, Kingfisher Biotech, Inc., Saint Paul, MN), and IL-10 (Bovine IL-10 DIY ELISA, Kingfisher Biotech, Inc.) were analyzed by commercially available ELISA kits following the manufacturers' instructions. Plasma concentrations of HP were determined by a colorimetric method described by Cooke and Arthington (2013). The intra- and inter-assay CV were 5.0 and 6.7% for cortisol, 3.7 and 8.1% for IL-10, 3.2 and 15.8% for TNF-α, 3.5 and 1.5% for HP, and 3.4 and 3.3% for LBP, respectively.

After thawing, skim milk samples were centrifuged at 15,000 × g for 45 min at 4 °C to collect the supernatant before analysis. Concentrations of TNF-α, IL-10, HP, and LBP were analyzed using the same approaches as described above. The intra- and inter-assay CV were 2.9 and 10.8% for IL-10, and 2.5 and 18.6% for TNF-α, and 1.8 and 4.4% for HP. The intra-CV of LBP assay was 11.2%.

Plasma, serum and milk samples collected before and after i.m. LPS were analyzed for TNF-α, IL-10, and HP as described above. For the plasma or serum samples, the intra- and inter-assay CV were 4.6 and 13.0% for IL-10, and 3.2 and 14.4% for TNF-α, 1.0 and 10.1% for HP. For the skim milk samples collected from LPS infused quarters, the intra-

and inter-assay CV were 3.8 and 9.2% for IL-10, and 3.0 and 18.7% for TNF-α, 1.6 and 3.6% for HP.

RNA extraction, cDNA synthesis, and quantitative RT-PCR

Total RNA from the mammary biopsy was extracted using TRI-reagent (Sigma-Aldrich, St. Louis, MO) and PureLink™ RNA Mini Kit (Invitrogen, Carlsbad, CA) with an on-column DNase treatment (PureLink™ DNase Set, Invitrogen) according to the manufacturer's instructions. Following extraction, the second DNase treatment was performed using a Turbo DNA-free kit (Invitrogen). The RNA samples were stored in -80 ℃ until analyses. The cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Foster City, CA). Primers were designed using PrimerQuest (Integrated DNA Technologies, Coralville, IA) (Table 3.2). The *GAPDH* was used as a housekeeping gene and target genes included tumor necrosis factor (*TNF1*), interleukin-10 (*IL10*), haptoglobin (*HP*), serum amyloid A 3 (*SAA3*). Real-time PCR was carried out using cDNA reversed transcribed from 10 ng of the purified RNA and Power SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA) on a StepOnePlus thermocycler (Applied Biosystems). Each reaction followed the condition: 95°C for 10 min for initial denaturation and enzyme activation, 40 cycles of 95 °C for 15 sec and 57 °C for 1 min for amplification. Melting curve analysis was performed for each reaction to ensure specificity of the reaction. The "no template controls" were included in all plates to detect contamination, primer dimers and misprimed products. The $2^{-\Delta\Delta CT}$ method, where CT is the threshold cycle, was used to calculate the relative gene expression. Samples collected d -9 of the experiment were use as calibrator samples.

Statistical analyses

PROC UNIVARIATE of SAS 9.4 (SAS Institute Inc., Cary, NC) was used to calculate THI. Repeated measures data including BW, BCS, vaginal temperature, respiration rate, DMI, milk yield, percentages and yield of milk components, feed efficiency (milk yield/DMI), hematological profiles, plasma, serum or milk concentrations of metabolites, hormones, inflammatory cytokines, and APP, were analyzed using PROC MIXED procedure of SAS 9.4. The statistical models included treatment, time and the interaction between treatment and time as the fixed variables. Cow within the treatment was included as a random variable. Data collected during the baseline (prior to the onset of environmental challenge) were included in the models as a covariate. For data collected after i.m. LPS, the data prior to the i.m. LPS were included in the SAS model as covariates. Least squares means ± standard error of the means were reported. Significance was declared when $P \leq 0.05$ and trends when $0.05 < P \leq 0.15$.

Results

Environmental challenge phase

During the environmental challenge, THI averaged 78.6 ± 4.5 and 78.1 ± 4.5 in pens housing NC and CL cows. The highest THI within a day was observed at 1500 – 1600 h and the lowest observed 0700 h (Figure 3.1). These data suggest that all cows were exposed to similar intensities of heat stress. Compared with CL cows, NC cows had higher ($P < 0.01$) vaginal temperature and respiration rate, but lower ($P \leq 0.03$) BW, DMI, milk yield, FCM, and ECM (Table 3.4, Figure3.2). However, no differences ($P \geq 0.18$) were observed between treatments for BCS, feed efficiency and concentrations of fat, protein,

lactose and solid-not-fat (**SNF**) (Table 3.4). A tendency of treatment by time interaction ($P = 0.11$) found in milk SCS. Non-cool cows had lower somatic cell score (SCS) on d 8 ($P = 0.03$) than CL cows. In contrast, NC cows had higher milk urea nitrogen (**MUN**) concentration ($P = 0.02$) compare with CL cows (Table 3.4). Because of the lower milk yield, NC cows had lower yields of milk protein ($P < 0.01$), lactose ($P = 0.01$) and tended to have lower SNF ($P = 0.07$) compared with CL cows (Table 3.4).

A treatment by time interaction ($P < 0.01$) was observed for plasma cortisol concentrations. Circulating cortisol concentrations were higher ($P = 0.04$) at d 1 for NC but lower ($P < 0.02$) at d 28 of the experiment compared with CL cows (Figure 3.3A). A treatment by time interaction ($P = 0.02$) was also observed for serum IL-10 concentration (Figure 3.3B), because serum IL-10 concentrations for NC tended to be lower at d 14 of the experiment compared with CL cows (Figure 3.3B). No differences were observed for treatment or interaction of treatment by time for circulating TNF-α, HP and LBP concentrations. Compared with CL cows, milk concentration of IL-10 tended to be higher ($P = 0.08$) in NC cows (Figure 3.4A). However, no treatment effect or treatment by time interactions ($P \geq 0.17$) were observed for milk concentrations of TNF-α, HP and LBP (Table 3.4, Figure 3.4B, C, D). In contrast, the yield of milk TNF-α was higher ($P < 0.01$) for CL cows at d 1 and tended to ($P \leq 0.11$) be higher on d 3 and 7 of the experiment compared with NC cows (treatment × time interaction, $P = 0.04$, Figure 3.4F). Compared with CL cows, NC cows resulted in higher ($P = 0.03$) milk HP yield but similar ($P = 0.49$) IL-10 yield. The distinct patterns between milk concentrations and yields of inflammatory products were because of the lower milk yield for NC cows compared with CL cows (Table 3.5).

No differences ($P > 0.10$) were observed in mammary gene expression of cytokines and APP (Table 3.6). A tendency for an interaction of treatment by time interaction ($P = 0.08$) for HP gene expression, however, no treatment effects or tendencies were identified at any time point by PDIFF or SLICE function of SAS (Figure 3.5B).

Intramammary LPS infusion

Regardless of the treatment, i.m. LPS reduced DMI and milk yield (time effect: $P < 0.01$). Compared with CL cows, milk yield and DMI of NC cows remained lower ($P < 0.01$) after i.m. LPS (Figure 3.6A, B). However, relative to the data collected before LPS infusion, NC cows had greater ($P = 0.01$) reduction in DMI on d 4 and tended ($P = 0.09$) to have greater reduction in DMI on d 2 after i.m. LPS compared with CL cows (treatment × time interaction: $P = 0.08$, Figure 3.6D). Both NC and CL cows had similar ($P \geq 0.78$) changes in milk yield relative to pre-infusion (Figure 3.6E). Respiration rate was higher ($P < 0.01$) for NC at 3 h relative to LPS infusion (Figure 3.6C). Vaginal temperature peaked at 6 h after i.m. LPS in both NC and CL cows. Before and after the peak vaginal temperature, the temperature was higher ($P < 0.01$) for NC cows compared with CL cows (Figure 3.7B). (Table 3.7)

Infusion of LPS decreased milk concentration of fat, lactose, and SNF but increased protein, and MUN concentration and SCC in LPS infused quarters (time: $P < 0.01$). No treatment or treatment by time interactions were observed for milk fat. Compared with CL cows, SCC and MUN tended to be higher ($P = 0.07$ and $P = 0.06$) for NC cows; however, milk SNF was lower ($P = 0.02$) for NC than CL cows. Milk protein percentage was higher at 12h ($P < 0.01$) and tended to higher at 24h ($P = 0.13$) relative to LPS infusion in NC compared with CL (Figure 3.8B). A tendency for a treatment by time interaction was

observed for milk lactose concentration ($P = 0.08$) because concentrations were higher for NC ($P < 0.01$) compared with CL 48 h after LPS infusion (Figure 3.8C).

