Long-term impacts of late-gestation maternal heat stress on growth performance, blood hormones and metabolites of newborn calves independent of maternal reduced feed intake

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ARTICLE INFO

ABSTRACT

The objective of this study was to evaluate the effects of heat stress in late gestation independent of maternal reduced feed intake on performance, blood hormones and metabolites, and immune responses of dairy calves from birth through weaning. A total of 30 multiparous Holstein cows at 45 d before expected calving were randomly assigned to one of 3 groups: (1) thermal neutral (CL, n = 10) conditions with ad libitum feed intake (10% of refusals on an as-fed basis); (2) pair-fed thermal neutral (CLPF, n = 10) conditions to reduce feed intake to levels similar to the heat stress (HS) group while reared under thermoneutral conditions (80% of the CL group); or (3) heat stress (HS, n = 10) conditions with ad libitum feed intake. Pair-feeding was conducted to quantify the confounding effects of dissimilar feed intake. Calves (10/group) born to cows that were exposed to cooling (IU-CL), pair-feeding (IU-CLPF), or heat stress (IU-HS) were used from birth through weaning. After birth, all the calves were managed under identical conditions. IU-HS calves had lower birth weight, and hip height at birth and 14 d of age. Compared with IU-CL and IU-CLPF calves, IU-HS calves had lower serum concentration of IgG and apparent efficiency of IgG absorption but higher serum insulin concentrations. Cortisol concentration in serum was higher in IU-HS and IU-CLPF calves compared to IU-CL calves. The neutrophil percentage was lower in IU-CL calves than in IU-HS and IU-CLPF calves. Neutrophil-lymphocyte ratio was higher in IU-HS calves compared to IU-CLPF and IU-CL calves. The mRNA expression of TNFα of IU-HS calves was downregulated compared with IU-CL and IU-CLPF calves. In summary, maternal HS during late gestation reduces calf birth weight and dramatically alters blood hormones and metabolites, but its effect on immune system function was not independent of maternal reduced feed intake.

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1. Introduction

Heat stress (HS) induces behavioral and metabolic changes in cattle exposed to HS conditions to reduce feed intake, activity, and metabolic rate in an attempt to reduce

internal metabolic heat production [1]. However, HS during pregnancy not only is affecting the performance of the cow but also can likely cause impaired performance of calves later in life [2–4]. In utero (IU) HS can have a lasting imprint on offspring growth, behavior, and metabolism and was shown to also impair the future HS response in a study that used pigs [5]. Programming results from adaptive alterations in gene expression patterns that occur in response to stressors and causes altered growth of specific organs and systems during their most critical time of development [6].

The IU exposure of calves to HS has been shown to induce fetal growth retardation by decreasing uterine blood flow [7] and placental weight [8]. In dairy cattle, calves born to cows under HS during late gestation were lighter at birth and through puberty [2,9,10], exhibited compromised passive immunity [2,3], and expressed lower peripheral blood mononuclear cell proliferation [2] compared to those born to cooled cows. Maternal HS also alters the inflammatory response of calves in early life [11]. Strong et al [4] reported that in utero HS calves had decreased mRNA expression of tumor necrosis factor α (TNF α) and toll-like receptor 2 (TLR2) compared with control calves. A recent study on the role of epigenetic modifications in developmental programming reported that maternal HS alters the DNA methylation of key regulatory gene pathways in the liver and mammary gland and programs their morphology in postnatal life, which may contribute to the reduced performance of prenatally heat stressed calves [12].

Heat stress has been widely shown to compromise feed intake of dairy cows [13] and many of the changes due to HS appear to be directly mediated by reduced feed consumption. Although the importance of late gestation HS has been documented in dairy calves, studies investigating the impacts of maternal HS independent of reduced feed intake are limited. Thus, the objectives of the present study were to investigate the effects of maternal HS independent of maternal reduced feed intake on growth performance, blood metabolites and hormones, and mRNA expression of genes involved in immune responses in Holstein dairy calves. Our hypothesis was that maternal HS independent of reduced feed intake during late gestation alters offspring growth, metabolic and hormonal responses from birth through weaning.

2. Materials and methods

The present study was conducted from 45 d before calving until 2 wk after weaning of calves, according to the guidelines of the Iranian Council of Animal Care [14] in a commercial dairy farm (ETKA Agri. Dairy Production Co., Gorgan, Iran), located 46 km from North of Gorgan, Iran (37° 8′ 2.98″ N 54° 37′ 8.73″ E), between August and December, 2017.

2.1. Animal, management, and groups

At 45 d before expected calving, a total of 30 multiparous Holstein cows were randomly assigned to 1 of 3 treatments (10 cows/group): (1) cooling and ad libitum feed intake (10% of refusals on an as-fed basis; CL); (2) cooling and pair-feeding (80% of the CL group; CLPF); and (3) heat stress and ad libitum feed intake (10% of refusals on an as-fed basis; HS). The reduction in feed intake of CLPF cows was calculated daily based on 80% of the average dry matter intake (DMI) of the CL group. There was no difference among the groups (mean ± SEM) in parity (2.5 ± 1.4) or BCS (3.2 ± 0.07) at the start of the experiment. All cows in the study calved within a 2 wk period. Cows were housed in an individual free-stall barn, bedded with straw, which for CLPF and CL cows consisting of shade, sprinklers, and fans that were turned on from 07:00 to 19:00 h, whereas HS cows were provided only with shade. During the experiment, all cows had free access to clean drinking water. Fans were installed in waiting areas, and sprinklers were applied behind the feed bunk. The diameter of the fans was 76 cm, and jet 0.47 cm^3 air per fan. Sprinklers had a capacity of 1.25 L of water per nozzle per minute. As cows demonstrated signs of calving, they were moved to individual maternity pens for delivery. Cows were dried 60 d before the expected calving. All cows were fed individually the same total mixed ration, 2 times a day (07:00, and 17:00 h), and orts were recorded on a daily basis before morning feeding. Rectal temperature (RT) was recorded for each cow daily at 14:00 h using a manual thermometer. Daily mean temperature and mean relative humidity were obtained from a meteorological station located 5 km away from the dairy farm. The temperature-humidity index (THI) was calculated according to [15]:

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

where T is air temperature (°C), and RH is the relative humidity percentage. Minimum and maximum temperature and relative humidity were recorded daily, and average THI calculated for each day.

2.2. Calves management

Newborn calves were separated from their dams immediately after birth and had their navel dipped with 2% iodine to prevent infection. At birth, calves were weighed and moved into the naturally ventilated barn and placed in individual pens (1.2 × 2.5 m) bedded with straw, which was replaced every 2 d. To eliminate the confounding effects of dam's treatments, all the calves received colostrum pooled from all the cows. Each calf was offered 3.5 L of colostrum from the colostrum pool by esophageal feeder within 4 h of birth and 12 h after the first feeding. The frozen colostrum (−20°C) was thawed at room temperature and subsequently warmed in a warm water bath to 40°C, and before feeding to calves, one sample (100 mL) was taken for further analysis. Calves received 5 L/d of whole milk in steel buckets twice daily at 06:00 and 18:00 from d 3 to weaning. All calves were weaned when they could consume 900 g starter/day for 3 consecutive days. The treatments assigned to calves, IU-HS (n = 10 born to HS cows), IU-CLPF (n = 10 born to CLPF cows), or IU-CL (n = 10 born to CL cows), reflected the treatments assigned to their dams during the dry period. All calves were fed the same starter diet formulated according to NRC (2001) at d 3 until the end of the experiment. The

study was terminated 2 wk after weaning. From day 1 to 2 wk after weaning, all the calves had free access to clean, fresh water, and a starter feed. Starter samples were collected on a weekly basis and stored at −20°C before chemical analysis. Ingredients and chemical composition of the starter feed are represented in Table 1.

