Pre- and postnatal heat stress abatement affects dairy calf thermoregulation and performance

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ABSTRACT

Prenatal heat stress during late gestation exerts long-term effects on growth and productivity of the dairy calf. Further, direct exposure to heat stress during the preweaning period impairs calf thermoregulation and performance. We examined the effects of heat stress abatement during the prenatal period, postnatal period, or both on calf performance. We hypothesized that calves exposed to pre- and postnatal heat stress abatement would perform most optimally in terms of thermoregulation, growth, and health responses when compared with calves that are heat-stressed at any time in the pre- or postnatal periods. Holstein calves born to heat-stressed (HT) or cooled (CL) dams during late gestation (44 ± 5 d; prenatal HT or CL) were exposed to heat stress or cooling postnatally for 56 d (postnatal HT or CL), resulting in 4 treatments: HT-HT, HT-CL, CL-HT, and CL-CL; $n = 12$ /treatment. Calves were administered 4 L of pooled colostrum and after 2 d of age allotted 10 L/d milk replacer and up to 3 kg/d concentrate in automatic feeder group pens ($n = 6$ /pen). Postnatal cooling was achieved by 2 fans (average wind speed 2 m/s). Thermoregulatory responses (respiration rate and heart rate; rectal, body, and skin temperature), feed intake, growth parameters including average daily gain and medication events were recorded, and blood samples were collected weekly. Thermoregulatory responses were lower in postnatal CL calves compared with postnatal HT. In the afternoon, HT-HT calves had the highest respiration rate and rectal temperature, HT-CL calves had the lowest respiration rate, and CL-HT calves had the lowest heart rate compared with the other treatment groups. Prenatal CL calves weighed more at birth and weaning with a tendency for greater average daily gain compared with prenatal HT calves,

whereas postnatal CL calves had increased milk replacer and concentrate intake and a tendency for reduced fever, infection, and total medication events relative to postnatal HT. Prenatal HT calves were esophageal tube fed more often than prenatal CL. Blood hematocrit and 24-h serum IgG concentration were greater in prenatal CL calves relative to prenatal HT. Prenatal heat stress abatement improves weight gain, hematocrit, and immunoglobulin transfer, whereas postnatal heat stress abatement modulates thermoregulatory responses, feed intake, and calf health. This study is the first to characterize the combined effects of pre- and postnatal heat stress or active cooling on the dairy calf.

Key words: in utero, hyperthermia, group housing, productivity

INTRODUCTION

High environmental temperature and humidity are detrimental to livestock productivity and economy. Heat stress in both lactating and nonlactating dairy cows could cost the US dairy industry almost \$2 billion annually (St-Pierre et al., 2003; Ferreira et al., 2016). While extensive literature has highlighted the negative consequences of heat stress on lactating and dry dairy cow productivity, health, and reproduction (Bernabucci et al., 2010; Tao and Dahl, 2013), less information is available characterizing its effect on the calf. Pre-weaned calves are often not considered for heat stress abatement due to their larger surface/mass ratio and smaller heat loads as a functionally nonruminant and nonlactating animal (Collier et al., 1982; Broucek et al., 2009; Dado-Senn et al., 2019). However, the consequences of heat stress on youngstock both before and after birth warrants further investigation as early-life experiences can have long-term consequences on future productivity (van der Linden et al., 2009; Soberon et al., 2012; Geiger et al., 2016).

The dry period coincides with late gestation when the fetus grows at the fastest rate and accumulates

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~60% of its birth weight (Bauman and Currie, 1980). Calves exposed to prenatal heat stress weigh less at birth, weaning, and through 1 yr of age, due in part to a shorter gestation length and retarded fetal growth (Tao et al., 2012; Monteiro et al., 2014, 2016a). Prenatal heat-stressed calves have compromised passive immune transfer, decreased total plasma protein and hematocrit, and impaired cellular immune functions (Tao et al., 2012). These calves are less likely to survive to their first lactation compared with prenatal cooled calves. If prenatal heat-stressed heifers do reach lactation, they produce approximately 2 to 5 kg/d less milk across the entire first lactation compared with heifers exposed to prenatal cooling, partially attributed to perturbations in mammary gland microstructure and cellular turnover (Monteiro et al., 2016b; Skibieli et al., 2018a).

Postnatal heat stress also affects the preweaned dairy calf. Although estimates for the calf environmental thermoneutral zone vary, the reported upper critical temperatures of the calf thermoneutral zone range from 26 to 32°C (Neuwirth et al., 1979; Spain and Spiers, 1996) and the physiological range of calf body temperatures (**BT**) lies between 38.1 to 39.2°C with diurnal variation (Moran, 2002; Piccione et al., 2003). High ambient temperature and humidity lead to elevated calf rectal temperatures, skin temperatures, and respiration rates, especially when placed in calf hutch environments without access to supplemental shade (Coleman et al., 1996; Spain and Spiers, 1996; Carter et al., 2014). Calves exposed to heat stress have a reduced ADG and weaning weight compared with calves provided active heat stress abatement or born in winter months (Hill et al., 2011; López et al., 2018). This is in part due to a decline in feed and nutrient intake and an increase in water intake (Beede and Collier, 1986; Broucek et al., 2008). In extreme cases, hyperthermia can lead to mortality (Stull et al., 2008). Most studies in dairy calves have focused on improving calf hutch material and design or shade supplementation (Spain and Spiers, 1996; Carter et al., 2014; Kovacs et al., 2018), and only one study has evaluated the effect of providing fans to combat heat stress (Hill et al., 2011).

Heat stress is not a short-term event, especially in the southern and southeastern United States, which can experience over 100 d of heat stress annually (Ferreira et al., 2016). Consequently, a significant number of calves might experience heat stress in both prenatal and postnatal environments. Many studies of postnatal calf heat stress do not take into account the prenatal exposure of the calf, so it is difficult to discern where performance responses originate (Broucek et al., 2008; Hill et al., 2011; López et al., 2018). This is relevant as recent literature demonstrates that prenatal stressors can profoundly affect fetal growth and organ develop-

ment (i.e., microstructure and function) with long-term consequences on health and production (Berghänel et al., 2017; Van Eetvelde and Opsomer, 2017; Reynolds et al., 2019). Heifers exposed to prenatal heat stress have reduced rectal temperatures and sweating rates and increased skin temperatures when exposed to heat stress as mature lactating cows compared with prenatally cooled counterparts (Ahmed et al., 2017). In the porcine model, offspring exposed to pre- and postnatal heat stress exhibited increased core BT, compromised thermotolerance, reduced whole-body water accretion, and reduced glucose concentrations, but thermoregulatory mechanisms differ greatly between the porcine and bovine models (Hillman, 2009; Johnson et al., 2013; Johnson and Baumgard, 2019). Currently no literature is available examining the combined effects of pre- and postnatal heat stress or heat stress abatement on the preweaned dairy calf.

The objective of this study was to determine whether prenatal heat stress or heat stress abatement would affect short-term thermoregulatory and productive responses in similar or opposing postnatal environments. We hypothesized that calves exposed to prenatal heat stress abatement and then exposed to a postnatally similar environment (i.e., postnatal heat stress abatement) would perform most optimally in terms of thermoregulation, growth, and health responses when compared with calves heat-stressed at any time in the pre- or postnatal periods.