Concentrations of milk fat, protein, lactose, MUN, and SNF decreased but SCC increased in the control quarter (without LPS infusion) (time: $P < 0.01$). Compared with CL cows, NC cows had lower milk concentrations of lactose ($P < 0.01$) and SNF ($P < 0.01$). In contrast, interaction of treatment by time were observed for milk concentrations of fat, SCC, and MUN. Milk fat was higher at 0, 24 144 h and tended to higher at 96 h after i.m. LPS (Figure 3.9A). Milk SCC had the interaction of treatment and time difference, however, no treatments effects on any time point in function of SAS (Figure 3.9B). Milk urea nitrogen tended to higher at 0, 3, 6 h and tended to lower at 24 h and lower at 144 h in the non-infused quarter of NC cows compared with CL cows (Figure 3.9E). (Table 3.8)

Intramammary LPS infusion reduced ($P < 0.01$) circulating concentrations of white blood cells, lymphocytes, neutrophils, monocytes, basophils, and eosinophils without regard to treatment. However, no treatment or treatment by time interaction ($P \geq 0.17$) was observed for hematological profiles except for circulating monocytes. Blood monocyte concentrations were lower for NC cows ($P = 0.04$), especially at 0 h relative to i.m. LPS (treatment × time interaction, $P = 0.11$) compared with CL (Table 3.9; Figure 3.10)

Intramammary LPS infusion did not affect circulating TNF-α regardless of treatment. Relative to pre-infusion level (0 h), LPS infusion slightly reduced serum IL-10 concentration at 24 h (time: $P = 0.12$) (Figure 3.11A) but increased plasma HP concentrations from 12-96 h post infusion (time: $P < 0.01$; Figure 3.11C). Peak concentration of plasma HP was observed at 48 h following infusion. No treatment or treatment by time interaction was observed for serum TNF-α concentration, but NC cows

had lower (treatment: $P < 0.01$) circulating IL-10 and tended to (treatment: $P = 0.07$) have lower HP concentration compared with CL cows after intramammary LPS infusion. Following LPS infusion, milk concentrations of IL-10, TNF-α, and haptoglobin increased (time: $P < 0.01$) regardless of treatment. Peak milk concertation was observed at 6 h for TNF-α, 12 h for IL-10 and 24 - 48 h for haptoglobin following LPS infusion (Figure 3.11D, E, F). Treatment by time interactions tended to differ ($P \leq 0.15$) for milk concentration IL-10 (Figure 3.11D) and TNF-α (Figure 3.11E). Compared with CL cows, milk concentration of IL-10 of NC cows was higher ($P = 0.01$) at 3 h and tended ($P = 0.14$) to be higher at 0 and 96 h relative to LPS infusion (Figure 3.11D). Milk TNF-α concentration was also higher ($P < 0.01$) at 3 h after LPS infusion in NC cows relative to CL cows (Figure 3.11E). Milk haptoglobin concentration tended to be higher (Treatment: $P = 0.10$) in NC compared with CL cows following i.m. LPS. (Table 3.10)

Discussion

Environmental challenge phase

Heat stress caused by interaction of high temperature and relative humidity leads to significant productive and physiological responses in dairy cows, such as increased body temperature and decreased DMI and milk yield during heat stress (West, 2003). In our study, both treatments were exposed to similar THI (daily average ≈ 78.4). However, compared with CL cows, NC cows had higher vaginal temperature and respiration rate. This indicates that deprivation of evaporative cooling increased the heat load of cows. The experimental model used in this study is suitable to examine the impact of heat stress on systemic and mammary inflammation of lactating dairy cows. Interestingly, the differences

in vaginal temperature and respiration rate between CL and NC cows were greater in the first week of the experiment. This suggests that cows have stronger physiological responses under acute heat stress compared with chronic heat stress.

Mammals produce large amounts of metabolic heat but have relatively small area of body surface resulting in difficult heat dissipation. This is particularly important for lactating dairy cows because of the increased metabolic heat production associated with high DMI and milk synthesis. To reduce the heat production, cows under heat stress have decreased their DMI that also reduced milk yield (Kadzere et al., 2002). When daily average THI increased from 68 to 78, DMI decreased by 9.6% and milk yield reduced by 21% (Rachid et al., 2002). Similarly, Gorniak et al. (2014) observed reduced DMI in lactating dairy cows when THI exceeds 60. Zimbelman et al. (2009) also reported that milk yield of lactating dairy cows starts to decline when daily average THI exceeds 68. Decreased nutrient intake is thought to account for 35 to 50% of the decrease in milk yield by heat stress (Rhoads et al., 2009; Wheelock et al., 2010). Additionally, cows tended to utilize energy and nutrient (e.g., glucose) for body functions rather than milk synthesis (Rhoads et al., 2009; Wheelock et al., 2010). However, efficiency measured as ECM to DMI was not different between CL and NC cows. This suggest that the reduced DMI account for most of the reduction in milk yield is response to heat stress in the current study. The lower DMI and milk yield of NC cows compared with CL cows are also consistent with previous research conducted in mid-lactation dairy cows (Weng et al., 2018; Marins et al., 2021, in press). Weng et al. (2018) reported that deprivation of evaporative cooling reduced DMI by 19% (21.6 vs. 26.7 kg/d) and milk yield by 27% (25.9 vs. 35.4 kg/d), and Marins et al. (2021, in press) reported that NC cows had 16% reduction

in DMI (24.1 vs. 28.8 kg/d) and 19% decrease in milk yield (30.4 vs. 37.4 kg/d) compared with CL cows.

The effect of heat stress on milk protein concentrations were inconsistently reported. Consistent with the current experiment, previous studies reported that NC and CL cows had similar milk protein concentrations (Chen et al., 1993; Chan et al., 1997; Weng et al., 2018; Marins et al., 2021, in press). In contrast, Gao et al. (2017) and Rhoads et al. (2009) reported that heat stress decreased milk protein concentrations compared with thermoneutrality. Interestingly, Osei-Amponsah et al. (2020) reported that when THI exceeds 83, the milk protein concentration increases in grazing dairy cows compared with lower THI (THI = 73 - 82 or ≤ 72). The effect of heat stress on milk protein yields is also inconsistent. Gao et al. (2017) reported decreased milk protein yield in cows under heat stress compared with thermal neutrality. However, others reported that protein yield was not influenced by deprivation of evaporative cooling (Chan et al., 1997). In this experiment, protein yield was lower for NC cows due to the lower milk yield compared with CL cows. Milk fat concentrations typically is decreased during summer compared with other seasons (Kadzere et al., 2002). In contrast, milk fat concentration was not affected by heat stress or deprivation of evaporative cooling in controlled studies (Shwartz et al., 2009; Weng et al., 2018), similar to the result observed in this current study. This discrepancy may indicate that factors other than heat stress contribute to the reduced milk fat concentration observed during summer. Milk lactose concentration was not different between treatments in this experiment. This is consistent with Gao et al. (2017) but different from Weng et al. (2018) who reported lower milk lactose concentration for NC compared with CL cows. Different experimental models, diets and management systems may result in the inconsistent impact

on milk composition by heat stress. In the current study, MUN was higher for NC than CL cows which is consistent with previous studies (Gao et al., 2017; Weng et al., 2018; Hou et al., 2021). The increased MUN with heat stress might result from altered N metabolism in the rumen, or increased amino acid deamination in the liver, or both (Ríus, 2019).

Circulating cortisol concentrations were higher in NC cows than CL cows immediately after deprivation of cooling suggesting increased cortisol release under acute heat stress, consistent with previous studies (Hall et al., 2018; Marins et al., 2021, in press). Following the initial increase, the plasma cortisol concentrations for NC cows returned to the similar concentration as CL cows. Interestingly, at d 28 of the experiment, circulating cortisol concentration was lower for NC cows compared with CL cows. Christison and Johnson (1972) reported that cortisol secretion increased when dairy cows were exposed to acute heat stress, but was depressed during chronic heat stress. Tumor necrosis factor-α is a pro-inflammatory cytokine and commonly used as a marker for inflammatory response. Compared with cows housed under temperate environment, circulating TNF-α concentration was higher in lactating dairy cows housed under heat stress condition (Min et al., 2016; Chen et al., 2018). In contrast, Safa et al. (2019) and Marins et al. (2021, in press) reported that deprivation of evaporative cooling reduced circulating TNF-α in lactating dairy cows. Liu et al. (2011) reported that, compared with thermal neutral mice, the TNF-α concentration in ileum tissue remains unchanged when mice were kept exposed in pre-warmed incubator (temperature 35.5±0.5℃, relative humidity 60±5%) until their rectal temperature reach to 39℃, 40℃, 41℃ and 42℃. But TNF-α increased when mice rectal temperature reached to 39℃, 40℃, 41℃ and 42℃ then cool in temperature of 25±0.5℃ for 6h. Interleukin-10 serves as an anti-inflammatory cytokine that is primarily

synthesized by monocytes, Th2 lymphocytes and B lymphocytes (de Waal Malefyt et al., 1992; Opal and DePalo, 2000). At d 14 of the experiment, NC cows tended to have a lower serum IL-10 concentration than CL cows, however, no differences were observed among treatments. These data were consistent with Marins et al. (2021, in press) who reported that deprivation of evaporative cooling did not affect circulating IL-10 in mid lactation dairy cows. In contrast, Zhang et al. (2014) reported that mid-lactating cows under high THI (THI=80.3) had higher circulating IL-10 than cows under low THI (THI=56.4). Acute phase response (**APR**) occurs when animals experience inflammation or are subjected to stress (Ceciliani et al., 2012; Cui et al., 2019). Acute phase proteins were synthesized during APR. The major function of APP includes opsonization of pathogens, eliminate toxic substrates and regulate innate immunity (Ceciliani et al., 2012). Cui et al. (2019) reported that serum HP and LBP concentrations increased when finishing pigs exposure to heat stress. In lactating dairy cows, plasma LBP and SAA concentrations increased when hyperthermia was induced by electric blanket (Al-Qaisi et al., 2020). However, in our currently study, both plasma HP and LBP concentrations were not impacted by treatments. Our results suggested that deprivation of evaporative cooling does not have affect systemic inflammation in healthy lactating dairy cows.

Haptoglobin is not only can be produced in the liver, but is also in the mammary gland (Hiss et al., 2004). Concentrations of APP in milk increase during mastitis, therefore APP are useful indicators of mammary infections or inflammation (Skinner et al., 1991; Paulina and Tadeusz, 2011). Milk TNF-α, HP and LBP concentrations were not different among treatments in this current study. These data indicate that deprivation of evaporative cooling did not induce inflammation in the lactating mammary gland. This is consistent

with our observation of similar milk SCS between CL and NC cows. The effect of evaporative cooling on the yield of milk cytokines and APP reflects the lower milk yield of NC cows compared with CL cows. Consistent with milk concentration, mammary gene expressions of TNF-α and APP were not different between treatments. The similar gene expression of IL-10 observed for both CL and NC cows may indicate that the trend of higher milk IL-10 concentration of NC compared with CL cows was not due to alterations that occurred on the mRNA level.

