Developing and validating a heat stress model and evaluating nutritional management strategies to mitigate heat stress and immune-challenges in dairy cows

by

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DEDICATION

I would like to dedicate this success to my parents Abdel-Nasser Al-Qaisi and Najah Al-Halawani, to whom I owe very much. I also dedicate this work to my lovely wife Ala’ Khaleel and my hero, my son, Nasser Al-Qaisi. To my siblings: Leena, Nader, and Ibrahim Al-Qaisi and all my family members in Jordan and USA.

This accomplishment is devoted to all of you.
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ABSTRACT

Heat stress (HS) negatively impacts animal productivity and welfare. Precisely studying HS typically requires expensive climate-controlled facilities, resources often inaccessible to most scientists. Thus, it is of interest to develop and validate alternative and cost-effective models to study HS and to assess nutritional HS mitigation strategies using this model in lactating dairy cows. Many of the negative consequences of HS appears to be mediated by intestine-derived lipopolysaccharide (LPS) and thus HS biology can be modeled by infusing LPS. Administrating LPS decreases circulating calcium (Ca) and decreases markedly both feed intake and milk yield in dairy cows. The current dissertation centered on developing, evaluating, and validating an alternative model to study HS and identifying nutritional management strategies to ameliorate negative consequences of both heat-stressed and immune-challenged dairy cows.

In studies 1 and 2 (Chapters 2 and 3), we evaluated the efficacy of using an electric heat blanket (EHB) as an alternative method to study HS and we observed similar changes in body temperature indices, production and metabolism to natural and climate-controlled HS experiments. Additionally, we validated the EHB model (using a pair-feeding design) and confirmed that lowered nutritional plane explains only ~50% of the decreased milk yield.

In study 3 (Chapter 4), we evaluated a dietary electrolyte, osmolyte, and energetic compounds (EOEC) on physiological parameters in heat-stressed cows using the EHB model. Feeding EOEC appears to increase heat dissipation by increasing skin temperature. Additionally, dietary EOEC altered metabolic and the blood gas profile in heat-stressed cows and thus is a promising nutritional strategy to utilize during the warm summer months.

In study 4 (Chapter 5), we examined a dietary *Saccharomyces cerevisiae* fermentation product (SCFP) on body temperature indices, metabolism, and acute phase protein response
(APPR) in heat-stressed dairy cows using the EHB model. Results demonstrated that HS caused an APPR and that feeding SCFP could be beneficial at reducing circulating cortisol and the APPR.

In study 5 (Chapter 6), we investigated the effects of providing an oral supplement containing Ca and live yeast on circulating Ca and production parameters in immune-challenged dairy cows. Results suggest that increased circulating Ca improves production parameters during inflammation. Overall, utilizing an oral supplement may be a valuable management strategy to improve animal welfare and productivity during and following immunoactivation.

In conclusion, employing the EHB model provides an excellent new platform for discovery research and for evaluating pragmatic HS mitigation strategies. Results demonstrated that feeding EOEC could benefit heat dissipation and metabolism. In addition, feeding SCFP may be useful at reducing the amount of “stress” and immune activation during HS. Furthermore, infusing i.v. LPS appears to be an effective technique to model hypocalcemia and to evaluate dietary strategies aimed at increasing circulating Ca in periparturient lactating dairy cows. Collectively, understanding the biology of HS is important for identifying mitigation strategies aimed at ameliorating the negative consequences of HS in dairy cow.
CHAPTER 1. LITERATURE REVIEW

Heat Stress

The term “stress” has been defined as the magnitude of forces external to the body leading to shifting its system from its resting state (Yousef, 1985; Kadzere et al., 2002). Increased ambient temperature requires an animal to make adjustments, which counteract physiological dysfunction and enable it to survive (Kadzere et al., 2002). In general, animals become hyperthermic when an imbalance between heat production (through both environmental conditions and internal thermogenesis) and dissipation occurs (Kadzere et al., 2002; Bernabucci et al., 2010). Heat stress (HS) represents one of the most important factors undermining global animal agriculture as it affects numerous production parameters such as milk production and composition, growth, and reproductive performance (Fuquay, 1981; Collier et al., 1982; Beede and Collier, 1986; Kadzere et al., 2002; Baumgard and Rhoads, 2013). The negative consequences of HS impose devastating economic losses, exceeding $1.5 billion in the United States dairy industry alone (Key and Sneeringer, 2014), and if the earth’s temperature continues to increase as anticipated the detrimental impacts will intensify (IPCC, 2007; Nardone et al., 2010). In addition to hindrances imposed by climate change, genetic selection for animal productivity leads to increased metabolic heat production and therefore increased susceptibility to HS (Brown-Brandl et al., 2004; Spiers et al., 2004; Baumgard and Rhoads, 2013; Das et al., 2016).

Minimizing the economic losses associated with HS is becoming more imperative due to rapid human population growth (occurring mainly in the tropical and subtropical areas), which increases resource demands (including animal lean tissue) and threatens food security, predominantly in developing countries (Baumgard and Rhoads, 2013). Therefore, further
investigation on how HS impedes animal productivity is crucial for developing and implementing effective mitigation strategies, which strengthen the global animal agriculture economy.

**Thermoregulation during Heat Stress in Dairy Cows**

Homeotherms utilize the thermoregulatory system to maintain body temperatures when environmental conditions are energetically suboptimal (Bligh and Harthoorn, 1965; Kadzere et al., 2002). Dairy cows achieve their maximum productivity when ambient temperatures range between 5 and 25°C, which is known as the thermoneutral zone (TNZ; Kadzere et al., 2002; Figure 1.1). This zone is characterized by minimum heat production and low physiological costs, allowing the animal to reach their full genetic potential (Kadzere et al., 2002). The TNZ differs according to physiological status of an animal’s (i.e., age, feed intake, insulation, and productivity) as well as environmental factors (i.e., ambient temperature, relative humidity, and solar radiation; Yousef, 1985; Kadzere et al., 2002). The TNZ is fenced by the lower critical temperature (LCT) and the upper critical temperature (UCT; Kadzere et al., 2002), and shifts outside the TNZ (above or below) will activate thermoregulatory mechanisms that are energetically expensive and will eventually hinder animal productivity. Thus, maintaining euthermia is imperative for maximizing dairy cow productivity (Fuquay, 1981; Kadzere et al., 2002).

Animals dissipate heat via four primary routes: conduction, convection, radiation, and evaporative heat loss (Kadzere et al., 2002). Conduction, convection, and radiation (as reviewed by Kadzere et al., 2002) represent sensible heat transfer systems, which require a temperature difference to facilitate heat flow into a cooled environment (Shearer and Beede, 1990; Kadzere et al., 2002; Collier and Gebremedhin, 2015). Conduction heat loss occurs via direct contact of two surfaces which differ in temperature (thus a gradient is present), this allows heat to flow from high to low temperature surfaces. In addition, this mechanism is dependent on the conductivity of the surface and the area of contact (Kadzere et al., 2002; Collier and Gebremedhin, 2015). When an
animal is standing, this route exchanges little heat, as animals are in direct contact with air, which has minimal thermal conductivity (Kadzere et al., 2002). From a management standpoint in dairy farms, it is vital to choose bedding material (e.g., ground limestone) that is characterized by high thermal conductivity allowing for maximum heat transfer (Kadzere et al., 2002).

**Figure 1.1.** Thermoneutral zone (Kadzere et al., 2002; Aggarwal and Upadhyay, 2013)

Convection is the transfer of heat between liquid or air and the animal. As previously mentioned, the temperature gradient is the primary determinant of heat transfer in this system, however, velocity of air movement also plays a role (Kadzere et al., 2002). Radiation is the amount of heat gained or lost by the animal through absorption or release of infrared radiant heat (Kadzere et al., 2002). Although the temperature of the object plays a key part in heat exchange, other factors such as hair coat texture and color contribute as well (Kadzere et al., 2002). When environmental conditions (including ambient temperature and relative humidity) become similar to the surface
temperature of the animal, the sensible routes of heat dissipation become ineffective as the thermal
gradient is reduced. Consequently, heat dissipation can only occur via insensible or latent heat
exchange (Bligh and Johnson, 1973).

Sweating and panting facilitate evaporative heat loss by expulsion of heat (energy) via a
vapor or pressure gradient from the skin and respiratory tract (Shearer and Beede, 1990; Kadzere
et al., 2002; Collier and Gebremedhin, 2015). Among livestock, horses and cattle are considered
sweating animals, while poultry, sheep, and pigs are panters (Collier and Gebremedhin, 2015).
Two types of sweating are utilized by dairy cows, 1) insensible sweating or perspiration which
takes place continuously and 2) thermal sweating which is the primary form of evaporative heat
loss when the ambient temperature increases (Kadzere et al., 2002). Panting is characterized by
increased respiration frequency and decreased tidal volume, resulting in increased upper
respiratory tract ventilation which facilitates heat dissipation (Collier and Gebremedhin, 2015).
However, during high relative humidity conditions, evaporative heat loss becomes ineffective, as
air becomes more saturated with water vapor. As a result, it is highly recommended to have a
combination of fans and sprinklers in high humidity areas to improve the efficiency of evaporative
cooling (Shearer and Beede, 1990).

**Effects of Heat Stress on Dairy Cows’ Productivity**

**Dry Matter Intake**

Thermal stress decreases dry matter intake (DMI) in dairy cows as reported in the literature
(Shanklin, 1963; Attebery and Johnson, 1969; Fuquay, 1981; Collier et al., 1982; Beede and
Collier, 1986). This reduction starts at an ambient temperature of 25-26°C and will be severe when
it exceeds 30°C (Beede and Collier, 1986; Kadzere et al., 2002). Decreased DMI is a strategy to
minimize metabolic heat production and is a conserved response across species during
hyperthermia (Collin et al., 2001; West, 2003; Baumgard and Rhoads, 2013). Heat stress directly
affects appetite through modulation of the rostral cooling center of the hypothalamus, which triggers the medial satiety center (Kadzere et al., 2002). In addition, respiration rates and water intake are increased during hyperthermia to decrease body temperature (Collier et al., 1982), and as a result, time spent eating is reduced (Collier et al., 1982) and gut fill may be increased (Beede and Collier, 1986; Armstrong, 1994).

Heat-stress induced decreases in DMI reduces gut motility and rumination which compromise rumen health and nutrient digestion (Beede and Collier, 1986). During HS, feed digestibility is increased likely due to decreased DMI caused decreased passage rate (Fuquay, 1981). Decreased DMI (specifically roughage) causes a reduction in volatile fatty acid (VFA) production and alterations in acetate to propionate ratios (Kadzere et al., 2002). Thus, it is important to utilize management practices (i.e., shading, cooling systems, nutritional strategies) that could mitigate the deleterious effects of HS on DMI.

**Milk Yield**

Environmentally induced hyperthermia decreases milk yield in dairy cows (Bianca, 1965; Fuquay, 1981; Rhoads et al., 2009; Baumgard and Rhoads et al., 2013). Dairy cows become more prone to HS as increased milk yield is accompanied with more metabolic heat production (Kadzere et al., 2002). In addition, Berman (2005) demonstrated that increasing milk yield from 35 to 45 kg/d increased the susceptibility of dairy cows to HS and decreases “threshold temperature” by 5°C. Traditionally, milk yield reductions were reported to occur when the temperature humidity index (THI) exceeded 72 (Armstrong, 1994) although more recent reports demonstrate decreased production at a THI of 68 (Zimbelman et al., 2009).

The basis for how HS limits milk production involves many mechanisms (as reviewed by Baumgard and Rhoads, 2013): 1) an alteration in endocrine profile which is characterized by marked changes in both catabolic and anabolic hormones, 2) multiple intracellular signaling
pathways which affect maintenance, productivity, and survival, and 3) direct impacts on secretory cells or mammary epithelial cells.

As stated earlier, environmentally induced hyperthermia causes a substantial decrease in DMI as an approach to reduce metabolic heat production, which was theorized to be the sole reason for milk yield reduction (Fuquay, 1981; Beede and Collier, 1986; West, 2003). However, by employing the pair-feeding design to exclude confounding effects of dissimilar nutrient intake, it has been identified that reduced DMI only explains ~50% of decreased milk yield in heat-stressed dairy cows (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011; Gao et al., 2017). Accordingly, the remaining decrease in production (direct effects) is mediated by the fact that heat-stressed animals exploit metabolic and physiologic adjustments (discussed below) to ameliorate the heat insult, independent of nutrient intake. These findings illustrate that hyperthermic dairy cows reduce milk yield by both direct (independent of nutrient intake) and indirect (via reduced feed intake; Baumgard and Rhoads, 2013) mechanisms.

Effects of Heat Stress on Postabsorptive Metabolism

Carbohydrate Metabolism

Hyperthermia was first demonstrated to reduce circulating glucose in cats following acute HS (Lee and Scott, 1916) and this has since been observed in livestock species including: cattle (Settivari et al., 2007; O’Brian et al., 2010), sheep (Achmadi et al., 1993; Mahjoubi et al., 2014), and pigs (Sanz Fernandez et al., 2014). According to Wheelock and colleagues (2010), HS decreased milk lactose production (200-400g) compared with pair-fed thermal neutral animals. Reasons for decreased lactose production could be explained partially by reduced blood glucose levels, as glucose is the precursor for lactose synthesis (Nafikov and Beitz, 2007). Another reason could be related to increased glucose utilization by extramammary tissue (Baumgard and Rhoads, 2013). However, other studies showed that HS increased circulating glucose in quails (El-Kholy...
et al., 2018), chickens (Garriga et al., 2006), and human athletes (Fink et al., 1975; Febbraio, 2001). Reasons for elevated glucose levels could be attributed to increased glycogen breakdown and gluconeogenesis (Collins et al., 1980; Febbraio, 2001). Further, the gene expression of pyruvate carboxylase, a key enzyme that regulates gluconeogenesis, is increased during HS conditions (Wheelock et al., 2008; O’Brien et al., 2008; Rhoads et al., 2011).

Insulin plays a key role in nutrient partitioning as it is the primary anabolic hormone controlling carbohydrate, lipid, and protein metabolism (see review by Baumgard et al., 2016). Although heat-stressed animals are in a catabolic state (i.e., reduced feed intake and negative energy balance) hyperinsulinemia is commonly observed in lactating dairy cows (Wheelock et al., 2010), growing calves (O’Brien et al., 2010), and pigs (Mayorga et al., 2018; Xin et al., 2018; He et al., 2019) during HS. Explanations for hyperinsulinemia during HS are not fully understood, but might be an important strategy to reduce heat production as glucose oxidation is energetically efficient (compared to fatty acid and amino acid oxidation; Baumgard and Rhoads, 2013) or may be related to the fact that leukocytes are insulin responsive (Calder et al., 2007; Maratou et al., 2007) and lipopolysaccharide appears to have a role in stimulating insulin secretion (Baumgard et al., 2016). Thus, heat-stressed animals appear to favor glucose as the primary fuel source (Baumgard and Rhoads, 2013).

**Lipid Metabolism**

During HS conditions, it has been documented that fat accumulation was increased in chickens (Geraert et al. 1996) and pigs (Collin et al., 2001; Renaudeau et al. 2014). Reasons for increased carcass lipid accumulation possibly stem from the lack of adipose tissue mobilization, as previous reports have showed that HS decreases circulating non-esterified fatty acid (NEFA) in lactating cows (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011; Gao et al., 2017), sheep (Sano et al., 1983), goats (Al-Dawood, 2017), chickens (Geraert et al., 1996), and
growing pigs (Mitev et al., 2005; Pearce et al., 2013). Furthermore, circulating NEFA was decreased following an epinephrine challenge in heat-stressed cows and pigs (Baumgard et al. 2011; Sanz Fernandez et al., 2015). Furthermore, Sanders and colleagues (2009) demonstrated that adipose tissue lipoprotein lipase is increased during HS conditions, which indicates an increased capability of the adipose tissue to uptake and store intestinal and hepatic-derived triglycerides. Despite a marked increase in catabolic and stress hormones during HS (e.g. circulating cortisol, epinephrine, and glucagon; Bianca, 1965; Beede and Collier, 1986), the lack of adipose tissue mobilization is unusual.

**Protein Metabolism**

Heat stress alters protein metabolism by decreasing protein accretion in a variety of species (Close et al., 1971; Lu et al., 2007). Environmentally induced hyperthermia reduces DNA and RNA synthesis (Henle and Leeper, 1979; Streffer, 1982). In addition, HS decreases casein synthesis within the mammary gland (Bernabucci et al., 2002). Moreover, HS increases muscle catabolism biomarkers such as plasma urea nitrogen in cows (Shawartz et al., 2009) and pigs (Pearce et al., 2013) and 3-methyl-histidine and creatine in heat-stressed lactating cows (Kamiya et al., 2006). Increased skeletal muscle mobilization likely occurs to support gluconeogenesis (Baumgard and Rhoads, 2013; Belhadj Slimen et al., 2016) and synthesis of acute phase proteins (Johnson, 2012).

Collectively, heat-stressed animals employ many postabsorptive metabolic changes independent of feed intake. These changes are presumably adaptive mechanisms to maintain euthermia, but they constrain animal productivity and are a remarkable economic burden. Understanding the mechanisms of how HS threatens animal performance is the prerequisite to generate mitigation strategies to improve animal welfare and agriculture economics. Table 1.1 summarizes the effects of HS on energetics parameters.
Table 1.1. Effects of heat stress on plasma energetics parameters

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Species</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>Chickens (uric acid)</td>
<td>↓ / ↑</td>
<td>42/45, 46, 82</td>
</tr>
<tr>
<td></td>
<td>Cows</td>
<td>↓ / ↑</td>
<td>74, 77/ 1, 27, 28, 37, 57, 58, 63, 72, 73, 75, 80, 83</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td>↑</td>
<td>25, 34</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>= / ↑</td>
<td>49 / 35, 59, 69</td>
</tr>
<tr>
<td></td>
<td>Rabbits</td>
<td>↑</td>
<td>22, 41</td>
</tr>
<tr>
<td>NEFA</td>
<td>Chickens</td>
<td>↓ / = / ↑</td>
<td>30/45 /11</td>
</tr>
<tr>
<td></td>
<td>Cows</td>
<td>↓ / = / ↑</td>
<td>1, 27, 36, 62, 63, 64, 66, 76, 80, 82/ 13, 33/ 57, 74, 77, 78</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td>↑</td>
<td>25, 34</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>↓ / = / ↑</td>
<td>32, 44, 53, 59, 68/ 49, 50/ 35</td>
</tr>
<tr>
<td></td>
<td>Rabbits</td>
<td>↑</td>
<td>15, 23, 24, 54</td>
</tr>
<tr>
<td>Glucose</td>
<td>Cats</td>
<td>↓</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>↓ / = / ↑</td>
<td>24, 30 / 42, 61/ 8, 9, 29, 31</td>
</tr>
<tr>
<td></td>
<td>Cows</td>
<td>↓ / = / ↑</td>
<td>1, 13, 14, 16, 27, 36, 57, 62, 63, 64, 72, 75, 76, 80, 83, 84/33, 74/ 28</td>
</tr>
<tr>
<td></td>
<td>Dogs</td>
<td>↓</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td>↑</td>
<td>3, 7, 20, 21, 54</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>↓ / = / ↑</td>
<td>69/35, 44, 49, 50, 79/ 32, 59, 60, 65</td>
</tr>
<tr>
<td></td>
<td>Rabbits</td>
<td>↑</td>
<td>48, 56</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>↓ / = / ↑</td>
<td>2, 6, 47, 71/ 5, 67/ 10, 17, 43</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
<td>↑</td>
<td>18, 19</td>
</tr>
<tr>
<td>Insulin</td>
<td>Cows</td>
<td>= / ↑</td>
<td>30/45, 46, 82</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td>=</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>↓ / = / ↑</td>
<td>50/44 / 12, 32, 35, 49, 59, 70, 81</td>
</tr>
<tr>
<td></td>
<td>Snake/Rats</td>
<td>↑</td>
<td>26/ 55, 79</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>↑</td>
<td>47, 67</td>
</tr>
</tbody>
</table>

*Adapted from (Sanders, 2010; Johnson, 2014; Pearce, 2014; Sanz-Fernandez, 2014; Abujamieh, 2015; Mayorga, 2017)*

1Non-esterified fatty acids

*Blood urea nitrogen*
Effects of Heat Stress on Gastrointestinal Tract

The gastrointestinal (GI) tract plays an imperative role in digesting and absorbing water and nutrients as well as provides a physical barrier which prevents the permeation of undesirable luminal content or proinflammatory molecules, such as pathogens, toxins, and antigens into the body (i.e., circulatory system; see review by Suzuki, 2013). This is largely achieved by linking the intestinal epithelial cells to each other by four major junctional complexes: tight junctions (TJ), adhesion junctions, desmosomes, and gap junctions (Barreau and Hugot, 2014). It has been reported that TJ have multiple protein complexes consisting of transmembrane proteins, including claudins and occludin (Suzuki, 2013). These protein complexes determine the selective paracellular permeability to solutes (Anderson and Van Itallie, 2009). Any disruptions in any of the aforementioned complexes can lead to increased intestinal permeability. Thus, many molecules will translocate including bile, hydrolytic enzymes, and endotoxin (i.e., lipopolysaccharide, LPS) into circulatory system and consequently cause immunoactivation and inflammation (Lambert, 2009).

It has been reported that HS increases intestinal permeability in rodents (Hall et al., 2001; Lambert et al., 2002; Prosser et al., 2004; Oliver et al., 2012), dairy cows (Koch et al., 2019), pigs (Pearce et al., 2013b; San-Fernandez et al., 2014), and humans (Lambert, 2004). According to Baumgard and Rhoads (2013), ruminants may be more susceptible to leaky gut than monogastrics as HS induced rumen acidosis (Kadzere et al., 2002) can also negatively alter intestinal barrier function (Plaizier et al., 2008). Additionally, HS negatively affects intestinal morphology by decreasing the villous height (Lambert et al., 2002; Yu et al., 2010; Abuajamieh et al., 2016). Many of the adverse effects of HS on productivity can be explained by impaired intestinal barrier integrity, which is likely as a result of thermoregulation or heat dissipation mechanisms (Hall et al., 2001; Baumgard and Rhoads, 2013). Heat-stressed animals diverted blood to the periphery in
order to maximize radiant heat loss and as a result the GI tract vasoconstricts to maintain blood pressure. Decreased blood flow to the splanchnic tissues causes hypoxia, enterocyte damage, and impaired epithelial integrity; a scenario that allows LPS and undoubtedly thousands of different types of antigens to infiltrate into circulation and consequently cause immunooactivation and inflammation (Lambert, 2009).

Systemic inflammation starts when a pathogen associated molecular patterns (PAMP) bind to a toll-like receptor (TLR). Lipopolysaccharide, a cell wall component of gram-negative bacteria that can be found in large quantities in the gut-lumen and is an example of a PAMP capable of eliciting an immune response (Ravin et al., 1960; Wizniter et al., 1960). The LPS is comprised of three primary parts: lipid A, a core polysaccharide, and an O polysaccharide. The lipid A moiety is responsible for the toxicity of gram-negative bacteria, as it interacts with receptors and initiates the immune response (Munford, 2005). Once LPS is recognized by TLR4 and other proteins such as LPS-binding protein (LBP), and cluster differentiation 14 (CD14), an intracellular signaling cascade is initiated, resulting in nuclear transcription factor kappa-B (NFkB) translocation and inflammatory cytokine transcription (Lu et al., 2008). There are many cytokines involved in inflammation such as tumor necrosis factor alpha (TNFα), interleukins (IL), and type-I interferons (Tak and Firestein, 2001). Table 1.2 summarizes the effects of HS on immune activation markers.

Translocation of intestinally-derived LPS during HS into the circulation was first demonstrated by Graber et al. (1971). Many additional studies have shown that circulating LPS is increased during HS in pigs (Pearce et al., 2013; Gabler et al., 2018), rodents (Hall et al., 2001; Lim et al., 2007), humans (Broke-Utne et al., 1988; Bouchama et al., 1991), monkeys (Gathiram et al., 1988), and chickens (Cronje, 2007). Immunoactivation caused by endotoxin infiltration likely contributes to the adverse effects of HS on animal productivity. Thus, understanding the
mechanisms and the pathophysiology of HS is imperative for developing managerial strategies, which improve animal welfare and minimize the economic losses imposed by HS.