MATERIALS AND METHODS

Experimental Design and Treatments

Prenatal Treatment. The experiment was conducted between May and November 2018 at the University of Florida Dairy Unit in Hague, Florida. All procedures were approved by the University of Florida Institutional Animal Care and Use Committee. Multiparous pregnant Holstein cows were randomly selected based on parity and mature-equivalent milk production and enrolled into a dry period treatment for 44 ± 5 d. Cows were housed in 2 separate sand-bedded freestall pens and either heat-stressed (**HT**; $n = 30$, access only to the shade of the barn) or cooled (**CL**; $n = 30$, access to the shade of the barn, plus fans and water soakers). Heat stress was assumed to be achieved at a temperature-humidity index (**THI**) ≥ 68 (Zimbelman et al., 2009). Fans ran continuously, and water soakers were activated for 1.5 min in 5-min intervals when ambient temperature exceeded 22.2°C. Ambient temperature and relative humidity of the barn were recorded every 15 min with Hobo Pro Series Temp Probes (Onset Computer Corp., Pocasset, MA) from June to October

2018. Vaginal temperatures were recorded once daily and respiration rate were recorded thrice weekly at 1300 h from the dam during the dry period. The THI of the barn was calculated according to Dikmen et al. (2008) for the duration of the prenatal treatment. Forty-eight bull and heifer calves born to these dams were used in this study. These calves experienced either heat stress (prenatal HT, $n = 24$) or cooling (prenatal CL, $n = 24$) conditions through the intrauterine environment during late gestation (44 ± 5 d). Twin calves weighing less than 35 kg or calves that died before 2 d of age were not enrolled in postnatal treatment and replaced with a new enrollee from the same prenatal treatment.

Postnatal Treatment. At 2 d of age (2.4 ± 0.2 d), calves were assigned to group automatic feeder sand-bedded pens under the shade of an open-sided barn with mobile curtains (postnatal HT, $n = 24$) or to group pens under shade plus fans (postnatal CL, $n = 24$) until complete weaning (56 d). One fan was positioned at the calf level (VMK42–2-O 107 cm barrel fan, Schaefer, Eau Claire, WI) and an auxiliary fan was posted above the pen (~ 1.8 m above the ground, 36DF 91 cm mounted direct flow circulation fan; Schaefer). All fans were placed on temperature-controlled timers that turned on when ambient temperature was greater than 20°C . The experimental design was a 2×2 factorial (prenatal and postnatal, 2 levels each) resulting in 4 treatments ($n = 12$ per treatment): (1) CL prenatally and postnatally (**CL-CL**), (2) CL prenatally and heat-stressed postnatally (**CL-HT**), (3) HT prenatally and CL postnatally (**HT-CL**), and (4) HT prenatally and postnatally (**HT-HT**). The arrangement of pens (8 pens, $n = 6$ calves per pen; 4.6×9.1 m, $6.97 \text{ m}^2/\text{calf}$) is depicted in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2019-17926>). Prenatal treatments were equally distributed per pen (i.e., one postnatal HT pen had $n = 3$ CL-HT and $n = 3$ HT-HT calves). Sex distribution per treatment were as follows: CL-CL = 7 bulls, 5 heifers; CL-HT = 8 bulls, 4 heifers; HT-CL = 6 bulls, 6 heifers; HT-HT = 5 bulls, 7 heifers.

Postnatal Calf Nutritional Management and Housing

Within 2 h of birth, calves were separated from their dams, weighed, and had their navels dipped prophylactically in 2% iodine. Pooled colostrum from the farm was fed to calves as 4 L in one feeding. Colostrum samples ($n = 4$ –6 per prenatal treatment) were collected for immunoglobulin analysis. From 0 to 2 d of age, calves were housed in individual pens for monitoring and fed 4 L/meal pasteurized milk at 0600 and 1600 h. After 2 d of age, calves were assigned to sand-bedded group automatic feeder pens and postnatal treatment

was initiated. Calves were allotted 10 L/d of milk replacer (**MR**, UF Special 28/15 Bova DFB Medicated, Southeast Milk, Okeechobee, FL) via automatic feeder (DeLaval CF1000X, DeLaval, Tumba, Sweden). Mixing rate for MR was 150 g/L. Calves were trained thrice daily to consume milk via automatic feeder until calf accessed the feeder without assistance. All calves were required to consume at least 4.5 L/d of MR upon veterinary recommendation. If a calf had not consumed at least 1 L of MR by 0900 h or 4.5 L of MR by 1900 h, 1.5 to 4.5 L of MR was administered either by bottle (if the calf was receptive) or by esophageal tube feeding (if the calf refused to suckle) in 1 to 3 feedings between 0900 and 1900 h. Bottle and tube feeding events and amounts fed were recorded. Milk weaning began at 42 d as a 10-d stepdown program as a 2-L reduction every 2 d from 10 to 0 L. Throughout the preweaning period, starter grain concentrate (Ampli-Calf Starter 20 Warm Weather, Purina Animal Nutrition LLC, Shoreview, MN) was provided in a step-up manner, starting at 0.2 kg/d with approximately 0.1 kg increase every other day starting at 21 d, ending at 3 kg/d. Water was provided ad libitum, and water intake was not recorded.

The automatic feeder recorded daily MR and concentrate intake (Förster Technik Software), which were averaged weekly for analysis. Samples of starter concentrate and MR were collected once each week and then pooled every 2 wk for chemical analysis for the duration of the experiment ($n = 5$ pooled starter concentrate, MR; Cumberland Valley Analytical Services Inc., Waynesboro, PA). The chemical compositions of the MR and concentrate can be found in Table 1. Vaccination protocol for the calves followed the standard operating procedures of the University of Florida Dairy Unit.

Environment and Thermoregulation Measurements

Environmental measurements were collected daily for each pen in the morning (**AM**, 0700 to 0800 h), afternoon (**AF**, 1300 to 1400 h), and evening (**PM**, 1900 to 2000 h) and averaged from 3 different locations within each pen (Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-17926>). Wind speed (m/s) and air flow (m^3/s) were recorded using an anemometer (MS6252A Digital Anemometer System, Proster, Miller Place, NY) placed at calf level and rotated until the maximum wind speed was located. Pen particle matter (fine and regular: **PM**_{2.5} and **PM**₁₀, respectively), number of particles (per/L), air quality index, formaldehyde, total volatile organic carbons, and environmental temperature, humidity, and THI were recorded using an air quality meter (LKC-1000S+ Air Quality Monitor, Tem-

Table 1. Nutrient and chemical composition of milk replacer (MR, n = 5 pooled samples) and starter concentrate (n = 5 pooled samples) fed to calves in group automatic feeder pens (n = 6/pen) for 56 d¹

Item	MR ²		Concentrate ³	
	Mean	SD	Mean	SD
Moisture (%)	3.70	0.16	12.66	0.32
DM (%)	96.30	0.16	87.34	0.32
Nutrient content (DM basis)				
CP (%)	27.70	0.39	22.70	0.24
Fat (%)	19.34	0.38	2.75	0.17
ADF (%)	0.64	0.50	15.70	0.66
NDFa ⁴ (%)	1.42	0.53	28.22	1.08
NFC (%)	—	—	38.19	0.91
Ash (%)	6.88	0.26	8.19	0.26
ME ⁵ (Mcal/kg)	4.82	0.02	2.75	0.02
Ca (%)	0.76	0.01	1.11	0.03
P (%)	0.69	0.01	0.62	0.01
Mg (%)	0.13	0.01	0.32	0.00
K (%)	1.57	0.01	1.66	0.02
Na (%)	0.49	0.01	0.58	0.02
Fe (mg/kg)	30.80	8.81	240.40	7.86
Mn (mg/kg)	8.80	1.30	102.40	4.28
Zn (mg/kg)	37.40	2.51	109.00	8.86
Cu (mg/kg)	4.00	1.00	21.00	1.22

¹Calves were allotted 10 L of MR and up to 3 kg of grain daily, and mixing rate for MR was 150 g/L. Weaning began at 42 d in a 10-d stepdown weaning program.