Amounts of starter feed offered were recorded daily and orts measured weekly. Metabolizable energy contents of the starter feed milk were estimated using NRC equations [1]. The intake of ME was calculated by dividing that of each feed (starter or milk) by its ME content. Rectal temperature was recorded daily for each calf using a manual thermometer (Qingdao Dacon Trading Co Ltd, Shandong, China) at 14:00 h.

In this study, calves were weighed at birth before colostrum feeding and then, BW and hip height (distance from base of the rear feet to hook bones), body length (distance between the points of shoulder and rump), body barrel (circumference of the belly before feeding), and heart girth (circumference of the chest) were measured every 15 d until 2 wk after weaning, according to methods described previously [16]. Average daily weight gain (kg/d), the gain-to-feed ratio [kg of BW gain/total DMI (DMI = milk DM + starter feed DMI)], and average feed efficiency (FE, kg of BW gain/kg of total DMI) were calculated. Fecal scores were recorded daily based on a 1 to 5 system (1 being normal, thick in consistency; 2 being normal but less thick; 3 being abnormally thin but not watery; 4 being watery; 5 being watery with abnormal coloring [modified from Kertz et al [17]]. Calves with a fecal score greater than 2 that were lethargic and had elevated RT were treated with oral electrolytes.

Table 1
Ingredient and chemical composition of starter feed.[a]

Item	
Ingredients	% of DM basis
Corn	45.00
Barley	10.00
Soybean meal	29.00
Wheat bran	10.18
Vitamin and mineral premix[b]	1.20
Calcium carbonate	1.10
Dicalcium phosphate	0.20
Sodium bicarbonate	0.02
Salt	0.20
Soybean oil	2.50
Binder	0.60
Total	100
Chemical composition	g/kg of DM
DM	90
Crude protein	18.32
Ether extract	3.54
Neutral detergent fiber	20.74
Acid detergent fiber	9.80
Ash	7.30
Metabolizable energy	2.90
Net energy$_{growth}$	1.30
Nonfiber carbohydrate[c]	50.10

[a] Diets were fed to calves from 2 d of age until 14 d after weaning.
[b] Mineral and vitamin premix included: 11.60 g of Cu/kg, 0.13 g of Co/kg, 9.40 g of Fe/kg, 0.14 g of I/kg, 5.4 g of Mn/kg, 0.04 g of Se/kg, 6.70 g of Zn/kg, 1,800 kIU of vitamin A/kg, 600 kIU of vitamin D/kg, 16 kIU of vitamin E/kg, 0.01 g of biotin/kg, 2.93 g of cobalamin/kg, 0.04 g of folic acid/kg, 1.77 g of niacin/kg, 1.32 g of pantothenic acid/kg, 0.24 g of pyridoxine/kg, 0.30 g of riboflavin/kg, 0.29 g of thiamin/kg.
[c] Calculated as dry matter − (neutral detergent fiber + crude protein + ether extract + ash) [1].

2.3. Sampling and laboratory analysis

All the laboratory analyses were performed at the Laboratory and Research, Department of Animal Science at the Gorgan University of Agriculture and Natural Resources, Gorgan, Iran. Weekly subsamples of feeds and refusals were mixed completely, dried at 55°C for 48 h, and ground to pass through a 1-mm screen in a Wiley mill (Ogawa Seiki Co. Ltd., Tokyo, Japan) before chemical analysis [18] for DMI (method 934.91), crude protein (method 988.05), lipids (method 920.39), Ash (method 942.05), and acid detergent fiber (method 973.18) contents. Neutral detergent fiber was analyzed without the use of sodium sulfite and with the inclusion of α-amylase [19].

Coccygeal blood samples (10 mL) from individual calves were collected 3 h after the morning feeding on d 0, 14, 21, 35, 50 (±3 d) in vacutainer collection tubes without additives. After collection, all the blood samples were maintained at room temperature for a minimum of 1 h to allow clotting to occur. At the same time, blood samples (10 mL) were collected in K3Ethylenediaminetetraacetic acid (EDTA)-coated vacutainer tubes and placed on ice immediately after collection. All blood samples were then centrifuged (1,500 × g for 20 min at 4°C). After centrifugation, serum was harvested from tubes without additives and stored at −20°C until analyzed for total antioxidant activity and malondialdehyde (MDA). Also, the plasma was harvested from K3EDTA vacutainer tubes and frozen at −20°C until the determination of glucose, cholesterol, triglycerides, urea, HDL, insulin, prolactin, and cortisol. Blood samples were also collected via jugular venipuncture in 6 mL K3EDTA vacutainer tubes and maintained on ice until analyzed for subsequent hematological analysis within 1 h.

Concentration of blood metabolites were determined enzymatically, using a spectrophotometer (PG instrument, T80, England), and commercially available kits [Pars Azmoon Company, Tehran, Iran; catalog numbers: glucose (1–500–017), cholesterol (1–500–010), nitrogen urea (1–400–029), triglyceride (1–500–032], according to the manufacturer's instruction. Total antioxidant activity was measured by the ferric-reducing antioxidant power (FRAP) method [20]. The level of lipid peroxidation was measured using the malondialdehyde method [21].

A commercially available ELISA kit (Cortisol ELISA RE52061, IBL International Corp., Toronto, ON, Canada) was used for the measurement of serum cortisol concentration (ng/mL) and followed the manufacturer's instructions. The intra-assay and interassay coefficients of variation (CVs) for serum cortisol were 5.2% and 7.9%, respectively. Serum insulin concentration was measured using a bovine-specific insulin ELISA (Mercodia, Uppsala, Sweden). Intra-assay and interassay CVs were 4.6% and 8.5%, respectively. Serum prolactin concentration was measured using an indirect competitive ELISA with a rabbit-anti-prolactin antibody [22]. Intra-assay variability was 3.1%, and interassay variability was 10.7%. The sensitivity of the assay was 0.7 ng/mL.

White blood cell (WBC) ($\times 10^3/\mu L$), the hematocrit percentage, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), the percentage of monocytes, neutrophils (PMN), and lymphocytes were measured using

a hematology cell counter (Abbott Cell Dyn 3700 Full Option). The neutrophil:lymphocyte cell ratio (N:L) was also calculated.

2.4. Colostrum and serum IgG

Individual subsamples (100 mL) of pooled colostrum were collected and frozen at −20°C. Colostrum samples were thawed in a warm water bath and thoroughly mixed before analysis. Representative colostrum samples were analyzed for concentration of fat, total protein, and total solids using a MilkoScan (Foss Electric, Hillerod, Denmark). Immunoglobulin G concentration was analyzed using a bovine ELISA kit for bovine IgG from Bio-X Diagnostics (Jemelle, Belgium) in colostrum (the colostrum fat was removed through centrifuging before freezing; colostrum IgG data were reported on a fat-free basis) and serum samples of calves. Colostrum and serum samples were thawed at 4°C overnight. All kit components were brought to 21°C before use. The wash buffer was diluted 20-fold with distilled water. A calibration curve was developed and samples were diluted at 1/1,000 in PBS according to the manufacturer's instructions. Diluted samples were added to the test plate in duplicate and incubated at 21°C for 1 h. The test plate was then washed 3 times with the wash buffer; the chromogen solution (100 μL) was added to each well and incubated away from light for approximately 10 min. Finally, stop solution (50 μL) was added to each well and the optical densities were recorded using a microplate spectrophotometer with a 450-nm filter. An interassay CV of <0.15 was observed. The concentration of IgG in samples was calculated from the standard reference curve containing known concentrations of IgG provided in the test kit. Colostrum composition included 5.04% of fat (SEM = 0.48), 11.39% of protein (SEM = 0.54), 2.52% of lactose (SEM = 0.06), 21.82% of total solids (SEM = 0.39), and 85.11 of IgG (SEM = 2.45).

