Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows


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ABSTRACT

Heat stress (HT) and photoperiod affect milk production and immune status of dairy cows. The objective was to evaluate the effects of HT abatement prepartum under controlled photoperiod on hepatic metabolic gene expression and cellular immune function of periparturient Holstein cows (n = 21). Cows were dried off 46 d before expected calving date and assigned to treatments by mature equivalent milk production. The treatments were 1) HT and 2) cooling (CL), both imposed during a photoperiod of 14L:10D. Rectal temperature was measured twice daily, whereas respiration rate was measured 3 times/wk at 1500 h during the entire dry period. After calving, cows were housed in a freestall barn with cooling, and milk yield was recorded daily up to 140 d in milk. Liver samples were taken at dry off, −20, 2, and 20 d relative to calving by biopsy. Under a similar schedule, neutrophil function was determined in blood of cows on HT (n = 12) and CL (n = 9). Blood samples were taken on −46, −32, −18, 0, 14, 28, and 42 d relative to calving for measurement of metabolites and were collected twice daily from −7 to 2 d relative to calving for prolactin (PRL) analysis. The HT cows had greater concentrations of PRL at 0 d relative to calving (150 vs. 93; SEM = 11 ng/mL) and had higher afternoon rectal temperatures (39.4 vs. 39.0; SEM = 0.04°C) and elevated respiration rates (78 vs. 56; SEM = 2 breaths/min) during the prepartum period compared with CL cows. Relative to HT cows, CL cows had greater hepatic expression of PRL-R, SOCS-3, and CAV-1 mRNA. Neutrophil oxidative burst was greater in CL cows relative to HT cows at 2 d (61 vs. 42; SEM = 6%) and at 20 d (62 vs. 49; SEM = 5%) relative to calving, and phagocytosis was greater in CL cows at 20 d (47 vs. 33; SEM = 4%) relative to calving compared with HT cows. Humoral response, as measured by IgG secretion against ovalbumin challenge, was greater for CL cows at −32 d (0.44 vs. 0.33; SEM = 0.05 OD) and −21 d (0.60 vs. 0.50 ± 0.04 OD) relative to calving compared with HT cows. These results suggest that HT abatement during the dry period improved innate and acquired immune status as measured by neutrophil function and immunoglobulin secretion against ovalbumin challenge, and altered hepatic gene expression related to PRL signaling in the periparturient period or subsequent lactation.

Key words: dairy cattle, heat stress, immune status, photoperiod

INTRODUCTION

Heat stress (HT) dramatically depresses milk production during lactation (Collier et al., 2006), and cooling cows during the dry period increases milk production in the subsequent lactation (Avendaño-Reyes et al., 2006; Adin et al., 2009; do Amaral et al., 2009). Abatement of HT during the dry period appears to improve the transition into lactation possibly through up-regulation of hepatic mRNA expression of genes involved in lipid metabolism (ACADVL), prolactin (PRL) signaling (SOCS-2), and mammary gland involution (IGFBP-5) compared with cows under HT (do Amaral et al., 2009). Nevertheless, data on the effects of HT on hepatic gene expression related to lipid metabolism (ACADVL, PPAR-A, and CPT1-A) and the prolactin signaling pathway (PRL-R, SOCS-1, SOCS-2, SOCS-3, CISH, and CAV-1) during the transition period are scarce.

Our previous results indicated that the elevated circulating PRL was associated with increased expression of the SOCS gene in the mammary gland and lymphocytes (Wall et al., 2005; do Amaral et al., 2010). Studies in rodents implicate hepatic elevation of SOCS 1 and SOCS 3 in the insulin resistance and hepatic steatosis observed in obesity (Ueki et al., 2005). In cattle, HT increased insulin secretion and decreased insulin sensitivity, hallmarks of insulin resistance (Itoh et al., 1998), and HT increased circulating concentrations of PRL (Johnson and Vanjonack, 1976). In addition to poten-
Heat stress impairs acquired immune function as measured by lymphocyte proliferation (Kamwanja et al., 1994; Lacetera et al., 2005, 2006). The mechanism whereby HT affects immune status may be mediated through changes in PRL-signaling pathways. For example, the greater concentrations of PRL in plasma of cows exposed to HT were associated with reduced lymphocyte proliferation compared with cooled cows (do Amaral et al., 2010). In addition, lymphocytes isolated from heat-stressed cows had lower mRNA expression of PRL-R, greater SOCS-1 mRNA, and tended to have greater SOCS-3 mRNA compared with cooled cows. Yet, little is known about the effects of HT during the entire dry period under a controlled photoperiod on neutrophil function and humoral immune response of periparturient dairy cows. We hypothesized that increases in PRL associated with HT during the dry period would depress PRL-signaling pathways in the liver and immune system and result in lower milk yield. Thus, the objectives were to evaluate the effects of HT abatement under controlled photoperiod during the entire dry period on hepatic metabolic gene expression, immune status of cows, and their subsequent lactational performance.