²UF Special 28/15 Bova DFB Medicated, Southeast Milk, Okeechobee, FL.

³Ampli-Calf Starter 20 Warm Weather; Purina Animal Nutrition LLC, Shoreview, MN.

⁴NDFa = ash-free NDF.

⁵Calculated according to NRC (2001).

top, Milpitas, CA). Additionally, ambient temperature and relative humidity data were recorded every 15 min using Hobo Pro Series Temp Probes.

Body temperature was recorded using a calibrated temperature probe (i-button DS1922-F5#, accuracy $\pm 0.065^{\circ}\text{C}$; Maxim, Irving, TX) attached to the tail with waterproof adhesive tape and veterinary wrap above the rectum. The I-buttons were replaced weekly. Thermoregulatory responses were obtained 3 times (AM, 0700 h; AF 1300 h; and PM, 1900 h) daily from d 2 to 56. Measurements included respiration rate (counting the number of flank movements in 30 s and multiplying by 2), rectal temperature (**RT**, using a rectal digital thermometer; SharpTemp V Large Animal Digital Thermometer, PBS Animal Health, Massillon, OH), skin temperature (**ST**, using an infrared thermometer (Raytek MiniTemp MT6 Infrared Thermometer, Instrumart, South Burlington, VT) placed 15 cm away from a 5-cm² shaved rump site, and heart rate (**HR**, using a stethoscope to count the number of resting heart beats in 30 s and multiplying by 2).

Growth and Health Responses

Growth and health scores were recorded weekly at d 0, 7, 14, 21, 28, 35, 42, 49, and 56 between 0800 and 0900 h. Body weight was recorded using a calibrated

electronic scale. Total and weekly ADG was calculated from BW and number of days between weighing. Hip height, hip width, and withers height were obtained with a measuring stick (Sullivan Supply, Dunlap, IA). Body condition score was visually assessed by the same individual each time. Health scores were recorded based on the University of Wisconsin–Madison School of Veterinary Medicine Calf Health Score rubric (McGuirk, 2008). Briefly, calves were scored for the presence and severity (0 = best to 3 = worst) for categories including eye, ear, nose, cough, coat, and dehydration. The same individual assigned scores each time. Medication (i.e., probiotic, nonsteroidal anti-inflammatory drug, or antibiotic) events for scours, fever (temperature $\geq 40^{\circ}\text{C}$), infection (horn bud, ear tag, or navel), sepsis, and respiratory disorders between 2 to 56 d of age were recorded.

Blood Sampling and Analyses

Blood samples were collected via jugular venipuncture at 24 ± 1 h of age (after colostrum feeding) and at d 7, 14, 21, 28, 35, 42, 49, and 56 between 0900 and 1000 h. Blood was obtained using serum and sodium heparin vacutainer blood collection tubes (BD catalog #366430 and 366480, Franklin Lakes, NJ). After collection, sodium heparin tubes were placed on ice and

serum tubes clotted for 20 min at room temperature. Within 1 h, all tubes were centrifuged at $3,000 \times g$ for 20 min to harvest plasma and serum fractions. Blood hematocrit was analyzed from whole blood, and total protein was quantified from fresh plasma using a digital Brix Refractometer (MA871, Milwaukee Instruments, Rocky Mount, NC). For hematocrit, capillary tubes (Fisher Scientific #22-362-566, Pittsburgh, PA) filled with whole blood were centrifuged at $2,240 \times g$ at 25°C for 3 min in a microhematocrit centrifuge and read using a circular hematocrit reader. Remaining serum and plasma samples were aliquoted and stored at -20°C until further analysis. Plasma concentrations of fatty acids, BHB, and glucose were determined by colorimetric, enzymatic, and GOD-PAP methods, respectively (kit #FA115, RB1007, and GL3981, Randox Laboratories Ltd., Crumlin, UK) by Randox Rx Daytona. The inter-assay coefficients of variation for fatty acid, BHB, and glucose analysis were 11.4, 5.7, and 8.7%, respectively.

Colostrum and Serum IgG

A subset of pooled colostrum samples ($n = 4$ to 6 per treatment) were collected before administration at 1 ± 0.2 h after birth, and serum was collected at 24 ± 1 h after birth ($n = 10$ /prenatal treatment). Total IgG concentration of colostrum and serum were measured by radial immunodiffusion assay (Triple J Farms, Bellingham, WA). Samples were diluted in 0.9% saline (serum 1:2, colostrum 1:5) to fall within the standard curve. Standard and samples were pipetted into the bovine anti-bovine antibody plate and incubated at room temperature in the dark for 25 h. The precipitin ring diameter was measured using a $7\times$ scale loupe (no. 1975, Peak Optics, GWJ Co., La Quinta, CA). Based on the standard diameter measurements, IgG concentration of each sample was calculated. The interassay coefficient of variation of the IgG assay was 0.8%.

Statistical Analysis

Environmental and air quality data were analyzed by pen using generalized linear mixed models using the MIXED procedure of SAS (v. 9.4, SAS Institute Inc., Cary, NC) with the main effects of pen (1–8), day (repeated measure), and their interaction. Birth weight and height, colostrum and d 1 serum IgG, and d 1 plasma total protein were analyzed using the MIXED procedure with the main effect of prenatal treatment (HT or CL), and calf ID nested within treatment as a random effect. Count data including feeding events, health scores, and health events were analyzed by PROC GLIMMIX. All remaining data were analyzed

as a 2×2 factorial using the MIXED procedure. Main effects included prenatal treatment (prenatal CL and HT), postnatal treatment (postnatal CL and HT), and their interactions (CL-CL, CL-HT, HT-CL, and HT-HT), time (hour, day, or week depending on the variable, as repeated measure), and their interactions. For all calf models, sex was included as a covariate. Calf ID nested within prenatal and postnatal treatment was used as a random effect, and the first-order autoregressive covariance structure (AR-1) was used as the covariate structure. Residuals were tested for normality and data were transformed if needed. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$. Data are presented as least squares means $\pm$ standard error, unless otherwise stated.