Table 1.2. Effects of heat stress on immune activation markers

<table>
<thead>
<tr>
<th>Species</th>
<th>Measure</th>
<th>Change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td>Plasma IL-6</td>
<td>Increase</td>
<td>4, 15</td>
</tr>
<tr>
<td>Humans</td>
<td>Plasma TNF-α</td>
<td>Increase</td>
<td>15</td>
</tr>
<tr>
<td>Humans</td>
<td>II-1 α, II-1β, IFN-γ</td>
<td>Increase</td>
<td>3, 4</td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>Serum IL-2</td>
<td>Acute Decrease</td>
<td>9</td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>Serum IFN-α</td>
<td>Decrease</td>
<td>9</td>
</tr>
<tr>
<td>Rats</td>
<td>Plasma IFN-γ, IL-2, IL-4, IL-10</td>
<td>Increase</td>
<td>8</td>
</tr>
<tr>
<td>Pigs</td>
<td>Tissue IL-8 and haptoglobin</td>
<td>Acute Increase, Increase</td>
<td>11</td>
</tr>
<tr>
<td>Pigs</td>
<td>Tissue Myeloperoxidase</td>
<td>Increase</td>
<td>11</td>
</tr>
<tr>
<td>Pigs</td>
<td>Plasma LBP</td>
<td>Increase</td>
<td>10</td>
</tr>
<tr>
<td>Pigs</td>
<td>Haptoglobin</td>
<td>Increase</td>
<td>12, 13</td>
</tr>
<tr>
<td>Broilers</td>
<td>Macrophage Activity</td>
<td>No Change</td>
<td>16</td>
</tr>
<tr>
<td>Broilers</td>
<td>Serum TNF-α and IL-2</td>
<td>Increase</td>
<td>1</td>
</tr>
<tr>
<td>Broilers</td>
<td>Intestinal II-1β</td>
<td>Increase</td>
<td>14</td>
</tr>
<tr>
<td>Mice</td>
<td>Hypothalamic II-1β</td>
<td>Increase</td>
<td>2</td>
</tr>
<tr>
<td>Baboons</td>
<td>Circulating IL-6</td>
<td>Increase</td>
<td>5</td>
</tr>
<tr>
<td>Baboons</td>
<td>Circulating C3, C4</td>
<td>Decrease</td>
<td>5</td>
</tr>
<tr>
<td>Rats</td>
<td>Serum TNF-α</td>
<td>Increase</td>
<td>6</td>
</tr>
<tr>
<td>Rats</td>
<td>Plasma II-1β, IL-6, TNF-α</td>
<td>Increase</td>
<td>7</td>
</tr>
</tbody>
</table>

*aAdapted from: Pearce, 2014
1Alhenaky et al., 2017
2Biedenkapp and Leon, 2013
3Bouchama et al., 1991
4Bouchama et al., 1993
5Bouchama et al., 2007
6Kluger et al., 1997
7Ji et al., 2014
8Liu et al., 2012
9Liu et al., 2013
10Mayorga et al., 2019
11Pearce et al., 2013b
12Santos et al., 2018
13Song et al., 2011
14Song et al., 2017
15Starkie et al., 2005
16Quinteiro-Filho et al., 2012

Hypocalcemia and Immunoactivation

Periparturient dairy cows can experience a myriad of metabolic disorders, and transient hypocalcemia represents one of the most common. Hypocalcemia is considered a gateway to other disorders such as ketosis, mastitis, and metritis, all of which compromise profitability and increase culling risks (DeGaris and Lean, 2008; Goff, 2008). After parturition, the mammary gland has a large calcium (Ca) demand, and proper parathyroid hormone (PTH) and calcitonin action is required to maintain eucalcemia (Horst et al., 2005). However, the mammary gland’s Ca uptake is so acute and extensive that it exceeds the homeostatic strategies employed to replenish circulating
Ca (Goff, 2008) and cows can either enter into clinical or subclinical hypocalcemia. It is estimated that subclinical hypocalcemia affects 25% of primiparous and 47-50% of multiparous dairy cows (Reinhardt et al., 2011; Oetzel, 2013). Although not overtly pathological, subclinical hypocalcemia has been associated with decreased productivity and other economically important phenotypes later in lactation (Goff, 2008, 2014; Oetzel, 2013). Different prophylactic and therapeutic strategies for preventing post-calving hypocalcemia include: feeding pre-calving acidifying rations (-DCAD), low Ca-diets (Thilsing-Hansen et al., 2002) or Ca chelating compounds (Goff, 2008). These dietary strategies have markedly reduced clinical rates of “milk fever”, but periparturient subclinical hypocalcemia remains a common post-calving “pathology”. Consequently, orally bolusing Ca following parturition has become a common management tactic (Oetzel and Miller, 2012; Oetzel, 2013).

Although the magnitude and extent differ, it is likely that all periparturient dairy cows (even seemingly healthy ones) experience some degree of inflammation (Trevisi et al., 2012; Bradford et al., 2015; Trevisi and Minuti, 2018) and immunoactivation decreases circulating Ca in a variety of species (Carlstedt et al., 2000; Shinozuka et al., 2018) including ruminants (Waldron et al., 2003a; Horst et al., 2018). Inflammation can be modeled by infusing LPS and this markedly reduces blood Ca in several species (Elsasser et al., 1996; Carlstedt et al., 2000; Toribio et al., 2005; Shinozuka et al., 2018) including dairy cows (Waldron et al., 2003a, Kvidera et al., 2017; Horst et al., 2018). Hypocalcemia is thought to compromise neutrophil function and thus increase susceptibility to infection (Kimura et al., 2006; Martinez et al., 2012, 2014) and therefore periparturient hypocalcemia is thought to be causal to other transition cow diseases (Kimura et al., 2006; Martinez et al., 2012, 2014). Table 1.3 summarizes the effects of endotoxin on circulating mineral concentrations.
Calcium plays a key role in muscle and nerve function (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016). Thus, reduced blood Ca likely compromises skeletal muscle strength and gastrointestinal motility and eventually induces negative transition-cow consequences such as decreased DMI, increased metabolic disorders, and decreased milk production (Oetzel, 2013). In addition, previous research reported that subclinical hypocalcemia impairs energy metabolism by decreasing insulin concentrations, which in turn allows for enhanced adipose tissue mobilization and thus increased ketone production (Martinez et al., 2012, 2014). Furthermore, all cows (even seemingly healthy ones) experience inflammation during the transition period (Trevisi et al., 2012; Bradford et al., 2015) and immunoactivation causes hypocalcemia and reduced DMI (Waldron et al., 2003a,b; Kvidera et al., 2017; Horst et al., 2018) as intracellular Ca signaling plays an important role in immune cell activation, which performs as a second messenger of signal transduction (Lewis, 2001; Kimura et al., 2006). Leukocyte activation initiates a signaling cascade leading to the release of Ca from the endoplasmic reticulum into the cytosol (Lewis, 2001). Consequently, increased cytosolic Ca levels triggers Ca influx from the extracellular space via Ca$^{2+}$ release-activated Ca$^{2+}$ channels (Lewis, 2001), which is likely responsible for systemic hypocalcemia following LPS infusion. Increased intracellular Ca plays a key role in cytokine production as well as cell proliferation (Kimura et al., 2006). Furthermore, another possible reason for developing hypocalcemia post-LPS infusion could be increased Ca accumulation in ascites fluid and liver tissue which has been reported previously in pigs (Carlstedt et al., 2000). Additionally, LPS increased circulating cortisol may contribute to hypocalcemia as reported by Waldron et al. (2003a), as glucocorticoids act like calcitonin as highlighted by Hirsch et al. (1998).
Table 1.3. The effects of endotoxin on minerals in various species

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Species</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Bovine</td>
<td>↓</td>
<td>2, 4, 6, 9, 10, 13, 15, 17</td>
</tr>
<tr>
<td></td>
<td>Dogs</td>
<td>↓</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Horses</td>
<td>↓</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>↓</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>↓</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>↓</td>
<td>14</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Cows</td>
<td>↓</td>
<td>4, 13, 17</td>
</tr>
<tr>
<td>Iron</td>
<td>Bovine</td>
<td>↓</td>
<td>9, 11</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>↓</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>↓</td>
<td>7, 8</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>↓</td>
<td>3</td>
</tr>
<tr>
<td>Zinc</td>
<td>Bovine</td>
<td>↓</td>
<td>9, 11</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>↓</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>↓</td>
<td>7, 8</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>↓</td>
<td>3</td>
</tr>
<tr>
<td>Sodium</td>
<td>Bovine</td>
<td>=</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>↑</td>
<td>11</td>
</tr>
<tr>
<td>Potassium</td>
<td>Bovine</td>
<td>=</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>↑</td>
<td>11</td>
</tr>
</tbody>
</table>

1Carlstedt et al., 2000  
2Elsasser et al., 1996  
3Failla et al., 1983  
4Griel et al., 1975  
5Holowaychuk et al., 2012  
6Horst et al., 2018  
7Johnson et al., 1993  
8Klasing et al., 1987  
9Konigsson et al., 2002  
10Kvidera et al., 2017  
11Lohuis et al., 1988  
12Naylor and Kronfeld, 1986  
13Sandstedt et al., 1984  
14Shinozuka et al., 2018  
15Tennant et al., 1973  
16Toribio et al., 2005  
17Waldron et al., 2003

Mitigation Strategies of Heat Stress

As discussed earlier, HS negatively impacts animal productivity and compromises welfare. Thus, it is paramount to employ heat abatement strategies that help animals to attain their production capacity during the hot summer months. Beede and Collier, (1986) identified three basic management practices to combat HS in dairy cows: 1) environmental modification by providing shade and evaporative cooling via fans and sprinklers 2) genetic development of more
heat-tolerant breeds, and 3) nutritional management strategies. The first approach is a cost-effective avenue to attenuate the effect of thermal stress and minimize economic losses imposed by HS. However, although several advanced management strategies (i.e., cooling systems, barn construction) have been implemented, the negative impacts of HS remain a major constraint to the global dairy industry (Burgos et al., 2007). The second strategy is difficult to achieve since animals are selected for maximum productivity of milk yield, which is a key contributor to increase metabolic heat production, resulting in increased susceptibility to HS (Brown-Brandl et al., 2004). Finally, different nutritional approaches have been implemented during HS such as increased energy density of the diet, reduced fiber content, or increased the inclusion level of fat. Additionally, there is a growing interest in implementing nutritional strategies aimed to alleviate the adverse consequences of HS in dairy cows. This section will focus on electrolyte and Saccharomyces cerevisiae fermentation product (SCFP) supplementation and their role in improving the performance of heat-stressed dairy cows.

**Electrolyte Supplementation**

Hot environmental conditions disturb electrolyte and acid-base balance of dairy cows due in large part to increased sweating and respiration rate as potassium (K) is the primary bovine cation lost in considerable amounts during sweating (Jenkinson and Mabon, 1973; Schneider et al., 1988; Tucker et al., 1988). Additionally, HS-induced increased respiration rate leads to excessive loss of carbon dioxide (CO₂) during panting and induces a reduction in partial pressure of CO₂ in blood. In turn, the kidney excretes bicarbonate (HCO₃⁻) and sodium (Na) to maintain net neutrality) into urine to maintain the constant ratio between the HCO₃ and CO₂ (20:1). As a result, HCO₃ in saliva is decreased and susceptibility to rumen acidosis is increased because blood HCO₃ is transferred into saliva on a concentration basis (Kadzere et al., 2002; West, 2003).
Therefore, it is advantageous to supplement electrolytes during hot climates to maintain systemic acid-base balance (Kadzere et al., 2002; West, 2003). In addition, electrolytes supplementation can regulate water balance (Schneider et al., 1986). Feeding electrolytes in forms of NaHCO₃ and KCl benefited heat-stressed dairy cows by increasing milk yield, regulating acid-base balance, and lowering body temperature (Coppock et al., 1982; Tucker et al., 1988; West et al., 1991, 1992). Although many beneficial results are observed, other reports have shown no improvement in DMI and milk yield (Schneider et al., 1988; Chan et al., 2005; Cabrera, 2014). In heat-stressed broilers, electrolyte supplementation improved body weight gain, feed conversion ratio, and reduced body temperature (Borges et al., 2003). In addition, feeding a high DCAD diet improved DMI and increased nighttime water intake in heat-stressed dairy goats (Nguyen et al., 2019). Thus, supplementing electrolytes is beneficial to maintain acid-base balance, as the primary electrolyte K is lost via sweating and Na via urine, and increase DMI, milk yield, and reduce body temperature during HS.

**Saccharomyces Cerevisiae Fermentation Product (SCFP) Supplementation**

Yeasts are classified within the fungi kingdom (Yoon and Stern, 1995) and are considered facultative anaerobes, capable of withstanding and growing in conditions with or without oxygen (Stone, 2006). Yeasts are extensively used in the baking industry, as they ferment sugar and starch into carbon dioxide leading to expansion of the dough (Schneiter, 2004). *Saccharomyces cerevisiae* is the species of yeast most commonly supplemented to dairy cows; it has shown beneficial effects in animal performance and is considered a “natural” alternative to growth-promoting antibiotics (Yoon and Stern, 1995; Broadway et al., 2015; Shurson, 2018). The first use of yeast as a supplement for dairy cows was reported by Eckles and Williams (1925). *Saccharomyces cerevisiae* can be supplemented in the diet as live cells or as a yeast culture (Broadway et al., 2015) comprising of a unique combination of live yeast and fermentation by-
products or metabolites (Poppy et al., 2012). Briefly, live yeast cells are inoculated with a culture media and they produce fermentation products (Shurson, 2018). These products include antioxidants, amino acids, B vitamins, soluble fiber, and other bioactive compounds presumably used as growth promoters for rumen microorganisms (Zaworski et al., 2014).

Yoon and Stern (1995) reviewed the potential mode of action for the yeast cultures and demonstrated improved removal of rumen oxygen and an increased the number of lactate utilizing bacteria. As a consequence, utilization of lactate increases and thus modulate ruminal pH and this could increase microorganism number (Newbold et al., 1998) in the rumen allowing for more ammonia to be incorporated into microbial protein, which will reflect positively on overall animal productivity.

Previous studies using the SCFP improved rumen pH (Erasmus et al., 2005), DMI (Dann et al., 2000), and milk yield (Harrison et al., 1988; Hippen et al., 2007; Ramsing et al., 2009), however, other studies have observed no differences in DMI and milk yield (Robinson, 1997; Schingoethe et al., 2004). Interestingly, previous studies have shown that feeding SCFP is most beneficial during challenging situations such as HS (Schingoethe et al., 2004; Zhu et al., 2016), dietary aflatoxin B1 challenge (Jiang et al., 2018), and the transition period (Dann et al., 2000; Zaworski et al., 2014; Shi et al., 2019).

Recently, feeding SCFP as an immunomodulator to bolster the immune function has received considerable attention (Broadway et al., 2015; Shurson, 2018). Zaworski et al. (2014) reported that transitioning dairy cows supplemented with SCFP had decreased somatic cell counts compared with controls. Additionally, SCFP supplementation reduced the acute phase protein response in dairy cows during the periparturient period (Knoblock et al., 2018), which likely indicates less systemic stress or quicker resolution of inflammation. Such responses may be
mediated by the immunomodulatory effect of SCFP through the cell wall components of yeast (β-glucan and mannan) which are thought to bolster the immune system by altering cytokine production and improving neutrophil function (as reviewed by Volman et al., 2008; Broadway et al., 2015). Furthermore, Gao et al. (2008) observed that feeding yeast culture in broiler chickens increased growth performance and altered the immune function by increasing antibody titers to Newcastle disease and lysozyme activity.

In summary, feeding SCFP improves rumen fermentation and overall animal productivity and appears to improve the inflammatory response (especially during challenging situations). Therefore, supplementing a SCFP would represent a feasible and plausible dietary strategy to improve rumen health and reduce the inflammatory response in heat-stressed dairy cows. Table 1.4 summarizes the effects of feeding SCFP on production and health parameters in various species.

Table 1.4. Effects of *Saccharomyces cerevisiae* fermentation product on production and health parameters in various species

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Species</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cows</td>
<td>↓/ =/↑</td>
<td>1, 10/ 2, 4, 6, 18, 21, 22, 26, 27/ 7, 8, 9</td>
</tr>
<tr>
<td>Milk yield</td>
<td>Cows</td>
<td>=/↑</td>
<td>2, 6, 10, 11, 20, 22/ 1, 4, 12, 13, 15, 16, 19, 23, 26, 27</td>
</tr>
<tr>
<td>Milk SCC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cows</td>
<td>↓ / =</td>
<td>5, 24, 25/ 8, 19</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Cows</td>
<td>↓</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>↓</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Turkeys</td>
<td>↓</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dry matter intake  
<sup>b</sup>Somatic cell counts  
<sup>c</sup>Acute phase protein response  
<sup>1</sup>Alshaikh et al., 2002  
<sup>2</sup>Arambel and Kent, 1990  
<sup>3</sup>Bartz et al., 2018  
<sup>4</sup>Bruno et al., 2009  
<sup>5</sup>Bluel, 2006  
<sup>6</sup>Cook et al., 2007  
<sup>7</sup>Carro et al., 1992  
<sup>8</sup>Dann et al., 2000  
<sup>9</sup>Erasmus et al., 1992  
<sup>10</sup>Erasmus et al., 2005  
<sup>11</sup>Harris et al., 1992  
<sup>12</sup>Harrison et al., 1988  
<sup>13</sup>Hippen et al., 2007  
<sup>14</sup>Knoblock et al., 2018  
<sup>15</sup>Kung et al., 1997  
<sup>16</sup>Lehloeny et al., 2008  
<sup>17</sup>Nelson et al., 2018  
<sup>18</sup>Rameshwar et al., 1998  
<sup>19</sup>Ramsing et al., 2009  
<sup>20</sup>Robinson et al., 1997  
<sup>21</sup>Robinson and Garrett, 1999  
<sup>22</sup>Schingoethe et al., 2004  
<sup>23</sup>Williams et al., 1991  
<sup>24</sup>Yuan et al., 2015  
<sup>25</sup>Zaworski et al., 2014  
<sup>26</sup>Zhu et al., 2016  
<sup>27</sup>Zhu et al., 2017
Limitations of Heat Stress Research

As discussed earlier, understanding the biological reasons why HS reduces production is a prerequisite to developing mitigation strategies aimed at ameliorating the negative consequences of environmental hyperthermia. Traditionally, environmental chambers have been required to design and conduct well-controlled HS studies in lactating dairy cows that employ thermal neutral as well as pair-fed controls. However, due to construction and operation costs, many institutions lack the necessary facilities or resources. Furthermore, accurately employing true thermal neutral controls is hard to obtain in natural HS studies, as the thermal neutral group is considered mildly hyperthermic. Additionally, the marked variation in ambient temperature from day to day allows for an inconsistent heat load pattern in the HS group. Thus, it is great of interest to look for an alternative and cost-effective model to study HS in lactating dairy cows.

Conclusion

Environmental hyperthermia has many detrimental effects on dairy cow productivity, including decreased milk yield and composition, growth, and reproduction. These negative effects impose a substantial economic burden on animal agriculture. Thus, understanding the mechanisms of how HS compromises animal productivity is an important prerequisite to developing and implementing mitigation strategies aimed at improving animal welfare and agriculture economics. Accurately examining the biological reasons by which HS jeopardizes animal performance requires environmental chambers, which most facilities lack. Therefore, developing and evaluating alternative and cost-effective models to study HS and to assess nutritional HS mitigation strategies using this model in lactating dairy cows may expand the accessibility to the environmental physiology discipline and advance research in thermal biology area.

The overall objectives of the current dissertation were 1) develop and validate alternative cost-effective HS models, 2) evaluate HS mitigation strategies especially nutritional utilizing the
alternative model, and 3) evaluate mitigation strategies to LPS and deleterious immune challenge effects.

References


CHAPTER 2. TECHNICAL NOTE: DEVELOPING A HEAT STRESS MODEL IN DAIRY COWS USING AN ELECTRIC HEAT BLANKET


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Abstract

Precisely studying the biological consequences of heat stress (HS) in agriculturally relevant animals typically requires expensive climate-controlled facilities, infrastructure inaccessible to most researchers. Thus, study objectives were to explore the efficacy of an electric heat blanket (EHB) as an alternative method for evaluating HS and to determine whether EHB-induced hyperthermia affects production parameters similar to natural HS. Lactating Holstein cows (n = 8; 133 ± 3 d in milk; 709 ± 31 kg; 2.6 ± 0.3 parity) were housed in individual box stalls and allowed to acclimate for 3 d. After acclimation, the trial consisted of 2 experimental periods (P). During P1 (3 d), cows were housed in thermoneutral conditions for collecting baseline data. During P2 (7 d) cows were fitted with an EHB. During the entire experiment cows were fed ad libitum, and dry matter intake (DMI) was recorded daily. Cows were milked twice daily (0600 and 1800 h) and milk samples were collected on d 2 and 3 of P1 and d 3 and 7 of P2. Rectal temperature, respiration rate, heart rate, and skin temperature were obtained twice daily (0600 and 1800 h) during both P1 and P2. Overall, there was an increase in rectal temperature and respiration rate at 0600 h (1.0°C and 25 breaths/min, respectively) and 1800 h (1.2°C and 29 breaths/min,
respectively) during P2. The EHB decreased DMI and milk yield (25 and 21%, respectively) by the end of P2. During P2, milk protein tended to decrease (4.4%) compared with P1. In contrast, milk urea nitrogen increased (33%) during P2 relative to P1. No other differences were observed in milk composition. In summary, our results indicate that employing an EHB affects physiological and production parameters similarly to natural HS (i.e., increased rectal temperature and respiration rate, decreased DMI and milk yield); thus, the EHB is an effective and inexpensive research tool for evaluating the biological consequences of HS in lactating dairy cows.

**Key words:** dairy cow, heat stress, electric blanket

**Technical note**

Heat stress (HS) is an annual environmental issue negatively affecting a variety of production parameters, including milk yield and composition, growth, and reproduction (Baumgard and Rhoads, 2013). Heat stress occurs when environmental conditions, coupled with internal thermogenesis, create a heat load that exceeds thermolytic capacity (Bernabucci et al., 2010). Dairy cows are more susceptible to HS than most farm animals due to their high metabolic heat production and low surface area:mass ratio (West, 2003; Liu et al., 2014). The economic burden of HS is more than $1.5 billion in the United States dairy industry alone (Key and Sneeringer, 2014). Although HS is an animal welfare and economic issue in developed nations, it is a key constraint to food security in many emerging economies (Baumgard and Rhoads, 2013). The negative consequences of HS will intensify if climate change and weather variability continue as predicted (Nardone et al., 2010) and genetic selection continues to emphasize economically important phenotypes, as these traits are accompanied by increased basal heat production (Spiers et al., 2004; Baumgard and Rhoads, 2013; Das et al., 2016).

Heat stress has indirect (via reduced feed intake) and direct effects on the endocrine system, metabolism, and immunoactivation (Baumgard and Rhoads, 2012, 2013). Heat-induced decreased
DMI only explains about 50% of the decreased milk yield (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011) and characterizing the underlying etiology behind the remaining decrease in production would presumably provide the necessary foundation to develop mitigation strategies. Thus, there remains an urgent need to thoroughly characterize the mechanisms by which HS decreases dairy cow productivity. Traditionally, environmental chambers have been required to design and conduct well-controlled HS studies in lactating dairy cows, especially if experimental objectives are to isolate the direct effects of HS. However, due to construction and operation costs, many institutions lack the necessary facilities or resources. Hence, our objectives were to explore the efficacy of using an electric heat blanket (EHB) as an alternative and cost-effective method to study HS and to determine whether EHB-induced hyperthermia affects physiological and production parameters similar to natural HS. If effective, this alternative model would broaden accessibility to the environmental physiology discipline and likely hasten advances in the field of thermal biology.

Lactating Holstein cows (n = 8; 133 ± 3 DIM; 709 ± 31 kg BW; parity 2.6 ± 0.3) were housed in sand and straw-bedded individual box stalls (4.57 × 4.57 m) within a naturally ventilated barn at the Iowa State University Dairy Farm (Ames) and were allowed to acclimate for 3 d. The trial included 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and housed in thermoneutral conditions for collecting baseline body temperature indices and production parameters (hence, each animal served as its own control). During P2 (7 d) cows were fitted with an EHB (1.87 × 1.59 m; 100% nylon with polyurethane coating; weight = 7.7 kg) consisting of 12 infrared heating pads as a heat source (Figure 2.1; Thermotex Therapy Systems Ltd. Calgary, AB, Canada); the EHB remained on the cows for the entirety of P2. The blanket was powered by a 110-V electrical cord that connected to the EHB at the withers. The power cord was positioned on the
ceiling in the center of the box stall in a mounted and retractable cord reel with auto rewind to facilitate unabated movement and natural behavior. The EHB had a surface area of 29,733 cm² that covered approximately 50% of the cow (assuming a 650- to 700-kg cow has a surface area of about 55,000 to 59,000 cm²; Elting, 1926; Berman, 2003). Ambient temperature and relative humidity were monitored and recorded every 10 min by a data logger (EL-USB-2 LCR, Lascar Electronics, Erie, PA) and condensed into a daily average. Cows were housed in thermoneutral ambient conditions throughout the experimental period (21.0 ± 0.6°C, 43.3 ± 1.4% relative humidity; 66 temperature-humidity index). All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

Cows were individually fed a TMR consisting primarily of corn silage (34% of diet DM) once daily (0800 h), and orts were measured daily before feeding. The TMR was formulated to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals, and vitamins. Cows were milked twice daily (0600 and 1800 h), and yield was recorded. Milk samples from each cow were collected on d 2 and 3 of P1 and on d 3 and 7 of P2. Samples were stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC-approved infrared analysis equipment and procedures (AOAC International, 1995).

During both P1 and P2, rectal temperature (Tr), skin temperature (Ts), respiration rate (RR), and heart rate (HR) were obtained twice daily (0600 and 1800 h). During the first 48 h of P2, body temperature indices were obtained hourly in order to monitor cow health and ensure animal safety (data not shown). Rectal temperatures were measured using a standard digital thermometer (M700 digital thermometer, GLA Agricultural Electronics, San Luis Obispo, CA). Skin temperatures were measured on the neck (not covered by the EHB) using an infrared
thermometer (IRT207 Heat Seeker 8:1 mid-range infrared thermometer, General Tools and Instruments, New York, NY). Respiration rates were determined by counting flank movements during a 15-s interval and multiplied by 4 to obtain breaths per minute. Heart rate was determined using a stethoscope placed over the left side of the rib cage behind the elbow, and heart beats were counted for a 15-s interval. This measurement was multiplied by 4 to obtain beats per minute.

Effects of day and period were assessed separately using the MIXED procedure of SAS (version 9.4; SAS Institute. Inc., Cary, NC). Dry matter intake, milk yield, body temperature indices, and milk composition during P2 were analyzed using repeated measures with an autoregressive covariance structure and day as the repeated effect. In addition, the effects of period were analyzed separately using the MIXED procedure of SAS with a diagonal covariance structure. Results are reported as least squares means and were considered different when $P \leq 0.05$ and a tendency if $0.05 < P < 0.10$.

The EHB caused an immediate increase in body temperature indices (Tr and RR), and the magnitude of increase was characteristic of cows experiencing seasonal HS (Kadzere et al., 2002). Overall and relative to P1, there was an increase in Tr and RR at both 0600 and 1800 h during P2 (0600 h; 1.0°C and 25 breaths/min, respectively; 1800 h; 1.2°C and, 29 breaths/min, respectively; $P < 0.01$; Figure 2.2). Heart rate measured at 0600 h tended to increase with time during P2 ($P = 0.06$; Table 2.1) but was unaffected at the 1800 h measurement ($P = 0.12$; Table 2.1). No differences were observed in Ts at both 0600 and 1800 h during P2 relative to P1 ($P = 0.63$; $P = 0.44$, respectively; Table 2.1).

Overall, DMI decreased during P2 compared with P1 ($P < 0.01$). By d 5 to 7 of P2, DMI was decreased 25% relative to P1 ($P = 0.02$; Figure 2.3A). Likewise, milk production was decreased overall during P2 compared with P1 (13%; $P < 0.01$), and the nadir (21%) occurred on
d 7 of P2 ($P = 0.05$; Figure 2.3B). Period 2 milk protein tended to decrease (4.4%; $P = 0.07$) compared with P1. In contrast, MUN increased during P2 (33%; $P < 0.01$) relative to baseline. No other differences were observed in milk content of fat, lactose, TS, and milk SCC during P2 ($P > 0.10$; Table 2.2).