The total IgG concentration of serum samples collected at 1 d of age was used to calculate the apparent efficiency of absorption (AEA), and serum volume was considered as 9% of the birth weight. The AEA was assessed in this study to determine how much IgG the calves in each treatment group absorbed into their blood before the closure of the gut at 24 h. Data used to calculate the AEA were the 24-h serum IgG concentration and total IgG mass fed in the first 24 h [feed 1 (0 h) and feed 2 (12 h)]. The following formula was used to calculate the AEA [23]:

$$AEA = [\text{serum IgG (mg/mL)} \times \text{plasma volume (L)/total IgG intake (mg)}] \times 100.$$

2.5. Polymorphonuclear leukocytes isolation, RNA extraction, and qPCR

Polymorphonuclear leukocytes were isolated based on procedures described previously [24]. Briefly, at days 0, 28, and 60 of age, blood (∼100 mL) was sampled in ACD Vacutainer (Becton, Dickinson) tubes and mixed well by inversion and placed on ice until isolation (∼1 h). Tubes were combined into three 50-mL conical tubes (Fisherbrand, Thermo Fisher Scientific Inc) and centrifuged at 918×g for 30 min at 4°C. After centrifugation, the plasma, buffy coat, and approximately one-third of the red blood cells (RBCs) were discarded. Twenty-five mL of deionized water at 4°C was added to lyse the RBC, followed by addition of 5 mL of 5× PBS at 4°C to restore an iso-osmotic environment. Samples were centrifuged at 200 × g for 10 min at 4°C, and the supernatant was decanted. Subsequently, samples were washed with 1× PBS and collected by centrifugation at 500 × g for 3 min at 4°C 3 times. The isolated polymorphonuclear leukocytes were immediately homogenized in 2 mL of TRIzol reagent (Invitrogen, Carlsbad, CA) with 1 μL of linear acrylamide (Ambion Inc., Austin, TX) using a Polytron power homogenizer (Kinematica, Bohemia, NY) at maximum speed. The suspension was then transferred equally into 2 RNA-free microcentrifuge tubes (2 mL; Fisher Scientific) and stored at −80°C until further analysis.

The mRNA abundance of GAPDH, TNFα, IL-6, IL-8, and TLR2 transcripts in bovine total blood leukocytes was determined by quantitative (q) PCR. The suspension of RNA and TRIzol reagent were thawed and, on centrifugation, total RNA was separated with chloroform followed by acid phenol: chloroform (Ambion Inc). Total RNA was then precipitated with isopropanol, and the RNA pellet was cleaned with 75% ethanol before reconstitution in RNA storage buffer (Ambion Inc) for storage at −80°C. The purity of RNA was confirmed by optical density (OD) 260 nm/OD 280 nm (NanoDrop ND-1000, NanoDrop Technologies, Rockland, DE). Quality of RNA evaluated via RNA integrity number in the 2100 Bioanalyzer (Agilent Technologies Inc, Santa Clara, CA) was 7.7 ± 0.2. All samples were incubated with DNase (Applied Biosystems, Foster City, CA) for 30 min at 37°C to remove genomic DNA, and subsequently heat denatured at 75°C for 15 min.

Real-time PCR was used to quantify TNFα, IL-6, IL-8, and TLR2. Reverse transcription was done with random hexamers in a 100 μL final volume that contained 50 U/mL Multi-Scribe reverse transcriptase, 25 mm MgCl2, 2.5 mM random hexamers, 0.4 U/mL RNAse inhibitor, 50 mM dNTPs, and TaqMan RT buffer (TaqMan reverse transcription reagents, Applied Biosystems, Foster City, CA). This mixture was incubated at 25°C for 10 min, heated to 37°C for 60 min and inactivated at 95°C for 5 min. The final volume was stored at −80°C until used. Real-time TaqMan PCR for the internal control and the genes of interest were run in separate wells.

The PCR reactions were placed into a 96-well plate and contained 900 nm of each primer, 250 nm of the TaqManMGBprobe and PCR Mastermix (TaqMan Universal PCR Mastermix, no AmpErase UNG, Applied Biosystems, Foster City, CA) and 5 μL of the diluted cDNA samples in a final volume of 50 μL. The samples and standards were amplified in an automated fluorometer (ABI Prism 7000 Sequence Detection System, Applied Biosystems, Foster City, CA) with an initial incubation of 2 min at 50°C and then heated for 10 min at 95°C for activation of the AmpliTac Gold, then 40 cycles of 15 s at 95°C and 60 s at 60°C. The final quantitation was done using a standard curve generated by dilutions of the gene of interest or the internal standard (GAPDH, ACTB, SDHA, B2M). Data are reported as relative transcription of

Table 2
Primer sequences of each gene target analyzed in polymorphonuclear leukocytes of calves born to heat-stressed or pair-fed to HS levels dams during the last 45 d prepartum.

| Gene symbol	Gene name	Primer	Sequence	Reference/accession no.
TNFα	Bos taurus tumor necrosis factor α	F	ACACCATGAGCACCAAAAGC	[25]
		R	AGGCACCAGCAACTTCTGGA	
TLR2	Bos taurus isolate 74 toll-like receptor 2	F	CTGTGTGCGTCTTCCTCAGA	AF368419
		R	TCAGGGAGCAGAGTAACCAGA	
IL8	Bos taurus IL 8	F	TCACACGGGTAACGAATGAA	NM_173925
		R	TTTGAGGTTGAGAAATGCCC	
IL6	Bos taurus IL 6	F	AACCACTGCTGGTCTTCTGG	[26]
		R	GTGTGTGGCTGGAGTGGTTA	
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	F	GGTCGGAGTGAACGGATTTG	[27]
		R	TGGCAACGATGTCCACTTTG	
ACTB	Beta-actin	F	CATGTACGTTGCTATCCAGGC	DQ178122
		R	CTCCTTAATGTCACGCACGAT	
SDHA	Succinate dehydrogenase A	F	GAACCGAAGATGGCAAGA	DQ178128
		R	CAGGAGATCCAAGGCAAA	
B2M	ß-2-microglobulin	F	GAGGCTATCCAGCGTACTCCA	DQ178123
		R	CGGCAGGCATACTCATCTTTT	

Abbreviation: HS, heat stress; F, forward; R, reverse.

the gene of interest relative to the internal standard. Primers and probes (Table 2) were generated by Primer-Express software (Applied Biosystems, Foster City, CA) using published sequences of the genes of interest.