RESULTS

Prenatal Heat Stress Abatement

Vaginal temperatures and respiration rate recorded from the dry pregnant dams were decreased in the CL relative to HT dams (vaginal temperatures: 38.9 vs. $39.1 \pm 0.1^{\circ}\text{C}$; respiration rate: 54.6 vs. 65.3 ± 1.2 bpm for CL vs. HT, respectively, $P < 0.01$). The THI of the CL and HT pens remained above 78 for the duration of the prenatal treatment exposure, thus dry period heat stress was achieved. Gestation length and dry period length did not differ between treatment groups (275 vs. 276 ± 1 d, $P = 0.38$; 43 vs. 45 ± 5 d, $P = 0.16$; for CL vs. HT, respectively).

Postnatal Pen Air Quality, THI, and Wind Speed

Postnatal CL pens equipped with fans had reduced particle pollution in the AM but not AF and PM, as evidenced by reduced fine and regular particle matter ($\text{PM}_{2.5}$, PM_{10}), lower number of overall particles, and lower air quality index when compared with HT pens with no fans (Table 2, AM: $P \leq 0.07$). Independent of treatment, 67% of air gas measurements (formaldehyde and total volatile organic carbons) fell below detectable limits and thus were not statistically analyzed.

Temperature-humidity index during AM, AF, and PM is reported in Table 2, and daily and hourly THI is shown in Figure 1. Environmental THI did not differ among treatment pens in the AM, AF, or PM (Table 2). Average daily THI remained above 68 for the duration of the experiment (Figure 1A). Hourly THI remained above 68 and lingered around 80 between 1000 to 1800 h, peaking at 83 at 1400 h (Figure 1B). Wind speed and air flow were higher during AM, AF, and PM in CL pens equipped with fans compared with HT pens with

no fans (Table 2, $P < 0.01$). Average wind speed in postnatal CL and HT pens was 2 versus 0.15 m/s, and average air flow was 1.98 versus 0.14 m³/s, respectively.

Postnatal Thermoregulatory Responses

Postnatal CL calves maintained a BT that was 0.5 to 1°C lower than calves exposed to postnatal HT throughout the day (Figure 1B, $P < 0.001$). Maximum BT for all calves was observed between 1500 to 1600 h and was 0.42°C greater in postnatal HT calves (37.90 vs. 38.32°C for postnatal CL vs. HT respectively, Figure 1B), while minimum BT occurred at 0700 h and was 0.96°C greater in postnatal HT calves (36.40 vs.

37.36°C, respectively). Thus, even though both treatments had lower BT in the morning, the drop from maximum to minimum BT was more pronounced in the postnatal CL compared with postnatal HT calves (1.50 vs. 0.96°C, respectively). Prenatal treatment ($P = 0.32$) and treatment interaction ($P = 0.56$) had no effect on calf BT. Week was significant ($P < 0.001$) whereby BT was lowest in wk 2 and 8 and highest in wk 3 to 6. Time (hour), prenatal treatment $\times$ time, and postnatal treatment $\times$ time were significant ($P < 0.001$) whereby BT was lowest and most disparate within treatments between 0600 and 0800 h and highest and least disparate within treatments between 1500 and 1600 h (Figure 1B).

Table 2. Air quality indicators, wind speed, and air flow between postnatal pens¹

Item	Postnatal pen ²		SEM	P-value ^{3,4,5}
	CL	HT		Post
Morning (AM), 0700 h ⁶				
Particle matter _{2.5} (µg/m ³)	15.11	16.42	0.51	0.07
Particle matter ₁₀ (µg/m ³)	21.39	23.36	0.72	0.05
Number of particles (per L)	22,139	24,224	753	0.05
Air quality index	21.17	23.25	0.72	0.04
Formaldehyde (mg/m ³)	ND ⁷	ND	—	—
Total volatile organic carbon (mg/m ³)	ND	ND	—	—
THI ⁸	73.31	73.84	0.28	0.17
Wind speed (m/s)	2.04	0.05	0.04	<0.01
Air flow (m ³ /s)	1.94	0.05	0.04	<0.01
Afternoon (AF), 1300 h				
Particle matter _{2.5} (µg/m ³)	10.15	10.68	0.31	0.23
Particle matter ₁₀ (µg/m ³)	14.11	14.90	0.42	0.18
Number of particles (per L)	14,606	15,492	441	0.16
Air quality index	13.81	14.67	0.43	0.16
Formaldehyde (mg/m ³)	ND	ND	—	—
Total volatile organic carbon (mg/m ³)	ND	ND	—	—
THI	81.88	81.85	0.22	0.94
Wind speed (m/s)	2.03	0.26	0.05	<0.01
Air flow (m ³ /s)	1.95	0.24	0.05	<0.01
Evening (PM), 1900 h				
Particle matter _{2.5} (µg/m ³)	11.25	11.28	0.39	0.95
Particle matter ₁₀ (µg/m ³)	15.80	15.79	0.55	0.98
Number of particles (per L)	16,349	16,411	574	0.94
Air quality index	15.54	15.62	0.56	0.92
Formaldehyde (mg/m ³)	ND	ND	—	—
Total volatile organic carbon (mg/m ³)	ND	ND	—	—
THI	78.86	78.81	0.27	0.89
Wind speed (m/s)	2.09	0.13	0.03	<0.01
Air flow (m ³ /s)	2.04	0.12	0.03	<0.01

¹At 2 d of age, calves born to either prenatal cooled (CL) or heat-stressed (HT) dams were enrolled into 8 sand-bedded group automatic feeder pens (4.6 $\times$ 9.1 m, n = 6 calves/pen, 6.97 m²/calf) with either access to 2 fans (postnatal CL, 4 pens) or access only to the shade of the barn (postnatal HT, 4 pens). Measurements were collected in the morning (AM, 0700 h), afternoon (AF, 1300 h), and evening (PM, 1900 h) and averaged for 3 locations within the pen (inside, center, and outside).

²CL = postnatal CL; HT = postnatal HT.

³Post = postnatal main effect.

⁴Significant ($P \leq 0.05$); tendency ($0.10 \geq P > 0.05$).

⁵No significant time effects or treatment $\times$ time interactions ($P > 0.05$).

⁶AM, AF, and PM measurements were analyzed separately.

⁷ND = not determinable.

⁸THI = temperature-humidity index.

Figure 1. (A) Average daily environmental temperature, humidity, and temperature-humidity index (THI) and (B) hourly THI and calf body temperature during the duration of the experiment. Dairy calves were exposed to a 2×2 factorial arrangement of prenatal (44 ± 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12$ /treatment). CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT. Body temperature is graphed using the LSM $\pm$ SE of the triple interaction (prenatal $\times$ postnatal $\times$ time), and THI is graphed over time. Arrows indicate time of thermoregulatory responses measurement. $\blacksquare/\bullet$: prenatal CL, $\square/\circ$: prenatal HT, $\bullet/\circ$: postnatal CL, $\blacksquare/\square$: postnatal HT, $\blacktriangle$: THI. Main effects and interactions were not significant unless otherwise noted.