In the present study, both Tr and RR were markedly increased by the EHB, indicating that we successfully implemented a stressful heat load. The magnitude of the average change in both Tr and RR (~1.1°C and 27 breaths/min) was similar to those achieved in climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011; Cowley et al., 2015). Although the extent of increased body temperature indices was expected, little or no signs of acclimation occurred with time. In other words, we expected Tr and RR to peak between d 1 and 2 and then gradually decrease; such changes indicative of acclimation have been observed in natural HS (Kadzere et al., 2002) and in HS studies conducted in environmental chambers (Rhoads et al., 2009; Wheelock et al., 2010). Reasons for the apparent lack of thermal acclimation are not clear, but the blanket obviously obstructed normal routes of heat dissipation (i.e., evaporative cooling via sweating) that are presumably key aspects of acclimation.

Interestingly, Ts was unaffected by the EHB, and this contradicts climate-controlled studies that report increased Ts (Rhoads et al., 2009; Shwartz et al., 2009). Interpreting skin temperature is difficult because it is a combination of the amount of radiant heat dissipated, ambient air temperature, wind speed, and water evaporation (Kadzere et al., 2002). During natural HS and in climate-controlled experiments, the temperature gradient between the skin and air is low or actually negative. This is not the case for the EHB model, as the blanket covers only the back and abdominal region (Figure 2.1; about 50% of the cow’s surface area) and does not fully cover the
neck (where Ts was measured). The ambient temperature during the experiment was 21°C, and thus the temperature gradient between skin and air was much greater than would be in a natural HS setting.

Heat stress induced by the EHB decreased DMI as expected, and this agrees with the literature (see reviews by Kadzere et al., 2002; West, 2003; Baumgard and Rhoads 2012, 2013). The gradual and progressive pattern of reduced feed intake is similar to that observed in climate-controlled chamber experiments (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Reduced DMI is a highly conserved response to HS and presumably serves as a survival strategy to decrease metabolic heat production (Kadzere et al., 2002; West, 2003; Baumgard and Rhoads, 2013). The EHB also decreased milk yield (21%); and this also was expected. Our previous reports indicate that reduced nutrient intake accounts for only 50% of the decreased milk yield, with the remaining portion due to changes in nutrient partitioning and immunoactivation (Baumgard and Rhoads, 2012, 2013). However, the current experiment did not use a pair-feeding design, and thus we are unable to confirm whether a similar feed intake:milk production relationship exists with the EHB.

Milk protein content tended to decrease during HS, and this agrees with the seasonal pattern of low-summer milk protein (Bouraoui et al., 2002) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011; Cowley et al., 2015). Exact reasons for why HS decreases milk protein content are not clear, but increased extramammary amino acid utilization (Gao et al., 2017) and a downregulation of mammary protein synthesizing machinery (Bernabucci et al., 2010; Cowley et al., 2015) are 2 probable explanations. The EHB increased MUN; and this agrees with previous climate-controlled studies (Wheelock et al., 2010; Cowley et al., 2015) and is likely the result of skeletal muscle mobilization to support
gluconeogenesis and acute phase protein synthesis (Baumgard and Rhoads, 2013). As opposed to the characteristic decrease in milk fat typically observed during the summer (Hays, 1926; Bouraoui et al., 2002), we did not detect EHB-induced decreased milk fat content. However, the lack of a HS effect on milk fat agrees with most climate-controlled HS reports (Rhoads et al., 2009; Shwartz et al., 2009; Cowley et al., 2015) and suggests that low-summer milk fat is not directly caused by HS.

In conclusion, employing the EHB increased body temperature indices (Tr and RR) and negatively affected production parameters. Thus, utilizing the EHB is a relatively low-cost and scientifically valuable, albeit unconventional, research technique to model and simulate HS in lactating dairy cows. Importantly, the EHB is likely not a good technique to study products whose mode of action is to facilitate heat dissipation via radiation, convection, or evaporation (vasodilatation at the periphery or sweating), as the blanket markedly interferes with normal routes of heat loss. However, if experimental objectives are to study the biological consequences of HS or to test products with activity within the gastrointestinal tract or via modifying metabolism, then the EHB is a feasible research strategy.

References


Table 2.1. Effects of an electric heat blanket-induced heat stress on physiological indicators in lactating Holstein cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day</th>
<th></th>
<th></th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P1*  1  2  3  4  5  6  7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR(^1), beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0600 h</td>
<td></td>
<td>74 . 82 88 77 85 83 81</td>
<td></td>
<td>3</td>
<td>0.06</td>
</tr>
<tr>
<td>1800 h</td>
<td></td>
<td>75 91 88 80 83 87 85 86</td>
<td></td>
<td>4</td>
<td>0.12</td>
</tr>
<tr>
<td>Ts(^2), °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0600 h</td>
<td></td>
<td>33.7 . 35.5 34.5 34.3 35.0 34.0 33.8</td>
<td></td>
<td>0.8</td>
<td>0.63</td>
</tr>
<tr>
<td>1800 h</td>
<td></td>
<td>35.0 35.7 34.8 35.2 35.6 35.7 35.6 36.9</td>
<td></td>
<td>0.6</td>
<td>0.44</td>
</tr>
</tbody>
</table>

\(^1\)Heart rate

\(^2\)Skin temperature

*Average of d 1 to 3 of period 1.
Table 2.2. Effect of an electric heat blanket-induced heat stress on milk variables in lactating Holstein cows

<table>
<thead>
<tr>
<th>Milk Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, %</td>
<td>3.91</td>
<td>4.04</td>
<td>0.20</td>
<td>0.66</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.03</td>
<td>2.90</td>
<td>0.05</td>
<td>0.07</td>
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<tr>
<td>Lactose, %</td>
<td>4.81</td>
<td>4.80</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Total solids, %</td>
<td>12.7</td>
<td>12.6</td>
<td>0.2</td>
<td>0.95</td>
</tr>
<tr>
<td>SCC, x 1,000 cells</td>
<td>91</td>
<td>106</td>
<td>24</td>
<td>0.66</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>12.8</td>
<td>17.0</td>
<td>0.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Figure 2.1. Holstein cow fitted with the electric heat blanket.
Figure 2.2. Effects of an electric heat blanket on (A) rectal temperature (Tr) at 0600 h, (B) Tr at 1800 h, (C) respiration rate (RR; bpm = breaths per minute) at 0600 h and (D) RR at 1800 h in lactating dairy cows. Values for P1 represent the average of the 3 d of period 1. Values with differing letters (a-c) denote differences ($P < 0.05$) between days. Cows were fitted with an electric heat blanket from d 1 through 7. Results are expressed as LSM ± SEM.
Figure 2.3. Effects of an electric heat blanket on (A) DMI and (B) milk yield in lactating dairy cows. Values for P1 represent the average of the 3 d of period 1 (pre-electric heat blanket). Values with differing letters (a-b) denote differences ($P < 0.05$) between days. Cows were fitted with an electric heat blanket from d 1 through 7. Results are expressed as LSM ± SEM.
CHAPTER 3. VALIDATING A HEAT STRESS MODEL: THE EFFECTS OF AN ELECTRIC HEAT BLANKET (EHB) AND NUTRITIONAL PLANE ON LACTATING DAIRY COWS

Modified from a paper to be submitted to the Journal of Dairy Science


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Abstract

The efficacy of an electric heat blanket (EHB) has previously been confirmed as an alternative method to pragmatically evaluate heat stress (HS). However, a pair-feeding design has not been employed with the EHB model. Therefore, study objectives were to determine nutritional plane’s contribution to altered metabolism and productivity during EHB-induced HS. Multiparous Holstein cows (n = 18) were subjected to 2 experimental periods (P): During P1 (4 d), cows were in thermoneutral conditions with ad libitum feed intake. During P2 (4 d), cows were assigned to 1 of 2 treatments: 1) thermoneutral conditions and pair-fed (PF; n = 8) or 2) EHB-induced HS (Thermotex Therapy Systems Ltd. Calgary, Canada) with ad libitum feed intake (n = 10). Overall, the EHB increased rectal temperature, vaginal temperature, skin temperature, respiration rate, and heart rate (1.4°C, 1.3°C, 0.8°C, 42 breaths/min, and 17 beats/min, respectively) relative to PF cows. The EHB reduced dry matter intake (DMI; 47%) and by design PF cows had a similar pattern and extent of decreased DMI. Milk yield decreased in EHB and PF cows by 27.3% (12.1 kg) and 13.4% (5.4 kg), respectively, indicating reduced DMI accounted for only ~50% of decreased milk synthesis. Milk fat content tended to increase (19%) in the EHB group, while in the PF cows it
remained similar relative to P1. During P2, milk protein and lactose content tended to decrease or decreased (1.30 and 2.2%, respectively) in both EHB and PF groups. During P2, milk somatic cell count increased (31%) in both groups relative to P1. Milk urea nitrogen remained unchanged in PF controls but increased (34.2%) in EHB cows relative to P1. The EHB decreased blood partial pressure of CO₂, total CO₂, HCO₃, and base excess levels (17, 16, 17, and 81%, respectively) when compared with the PF cows. The EHB tended to increase hematocrit (indicator of dehydration; 6.3%) compared with PF controls. During P2, both the EHB and PF cows had a similar decrease (4%) in plasma glucose content, but no differences in circulating insulin were observed. However, a group by day interaction was detected for plasma non-esterified fatty acids; as they progressively increased in the PF controls but remained unaltered in the EHB cows. Blood urea nitrogen increased in the EHB cows (61%) compared with the PF controls. In summary, utilizing the EHB model indicates that reduced nutrient intake only explains about 50% of the decrease in milk yield during HS and the post-absorptive changes in nutrient partitioning are similar to those obtained in climate-controlled chamber studies. Consequently, the EHB is a reasonable and economically feasible model to study environmental physiology of dairy cows.

**Key words:** heat stress, pair feeding, dairy cow

**Introduction**

Heat stress (HS) imposes a major hurdle to efficient livestock productivity. In the American dairy industry alone, environmental hyperthermia costs more than $1.5 billion annually (Key and Sneeringer, 2014). This financial burden is explained by reduced milk yield, impaired growth rates, decreased reproductive performance, and compromised health (Kadzere et al., 2002; Baumgard and Rhoads, 2013). Therefore, HS limits the production of high quality protein for human consumption, compromises farm profitability and is a serious food security issue (particularly in many developing countries; Baumgard and Rhoads, 2013).
During HS, dairy cows voluntarily reduce feed intake, and this is presumably a key strategy to decrease metabolic heat production (Kadzere et al., 2002; West, 2003; Baumgard and Rhoads, 2013). It has traditionally been thought that reduced feed intake is responsible for decreased milk production (Fuquay, 1981; Collier et al., 1982; West, 2003). However, our previous experiments utilizing a pair-feeding design demonstrated that hypophagia only explains about 50% of the decreased milk yield during HS (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011), indicating HS decreases milk yield by both indirect (via reduced feed intake) and direct effects (Baumgard and Rhoads, 2012, 2013). Independent of reduced nutrient consumption, HS directly alters postabsorptive carbohydrate, lipid, and protein metabolism, which is primarily characterized by increased circulating insulin, blunted adipose tissue mobilization, and increased plasma markers of muscle catabolism (Baumgard and Rhoads, 2013). The aforementioned energetic changes (seemingly reflective of anabolic metabolism) are bioenergetically difficult to explain as HS is a hypercatabolic and life-threatening condition. Thus, having a better understanding of how and why HS initiates these unique alterations to nutrient partitioning is likely a prerequisite to developing effective mitigation strategies.

Accurately parsing between the direct and indirect consequences of HS requires environmental chambers. This is primarily because obtaining a true thermoneutral environment in a natural setting is difficult and these studies rely on active cooling to keep the controls from becoming exceedingly hyperthermic. Further, daily variation in ambient conditions (temperature, humidity, wind, solar radiation etc.) create inconsistent heat-loads during the experiment. However, most institutions do not have environmental chambers large enough for cattle due to construction costs and operational expenses. Thus, we developed a HS model that employs an electric heat blanket (EHB) as a cost-effective way to induce HS (based upon thermal indices and
production phenotypes) in dairy cows (Al-Qaisi et al., 2019). However, we have not determined if the direct effects of EHB-induced HS alter metabolism similarly to the way it does in climate-controlled experiments. Therefore, study objectives were to determine nutritional plane’s contribution to altered metabolism and productivity in the EHB model.

**Materials and Methods**

**Animals and Experimental Design**

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Eighteen lactating Holstein cows (140 ± 10 DIM; 674 ± 15 kg of BW; parity 2.3 ± 0.1) were housed in sand and straw-bedded individual box stalls (4.57 × 4.57 m) within a naturally ventilated barn at the Iowa State University Dairy Farm (Ames) and were allowed 4 d to acclimate. The trial consisted of 2 experimental periods (P). During P1 (4 d), cows were housed in thermoneutral conditions (21.0 ± 0.3°C, 63.0 ± 0.6% relative humidity; 67 temperature-humidity index) with ad libitum feed intake. During P2 (4 d), cows were assigned to 1 of 2 groups: 1) thermoneutral conditions and pair-fed (PF; n = 8) or 2) HS induced artificially by an EHB with ad libitum feed intake (EHB; n = 10). Cows were fitted with an EHB consisting of 12 infrared heating pads as a heat source (Thermotex Therapy Systems Ltd. Calgary, Canada); the EHB remained on the cows for the entirety of P2. The blanket was powered by a 110-V electrical cord that connected to the EHB at the withers as previously described (Al-Qaisi et al., 2019). One cow from the EHB group was excluded from the trial due to illness unrelated to the blanket and her data was not incorporated in the final data set. During P2, the PF cows were pair-fed to their EHB counterparts to eliminate the confounding effects of dissimilar nutrient intake as we have previously described (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). In brief, the P1 feed intake was averaged for each cow and used as a baseline. For each EHB cow, the decrease in feed intake during P2 was calculated as the percentage of feed intake reduction relative to P1 for each day of
heat exposure. The percentage of feed intake reduction was averaged for all cows in the EHB group per day of heat exposure and applied individually to the baseline of each cow in the PF group. The daily amount of feed provided to the PF group was divided equally into 2 portions during P2 (~0800 and 1800 h) to minimize large metabolic changes associated with gorging.

Ambient temperature and relative humidity were monitored and recorded every 10 min by a data logger (EL-USB-2 LCR, Lascar Electronics, Erie, PA) and condensed into a daily average. Cows were housed in thermoneutral ambient conditions throughout P2 (23.2 ± 0.1°C, 59.4 ± 0.4% relative humidity; 70 temperature-humidity index).

Cows were individually fed a TMR once daily (0800 h), and orts were measured prior to feeding. The TMR was formulated to meet or exceed the predicted requirements (NRC, 2001; Table 3.1) of energy, protein, minerals, and vitamins for lactating cows. Cows were milked twice daily (0600 and 1800 h), and yield was recorded. Milk samples from each cow were collected daily during both experimental periods. Samples were stored at 4°C with a preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC-approved infrared analysis equipment and procedures (AOAC International, 1995).

During both P1 and P2, rectal temperature (Tr), skin temperature (Ts), respiration rate (RR), and heart rate (HR) were obtained twice daily (0600 and 1800 h). Rectal temperatures were measured using a standard digital thermometer (M700 digital thermometer, GLA Agricultural Electronics, San Luis Obispo, CA). Skin temperatures were measured on the neck using an infrared thermometer (IRT207 Heat Seeker 8:1 mid-range infrared thermometer, General Tools and Instruments, New York, NY). Respiration rates were determined by counting flank movements during a 15-s interval and multiplied by 4 to obtain breaths per minute. Heart rate was determined
using stethoscope placed over the left side of the rib cage behind the elbow, and heart beats were counted for a 15-s interval. This measurement was multiplied by 4 to obtain beats per minute.

Continuous vaginal temperatures ($T_v$) were obtained via a calibrated temperature logger (iButton DS 1921, Maxim Integrated, San Jose, CA) fitted in a hollowed-out space in the center of a blank controlled internal drug release (CIDR; Zoetis, Parsippany, NJ) and inserted into the vagina with an applicator. Loggers were fixed in the CIDR using a silicone aquarium sealant (Aqueon, Franklin, WI). Vaginal temperatures were obtained every 10 min for 6 consecutive days (from P1 d3 to P2 d4). Time of CIDR insertion and removal were recorded. Data collected within the first hour of CIDR insertion were removed to guarantee the precision of the temperature measurements.

Blood samples were collected via coccygeal venipuncture (plasma, K2EDTA tube; serum, serum tube; BD vacutainers, Franklin Lakes, NJ) on d 2 and 4 of both P1 and P2 following the morning milking. Serum samples were allowed to clot at room temperature for 1 h prior to centrifugation. Plasma and serum were harvested following centrifugation at 1500 × g for 15 min at 4°C and were subsequently frozen at -20°C until analysis.

Plasma insulin, non-esterified fatty acids (NEFA), and BUN concentrations were determined using commercially available kits according to manufacturers’ instructions (insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; BUN, Teco Diagnostics Anaheim, CA). The inter and intra-assay coefficients of variation for insulin, NEFA, and BUN assays were 11.5 and 4.2%, 4.5 and 5.3%, 10.0 and 5.9%, respectively.

Blood gas analysis and circulating glucose were measured on fresh blood collected on d 4 of P1 and d 2 and 4 of P2 into lithium heparin tubes and assayed immediately using an i-STAT handheld blood analyzer (CG8+ cartridge; MN:300-G; Abbott Point of Care Inc., Abbot Park, IL).
**Statistical Analysis**

Data were statistically analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Body temperature indices, production parameters, and blood metabolites were analyzed using the MIXED procedure of SAS with an auto regressive covariance structure and day of the experiment as the repeated effect. The model included group, day, and group by day interaction. Each specific variable’s P1 value served as a covariate. In addition, the effects of period were analyzed separately using the MIXED procedure of SAS. The model included group, period, and their interaction and cow was included as a random effect. Results are reported as least squares means and were considered different when \( P \leq 0.05 \) and a tendency if \( 0.05 < P \leq 0.10 \).

**Results**

During P1, all body temperature indices were similar for cows destined to be in the PF and EHB groups. As expected, during P2, the EHB markedly increased Tr and Tv (1.4 and 1.3°C, respectively; \( P < 0.01 \); Figure 3.1) relative to PF cows. Similarly, Ts increased (0.8°C; \( P < 0.01 \); Table 3.2) in the EHB cows when compared with the PF controls. Furthermore, HS conditions induced by the EHB increased RR and HR (42 breaths/min and 17 beats/min, respectively; \( P < 0.01 \); Table 3.2) relative to PF group.

Overall during P2, the EHB reduced DMI (47%; \( P < 0.01 \)) relative to P1; and by experimental design, the PF cows had similar pattern and extent of decreased DMI (Table 3.3; Figure 3.2A). Milk yield decreased (\( P = 0.03 \)) in EHB and PF cows by 27.3% (12.1 kg) and 13.4% (5.4 kg), respectively compared with P1 (Table 3.3; Figure 3.2B). Milk fat content tended to increase (19%; \( P = 0.08 \); Table 3.3) in the EHB cows, while it remained similar in the PF controls relative to P1. During P2, milk protein content tended to decrease (1.30%; \( P = 0.09 \); Table 3.3) in both EHB and PF groups. In addition, both EHB and PF cows had decreased (\( P < 0.01 \)) milk lactose content (4.96% in P1 vs. 4.85% in P2; Table 3.3). During P2, milk SCC increased (31%;
During P2, circulating glucose decreased progressively with time ($P = 0.02$; Figure 3.3A) similarly between groups ($P = 0.87$; Figure 3.3A). Compared with P1, circulating insulin decreased (44%; $P < 0.01$; Figure 3.3B) and this decrease was similar between groups during P2 ($P = 0.31$; Figure 3.3B). A group by day interaction was observed on circulating NEFA; as it progressively increased in the PF controls but remained unchanged in the EHB cows during P2 ($P = 0.03$; Figure 3.3C). In addition, a group by period interaction was detected for plasma BUN levels, as it remained stable in the PF controls but increased in the EHB cows (37%; $P < 0.01$; Table 3.3).

During P2, decreased partial pressure of CO$_2$, HCO$_3$, total CO$_2$, and base excess levels (17, 16, 17, and 81%, respectively; $P < 0.01$; Figure 3.4A-D) was observed in the EHB cows compared with PF controls. During P2, hematocrit tended to increase (6.3%; $P = 0.10$; Figure 3.5) in the EHB cows relative to PF controls. Hemoglobin increased in both EHB and PF groups (3.1%; $P = 0.05$; Table 3.4) relative to P1. Circulating sodium remained unchanged in the EHB cows but decreased in the PF controls (1.5%; $P < 0.01$; Table 3.4) relative to P1. Furthermore, no group differences were observed on ionized calcium and other iSTAT blood parameters during P2 ($P > 0.10$; Table 3.4).

**Discussion**

Suboptimal environmental conditions are detrimental to farm animal productivity. When the ambient temperature is below or above the thermoneutral zone, efficiency and profitability are compromised because nutrients are diverted away from productive purposes in order to maintain euthermia (Baumgard and Rhoads, 2012). Accurately studying HS typically requires expensive climate-controlled facilities (especially if experimental objectives are to distinguish between the
direct and indirect effects of HS); infrastructure inaccessible to most scientists. Thus, we have developed a model utilizing an EHB and demonstrated that it is an effective and pragmatic technique to study HS in dairy cows (Al-Qaisi et al., 2019). Utilizing the EHB broadens the accessibility of the thermal biology discipline as it is relatively easy and cheap and is conducive to flexible experimental designs. However, quantifying the contribution of direct and indirect (i.e. reduced feed intake) effects of HS has not been determined in the EHB model. Thus, it is of interest to determine nutritional plane’s contribution to altered metabolism and productivity in the EHB model.

In the current study, the EHB caused marked hyperthermia, as demonstrated by an increase in all body temperature variables relative to both P1 and the PF controls, confirming that the EHB is capable of implementing a substantial heat load. The magnitude of changes in the thermal indices agrees with our previous EHB study (Al-Qaisi et al., 2019) and climate-controlled experiments (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011; Cowley et al., 2015). However, from a thermal indices perspective, “acclimation” was not observed as Tr, Tv and RR remained equally increased at the end of P2 as it did on d 1 of P2. Although consistent with our previous EHB experiment (Al-Qaisi et al., 2019), a lack of acclimation differs from what is normally observed in natural HS (Kadzere et al., 2002) and climate-controlled experiments of HS (Rhoads et al., 2009; Wheelock et al., 2010). Reasons for the discrepancies are not entirely clear, but the length of P2 was shorter than in previous climate-controlled experiments (4 vs. 7-10 d) and maybe there was insufficient time to express the acclimation phenotype. Further, it is very likely that upregulated heat dissipation mechanisms (i.e. sweating) are a key component of “acclimation” and obviously the blanket would interfere with this strategy.
As expected, the EHB decreased DMI (47%); by experimental design, the PF group had similar pattern of decreased DMI during P2, and this supports our recent results (Al-Qaisi et al., 2019). Reduced DMI is a common response during HS and it likely represents as a survival strategy to decrease metabolic heat production (Collin et al., 2001; Kadzere et al., 2002; Baumgard and Rhoads, 2013). Milk yield was decreased in the EHB and PF group by 27.3 and 13.4%, respectively, relative to P1, indicating that reduced DMI accounted for only ~50% of decreased milk yield. This is consistent with HS studies conducted in environmental chambers (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011; Cowley et al., 2015; Gao et al., 2017). Thus, by employing the PF design, we were able to confirm a similar nutrient intake:milk production relationship exists with the EHB model.

During P2, the EHB cows had increased milk fat content (19%; Table 3.3) and this corroborates most HS studies conducted in environmental chambers (Regan and Richardson, 1938; Rhoads et al., 2009). Contrarily, milk fat content typically decreases during the warm summer months (Hays, 1926; Huber, 1996; Bouraoui et al., 2002). Additionally, some data generated from environmental chambers demonstrated milk fat content did not change in heat-stressed cows (Shwartz et al., 2009; Cowley et al., 2015). Regardless, this suggests factors other than HS cause low summer milk fat. Milk lactose concentrations slightly decreased in both EHB and PF groups (2.2%; Table 3.3) during P2, which agrees with previous reports (Nardone et al., 1997; Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010). Reasons explaining the decreased milk lactose content are not clear, but there appears to be an increase in non-mammary glucose utilization during HS and this restructuring in the hierarchy of glucose trafficking may help explain the decrease in lactose content. During P2, milk protein content decreased in both groups, which agrees with recent EHB study (Al-Qaisi et al., 2019) and previous climate-controlled experiments.
(Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Cowley et al., 2015), which likely indicates that protein synthesis in the mammary gland is downregulated (Cowley et al., 2015) or AA are partitioned away from mammary gland (Gao et al., 2017). Additionally, the EHB increased MUN, which agrees with our recent results (Al-Qaisi et al., 2019) and previous reports from HS studies in environmental chambers (Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017). This likely stems from the mobilization of skeletal muscle for supplying the precursors for gluconeogenesis and acute phase protein synthesis (Baumgard and Rhoads, 2013).

As mentioned above, direct effects of HS (independent of feed intake) are characterized by postabsorbive changes in carbohydrate, lipid, and protein metabolism. In the present study, circulating glucose was decreased for both the EHB and PF cows during P2. This response corroborates previous HS results in dairy cows (Itoh et al., 1998; Rhoads et al., 2009; Wheelock et al., 2010), sheep (Achmadi et al., 1993), and pigs (Sanz Fernandez et al., 2014). Exact reasons for why HS decreases blood glucose are not fully elucidated, but reduced DMI and increased glucose uptake by the immune system (Kvidera et al., 2017) are two possible explanations. Immune activation is likely because HS reduces intestinal barrier integrity; a scenario that allows lipopolysaccharide (and presumably thousands of potential antigens) infiltration into local and systemic circulation (Lambert, 2009; Baumgard and Rhoads, 2013; Sanz Fernandez et al., 2014).