**MATERIALS AND METHODS**

**Animals, Treatments, and Sampling**

The experiment was conducted at the University of Florida Dairy Research Unit (Hague, FL) from May 2008 until November 2008. All experimental animals were handled according to the guidelines approved by the University of Florida IFAS Animal Research Committee. Cows were dried off 46 d before expected calving according to the standard protocol of the University of Florida Dairy Research Unit, by intramammary infusion of each quarter with antibiotic (Quartermaster, Pfizer Animal Health, Kalamazoo, MI) and cessation of milking. The treatments were 1) HT (n = 12) and 2) cooling (CL; n = 9) imposed during the dry period only. Mature-equivalent milk production was similar between treatment groups. Parities were similar between treatments (1.7 ± 1.1 lactations for CL and 1.7 ± 0.9 lactations for HT). Fans and sprinklers were used as the cooling system and both turned on automatically whenever the ambient temperature exceeded 21.0°C. Treatments started at dry-off (from May 14, 2008, until July 30, 2008) and ended at calving (June 21, 2008, until September 11, 2008). Both treatments were exposed to a similar photoperiod of 14L:10D provided using metal halide lights at approximately 250 lx at eye level of the cow. The lights were on from 0600 until 2000 h for both treatments. During the entire dry period, rectal temperature was measured twice daily at 0700 and 1500 h, whereas respiration rate was measured 3 times/wk at 1500 h. Respiration rates were measured by counting flank movements for 60 s. Cows were housed in a sand-bedded, freestall barn equipped with fans, sprinklers, and Calan gates (American Calan Inc., Northwood, NH). Only the CL cows were cooled with fans and sprinklers. An adjacent sand-bedded pen with shade and water was used for calving when signs of parturition were detected. After calving, all cows were housed in the same sand-bedded, freestall barn equipped with fans and sprinklers automatically turned on whenever ambient temperature exceeded 21°C. Barn temperature and relative humidity were measured using the Hobo Pro series Temp probe (Onset Computer Corp., Pocasset, MA). Temperature and humidity index (THI) was calculated using the following equation: 

\[
THI = [0.8 \times \text{ambient temperature (°C)}] + \left[\left(\frac{\% \text{ relative humidity}}{100}\right) \times (\text{ambient temperature} - 14.4)\right] + 46.4
\]

(Buffington et al., 1981). Calan gates were used to measure individual feed intakes. Milk yield was recorded daily up to 140 DIM. Dry cows were fed a TMR (Table 1) formulated according to NRC requirements (NRC, 2001) once daily at 0900 h, whereas milking cows were fed TMR twice daily at 0800 and 1200 h to allow 5 to 10% feed refusals daily. Daily DMI was measured from dry off to 42 d relative to calving. The DM content of corn silage was adjusted weekly to maintain the formulated forage-to-concentrate ratio in the ration. Cows were milked twice daily at 0800 and 2000 h. Cows were weighed and scored for body condition (Edmonson et al., 1989) at −46, −32, −18, 0, 14, 28, and 42 d relative to calving before feeding during the dry period, and after the 0800 h milking and before feeding during lactation.

**Sample Collection**

Milk samples were collected weekly from 2 consecutive milkings using bronopol B-14 as a preservative and analyzed for fat, true protein, and SCC by Southeast Milk Laboratory (Belleview, FL) using a Bentley 2000 NIR analyzer (Bentley Instruments, Chaska, MN). Final concentrations were calculated after adjusting for
milk production and concentrations measured during the morning and evening milkings. Blood (10 mL) was collected at 0700 h at −46, −32, −18, 0, 14, 28, and 42 d relative to calving from coccygeal vessels into sodium heparinized tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for glucose, NEFA, BHBA, and BUN analyses. In addition, blood samples were taken twice daily (0700 and 1500 h) from −7 until 2 d relative to calving for detecting the PRL surge around parturition. Upon collection, samples were immediately placed on ice until centrifugation at 2,619 × g at 5°C for 30 min. Plasma was separated and stored at −20°C for subsequent metabolite and PRL analyses.