The effect of pre- and postnatal treatment and their interactions on thermoregulatory responses is depicted in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17926>) and Figure 2. A prenatal $\times$ postnatal interaction effect was observed for respiration rate in the AM, AF, and PM (all $P \leq 0.01$), whereby HT-CL calves had lower respiration rate in the AM and PM, and HT-HT calves had higher respiration rate in the AF relative to the other groups (Figure 2A). Rectal temperature was reduced in postnatal CL calves in the AM, AF, and PM by an average of 0.13°C (all $P < 0.01$) compared with postnatal HT. Further, HT-HT calves had significantly higher RT compared with CL-CL, CL-HT, and HT-CL calves in the AM and AF (Figure 2B). Postnatal CL significantly lowered calf ST in the AM, AF, and PM by an average of 1.79°C (all $P < 0.01$) compared with postnatal HT (Figure 2C). There was a significant interaction or a tendency for a prenatal $\times$ postnatal interaction for HR in the AM ($P = 0.08$), AF ($P < 0.01$), and PM ($P = 0.08$) whereby the CL-HT calves had a lower HR compared with other treatment

groups (Figure 2D). For all thermoregulatory responses at AM, AF, and PM, a significant effect of day ($P < 0.01$, data not shown) was observed, but no treatment by day interactions ($P > 0.11$).

Milk and Concentrate Intake

Postnatal CL calves had greater mean MR and concentrate DMI, leading to higher overall ME, CP, and fat intake compared with postnatal HT calves (Table 3, $P \leq 0.06$). However, a tendency was observed for a postnatal treatment $\times$ time interaction for total DMI, ME, CP, and fat intake ($P < 0.08$). Specifically, postnatal CL calves increased MR consumption during most of the preweaning period (wk 3, 4, 5, and 6, Figure 3A). Postnatal CL calves also had greater starter concentrate intake during the preweaning period (wk 3, 4, 5, and 6) and during and after weaning (wk 7 and 8) once milk consumption was restricted and terminated (Figure 3B). There was no prenatal treatment or prenatal $\times$ postnatal interaction on intake (Table 3), but a significant effect of week ($P < 0.01$) was observed.

Growth Parameters

Prenatal CL calves weighed 2.4 kg more at birth (42.48 vs. 40.08 ± 0.77 kg for prenatal CL vs. HT respectively, $P = 0.03$) and 6.6 kg more at weaning (81.61 vs. 75.02 ± 1.93 kg, $P = 0.02$), with a tendency for increased overall ADG across 56 d (0.68 vs. 0.62 ± 0.02 kg, $P = 0.10$) compared with prenatal HT calves (Table 4). Relative to prenatal HT, prenatal CL calves consistently weighed more over time, regardless of postnatal treatment (Figure 4A). There was no effect of postnatal treatment ($P = 0.49$), prenatal $\times$ postnatal interaction ($P = 0.38$), or treatment $\times$ time interaction for BW ($P = 0.34$), but BW increased over time ($P < 0.01$).

Weekly ADG varied greatly from week to week with a tendency for a prenatal effect and a significant postnatal treatment $\times$ time interaction (Figure 4B, $P = 0.09$ and $P = 0.02$, respectively). Postnatal HT calves had greater rates of gain only during wk 2 and 3 compared with postnatal CL calves ($P \leq 0.04$). Meanwhile, prenatal CL calves had greater rates of gain during wk 8 compared with prenatal HT calves ($P = 0.01$). Indeed, HT-HT calves had negative ADG around weaning when all other groups were positive (Figure 4B, >263 g/d difference; $P < 0.13$ for HT-HT vs. CL-CL, CL-HT, and HT-CL calves). Following a similar pattern, gain-to-feed ratio also varied over time (Figure 4C). Notably, wk 2 HT-CL calves had a negative gain-to-feed ratio when all other groups were positive ($P \leq 0.01$) and wk 8 HT-HT calves had a negative gain-to-feed ratio when all other groups were positive ($P <$

0.07). The most feed-efficient calves were the CL-HT group (Table 3, 0.67 vs. 0.56 , 0.55 , and 0.59 ± 0.04 kg of BW/kg of intake for CL-HT, CL-CL, HT-CL, and HT-HT, respectively, $P < 0.14$), and ADG/ME, ADG/CP, and ADG/fat ratios followed a comparable trend (Table 3).

Hip height, withers height, and hip width did not differ among treatments at birth or at weaning, but there was a significant overall hip height increase in postnatal HT calves when compared with postnatal CL calves (Table 4, $P = 0.01$). Body condition score and other overall height and width changes did not differ among treatments.

Calf Health, Medication Events, and Management Interventions

Health scores measuring ear, coat, dehydration status, nose, and cough characteristics did not differ between treatments ($P > 0.99$, data not shown). Postnatal CL calves had a tendency for reduced medication

events for fever and infection in comparison to postnatal HT calves (Table 5, $P = 0.08$). Medication events for scours, sepsis, and respiratory disorders did not differ among treatments. The total number of medication events tended to be reduced from 3 to 2 total medications in postnatal CL calves compared with HT calves (1.8 vs. 2.6 ± 0.3 events for postnatal CL vs. HT, respectively, $P = 0.07$). If a calf was not consuming MR via autfeeder during training, MR was administered by bottle if the calf was receptive or by esophageal tube feeding if the calf refused to suckle. Prenatal treatment tended to influence number of esophageal tube feedings, where prenatal HT calves were tube fed approximately 2 times more than prenatal CL calves (Table 5, 0.67 vs. 1.88 ± 0.45 events for prenatal CL vs. HT respectively, $P = 0.06$).

Blood and Colostrum Analyses

Blood hematocrit percentage was greater in prenatal CL calves compared with prenatal HT calves, particu-

Figure 2. Morning (AM, 0700 h), afternoon (AF, 1300 h), and evening (PM, 1900 h) thermoregulatory responses including (A) respiration rate, (B) rectal temperature, (C) shaved rump skin temperature, and (D) heart rate of dairy calves were exposed to a 2×2 factorial arrangement of prenatal (44 ± 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12$ /treatment). CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT. AM, AF, and PM thermoregulatory responses were analyzed separately. Different letters (a–c) indicate a significance ($P \leq 0.05$) between individual treatments (i.e., CL-CL vs. CL-HT) within each time of the day. Dashed line in (B) indicates upper physiological range of calf rectal temperature (Moran, 2002; Piccione et al., 2003). Data are graphed using the LSM $\pm$ SE of the prenatal $\times$ postnatal interaction. Interactions between prenatal or postnatal treatments and time were not significant.

Table 3. Milk replacer, concentrate, total feed intake, and feed efficiency of dairy calves exposed to a 2 × 2 factorial arrangement of prenatal (44 ± 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; n = 12/treatment)¹