Insulin plays a key role in nutrient partitioning as it is the major acute anabolic hormone controlling carbohydrate, lipid, and protein metabolism (as reviewed by Baumgard et al., 2016). Our previous results showed that insulin concentrations increased in lactating dairy cows (Wheelock et al., 2010), growing calves (O’Brien et al., 2010), pigs (Pearce et al., 2013; Mayorga et al., 2018), and snakes (Gangloff et al., 2016) during HS. Circulating insulin was decreased for both the EHB and PF cows during P2 compared with P1. Although circulating insulin did not differ
significantly between groups in this study, the EHB cows had numerically increased (27%) insulin levels compared with the PF group. Reasons for not observing a statistical effect in circulating insulin in this experiment are not clear, but insulin concentrations were determined just once during P2 (i.e., d 4) and this may have been too infrequent to make meaningful biological interpretations.

Suboptimal feed intake in lactating cows induces homeorhetic changes to support the physiological state of lactation. This is primarily characterized by enhanced adipose tissue lipolysis and increased use of NEFA as an energy source; a key mechanistic strategy which spares glucose for milk synthesis (Bauman and Currie, 1980; Baumgard et al., 2017). In the current study, the PF cows had markedly increased circulating NEFA as expected since they were on a lower plane of nutrition. However, circulating NEFA in the EHB cows remained at basal levels despite the reduction in DMI, which supports ours and others previous ruminant (Sano et al., 1983; Itoh et al., 1998; Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Al-Dawood, 2017) and monogastric (Geraert et al., 1996, Pearce et al., 2013, Sanz Fernandez et al., 2015) results. Lack of a NEFA response during HS is often attributed to increased circulating insulin (Baumgard and Rhoads, 2013) as it is a potent antilipolytic hormone (Vernon, 1992). As already mentioned, circulating insulin did not differ between treatments so the signals preventing adipose tissue mobilization is likely redundant and this complexity requires further exploration.

As stated earlier, protein metabolism is also affected during a heat load. Skeletal muscle proteolysis is ostensibly increased during HS, as indicated by elevated plasma markers of muscle catabolism such as creatine, 3-methylhistadine, and BUN (Baumgard and Rhoads, 2013; Gao et al., 2017; Conte et al., 2018). In agreement with HS studies conducted in environmental chambers (Rhoads et al., 2009; Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017), the EHB caused
increased BUN levels (37%) during P2; a product of AA deamination stemming from the need to provide AA for gluconeogenesis and acute phase protein synthesis (Baumgard and Rhoads, 2013).

Evaporation is the primary route of heat dissipation when ambient temperatures near an animal body temperature, and this is predominantly achieved by two routes: sweating and panting (hyperventilation; Kadzere et al., 2002). During HS, the amount of carbon dioxide exhaled through rapid RR rates is increased, and thus panting eventually decreases blood CO₂ leading to transient respiratory alkalosis (Kadzere et al., 2002; Conte et al., 2018). In order to keep a constant ratio of HCO₃:CO₂ (20:1) to maintain the primary buffering system in the blood, heat-stressed cows increase HCO₃ secretion in the urine (Kadzere et al., 2002; Conte et al., 2018). Consequently, the salivary HCO₃ pool, which is essential to maintain a healthy rumen pH, is decreased, making the heat-stressed dairy cow more prone to rumen acidosis (Conte et al., 2018). In the current study, the EHB decreased partial pressure of CO₂, HCO₃, total CO₂, and base excess levels relative to PF controls, which agrees with others (Schneider et al., 1984; West et al., 1991). Additionally, hematocrit tended to increase in the EHB group relative to their PF counterparts, which likely indicates EHB cows become mildly dehydrated and this agrees with previous reports (Michel et al., 2007; Das et al., 2016). Consequently, the aforementioned variables also indicate that the EHB induces HS in a very similar way as climate-controlled chambers and natural HS.

Conclusions

Understanding the biological reasons why HS reduces production is a prerequisite to developing mitigation strategies aimed at reducing the economic losses to the global dairy industry. This study confirms that the EHB is an effective model to study HS in dairy cows as indicated by increased body temperature variables and reduced production parameters. By employing the PF design, we clearly illustrated that a lowered nutritional plane explains only
approximately 50% of the decreased milk yield in heat-stressed cows, which is remarkably similar to previous environmental chamber studies. In addition, the EHB blunted adipose tissue mobilization, increased plasma biomarkers of muscle catabolism and altered blood gas variables similar to natural and climate-controlled chamber HS studies. Consequently, the EHB is an alternative model to implement discovery-based research and to evaluate nutritional HS mitigation strategies.

References


Table 3.1. Ingredients and composition of diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>EHB</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>30.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Baleage</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>12.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Ground Corn</td>
<td>28.6</td>
<td>30</td>
</tr>
<tr>
<td>Mineral and protein mix</td>
<td>4.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Whole Cottonseed</td>
<td>-</td>
<td>3.7</td>
</tr>
<tr>
<td>Corn Gluten Feed</td>
<td>6.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Soy Plus</td>
<td>7.1</td>
<td>4.6</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Straw</td>
<td>3.2</td>
<td>-</td>
</tr>
<tr>
<td>Bypass Fat</td>
<td>0.8</td>
<td>-</td>
</tr>
</tbody>
</table>

Chemical analysis
<table>
<thead>
<tr>
<th>Starch</th>
<th>EHB</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26.6</td>
<td>27.7</td>
</tr>
<tr>
<td>CP</td>
<td>16.6</td>
<td>17.5</td>
</tr>
<tr>
<td>NDF</td>
<td>32.3</td>
<td>27.7</td>
</tr>
<tr>
<td>ADF</td>
<td>21.4</td>
<td>17.9</td>
</tr>
<tr>
<td>NEr Mcal/kg DM</td>
<td>1.61</td>
<td>1.67</td>
</tr>
</tbody>
</table>

1Values represent an average of ration nutrient summary reports collected throughout the trial. Diet dry matter averaged 50.45% for the EHB and 46.43% for the PF ration.

2Average nutrient levels for EHB ration: 4.82% Fat, 0.85% Ca, 0.39% P, 0.35% Mg, 0.22% S, 1.31% K, 0.49% Na, 0.61% Cl, 86.92 ppm of Zn, 51.26 ppm of Mn, 4.05 ppm of Fe, 15.62 ppm of Cu, 0.87 ppm of Co, 0.38 ppm Se, 0.87 ppm of I, 14,977 IU/kg of vitamin A, 1415 IU/kg of vitamin D, and 41.6 IU/kg of vitamin E. Average nutrient levels for PF ration: 4.95% Fat, 0.89% Ca, 0.41% P, 0.35% Mg, 0.22% S, 1.41% K, 0.48% Na, 0.64% Cl, 86.88 ppm of Zn, 51.24 ppm of Mn, 4.05 ppm of Fe, 15.62 ppm of Cu, 0.87 ppm of Co, 0.38 ppm Se, 0.87 ppm of I, 14,977 IU/kg of vitamin A, 1415 IU/kg of vitamin D, and 41.6 IU/kg of vitamin E.
Table 3.2. Effects of pair-feeding (PF) or electric heat blanket (EHB) on physiological indicators in lactating Holstein cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Period 2&lt;sup&gt;2&lt;/sup&gt;</th>
<th>P-value</th>
<th>Group × Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (TN)</td>
<td>Group 2 (TN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin temperature, °C</td>
<td>31.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>-<sup>c</sup>Values within row of each variable with differing superscripts indicate statistical difference (P < 0.05).

<sup>1</sup>During period 1, cows in both PF and EHB were treated similarly housed in thermal neutral conditions (TN) and fed ad libitum.
Table 3.3. Effects of pair-feeding (PF) or electric heat blanket (EHB) on production and metabolism variables in lactating Holstein cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th></th>
<th>Period 2</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group 1 (TN)</td>
<td>Group 2 (TN)</td>
<td>Group 1 (PF)</td>
<td>Group 2 (EHB)</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td></td>
<td>25.5</td>
<td>27.7</td>
<td>14.0</td>
<td>14.8</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td></td>
<td>40.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.0</td>
<td>14.8</td>
</tr>
<tr>
<td>Milk variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td></td>
<td>3.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein, %</td>
<td></td>
<td>3.22</td>
<td>2.95</td>
<td>3.15</td>
<td>2.94</td>
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<tr>
<td>Lactose, %</td>
<td></td>
<td>4.97</td>
<td>4.94</td>
<td>4.87</td>
<td>4.83</td>
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<td>SCC, ×1,000 cells</td>
<td></td>
<td>40</td>
<td>25</td>
<td>46</td>
<td>39</td>
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<tr>
<td>MUN, mg/dL</td>
<td></td>
<td>13.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>BUN, mg/dL</td>
<td></td>
<td>10.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a-c</sup>Values within row of each variable with differing superscripts indicate statistical difference (P < 0.05).

<sup>1</sup>During period 1, cows in both PF and EHB were treated similarly housed in thermal neutral conditions (TN) and fed ad libitum.

<sup>2</sup>During period 2, cows pair-fed and kept in TN conditions or fitted with electric heat blanket (EHB) and fed ad libitum.
Table 3.4. Effects of pair-feeding (PF) or electric heat blanket (EHB) on blood variables in lactating Holstein cows

<table>
<thead>
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<th>Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>SEM</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>60.75</td>
<td>66.20</td>
<td>19.20</td>
</tr>
<tr>
<td>sO₂, %</td>
<td>77.90</td>
<td>80.90</td>
<td>5.90</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>8.50</td>
<td>9.12</td>
<td>0.23</td>
</tr>
<tr>
<td>iCa, mmol/L</td>
<td>1.23</td>
<td>1.23</td>
<td>0.02</td>
</tr>
<tr>
<td>K, mmol/L</td>
<td>4.20</td>
<td>4.41</td>
<td>0.06</td>
</tr>
<tr>
<td>Na, mmol/L</td>
<td>136.5a</td>
<td>135.2a</td>
<td>0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.47</td>
<td>7.44</td>
<td>0.02</td>
</tr>
</tbody>
</table>

a-b Values within row of each variable with differing superscripts indicate statistical difference (P < 0.05).

1During period 1, cows in both PF and EHB were treated similarly housed in thermal neutral conditions (TN) and fed ad libitum.

2During period 2, cows pair-fed and kept in TN conditions or fitted with electric heat blanket (EHB) and fed ad libitum.
Figure 3.1. Effects of pair-feeding (PF) or electric heat blanket (EHB) on (A) rectal temperature (Tr) and (B) vaginal temperature (Tv) in lactating Holstein cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
Figure 3.2. Effects of pair-feeding (PF) or electric heat blanket (EHB) on (A) DMI and (B) milk yield in lactating Holstein cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
Figure 3.3. Effects of pair-feeding (PF) or electric heat blanket (EHB) on circulating (A) glucose, (B) insulin, and (C) non-esterified fatty acid (NEFA) in lactating Holstein cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
Figure 3.4. Effects of pair-feeding (PF) or electric heat blanket (EHB) on blood (A) partial pressure of carbon dioxide (pCO₂), bicarbonate (HCO₃⁻), total carbon dioxide (TCO₂), and (D) base excess levels in lactating Holstein cows. Results are expressed as LSM ± SEM.
Figure 3.5. Effects of pair-feeding (PF) or electric heat blanket (EHB) on blood hematocrit levels in lactating Holstein cows. Results are expressed as LSM ± SEM.
CHAPTER 4. EFFECTS OF DIETARY ELECTROLYTES, OSMOLYTES AND ENERGETIC COMPOUNDS ON BODY TEMPERATURE INDICES IN HEAT-STRESSED LACTATING COWS


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Abstract

Heat stress (HS) negatively impacts production and physiological parameters in dairy cows. Electrolyte supplementation may improve acid-base balance and ultimately reduce body temperature. Therefore, study objectives were to determine the effects of a product containing electrolytes, osmolytes, and energetic compounds (EOEC) on body temperature indices in heat-stressed Holstein cows. Nineteen multiparous, lactating cows were housed in individual box stalls and randomly assigned to 1 of 2 dietary treatments: 1) a control diet (n = 9) or 2) a control diet supplemented with 113 g/d of EOEC (n = 10; Bovine BlueLite Pellets; TechMix, LLC., Stewart, MN) that was top-dressed once daily. The trial consisted of 2 experimental periods (P). During P1 (4 d), cows were fed their respective treatments and housed in thermoneutral conditions for collecting baseline data. During P2 (4 d), HS was artificially induced using an electric heat blanket (EHB; Thermotex Therapy Systems Ltd. Calgary, Canada). Vaginal temperature (Tv) was measured every 10 min using a data logger (iButton DS1921, Maxim Integrated, San Jose, CA). Overall, HS increased rectal temperature (Tr), Tv, skin temperature (Ts), respiration rate (RR), and
heart rate (HR) (1.8°C, 1.5°C, 2.0°C, 2-fold, and 12 bpm, respectively) relative to P1. There were no dietary treatment differences in Tr, Tv, RR, and HR; however, EOEC-supplemented cows had increased (0.8°C) Ts relative to CON cows. Compared to P1, HS decreased DMI and milk yield (45 and 27%, respectively) similarly amongst treatments. Relative to P1, HS tended to increase circulating glucose in both treatments on d 2 of P2 and it decreased more rapidly in CON compared to the EOEC-fed cows by the end of P2. Relative to P1, circulating insulin decreased (41%) in CON cows, whereas it remained unaffected in EOEC-supplemented cows, resulting in a 2-fold increase in EOEC-fed cows compared with CON. Relative to P1, HS increased circulating non-esterified fatty acids (NEFA; 63%). Further, there tended to be a treatment by day interaction on circulating NEFA, as it decreased from d 2 to 4 of P2 in EOEC-fed cows, while it continued to increase in CON (39% decrease in EOEC vs. CON cows on d 4). Relative to P1, HS decreased blood partial pressure of carbon dioxide (pCO2; 19%), but EOEC supplementation attenuated the decrease (12%) relative to CON. Relative to P1, HS increased hematocrit levels (7%), but it was unaffected by dietary treatment. In summary, feeding EOEC altered some key aspects of energetic metabolism and increased Ts. Increased Ts suggest that EOEC enhances heat dissipation, likely via increased sweating.

**Key words:** heat stress, hydration, electrolyte, insulin
**Introduction**

Exposure to elevated ambient temperatures coupled with high humidity and poor heat dissipation, jeopardizes animal welfare and compromises productivity (Kadzere et al., 2002). Although several advanced management practices (i.e., cooling systems) have been developed and utilized, the negative impacts of heat stress (HS) continues to be a hurdle to profitable production. For example, HS is an economic burden to the global animal agriculture industries, and it costs more than $1.5 billion in the United States dairy industry alone (Key and Sneeringer, 2014). These fiscal losses stem from reduced milk yield and composition, growth, reproduction, and compromised health (Baumgard and Rhoads, 2013).

Environmental hyperthermia causes a substantial decrease in feed intake, a survival tactic implemented to presumably reduce metabolic heat production (Collin et al., 2001; West, 2003). Furthermore, HS alters carbohydrate, lipid, and protein metabolism independent of feed intake (Baumgard and Rhoads, 2013). Nutritional strategies are a flexible and cost-effective approach to mitigate HS and are employable by both large and small producers. Therefore, identifying nutritional tools aimed at improving overall dairy welfare and reducing the adverse consequences of HS on performance is important for sustainable farming.

Heat-stressed dairy cows utilize panting and sweating to dissipate heat via evaporative cooling (Kadzere et al., 2002). Cows, like almost all mammals, utilize a strict ratio (20:1) of pCO₂ to HCO₃⁻ as a key circulating buffering system. High respiration rates increase the amount of carbon dioxide (CO₂) exhaled and thus reduce blood pCO₂, and consequently the ratio is strained, and it induces temporary respiratory alkalosis (Kadzere et al., 2002). In an attempt to maintain the ratio, HCO₃⁻ is secreted into the urine (Kadzere et al., 2002). As a result, saliva HCO₃⁻ content decreases (because blood HCO₃⁻ transfer to saliva is concentration dependent) and thus the susceptibility to
rumen acidosis increases (Kadzere et al., 2002; West, 2003). Additionally, HS causes a significant amount of potassium (K) loss via sweating (Jenkinson and Mabon, 1973; Schneider et al., 1988; Tucker et al., 1988). Furthermore, Collier et al. (1982) demonstrated that HS decreased ruminal K and Na concentrations. Thus, it is typically recommended to increase the dietary mineral content (mainly Na and K) during warm summer conditions (NRC, 2001; West, 2003). Although inconsistent (Schneider et al., 1988; Chan et al., 2005; Cabrera, 2014), others have shown that supplementing electrolytes in the form of NaHCO$_3$ and KCl benefited heat-stressed dairy cows by increasing milk yield and lowering body temperature (Coppock et al., 1982; Tucker et al., 1988; West et al., 1992). Dietary betaine (an osmolyte) has also been shown to improve productivity in heat-stressed chickens (Shakeri et al., 2018), pigs (Mendoza et al., 2017) and cows (Zhang et al., 2014; Dunshea et al., 2019) and the mechanism is thought to be at the intestine level (Cronje, 2005) and by reducing metabolic heat production (Moeckel et al., 2002).

We hypothesized that providing dietary electrolytes, osmolytes and simple energetic compounds could potentially reduce body temperature indices and increase lactation performance in dairy cows exposed to HS. Therefore, study objectives were to determine the effects of a product containing electrolytes, osmolyte, and energetic compounds (EOEC) on body temperature indices in heat-stressed lactating Holstein cows.

**Materials and Methods**

**Animals and Experimental Design**

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Twenty multiparous, lactating Holstein cows (151 ± 8 DIM; 699 ± 13 kg of BW) were used in an experiment conducted in 2 replicates. Cows were housed in sand and straw-bedded individual box stalls (4.57 × 4.57 m) within a naturally ventilated barn at the Iowa State University.
Dairy Farm (Ames). Cows were acclimated for 4 d and then randomly assigned to 1 of 2 dietary treatments: 1) a control diet (n = 9) or 2) a control diet supplemented with 113 g/d of a product containing electrolytes, osmolytes, and energetic compounds (EOEC; n = 10; Bovine Bluelite pellets; Techmix LLC., Stewart, MN) top-dressed once daily. The trial consisted of 2 experimental periods (P). During P1 (4 d), cows were fed their respective dietary treatments and housed in thermoneutral (TN) conditions for collection of baseline body temperature indices and production parameters. During P2 (4 d), HS was artificially induced using an electric heat blanket (EHB) consisting of 12 infrared heating pads as a heat source (Thermotex Therapy Systems Ltd. Calgary, Canada), powered by a 110-V electrical cord that connected to the EHB at the withers as previously described (Al-Qaisi et al., 2019a). The EHB remained on the cows for the entirety of P2. One cow from the CON treatment was excluded from the trial due to illness unrelated to the blanket and her data was not incorporated in the final data set.

Ambient temperature and relative humidity in the barn were monitored and recorded every 10 min by a data logger (EL-USB-2 LCR, Lascar Electronics, Erie, PA) and condensed into a daily average. Cows were housed in TN conditions throughout the experimental period (23.0 ± 0.3°C, 65.5 ± 0.8% relative humidity).

Cows were individually fed a TMR consisting primarily of corn silage once daily (0800 h) and orts were measured prior to feeding. The TMR was formulated to meet or exceed the predicted requirements (NRC, 2001; Table 4.1) of energy, protein, minerals, and vitamins for lactating cows. Cows were milked twice daily (0600 and 1800 h) and yield was recorded. Milk samples from each cow were collected daily during both experimental periods. Samples were stored at 4°C with a preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) until analysis by Dairy Lab
Services (Dubuque, IA) using AOAC-approved infrared analysis equipment and procedures (AOAC International, 1995).

During both P1 and P2, rectal temperature (Tr), skin temperature (Ts), respiration rate (RR), and heart rate (HR) were obtained twice daily (0600 and 1800 h). Rectal temperatures were measured using a standard digital thermometer (M700 digital thermometer, GLA Agricultural Electronics, San Luis Obispo, CA). Skin temperatures were measured on the neck using an infrared thermometer (IRT207 Heat Seeker 8:1 mid-range infrared thermometer, General Tools and Instruments, New York, NY). Respiration rates were determined by counting flank movements during a 15-s interval and multiplied by 4 to obtain breaths per minute (bpm). Heart rate was determined using stethoscope placed over the left side of the rib cage behind the elbow, and heart beats were counted for a 15-s interval. This measurement was multiplied by 4 to obtain beats per minute (bpm).

Continuous vaginal temperatures (Tv) were obtained via a calibrated temperature logger (iButton DS 1921, Maxim Integrated, San Jose, CA) fitted in a hollowed-out space in the center of a blank controlled internal drug release (CIDR; Zoetis, Parsippany, NJ) and inserted into the vagina with an applicator. Loggers were fixed in the CIDR using a silicone aquarium sealant (Aqueon, Franklin, WI). Vaginal temperatures were obtained every 10 min for 6 consecutive days (from P1 d3 to P2 d4).

Blood samples were collected via coccygeal venipuncture (plasma, K2EDTA tube; serum, serum tube; BD vacutainers, Franklin Lakes, NJ) on d 2 and 4 of both P1 and P2 following the morning milking. Serum samples were allowed to clot at room temperature for 1 h prior to centrifugation. Plasma and serum were harvested following centrifugation at 1500 × g for 15 min at 4°C and were subsequently frozen at -20°C until analysis.
Plasma non-esterified fatty acids (NEFA), blood urea nitrogen (BUN), and insulin concentrations were determined using commercially available kits according to manufacturers’ instructions (NEFA, Wako Chemicals USA, Richmond, VA; BUN, Teco Diagnostics Anaheim, CA; insulin, Mercodia AB, Uppsala, Sweden). The inter and intra-assay coefficients of variation for NEFA and BUN were 4.5 and 5.0% and 4.8 and 4.5%, respectively. The intra assay coefficient of variation for insulin was 5.0%.

Blood gases and circulating glucose were measured on fresh blood collected on d 4 of P1 and d 2 and 4 of P2 into lithium heparin tubes and assayed immediately using an i-STAT handheld blood analyzer (CG8+ cartridge; MN:300-G; Abbott Point of Care Inc., Abbot Park, IL).

Statistical Analysis

Data were statistically analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Body temperature indices, production parameters, and blood metabolites were analyzed using the MIXED procedure of SAS with an auto regressive covariance structure and day of the experiment as the repeated effect. The model included treatment, day, and treatment by day interaction. Each specific variable’s P1 value served as a covariate. In addition, the effects of period were analyzed separately using the MIXED procedure of SAS. The model included treatment, period, and their interaction and cow was included as a random effect. Results are reported as least squares means and were considered different when $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

As expected, HS markedly increased Tr (1.8°C), Tv (1.5°C), Ts (2.0°C), RR (43 bpm), and HR (12 bpm) during P2 compared with P1 ($P < 0.01$; Table 4.2; Figure 4.1). There were no treatment differences in Tr, Tv, RR, and HR ($P > 0.40$; Table 4.2). However, EOEC-supplemented cows had increased Ts compared to CON cows (0.8°C; $P = 0.04$; Figure 4.1).
Regardless of dietary treatments, HS decreased DMI (45%; \( P < 0.01 \); Figure 4.2A) during P2 relative to P1 and the decrease was most prominent on d 4 of P2 (52%; \( P < 0.01 \); Figure 4.2A). Overall and relative to P1, milk production decreased (27%; \( P < 0.01 \)) and reached nadir on d 4 of P2 (38%; \( P < 0.01 \); Figure 4.2B) but was unaffected by dietary treatment (\( P > 0.30 \); Figure 4.2B). Regardless of dietary treatment, HS increased milk fat content (16%; \( P < 0.01 \); Table 4.3) and HS decreased milk protein and lactose content (1.8 and 2.0%, respectfully; \( P < 0.05 \); Table 4.3) relative to P1. Heat stress increased SCC and MUN (63 and 35%, respectively, \( P < 0.01 \); Table 4.3) compared to P1 similarly amongst treatments.

Overall, relative to P1, HS tended to increase circulating glucose in both treatments and the increase was most pronounced on d 2 of P2. By the end of P2, glucose from both treatments decreased, but tended to be increased in the EOEC-supplemented cows compared to the CON cows (5%; \( P = 0.07 \); Figure 4.3A). A treatment by period interaction was detected for circulating insulin, as its concentration did not change during P2 in EOEC-fed cows but decreased in CON cows relative to P1 (41%; \( P = 0.04 \); Figure 4.3B). Relative to P1, HS increased circulating NEFA (63%; \( P < 0.01 \); Figure 4.3C). There was a treatment by day interaction detected for NEFA such that it decreased from d 2 to 4 of P2 in EOEC-supplemented cows, while it continued to increase in CON cows (\( P = 0.06 \); Figure 4.3C). Heat stress increased BUN (35%; \( P < 0.01 \); Figure 4.3D) but it was unaffected by dietary treatment (\( P = 0.21 \); Figure 4.3D).

During P2, HS did not affect blood pH (\( P > 0.26 \); Table 4.4), but overall the EOEC-supplemented cows had decreased blood pH compared with CON cows (\( P = 0.02 \); Table 4.4). Overall, HS decreased pCO\(_2\) levels (19%; \( P < 0.01 \); Figure 4.4) relative to P1, but EOEC supplementation attenuated the decrease relative to CON cows during P2 (\( P = 0.04 \); Figure 4.4). Heat stress decreased circulating HCO\(_3^-\), total CO\(_2\), base excess, and Na (15, 15, 78, and 1%,
respectively, \( P < 0.01 \); Table 4.4) but no dietary treatment differences were observed on HCO\(_3\), total CO\(_2\), or base excess; however, circulating Na was overall increased in EOEC-supplemented cows in both periods (\( P = 0.02 \); Table 4.4). In contrast, HS increased circulating partial pressure of oxygen (pO\(_2\)), soluble oxygen (sO\(_2\)), hematocrit, hemoglobin (67, 12, 7, and 7%, respectively, \( P \leq 0.05 \); Table 4.4), but these parameters were unaffected by dietary treatment. Neither HS nor dietary treatment altered circulating K and ionized calcium (\( P > 0.14 \); Table 4.4).