Intramammary LPS infusion

Milk yield and DMI were reduced by i.m. LPS, consistent with previous studies (Silanikove et al., 2011, Marins et al., 2019). Reduced milk yield following mammary inflammation results from both systemic effects, e.g., reduced DMI, and local effects such as apoptosis and cells damage of the mammary gland (Zhao and Lacasse, 2008; Ballou, 2012). Relative to the pre-infusion levels, NC cows had a similar reduction in milk yield but greater decrease in DMI compared with CL cows. This indicates that heat stress does not affect the milk yield response but results in stronger systemic responses, e.g., DMI, in lactating cows to mammary inflammation induced by LPS. As expect, milk SCC increased in LPS infused quarter. It is commonly observed that circulating immune cells, especially neutrophils, are recruited to the site of infection in response to invading pathogens (Alhussien and Dang, 2018). Interestingly, NC cows tended to have higher SCC in the LPS infused quarter than CL cows. It is important to note that two NC cows had milk samples collected at 12 h post infusion that had SCC higher than the detection limit, therefore, not included in the final analysis. The data suggest a greater immune cell influx into the mammary gland of NC cows than CL cows following i.m. LPS.

Regardless of the treatment, milk fat concentration in both LPS infused and non-infused quarters decreased to the lowest concentration at 6 h relative to i.m. LPS, suggesting reduced mammary fat synthesis. Following i.m. LPS, plasma non-esterified fatty acids concentration reduces following infusion (Pires et al., 2019; Marins et al., 2020). This may inhibit incorporation of pre-formed fat into milk fat in the mammary gland. The fact that no differences were observed among treatments for milk fat concentration following LPS infusion suggested that deprivation of evaporative cooling did not affect on milk fat synthesis in the inflamed mammary gland. The increased milk protein concentration in LPS infused quarter after infusion in both treatments was consistent with previous studies (Aditya et al., 2017; Marins et al., 2019; Salama et al., 2020). As discussed by Marins et al. (2019), the increased milk protein concentration after mammary inflammatory challenge is due to a combination of the dilution effect by reduced milk yield, increased serum protein influx because of disrupted mammary epithelial junction (Auldist et al., 1995; Stelwagen and Singh, 2014), and the enhanced synthesis of antibacterial proteins and inflammatory products (Lohuis et al., 1988; Isobe et al., 2009; Gott et al., 2015) as indicated by the increased milk concentration of inflammatory cytokines and APP. The greater milk protein concentrations in the LPS infused quarters of NC cows compared with CL cows at 12 and 24 h following infusion may be partially due to the greater APP (e.g., haptoglobin) synthesis.

Intramammary LPS infusion reduced milk lactose concentration in LPS infused quarters until 12 h following infusion regardless of treatment. This is partially because of the disrupted mammary epithelial junction during mastitis (Kobayashi et al., 2013). Marins et al. (2019) indicated that plasma lactose increases and peaks at 3 h after intramammary

LPS infusion. Additionally, Waldron et al. (2006) estimated that mammary inflammation induced by i.m. LPS resulted in ~850 g glucose consumption by tissues rather than mammary gland within 14 h post infusion in early lactating cows. This also limits lactose synthesis within the mammary gland. Marins et al. (2020) reported that NC cows had lower circulating glucose concentrations but stronger insulin release following intramammary LPS infusion compared with CL cows. These altered metabolic responses may explain the lower milk lactose concentration in non-infused quarter of NC cows than CL cows. In LPS infused quarters, the greater milk lactose concentration observed for NC cows at 48 h after infusion compared with CL cows may be due to local effects within the mammary gland rather than systemic metabolic responses. In this current study, deprivation of evaporative cooling increased MUN concentrations in LPS infused quarter. The result was consistent with the previous report of Marins et al. (2019) and suggested that altered ruminal and peripheral protein metabolism by heat stress during mammary inflammation induced by LPS (Perkins et al., 2002; Marins et al., 2019).

In this current study, body temperature as measured by vaginal temperature peaked (41.1°C) at 6 h following i.m. LPS. The result was consistent with a previous study (Marins et al., 2019). Compared with CL cows, NC cows had higher vaginal temperature before and after i.m. LPS due to the greater heat load. However, both groups of cows reached a similar peak of vaginal temperature following i.m. LPS, indicating similar fever development. Intramammary LPS infusion did not affect serum TNF-α and IL-10 concentrations regardless of treatments. Ning et al. (2018) reported that TNF-α concentration was not altered in blood collected from jugular and mammary veins following intravenous LPS infusion. However, concentrations of IL-1β, IL-6 and IL-8 and

APP were increased in both jugular and mammary vein after intravenous LPS infusion. Lehtolainen et al. (2004) also reported that TNF-α was not detected or was present in low concentrations in serum after i.m. LPS. However, Johnzon et al. (2018) reported that TNF-α increased in both serum and milk when the dose of infused LPS into the mammary gland increased to 200 μg. The smaller dose of LPS (10 μg) used in this current experiment may prevent us from observing the increased circulating TNF-α. Plasma HP concentration increased and peaked at 48 h following i.m. LPS, consistent with Paulina and Tadeusz (2011) and Suojala et al. (2008). Vels et al. (2009) and Zarrin et al. (2014) also reported upregulated hepatic HP gene expression following LPS induced mastitis. These data suggest upregulated systemic APR during LPS induced mammary inflammation. Interestingly, NC cows had lower circulating IL-10 and HP concentrations, indicating a weaker systemic inflammatory response after i.m. LPS compared with CL cows. However, the mechanisms and impacts of this effect are not clear. This deserves further investigation.

In contrast with blood concentrations, milk concentrations of TNF-α, IL-10 and HP were profoundly increased after i.m. LPS, confirming upregulated mammary inflammation. Similarly, Perkins et al. (2002) and Suojala et al. (2008) reported increased milk inflammatory cytokine and APP concentrations after intramammary endotoxin or E. coli infusion. Schmitz et al. (2004) and Zarrin et al. (2014) also reported upregulated mammary gene expression of TNF-α, IL10, HP and milk amyloid A after i.m. LPS. Interestingly, the mammary gland of NC cows had a more rapid increase in milk TNF-α and IL-10 at 3 h following i.m. LPS. Coupled with the greater milk HP concentration, these data suggest that the mammary gland under NC cows has faster and stronger inflammatory responses to LPS compared with CL cows. These changes explain the greater milk SCC of

NC cows compared with CL cows following intramammary LPS infusion observed in this experiment. Mammary inflammatory responses to infection are initiated by resident macrophage or mammary epithelial cells that recognize and bind pathogen-associated molecular patterns of bacterial molecules (Schukken et al., 2011; Wellnitz and Bruckmaier, 2012). Marins et al. (2021, in press) reported that peripheral blood mononuclear cells (PBMC) isolated from NC lactating dairy cows had greater TNF-α and IL-10 production when stimulated with LPS *in vitro* compared with CL cows. In miniature pigs, heat stress up-regulates the gene expression of toll-like receptor-4 in PBMC (Ju et al., 2014). Similarly, peritoneal macrophages isolated from heat-stressed mice produced more TNF-α after in vitro LPS stimulation compared with mice under thermal neutrality (Lee et al., 2012). The results suggest that heat stress upregulates the inflammatory responses of immune cells to LPS, such as resident macrophage within the mammary gland. However, it is important to note that bovine mammary epithelial cells also express toll-like receptor-4 and are able to produce inflammatory cytokines and chemokines (Okada et al., 1999; Pareek et al., 2005; McClenahan et al., 2006; Ibeagha-Awemu et al., 2008). Thus, the possibility that the inflammatory responses of mammary epithelial cells to LPS or bacterial stimulation is also upregulated by heat stress cannot be excluded.

Conclusions

In our current experiment, deprivation of evaporative cooling during summer significantly impairs lactating dairy cows' performance. In contrast, circulating and milk concentrations of cytokines and acute phase protein were similar between NC and CL cows, suggesting unchanged systemic and mammary inflammation. However, deprivation

of evaporative cooling resulted in a faster and stronger mammary inflammatory response following i.m. LPS, which resulted in greater increase in milk SCC. The results indicated that heat stress combined with intramammary infection led to stronger mammary inflammatory response. Future research utilizing intramammary bacterial infusion models are warranted.

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Table 3.1. Ingredient composition of experimental diets

Ingredient	% of DM
Oat silage	14.13
Corn silage	28.27
Bermuda grass hay	3.53
Brewers grain, wet	10.60
Molasses	3.53
Ground corn	22.08
Energy booster 100[1]	1.32
Citrus pulp	1.77
Amino plus[2]	3.09
Soybean meal	8.39
Salt, white	0.27
CalMin[3]	0.35
Calcium carbonate	0.44
Sodium bicarbonate	0.35
Magnesium oxide	0.35
Dynamate[4]	0.11
Diamond V XP[5]	0.20
OmniGen-AF[6]	0.21
ClariFly[7]	0.04
Rumensin[8]	0.29
AB-20[9]	0.41
Trace-mineral-vitamin premix[10]	0.27

[1]Hydrogenatted fatty acid supplement, MilkSepcialties Global Inc., Eden Prairie, MN.
[2]Expeller soybean meal, Ag Processing Inc., Omaha, NE.
[3]Calcareous marine algae (Celtic Sea Minerals, Ireland)
[4]Potassium magnesium sulfate (Mosaic Co. Inc., Plymouth, MN)
[5]Yeast culture (Diamond V. Inc., Cedar Rapids, IA)
[6]Immune stimulant (Phibro Animal Health Corp., Teaneck, NJ)
[7]Larvicide (Central Life Science, Schaumburg, IL)
[8]Monensin (Elanco, Greenfield, IN).
[9]Bentonite adsorbent (Phibro Animal Health, Teaneck, NJ)
[10]Mineral-vitamin premix contained (DM basis): 26.1% Ca; 0.38% Mg; 1.76% S; 144 ppm Co; 9,523 ppm Cu; 1,465 ppm Fe; 842 ppm I; 28,617 ppm Mn; 220 ppm Se; 25,343 ppm Zn; 4,210,830 IU/kg Vitamin A; 1,684,330 IU/kg Vitamin D; 21,045 IU/kg Vitamin E

Table 3.2. Protein names, gene symbols, accession numbers, primer sequences and amplicon sizes of genes examined.