Representative feed samples of corn silage, bermudagrass hay, alfalfa hay, and concentrate mixes were collected weekly and composited monthly. Composites were ground through a 1-mm Wiley mill screen (A. H. Thomas, Philadelphia, PA) and analyzed for mineral and fat composition, NDF, ADF, and CP (Dairy One, Ithaca, NY).

On −46, −20, 2, and 20 d relative to calving, liver samples (approximately 400 mg) were collected via biopsy, rinsed with sterile saline, snap frozen in liquid N, and stored at −80°C until analyzed for mRNA transcript abundance.

### Neutrophil Function

Blood (6 mL) was collected in Vacutainer tubes containing acid citrate dextrose. Samples were collected from coccygeal vessels at −46, −20, 2, and 20 d relative to calving at 1000 h. Neutrophil abundance and function were assessed within 3 h of blood collection. Hematology analysis was done on all blood samples using an Advia 120 Hematology System (Siemens Healthcare Diagnostics, Deerfield, IL). To measure phagocytosis...
and oxidative burst of neutrophils, whole blood (100 μL) was pipetted into each of 3 tubes. Then, 10 μL of 50 μM dihydrorhodamine 123 (Sigma-Aldrich, St. Louis, MO) was added to all tubes. Tubes were vortexed and incubated at 37°C for 10 min with constant rotation using the Clay Adams nutator (BD, San Jose, CA). Ten microliters of a 20 μg/mL solution of phorbol 12-myristate, 13-acetate (Sigma-Aldrich) was added to the second tube only. An *Escherichia coli* bacterial suspension (10⁶ cells/mL) labeled with propidium iodide (Sigma-Aldrich) was added to the third tube to establish a bacteria-to-neutrophil ratio of 40:1. Tubes were vortexed and incubated at 37°C for 30 min with constant rotation using the Clay Adams nutator. Then, all tubes were removed and placed immediately on ice to stop phagocytosis and oxidative burst activity. Tubes were processed in a Q-Prep Epics immunology workstation (Beckman Coulter, Fullerton, CA) on the 35-s cycle. Cold distilled water (500 μL) and 0.4% trypan blue (10 μL) were added to each tube, and tubes were vortexed and incubated at 37°C for 10 min with constant rotation using the Clay Adams nutator. Then, all tubes were removed and placed immediately on ice to stop phagocytosis and oxidative burst activity. Tubes were processed in a Q-Prep Epics immunology workstation (Beckman Coulter, Fullerton, CA) on the 35-s cycle. Cold distilled water (500 μL) and 0.4% trypan blue (10 μL) were added to each tube, and tubes were vortexed and kept on ice; 10,000 cells were read on the Facsort flow cytometer (BD Biosciences). The amount of bacteria that each neutrophil phagocytized was measured by median fluorescence intensity using the flow cytometer (Givan, 2001).