**Discussion**

Environmentally-induced hyperthermia undermines animal productivity and imposes huge economical constraints on the global dairy industry (Kadzere et al., 2002). Part of the negative effects of HS on productivity can be explained by an imbalance in the acid-base status due in large part to increased sweating and respiration rates (West et al., 1991; Kadzere et al., 2002). Potassium, is lost in considerable amounts during ruminant sweating (Jenkinson and Mabon, 1973; Schneider et al., 1988; Tucker et al., 1988). Additionally, HS-induced increased respiration rate leads to a reduction in circulating pCO\(_2\). To compensate for the reduced CO\(_2\), the kidney excretes HCO\(_3\) (and Na to maintain net neutrality) into urine to conserve the constant ratio between HCO\(_3\) and CO\(_2\) (20:1; Kadzere et al., 2002). As a result, saliva HCO\(_3\) is decreased and susceptibility to rumen acidosis is increased (Kadzere et al., 2002; West, 2003). Supplementing electrolytes during HS might represent an opportunity to improve productivity and additionally regulate water balance thereby improving hydration (Schneider et al., 1986; Kadzere et al., 2002; West, 2003). Although variable (Schneider et al., 1988; Chan et al., 2005; Cabrera, 2014), feeding electrolytes in the form of NaHCO\(_3\) and KCl benefited heat-stressed dairy cows by lowering body temperature, and increasing DMI and milk yield (Coppock et al., 1982; Tucker et al., 1988; West et al., 1991, 1992).

Osmolytes represent an additional strategy for ameliorating the negative consequences of HS. Betaine is an osmolyte that has a unique structure known as zwitterion (Lever and Slow, 2010)
that includes polar regions which enables it to have an osmoregulatory role by retaining intracellular water, which aids in regulating cellular water balance (Ratriyanto and Mosenthin, 2018; Dunshea et al., 2019). In addition, betaine decreases the need for ion pumping which could eventually reduce metabolic heat production (Moeckel et al., 2002; DiGiacomo et al., 2016). Further, betaine has been shown to improve intestinal barrier function in multiple HS models (Cronje, 2005, 2007). Thus, we hypothesized that feeding a product containing electrolytes and betaine would assist in mitigating the negative consequences of HS on dairy cow productivity.

In the present study, the EHB successfully created a meaningful heat load, as indicated by a marked increase in all body temperature variables (Table 4.2; Figure 4.1); and the magnitude of the changes agrees with our recent EHB trial (Al-Qaisi et al., 2019) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). No differences were observed between dietary treatment on Tr, Tv, RR, or HR, which is consistent with Cabrera (2014) who supplemented a similar product used in this experiment. However, EOEC-supplemented cows had increased Ts. Although Ts can be difficult to interpret as it is heavily influenced by multiple biological and environmental variables (Mayorga et al., 2019), it can indicate increased heat dissipation (Blatteis, 1998), presumably because of increased sweating. Although not measured in the current experiment, increased sweating could be envisioned because K is the primary electrolyte used in ruminant sweating and the EOEC product contained a substantial amount of this ion. Increased heat dissipation would theoretically decrease other body temperature indices, but the EHB blocks 50% of the cow’s surface area (Al-Qaisi et al., 2019a) and thus the unique model utilized may have limited the ability to statistically detect meaningful differences in thermal metrics. Additional reasons for the lack of differences in most of the body temperature variables could be related to the fact that DMI and milk yield were similar.
between treatments (discussed below), as both represent major sources of basal heat production.

Heat stress sharply decreased DMI (45%) relative to P1, which corroborates a previous EHB study (Al-Qaisi et al., 2019) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Cowley et al., 2015). Decreased DMI is a strategy to minimize metabolic heat production and is a highly conserved response across species (Collin et al., 2001; West, 2003; Baumgard and Rhoads, 2013). In agreement with previous reports (Schneider et al., 1988; Cabrera, 2014), DMI was similar between dietary treatments. However, West et al. (1991) found that DMI increased with dietary electrolyte supplementation. As expected, HS decreased milk yield (27%), which agrees with our recent EHB reports (Al-Qaisi et al., 2019a,b) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Cowley et al., 2015). However, milk yield was unaffected by dietary treatment, which is consistent with previous studies (Schneider et al., 1988; Chan et al., 2005; Cabrera, 2014). In contrast, West et al. (1991) observed a linear increase in milk yield with increasing dietary electrolytes. It was not too surprising that we did not observe treatment differences in production responses (DMI and milk yield) as the experiment was designed with the emphasis on physiological and body temperature indices and knowingly underpowered for production metrics. Regardless, reasons for discrepancies in production responses to feeding electrolytes are unclear, but could be attributed to differences in inclusion levels, electrolyte type, basal diet, small sample sizes, or severity of HS.

Milk fat percentage was not affected by dietary treatment, which is consistent with previous reports that feed supplemental electrolytes during HS (West et al., 1991, 1992; Chan et al., 2005; Cabrera, 2014). On the other hand, HS increased milk fat content (16%) relative to P1, which corroborates previous HS reports (Regan and Richardson, 1938; Rhoads et al., 2009; Baumgard et al., 2011). In agreement with previous HS studies (Shwartz et al., 2009; Baumgard et al., 2011;
Al-Qaisi et al., 2019a), milk protein content decreased (2%) during HS, but it was unaltered by dietary treatment, which is consistent with others (West et al., 1992; Chan et al., 2005; Cabrera, 2014). Decreased milk protein is likely due to increase extramammary AA utilization (Gao et al., 2017) or decreased functionality of milk protein synthesizing machinery (Bernabucci et al., 2010; Cowley et al., 2015). Although not effected by treatment, HS decreased milk lactose content (2%), which is in accord with previous HS studies (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010). Reasons for decreased milk lactose content is probably due to an increase in extramammary glucose utilization during HS (Baumgard and Rhoads, 2013). During P2, HS increased MUN (35%), which agrees with previous HS studies (Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017; Al-Qaisi et al., 2019a,b), but it was unaffected by dietary treatment. Increased MUN is likely originating from breakdown of skeletal muscle to provide amino acids for gluconeogenesis and acute phase proteins (Johnson, 2012; Baumgard and Rhoads, 2013).

Interestingly, irrespective of dietary treatment, HS increased SCC (63%) relative to P1, which agrees with a recent HS report (Safa et al., 2018). Coincidently, increased milk SCC also occurs in endotoxin challenge experiments (Shuster et al., 1991; Horst et al., 2018), suggesting that the increased SCC during HS likely occurs due to impaired intestinal epithelial integrity which permits pathogen infiltration (i.e., lipopolysaccharide) resulting in immunoactivation and inflammation (Cronje, 2005; Lambert, 2009; Baumgard and Rhoads, 2013).

Heat stress decreased circulating glucose at the end of P2 in CON cows, which corroborates with previous HS studies in cows (Itoh et al., 1998; Shwartz et al., 2009; Wheelock et al., 2010; Calamari et al., 2018; Al-Qaisi et al., 2019b) sheep (Achmadi et al., 1993; Al-Mamun et al., 2017), and pigs (Sanz Fernandez et al., 2014; Seelenbinder et al., 2018). Decreased glucose levels are mediated by both decreased DMI and increased utilization of glucose by activated leukocytes.
(Kvidera et al., 2017). Not too surprisingly, glucose concentrations tended to be increased (5%) in EOEC-supplemented cows compared with CON cows, and this is presumably explained by the fact that sugars (i.e., dextrose) are an ingredient in the EOEC supplement. In the present study, the CON cows had decreased circulating insulin (41%) relative to P1, while it remained at a similar concentration in the EOEC-supplemented cows (resulting in a 2-fold difference between treatments during P2). Treatment differences in glucose levels are most likely responsible for increased circulating insulin in the EOEC-fed cows. Heat stress slightly increased circulating NEFA relative to P1, which is not entirely consistent with our previous HS studies (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010). The aforementioned experiments employed a pair-feeding design, while the study herein did not utilize this scientific approach. Regardless, the increase in NEFA during HS was attenuated with EOEC supplementation. Reduced NEFA is likely explained by the increase in insulin as it is a potent antilipolytic hormone (Vernon, 1992). From a metabolic perspective, it is clear that the EOEC was affecting energetics and these changes have implications to multiple scenarios in addition to HS (i.e. transition cow, off-fed events, shipping etc.).

In agreement with previous HS studies (Rhoads et al., 2009; Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017), HS increased BUN levels (37%) during P2. Blood urea nitrogen is an indirect indicator of skeletal muscle catabolism, which likely occurs to provide amino acids to support gluconeogenesis and acute phase protein synthesis (Baumgard and Rhoads, 2013). The BUN response closely resembles temporal pattern of MUN and this was expected, as urea diffuses from blood into milk based upon a concentration dependent process (Roseeler et al., 1993). Despite the effects of EOEC on energetic metabolism, no treatment differences were detected in BUN or
MUN, which is kind of unexpected as increased insulin should reduce skeletal muscle mobilization (Baumgard and Rhoads, 2013).

Evaporative heat loss (i.e., sweating and panting) is initiated when euthermia cannot be achieved via sensible routes of heat transfer (Kadzere et al., 2002). As previously mentioned, HS-increased respiration rates, and panting alters blood gases. Herein we observed many of the well-characterized (Schneider et al., 1986; Kadzere et al., 2002; Michel et al., 2007; Das et al., 2016; Conte et al., 2018) HS-induced changes in blood gases; decreased pCO$_2$, HCO$_3$, total CO$_2$, and base excess and increased hematocrit, although no changes were observed in blood pH. Elevated hematocrit suggests mild dehydration, which is consistent with previous reports (Michel et al., 2007; Das et al., 2016; although it appears that our hematocrit levels mildly lower than expected, George et al., 2010). In contrast to our hypothesis, EOEC supplementation did not influence hematocrit levels. Supplementing EOEC decreased blood pH and increased pCO$_2$ (12%) relative to CON cows, which suggest that it attenuated respiratory alkalosis and the strain on pCO$_2$ during HS. The changes in pH and pCO$_2$ could be attributed to the effects of the electrolyte on improving acid-base balance. Heat stress decreased (1%) circulating Na but it was increased in EOEC-supplemented cows and this could be explained by the fact that Na is an ingredient in the EOEC product (i.e., NaHCO$_3$). Although we hypothesized that increased K status is the reason for increased Ts because of increased sweating (discussed above), circulating K did not change between treatments and during HS. Reasons for not detecting treatment differences in circulating K maybe explained by the fact that we just measured “concentrations” and not “flux”. Further research is needed to examine the effects of dietary EOEC on blood gas parameters during HS.

Conclusions

Employing the EHB increased all body temperature variables and caused a substantial reduction in production parameters (i.e., DMI and milk yield). In addition, HS negatively affected
blood gas parameters and altered acid-base balance. Feeding EOEC could be beneficial during HS, as it ostensibly increased heat dissipation by increasing Ts. Additionally, dietary EOEC increased circulating levels of glucose and insulin, decreased NEFA levels, and reduced the strain on pCO₂ during HS. Collectively, feeding EOEC altered the metabolic and blood gas profile in heat-stressed dairy cows.

References


### Table 4.1. Ingredients and composition of diet

<table>
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<tr>
<th>Ingredients</th>
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Chemical analysis, % of DM

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<tr>
<td>NE₄ Mcal/kg DM</td>
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¹Values represent an average of ration nutrient summary reports collected throughout the trial. Diet dry matter averaged 50.45%.

²Average nutrient levels: 4.82% Fat, 0.85% Ca, 0.39% P, 0.35% Mg, 0.22% S, 0.61% Cl, 86.92 ppm of Zn, 51.26 ppm of Mn, 4.05 ppm of Fe, 15.62 ppm of Cu, 0.87 ppm of Co, 0.38 ppm Se, 0.87 ppm of I, 6807.5 IU/kg of vitamin A, 1415 IU/kg of vitamin D, and 41.6 IU/kg of vitamin E.
Table 4.2: Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on body temperature variables in lactating dairy cows

<table>
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<th>Parameter</th>
<th>Period 1&lt;sup&gt;1&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Rectal temperature, °C</td>
<td>38.4  38.4</td>
<td>40.1  40.3</td>
<td>0.1</td>
<td>0.14</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>Vaginal temperature, °C</td>
<td>38.2  38.2</td>
<td>39.6  39.8</td>
<td>0.1</td>
<td>0.34</td>
<td>&lt;0.01</td>
<td>0.26</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>47   47</td>
<td>90   89</td>
<td>2</td>
<td>0.75</td>
<td>&lt;0.01</td>
<td>0.71</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>80   81</td>
<td>92   94</td>
<td>2</td>
<td>0.52</td>
<td>&lt;0.01</td>
<td>0.88</td>
</tr>
</tbody>
</table>

<sup>1</sup>During period 1, cows in both treatments were housed in thermo neutral conditions and allowed to eat ad libitum.

<sup>2</sup>During period 2, all cows were fitted with an electric heat blanket and allowed to eat ad libitum.

<sup>3</sup>PER = Period.
### Table 4.3: Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on production variables in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Period 2&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM</th>
<th>Treatment</th>
<th>PER</th>
<th>Treatment × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>27.9</td>
<td>15.1</td>
<td>0.7</td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>OEOC</td>
<td>27.6</td>
<td>15.6</td>
<td></td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>45.2</td>
<td>34.2</td>
<td>2.2</td>
<td>0.42</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>OEOC</td>
<td>43.7</td>
<td>31.0</td>
<td></td>
<td>0.42</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>Milk variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>3.43</td>
<td>4.07</td>
<td>0.22</td>
<td>0.12</td>
<td>&lt;0.01</td>
<td>0.58</td>
</tr>
<tr>
<td>OEOC</td>
<td>3.96</td>
<td>4.47</td>
<td></td>
<td>0.12</td>
<td>&lt;0.01</td>
<td>0.58</td>
</tr>
<tr>
<td>Protein, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>2.95</td>
<td>2.94</td>
<td>0.08</td>
<td>0.29</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>OEOC</td>
<td>3.11</td>
<td>3.01</td>
<td></td>
<td>0.29</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Lactose, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>4.94</td>
<td>4.83</td>
<td>0.06</td>
<td>0.97</td>
<td>&lt;0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>OEOC</td>
<td>4.93</td>
<td>4.84</td>
<td></td>
<td>0.97</td>
<td>&lt;0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>SCC, × 1000 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>25</td>
<td>39</td>
<td>19</td>
<td>0.19</td>
<td>&lt;0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>OEOC</td>
<td>50</td>
<td>83</td>
<td></td>
<td>0.19</td>
<td>&lt;0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>11.8</td>
<td>16.0</td>
<td>0.5</td>
<td>0.99</td>
<td>&lt;0.01</td>
<td>0.83</td>
</tr>
<tr>
<td>OEOC</td>
<td>11.9</td>
<td>15.9</td>
<td></td>
<td>0.99</td>
<td>&lt;0.01</td>
<td>0.83</td>
</tr>
</tbody>
</table>

<sup>1</sup>During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.

<sup>2</sup>During period 2, all cows were fitted with an electric heat blanket and allowed to eat ad libitum.

<sup>3</sup>PET = Period.
Table 4.4: Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on circulating physiological parameters in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th></th>
<th>Period 2</th>
<th></th>
<th>SEM</th>
<th>Treatment</th>
<th>PER</th>
<th>Treatment × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.44</td>
<td>7.39</td>
<td>7.45</td>
<td>7.42</td>
<td>0.02</td>
<td>0.02</td>
<td>0.26</td>
<td>0.56</td>
</tr>
<tr>
<td>pO₂, mmHg</td>
<td>66.2</td>
<td>53.1</td>
<td>100.1</td>
<td>97.6</td>
<td>17.1</td>
<td>0.71</td>
<td>0.03</td>
<td>0.75</td>
</tr>
<tr>
<td>sO₂, %</td>
<td>81.1</td>
<td>75.1</td>
<td>87.7</td>
<td>87.0</td>
<td>5.0</td>
<td>0.55</td>
<td>0.05</td>
<td>0.57</td>
</tr>
<tr>
<td>TCO₂, mmol/L</td>
<td>31.6</td>
<td>31.1</td>
<td>26.4</td>
<td>26.9</td>
<td>0.6</td>
<td>0.98</td>
<td>&lt;0.01</td>
<td>0.39</td>
</tr>
<tr>
<td>HCO₃, mmol/L</td>
<td>30.3</td>
<td>29.7</td>
<td>25.3</td>
<td>25.8</td>
<td>0.6</td>
<td>0.88</td>
<td>&lt;0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>Base excess, mmol/L</td>
<td>6.2</td>
<td>4.9</td>
<td>1.3</td>
<td>1.1</td>
<td>0.7</td>
<td>0.35</td>
<td>&lt;0.01</td>
<td>0.41</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>26.7</td>
<td>25.7</td>
<td>28.0</td>
<td>28.0</td>
<td>0.5</td>
<td>0.36</td>
<td>&lt;0.01</td>
<td>0.34</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>9.1</td>
<td>8.7</td>
<td>9.5</td>
<td>9.5</td>
<td>0.2</td>
<td>0.29</td>
<td>&lt;0.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Na, mmol/L</td>
<td>135.3</td>
<td>136.6</td>
<td>134.3</td>
<td>135.0</td>
<td>0.4</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>0.35</td>
</tr>
<tr>
<td>K, mmol/L</td>
<td>4.4</td>
<td>4.5</td>
<td>4.3</td>
<td>4.4</td>
<td>0.1</td>
<td>0.52</td>
<td>0.20</td>
<td>0.73</td>
</tr>
<tr>
<td>iCa, mmol/L</td>
<td>1.23</td>
<td>1.24</td>
<td>1.22</td>
<td>1.23</td>
<td>0.02</td>
<td>0.55</td>
<td>0.61</td>
<td>0.85</td>
</tr>
</tbody>
</table>

1During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.
2During period 2, all cows were fitted with electric heat blanket and allowed to eat ad libitum.
3PER = Period.
Figure 4.1. Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on skin temperature (Ts) in lactating dairy cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
Figure 4.2. Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on (A) DMI and (B) milk yield in lactating dairy cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
Figure 4.3. Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on circulating (A) glucose, (B) insulin, (C) non-esterified fatty acid (NEFA), and (D) blood urea nitrogen (BUN) in lactating dairy cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
**Figure 4.4.** Effects of electrolyte, osmolyte, and energetic compounds (EOEC) supplementation on partial pressure of CO₂ (pCO₂) in lactating dairy cows. Values for P1 represent the average of the 4 d of period 1. Results are expressed as LSM ± SEM.
CHAPTER 5. EFFECTS OF A SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCT ON HEAT-STRESSED DAIRY COWS

Modified from a paper to be submitted to the Journal of Dairy Science

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*Department of Animal Science, Iowa State University, Ames, Iowa, 50011
†Diamond V, Cedar Rapids, Iowa, 52404

Abstract

Study objectives were to evaluate the effects of supplementing a Saccharomyces cerevisiae fermentation product (SCFP) on body temperature indices, metabolism, the acute phase protein response (APPR), and production parameters during heat stress (HS). Twenty multiparous, lactating Holstein cows (675 ± 12 kg BW; 144 ± 5 d in milk; parity 2.3 ± 0.1) were utilized in an experiment conducted in 2 replicates (10 cows/replicate). Cows were randomly assigned to 1 of 2 dietary treatments: 1) a control diet (CON; n=10) or 2) a control diet supplemented with 19 g/d of SCFP (n=10; NutriTek; Diamond V, Cedar Rapids, IA). Cows were fed their respective diets for 21 d prior to study initiation and the trial consisted of 2 experimental periods (P). During P1 (4 d), cows were fed ad libitum and housed in thermoneutral conditions for collecting baseline data. During P2 (7 d), HS was artificially induced using an electric heat blanket (EHB; Thermotex Therapy Systems Ltd. Calgary, AB, Canada). Cows were fitted with the EHB for the entirety of P2. Rectal temperature (Tr), respiration rate (RR), and skin temperature (Ts) were obtained twice daily (0600 and 1800 h) during both periods. Overall, HS increased Tr, Ts, and RR, (1.4°C, 4.8°C,
and 54 breaths/min, respectively) relative to P1, but there were no dietary treatment differences detected. Compared with P1, HS decreased DMI and milk yield (36 and 26%, respectively), and the reductions were similar between dietary treatments. Relative to P1, HS increased milk fat content and milk urea nitrogen (17 and 30%, respectively) and decreased milk protein and lactose composition (7 and 1.7%, respectively). Milk somatic cell count was not affected by HS but tended to be decreased (19%) in SCFP fed cows. Overall, HS increased (52%) cortisol concentrations in CON but circulating cortisol did not increase in SCFP fed cows. Heat stress increased circulating lipopolysaccharide binding protein and serum amyloid A (SAA; 2- and 4-fold; respectively) and SCFP supplementation tended to decrease peak SAA (~33%) relative to CON cows. Relative to P1, HS did not influence circulating white blood cells and neutrophils, but they were increased (~9 and ~26%, respectively) in SCFP fed cows compared with CON. In conclusion, HS initiated an APRR and feeding SCFP blunted the cortisol and SAA concentrations and altered some key leukocyte dynamics during HS.

**Key words:** electric heat blanket, *Saccharomyces cerevisiae* fermentation product, immune activation

**Introduction**

Heat stress (HS) jeopardizes animal welfare and compromises productivity (Baumgard and Rhoads, 2013). Despite implementing advanced management strategies (i.e., cooling systems, barn construction), HS remains a major constraint to the global dairy industry. Heat stress is an economic burden to all animal agriculture and it costs > $1.5 billion in the United States dairy industry alone (Key and Sneeringer, 2014); losses explained by reduced milk yield, impaired growth rates, decreased reproduction, and compromised health (Baumgard and Rhoads, 2013). Many of the negative effects of HS on productivity result from compromised intestinal barrier integrity (Baumgard and Rhoads, 2013). Heat-stressed animals redistribute blood to the periphery
in order to increase radiant heat loss and consequently the gastrointestinal tract vasoconstricts to maintain blood pressure. Reduced blood flow to the splanchnic tissues causes hypoxia, enterocyte damage, and impaired epithelial barrier function; a scenario that allows lipopolysaccharide (LPS) and undoubtedly thousands of different types of antigens to infiltrate into circulation and subsequently cause immunoactivation and inflammation (Lambert, 2009).

Although inconsistent (Robinson, 1997; Schingoethe et al., 2004), supplementing Saccharomyces cerevisiae fermentation product (SCFP) has been shown to positively affect rumen pH, DMI, milk yield, fermentation patterns, and immune function (Erasmus et al., 2005; Desnoyers et al., 2009; Zaworski et al., 2014; Yuan et al., 2015). Additionally, previous studies demonstrated that feeding SCFP has beneficial effects on key metrics of dairy cow production during HS (Bruno et al., 2009; Zhu et al., 2016). Hence, we hypothesized that supplementing a SCFP would alleviate or at least partially ameliorate the negative consequences of HS on key immune and stress barometers in dairy cows. Therefore, study objectives were to evaluate effects of a SCFP on body temperature indices, metabolism, and the acute phase protein response (APPR) in heat-stressed lactating dairy cows.

**Materials and Methods**

**Animals and Experimental Design**

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Twenty multiparous, lactating Holstein cows (675 ± 12 kg BW; 144 ± 5 DIM; parity 2.3 ± 0.1) were utilized in an experiment conducted in 2 replicates (10 cows/replicate). Cows were randomly assigned to 1 of 2 dietary treatments: 1) a control diet (n = 10) or 2) a control diet supplemented with 19 g/d of SCFP (n = 10; NutriTek; Diamond V, Cedar Rapids, IA). Cows were fed their respective diets for 21 d prior to study initiation during which individual animal feed
intake was not measured. Following the initial feeding phase, cows were housed in individual box stalls (4.57 × 4.57 m) at the Iowa State University Dairy Farm (Ames, IA). Cows were allowed to acclimate for 3 d during which they were implanted with jugular catheters as previously described (Baumgard et al., 2011). The trial consisted of 2 experimental periods (P). During P1 (4 d), cows were fed ad libitum and housed in thermoneutral (TN) conditions for collection of baseline body temperature indices and production parameters. During P2 (7 d), HS was artificially induced via an electric heat blanket (EHB), which cows were fitted with for the entirety of P2; the EHB consists of 12 infrared heating pads as a heat source (Therмотex Therapy Systems Ltd. Calgary, AB, Canada). The blanket was powered by a 110-volt electrical cord that connected to the EHB at the withers as previously described (Al-Qaisi et al., 2019). Cows were exposed to TN conditions throughout P2 (19.1 ± 0.3°C; 64.6 ± 0.6% relative humidity; 64.8 temperature-humidity index). Temperature and humidity were monitored and recorded every 10 min by a data logger (Lascar EL-USB-2-LCD; Erie, PA) and then condensed into daily averages.

All cows were fed a TMR (consisting primarily of corn silage and ground corn) once daily (0800 h) and orts were recorded. The TMR was formulated to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NRC, 2001; Table 5.1). Throughout the experiment, cows were milked twice daily (0600 and 1800 h) and yield was recorded. Milk samples from each cow were obtained daily during both experimental periods. Samples were stored at 4 °C with a preservative (bronopol tablet; DandF Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved infrared analysis equipment and procedures (AOAC International, 1995). In addition, milk samples were collected on d 4 of P1 and d 7 of P2 for milk fatty acid profile analysis. Total lipids were extracted according to previously described methods (Hara and Radin, 1978). Fatty acid methyl esters were
transesterified with sodium methoxide as described by Chouinard et al., (1999). Gas chromatography (GC; model 3800; Varian Analytical Instruments, Walnut Creek, CA) was utilized for sample analysis. Briefly, 1µL of fatty acid methyl esters was injected into the GC with split ratio 99. Fatty acid methyl esters (FAME) standards gas-liquid chromatography GLC461 (Nu-Chek Prep, Inc., Elysian, MN) were used to determine peak identification and quantification. The carrier gas was helium. Injector and detector temperatures were 220°C and helium flow rate was 1.1 mL/min.

During both P1 and P2, rectal temperature (Tr), skin temperature (Ts), respiration rate (RR), and heart rate (HR) were obtained twice daily (0600 and 1800 h). Rectal temperature was measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA). Skin temperature was measured on the neck (not covered by the EHB) using an infrared thermometer (IRT207: The heat seeker 8:1 Mid-Range Infrared Thermometer, general tool, New York, NY). Respiration rate was determined by counting flank movements during 15-s intervals and multiplying by 4 to obtain breaths per minute (bpm). Heart rate was determined using a stethoscope placed over the left side of the rib cage behind the elbow and heart beats were counted for a 15-s interval; this measurement was multiplied by 4 to obtain beats per minute (bpm).