Protein name	Gene symbol	Accession number	Primer[1]	Sequence (5' - 3')	Amplicon size, bp
GADPH	*GADPH*	NM_001034034	F	CCCTTCATTGACCTTCACTAC	115
			R	TGGCCTTTCCATTGATGAC	
Tumor necrosis factor	*TNF*	NM_173966.3	F	ACACTCAGGTCCTCTTCTC	142
			R	GGTTGTCTTCCAGCTTCAC	
Interleukin 10	*IL10*	NM_174088.1	F	CCTTGTCGGAAATGATCCAG	148
			R	GGCAGAAAGCGATGACAG	
Haptoglobin	*HP*	NM_001040470.2	F	GGACAGAAACTCCCTGAATG	134
			R	TGCTGGGAGACCATCTT	
Serum amyloid A 3	*SAA3*	NM_181016.3	F	CTTTCCACGGGCATCATT	139
			R	CCCTGTAGTTGGCTTCTTTC	

[1]F = forward; R = reverse

Table 3.3. Chemical composition of the experimental diet.

Composition	% of DM
CP	16.6 ± 0.3[1]
NDF	44.4 ± 2.8
ADF	17.7± 1.0
Starch	19.3 ± 1.8
Sugar	4.7 ± 0.8
EE[2]	4.4 ± 2.8
Ash	8.7 ± 0.8

[1]Mean ± Standard Deviation
[2]EE = Ether extract

Table 3.4. Respiration rate (RR), vaginal temperature (VT), body weight (BW), body condition score (BCS), dry matter intake (DMI), milk yield and milk composition of lactating Holstein cows provided evaporative cooling (CL, n = 9) or not (NC, n = 9)

Parameter	Treatment			*P*-value		
	CL	NC	SEM	C[1]	T	C×T
RR[2], breath/min	62	72	1.53	<0.01	<0.01	<0.01
VT[2], ℃	38.92	39.59	0.09	<0.01	<0.01	<0.01
BW[2], kg	643.81	635.05	2.82	0.04	0.24	<0.01
BCS[2]	2.69	2.69	0.25	0.92	0.77	1.00
DMI[2], kg/d	25.27	22.32	0.44	<0.01	<0.01	0.03
Milk yield, kg/d	33.64	28.75	0.76	<0.01	0.09	0.54
3.5% FCM[3], kg/d	33.59	27.01	1.78	0.02	0.04	0.43
ECM[4], kg/d	32.73	26.06	1.49	<0.01	0.07	0.40
Feed efficiency[5]	1.27	1.18	0.09	0.52	<0.01	0.19
Milk composition						
Fat, %	3.56	3.98	0.23	0.22	0.22	0.53
Fat, kg/d	1.18	0.98	0.09	0.17	0.06	0.39
Protein, %	2.76	2.67	0.07	0.35	<0.01	0.71
Protein, kg/d	0.90	0.72	0.03	<0.01	0.11	0.49
Lactose, %	4.77	4.72	0.06	0.51	0.01	0.51
Lactose, kg/d	1.54	1.30	0.05	0.01	0.06	0.15
SNF, %	8.50	8.27	0.11	0.18	<0.01	0.36
SNF, kg/d	2.68	2.36	0.11	0.07	0.02	0.34
MUN, mg/L	11.78	13.51	0.46	0.02	<0.01	0.25
SCS[6]	3.10	2.20	0.44	0.18	0.47	0.11

[1]C: Cooling; T: Time
[2]Calculated with 15 cows each treatment
[3]3.5% FCM = (0.4324 × kg of milk yield) + (16.216 × kg of milk fat yield)
[4]ECM = (0.327 × kg of milk yield) + (12.95 × kg of milk fat yield) + (7.20 × kg of milk protein yield)
[5]ECM/DMI
[6]SCS = Log_2(SCC/100) + 3

Table 3.5. Blood concentration of cortisol and inflammatory products and milk concentration and yield of inflammatory products of lactating Holstein cows exposed to evaporative cooling (CL, n = 9) or not (NC, n = 9).

	Treatment			*P*-value		
	CL	NC	SEM	C[1]	T	C×T
Blood						
Cortisol, ng/mL	14.13	13.13	1.10	0.54	<0.01	<0.01
IL-10, ng/mL	3.50	2.56	0.67	0.34	<0.01	0.02
TNF-α, ng/mL	5.12	4.44	0.68	0.50	0.63	0.63
Haptoglobin, μg/ml	37.77	34.64	7.45	0.77	0.01	0.62
LBP, μg/ml	2.69	2.99	0.41	0.61	0.05	0.28
Milk						
IL-10, ng/mL	0.11	0.18	0.03	0.08	<0.01	0.40
IL-10, ng/per milking	1.91	2.37	0.46	0.49	<0.01	0.52
TNF-α, ng/mL	4.41	4.05	0.45	0.58	0.04	0.17
TNF-α, ng/ per milking	65.56	49.49	6.25	0.09	0.13	0.04
Haptoglobin, μg/ml	82.77	81.54	4.58	0.85	0.64	0.70
Haptoglobin, μg/ milking	1344.5	1148.5	60.84	0.03	0.37	0.63
LBP, μg/ml	0.18	0.18	<0.01	0.55	0.15	0.55
LBP, μg/ milking	6.74	6.56	0.98	0.90	0.99	0.75

[1]C: Cooling; T: Time

Table 3.6. Relative gene expression of mammary tissue of cows provided evaporative cooling (CL, n = 6) or not (NC, n = 6)

	Treatment			*P*-value		
	CL	NC	SEM	C[1]	T	C×T
TNF	1.16	1.37	0.50	0.78	<0.01	0.74
IL10	1.50	1.22	0.70	0.78	0.02	0.33
Haptoglobin	3.65	1.83	3.32	0.70	<0.01	0.09
Serum amyloid A	1.55	2.01	1.83	0.86	<0.01	0.19

[1]C: Cooling; T: Time

Table 3.7. Respiration rate (RR), vaginal temperature (VT), dry matter intake (DMI), and milk yield of cows provided evaporative cooling (CL, n = 7) or not (NC, n = 7) in response to intramammary lipopolysaccharide (LPS) infusion

	Treatment			*P*-value		
	CL	NC	SEM	C[1]	T	C×T
RR, breath/min	63	72	2.32	0.01	<0.01	0.01
VT, ℃	39.44	40.21	0.20	0.03	<0.01	<0.01
DMI, kg/d	22.43	18.19	0.35	<0.01	<0.01	0.06
DMI, % of baseline	89.67	85.78	1.99	0.19	<0.01	0.08
Milk yield, kg/d	31.75	23.47	0.63	<0.01	<0.01	0.60
Milk yield, % of baseline	88.6	88.51	2.32	0.99	<0.01	0.78

[1]C: Cooling; T: Time

Table 3.8. Milk composition of lactating Holstein cows provided evaporative cooling (CL, n = 7) or not (NC, n = 7). Milk samples were collected from LPS infusion and non-infusion quarters at 3, 6, 12, 24, 48, 96, and 144 h relative to infusion

	Treatment			*P*-value		
	CL	NC	SEM	C[1]	T	C×T
LPS infused quarter						
Fat, %	5.96	4.87	0.53	0.24	<0.01	0.31
Protein, %	2.89	3.04	0.10	0.33	<0.01	0.05
Lactose, %	3.99	4.04	0.08	0.69	<0.01	0.08
SCC, 10^3 cells/mL	4.23	5.87	0.59	0.07	<0.01	0.61
SNF, %	7.97	7.14	0.17	0.02	<0.01	0.09
MUN, mg/dL	9.20	13.62	1.2	0.06	<0.01	0.60
Control quarter						
Fat, %	6.91	5.53	0.39	0.46	<0.01	0.07
Protein, %	2.52	2.52	0.02	0.88	<0.01	0.66
Lactose, %	4.57	4.44	0.02	<0.01	<0.01	0.47
SCC, 10^3 cells/mL	0.22	0.24	0.03	0.77	<0.01	0.14
SNF, %	7.99	7.74	0.04	<0.01	<0.01	0.79
MUN, mg/l	11.34	11.22	0.28	0.78	<0.01	<0.01

[1]C: Cooling; T: Time

Table 3.9. Hematological profile of cows provided evaporative cooling (CL, n = 7) or not (NC, n = 7) in response to intramammary LPS infusion

				P-value		
	CL	NC	SEM	C[1]	T	C×T
Hematological profile, 10^3 cells/µL						
White blood cells	6.36	8.12	0.90	0.19	<0.01	0.42
Lymphocyte	3.22	5.19	2.99	0.73	<0.01	0.17
Neutrophil	2.62	2.36	0.30	0.51	<0.01	0.33
Monocyte	0.21	0.21	0.02	0.94	<0.01	0.11
Eosinophil	0.19	0.24	0.03	0.27	<0.01	0.68
Basophil	0.05	0.06	0.009	0.23	<0.01	0.32

[1]C: Cooling; T: Time.

Table 3.10. Blood and milk concentrations of inflammatory cytokines and haptoglobin of lactating Holstein cows provided evaporative cooling (CL, n = 7) or not (NC, n = 7) in LPS infusion period of experiment. Blood and milk were collected at -4, 0, 3, 6, 12, 24, 48, 96, and 144 h relative to infusion.