Item	Prenatal × postnatal treatment ²				SEM	P-value ^{3,4,5}		
	CL-CL	CL-HT	HT-CL	HT-HT		Pre	Post	Pre × Post
Milk replacer intake								
DM (kg/d)	0.92	0.86	0.89	0.82	0.03	0.21	0.03	0.72
ME (Mcal/d)	4.41	4.15	4.29	3.93	0.14	0.21	0.03	0.71
CP (g/d)	253	239	246	226	7.82	0.20	0.03	0.71
Fat (g/d)	177	166	172	157	5.41	0.21	0.02	0.67
Concentrate intake								
DM (kg/d)	0.14	0.06	0.11	0.06	0.01	0.38	<0.01	0.73
ME (Mcal/d)	0.37	0.16	0.31	0.14	0.04	0.30	<0.01	0.90
CP (g/d)	39	31	37	26	4.92	0.40	0.06	0.64
Fat (g/d)	3.69	1.80	3.14	1.31	0.43	0.17	<0.01	0.66
Total intake ⁶								
DM (kg/d)	1.26	1.11	1.29	1.05	0.06	0.79	<0.01	0.42
ME (Mcal/d)	5.42	4.81	5.48	4.62	0.20	0.76	<0.01	0.55
CP (g/d)	334	295	340	280	14.01	0.78	<0.01	0.46
Fat (g/d)	191	171	190	168	6.71	0.74	<0.01	0.92
Feed efficiency ⁷								
ADG/DMI (kg/kg)	0.56	0.67	0.55	0.59	0.04	0.25	0.08	0.41
ADG/ME (kg/Mcal)	0.12	0.15	0.12	0.12	0.01	0.12	0.17	0.25
ADG/CP (kg/kg)	2.08	2.47	2.03	2.14	0.15	0.20	0.10	0.34
ADG/fat (kg/kg)	3.54	4.44	3.50	3.38	0.25	0.03	0.14	0.04

¹Dry matter intake was recorded daily and averaged by week for analysis. Metabolizable energy, CP, and fat were determined from sample chemical analysis.

²CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT.

³Pre = prenatal main effect; Post = postnatal main effect; Pre × Post = prenatal × postnatal interaction effect.

⁴Significant ($P \leq 0.05$); tendency ($0.10 \geq P > 0.05$).

⁵Time was significant for all variables ($P < 0.01$).

⁶Postnatal × time interaction ($P < 0.08$) for total intake variables. No other treatment × time interactions were significant.

⁷Postnatal × time interaction and prenatal × postnatal × time interaction ($P < 0.04$) for efficiency variables.

larly from d 21 to 49 (Figure 5A, $P = 0.02$). A tendency was observed for postnatal CL calves to have a greater hematocrit compared with postnatal HT calves ($P = 0.10$). Plasma total protein varied over time ($P < 0.01$) but was not different among treatments (Figure 5B). A prenatal × postnatal treatment interaction ($P = 0.04$) was observed for plasma fatty acid as HT-HT calves had significantly higher fatty acid levels compared with CL-HT calves (Supplemental Table S2, <https://doi.org/10.3168/jds.2019-17926>; $P = 0.03$). Changes in BHB concentration were only detectable after weaning at 49 and 56 d, where postnatal CL calves had greater BHB levels compared with postnatal HT calves (Supplemental Table S2; $P < 0.01$). Glucose concentrations varied over time (Supplemental Table S2), but no difference was observed between treatments. The IgG concentration of pooled colostrum administered to calves did not differ among treatments (Figure 5C), but serum concentration of IgG 24 h after colostrum consumption was significantly higher in prenatal CL calves relative to prenatal HT calves ($P = 0.05$). No difference was observed in 24-h plasma total protein concentration.

DISCUSSION

Previous studies highlight the negative effects of heat stress on calf productivity when exposure occurs prenatally during late gestation or postnatally during the preweaning period (Tao and Dahl, 2013; Roland et al., 2016; Dahl et al., 2019). However, calves often experience heat stress across both developmental windows. After calves are exposed to heat stress or cooling through the in utero environment, the physiological consequences of similar or opposing postnatal thermal environments are unknown. Herein, we demonstrate that prenatal and postnatal heat stress abatement improves calf thermoregulatory responses and growth and health outcomes.

In this study, all calves were exposed to similar levels of elevated THI across both the prenatal and postnatal periods, but respiration rate and RT were reduced in CL relative to HT dams (i.e., prenatal CL vs. HT calves) and in postnatal CL relative to HT calves, indicating effective heat stress abatement for prenatal or postnatal CL calves. Although THI thresholds have been well established for lactating and dry cows, no

such threshold exists for the dairy calf (Broucek et al., 2009; Zimbelman et al., 2009; Kovacs et al., 2018). Additionally, although the use of fans in combination with water soakers or sprinklers is recognized as a successful dairy cow heat abatement technique (Collier et al., 2006), most heat stress literature in calves focuses on the provision of shade or hutch material and design (Coleman et al., 1996; Spain and Spiers, 1996) with fewer data on the effect of active heat stress abatement (i.e., fans or soakers; Hill et al., 2011). This is the first

study to evaluate fan usage for heat stress abatement in group-housed calves.

In the postnatal CL pens, providing 2 fans between 91 to 107 cm, one at calf level and one placed above, led to an average pen windspeed of 2 m/s and air flow of $\sim 2 \text{ m}^3/\text{s}$, which is equivalent to windspeed recommendations of the Wisconsin Dairyland Initiative for lactating dairy cows (University of Wisconsin-Madison Dairyland Initiative, 2019). We determined that the air quality in our open-sided group automatic feeder pens, regardless of treatment, was within safe levels as recommended by the US Environmental Protection Agency (AirNow, 2019; US Environmental Protection Agency, 2019).

Postnatal CL calves had significantly lower thermoregulatory responses, such as BT, respiration rate, RT, and ST compared with postnatal HT calves, in accordance with previous studies of shade heat stress abatement for hutch-housed dairy calves (Coleman et al., 1996; Spain and Spiers, 1996; Peña et al., 2016; Kovacs et al., 2018). Notably, even though postnatal HT calves in the current study were under chronic heat stress, they had access to the shade of the barn; thus, maximum values and ranges between treatment groups are less severe when compared with an acute heat stress shade versus no shade study (Kovacs et al., 2018). Regardless, in the evening when heat loads were highest, postnatal HT calves had RT that were above the physiological range of calf rectal temperatures (Moran, 2002), which could have negative consequences on normal physiological functions (Yousef, 1985).

Further, interactions between prenatal and postnatal environments were observed. For example, HT-HT calves had the highest RT and respiration rate in the AF compared with other treatment groups. This is incongruous with a study in dairy cattle whereby heifers exposed to prenatal heat stress had reduced RT and no differences in respiration rate when exposed to heat stress as mature lactating cows compared with prenatally cooled heifers (Ahmed et al., 2017). However, the increase in RT in the present experiment is consistent with a study in pigs whereby in utero heat-stressed pigs had a greater increase in RT when exposed to heat stress in adolescence compared with pigs exposed to thermoneutral conditions in either or both of those periods (Johnson et al., 2013). Differences between models can be attributed to species, age, and body size at postnatal heat stress exposure, or difference in physiological state (Broucek et al., 2009; Hillman, 2009). In line with our hypothesis, CL-CL calves were expected to have the lowest respiration rate. However, HT-CL calves had the lowest respiration rate in the morning and evening compared with all other treatment groups. It is tempting to speculate that in utero programming

Figure 3. (A) Milk replacer and (B) concentrate intake of dairy calves were exposed to a 2×2 factorial arrangement of prenatal (44 $\pm$ 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12$ /treatment). CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT. The dash in the x-axis indicates start of weaning at 42 d. * and # indicate significance ($P \leq 0.05$) and tendencies ($0.10 \geq P > 0.05$) for postnatal treatment, respectively. Data are graphed using the LSM $\pm$ SE of the triple interaction (prenatal $\times$ postnatal $\times$ time). ■/●: prenatal CL, □/○: prenatal HT, ●/○: postnatal CL, ■/□: postnatal HT. Main effects and interactions were not significant unless otherwise noted.