Blood samples were collected daily at 0600 h during both P1 and P2 from the catheter and divided equally between a tube containing K2EDTA (BD, Franklin Lakes, NJ; for plasma collection) and an empty glass tube (for serum collection). Serum samples were allowed to clot at room temperature for 1 h prior to centrifugation. Plasma and serum were harvested following centrifugation at 1,500 × g for 15 min at 4°C and were subsequently frozen at -20°C until analysis. In addition, samples for complete blood count (CBC) analysis were collected on d 3 and 4 of P1 and d 1, 3, 5, and 7 of P2. A 3 mL blood sample was collected from the catheter into a tube
containing an anticoagulant (K₂EDTA; BD Franklin Lakes, NJ) and stored at 4°C for ~12 h before submitting to the Iowa State University’s Department of Veterinary Pathology for analysis.

Plasma glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), blood urea nitrogen (BUN), LPS-binding protein (LBP), serum amyloid A (SAA), and cortisol concentrations were determined using commercially available kits according to manufacturers’ instructions (glucose, Wako Chemicals USA, Richmond; insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; BHB, Pointe Scientific Inc., Canton, MI; BUN, Teco Diagnostics Anaheim, CA; LBP, HK 503, Hycult Biotechnology, Uden, the Netherlands; SAA, Tridelta Development Ltd., Kildare, Ireland; cortisol, Enzo Life Sciences, Farmingdale, NY). The inter- and intra-coefficients of variation for glucose, insulin, NEFA, BHB, BUN, LBP, SAA, and cortisol were 4.1 and 2.9 %, 10.1 and 3.6%, 9.8 and 4.0%, 1.4 and 4.3%, 4.5 and 3.3%, 6.2 and 3.8%, 13.9 and 3.5%, 8.4 and 5.3%, respectively.

Statistical Analysis

Each animal’s respective response variable was analyzed using repeated measures with an autoregressive covariance structure for DMI, milk yield and composition, blood metabolites, inflammatory biomarkers, and CBC. The repeated effect was day of P2 and each specific variable’s average P1 value served as a covariate. Effects of treatment, day, and their interaction were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Additionally, the effects of period were analyzed separately using the MIXED procedure of SAS. The model included treatment, period, and their interaction and cow was the random effect. Results are reported as least squares means and were considered different when $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

As expected, overall HS increased Tr (1.4°C), RR (54 bpm), and Ts (4.8°C) throughout P2 compared with P1 ($P < 0.01$; Table 5.2). There were no dietary treatment detectable differences in
Tr, RR, and Ts ($P > 0.23$; Table 5.2), but, a treatment by period interaction was detected on HR, as it increased in both treatments relative to P1, but the magnitude of increase (P1 vs. P2) was slightly less severe in SCFP-fed cows compared with CON cows ($P < 0.01$; Table 5.2).

During P2, HS markedly decreased DMI (36%; $P < 0.01$; Table 5.3; Figure 5.1A) similarly between dietary treatments relative to P1 and the decrease was most prominent on d 4 of P2 (~46%; $P < 0.01$; Figure 5.1A). Relative to P1, HS decreased milk production (~26%; $P < 0.01$; Table 5.3; Figure 5.1B) and this reduction was most pronounced on d 5 of P2 (33%; $P < 0.01$; Figure 5.1B); however, no dietary differences were observed ($P > 0.60$; Figure 5.1B). Regardless of treatment, HS increased milk fat content and MUN (17 and 30%, respectively; $P < 0.01$; Table 5.3; Figure 5.2A) relative to P1. In contrast, milk protein and lactose concentrations were moderately decreased (7 and 1.4%, $P < 0.01$, respectively; Table 5.3) relative to P1 and neither variable influenced by dietary treatment. Milk SCC was not affected by HS but tended to decrease in SCFP-fed cows relative to CON cows (19%; $P = 0.07$; Figure 5.2B).

Relative to P1, HS decreased the content of de novo ($\leq$ C14:1) and mixed (C16:0 and 16:1) fatty acids (24.2 and 5.4%; $P < 0.01$; Table 5.4), and the milk content of pre-formed fatty acids ($\geq$C17:0) increased (20%; $P < 0.01$; Table 5.4). Regardless of dietary treatment, HS decreased glucose (~4%; $P < 0.01$; Table 5.5), increased NEFA, BHB, and BUN concentrations (~50, ~38, and ~18%; $P < 0.01$, respectively; Table 5.5), and did not affect circulating insulin ($P > 0.25$; Table 5.5) compared with P1. However, HS increased the insulin:DMI (77.5%; $P < 0.01$; Table 5.5) during P2 relative to P1, but this ratio was not influenced by dietary treatment. Heat stress increased cortisol concentrations (52%; $P < 0.05$; Figure 5.3) in CON, but circulating cortisol remained unaffected by HS in the SCFP-fed cows (Figure 5.3).
Overall during P2, circulating LBP and SAA increased (2- and 4-fold, respectively; Figure 5.4) relative to P1 ($P > 0.19$; Figure 5.4). While no overall dietary treatment differences were observed, but post-hoc analysis revealed a tendency for decreased circulating SAA on d 5 of P2 in SCFP-fed cows (~33%; $P = 0.07$; Figure 5.4B) relative to CON cows. Overall and relative to P1, HS did not influence circulating white blood cells and neutrophils, but both were increased (~9 and ~26%; $P \leq 0.05$; Figure 5.5A and B) in SCFP compared with CON cows during P2. Regardless of dietary treatments, HS decreased circulating red blood cells, lymphocytes, basophils, hemoglobin, and hematocrit (3, 7, 14, 3, and 4%; $P < 0.01$; Table 5.6). There was a treatment by period interaction on circulating platelets, as it remained unchanged in CON cows, but increased in SCFP-fed cows during P2 (31%; $P = 0.05$; Table 5.6).

**Discussion**

Many important production traits are adversely impacted by HS and thus this abiotic stressor is an enormous financial burden on the dairy industry (Key and Sneeringer, 2014). Several management strategies (i.e., cooling systems, etc.) have been implemented to ameliorate the negative consequences of HS, but hindered animal performance remains a problem during the warm summer months (Armstrong, 1994). The deleterious effects of HS on productivity are likely mediated by reduced intestinal barrier integrity (Hall et al., 2001; Lambert et al., 2002; Baumgard and Rhoads, 2013). Impaired epithelial integrity allows for luminal contents (i.e. lipopolysaccharide and other toxins) to translocate into the portal and systemic circulation and these antigens stimulate immune and inflammatory responses (Cronje, 2005; Lambert, 2009; Baumgard and Rhoads, 2013). The immune system is energetically expensive and when it is engaged, it rearranges the hierarchy of nutrient partitioning away from milk synthesis (Kvidera et al., 2017). Therefore, dietary interventions that target intestinal toxin/pathogen binding, intestinal barrier function or immune system modulation may improve productivity.
Although not always observed (Robinson, 1997; Schingoethe et al., 2004), feeding SCFP has been shown to improve DMI, milk yield, feed efficiency, and rumen pH (Erasmus et al., 2005; Desnoyers et al., 2009; Poppy et al., 2012; Zaworski et al., 2014; Acharya et al., 2017). Additionally, previous studies have shown that feeding SCFP has beneficial effects on dairy cow performance during challenging situations such as HS (Schingoethe et al., 2004; Zhu et al., 2016), dietary aflatoxin challenge (Jiang et al., 2018), and the transition period (Dann et al., 2000; Zaworski et al., 2014; Shi et al., 2019). Recently, feeding SCFP as an immunomodulator to enhance immune function has received considerable attention (Broadway et al., 2015; Shurson, 2018).

In the current study, HS was successfully induced via the EHB as demonstrated by marked increases in all body temperature variables evaluated (Table 5.2). This is consistent with our previous EHB studies (Al-Qaisi et al., 2019a,b) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Although no dietary treatment differences were observed on Tr, Ts, or RR during P2, the magnitude of increase of HR (P1 vs. P2) was less severe in SCFP-supplemented cows compared with CON cows. Whether this response in HR is biologically meaningful is unclear. Regardless, the fact that there were no dietary treatment differences in most of the body temperature indices is not too surprising as DMI and milk yield (two key contributors to basal heat production) were similar between treatments.

In agreement with our previous EHB studies (Al-Qaisi et al., 2019a,b) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Cowley et al., 2015), HS markedly decreased DMI (36%) relative to P1. Decreased DMI represents a strategy to reduce metabolic heat production and it is a conserved response across species during HS (Collin et al., 2001; Kadzere et al., 2002; Baumgard and Rhoads, 2013). During P2, DMI was similar between
dietary treatments and this agrees with previous SCFP reports (Schingoethe et al., 2004; Bruno et al., 2009; Zhu et al., 2016), but disagrees with others (Dann et al., 2000). Reasons for inconsistencies in DMI are unclear, but it could be related to many factors including stage of lactation, as cows in this experiment were in mid to late lactation and the improved SCFP-induced DMI improvements are typically seen in early lactation (Poppy et al., 2012).

Not unexpectedly, HS decreased milk yield (26%) relative to P1, which corroborates our previous EHB studies (Al-Qaisi et al., 2019a,b) and climate-controlled experiments (Rhoads et al., 2009; Shwartz et al., 2009; Cowley et al., 2015). However, no dietary treatment differences were observed on milk yield during HS, which agrees with Schingoethe and colleagues (2004). Other studies (Bruno et al., 2009; Zhu et al., 2016), however, reported positive effects of SCFP supplementation during HS on milk yield. Lack of dietary treatment differences in overall productivity (i.e., DMI and milk yield) in this study is not unanticipated as the experiment was powered for metabolic and immune activation markers and knowingly underpowered for production metrics. It would be of interest to evaluate SCFP’s potential to improve economically important phenotypes during HS in the future.

Dietary treatment had no effect on milk fat percentage, which agrees with previous reports that evaluated SCFP during HS (Schingoethe et al., 2004; Zhu et al., 2016). However, HS increased milk fat content (17%) relative to P1, which is consistent with previous results (Regan and Richardson, 1938; Rhoads et al., 2009; Baumgard et al., 2011; Al-Qaisi et al., 2019b). This is interesting as normally milk fat content has a seasonal pattern and is typically the lowest during the warm summer months (Hays, 1926; Huber, 1996; Bouraoui et al., 2002). Reasons why there are discordant effects between natural HS and HS models is not clear, but most HS models are acute and intense (i.e. less than 10 d) and maybe the low summer milk fat is part of “acclimation”;
a physiological state that is not fully obtained in the climate chamber/EHB models. Regardless of dietary treatment, HS decreased the content of de novo derived fatty acids and increased the percentage of preformed fatty acids, and this altered profile during HS agrees with previous reports (Moody et al., 1971; Bandaranayaka and Holmes, 1976). Interestingly, the aforementioned changes in milk fatty acid origin (decreased and increased de novo and preformed, respectively) is normally associated with diet-induced milk fat depression (Bauman et al., 2008), not increased milk fat content as observed herein (Figure 5.2A) and other HS models (Regan and Richardson, 1938; Rhoads et al., 2009). Clearly, a better understanding of how milk fat synthesis is regulated during HS is warranted.

Heat stress decreased milk protein content (7%), which agrees with our previous HS results (Shwartz et al., 2009; Baumgard et al., 2011; Al-Qaisi et al., 2019a), but it was unaffected by dietary treatment and this also agrees with previous reports (Schingoethe et al., 2004; Zhu et al., 2016). Reduced milk protein composition is likely due to increased AA utilization for extra-mammary purposes (Gao et al., 2017) and potentially the direct effects of HS on mammary protein synthesis (Bernabucci et al., 2010; Cowley et al., 2015). The direct effects of HS on milk protein synthesis maybe mediated by either antigen (i.e., LPS) as mammary epithelial cells have the toll-like receptor-4 (Ibeagha-Awemu et al., 2008) or inflammation as this milk component is also markedly decreased in LPS infusion models (Hinz et al., 2012; Ning et al., 2018).

In agreement with previous HS studies (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010; Al-Qaisi et al., 2019b), HS decreased milk lactose content, but this effect was not rescued by SCFP, which corroborates previous results (Schingoethe et al., 2004; Zhu et al., 2016). Reasons for the decreased milk lactose content could be attributed to increase on extra-mammary glucose utilization during HS (discussed below).
Heat stress increased MUN and this corroborates previous HS studies (Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017; Al-Qaisi et al., 2019a,b). The increased MUN is presumably because heat-stressed cows were mobilizing skeletal muscle to provide amino acids for the production of acute phase proteins (Johnson, 2012) and glucose (Baumgard and Rhoads, 2013). Feeding SCFP had no detectable effects on MUN and this contradicts recent reports indicating that SCFP linearly decreased MUN during natural HS (Zhu et al., 2016) and during the transition period (Shi et al., 2019). The inconsistencies between studies might be due to different stages of lactation, HS severity, or different feed composition. Interestingly, SCC tended to decrease (19%) in SCFP-fed cows relative to CON cows during HS, which is consistent with transition period studies (Bluel, 2006; Zaworski et al., 2014; Yuan et al., 2015). However, other studies (Dann et al., 2000; Ramsing et al., 2009) reported no effect of SCFP supplementation on milk SCC. Reasons for reduced SCC in SCFP-fed cows are not fully understood, but likely involve the immunomodulatory effects of SCFP (β-glucan and mannan) and the basal levels of immune activation (systemic and intramammary; as reviewed by Volman et al., 2008; Broadway et al., 2015). Further investigating the effect of dietary SCFP on mammary health is of particular interest.

Dietary treatment did not affect circulating glucose, however, HS decreased glucose levels (~4%) relative to P1, which is similar to previous HS studies in cows (Itoh et al., 1998; Shwartz et al., 2009; Wheelock et al., 2010; Al-Qaisi et al., 2019b) sheep (Achmadi et al., 1993), and pigs (Sanz Fernandez et al., 2014). Presumably, glucose concentrations are reduced because of decreased propionate delivery to the liver (i.e. decreased DMI) and increased glucose uptake by activated leukocytes (discussed below), which utilize copious amounts of glucose (Kvidera et al., 2017). Furthermore, reduced blood glucose levels are likely responsible (at least partially) for
decreased milk lactose content, as glucose is the precursor for lactose synthesis (Nafikov and Beitz, 2007).

In the current study, no dietary treatment differences were observed in the insulin: DMI, or in circulating insulin, NEFA, BHB, and BUN during HS. However, despite the marked reduction in DMI during P2, HS increased the insulin:DMI ratio (76%) and this agrees with previous HS work in ruminants and pigs (O’Brien et al., 2010; Wheelock et al., 2010; Sanz Fernandez et al., 2015; Mayorga et al., 2018). Reasons for the insulin response during HS are not fully understood but may be related to the fact that leukocytes are insulin responsive (Calder et al., 2007; Maratou et al., 2007) and LPS appears to have a role in stimulating insulin secretion (Baumgard et al., 2016). Additionally, HS slightly increased circulating NEFA, which disagrees with our previous HS reports (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010) indicating a blunted basal and stimulated NEFA concentration during HS. Reasons for inconsistencies in circulating NEFA is likely due to the fact that current study did not employ the pair-feeding design as in other experiments. The increase in NEFA described herein (50%) is marginal compared to the large increases occurring in thermal neutral negative energy balance like those described in the transition period (Grummer, 1993, 1995) or during feed restriction (Grummer et al., 1994, Carlson et al., 2006). Furthermore, HS increased circulating BHB (38%), which agrees with other HS studies in cattle (Ronchi et al., 1999; Abeni et al., 2007). Reasons for increased circulating BHB during HS remain unclear as it could be due to increased pool entry (from the GIT or the liver) or decreased removal from the plasma pool. But similar to NEFA, the increase in BHB is likely biologically inconsequential, especially considering the magnitude of change in BHB concentration (> 4-fold) that normally accompanies the transition period (Bobe et al., 2004; McArt et al., 2015). In agreement with HS studies conducted in environmental chambers (Rhoads et al., 2009; Wheelock
et al., 2010; Cowley et al., 2015; Gao et al., 2017), HS caused increased BUN levels (18%), which is likely due to increased AA utilization for gluconeogenesis and production of acute phase protein (Baumgard and Rhoads, 2013). Because BUN transfers into milk on a concentration gradient (Roseeler et al., 1993), it is not surprising that the temporal pattern of both BUN and MUN (described above) reflect each other.

As mentioned earlier, impaired intestinal barrier integrity is common during acute HS (Lambert et al., 2002; Pearce et al., 2013). Increased intestinal permeability leads to LPS translocation into portal and systemic circulation which upon recognition by immune cells triggers an inflammatory response characterized by the production of acute phase proteins (Ceciliani et al., 2012). This APPR has an imperative role in pathogen opsonization, removal of toxic substances, and in regulating the innate immune system (Ceciliani et al., 2012). In the present study, HS increased circulating LBP and SAA (2- and 4-fold, respectively) relative to P1, which likely indicates an increased inflammatory response during HS and this agrees with our recent report in pigs (Mayorga et al., 2019). Interestingly, circulating SAA tended to decrease on d 5 in SCFP-fed cows during HS relative to CON cows. This is consistent with a previous study in which SCFP supplementation reduced APPR during the periparturient period (Knoblock et al., 2018). Reasons for reduced circulating SAA are not well-defined, but could be related to the immunomodulatory effect of SCFP. As mentioned earlier, β-glucans and mannans putatively have a positive impact on the immune system through altered cytokine production and pathogen removal from the digestive tract, thereby reducing bacteria attachment and colonization (Volman et al., 2008; Nocek et al., 2011; Broadway et al., 2015). Despite the apparent decrease in peak SAA, circulating white blood cells and neutrophils were increased (9 and 26%; respectively) in SCFP-fed cows compared with CON cows. Accurately interpreting these aforementioned discordant patterns is difficult, but
results certainly suggest that SCFP has immunomodulatory effects during HS. Further investigating the effect of SCFP on immune system metrics during HS is of particular interest.

Not surprisingly, HS increased cortisol concentrations and this was especially apparent in the CON cows. Elevated cortisol is a classic endocrine response to stress (Binsiya et al., 2017; Sejian et al., 2018) and it corroborates previous HS studies in dairy cows (Titto et al., 2017; Hall et al., 2018) and calves (Lopez et al., 2018). Interestingly, SCFP-fed cows did not have an increase in circulating cortisol during HS, and this is consistent with previous studies where SCFP supplementation reduced circulating cortisol levels in transition dairy cows (Zaworski et al., 2014), heat-stressed broiler chickens (Nelson et al., 2018) and turkeys (Bartz et al., 2018). Reasons for reduced circulating cortisol following SCFP supplementation are not fully understood. Increased glucocorticoids are thought to negatively affect neutrophil function (Burton et al., 1995, 2005), thus the blunted cortisol response reported herein could have a large impact on a variety of on-farm situations characterized by immune dysfunction (i.e. the transition period; Trevisi et al., 2012). Further research is needed to explain the mechanism (and consequences of) by which SCFP supplementation modifies the stress axis.

**Conclusions**

Utilizing the EHB increased all body temperature variables, caused inflammation, and reduced production parameters (i.e., DMI and milk yield). Herein, we demonstrated that feeding SCFP could be beneficial at reducing circulating cortisol and the APPR and increasing key leukocytes during stressful conditions. Overall, feeding SCFP altered the metabolic and immune-profile in heat-stressed dairy cows and these changes are presumably reflective of a reduced “stressed” state.
References


Table 5.1. Ingredients and composition of diets\(^1\)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CON(^3) % of DM</th>
<th>SCFP(^4) % of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>24.1</td>
<td>24.1</td>
</tr>
<tr>
<td>Baleage</td>
<td>19.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Ground Corn</td>
<td>27.6</td>
<td>27.6</td>
</tr>
<tr>
<td>Mineral and Protein mix</td>
<td>7.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Whole Cottonseed</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Corn Gluten Feed</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Soy Plus</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Chemical analysis

<table>
<thead>
<tr>
<th></th>
<th>CON(^3)</th>
<th>SCFP(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>28.2</td>
<td>29.8</td>
</tr>
<tr>
<td>CP</td>
<td>16.9</td>
<td>17.0</td>
</tr>
<tr>
<td>NDF</td>
<td>27.6</td>
<td>25.9</td>
</tr>
<tr>
<td>ADF</td>
<td>19.3</td>
<td>18.0</td>
</tr>
<tr>
<td>NE(_L) Mcal/kg DM</td>
<td>1.66</td>
<td>1.68</td>
</tr>
</tbody>
</table>

\(^1\)Values represent an average of ration nutrient summary reports collected throughout the trial. Diet dry matter averaged 53.30\% for the control and 55.99\% for the SCFP ration.

\(^2\)Average nutrient levels for both rations: 5.06\% Fat, 0.91\% Ca, 0.39\% P, 0.36\% Mg, 0.21\% S, 1.22\% K, 0.42\% Na, 0.63\% Cl, 78.58 ppm of Zn, 45.66 ppm of Mn, 3.54 ppm of Fe, 13.62 ppm of Cu, 0.76 ppm of Co, 0.16 ppm Se, 0.76 ppm of I, 6167.2 IU/kg of vitamin A, 1233.45 IU/kg of vitamin D, and 24.66 IU/kg of vitamin E.

\(^3\)CON = control diet.

\(^4\)SCFP = \textit{Saccharomyces cerevisiae} fermentation product diet.
Table 5.2: Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on body temperature variables in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th></th>
<th>Period 2</th>
<th></th>
<th>SEM</th>
<th>Treatment</th>
<th>PER</th>
<th>Treatment × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperature, °C</td>
<td>CON 38.4</td>
<td>SCFP 38.4</td>
<td>CON 39.7</td>
<td>SCFP 39.9</td>
<td>0.1</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Skin temperature, °C</td>
<td>CON 29.3</td>
<td>SCFP 28.8</td>
<td>CON 33.8</td>
<td>SCFP 33.9</td>
<td>0.3</td>
<td>0.57</td>
<td>&lt;0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>CON 34</td>
<td>SCFP 36</td>
<td>CON 87</td>
<td>SCFP 91</td>
<td>2</td>
<td>0.31</td>
<td>&lt;0.01</td>
<td>0.53</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>CON 79(^{c})</td>
<td>SCFP 82(^{bc})</td>
<td>CON 87(^{a})</td>
<td>SCFP 85(^{a})</td>
<td>2</td>
<td>0.83</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(^{a-c}\)Values within a row of with differing superscripts indicate statistical difference (\(P < 0.05\)).

\(^{1}\)During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.

\(^{2}\)During period 2, all cows were fitted with an electric heat blanket and allowed to eat ad libitum.

\(^{3}\)PER = Period.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
<th>SEM</th>
<th>Treatment</th>
<th>PER</th>
<th>Treatment × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DMI, kg/d</strong></td>
<td>CON</td>
<td>SCFP</td>
<td>CON</td>
<td>SCFP</td>
<td>SEM</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>25.1</td>
<td>25.5</td>
<td>15.7</td>
<td>16.4</td>
<td>0.7</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Milk yield, kg/d</strong></td>
<td>43.8</td>
<td>43.5</td>
<td>32.9</td>
<td>32.0</td>
<td>1.4</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Milk variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.80</td>
<td>3.96</td>
<td>4.56</td>
<td>4.52</td>
<td>0.20</td>
<td>0.81</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.12</td>
<td>3.11</td>
<td>2.90</td>
<td>2.90</td>
<td>0.06</td>
<td>0.97</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.72</td>
<td>4.78</td>
<td>4.64</td>
<td>4.73</td>
<td>0.05</td>
<td>0.25</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>13.2</td>
<td>11.9</td>
<td>16.4</td>
<td>16.3</td>
<td>0.5</td>
<td>0.28</td>
</tr>
</tbody>
</table>

1During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.
2During period 2, all cows were fitted with an electric heat blanket and allowed to eat ad libitum.
3PER = Period.
Table 5.4: Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on milk fatty acid in lactating dairy cows

<table>
<thead>
<tr>
<th>Fatty acid, %</th>
<th>Period 1</th>
<th>Period 2</th>
<th>SEM</th>
<th>Trt</th>
<th>PER</th>
<th>Trt × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>SCFP</td>
<td>CON</td>
<td>SCFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;1:0&lt;/sub&gt;</td>
<td>0.96</td>
<td>1.19</td>
<td>1.07</td>
<td>1.11</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>C&lt;sub&gt;6:0&lt;/sub&gt;</td>
<td>0.88</td>
<td>0.93</td>
<td>0.85</td>
<td>0.82</td>
<td>0.05</td>
<td>0.84</td>
</tr>
<tr>
<td>C&lt;sub&gt;8:0&lt;/sub&gt;</td>
<td>0.67</td>
<td>0.67</td>
<td>0.58</td>
<td>0.55</td>
<td>0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>C&lt;sub&gt;10:0&lt;/sub&gt;</td>
<td>2.14</td>
<td>2.09</td>
<td>1.51</td>
<td>1.43</td>
<td>0.12</td>
<td>0.63</td>
</tr>
<tr>
<td>C&lt;sub&gt;12:0&lt;/sub&gt;</td>
<td>3.04</td>
<td>2.87</td>
<td>1.88</td>
<td>1.80</td>
<td>0.14</td>
<td>0.44</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>8.82</td>
<td>10.43</td>
<td>7.79</td>
<td>7.55</td>
<td>0.78</td>
<td>0.41</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1&lt;/sub&gt;</td>
<td>0.90</td>
<td>0.81</td>
<td>0.56</td>
<td>0.57</td>
<td>0.05</td>
<td>0.57</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;</td>
<td>1.29</td>
<td>1.05</td>
<td>0.69</td>
<td>0.72</td>
<td>0.09</td>
<td>0.23</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt;</td>
<td>35.31</td>
<td>34.46</td>
<td>32.28</td>
<td>32.59</td>
<td>0.65</td>
<td>0.73</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:3&lt;/sub&gt;</td>
<td>1.99</td>
<td>1.82</td>
<td>2.44</td>
<td>2.27</td>
<td>0.18</td>
<td>0.35</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;, cis-9</td>
<td>0.42</td>
<td>0.38</td>
<td>0.39</td>
<td>0.35</td>
<td>0.04</td>
<td>0.43</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;, cis-11</td>
<td>0.52</td>
<td>0.50</td>
<td>0.36</td>
<td>0.28</td>
<td>0.04</td>
<td>0.29</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;, cis-12</td>
<td>0.09</td>
<td>0.05</td>
<td>0.14</td>
<td>0.10</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;, cis-13</td>
<td>0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;, trans-6</td>
<td>0.02</td>
<td>0.06</td>
<td>0.10</td>
<td>0.13</td>
<td>0.03</td>
<td>0.28</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;, trans-11, trans-10</td>
<td>2.30</td>
<td>2.15</td>
<td>1.73</td>
<td>1.51</td>
<td>0.21</td>
<td>0.48</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt;, trans-12</td>
<td>0.37</td>
<td>0.53</td>
<td>0.24</td>
<td>0.24</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt;, trans-15</td>
<td>0.54</td>
<td>0.33</td>
<td>0.67</td>
<td>0.40</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:3&lt;/sub&gt;</td>
<td>2.83</td>
<td>2.71</td>
<td>3.25</td>
<td>2.95</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:3&lt;/sub&gt;</td>
<td>0.55</td>
<td>0.52</td>
<td>0.61</td>
<td>0.57</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>C&lt;sub&gt;19:0&lt;/sub&gt;, cis-9, trans-11</td>
<td>0.47</td>
<td>0.52</td>
<td>0.42</td>
<td>0.36</td>
<td>0.04</td>
<td>0.86</td>
</tr>
<tr>
<td>CLA</td>
<td>0.09</td>
<td>0.06</td>
<td>0.10</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>USA/SA</td>
<td>0.18</td>
<td>0.22</td>
<td>0.21</td>
<td>0.21</td>
<td>0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>De novo FA&lt;sup&gt;5&lt;/sup&gt;</td>
<td>17.59</td>
<td>19.11</td>
<td>14.30</td>
<td>13.90</td>
<td>1.00</td>
<td>0.61</td>
</tr>
<tr>
<td>Mixed FA&lt;sup&gt;6&lt;/sup&gt;</td>
<td>37.30</td>
<td>36.30</td>
<td>34.73</td>
<td>34.86</td>
<td>0.58</td>
<td>0.54</td>
</tr>
<tr>
<td>Preformed FA&lt;sup&gt;7&lt;/sup&gt;</td>
<td>38.16</td>
<td>38.93</td>
<td>45.85</td>
<td>46.16</td>
<td>1.08</td>
<td>0.64</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values within a row of with differing superscripts indicate statistical difference (*P* < 0.05).