	Treatment			*P*-value		
	CL	NC	SEM	C[1]	T	C×T
Blood						
IL10, ng/mL	4.17	2.70	0.37	<0.01	0.12	0.85
TNF-α, ng/mL	3.00	2.22	0.60	0.37	0.56	0.57
Haptoglobin, μg/ml	41.49	26.23	5.70	0.07	<0.01	0.37
Milk						
IL10, ng/mL	0.39	0.54	0.06	0.07	<0.01	0.11
TNF-α, ng/mL	4.08	5.71	0.82	0.18	<0.01	0.15
Haptoglobin, μg/ml	94.62	106.85	5.05	0.10	<0.01	0.29

[1]C: Treatment; T: Time

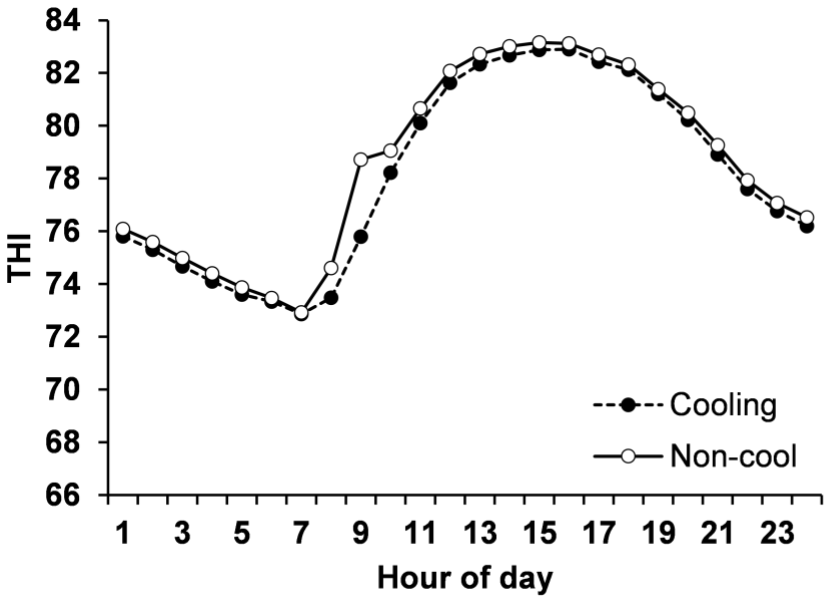


Figure 3.1. Temperature-humidity index of cows exposed to evaporative cooling (solid circle [●]) or non-cool (open circle [○]) in environmental challenge phase.

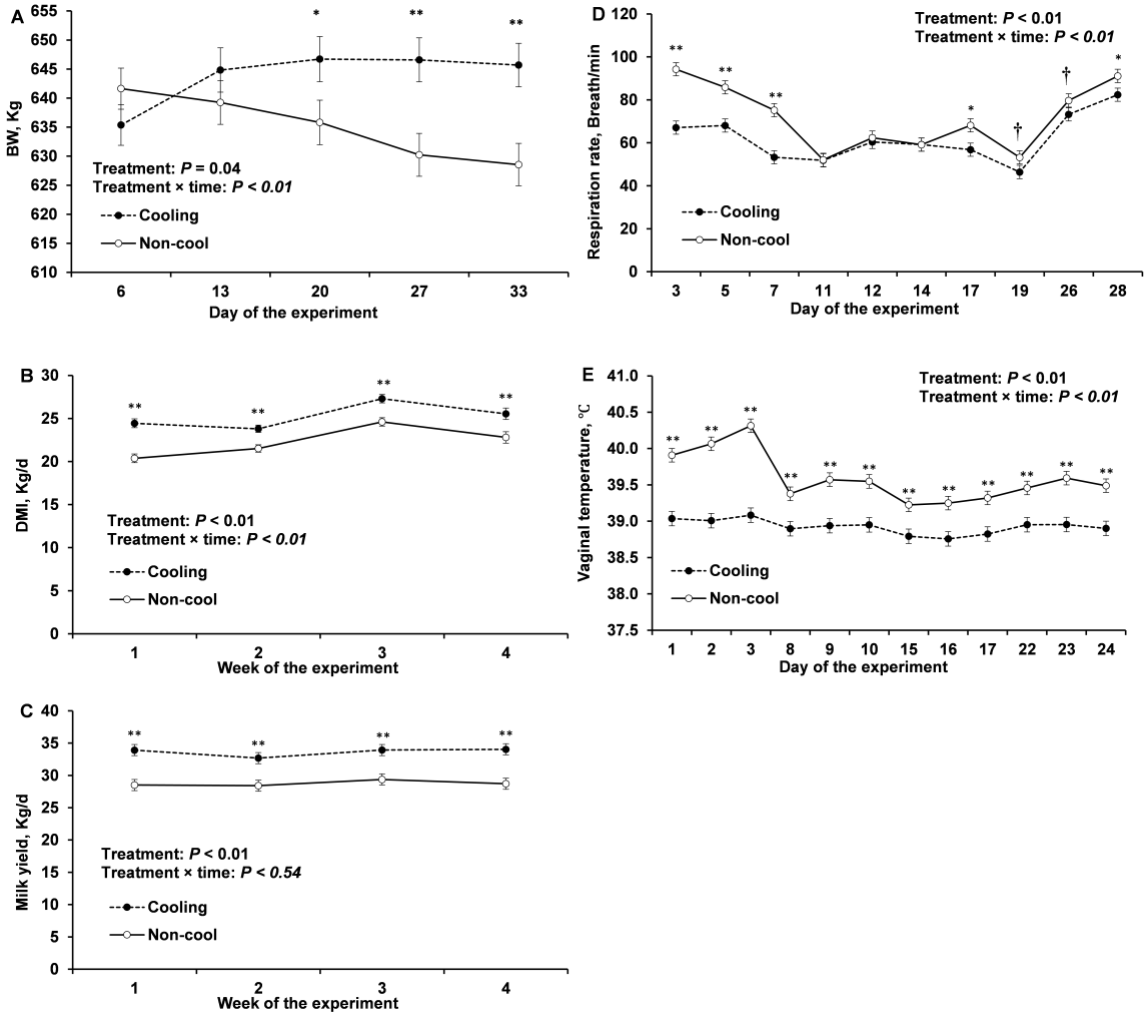


Figure 3.2. Body weight (A), DMI (B), milk yield (C), respiration rate (D), and vaginal temperature (E) of cows exposed to evaporative cooling (solid circle [●]) or non-cool (open circle [○]) in environmental challenge phase.

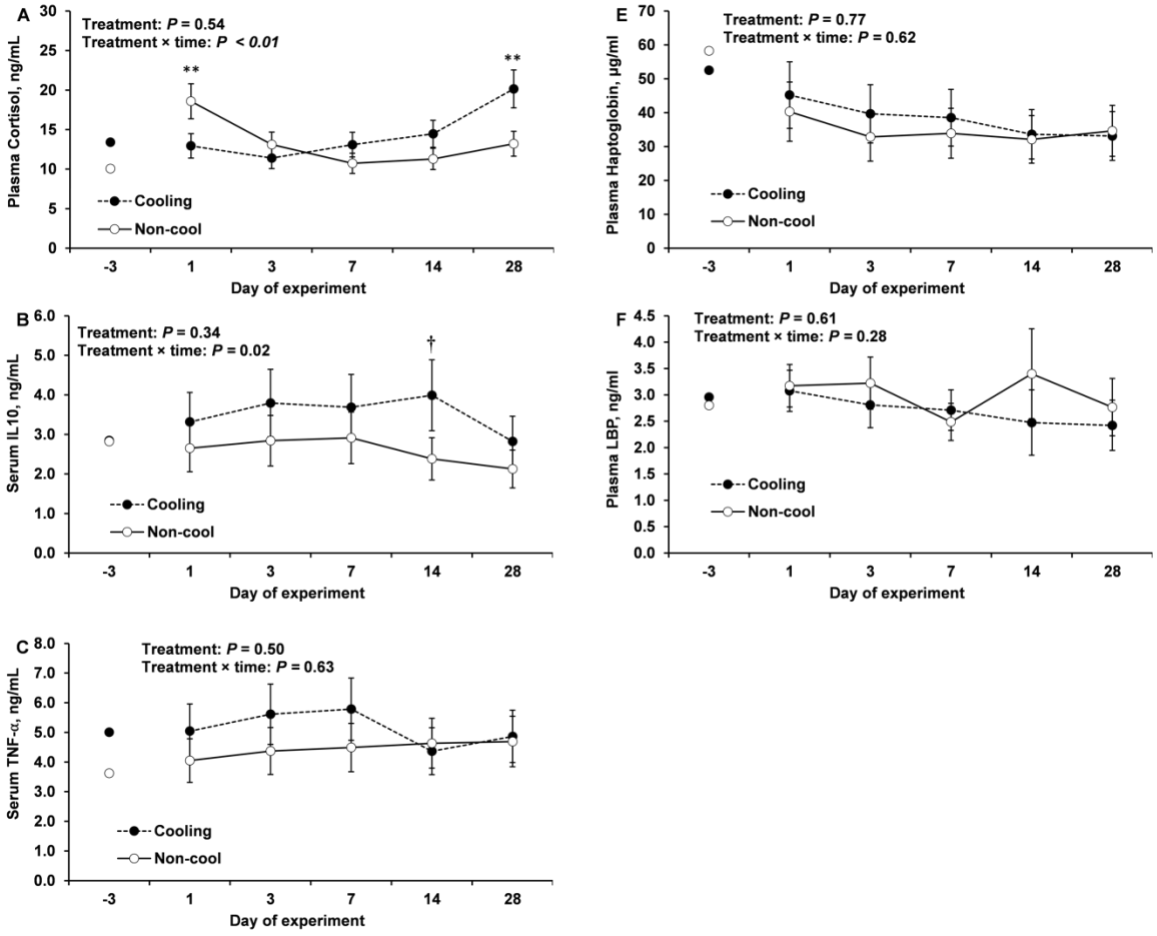


Figure 3.3. Blood concentrations of cortisol (A), IL-10 (B), TNF-α (C), and haptoglobin (D) and LBP (F) of cows exposed to evaporative cooling (solid circle [●]) or not (open circle [○]). ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.15$.