**Ovalbumin Challenge**

All cows were injected s.c. with 1 mg of ovalbumin (Sigma-Aldrich) diluted in Quil A adjuvant (0.5 mg of Quil A/mL of PBS; Accurate Chemical & Scientific Corp., Westbury, NY) at −46, −32, and 0 d relative to calving. Blood samples for serum analysis of IgG were collected at −46, −32, −21, 0, 7, 14, 21, 28, 35, and 42 d relative to calving for cows. All samples were taken in Vacutainer tubes (Becton Dickinson) containing no anticoagulant. Serum concentration of anti-ovalbumin IgG was measured by ELISA as described by Mallard et al. (1997). Briefly, flat-bottomed, 96-well polystyrene plates (Immulon 2, Dynex Tech., Chantilly, VA) were coated with a solution of ovalbumin dissolved in carbonate-bicarbonate coating buffer (1.4 mg of ovalbumin/mL of carbonate-bicarbonate buffer). Plates were incubated at 4°C for 24 h, and then washed with PBS and 0.05% Tween-20 solution (pH = 7.4). Plates were blocked with a PBS-3% Tween-20-BSA (Sigma-Aldrich) solution and incubated at room temperature for 1 h. Plates were washed, and diluted sera samples and control sera (1/50 and 1/200) were added in duplicate using a quadrant system (Wright, 1987). Positive and negative control sera to anti-ovalbumin IgG were obtained from a pool of sera of known high (21 d after the third injection of ovalbumin) and low (pre-ovalbumin injection) concentrations, respectively. All samples from the same cows were analyzed in the same plate, and plates contained a balanced number of animals from each treatment. Plates were incubated at room temperature for 2 h and washed with the described previously buffer solution. Subsequently, alkaline phosphatase-conjugated rabbit anti-bovine IgG whole molecule (Sigma-Aldrich) was dissolved in wash buffer, added to the plates and incubated for 2 h at room temperature. After incubation, plates were washed 4 × and substrate solution (p-nitrophenyl phosphate disodium; Sigma-Aldrich) was added, and plates were incubated at room temperature for 30 min. Plates were read on an automatic ELISA plate reader (MRX Revelation, Dynex Technologies Inc.) and the optical density was recorded at 405 nm and the reference at 650 nm. Prior to the initiation of experimental serum sample analyses, 3 plates containing positive controls for anti-ovalbumin IgG were analyzed at 1/50 and 1/200 dilutions to calculate an initial mean and SD. Further positive controls obtained from plates containing experimental samples were subsequently added to calculate the total mean and SD. Plates with positive control means above or below 1.5 SD of total accumulated positive controls were repeated. Interassay CV for the positive control samples was 9%. Plates were also repeated when the CV of positive control samples exceeded 20% within a plate. A correction factor was calculated for each plate by dividing the total mean from the accumulated positive control results to the total mean of the positive controls from each plate. Experimental sample results were obtained from the product of the sum of the average of each duplicated sample dilution by the correction factor of each plate.

**Analytical Procedures**

Plasma concentrations of PRL were measured on samples taken twice daily from −7 to 2 d relative to calving (do Amaral et al., 2009). Plasma concentrations of NEFA (NEFA-HR(2) kit; Wako Fine Chemical Industries USA Inc., Dallas, TX) and BHBA (Autokit 3-HB, Wako Fine Chemical Industries USA Inc.) were determined on samples taken once every 2 wk from dry off until 42 d postpartum. Under a similar schedule, a Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to determine concentrations of plasma BUN (a modification of Coulombe and Favreau, 1963; and Marsh et al., 1965) and plasma glucose (a modification of Gochman and Schmitz, 1972).

**Reverse Transcription-PCR**

Total RNA was extracted from liver using the RNeasy Midi Kit (Qiagen Inc., Valencia, CA) and the recom-
mended method for isolation of RNA from fatty animal tissues (Qiagen Inc.). Quality of RNA was assessed using the Agilent 2100 Bioanalyzer with RNA 6000 Nano LabChip Kits (Agilent Technologies, Palo Alto, CA), and RNA concentration was determined using an ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE). Relative gene expression for prolactin receptor (PRL-R), suppressors of cytokine signaling (SOCS-1, SOCS-2, and SOCS-3), cytokine inducible SH2-containing protein (CISH), caveolin-1 (CAV-1), IGFBP-5, heat shock protein A-5 (HSPA-5), ACADVL, peroxisome proliferator-activated receptor (PPAR-A), and carnitine palmitoyltransferase 1-A (CPT1-A) was assessed by real-time quantitative reverse transcription (RT)-PCR using an iCycler iQ or MyiQ Realtime PCR Detection System (Bio-Rad Laboratories, Hercules, CA). Primer sequences, reverse transcription, and PCR assay methods were conducted as described previously (do Amaral et al., 2009, 2010). Primer sequences and annealing temperatures not described previously were as follows: (CAV-1, 60°C) 5′-CACTTCTCTCCTCCCT-CAC-3′ (sense), 5′-CCGGTATGGATAGTTTG-3′ (antisense); (CPT1-A, 59.2°C) 5′-ACATTCTCG-GTAGGCAC-3′ (sense) and 5′-TGATGATGTCG-GTCATTTGCT-3′ (antisense); and (PPAR-A, 59.2°C), 5′-GTGCGCTTTCAGTTGGATGT-3′ (sense) and 5′-CGGTTCCGAATCTTTCTAGG-3′ (antisense).