2.6. Statistical analysis

Data were analyzed by fitting the mixed-effects model using the PROC MIXED procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC), with time as repeated measures. The model consisted of treatment, time (sampling date), and the treatment × time interaction as the fixed effects, and calves as the random effect. Skeletal growth data were analyzed using the aforementioned model without the effect of time. Before analysis, all data were tested for normality of distribution by evaluating the Shapiro–Wilk statistic using the UNIVARIATE procedure of SAS. Where appropriate, data were transformed using a log10 transformation. Birth BW was not included as a covariate for AEA comparison between treatments because it was included in the AEA calculation [23]. Initial values of body measurements were considered as a covariate of the body measurements analysis. Relative gene expression of TNFα, TLR2, IL8, and IL6 were analyzed as repeated measures using the mixed model procedure of SAS; the model included treatment, time, and the treatment × time interaction. The Tukey-Kramer adjustment was applied to account for multiple comparisons. The threshold of significance was set at $P \leq 0.05$; trends were declared at $0.05 < P \leq 0.10$.
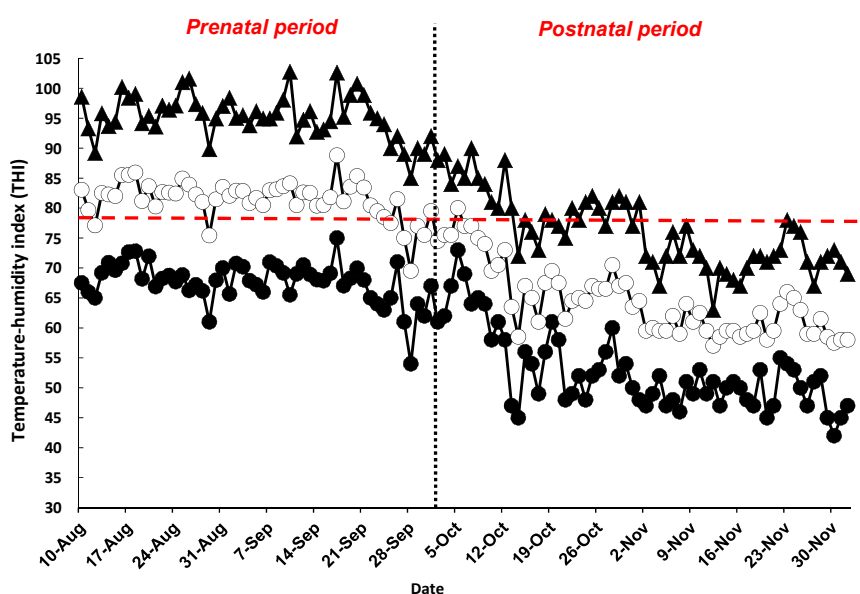
Fig. 1. Minimum (—●—), average (—○—), and maximum (—▲—) temperature-humidity index (THI) over the experiment period. Dashed lines represent THI ≥78.

3. Results

3.1. Prepartum period

Figure 1 represents the minimum, average, and maximum THI data during the prenatal period (45 d before birth) and postnatal life (birth to 2 wk after weaning). Heat-stressed cows had a higher RT (39.37 ± 0.32 vs 38.84 ± 0.30 and $38.77 \pm 0.61°C$; $P < 0.01$), and respiratory rate (81.58, vs 62.20 and 62.04 breaths/min; $P < 0.01$) than CLPF and CL cows. As compared with the CL cows (mean $\pm$ SD), heat-stressed cows showed reduced ($P < 0.01$) DMI during the experiment (12.37 ± 1.58 vs 10.47 ± 1.08 kg/d, respectively); however, DMI for HS and CLPF cows were similar by design during the experiment (10.47 ± 1.08 vs 10.28 ± 1.21 kg/d, respectively).

3.2. BW and growth performance

The data for BW and skeletal growth of calves are presented in Table 3. Calves born to IU-HS cows had lower birth weight than those of in IU-CL and IU-CLPF group ($P = 0.02$). There was no difference among the groups for weaning and final weight. Body length, body barrel, and heart girth of the calves did not differ among the groups.

However, hip height was lower in IU-HS calves compared with the other groups at birth ($P = 0.04$) and day 14 ($P = 0.03$) after birth. Also, hip height tended to be greater on days 21 and 35 ($P = 0.07$ and $P = 0.09$) after birth.

Results on starter feed intake, ME intake, average daily gain (ADG), feed efficiency, fecal score, and abnormal fecal days are presented in Table 4. Starter feed intake (kg/d and % of BW, Fig. 2A) and ME intake (Fig. 2B) did not differ among the groups; however, IU-HS calves had decreased starter feed intake at 4 and 6 wk after birth ($P < 0.05$). The fecal score did not differ between the groups; however, IU-HS calves tended ($P = 0.09$) to have more abnormal fecal days compared to other groups. Average daily gain, ME intake, ADG/ME intake, feed efficiency, and weaning age of calves did not differ among the groups.

The RT in IU-HS calves was greater at birth compared with the other groups ($P < 0.05$). However, there were no differences among the groups in RT between 2 and 10 wk of age (Fig. 3).

3.3. Blood metabolites and hormones

Calves exposed to IU-HS in utero had lower ($P = 0.05$) AEA compared with the other groups (Fig. 4). The blood parameters are presented in Table 5. The IU-HS calves had lower serum IgG concentrations compared with the other groups ($P < 0.01$). According to our results, serum IgG concentrations were affected by maternal heat stress

Table 3
Effects of maternal heat stress and or maternal pair-feeding on body weight and skeletal growth of calves (n = 10 per treatment).

Item	Treatment			Pooled SEM	Group
	IU-CL	IU-CLPF	IU-HS		
BW (kg)					
Birth	37.0[a]	36.6[a]	30.7[b]	1.08	0.02
Weaning[a]	83.8	81.83	80.1	2.24	0.52
Final	103.8	104.0	103.6	1.37	0.97
Hip height (cm)					
0 d	84.2[a]	85.0[a]	77.2[b]	2.13	0.04
14 d	87.0[a]	87.3[a]	79.7[b]	2.13	0.03
21 d	89.7	89.8	83.0	2.23	0.07
35 d	93.0	92.8	86.8	2.10	0.09
50 d	97.0	96.2	91.0	2.12	0.16
Body length (cm)					
0 d	80.3	81.0	80.7	1.77	0.96
14 d	87.8	87.5	86.3	1.69	0.81
21 d	91.3	90.2	89.2	1.61	0.64
35 d	94.0	93.0	91.1	9.04	0.37
50 d	98.0	98.8	95.2	9.47	0.33
Body barrel (cm)					
0 d	71.5	71.4	69.7	1.43	0.60
14 d	79.7	79.8	78.5	1.95	0.87
21 d	85.7	86.0	85.7	2.14	0.99
35 d	93.2	94.7	88.8	9.62	0.55
50 d	102.0	104.2	102.3	10.0	0.38
Heart girth (cm)					
0 d	75.8	75.4	74.7	1.58	0.87
14 d	83.7	83.33	82.8	1.37	0.91
21 d	89.8	89.67	88.3	1.46	0.73
35 d	94.8	95.17	90.1	9.49	0.50
50 d	101.7	102.0	85.0	9.92	0.40

Abbreviations: CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress.

[a-b]Means within a row with different superscripts are significantly different ($P < 0.05$).

[a] Calves were weaned when they could consume 900 g starter/day for 3 d continuously.

Table 4
Effects of maternal heat stress and or maternal pair-feeding on starter intake, metabolizable energy (ME) intake, average daily gain (ADG), feed efficiency, fecal score, and weaning age of calves (n = 10 per treatment).

Item	Treatment[a]			Pooled SEM	P-value[b]		
	IU-CL	IU-CLPF	IU-HS		G	T	G × T
Starter intake (kg/d)	1.29	1.21	1.16	0.44	0.17	<0.01	0.24
Starter intake (% of BW)	1.29	1.16	1.21	0.04	0.13	<0.01	0.23
ME intake (Mcal/d)	6.05	6.03	5.84	0.04	0.10	<0.01	0.28
Average daily gain (kg/d)	0.63	0.70	0.65	0.02	0.20	0.01	0.46
ADG/ME intake (kg/Mcal)[c]	0.08	0.09	0.07	0.002	0.30	0.56	0.23
Feed efficiency [d]	0.54	0.54	0.53	0.01	0.96	0.31	0.11
Fecal score [e]	1.95	1.93	1.99	0.03	0.61	0.98	0.42
Abnormal fecal days, no.[f]	4.53	4.80	5.10	0.43	0.09	–	–
Weaning age[g]	74.2	78.5	80.5	2.58	0.24	–	–

Abbreviations: CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress.