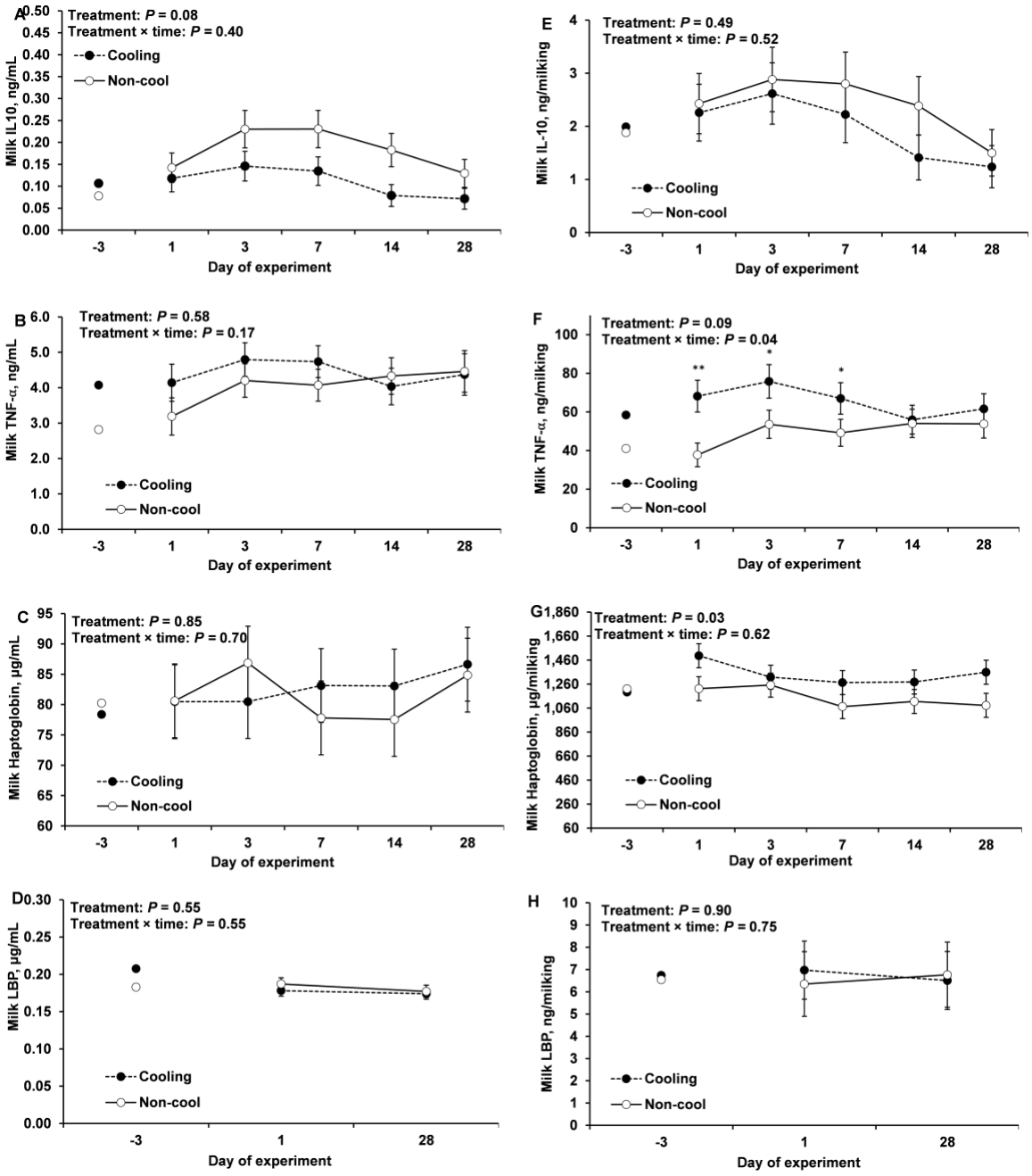


Figure 3.4. Milk concentrations of IL-10 (A), TNF-α (B), haptoglobin (C), LBP(D) and yields of IL-10 (E), TNF-α (F), haptoglobin (G) and LBP (H) of cows exposed to evaporative cooling (solid circle [●]) or not (open circle [○]). ** P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.15.

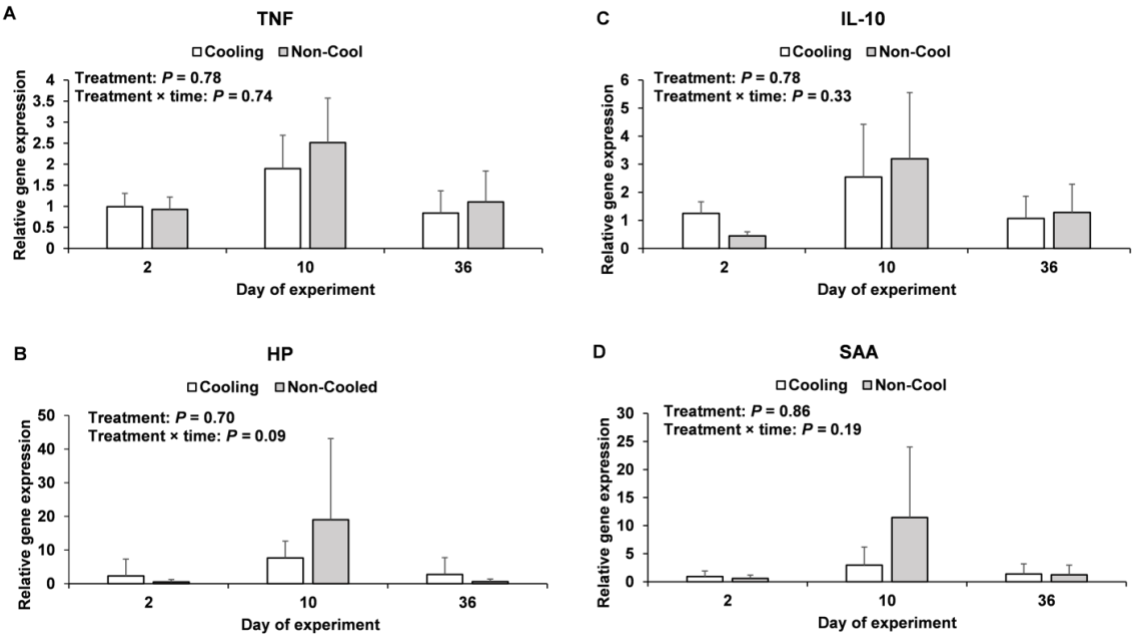


Figure 3.5. Relative gene expression of mammary tissue of cows exposed to evaporative cooling (n=6) or not (n=6). TNF (A), HP (B), IL-10 (C), SAA (D). Error bars represent SEM.

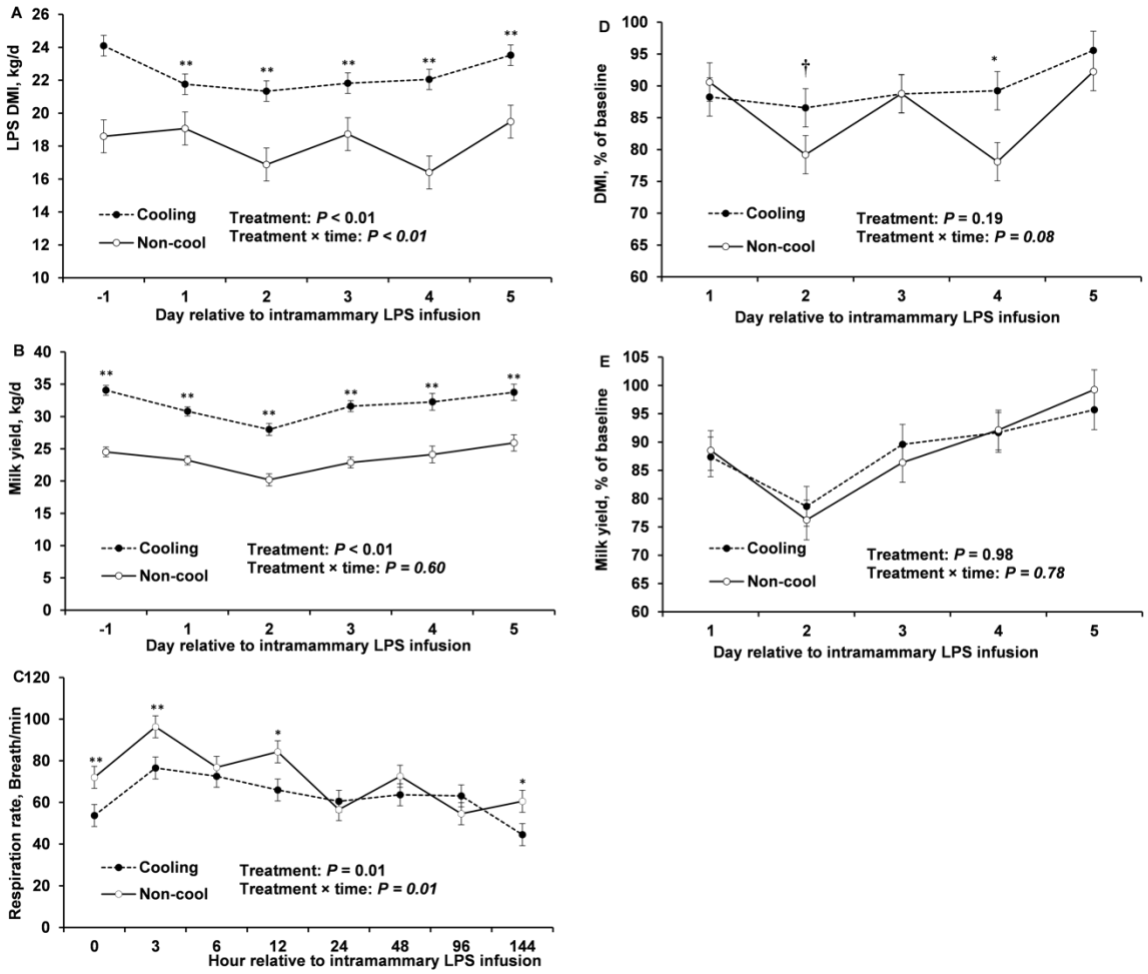


Figure 3.6. DMI (A), milk yield (B), respiration rate (C), LPS DMI relative to baseline (D), and LPS milk yield relative to baseline (E) of cows exposed to evaporative cooling (solid circle [●]) or not (open circle [○]) in LPS challenge phase. ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.15$.