The relative abundance of each gene of interest was calculated using qBasePlus Version 1.4 Software (Biogazelle, Ghent, Belgium). In this analysis, each gene of interest was normalized to 2 reference genes (M value = 0.349), including hydroxymethylbilane synthase (HMBS) and ribosomal protein S9 (RPS9), using the geometric mean of the corrected reference gene relative quantities. Normalization accounted for differences in amplification efficiency (E) of each gene assay (range of 1.872 to 2.098) determined from the slope of a 5-point standard curve of 10^2 to 10^7 molecules of purified PCR target analyzed concurrently with experimental samples, where E = 10^{−1/slope} − 1. Primer sequences and assay conditions for HMBS and RPS9 are described in do Amaral et al. (2010).

**Statistical Analysis**

Repeated-measures data (DMI, milk yield, BW, BCS, PRL, glucose, NEFA, BHBA, BUN, neutrophil phagocytosis and oxidative burst, IgG, and mRNA expression) were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). For hepatic gene expression, the first sample taken at −46 d relative to calving (i.e., dry off) was considered as the baseline and all data were expressed relative to the baseline value. The model included fixed effects of treatment, time, and treatment × time interaction, and the random effect of cow.

Data were tested to determine the structure of best fit, namely first-order autoregressive, heterogeneous AR(1), compound symmetry, or heterogeneous CS, as indicated by a lower Schwartz Bayesian information criterion value (Littell et al., 1996). Standard error of the mean is reported.

**RESULTS**

**Rectal Temperature, Barn Temperature, and Relative Humidity**

As expected, rectal temperatures during the morning (38.84 vs. 38.70°C; SEM = 0.04°C; P = 0.01) and afternoon (39.41 vs. 39.01°C; SEM = 0.04°C; P < 0.001) periods were greater for HT cows than for CL cows. In addition, HT cows had greater respiration rates (78 vs. 56 breaths/min; SEM = 2 breaths/min; P < 0.001) despite similar barn temperature and relative humidity (P ≥ 0.15; Figure 1) and THI (77.6 and 77.1, respectively, for HT and CL).

**Dry Period Length and Calf Weight**

Cows exposed to HT tended to have a dry period 7 d shorter (39 vs. 46 d; P = 0.08) than that of CL cows. Calves from HT cows were 5 kg lighter than calves of CL cows (39.5 vs. 44.5 kg; SE = 1.7 kg; P = 0.05).

**Milk Production, Milk Composition, and DMI**

Cows exposed to CL tended to produce more milk compared with HT cows (P = 0.09; Table 2). The difference in milk production tended to be greater when adjusted for fat concentration (4.9 kg/d of 3.5% FCM; Table 2) and was due to differences in FCM yield during wk 11 to 20 of lactation (Figure 2). Cows exposed to CL had greater concentrations (P = 0.01) and yield (P = 0.02) of milk fat relative to HT cows (Table 2). No difference was observed between treatments in the percentage (P = 0.41) or yield (P = 0.67) of protein in milk (Table 2). Feed efficiency during the postpartum period did not differ (P = 0.78) between treatments (Table 2). Average BW (P = 0.79) and BCS (P = 0.81) from dry off until 42 d relative to calving did not differ between treatments (Table 2). Average DMI from dry off until 42 d did not differ between treatments (P = 0.17; Table 2). Cows exposed to HT during the dry period had similar DMI (as % of BW; Figure 3) to CL cows from dry off until −14 d relative to calving. As parturition approached, HT
cows had lower \((P = 0.03; \text{Figure 3})\) DMI (as % of BW) at calving but similar intakes at 14, 28, and 42 d compared with CL cows.

**Plasma PRL and Metabolites**

Cows exposed to HT had greater concentrations of PRL at 0 d relative to calving compared with CL cows (150 vs. 93 ng/mL; SEM = 11 ng/mL; \(P = 0.02; \text{Figure 4})\). Plasma concentrations of glucose (70 vs. 69 mg/dL; SEM = 1 mg/dL; \(P = 0.65\)), BUN (8.9 vs. 9.6 mg/dL; \(P = 0.28\)), NEFA (421 vs. 354 μEq/L; SEM = 41 μEq/L; \(P = 0.23\)), and BHBA (6.9 vs. 5.8 mg/dL; SEM = 0.7 mg/dL; \(P = 0.29\)) from dry off until 42 d did not differ between treatments. 