[a] Treatment: IU-CL calves born to cows with cooling and ad libitum feed intake, IU-CLPF calves born to cows with cooling and pair-fed to HS levels, and IU-HS calves born to cows in heat stress.

[b] G = group effect; T = time effect; G × T = group by time interaction.

[c] Average daily gain/metabolizable energy intake.

[d] Feed efficiency = kg of BW gain/kg of total DMI.

[e] Feces were scored daily using a 1 to 5 scale, where 1 = normal, thick in consistency; 2 = normal, but less thick; 3 = abnormally thin but not watery; 4 = watery; 5 = watery with abnormal coloring.

[f] Days with fecal score >2.

[g] Calves weaned when they could consume 900 g starter/day for 3 d continuously.
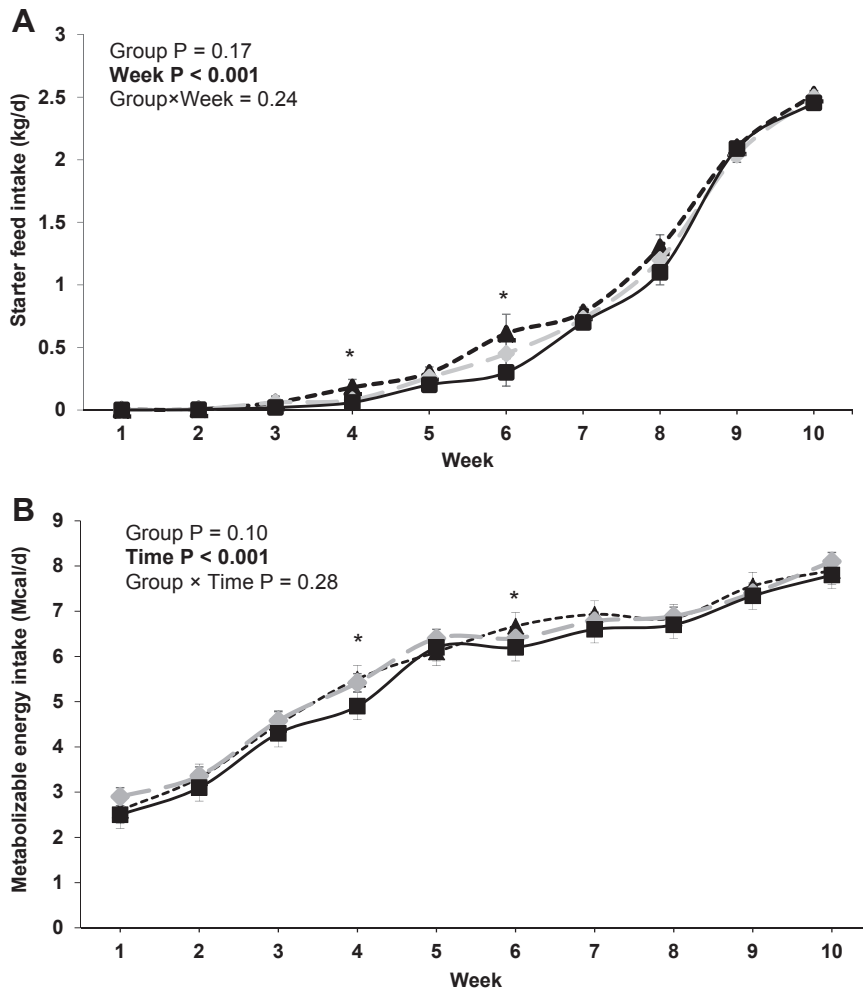
Fig. 2. Effects of maternal heat stress and or maternal pair-feeding on (A) starter feed intake (kg/d) and (B) metabolizable energy intake (Mcal/d) of dairy calves (n = 10 per treatment). Groups were IU-CL calves born to cows with cooling and ad libitum feed intake (· -▲- ·), IU-CLPF calves born to cows with cooling and feed restricted to HS levels of intake (· -♦- ·), and IU-HS calves born to cows in heat stress (—■—). Symbols indicate a difference (*$P < 0.05$) between the groups at a given time. Error bars indicate SEM. CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress.

independent of maternal reduced feed intake. There was no difference in blood concentrations of cholesterol, triglyceride, urea, HDL, MDA, prolactin, or glucose among the groups. There was no difference in serum FRAP concentrations among the groups; however, during the experiment, the IU-HS group had the lowest total antioxidant. Plasma cortisol concentrations were higher in the IU-HS and IU-CLPF groups compared to the IU-CL group. The IU-HS calves had higher plasma insulin concentrations than the IU-CLPF and IU-CL groups ($P = 0.03$).

There was no difference between the 3 groups in plasma WBC count. The IU-CLPF calves had an increased percentage of eosinophils compared with the other groups ($P = 0.04$). Eosinophil percentage was higher in the IU-CLPF group than in the IU-HS and IU-CL groups ($P < 0.01$). The neutrophil percentage was lower in the IU-CL group compared with the other groups ($P < 0.01$). By contrast, lymphocyte percentage decreased by the effect of maternal HS and nutritional deprivation than those in the IU-CL

group ($P < 0.01$). Neutrophil-lymphocyte ratio was higher in the IU-HS group compared with the other groups ($P = 0.01$). We did not observe any changes in PLT, MCT, MCV, MCH, MCHC, and the percentage of monocytes.

We observed no overall differences in the mRNA expression of *TLR2*, *IL8*, and *IL6* among the groups (Table 6). However, the mRNA abundance of *IL6* tended to be greater (Table 6; $P = 0.08$) in the IU-CL group compared with the other groups. Interaction among group and time was found for *IL6* ($P < 0.01$). The mRNA abundance of *TNFα* was lower in the IU-HS group compared with the other groups (Table 6; $P < 0.05$).
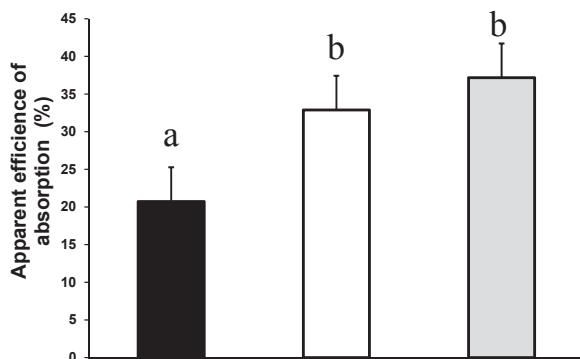
4. Discussion