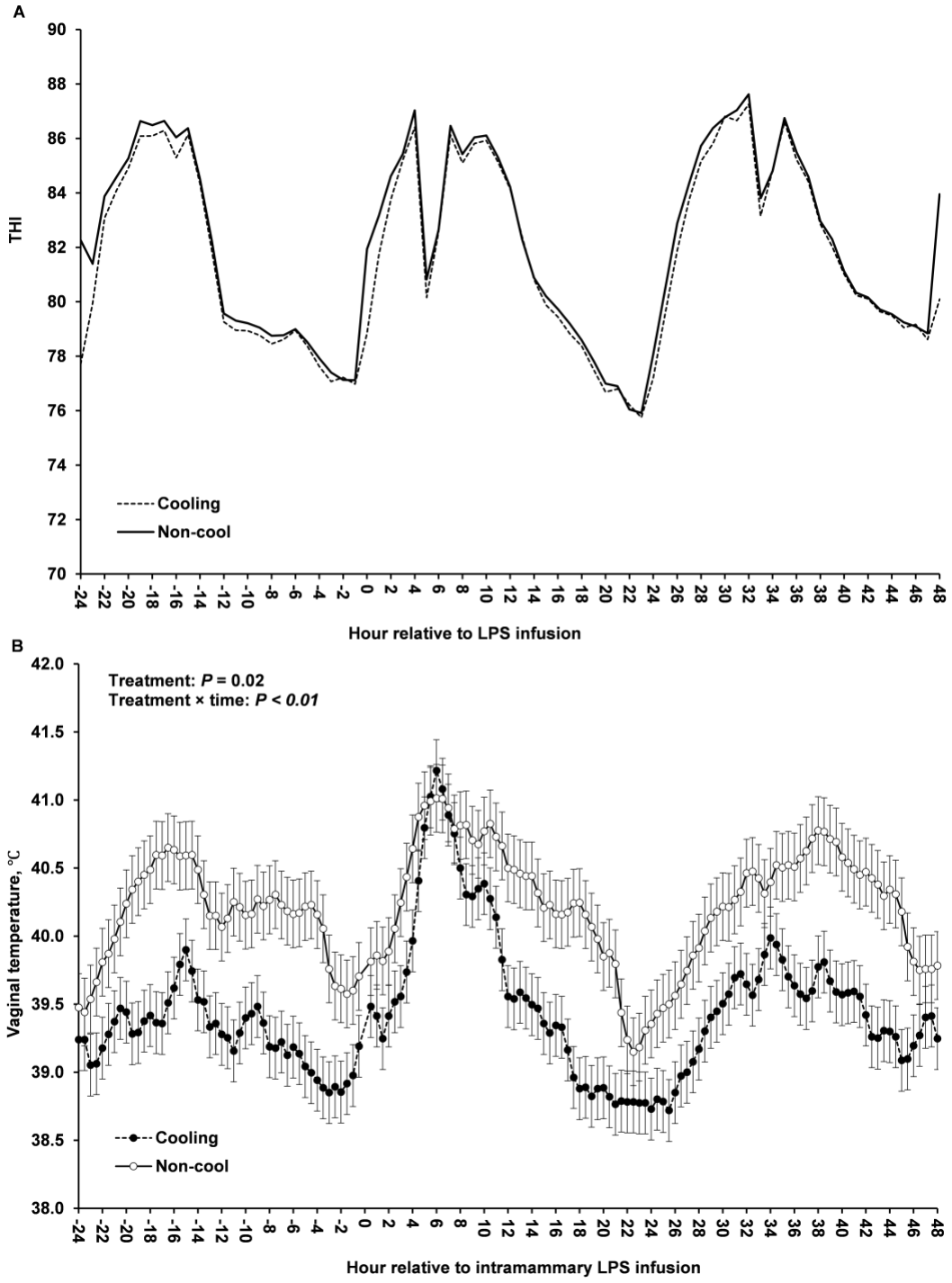
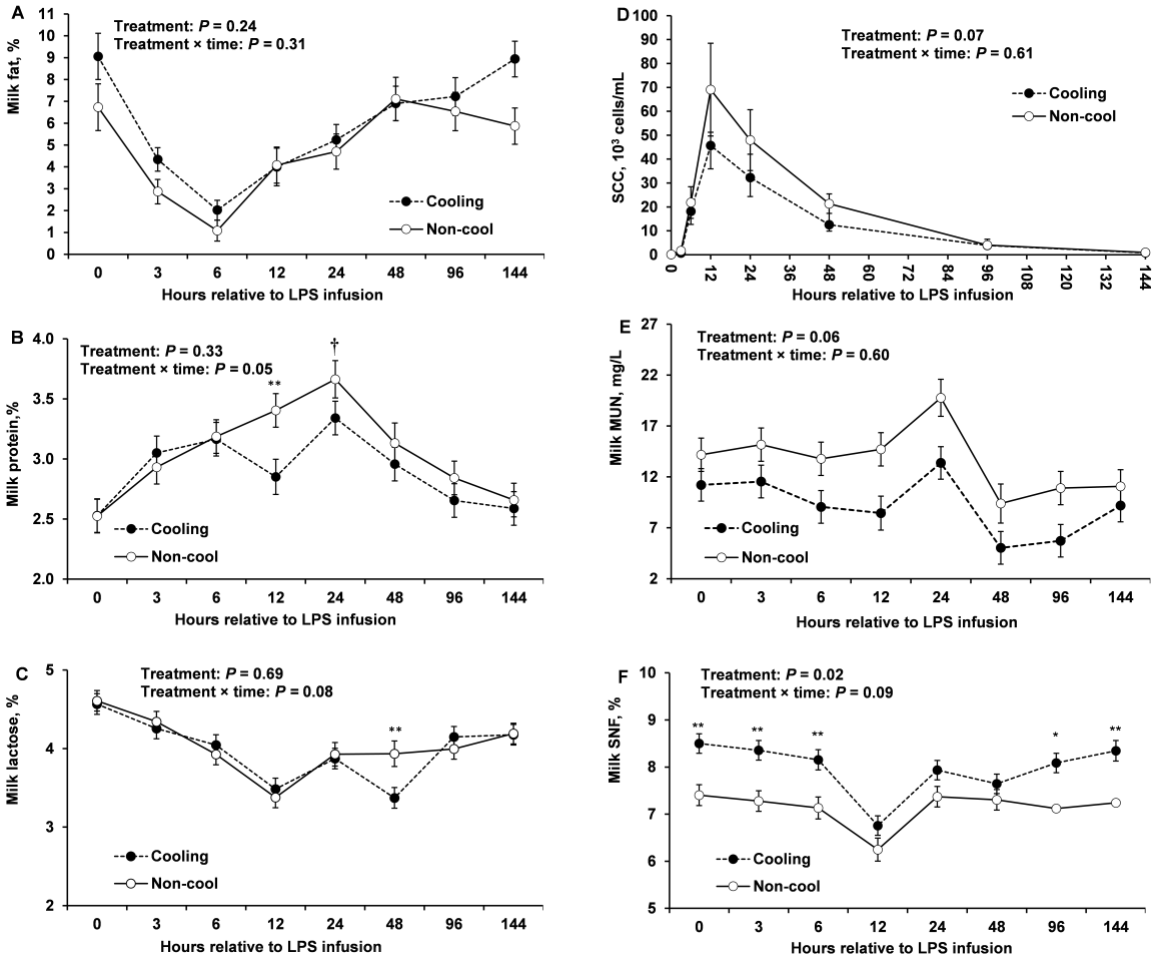
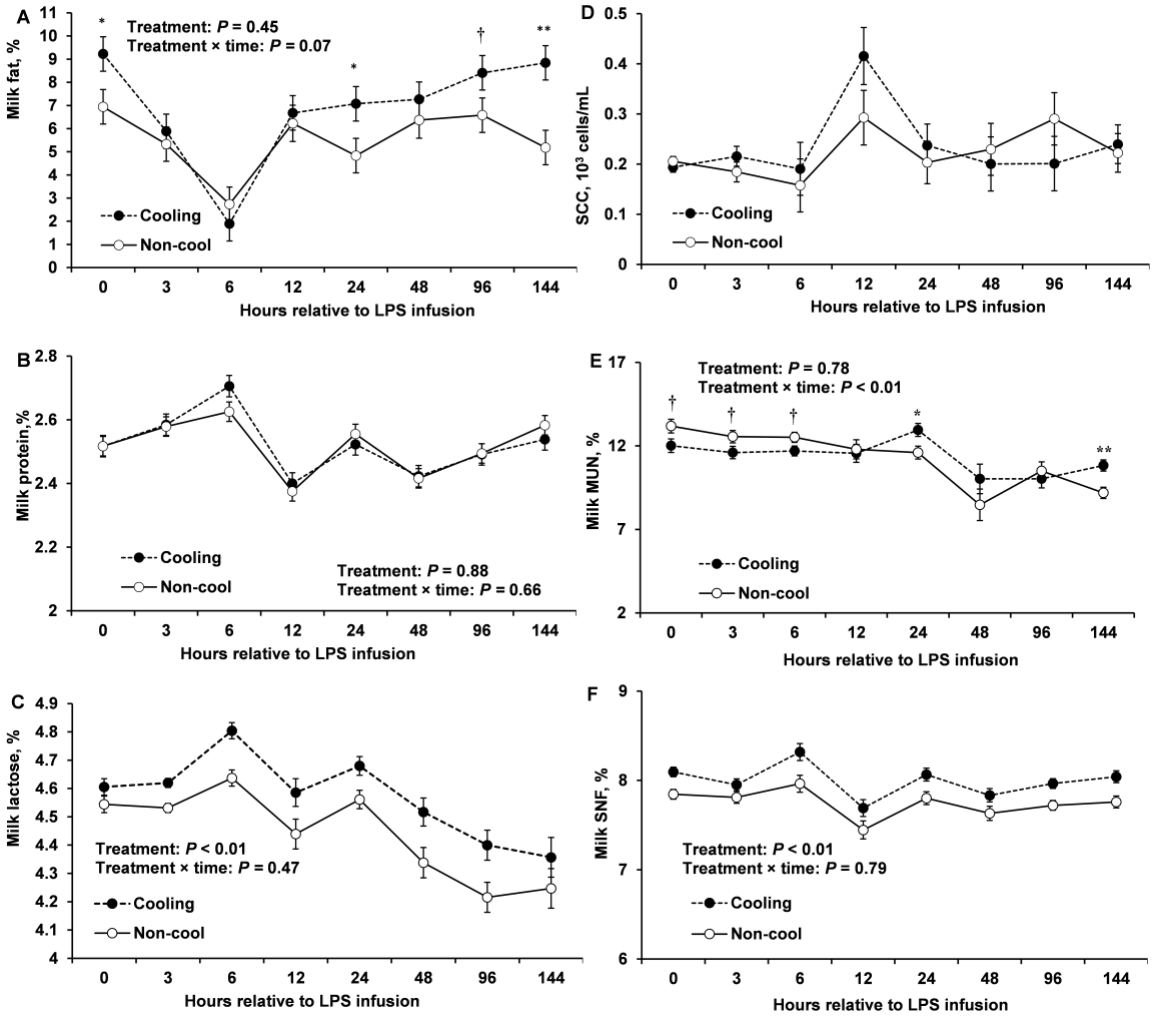


Figure 3.7. THI (A) and vaginal temperature (B) of cows exposed to evaporative cooling (solid circle [●]) or not (open circle [○]) before and after intramammary LPS infusion.