**Hepatic Gene Expression**

Mean hepatic mRNA expression of \(PRL-R\) (157 vs. 87% of baseline for CL and HT cows; SEM = 27; \(P = 0.05; \text{Table 3}\)), \(SOCS-3\) (377 vs. 153% of baseline for CL and HT cows; SEM = 120; \(P = 0.001; \text{Table 3}\)), and \(CAV-1\) (115 vs. 88% of baseline for CL and HT cows; SEM = 16; \(P = 0.03; \text{Table 3}\)) were greater for CL cows compared with HT cows. The average mRNA expression of \(SOCS-1\) (177 vs. 124% of baseline for CL and HT cows; SEM = 54; \(P = 0.31\)), \(SOCS-2\) (194 vs. 114% of baseline for CL and HT cows; SEM = 39; \(P = 0.85; \text{Table 3}\)), and \(IGFBP-5\) (120 vs. 136% of baseline for CL and HT cows; SEM = 27; \(P = 0.81\)) did not differ between treatments.

Mean hepatic mRNA expression across treatments of \(HSPA-5\) (93, 222, and 165% of baseline for \(-20\) and 20 d relative to calving; SEM = 81; \(P = 0.05\)), \(ACADVL\) (87, 130, and 97% of baseline for \(-20, 2\) and 20 d relative to calving; SEM = 18; \(P = 0.02\)), and \(CPT1-A\) (83, 103, and 84% of baseline for \(-20, 2\) and 20 d relative to calving; SEM = 11; \(P = 0.04\)) was greater at 2 d relative to calving compared with that at \(-20\) and 20 d relative to calving. In contrast, mean

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**Table 2.** Milk yield, 3.5% FCM yield, 3.5% FPCM yield, ECM yield, milk components, and feed efficiency for cows exposed to heat stress (n = 9) or cooling (n = 7) during a targeted 46-d dry period

<table>
<thead>
<tr>
<th>Variable</th>
<th>Heat stress</th>
<th>Cooling</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, kg/d</td>
<td>32.2</td>
<td>34.5</td>
<td>1.5</td>
<td>0.09</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>30.8</td>
<td>35.5</td>
<td>1.8</td>
<td>0.07</td>
</tr>
<tr>
<td>3.5% FPCM, kg/d</td>
<td>30.4</td>
<td>34.4</td>
<td>3.4</td>
<td>0.11</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>30.7</td>
<td>34.7</td>
<td>1.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.3</td>
<td>3.8</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.0</td>
<td>2.8</td>
<td>0.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Fat yield, kg/d</td>
<td>1.0</td>
<td>1.3</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Protein yield, kg/d</td>
<td>0.9</td>
<td>1.0</td>
<td>0.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Feed efficiency(^{4})</td>
<td>2.1</td>
<td>2.0</td>
<td>0.2</td>
<td>0.78</td>
</tr>
<tr>
<td>BW, kg</td>
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<td>659</td>
<td>23</td>
<td>0.79</td>
</tr>
<tr>
<td>BCS</td>
<td>3.3</td>
<td>3.3</td>
<td>1.0</td>
<td>0.81</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>8.4</td>
<td>9.8</td>
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<td>0.17</td>
</tr>
<tr>
<td>Prepartum</td>
<td>14.5</td>
<td>15.8</td>
<td>1.0</td>
<td>0.40</td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
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</table>

\(^{1}\)3.5% FCM = \((0.4324 \times \text{milk yield}) + (16.216 \times \text{milk fat yield})\); 3.5% FCM was calculated until 140 DIM.

\(^{2}\)3.5% fat- and protein-corrected milk = \((12.82 \times \text{kg of fat}) + (7.1 \times \text{kg of protein}) + (0.325 \times \text{kg of milk})\); 3.5% FPCM was calculated until 140 DIM.

\(^{3}\)ECM = \((0.327 \times \text{kg of milk}) + (12.95 \times \text{kg of fat}) + (7.20 \times \text{kg of protein})\) (Tyrrell and Reid, 1965); ECM was calculated until 140 DIM.

\(^{4}\)Feed efficiency = kg of 3.5% FCM/kg of DMI. Feed efficiency was calculated daily from calving until 42 d relative to calving.
hepatic mRNA expression of \( \text{PPAR-A} \) (96, 90, and 76\% of baseline for \(-20, 2 \) and \( 20 \) d relative to calving; \( \text{SEM} = 6; \text{P} = 0.02 \)) was greater at \(-20 \) and \( 2 \) d compared with that at \( 20 \) d relative to calving.