All dry cows in our experiment were exposed to the same environmental HS during the prepartum period, but according to our results, the evaporative cooling system improved RT and respiratory rate of CL cows. These
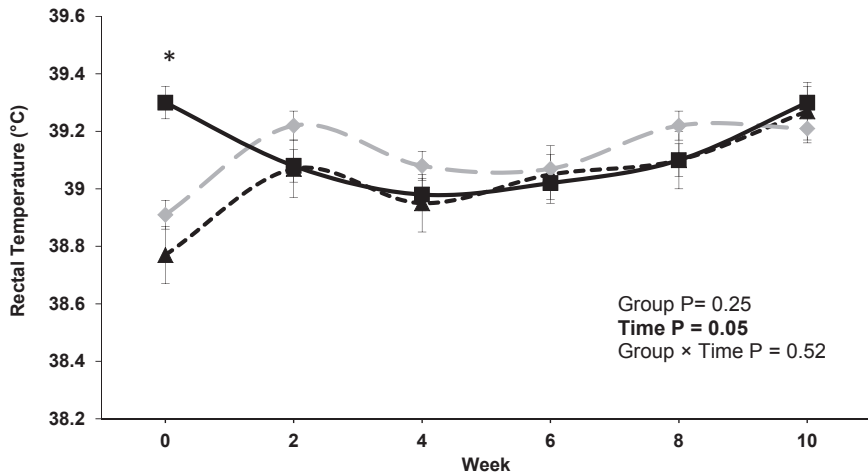
Fig. 3. Effects of maternal heat stress and or maternal pair-feeding on rectal temperature (°C) of dairy calves (n = 10 per treatment). Groups were IU-CL calves born to cows with cooling and ad libitum feed intake (—■—), IU-CLPF calves born to cows with cooling and feed restricted to HS levels of intake (·-♦-·), and IU-HS calves ad libitum born to cows in heat stress (·-▲-·). Symbols indicate a difference (*P < 0.05) between the groups at a given time. Error bars indicate SEM. CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress.

differences in physiological indicators of HS suggest that the experimental model produced a maternal uterine environment with hyperthermia [2], and therefore, an effective HS and undernutrition were achieved. In the present study, calves exposed to IU HS had a significantly lower birth weight, but their weaning and final weight were similar among the groups. This result is consistent with previous studies [2,10] in which maternal HS calves had a lower birth weight. Our results indicated that reduction in birth BW is independent of maternal reduced feed intake. Monteito et al [3] reported that calves born to heat-stressed cows are lighter at birth, and this difference in weight continues through puberty. The lower birth weight associated with maternal late gestation HS could be due to a combination of shorter gestation length, the direct impact of fetal hyperthermia, or fetal growth retardation

[2]. Maternal suboptimal nutrition during pregnancy may result in intrauterine growth restriction and newborns with lower birth BW [28]. In this regard, in the present study, different birth weight between treatments suggests that the extent of changes in the uterine thermo-environment and placenta function was sufficient to reduce the growth of the fetus, but the restriction in feed intake did not cause a decrease in birth weight.

In the present study, IU-HS calves had a lower starter feed intake and ME intake at 4 and 6 wk of age. However, total starter feed intake was similar among the groups. These results are in contrast with results from Monteiro et al [9], in which HS calves had lower starter feed intake and, as a consequence, lower ADG and BW compared with those from CL dry cows. However, we did not observe any difference in ADG and feed efficiency between the groups. These results may be explained by the fact that maternal HS exerts carryover effects on calf postnatal growth and perhaps on nutrient absorption and utilization [3]. Tudor [29] reported that feed restriction in the last trimester of pregnancy decreased the calf birth weight of beef cattle, although the energy intake of cows was severely restricted in that study.

In the present study, all the calves received the same pooled colostrum to avoid the confounding effect of colostrum quality on the absorptive ability of the neonatal calves. The lower total serum IgG during the first day after birth in IU-HS calves suggests that maternal HS negatively affects the IgG transfer from colostrum to the circulation of newborn calves independent of maternal reduced feed intake. Also, lower AEA in the IU-HS group compared with the IU-CL and IU-CLPF groups indicates a compromised passive immune transfer of IU-HS calves that is likely due to an impaired capacity for IgG absorption in the intestine. Similar effects of maternal HS on calf serum IgG and AEA have been described in studies by Tao et al [2] and Monteiro et al [10] that found maternal HS during late gestation of dairy cows reduced the circulating IgG compared with those from cows under thermoneutral conditions. Machado-Neto et al [30] also reported that piglets from late

Fig. 4. Effects of maternal heat stress and or maternal pair-feeding on apparent efficiency of absorption percentage (AEA %) of calves (n = 10 per treatment). Groups were as follows: (IU-CL) calves born to cows with cooling and ad libitum feed intake (▨), IU-CLPF calves born to cows with cooling and feed restricted to HS levels of intake (☐), and IU-HS calves born to cows in heat stress (■). Different lowercase letters (a, b) stand for differences among the group. Error bars indicate SEM. CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress.

Table 5
Effects of maternal heat stress and or maternal pair-feeding on blood metabolites and hormones of calves (n = 10 per treatment).

Item	Treatment[c]			SEM	P-value		
	IU-CL	IU-CLPF	IU-HS		G	T	G × T
IgG (mg/dL)	2,081[a]	2,240[a]	1,112[b]	240	0.003	<0.01	0.47
Cholesterol (mg/dL)	99.0	100.6	99.9	1.72	0.81	0.19	0.08
TG (mg/dL)	26.27	26.02	25.45	0.73	0.79	0.14	0.29
Urea (mg/dL)	14.62	14.90	15.07	0.48	0.80	<0.01	0.95
HDL (mg/dL)	42.06[b]	47.69[b]	53.25[a]	0.99	0.13	<0.01	0.05
Malondialdehyde (nmol/L)	1.80	1.81	1.74	0.05	0.63	<0.01	0.79
Prolactin (ng/mL)	9.66	9.01	8.77	0.95	0.51	0.86	0.64
FRAP (mmol/L)	0.47	0.45	0.41	0.01	0.23	<0.01	0.73
Cortisol (ng/mL)	30.9[b]	37.9[a]	38.8[a]	2.11	0.02	<0.01	0.003
Insulin (IU/ML)	20.7[b]	19.4[b]	26.8[a]	2.00	0.03	0.13	0.69
Glucose (mg/dL)	100.1	99.1	97.8	1.08	0.64	0.01	0.08
Hematological variables							
WBC (×10³/μL)	11.7	12.0	11.0	1.05	0.79	<0.01	0.69
Neutrophil (%)	36.7[b]	42.8[a]	46.0[a]	1.90	0.003	0.27	0.91
Lymphocyte (%)	60.4[a]	53.8[b]	50.6[b]	2.79	0.002	0.16	0.87
Eosinophil (%)	1.08[b]	1.46[a]	1.12[b]	0.13	0.04	0.46	0.95
Neutrophil/Lymphocyte[d]	0.64[c]	0.87[b]	1.11[a]	0.11	0.01	0.06	0.62
Monocytes (%)	1.93	1.97	2.07	0.17	0.84	0.01	0.94
Platelet	447	457	488	25.4	0.48	<0.01	0.82
Hematocrit	17.7	16.9	15.8	0.71	0.19	<0.01	0.98
MCV	40.6	41.5	42.4	0.63	0.14	0.23	0.81
MCH	19.2	19.6	20.7	0.64	0.23	<0.01	0.40
MCHC	47.0	47.5	48.7	1.56	0.71	<0.01	0.60

Abbreviations: CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress; G, group effect; G × T, group by time interaction; FRAP, ferric reducing antioxidant power; MCV, mean corpuscular volume; MCH, mean cell hemoglobin; MCHC, mean corpuscular hemoglobin concentration; T, time effect.
[a,b] Means within a row with different superscripts are significantly different (P < 0.05).
[c] Treatment: IU-CL calves born to cows with cooling and pair-fed to HS levels, and IU-CLPF calves born to cows with cooling and restricted intake, and IU-HS calves born to cows in heat stress.
[d] Calculated by dividing neutrophil count by lymphocyte count.

gestation heat-stressed sows had lower circulating IgG compared with those from sows under thermoneutral conditions. Ahmed et al [31] suggested that impaired passive immune transfer due to maternal HS is a consequence of reduced enterocyte turnover in the small intestine over the first days of the calf's life. On the other hand, in maternal heat-stressed calves, the lower birth BW caused by intrauterine growth retardation might also lead to impaired development of the fetal small intestine, resulting in reduced surface area for postnatal absorption [32]. In

Table 6
Effects of maternal heat stress and or maternal pair-feeding on the mRNA abundance of genes of (A) toll-like receptor 2 (TLR2), (B) tumor necrosis factor α (TNFα), (C) IL-8, (D) IL-6, and IL6 in polymorphonuclear leukocytes of dairy calves (n = 10 per treatment).