Table 4. Birth, weaning, and daily gains of BW and height for dairy calves exposed to a 2×2 factorial arrangement of prenatal (44 ± 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12/\text{treatment}$)

Item	Prenatal × postnatal treatment ¹				SEM	<i>P</i> -value ^{2,3}		
	Prenatal CL		Prenatal HT			Pre	Post	Pre × Post
	CL-CL	CL-HT	HT-CL	HT-HT				
Birth ⁴								
BW (kg)	42.48		40.08		0.77	0.03	—	—
Hip height (cm)	80.54		80.26		0.60	0.75	—	—
Withers height (cm)	75.29		75.09		0.57	0.81	—	—
Hip width (cm)	17.02		17.29		0.25	0.45	—	—
Weaning								
BW (kg)	79.96	83.24	75.54	74.50	2.73	0.02	0.68	0.44
Hip height (cm)	93.44	94.92	92.89	93.41	0.96	0.29	0.31	0.61
Withers height (cm)	86.90	88.97	87.47	87.08	0.90	0.47	0.36	0.18
Hip width (cm)	21.27	22.33	21.18	21.16	0.46	0.18	0.27	0.25
Overall								
ADG (kg)	0.70	0.66	0.62	0.62	0.04	0.10	0.61	0.66
BCS	2.55	2.63	2.54	2.55	0.03	0.16	0.19	0.31
Hip height change (cm)	12.81	14.51	13.43	14.43	0.52	0.61	0.01	0.50
Withers height change (cm)	13.20	12.47	13.12	12.23	0.78	0.84	0.31	0.92
Hip width change (cm)	5.46	5.40	4.62	5.10	0.33	0.10	0.53	0.42

¹CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT.

²Pre = prenatal main effect; Post = postnatal main effect; Pre $\times$ Post = prenatal $\times$ postnatal interaction effect.

³Significant ($P \leq 0.05$); tendency ($0.10 \geq P > 0.05$).

⁴Birth variables were analyzed only for prenatal treatment because postnatal treatment had not yet been applied.

of pulmonary physiology under heat stress may contribute to these differences, but this warrants additional investigation. Finally, CL-HT calves had significantly lower HR throughout the day compared with all other treatment groups. The reason for the reduced HR in CL-HT calves is unclear but could be due to altered activity levels postnatally (Laporta et al., 2017) or differences in vasculature development in utero (Louey and Thornburg, 2005). Although studies demonstrate the effect of in utero stressors on respiratory and cardiovascular disease outcomes (Louey and Thornburg, 2005; Manuck et al., 2016), further work is needed to determine the mechanisms through which in utero heat stress might be programming organ systems to accommodate short-term pulmonary and cardiovascular homeostasis.

Postnatal CL calves consumed more MR before weaning and more starter concentrate across preweaning and weaning periods. However, this increase in postnatal DMI did not translate to improved BW gain, which was driven entirely by prenatal heat stress abatement. Prenatal CL calves weighed 2.4 kg more at birth and 6.6 kg more at weaning and had a 10% increase in ADG compared with prenatal HT calves. These data are in accordance with literature that demonstrates an increase in BW at birth, weaning, and 1 yr of life for heifers born to dry period CL dams compared with calves born to dry period HT dams, due in part to

a shorter gestation length and retarded fetal growth (Tao and Dahl, 2013; Monteiro et al., 2014, 2016b). However, data are conflicting on the effect of postnatal heat stress on feed intake and BW gain. Calves born in summer in both temperate (Slovakia; Broucek et al., 2008) and tropical (Mexico; López et al., 2018) climates have reduced birth and weaning weight, ADG, and feed intake compared with those born in fall or winter, but it is unclear if these differences are due to pre- or postnatal heat stress exposure. A study during the summer in Ohio reported that individually housed calves cooled with a fan and shade had improved ADG and feed efficiency and reduced respiration rate when compared with calves housed identically but with no fans (Hill et al., 2011). However, similar to our results, other studies from warmer climates in shaded versus unshaded hutch environments found no difference in ADG (Coleman et al., 1996; Spain and Spiers, 1996; Manriquez et al., 2018). Differences between outcomes may be due to type of heat stress abatement (i.e., season vs. shade vs. shade plus fans) and the intensity and duration of the heat stress event of each particular trial. In the current study, calves were exposed to chronic, intense levels of heat stress even at night, with either access to shade and fans or only shade. Postnatal heat stress abatement promoted improved thermoregulation and feed intake in our group-housed calves, but this did not lead to greater BW gains. We postulate that even though im-

Figure 4. Weekly (A) BW, (B) ADG, and (C) gain-to-feed ratio of dairy calves were exposed to a 2×2 factorial arrangement of prenatal (44 ± 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12/\text{treatment}$). CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT. The dash in the x-axis indicates start of weaning at 42 d. † and ‡ indicate significance ($P \leq 0.05$) and tendencies ($0.10 \geq P > 0.05$) for prenatal treatment, respectively. * and # indicate significance ($P \leq 0.05$) and tendencies ($0.10 \geq P > 0.05$) for postnatal treatment, respectively. Data are graphed using the LSM $\pm$ SE of the triple interaction (prenatal $\times$ postnatal $\times$ time). ■/●: prenatal CL, □/○: prenatal HT, ●/○: postnatal CL, ■/□: postnatal HT. Main effects and interactions were not significant unless otherwise noted.

mediate growth responses are not evident in our study, improvements in feed and energy intake may promote immune system and mammary development that could later translate to production responses when heifers become lactating cows (Heinrichs and Heinrichs, 2011; Soberon et al., 2012; Geiger et al., 2016). However, this hypothesis remains to be tested.

Alterations in feed intake were reflected in blood metabolite profiles. Whereas few postnatal heat stress studies have analyzed feed intake, none reported differences in blood metabolites (Coleman et al., 1996; Hill et al., 2011). In the current study, postnatal CL calves had greater BHB levels compared with postnatal HT calves around weaning. This is likely a result of increased concentrate intake which stimulates rumen microbe and VFA production and subsequently initiates rumen development. Also, even though no pattern was discernable for blood glucose, HT-HT calves had significantly higher plasma fatty acid concentrations relative to CL-HT calves. It is possible that CL-HT calves can better use fat as an energy source, as evidenced by an improved ADG/fat ratio. In the porcine model, in utero heat-stressed pigs under postnatal heat stress had reduced blood glucose concentration relative to in utero thermoneutral pigs under postnatal heat stress, whereas plasma fatty acids did not differ (Johnson et al., 2015). However, previous studies from our group reported that prenatal HT calves had no difference in blood glucose but had greater fatty acid and BHB plasma concentrations at 32 d (Tao and Dahl, 2013; Monteiro et al., 2016a). Differences between studies can be attributed to variations in feed intake, timing and intensity of heat stress, or species metabolism. In accord with previous prenatal heat stress studies, prenatal HT calves had significantly reduced hematocrit percentage across the preweaning period, suggesting adaptation to the postulated reduced uterine oxygen supply caused by in utero heat stress (Tao et al., 2012; Monteiro et al., 2016a).