<sup>1</sup>During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.

<sup>2</sup>During period 2, all cows were fitted with an electric heat blanket and allowed to eat ad libitum.

<sup>3</sup>Trt = Treatment.

<sup>4</sup>PER = Period.

<sup>5</sup>De novo FA: sum of C4 to C14:1 fatty acid.

<sup>6</sup>Mixed FA: sum of C16 to C16:1 fatty acid.

<sup>7</sup>Preformed FA: sum of C18 to C20:4 fatty acid.
Table 5.5: Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on blood metabolites in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th></th>
<th>Period 2</th>
<th></th>
<th>SEM</th>
<th>Treatment</th>
<th>PER</th>
<th>Treatment × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>CON 72.0</td>
<td>SCFP 73.1</td>
<td>CON 68.9</td>
<td>SCFP 70.7</td>
<td>1.7</td>
<td>0.50</td>
<td>&lt;0.01</td>
<td>0.74</td>
</tr>
<tr>
<td>Insulin, µg/L</td>
<td>0.48</td>
<td>0.52</td>
<td>0.54</td>
<td>0.58</td>
<td>0.07</td>
<td>0.60</td>
<td>0.25</td>
<td>0.99</td>
</tr>
<tr>
<td>Insulin:DMI</td>
<td>0.019</td>
<td>0.021</td>
<td>0.035</td>
<td>0.036</td>
<td>0.003</td>
<td>0.73</td>
<td>&lt;0.01</td>
<td>0.81</td>
</tr>
<tr>
<td>NEFA, µEq/L</td>
<td>175.8</td>
<td>176.0</td>
<td>267.1</td>
<td>260.0</td>
<td>22.0</td>
<td>0.89</td>
<td>&lt;0.01</td>
<td>0.85</td>
</tr>
<tr>
<td>BHB, mg/dL</td>
<td>5.8</td>
<td>6.3</td>
<td>8.3</td>
<td>8.4</td>
<td>0.5</td>
<td>0.61</td>
<td>&lt;0.01</td>
<td>0.51</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>11.2</td>
<td>9.8</td>
<td>12.7</td>
<td>12.1</td>
<td>0.6</td>
<td>0.13</td>
<td>&lt;0.01</td>
<td>0.33</td>
</tr>
</tbody>
</table>

1During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.
2During period 2, all cows were fitted with an electric heat blanket and allowed to eat ad libitum.
3PER = Period.
Table 5.6: Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on complete blood cell parameters in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Period 2&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM</th>
<th>Treatment</th>
<th>PER&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Treatment × PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cells, ×10&lt;sup&gt;6&lt;/sup&gt;/μL</td>
<td>6.3 6.7</td>
<td>6.2 6.4</td>
<td>0.14</td>
<td>0.17</td>
<td>&lt;0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>Platelets, ×10&lt;sup&gt;3&lt;/sup&gt;/μL</td>
<td>267&lt;sup&gt;ab&lt;/sup&gt; 320&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263&lt;sup&gt;ab&lt;/sup&gt; 221&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30</td>
<td>0.88</td>
<td>&lt;0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Monocytes, ×10&lt;sup&gt;3&lt;/sup&gt;/μL</td>
<td>0.41 0.41</td>
<td>0.42 0.35</td>
<td>0.04</td>
<td>0.47</td>
<td>0.30</td>
<td>0.18</td>
</tr>
<tr>
<td>Lymphocytes, ×10&lt;sup&gt;3&lt;/sup&gt;/μL</td>
<td>5.32 3.54</td>
<td>5.08 3.19</td>
<td>1.20</td>
<td>0.29</td>
<td>&lt;0.01</td>
<td>0.64</td>
</tr>
<tr>
<td>Eosinophils, ×10&lt;sup&gt;3&lt;/sup&gt;/μL</td>
<td>0.49 0.42</td>
<td>0.46 0.44</td>
<td>0.08</td>
<td>0.70</td>
<td>0.96</td>
<td>0.55</td>
</tr>
<tr>
<td>Basophils, ×10&lt;sup&gt;3&lt;/sup&gt;/μL</td>
<td>0.07 0.07</td>
<td>0.06 0.06</td>
<td>0.01</td>
<td>0.83</td>
<td>&lt;0.01</td>
<td>0.79</td>
</tr>
<tr>
<td>Hemoglobin, gm/dL</td>
<td>10.9 11.2</td>
<td>10.7 10.8</td>
<td>0.1</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>29.8 30.7</td>
<td>28.9 29.4</td>
<td>0.4</td>
<td>0.19</td>
<td>&lt;0.01</td>
<td>0.41</td>
</tr>
</tbody>
</table>

<sup>a</sup>bValues within a row of with differing superscripts indicate statistical difference (*P* = 0.05).

<sup>1</sup>During period 1, cows in both treatments were housed in thermal neutral conditions and allowed to eat ad libitum.

<sup>2</sup>During period 2, all cows were fitted with electric heat blanket and allowed to eat ad libitum.

<sup>3</sup>PER = Period.
Figure 5.1. Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on (A) DMI and (B) milk yield in heat-stressed lactating dairy cows. The mean of Period 1 is represented by (P1) on the X-axis. Results are expressed as LSM ± SEM and the P-values are from the statistical analysis of P2.
Figure 5.2. Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on (A) milk fat and (B) somatic cell count in heat-stressed lactating dairy cows. The mean of Period 1 is represented by (P1) on the X-axis. Results are expressed as LSM ± SEM and the P-values are from the statistical analysis of P2.
**Figure 5.3.** Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on circulating cortisol in heat-stressed lactating dairy cows. The mean of Period 1 is represented by (P1) on the X-axis. Results are expressed as LSM ± SEM and the P-values are from the statistical analysis of P2.
Figure 5.4. Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on circulating (A) LPS-Binding protein and (B) serum Amyloid A in heat-stressed lactating dairy cows. The mean of Period 1 is represented by (P1) on the X-axis. Results are expressed as LSM ± SEM and the P-values are from the statistical analysis of P2.
**Figure 5.5.** Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) on circulating (A) white blood cell and (B) neutrophils in heat-stressed lactating dairy cows. The mean of Period 1 is represented by (P1) on the X-axis. Results are expressed as LSM ± SEM and the P-values are from the statistical analysis of P2.
CHAPTER 6. EFFECTS OF AN ORAL SUPPLEMENT CONTAINING CALCIUM AND LIVE YEAST ON CIRCULATING CALCIUM AND POST-ABSORPTIVE METABOLISM AND PRODUCTION FOLLOWING INTRAVENOUS LIPOPOLYSACCHARIDE INFUSION IN DAIRY COWS

Modified from a paper to be submitted to Research in Veterinary Science

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\textsuperscript{a}Department of Animal Science, Iowa State University, Ames 50011

\textsuperscript{b}TechMix, LLC, Stewart, MN 55385, USA

Abstract

Administrating lipopolysaccharide (LPS) decreases circulating calcium (Ca) and markedly reduces feed intake and milk yield in lactating cows. Feeding live yeast can increase appetite in non-LPS challenged cows, but whether supplemental Ca and live yeast benefit immune-challenged cows is unknown. Study objectives were to evaluate the effects of providing an oral supplement containing soluble Ca, live yeast, and other micronutrients on circulating Ca, inflammation and production parameters in lactating dairy cows. Twelve multiparous lactating Holstein cows were housed in individual box stalls and allowed 4 d to acclimate. The trial consisted of 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and baseline data was collected. At the beginning of P2 (which lasted 96 h), all cows were i.v. challenged with 0.375 \( \mu \)g/kg BW LPS.
Cows were assigned randomly to 1 of 2 treatments: 1) control (CON; no bolus; n=6) or 2) an oral bolus containing Ca and live yeast (CLY; YMCP Vitall 44.718 g of elemental Ca; Techmix, LLC., Stewart, MN; n=6), administered -0.5 and 6.5 h relative to LPS infusion. Blood samples were collected -1, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 6.5, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 h relative to LPS infusion. Following LPS administration, circulating ionized Ca decreased in both treatments but supplemental CLY ameliorated the hypocalcemia (48 h area under the curve: -10.8 vs. -1.9 mmol/L × h; \(P<0.01\)). Lipopolysaccharide decreased dry matter intake (DMI; 60%) similarly for both treatments on d 1, but overall (d 1-4) DMI tended to be reduced less (14 vs. 30%; \(P=0.06\)) in CLY supplemented vs CON cows. Lipopolysaccharide reduced milk yield (70%; \(P<0.01\)) from 12-24 h relative to baseline, but throughout P2, milk yield from CLY supplemented cows was increased (38%; \(P=0.03\)) relative to CON cows. Relative to baseline, infusing LPS increased (55%) circulating glucose from 0 to 2 h post-LPS administration (\(P<0.01\)) and then glucose progressively decreased similarly in both treatments between 3 and 12 h post LPS-infusion (17%; \(P>0.40\)). There tended to be a treatment by time interaction on milk urea nitrogen and milk somatic cell count (\(P<0.10\)), as both progressively increased in both treatments for 24 and 48 h, respectively but peaked lower and decreased faster in the CLY supplemented compared to the CON cows. Overall during P2, circulating LPS-binding protein and serum amyloid A increased (3- and 4-fold, respectively, \(P<0.01\)), but were unaffected by treatment (\(P>0.68\)). Furthermore, hyperinsulinemia was observed in both treatments at 24 h post-LPS infusion (increased 3-fold relative to baseline; \(P<0.01\)) but was unaffected by treatment (\(P=0.35\)). In conclusion, providing an oral supplement containing Ca and live yeast prior to and following LPS administration markedly ameliorated LPS-induced hypocalcemia, altered energetic metabolism and improved DMI and milk yield.
Key Words: calcium, lipopolysaccharide, dairy cow, yeast

Introduction

Periparturient dairy cows can experience a myriad of metabolic disorders, and transient hypocalcemia represents one of the most common. Subclinical hypocalcemia is purportedly a gateway to other disorders such as ketosis, mastitis, and metritis, all of which compromise profitability and increase culling risks (DeGaris and Lean, 2008; Goff, 2008). After parturition, the mammary gland has a large calcium (Ca) demand, and proper parathyroid hormone (PTH) and calcitonin action is required to maintain eucalcemia (Horst et al., 2005). However, the mammary gland’s Ca uptake is so acute and extensive that it exceeds the homeostatic strategies employed to replenish circulating Ca (Goff, 2008) and cows can either enter into clinical or subclinical hypocalcemia. Although not overtly pathological, subclinical hypocalcemia has been associated with decreased productivity and other economically important phenotypes later in lactation (Goff, 2008, 2014; Oetzel, 2013). Different prophylactic and therapeutic strategies for preventing post-calving hypocalcemia include: feeding pre-calving acidifying rations (-DCAD), low Ca-diets (Thilsing-Hansen et al., 2002) or Ca chelating compounds (Goff, 2008). These dietary strategies have markedly reduced clinical rates of “milk fever”, but periparturient subclinical hypocalcemia remains a common post-calving “pathology”. Consequently, orally bolusing Ca following parturition has become a common management tactic (Oetzel and Miller, 2012; Oetzel, 2013) and preferably proven over intravenous Ca administration (Wilms et al., 2019).

Although the magnitude and extent differ, it is likely that all periparturient dairy cows (even seemingly healthy ones) experience some degree of inflammation (Trevisi et al., 2012; Bradford et al., 2015) and immunoactivation decreases circulating Ca in a variety of species (Carlstedt et al., 2000; Shinozuka et al., 2018) including ruminants (Waldron et al., 2003a; Horst et al., 2018). Inflammation can be experimentally modeled by administering lipopolysaccharide (LPS) and this
markedly reduces blood Ca in several species (Elsasser et al., 1996; Carlstedt et al., 2000; Toribio et al., 2005; Shinozuka et al., 2018) including dairy cows (Waldron et al., 2003a, Kvidera et al., 2017; Horst et al., 2018). Hypocalcemia is thought to compromise neutrophil function and thus increase susceptibility to infection (Kimura et al., 2006; Martinez et al., 2012, 2014) and therefore periparturient hypocalcemia is thought to be causal to other transition cow diseases (Kimura et al., 2006; Martinez et al., 2012, 2014).

Although variable (Arambel and Kent, 1990; Kung et al., 1997), live yeast supplementation is thought to positively affect rumen pH, fermentation patterns, dry matter intake (DMI), and lactation performance (Desnoyers et al., 2009; Ramsing et al., 2009; Broadway et al., 2015) and may even benefit immune function (Broadway et al., 2015). Incidentally, the aforementioned variables are negatively affected in both poorly transitioning and experimentally induced immunoactivated (i.e. LPS) dairy cows (Drackely, 1999; Waldron et al., 2003b; Kvidera et al., 2017). Thus, we hypothesized that supplementing both Ca and live yeast may ameliorate the negative consequences of an activated immune-system in dairy cows. Study objectives were to evaluate the effects of providing an oral supplement primarily containing soluble Ca and live yeast on circulating Ca, energetic metabolism, leukocyte dynamics and production parameters in lactating dairy cows. Further, we wanted to explore if i.v. LPS administration could be used to model periparturient hypocalcemia.

**Materials and Methods**

**Animals and Experimental Design**

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Twelve non-pregnant lactating Holstein cows (760 ± 13 kg BW; 269 ± 20 DIM; parity 2.7 ± 0.2) were utilized and housed in individual box-stalls (4.57 × 4.57 m) at the Iowa State University Dairy Farm. Cows were allowed 4 d to acclimate during which they were implanted
with jugular catheters as previously described (Baumgard et al., 2011). The trial consisted of 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and baseline data was collected (for covariate analysis). At the beginning of P2, which lasted 96 h, all cows were i.v. challenged with 0.375 µg/kg BW LPS. The LPS dose was selected based on the magnitude of hypocalcemia observed in a previous report (Horst et al., 2018). Cows were randomly assigned to 1 of 2 treatments: 1) control (CON; no bolus; n = 6) or 2) an oral bolus containing Ca and live yeast, administrated -0.5 and 6.5 h relative to LPS infusion (CLY; YMCP Vitall; 44.72 g of (CaCl₂; 30.82 g) and (CaCO₃; 13.88 g); Saccharomyces cerevisiae 30 billion cfu; TechMix. LLC., Stewart, MN; n = 6). A stock solution of LPS (Escherichia coli O55:B5; Sigma Aldrich, St. Louis, MO) was created at a concentration of 100 µg/mL, passed through a 0.2 µm sterile syringe filter (Thermo Scientific; Waltham, MA), and stored in a sterile glass bottle 24 h prior to P2. The total volume of LPS solution administrated was approximately 3 mL.

**Diet and Daily Measurements**

Cows were individually fed a TMR consisting primarily of corn silage once daily (0800 h) and orts were measured before feeding. The TMR was formulated to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NRC, 2001; Table 6.1). To eliminate the effects of dissimilar nutrient intake and to isolate the effects of the oral supplement, cows were fasted for the first 12 h of P2. Cows were milked twice daily (0600 and 1800 h) and yield was recorded at each milking. A milk sample for composition analysis was collected at each milking and stored at 4°C with a preservative (bronopol tablet; DandF Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved infrared analysis equipment and procedures (AOAC International, 1995).

Rectal temperature (Tr), respiration rate (RR), and heart rate (HR) were obtained at -1, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 6.5, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 h relative to LPS infusion.
Rectal temperature was measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA). Heart rate was determined using a stethoscope placed over the left side of the rib cage behind the elbow. Respiration rate was determined by observing flank movements. Heart rate and respiration rate were measured during a 15 sec interval and were later transformed to beats/min and breaths/min (bpm), respectively.

**Blood Sample Collection**

Blood samples (~10 mL each) were obtained daily at 0600 h during P1 and at -1, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 6.5, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 h relative to LPS infusion during P2. Samples were collected from the catheter and divided equally between a tube containing K2EDTA (BD, Franklin Lakes, NJ; for plasma collection) and an empty glass tube (for serum collection). Serum samples were allowed to clot at room temperature for 1 h prior to centrifugation. Plasma and serum were harvested following centrifugation at 1,500 × g for 15 min at 4°C and were subsequently frozen at -20°C until analysis. Blood ionized calcium (iCa) and glucose were measured using an iStat handheld machine and cartridge (CG8+; Abbott Point of Care, Princeton, NJ) and were obtained at the same time points mentioned earlier relative to LPS infusion during P2. Administering LPS occurred immediately following the morning milking and 0 h blood sample collection. Blood samples for complete blood count (CBC) analysis were collected at 0, 1, 2, 3, 6, 9, 12, 24, 48, 72, and 96 h relative to LPS administration and stored at 4°C for ~12 h before submitting to the Iowa State University’s Department of Veterinary Pathology.

**Sample Analyses**

Plasma insulin, parathyroid hormone (PTH), LPS-binding protein (LBP), and serum amyloid A (SAA) concentrations were determined using commercially available kits according to manufacturers’ instructions (insulin, Mercodia AB, Uppsala, Sweden; PTH, Bovine intact PTH ELISA, Immutopics, San Clemente, CA; LBP, Hycult Biotechnology, Uden, the Netherlands;
SAA, Tridelta Development Ltd., Kildare, Ireland). The inter- and intra-assay coefficients of variation for PTH, LBP, and SAA were 8.4 and 11.8%, 5.4 and 8.2%, and 23.4 and 6.4%, respectively. The intra-assay coefficient of variation for insulin was 6.6%.

**Calculations and Statistical Analysis**

Area under the curve (AUC) for iCa was calculated through 96 h post-LPS by linear trapezoidal summation between successive pairs of iCa levels and time coordinates after subtracting baseline values as we have previously described (Baumgard et al., 2002).

Each animal’s respective response variable was analyzed using repeated measures with an autoregressive covariance structure for DMI, milk yield and composition, inflammatory biomarkers, and insulin, and spatial power law structure for RR, Tr, PTH, CBC and iSTAT parameters. A logarithmic transformation was performed for PTH data. The repeated effect was either day/time relative to LPS infusion. Each specific variable’s pre-infusion value served as a covariate. Effects of treatment, time (h or d), and treatment by time (h or d) interaction were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Furthermore, the effects of period (Pre-LPS vs. post-LPS infusion) were analyzed separately using the MIXED procedure of SAS. The model included treatment, time (pre vs post-LPS infusion), and their interaction as fixed effects and each individual cow as the random effect. Results are reported as least squares means and were considered different when $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

**Results**

Following LPS administration, circulating iCa decreased in both treatments, but CLY ameliorated the hypocalcemia (469% by 48 h AUC: -10.8 vs. -1.9 mmol/L × h; $P < 0.01$; Figure 6.1A). During P2, blood pH decreased (0.2%; $P = 0.03$; Table 6.2) post-LPS infusion but was not affected by treatments ($P = 0.19$; Table 6.2). Base excess, bicarbonate ($\text{HCO}_3$), and total carbon dioxide ($\text{TCO}_2$) tended to decrease (70, 9, and 8%, respectively; $P \leq 0.10$; Table 6.2) in CLY-
supplemented cows, while it remained constant in CON cows relative to baseline values. Regardless of treatment, administrating LPS decreased soluble oxygen ($sO_2$; 6.5%; $P = 0.05$; Table 6.2) but did not influence circulating sodium, potassium, partial carbon dioxide ($pCO_2$), and partial oxygen ($pO_2$; $P > 0.48$; Table 6.2). Mild hyperthermia ($+0.21^\circ C$ relative to baseline) was observed similarly in both treatments throughout P2 ($P < 0.01$; Figure 6.1B). Respiration rate increased 31 bpm between 0.5 to 1 h post-LPS infusion ($P < 0.01$; data not shown) compared to baseline values and HR increased 7 bpm post-LPS infusion relative to baseline ($P = 0.02$; data not shown). However, no effects of CLY supplementation were observed on Tr, RR or HR ($P > 0.17$).

Lipopolysaccharide administration markedly decreased DMI (60%; $P < 0.01$) similarly for both treatments on d 1; however, overall (d1-4) DMI tended to be less reduced (14 vs 30%; $P = 0.06$) in CLY supplemented cows when compared to controls (Figure 6.2A). As expected, LPS reduced milk yield (70% from 12-24 h; $P < 0.01$; Figure 6.2B) relative to baseline. Throughout P2, milk yield from CLY supplemented cows was increased (38%; $P = 0.03$) relative to CON cows (Figure 6.2B). Administrating LPS increased milk fat and protein content (30 and 22%; respectively; $P \leq 0.03$; Table 6.3) in CON cows, while both milk components did not change in CLY supplemented cows relative to P1. A treatment by time interaction was detected on milk lactose content, as it decreased in both treatments relative to baseline values ($P < 0.01$; Table 6.3), but the magnitude of decrease (P1 vs. P2) was less severe in the CLY supplemented compared to the CON cows. There tended to be a treatment by time interaction on milk urea nitrogen and milk somatic cell count, as both progressively increased in both treatments for 24 and 48 h, respectively ($P < 0.10$; Figure 6.2C and 6.2D, respectively) but peaked lower and decreased faster in the CLY supplemented compared to the controls.
Irrespective of treatment, infusing LPS increased (55%) circulating glucose from 0 to 2 h post-LPS administration ($P < 0.01$; Figure 6.3A) compared with baseline values. Circulating glucose decreased similarly in both treatments between 3 and 12 h post-LPS infusion (17%; $P = 0.40$; Figure 6.3A) compared to baseline. Hyperinsulinemia was observed in both treatments at 24 h post-LPS infusion (increased 3-fold relative to baseline; $P < 0.01$; Figure 6.3B). However, no effects of CLY supplementation on were observed on circulating insulin ($P = 0.35$; Figure 6.3B). Overall during P2, circulating PTH increased (24%; $P < 0.01$; P1vs.P2; Figure 6.4) post-LPS infusion relative to baseline levels; however, compared with CON, circulating PTH tended to be decreased in CLY-supplemented cows during P2 (12%; $P = 0.10$; Figure 6.4).

Overall during P2, circulating LBP and SAA increased following LPS administration (3- and 4-fold, respectively; Figure 6.5A and 6.5B) relative to baseline values, but were unaffected by dietary treatment ($P > 0.68$). Administrating LPS decreased (73% for the first 3 h) circulating white blood cells (WBC) counts, after which cell counts progressively increased with time (35% from 12-48 h, relative to baseline; $P < 0.01$; data not shown). Circulating neutrophils exhibited a similar pattern to WBC as they initially decreased for the first 3 h following LPS administration (94%; $P < 0.01$; Figure 6.5C). Likewise, circulating lymphocytes and monocytes initially decreased for the first 3 h following LPS administration (57 and 89%, respectively; $P < 0.01$) and gradually increased with time ($P < 0.01$; data not shown). However, no treatment differences were observed on circulating WBC, neutrophils, lymphocytes, and monocytes (Table 6.4).

Circulating eosinophils initially decreased (69% for 3 h; $P < 0.01$) post-LPS infusion and then gradually returned to baseline values with time (Figure 6.6A). Relative to CON cows, CLY-supplemented cows had increased circulating eosinophils during P2 (30%; $P < 0.01$) and this was especially apparent after the 3rd h (Figure 6.6A). Administrating LPS decreased circulating
platelets in both treatments relative to baseline, and CLY supplementation alleviated this response (17%; $P = 0.04$) and the differences primarily existed following the 10th h (Figure 6.6B). In contrast, LPS administration increased or tended to increase circulating hemoglobin and hematocrit (5 and 4%; $P < 0.05$ and $P = 0.10$, respectively; Table 6.4) relative to baseline but both metrics were unaffected by treatments. Relative to baseline, infusing LPS tended to decrease circulating red blood cells (4%; $P = 0.08$; Table 6.4), but it was not affected by treatment.