Item	Treatment[c]			SEM	P-value		
	IU-CL	IU-CLPF	IU-HS		G	T	G × T
Tumor necrosis factor α	1.45	1.35	1.10	0.05	<0.01	<0.01	0.97
Toll-like receptor 2	1.32	1.25	1.19	0.08	0.32	0.08	0.19
Interleukin-6	1.16[b]	1.26[a]	1.27[a]	0.03	0.08	0.10	<0.01
Interleukin-8	1.54	1.47	1.47	0.03	0.23	<0.01	0.16

Abbreviations: CL, thermal neutral; CLPF, pair-fed thermal neutral; HS, heat stress; G, group effect; T, time effect; G × T, group by time interaction. Data are from samples at d 0, 28, 60 of age.
[a,b] Means within a row with different superscripts are significantly different (P < 0.05).
[c] Treatment: IU-CL calves born to cows with cooling and ad libitum feed intake, IU-CLPF calves born to cows with cooling and restricted intake, and IU-HS calves born to cows in heat stress.

calves born to cows that experienced HS during late gestation, the lack of passive transfer is responsible for lower IgG concentrations rather than the colostrum quality [9]. In contrast to the results of Tao et al [2], we observed no differences in hematocrit among the groups. In that study, the IU HS calves had lower hematocrit levels at birth before colostrum feeding compared with calves cooled in utero. Such inconsistencies may be explained by the fact that in the study by Tao et al [2], all calves received fresh colostrum from their own dams, instead of frozen pooled colostrum as in the present study, which may explain the differences in blood hematocrit.

At the first line of defense against bacterial infection, the phagocytic and oxidative burst of neutrophils provides valuable information on the functional activity of these immune cells [33]. Oxidative burst is a process in which the pathogen is killed by toxic reactive oxygen species after it has been phagocytized by a neutrophil or macrophage [34]. It has been established that due to the increase in the number of neutrophils and the decrease in lymphocytes, the N/L is a suitable measure often used to assess the stress response. We found a greater cellular immune response in terms of neutrophil activation in the IU-HS and IU-CLPF groups. The increase in blood neutrophils may be related to the effect of maternal HS [35]. However, lymphocyte percentage decreased in the IU-HS and IU-CLPF groups. An increased neutrophil and decreased lymphocyte and eosinophil percentage was also reported to occur after many types of environmental stressors [36]. The higher percentage of neutrophils in the IU-HS and IU-CLPF groups,

along with the lower N/L ratio in the IU-HS group, indicates that prenatal HS continued to carry over to the neonate.

Traditionally, assessment of physiological stress has been estimated by measuring levels of adrenal hormones, mainly cortisol [37]. In our experiment, the IU-HS calves had an elevated cortisol concentration compared with the IU-CL group, but this effect was not independent of maternal reduced feed intake. It has been suggested that alterations in the fetal environment program the fetal hypothalamic-pituitary-adrenal (HPA) axis activity to prepare the neonate to survive in a stressful postnatal environment [38]. Such programming may include mechanisms that lead to alterations in the circulating concentration of catecholamines and cortisol [39]. Maternal HS can alter endocrine dynamics and reduce immune function [2]. The endocrine system, especially the HPA axis, plays an important role in modulating immune function [38]. Although cortisol may not directly inhibit neutrophil function, it seems to moderate immune function in another manner via inducing the apoptosis of lymphoid cells, and inducing a shift from a CD4+- to a CD8+- dominant pattern of immunity [40]. Machado-Nato et al [30] reported an increased serum cortisol concentration in sows and their piglets due to late gestation HS, which is a possible physiological reason for enterocyte maturation and reduced colostral IgG absorption of maternal heat-stressed piglets. Therefore, more studies are needed to evaluate the effects of maternal HS in late gestation on cortisol responses and it is a relation with the compromised immune system caused by maternal HS.

The altered metabolic response of offspring to maternal HS was observed as the alteration in plasma insulin concentration. The plasma insulin concentration was elevated after birth in newborn calves until 2 wk of age. Coupled with similar glucose concentrations, the higher insulin level in the IU-HS group is consistent with previous studies [3,41] that calves born to HS dry cows have higher plasma insulin concentration after colostrum ingestion compared with those from CL cows. It seems that the altered uterine environment along with fetal hyperthermia for the growing fetus, due to maternal acute HS, may have different impacts on peripheral insulin action. By contrast, Limesand et al [42] reported that ovine fetuses experiencing maternal HS during early gestation to mid-gestation had lower insulin concentration compared with the CL group. But in interpreting the differences among the studies in insulin responses, one has to be cautious because in our study all the calves consumed the same pooled colostrum to confound the different maternal effects on colostrum composition. In our study, the IU-HS group was exposed to acute HS in late gestation which may cause different metabolic responses compared with chronic HS. However, larger-scale studies are warranted to confirm or eliminate any carryover effect of maternal HS on metabolic responses.

Cytokines are a class of small proteins that are considered to be key mediators of inflammation, which regulate the development of inflammation and have the ability to increase the endothelial expression of cell adhesion molecules [33]. In our study, the IU-HS group had a lower abundance of *TNFα* mRNA than those in the IU-CLPF and IU-CL groups. Tumor necrosis factor α is a proinflammatory cytokine involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. Similar to our findings, Strong et al [4] reported that maternal HS during late gestation in dairy cows decreased expression of *TNFα* and *TLR2* compared with control calves. Also, Couret et al [43] found that maternal stress during late gestation decreased TNF-α production in piglets. Corroborating our results, Strong et al [4] hypothesized that the lower mRNA abundance of *TNF-α* could be related to an increase in circulating stress hormones in the calves exposed to maternal HS.

5. Conclusions

In conclusion, maternal HS significantly impairs the birth weight of neonate calves independent of reduced maternal feed intake. Calves from CL cows, independent of reduced maternal feed intake, were larger and had more IgG in the circulation compared with those calves born to heat-stressed cows. Maternal HS during the dry period reduced the AEA of immunoglobulins in the neonatal calves, but its effect was not independent of maternal reduced feed intake. The results from the metabolic variables assessed showed that calves born to dry period HS cows had greater blood insulin concentrations during the first 3 wk of life compared with those from CL cows. These results suggest that both maternal HS and maternal reduced feed intake resulted in compromised immune function in dairy calves.

CRediT authorship contribution statement

S.M.M. Seyed. Almoosavi: Conceptualization, Data curation, Writing - original draft. **T. Ghoorchi:** Conceptualization, Investigation, Supervision. **A.A. Naserian:** Conceptualization, Investigation, Supervision. **S.S. Ramezanpor:** Methodology. **M.H. Ghaffari:** Writing - review & editing.

Acknowledgments

References

[1] NRC N. Nutrient requirements of dairy cattle. National Research Council; 2001.
[2] Tao S, Monteiro A, Thompson I, Hayen M, Dahl G. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J Dairy Sci 2012;95:7128–36.

[3] Monteiro A, Tao S, Thompson I, Dahl G. In utero heat stress decreases calf survival and performance through the first lactation. J Dairy Sci 2016;99:8443–50.