Neutrophil Phagocytosis and Oxidative Burst

Neutrophil function, as measured by phagocytosis and oxidative burst, did not differ between treatments during the dry period when treatments were imposed. However, as the suppression of the immune system developed around parturition, cows exposed to CL had greater oxidative burst at \( 2 \) and \( 20 \) d and greater phagocytosis at \( 20 \) d compared with HT cows (Figure 5).

IgG Response to Ovalbumin Challenge

The acquired immune system was affected by HT (Figure 6). As expected, immunoglobulin levels against ovalbumin were low and did not differ between treatments at dry off (\(-46 \) d). After the first ovalbumin challenge at dry off, CL cows produced more IgG at \(-32 \) d relative to calving compared with HT cows (\( P = 0.05 \)). In addition, the response at \(-21 \) d to the second ovalbumin challenge at \(-32 \) d relative to calving was greater for CL cows relative to HT cows (\( P = 0.05 \)). However, after the third challenge at \( 0 \) d relative to calving, no differences were detected between treatments, but treatments were imposed only during the dry period.

DISCUSSION

In the present experiment, several factors indicated that HT abatement was achieved. First, as expected, rectal temperature was reduced in CL cows compared with HT cows, confirming the effectiveness of the evaporative cooling system despite similar barn temperature and relative humidity. Second, similar to other

<table>
<thead>
<tr>
<th>Gene</th>
<th>Heat stress</th>
<th></th>
<th>Cooling</th>
<th></th>
<th></th>
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<td></td>
<td>(-20 ) d</td>
<td>( 2 ) d</td>
<td>( 20 ) d</td>
<td>(-20 ) d</td>
<td>( 2 ) d</td>
<td>( 20 ) d</td>
</tr>
<tr>
<td>PRL-R</td>
<td>87</td>
<td>88</td>
<td>86</td>
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<tr>
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<td>134</td>
<td>99</td>
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<td>117</td>
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<td>SOCS-3</td>
<td>105</td>
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<td>125</td>
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<td>SOCS-2</td>
<td>133</td>
<td>93</td>
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<td>CISH</td>
<td>136</td>
<td>124</td>
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<sup>1</sup>Trt = heat stress or cooling; Day = day of sampling.

reports, CL cows had lower respiration rates relative to HT cows (Adin et al., 2009), and HT cows had lower calf BW (Wolfenson et al., 1988; Adin et al., 2009; do Amaral et al., 2009) and greater PRL concentrations in plasma (Collier et al., 1982; do Amaral et al., 2009, 2010). Thus, this model was appropriate to investigate the effects of cooling on hepatic gene expression and immunological measures of periparturient dairy cows.

Our data support that of Adin et al. (2009) who cooled cows in a closed tie-stall barn for the entire dry period in one experiment where rectal temperatures in cooled and noncooled cows were \(<39.0^\circ\text{C}\) and rectal temperature means differed by only 0.2°C with no difference in milk yields. It is important to emphasize that do Amaral et al. (2009) measured milk yield until 210 DIM, and most of the differences between treatments appeared after 70 DIM. In the present experiment, the total heat load was greater for both groups as evidenced by rectal temperatures \(>39.0^\circ\text{C}\) in both HT and CL cows, which likely explains the milk yield response compared with that observed by do Amaral et al. (2009) [4.9 kg/d difference in 3.5% FCM between treatments (35.5 vs. 30.8 kg/d of 3.5% FCM for CL and HT cows, respectively)]. In addition, CL cows had greater milk fat concentration and yield in the subsequent lactation relative to HT cows, supporting do Amaral et al. (2009), who showed that dry cows exposed to cooling (fans and sprinklers) for the entire dry period had greater milk fat concentration and yield compared with heat-stressed cows.

Average DMI from dry off until 42 d did not differ between treatments. When adjusted for BW, HT cows had lower DMI (% of BW) at 0 d relative to calving. It seems that rectal temperatures above 39.0°C impaired DMI in both treatments, but the effect was more pronounced in HT cows because other stress, perhaps related to parturition, was coupled with HT. In contrast, do Amaral et al. (2009) reported that CL cows had reduced DMI (as % of BW) at 0 and 14 DIM, likely caused by the lesser heat load as indicated by lower rectal temperatures of cows and lower barn temperature (28°C) compared with that in the present study (30°C).