**Discussion**

Colostrogenesis requires copious Ca, a quantity equivalent to 7 to 10 times the total amount of Ca in blood (Horst et al., 2005) and it is estimated that subclinical hypocalcemia affects 25% of primiparous and 47-50% of multiparous periparturient dairy cows (Reinhardt et al., 2011; Oetzel, 2013). Calcium plays a key role in muscle and nerve function (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016). Thus, reduced blood Ca likely compromises skeletal muscle strength and gastrointestinal motility and is thought to be causal to poor transition cow performance (decreased DMI and milk yield, ketosis, retained placenta, etc., Oetzel and Miller, 2012; Oetzel, 2013). In addition, previous research reported that subclinical hypocalcemia decreases insulin concentrations, which in turn allows for enhanced adipose tissue mobilization and thus increased ketone production (Martinez et al., 2012, 2014). Furthermore, all cows (even seemingly healthy ones) experience some degree of inflammation during the transition period (Trevisi et al., 2012; Bradford et al., 2015) and immunoactivation causes hypocalcemia and reduced DMI (Waldron et al., 2003a,b; Kvidera et al., 2017; Horst et al., 2018) as intracellular Ca signaling plays an important role in immune cell activation (Lewis, 2001; Kimura et al., 2006). It has been shown that supplementing yeast products can improve feed intake in apparently healthy cows (Wohlt et al., 1991, 1998) and modulate the immune function in transition cows (Yuan et al., 2015a). Consequently, it is of interest to evaluate the effects of an oral supplement containing both Ca and
live yeast on circulating Ca, immune system metrics, energetic metabolism and production parameters in immune-challenged lactating dairy cows.

In the current study, administrating LPS markedly increased circulating acute phase proteins (including LBP and SAA), induced mild hyperthermia, increased respiration rate and caused leukopenia followed by leukocytosis; metrics indicating successful immunoactivation. Circulating iCa markedly decreased in both treatments following LPS infusion, but supplemental CLY ameliorated the hypocalcemia. The improved iCa status with the oral bolus corroborates previous experiments evaluating supplementing oral Ca in transition cows (Goff and Horst, 1993, 1994; Martinez et al., 2016a,b), but disagrees with another report evaluating blood Ca following oral Ca propionate supplementation (Stokes and Goff, 2001). Reasons for the differences in circulating Ca responses following oral supplementation is not clear but may be due to differences in Ca salts used between experiments. Regardless, reasons for decreasing iCa post-LPS infusion remain unclear but may be attributed to the fact that Ca is required for immune cell activation (Lewis, 2001; Kimura et al., 2006). Leukocyte activation initiates a signaling cascade leading to the release of Ca from the endoplasmic reticulum into the cytosol (Lewis, 2001). Consequently, increased cytosolic Ca levels triggers Ca influx from the extracellular space via Ca$^{2+}$ release-activated Ca$^{2+}$ channels (Lewis, 2001), which is likely partially responsible for systemic hypocalcemia following LPS infusion. Increased intracellular Ca plays a key role in cytokine production and cell proliferation (Kimura et al., 2006). Furthermore, another possible reason for developing LPS-induced hypocalcemia could be increased Ca accumulation in ascites and liver, which has been reported previously in pigs (Carlstedt et al., 2000). Additionally, LPS hypercortisolemia may contribute to hypocalcemia as reported by Waldron et al. (2003a), as
glucocorticoids act like calcitonin as highlighted by Hirsch et al. (1998). Further research is needed to identify the mechanism(s) and rationale for immune activation-induced hypocalcemia.

As expected, LPS infusion markedly decreased DMI and milk yield and this agrees with previous LPS experiments (Waldron et al., 2003b; Kvidera et al., 2017; Horst et al., 2018). Interestingly, DMI tended to increase in CLY administered cows post-LPS infusion compared with controls. Furthermore, we observed an improvement in milk yield in CLY compared to CON cows, which disagrees with previous Ca bolus studies in transition cows (Stokes and Goff, 2001; Oetzel and Miller, 2012; Martinez et al., 2016b). Unfortunately, reasons for improved production parameters (i.e. DMI, milk yield) are not clear, as we were unable to isolate the effects of Ca and yeast. However, yeast supplementation has previously been demonstrated to positively affect rumen pH, improve nutrient utilization, DMI, fermentation patterns, and lactation performance (Desnoyers et al., 2009; Ramsing et al., 2009; Zaworski et al., 2014; Broadway et al., 2015). However, other studies did not observe changes in DMI (Swartz et al., 1994; Robinson, 1997; Yuan et al., 2015b) or milk yield following yeast supplementation (Arambel and Kent, 1990; Yuan et al., 2015b). It is of interest to further isolate the effects of yeast and Ca on production performance in immune-challenged dairy cows.

Overall, infusing LPS increased milk fat, which is likely a dilution effect of severely decreased milk yield and this is consistent with our previous studies (Kvidera et al., 2017; Horst et al., 2018). Infusing LPS increased milk somatic cell count, which agrees with previous endotoxin challenge experiments (Shuster et al., 1991; Kvidera et al., 2017; Horst et al., 2018). Increased somatic cell count likely occurs because LPS impairs mammary epithelial barriers (Wellnitz et al., 2016). Interestingly, somatic cell count tended to decrease in CLY cows relative to CON cows, indicating that either Ca or live yeast (or both) may have an immunomodulatory
effect in the mammary gland. In a recent study, Yuan et al., (2015a) demonstrated that yeast supplementation decreased somatic cell linear score in transition cows. The exact mechanisms by which yeast influences immune function are not fully understood, but it could be related to the β-glucan originating from cell wall components of yeast which appears to beneficially effect the innate immune system through altered cytokine production and improved neutrophil function (as reviewed by Volman et al., 2008; Broadway et al., 2015).

Infusing LPS increased MUN, which is consistent with our recent i.v. LPS report (Horst et al., 2018). Reasons for increased MUN likely stems from skeletal muscle proteolysis to provide amino acids for acute phase protein production (Reed et al., 1994). Interestingly, MUN was reduced in CLY cows compared with controls, which suggests less skeletal muscle breakdown. Another explanation could be related to the role live yeast has on improving intestinal nutrient utilization. Reducing MUN can be suggestive of increased nitrogen utilization efficiency through enhanced ammonia incorporation into microbial protein (Erasmus et al., 1992).

The process of immunoactivation is energy-demanding and requires the application of homeorhetic strategies and metabolic alterations that shunt energy and amino acids towards immune cells and ultimately away from production purposes (Reeds et al., 1994; Kvidera et al., 2017). In the present study, administering LPS caused a biphasic response in circulating glucose, with initial hyperglycemia (2 h post-LPS infusion) followed by hypoglycemia and this agrees with previous reports (Waldron et al., 2003a; Kvidera et al., 2017; Horst et al., 2018). During the hyperglycemic phase, hepatic glucose output coupled with systemic insulin resistance likely exceeds leukocyte glucose utilization, however, with time endogenous glucose production and reduced tissue uptake is insufficient to maintain euglycemia (Lang and Dobrescu, 1991; Lang et al., 1993; Kvidera et al., 2017). Hyperinsulinemia is another well-known response following LPS.
administration, this increase is paradoxical considering the catabolic state and this agrees with previous studies (Waldron et al., 2003b; Kvidera et al., 2017; Horst et al., 2018). This increase could be attributed to the importance of insulin for glucose uptake by immune cells (as reviewed by Baumgard et al., 2016). Supplementing CLY did not affect circulating insulin, which is surprising as Ca has a vital role in pancreatic insulin secretion (Satin, 2000; Rorsman and Ashcroft, 2018).

The parathyroid gland plays a substantial role in Ca homeostasis by secreting PTH during hypocalcemia (Patt and Luckhardt, 1942; Goff et al., 1991; Horst et al., 2005). In response to hypocalcemia, PTH increases bone resorption mechanisms, acts on the kidney to decrease Ca excretion, and stimulates the synthesis of 1,25-dihydroxyvitamin D which increases intestinal Ca absorption. All of the aforementioned actions are aimed at recovering eucalcemia (Garabedian et al., 1972; Goff, 2018). Overall, infusing LPS increased circulating PTH, which agrees with previous LPS study in horses (Toribio et al., 2005). Not surprisingly, circulating PTH from CLY supplemented cows was suppressed relative to CON, presumably due to the increased circulating iCa in CLY supplemented cows.

Administrating LPS induced leukopenia during the first 3 h post-LPS infusion and circulating neutrophils, lymphocytes, and monocytes followed a similar temporal pattern. This aligns with previous LPS studies (Griel et al., 1975; Bieniek et al 1998; Horst et al., 2018). Leukopenia may indicate leukocyte infiltration into a site of presumed infection (Horst et al., 2018). Furthermore, administrating LPS decreased circulating platelets in both treatments relative to baseline values. In agreement with previous studies supplementing live yeast (Yuan et al., 2015b), circulating platelets increased following CLY supplementation. Moreover, Kim et al., (2011) showed that supplementing yeast to neonatal vaccine-challenged calves increased platelet
counts. Recent literature suggests that platelets have an essential role in modulating the inflammatory response and pathogen killing (Jenne et al., 2013). Similarly, we discovered that CLY-fed cows had increased circulating eosinophils relative to controls. This is inconsistent with Yuan et al., (2015b) that did not observe any differences in eosinophils concentrations following yeast supplementation in transition dairy cows. Reasons of increased eosinophil counts are less clear, but it could be related to the immunomodulatory effects live yeast supplementation. Further research is needed to determine the effects of live yeast on platelets and eosinophils of immune-challenged dairy cows.

**Conclusion**

Providing an oral Ca and live yeast supplement prior to and following LPS administration markedly ameliorated LPS-induced hypocalcemia, improved DMI and milk yield, increased circulating eosinophils and platelets, and decreased circulating PTH. Overall, utilizing an oral supplement may be a valuable management strategy to improve animal welfare and productivity during and following immunoactivation. Additionally, infusing i.v. LPS appears to be an effective technique to model hypocalcemia and to evaluate dietary strategies aimed at increasing circulating Ca in periparturient lactating dairy cows. However, further research is warranted to isolate the effects of Ca and live yeast on immune-challenged dairy cows.

**References**


Table 6.1. Ingredients and composition of diet\(^1\)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% of DM(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>33.6</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>13.1</td>
</tr>
<tr>
<td>Ground Corn</td>
<td>21.5</td>
</tr>
<tr>
<td>Mineral and Crude Protein Mix</td>
<td>11.9</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>6.1</td>
</tr>
<tr>
<td>Corn Gluten Feed</td>
<td>5.6</td>
</tr>
<tr>
<td>Whole Cottonseed</td>
<td>4.4</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.9</td>
</tr>
<tr>
<td>Soy Plus</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Chemical analysis

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>26.0</td>
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<tr>
<td>CP</td>
<td>18.2</td>
</tr>
<tr>
<td>ADF</td>
<td>19.3</td>
</tr>
<tr>
<td>NDF</td>
<td>29.5</td>
</tr>
<tr>
<td>NE(_L) Mcal/kg DM</td>
<td>1.67</td>
</tr>
</tbody>
</table>

\(^1\)Values represent an average of ration nutrient summary reports collected throughout the trial. Diet dry matter averaged 50.99%.

\(^2\)Average nutrient levels: 4.83% Fat, 0.95% Ca, 0.44% P, 0.36% Mg, 0.21% S, 1.26% K, 0.53% Na, 0.62% Cl, 78.37 ppm of Zn, 44.26 ppm of Mn, 3.51 ppm of Fe, 13.49 ppm of Cu, 0.75 ppm of Co, 0.32 ppm Se, 0.75 ppm of I, 14370.18 IU/kg of vitamin A, 1221.66 IU/kg of vitamin D, and 38.43 IU/kg of vitamin E.
Table 6.2. Effects of an oral supplement containing calcium and live yeast (CLY) on physiological parameters in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-LPS infusion</th>
<th>Post-LPS infusion</th>
<th>SEM</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>CLY</td>
<td>CON</td>
<td>CLY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base excess, mmol/L</td>
<td>2.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.93</td>
<td>0.72</td>
</tr>
<tr>
<td>HCO₃&lt;sup&gt;-&lt;/sup&gt;, mmol/L</td>
<td>27.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.87</td>
<td>0.73</td>
</tr>
<tr>
<td>K, mmol/L</td>
<td>3.9</td>
<td>3.9</td>
<td>4.0</td>
<td>3.9</td>
<td>0.1</td>
<td>0.85</td>
</tr>
<tr>
<td>Na, mmol/L</td>
<td>136.2</td>
<td>136.4</td>
<td>135.5</td>
<td>136.9</td>
<td>0.8</td>
<td>0.38</td>
</tr>
<tr>
<td>pCO₂, mmHg</td>
<td>43.26</td>
<td>45.00</td>
<td>42.93</td>
<td>43.73</td>
<td>1.47</td>
<td>0.49</td>
</tr>
<tr>
<td>pH</td>
<td>7.41</td>
<td>7.41</td>
<td>7.40</td>
<td>7.39</td>
<td>0.01</td>
<td>0.66</td>
</tr>
<tr>
<td>pO₂, mmHg</td>
<td>35.44</td>
<td>38.05</td>
<td>34.65</td>
<td>38.87</td>
<td>3.63</td>
<td>0.42</td>
</tr>
<tr>
<td>sO₂, %</td>
<td>66.94</td>
<td>70.00</td>
<td>63.11</td>
<td>64.96</td>
<td>2.66</td>
<td>0.45</td>
</tr>
<tr>
<td>TCO₂, mmol/L</td>
<td>28.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90</td>
<td>0.78</td>
</tr>
</tbody>
</table>

<sup>a</sup>bValues within row of each variable with differing superscripts indicate statistical difference.

<sup>1</sup>CON = control; CLY = calcium + live yeast bolus.

<sup>2</sup>Pre-LPS vs. Post-LPS infusion.
Table 6.3. Effects of an oral supplement containing calcium and live yeast (CLY) on milk composition in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-LPS infusion</th>
<th>Post-LPS infusion&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>CLY</td>
<td>CON</td>
<td>CLY</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.62&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.17&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>c</sup>Values within row of each variable with differing superscripts indicate statistical difference (P < 0.05).
<sup>1</sup>CON = control; CLY = calcium + live yeast bolus.
<sup>2</sup>Pre-LPS vs. Post-LPS infusion.
Table 6.4. Effects of an oral supplement containing calcium and live yeast (CLY) on complete blood cell count in lactating dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-LPS infusion</th>
<th>Post-LPS infusion</th>
<th>SEM</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells, × 10^3/µL</td>
<td>8.1</td>
<td>9.1</td>
<td>6.3</td>
<td>8.9</td>
<td>1.8</td>
<td>0.42</td>
</tr>
<tr>
<td>Red blood cells, × 10^6/µL</td>
<td>6.2</td>
<td>6.3</td>
<td>6.62</td>
<td>6.4</td>
<td>0.3</td>
<td>0.87</td>
</tr>
<tr>
<td>Monocytes, × 10^3/µL</td>
<td>0.17</td>
<td>0.23</td>
<td>0.22</td>
<td>0.23</td>
<td>0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>Lymphocytes, × 10^3/µL</td>
<td>4.1</td>
<td>5.7</td>
<td>2.9</td>
<td>5.0</td>
<td>1.4</td>
<td>0.34</td>
</tr>
<tr>
<td>Basophils, × 10^3/µL</td>
<td>0.05</td>
<td>0.07</td>
<td>0.04</td>
<td>0.06</td>
<td>0.02</td>
<td>0.40</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>10.9</td>
<td>10.5</td>
<td>11.6</td>
<td>10.8</td>
<td>0.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>30.3</td>
<td>29.6</td>
<td>32.1</td>
<td>30.0</td>
<td>1.0</td>
<td>0.28</td>
</tr>
</tbody>
</table>

1CON = control; CLY = calcium + live yeast bolus.
2Pre-LPS vs. Post-LPS infusion.
Figure 6.1. Effects of an oral supplement containing calcium and live yeast (CLY) on (A) circulating ionized calcium (iCa) and (B) rectal temperature (Tr) in lactating dairy cows. Results are expressed as least squares means ± SEM *denotes differences (P < 0.05) and † denotes tendencies (0.05 < P ≤ 0.10).
Figure 6.2. Effects of an oral supplement containing calcium and live yeast (CLY) on (A) DMI, (B) milk yield, (C) milk urea nitrogen (MUN), and (D) somatic cell counts in lactating dairy cows. Results are expressed as least squares means ± SEM. * denotes differences ($P < 0.05$) and † denotes tendencies ($0.05 < P \leq 0.10$).
Figure 6.3. Effects of an oral supplement containing calcium and live yeast (CLY) on circulating (A) glucose, and (B) insulin in lactating dairy cows. Results are expressed as least squares means ± SEM.
**Figure 6.4.** Effects of an oral supplement containing calcium and live yeast (CLY) on circulating parathyroid hormone (PTH) in lactating dairy cows. Results are expressed as least squares means ± SEM.
Figure 6.5. Effects of an oral supplement containing calcium and live yeast (CLY) on circulating (A) LPS binding protein (LBP), (B) serum amyloid A (SAA), and (C) neutrophils in lactating dairy cows. Results are expressed as least squares means ± SEM.
Figure 6.6. Effects of an oral supplement containing calcium and live yeast (CLY) on circulating (A) eosinophils, and (B) platelets in lactating dairy cows. Results are expressed as least squares means ± SEM.
CHAPTER 7. SUMMARY AND CONCLUSIONS

Environmental hyperthermia has many detrimental effects on dairy cow productivity, including decreased milk yield and composition, growth, and reproduction. These negative effects impose a substantial economic burden on animal agriculture. In fact, the estimated annual economic losses due to HS exceeds $1.5 billion in the U.S. dairy industry alone (Key and Sneeringer, 2014). Thus, understanding the mechanisms of how heat stress (HS) compromises animal productivity is an important prerequisite to developing and implementing mitigation strategies aimed at improving animal welfare and agriculture economics.

Accurately studying the biological reasons by which HS undermines animal performance requires environmental chambers. However, most institutions lack those necessary facilities or resources. Hence, it is imperative to develop and validate alternative and cost-effective models to study HS, thus allowing a platform to assess nutritional HS mitigation strategies using these models in lactating dairy cows. Many of the negative consequences of HS on animal performance appear to be mediated by intestine-derived lipopolysaccharide (LPS) and thus HS biology can be modeled by infusing LPS. Administrating LPS markedly reduces feed intake, milk yield, and reduces blood Ca in several species (Elsasser et al., 1996; Carlstedt et al., 2000; Toribio et al., 2005; Shinozuka et al., 2018) including dairy cows (Waldron et al., 2003, Kvidera et al., 2017; Horst et al., 2018). Overall, the experiments outlined in this dissertation centered on developing, evaluating, and validating of an alternative model to study HS and on identifying nutritional management strategies to ameliorate negative consequences of both HS and immune-challenged lactating dairy cows.

In Chapter 2, we evaluated the efficacy of using an electric heat blanket (EHB) as an alternative and cost-effective method to study HS and determined whether EHB-induced
hyperthermia affects physiological and production parameters similar to natural HS. This was the first proof of concept study examining this model. Results obtained from this study indicated the EHB increased body temperature indices (i.e., rectal temperature and respiration rate), reduced DMI and milk yield, and altered milk composition similar to natural and climate-controlled HS studies.

Thermal stress markedly decreases DMI as a strategy to reduce metabolic heat production and it is a conserved response across species and phyla (Collin et al., 2001; West, 2003; Baumgard and Rhoads, 2013). It had been assumed that reduced feed intake is the sole reason for decreased growth and milk yield (Fuquay, 1981; West, 2003). However, by employing a pair-feeding design, previous climate-controlled HS experiments showed that reduced feed intake only accounts for about 50% of the decreased milk yield during HS (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). The remaining decrease in production is mediated by the direct effects of HS on postabsorptive carbohydrate, lipid, and protein metabolism. However, to differentiate between direct and indirect effects (via reduced feed intake) of EHB-induced hyperthermia, a pair-feeding approach was required to eliminate the confounding effects of dissimilar nutrient intake. Thus, objectives of Chapter 3 were to determine nutritional plane’s contribution to altered metabolism and productivity in the EHB model. By using the pair-feeding design, we clearly illustrated that a lowered nutritional plane explains only approximately 50% of the decreased milk yield in heat-stressed cows, with the remaining portion due to changes in postabsorptive metabolism. In addition, the EHB blunted adipose tissue mobilization and increased plasma biomarkers of muscle catabolism. Results from this study validated the use of the EHB as a model to simulate HS and to evaluate nutritional HS mitigation strategies.
In Chapter 4, we investigated the effects of a feed additive including electrolytes, osmolytes, and energetic compounds (EOEC) on body temperature indices in heat-stressed lactating Holstein cows. Previous reports have shown that supplementing electrolytes in the form of NaHCO$_3$ and KCl benefited heat-stressed dairy cows by increasing milk yield, regulating acid-base balance, and lowering body temperature (Coppock et al., 1982; Tucker et al., 1988; West et al., 1992). In addition, dietary betaine, a naturally occurring osmoregulator, has been shown to improve productivity in heat-stressed cows (Zhang et al., 2014; Dunshea et al., 2019) and other farm animals (Mendoza et al., 2017; Shakeri et al., 2018). We hypothesized that providing dietary EOEC could potentially reduce body temperature indices and increase lactation performance in dairy cows exposed to HS. Utilizing the EHB, we observed an increase in all body temperature variables and a marked decrease in both DMI and milk yield. Feeding EOEC appeared to increase heat dissipation by increasing skin temperature. Furthermore, dietary EOEC increased circulating glucose and insulin, decreased NEFA, and reduced the strain on pCO$_2$. In conclusion, feeding EOEC altered the metabolic and blood gas profile in heat-stressed dairy cows and these are ostensible reflections of HS amelioration.

The deleterious effects of HS on animal performance are likely mediated by reduced intestinal barrier integrity (Hall et al., 2001; Lambert et al., 2002; Baumgard and Rhoads, 2013; Koch et al., 2019). During HS, animals redistribute blood to the periphery to increase radiant heat loss and consequently the gastrointestinal tract vasoconstricts to maintain blood pressure. Reduced blood flow to the splanchnic tissues causes hypoxia, enterocyte damage and impaired epithelial integrity; the subsequent translocation of luminal contents (i.e., lipopolysaccharide and other toxins) into the portal and systemic circulation stimulate immune and inflammatory responses (Cronje, 2005; Lambert, 2009; Baumgard and Rhoads, 2013). The immune system is energetically
expensive. When activated it rearranges the hierarchy of nutrient partitioning away from milk synthesis (Kvidera et al., 2017). Therefore, dietary interventions that target intestinal toxin/pathogen binding, the maintenance intestinal barrier function or immune system modulation may improve productivity.

Recently, feeding *Saccharomyces cerevisiae* fermentation product (SCFP) has received considerable attention due to its role at modulating the immune system (Broadway et al., 2015). Feeding SCFP has been shown to improve DMI, milk yield, feed efficiency, and rumen pH (Erasmus et al., 2005; Desnoyers et al., 2009; Poppy et al., 2012; Zaworski et al., 2014; Acharya et al., 2017). Thus, in Chapter 5, we examined the effects of a SCFP on body temperature indices, metabolism, and APPR in heat-stressed lactating dairy cows. We hypothesized that supplementing a SCFP would alleviate or at least partially ameliorate the negative consequences of HS on key immune and stress biomarkers in heat-stressed dairy cows. Results obtained from this experiment demonstrated that the EHB increased all body temperature variables, induced inflammation (i.e., increased circulating LBP and SAA), and reduced production parameters (i.e., DMI and milk yield). Additionally, we demonstrated that feeding SCFP could be beneficial at reducing circulating cortisol and the APPR, and increased key leukocytes during stressful conditions. Overall, feeding SCFP altered the metabolic and immune-profile in heat-stressed dairy cows and these changes are presumably reflective of a reduced “stressed” state.

Subclinical hypocalcemia is a common metabolic disorder affecting periparturient dairy cows and is typically observed within the first days of lactation (Reinhardt et al., 2011; Oetzel and Miller, 2012). Colostrogenesis requires copious Ca and it is estimated that subclinical hypocalcemia affects 25% of primiparous and 47-50% of multiparous periparturient dairy cows.
Consequently, orally bolusing Ca following parturition has become a common management tactic (Oetzel and Miller, 2012; Oetzel, 2013).

Although the magnitude and extent differ, it is likely that all periparturient dairy cows (even seemingly healthy ones) experience some degree of inflammation (Trevisi et al., 2012; Bradford et al., 2015) and immunoactivation decreases circulating Ca in a variety of species (Carlstedt et al., 2000; Shinozuka et al., 2018). Immunoactivation can be experimentally modeled by administering LPS and this markedly reduces blood Ca in several species including dairy cows (Waldron et al., 2003, Kvidera et al., 2017; Horst et al., 2018) as intracellular Ca signaling plays an important role in immune cell activation (Lewis, 2001; Kimura et al., 2006). Previous research demonstrated that supplementing yeast products could improve feed intake in healthy cows (Wohlt et al., 1991, 1998) and modulate the immune function in transition cows (Yuan et al., 2015). Therefore, objectives of Chapter 6 were to evaluate the effects of an oral supplement containing both Ca and live yeast on circulating Ca, immune system metrics, energetic metabolism, and production parameters in immune-challenged lactating dairy cows. Results presented in this study showed that providing an oral Ca and live yeast supplement prior to and following LPS administration markedly ameliorated LPS-induced hypocalcemia, improved DMI and milk yield, and decreased circulating PTH. Collectively, utilizing an oral Ca and live yeast supplement could be a valuable management strategy to improve animal welfare and productivity during and following immunoactivation.

In conclusion, employing the EHB model provides an excellent new platform for discovery research and for evaluating pragmatic HS mitigation strategies. In addition, infusing i.v. LPS appears to be an effective technique to model hypocalcemia and to evaluate dietary strategies aimed at increasing circulating Ca in periparturient lactating dairy cows. However, further research
is warranted to isolate the effects of Ca and live yeast on immune-challenged dairy cows. Overall, HS and LPS infusion have similar deleterious effects on animal health and productivity. Figure 7.1 summarizes the events that causes reduced production and health during HS in dairy cows.

**Future Research**

1. Evaluating more nutritional mitigation strategies by utilizing the EHB model.

2. Evaluating the intestinal epithelial integrity by feeding Cr-EDTA using the EHB model.

3. Further investigating the dietary EOEC on blood gas parameters.

4. Evaluating the dietary EOEC with higher dose level on body temperature indices and production parameters in lactating dairy cows.

5. Evaluating the dietary betaine alone on body temperature indices in lactating dairy cows, since it was difficult to isolate between the effects of electrolytes and betaine in dairy cows in the current dissertation.

6. Investigating the effects of dietary SCFP on production parameters with larger sample size.

7. Further research is needed to understand the mechanisms behind how dietary SCFP blunted circulating cortisol.

8. Evaluating the effects of an oral supplement containing soluble Ca only on immune-challenged dairy cows.

Figure 7.1. Dairy cow responses to heat stress.
References