[4] Strong R, Silva E, Cheng HW, Eicher S. Acute brief heat stress in late gestation alters neonatal calf innate immune functions. J Dairy Sci 2015;98:7771–83.

[5] Johnson JS, Boddicker RL, Sanz-Fernandez MV, Ross JW, Selsby JT, Lucy MC, Safranski TJ, Rhoads RP, Baumgard LH. Effects of mammalian in utero heat stress on adolescent body temperature. Int J Hyperther 2013;29:696–702.

[6] Mastorci F, Vicentini M, Viltart O, Manghi M, Graiani G, Quaini F, Meerlo P, Nalivaiko E, Maccari S, Sgoifo A. Long-term effects of prenatal stress: Changes in adult cardiovascular regulation and sensitivity to stress. Neurosci Biobehav Rev 2009;33:191–203.

[7] Oakes GK, Walker AM, Ehrenkranz RA, Cefalo RC, Chez RA. Utero-placental blood flow during hyperthermia with and without respiratory alkalosis. J Appl Physiol 1976;41:197–201.

[8] Alexander G, Williams D. Heat stress and development of the conceptus in domestic sheep. J Agric Sci 1971;76:53–72.

[9] Do Amaral B, Connor E, Tao S, Hayen M, Bubolz J, Dahl G. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. J Dairy Sci 2011;94:86–96.

[10] Monteiro A, Tao S, Thompson I, Dahl G. Effect of heat stress during late gestation on immune function and growth performance of calves: Isolation of altered colostral and calf factors. J Dairy Sci 2014; 97:6426–39.

[11] Merlot E, Quesnel H, Prunier A. Prenatal stress, immunity and neonatal health in farm animal species. Animal 2013;7:2016–25.

[12] Skibiel AL, Peñagaricano F, Amorín R, Ahmed BM, Dahl GE, Laporta J. In utero heat stress alters the offspring epigenome. Sci Rep 2018;8: 14609.

[13] Van laer E, Tuyttens FAM, Ampe B, Sonck B, Moons CPH, Vandaele L. Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate. Animal 2015;9:1547–58.

[14] Iranian Council of Animal Care. Guide to the Care and Use of Experimental Animals, 1. Isfahan, Iran: Isfahan University of Technology; 1995.

[15] Dikmen S, Hansen P. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J Dairy Sci 2009;92:109–16.

[16] Khan ZI, Ashraf M, Hussain A. Evaluation of macro mineral contents of forages: Influence of pasture and seasonal variation. Asian-Australasian J Anim Sci 2007;20:908–13.

[17] Kertz A, Chester-Jones H. Invited review: Guidelines for measuring and reporting calf and heifer experimental data. J Dairy Sci 2004;87: 3577–80.

[18] Satchithanandam S, Fritsche J, Rader JI. Extension of aoac official method 996.01 to the analysis of standard reference material (srm) 1846 and infant formulas. J Aoac Int 2001;84:805–13.

[19] Van Soest Pv, Robertson J, Lewis B. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.

[20] Benzie IF, Strain J. [2] ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology. Elsevier; 1999. p. 15–27.

[21] Hong Y-L, Yeh S-L, Chang C-Y, Hu M-L. Total plasma malondialdehyde levels in 16 taiwanese college students determined by various thiobarbituric acid tests and an improved high-performance liquid chromatography-based method. Clin Chem 2000;33:619–25.

[22] Kollmann MT, Locher M, Hirche F, Eder K, Meyer HHD, Bruckmaier RM. Effects of tryptophan supplementation on plasma tryptophan and related hormone levels in heifers and dairy cows. Domest Anim Endocrin 2008;34:14–24.

[23] Quigley Iii J, Drewry J. Nutrient and immunity transfer from cow to calf pre-and postcalving. J Dairy Sci 1998;81:2779–90.

[24] Moyes AJ, Khambata RS, Villar I, Bubb KJ, Baliga RS, Lumsden NG, Xiao F, Gane PJ, Rebstock A-S, Worthington RJ. Endothelial c-type natriuretic peptide maintains vascular homeostasis. J Clin Invest 2014;124:4039–51.

[25] Bienhoff S, Allen G. Quantitation of bovine TNF-a mRNA by reverse transcription and competitive polymerase chain reaction amplification. Vet Immunol Immunop 1995;44:129–40.

[26] Pareek R, Wellnitz O, Van Dorp R, Burton J, Kerr D. Immunorelevant gene expression in LPS-challenged bovine mammary epithelial cells. Theor Appl Genet 2005;46:171–7.

[27] Taylor D, Badiani P, Weston K. A dominant interfering Myb mutant causes apoptosis in T cells. Gene Dev 1996;10:2732–44.

[28] Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod 2010;83: 325–31.

[29] Tudor G. Effect of pre-and post-natal nutrition on the growth of beef cattle i. The effect of nutrition and parity of the dam on calf birth weight. Aust J Agric Res 1972;23:389–95.

[30] Machado-Neto R, Graves CN, Curtis SE. Immunoglobulins in piglets from sows heat-stressed prepartum. J Anim Sci 1987;65:445–55.

[31] Ahmed R, Abdel-Latif M, Mahdi EA, Khalid A. Immune stimulation improves endocrine and neural fetal outcomes in a model of maternofetal thyrotoxicosis. Int Immunopharmacol 2015;29: 714–21.

[32] Laporta J, Fabris T, Skibiel A, Powell J, Hayen M, Horvath K, Miller-Cushon E, Dahl G. In utero exposure to heat stress during late gestation has prolonged effects on the activity patterns and growth of dairy calves. J Dairy Sci 2017;100:2976–84.

[33] Kampen AH, Tollersrud T, Lund A. Flow cytometric measurement of neutrophil respiratory burst in whole bovine blood using live staphylococcus aureus. J Immunol Methods 2004;289:47–55.

[34] Elbim C, Lizard G. Flow cytometric investigation of neutrophil oxidative burst and apoptosis in physiological and pathological situations. Cytometry A 2009;75:475–81.

[35] Mitlohner F, Galyean M, McGlone J. Shade effects on performance, carcass traits, physiology, and behavior of heat-stressed feedlot heifers. J Anim Sci 2002;80:2043–50.

[36] Radostist D, Blood D, Gay C. Veterinary medicine. 8ᵃ. London UK: Bailliere Tindal; 1763.

[37] Veissier I, De La Fe AR, Pradel P. Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. Appl Anim Behav Sci 1998;57:35–49.

[38] Matthews SG. Early programming of the hypothalamo–pituitary–adrenal axis. Trends Endocrinol Metab 2002;13:373–80.

[39] Littlejohn B, Price D, Banta J, Lewis A, Neuendorff D, Carroll J, Vann R, Welsh Jr T, Randel R. Prenatal transportation stress alters temperament and serum cortisol concentrations in suckling brahman calves. J Anim Sci 2016;94:602–9.

[40] Buford TW, Willoughby DS. Impact of DHEA (S) and cortisol on immune function in aging: a brief review. Appl Physiol Nutr Me 2008;33:429–33.

[41] Tao S, Dahl G. Invited review: heat stress effects during late gestation on dry cows and their calves. J Dairy Sci 2013;96:4079–93.

[42] Limesand SW, Rozance PJ, Zerbe GO, Hutton JC, Hay WW. Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. Endocrinology 2006;147: 1488–97.

[43] Couret D, Jamin A, Kuntz-Simon G, Prunier A, Merlot E. Maternal stress during late gestation has moderate but long-lasting effects on the immune system of the piglets. Vet Immunol immunopathology 2009;131:17–24.