Beyond metabolic and growth response, calf heat stress abatement may promote health and ease of management. Colostrum passive transfer of immunoglobulins is essential for calf health and survival, and its acquisition is impaired by prenatal heat stress (Tao et al., 2012). Indeed, prenatal HT calves undergo compromised passive immune transfer potentially through impaired small intestinal absorption or intestine surface area, and not because of colostrum quality (Monteiro et al., 2014). In our study, there was a positive effect of postnatal CL whereby postnatal CL calves had a tendency for reduced number of fever, infection, and total medication events. Similar studies assessing health outcomes in calves under shaded heat stress abatement found discrepancies in health scores and an increase

Table 5. Medication and assisted feeding events for dairy calves exposed to a 2×2 factorial arrangement of prenatal (44 ± 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12/\text{treatment}$)

Item	Prenatal $\times$ postnatal treatment ¹				SEM	<i>P</i> -value ^{2,3}		
	CL-CL	CL-HT	HT-CL	HT-HT		Pre	Post	Pre $\times$ Post
Medication events (count)								
Scours	1.25	1.18	1.18	1.09	0.32	0.81	0.80	0.97
Fever	0.08	0.47	0.18	0.37	0.15	0.69	0.08	0.48
Infection	0.13	0.48	0.29	0.52	0.17	0.39	0.08	0.50
Sepsis	0.16	0.11	0.24	0.18	0.11	0.46	0.62	0.97
Respiratory	0.00	0.00	0.00	0.34	0.04	0.99	0.99	0.99
Total treatments	1.92	2.64	1.71	2.66	0.45	0.79	0.07	0.76
Feeding assistance (count) ⁴								
Esophageal tube feeding	1.06	0.43	2.65	1.33	0.67	0.06	0.14	0.84

¹CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT.

²Pre = prenatal main effect; Post = postnatal main effect; Pre $\times$ Post = prenatal $\times$ postnatal interaction effect.

³Significant ($P \leq 0.05$); tendency ($0.10 \geq P > 0.05$).

⁴Calves under 4.5 L of daily milk replacer consumption were supplemented up to 4.5 L by bottle if accepted or tubing if unmotivated to eat.

in respiratory disease and total medication events compared with nonshaded counterparts, opposing the findings of the current study, but differences may be attributed to calf housing type (Peña et al., 2016; Roland et al., 2016; Manriquez et al., 2018). Moreover, long-term economic and productive implications to medication may occur. For instance, Soberon et al. (2012) found that increasing number of antibiotic treatments to dairy calves reduced first-lactation milk production by 500 kg.

Feeding motivation is an important consideration in calf health and management. Prenatal HT calves required more assistance to meet the minimum requirement of 4.5 L/d milk intake, thus were esophageal tube fed more often than prenatal CL calves throughout the experiment. A calf refusing to suckle and requiring esophageal tube feeding is considered anorexic, which has obvious welfare, health, and nutritional implications (Brisville et al., 2013). Esophageal feeding is a concern from a management standpoint, as the process is labor-intensive and improper technique can damage the calf larynx and esophagus (Stull and Reynolds, 2008). Anorexia has been documented in cases of diarrhea (Constable, 2004), toxicosis (Blanchard et al., 1993), and cloning (Brisville et al., 2013) but never in heat-stressed calves. Indeed, it has been suggested that heat-stressed dairy cattle may be experiencing hunger despite voluntary DMI decrease, incongruous to our results (Polsky and von Keyserlingk, 2017). We believe discrepancies arise due to the nature of heat-stress exposure, such that in utero heat stress may program the calf to have an altered metabolism that could reduce feeding motivation.

It is evident from the current study that both prenatal and postnatal heat stress abatement, separately, are

beneficial for certain aspects of dairy calf thermoregulation, growth, and health. However, this only partially supports our initial hypothesis that calves exposed to prenatal and postnatal heat stress abatement (i.e., CL-CL) would perform most optimally in all aspects compared with calves' heat stressed at any time (i.e., pre- or postnatal or both). Although CL-CL calves often had the lowest thermoregulatory responses and greatest intake numerically, they were not significantly different from HT-CL calves. A growing body of literature has established that the environment experienced in utero, whether positive or negative, can profoundly affect fetal growth and development (i.e., organ structure and function) and exert long-term effects on disease and productive responses when exposed to similar or opposing postnatal environments (Berghänel et al., 2017; Van Eetvelde and Opsomer, 2017; Reynolds et al., 2019). Indeed, work from our group has demonstrated that in utero heat stress alters the offspring epigenome in the mammary gland and liver, perhaps in preparation for a heat stress environment postnatally, leading to negative production outcomes later in life (Monteiro et al., 2016b; Skibieli et al., 2018b). Although determining the mechanisms behind in utero programming and outcomes depending on postnatal environment is beyond the scope of the current paper, in the future we will explore the underlying molecular mechanisms leading to the phenotypic differences we observe in the current study and highlight which combination of heat stress abatement is ideal for long-term productive success.

CONCLUSIONS

Herein, we demonstrate that postnatal heat stress induces increases in thermoregulatory responses, but

Figure 5. Weekly (A) blood hematocrit percentage and (B) plasma concentrations of total protein of dairy calves exposed to a 2×2 factorial arrangement of prenatal (44 $\pm$ 5 d) and postnatal (56 d) cooling (CL) or heat stress (HT; $n = 12$ /treatment). CL-CL = prenatal CL and postnatal CL; CL-HT = prenatal CL and postnatal HT; HT-CL = prenatal HT and postnatal CL; HT-HT = prenatal HT and postnatal HT. The dash in the x-axis indicates start of weaning at 42 d. (C) Total IgG concentration of colostrum ($n = 4$ prenatal CL, $n = 6$ prenatal HT; 2 h after birth) and serum ($n = 10$ /prenatal treatment; 24 h after birth) and total protein concentration of plasma (24 h after birth) of calves exposed to prenatal cooling or heat stress. † and ‡ indicate significance ($P \leq 0.05$) and tendencies ($0.10 \geq P > 0.05$) for prenatal treatment, respectively. * and # indicate significance ($P \leq 0.05$) and tendencies ($0.10 \geq P > 0.05$) for postnatal treatment, respectively. (A) and (B) are graphed using the LSM $\pm$ SE of the triple interaction (prenatal $\times$ postnatal $\times$ time), and (C) is graphed as prenatal treatment. ■/●: prenatal CL, □/○: prenatal HT, ●/□: postnatal CL, ■/□: postnatal HT. Main effects and interactions were not significant unless otherwise noted.

that the use of calf-level fans in group feeding pens was effective in lowering those responses. Prenatal heat stress abatement improves BW gains from birth to weaning, circulating IgG, hematocrit, and reduces the need for esophageal tube feeding. Postnatal heat stress abatement moderates feed intake, calf health, and thermoregulatory responses. Specific prenatal $\times$ postnatal thermoregulatory interactions are reported including lower respiration rate in HT-CL calves, higher RT in HT-HT calves, and lower HR in CL-HT calves. Although CL-CL calves did not perform most optimally compared with other treatment groups, taken together our results suggest that providing heat stress abatement during both pre- and postnatal development will promote calf growth and improve welfare. This paper is the first to characterize the combined effects of pre- and postnatal heat stress or active cooling on the preweaned dairy calf. Future studies will assess the long-term effects of calf heat abatement on productive and reproductive performance.

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