Concentrations of glucose, BUN, NEFA, and BHBA did not differ between treatments from dry off until 42 DIM. Because NEFA and BHBA are metabolically related to responses to intake and no differences were detected in DMI prepartum, the lack of response was expected. In contrast, do Amaral et al. (2009) reported that CL cows had greater NEFA and BHBA relative to HT cows postpartum, and those metabolite shifts were linked to intake depression at 0 and 14 d postpartum. Others have reported no effect of HT on NEFA concentrations in plasma (Collier et al., 1982; Urdaz et al., 2006).

**Figure 4.** Effect of cooling (CL, ■; n = 9) or heat stress (HT, ○; n = 12) during a 46-d dry period on prolactin (PRL) concentrations in plasma. Cows exposed to HT had greater PRL concentrations at 0 d relative to calving compared with CL cows. \(^*P = 0.02\); SEM = 11 ng/mL. Cows were housed in the same barn equipped with fans and sprinklers after parturition.

**Figure 5.** Effect of cooling (CL, ■; n = 9) or heat stress (HT, ○; n = 12) during a 46-d dry period on the neutrophil oxidative burst (a) and phagocytosis (b) before and after calving. Cows exposed to CL had greater neutrophil oxidative burst at 2 and 20 d \((P = 0.04; \text{SEM } = 3\%\) and greater neutrophil phagocytosis at 20 d \((P = 0.05; \text{SEM } = 5\%)\) compared with HT. \(^*P < 0.05\). Cows were housed in the same barn equipped with fans and sprinklers after parturition.
In terms of the effects of HT and CL on hepatic gene expression, to our knowledge, this is the first study to report an effect of HT abatement during the entire dry period on hepatic mRNA expression of PRL-R of periparturient dairy cows. The previous observation that HT increases PRL (Johnson and Vanjonack, 1976; Collier et al., 1982), combined with the positive relationship of PRL-R and milk yield observed under variable photoperiod treatments during the dry period (Auchtung et al., 2005; Velasco et al., 2008), led us to focus on PRL signaling as a potential mechanism to explain the negative aspects of heat stress during the dry period. Animals exposed to CL had greater immunoglobulin production at −32 and −21 d relative to calving (P = 0.05; SEM = 0.04 OD) compared with HT. *P ≤ 0.05. Cows were housed in the same barn equipped with fans and sprinklers after parturition. OD = optical density.

Figure 6. Effect of cooling (CL, ■; n = 9) or heat stress (HT, ○; n = 12) during a 46-d dry period on the immunoglobulin production against ovalbumin challenge. Ovalbumin challenge was done before blood sampling at −46, −32, and 0 d relative to calving. Cows exposed to CL had greater immunoglobulin production at −32 and −21 d relative to calving (P = 0.05; SEM = 0.04 OD) compared with HT. *P ≤ 0.05. Cows were housed in the same barn equipped with fans and sprinklers after parturition. OD = optical density.

The transition period is the most challenging time for the immune status of dairy cows and is generally associated with immunosuppression (Goff and Horst, 1997). Neutrophils are the first line of defense against bacterial infection and their phagocytic and oxidative burst activities provide valuable information on the functional activity of these immune cells (Kampen et al., 2004). Management strategies to improve neutrophil function during the transition period will likely reduce the incidence of disease and improve transition into lactation. To our knowledge, this is the first study to evaluate the effect of HT abatement during the entire dry period under controlled photoperiod on neutrophil function of periparturient dairy cows. During the dry period when HT abatement was imposed, neutrophil phagocytosis and oxidative burst did not differ between treatments. However, after the immunosuppressive state of parturition, neutrophils isolated from HT cows
had lower oxidative burst (at 2 and 20 d relative to calving) and lower phagocytic activity at 20 d relative to calving compared with CL cows. Thus, it appears that a carryover effect of HT abatement during the dry period existed into lactation.

Another part of the immune system is the humoral response, which involves production of antibodies against an antigen. In the present study, cows exposed to HT during the dry period had lower humoral responses at −32 and −21 d relative to calving compared with CL cows. This is evidence that HT impairs antibody production of periparturient dairy cows. We suggest that this response is linked to disturbed PRL signaling through PRL-R. In summary, HT abatement during the dry period improves the transition into lactation, and the measured changes in expression of genes involved in the PRL signaling pathway are consistent with mechanisms that may support milk yield. In addition, improvements of the innate and acquired immune systems were observed in CL cows. Heat stress abatement during the dry period is a promising management strategy to improve transition into lactation; however, studies on the effect of HT abatement during the entire dry period on incidence of periparturient diseases are needed to confirm the practical application of these findings.

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