Figuure 3.8. Milk concentrations of fat (A), protein (B), lactose (C), SCC (D), MUN (E), SNF (F) in quarters infused with LPS of cooling (solid circle [●]) or not (open circle [○]). ** P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.15.



Figuure 3.9. Milk concentrations of fat (A), protein (B), lactose (C), SCC (D), MUN (E), SNF (F) in quarters without LPS infusion of cooling (solid circle [●]) or not (open circle [○]). ** P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.15.

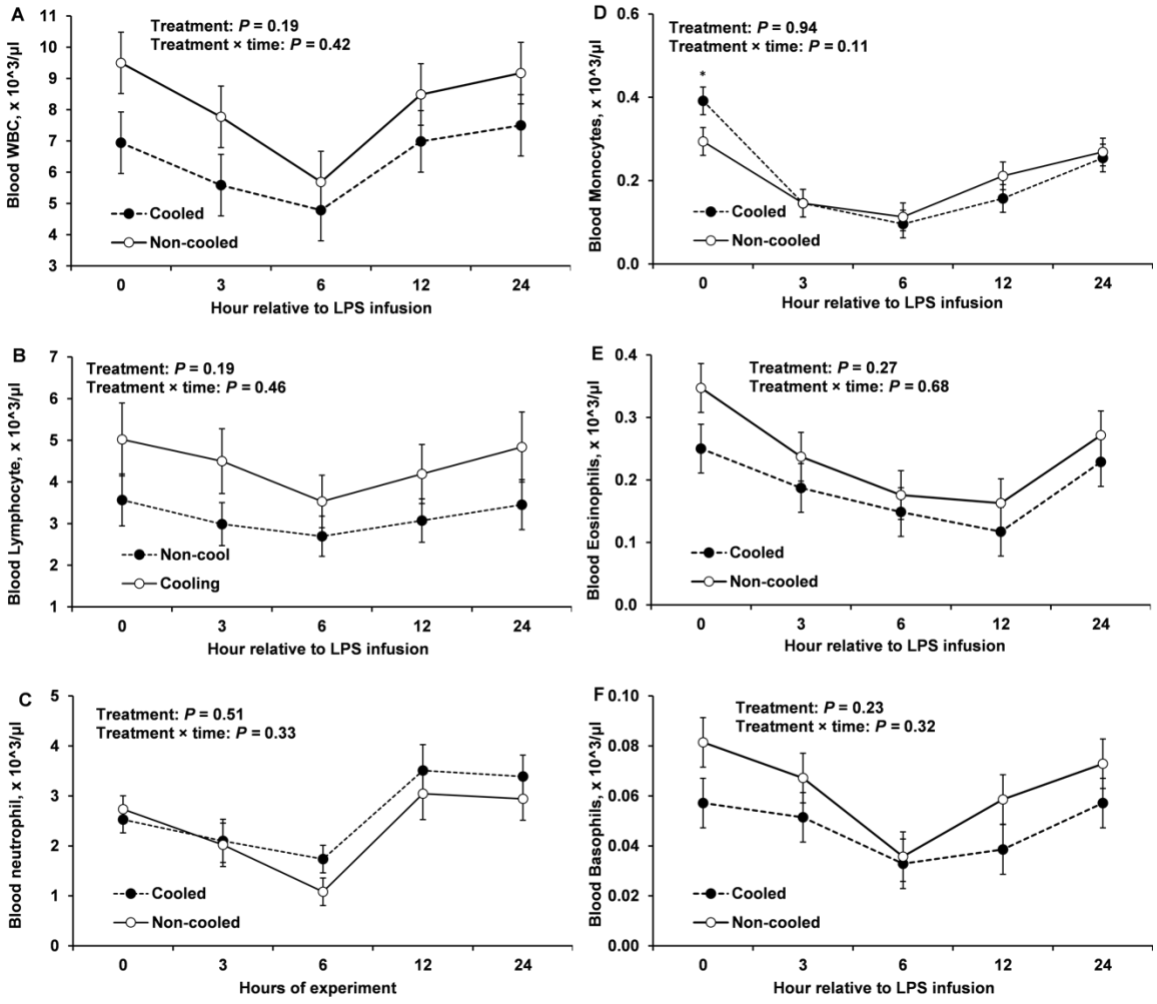


Figure 3.10. Blood concentration of white blood cell (WBC) (A), lymphocyte (B), neutrophil (C), monocyte (D), eosinophils (E), basophils (F) of cows exposed to evaporative cooling (solid circle [●]) or not (open circle [○]) in response to intramammary LPS infusion. * $P \leq 0.05$.

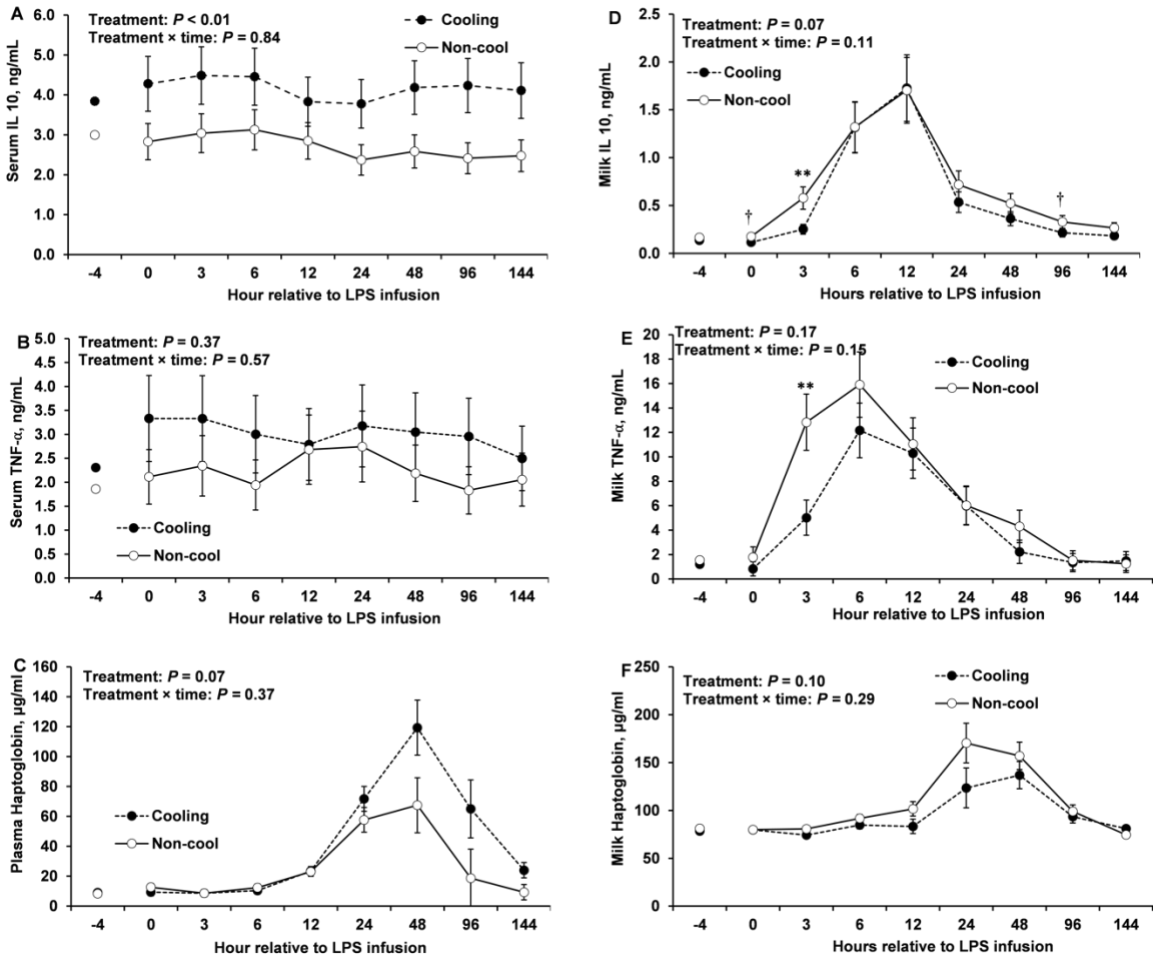


Figure 3.11. Blood concentrations of IL-10 (A), TNF-α (B), haptoglobin (C), and milk concentrations of IL-10 (D), TNF-α (E), and haptoglobin (F) of cows exposed to evaporative cooling (solid circle [●]) or not (open circle [○]) in response to intramammary LPS infusion. ** P ≤ 0.01, * P ≤ 0.05, † P ≤ 0.15.

CHAPTER 4

CONCLUSIONS

Heat stress is a critical issue in the dairy industry. It not only affects cow performance but also related to the economic loss of the dairy producers. It is well recognized that cows raised in summer had higher milk SCC and incidence of environmental mastitis. However, the biological mechanisms have not been well studied and discovered. In this study, we demonstrated that deprivation of evaporative cooling does not affect basal systemic and mammary inflammation. These data suggest that heat stress per se has no impact on inflammatory responses, and is not related to increased milk SCC of healthy lactating dairy cows. However, following intramammary LPS infusion, NC cows had faster increase in IL-10 and TNF-α concentrations and greater haptoglobin concentration in the milk than CL cows. These data indicate that heat stress enhances mammary inflammatory responses to infection in lactating dairy cows. This results in a greater increase in milk SCC and more severe disease symptoms of subclinical and clinical mastitis. The results obtained in this experiment provide an alternative explanation of the increased SCC and incidence of mastitis observed in lactating dairy cows during summer.