Effect of maternal heat stress during the dry period on growth and metabolism of calves

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ABSTRACT

Preliminary studies suggest that maternal heat stress (HS) during late gestation exerts carryover effects on a calf's insulin response after weaning, but a comprehensive evaluation of how maternal HS affects calf intake, growth and metabolic response from birth to weaning is lacking. Our objective was to evaluate the effects of maternal HS during the dry period on dry matter intake, growth and metabolism from birth to weaning. After birth, 20 heifers born to either HS (n = 10) or cooled (CL, n = 10) dry cows were immediately separated from their dams and fed 3.8 L of colostrum from a common pool within 4 h of birth. All heifers were managed identically and weaned at 49 d of age (DOA). Calf starter intake was recorded daily, and body weight was assessed at birth and every 2 wk from birth to 56 DOA. Blood samples were collected twice a week until 56 DOA to assess hematocrit and concentrations of insulin and metabolites. To evaluate metabolic responses to maternal HS, a glucose tolerance test, insulin and epinephrine challenge were performed on 3 consecutive days for all heifers at 8, 29, and 57 DOA. Maternal HS during the dry period did not affect heifer birth weight. Compared with HS, CL calves consumed more starter (0.53 vs. 0.34 kg/d) from birth to 56 DOA and were heavier (71.7 vs. 61.4 kg) at 56 DOA. Relative to HS calves, CL calves tended to have higher hematocrit (27.4 vs. 24.7%). There were no differences between treatments in plasma concentrations of insulin and glucose, but HS calves had higher NEFA and BHBA concentrations after 32 DOA. Compared with CL, HS calves had a faster glucose clearance after a glucose tolerance test and a

slower insulin clearance after an insulin challenge. In conclusion, maternal HS during late gestation reduces calf starter intake and growth, alters blood metabolite profile and increases non-insulin dependent glucose uptake.

Key words: heat stress, glucose metabolism, dairy calves

INTRODUCTION

Heat stress (**HS**) during the dry period has tremendous effects on a cow's lactational performance in the subsequent lactation and on her immune competence and metabolic adaptation during the transition period (do Amaral et al., 2010; 2011; Tao et al., 2012b). Additionally, the impacts of late gestation maternal HS are carried over to postnatal life of the calf. Previous studies indicate that a calf that experiences in utero heat stress has impaired passive and cell-mediated immune function before weaning (Tao et al., 2012a; Monteiro et al., 2014), suggesting that maternal HS during the dry period compromises the health of the offspring. Late gestation HS also exerts long term impacts on progeny performance up to and through the first lactation. Indeed, heifers born to heat-stressed dams have a greater chance of leaving the herd before puberty due to sickness, malformation or growth retardation compared with those from cooled dry cows (Monteiro et al., 2013). Interestingly, heifers born to HS dry cows also had a greater number of services per conception confirmed 30 d after insemination and lower milk production in the first lactation relative to those from CL cows (Monteiro et al., 2013). However, the physiological mechanisms of the impacts of maternal thermal insult on postnatal performance of the offspring are not clear.

Maternal heat stress also alters postnatal metabolism of the offspring. Lambs born to ewes heat-stressed in mid gestation develop higher insulin response to glucose (Yates et al., 2011) and lower lipolytic response to adrenergic stimulation compared with those from thermo-neutral

dams (Chen et al., 2010). In the dairy calf, although growth rates were similar during the pre-pubertal period (Monteiro et al., 2013), calves born to HS dry cows have enhanced glucose uptake after weaning, as evidenced by more rapid glucose clearance after a glucose tolerance test and an insulin challenge (Tao et al., 2014). However, it is unknown if this altered glucose metabolism after weaning by prenatal HS is developed in utero or during the postnatal stage due to variable nutrient consumption. Tao and Dahl (2013) observed that calves born to HS dry cows had a higher serum insulin concentration after colostrum ingestion during the first days of life compared with those from CL cows, which suggests a stronger pancreatic response to colostral lactose ingestion or a greater insulin resistance in peripheral tissues. Additionally, the impacts of maternal HS on calf starter intake, blood metabolites and insulin during the pre-weaning period are unknown. Our hypothesis was that maternal HS during the dry period alters calf metabolism during the pre-weaning period. The objective of the present study was to examine the impact of maternal HS during late gestation on heifer calf dry matter intake, growth and metabolism.

MATERIALS AND METHODS

Animals and Experimental Design

The University of Florida Institute of Food and Agricultural Sciences Animal Research Committee approved the treatments and animal handling prior to beginning the trial. The animal study was conducted at the Dairy Unit and Calf Unit of the University of Florida from August to December, 2014. Briefly, at approximately 45 d before expected calving, multiparous Holstein cows were dried-off and randomly assigned to one of two groups, HS or cooling (**CL**). All cows were housed in the same barn, but the stall areas for CL cows were equipped with fans and soakers, whereas those for HS were not. The ambient temperature and relative humidity in the stall areas for HS and CL cows were measured every 15 min by Hobo Pro Series Temp probes (Onset Computer Corporation, Pocasset, MA) during the entire dry period, and the temperature-

humidity index (**THI**) was calculated based on Dikmen et al. (2008). Rectal temperature was assessed (1430 h) and respiratory rate was determined (1500 h) on a daily basis during the dry period.

Only heifer calves (HS, n = 10 and CL, n = 10) were enrolled in the current study. After calving, all calves were immediately removed from their dams and fed 3.8 L of colostrum from the same pool within 4 h after birth by esophageal feeder. Calves were housed in individual wire hutches on sand bedding and managed in the same manner thereafter. The day of birth was considered as 0 days of age (**DOA**).

Intake, Growth Measures and Sample Collection

From 1 DOA, calves were fed 6 L/d of pasteurized milk divided into two equal feedings, in the morning (0700 h) and in the afternoon (1700 h), until 41 DOA, and then only in the morning (3 L/d) until weaning at 49 DOA. Samples of pasteurized milk were collected thrice weekly (AM and PM; Sun, Tue, Thu) throughout the study and analyzed for concentrations of lactose (4.7 ± 0.32%), fat (3.25 ± 0.42%), protein (3.37 ± 0.24%) and SCC (855 ± 447 × 10^3/mL) by a Bentley 2000 NIR analyzer at the DHIA Laboratory (Belleview, FL). Calf starter (Cornerstone, Purina Feed, Grey Summit, MO) and water were offered ad libitum starting at 1 DOA. The amount of starter offered and refused (~10%) were recorded daily for each calf. Samples of the starter were collected once weekly and dried at 55 °C for 48 h to determine the moisture content.

The BW, withers height, heart girth and hip height were measured at birth, 14, 28, 42 and 56 DOA, before morning feeding to evaluate growth. Blood samples were collected via jugular venipuncture into sodium-heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) at 1, 4, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49, 53, and 56 DOA before morning feeding and immediately placed in ice. Hematocrit was assessed and then samples were centrifuged at 2,619 × g at 4 °C for 30 min and plasma collected.

Metabolic Tests

All heifers were subjected to the metabolic tests consisting of an intravenous glucose tolerance test (**GTT**), insulin challenge (**IC**) and adrenaline challenge (**AC**) at 8, 29 and 57 DOA. The three metabolic tests were performed on 3 consecutive d in a randomized sequence. The actual dates were not different between treatments ($P > 0.7$) and averaged 8.8 ± 0.4, 30.1 ± 0.4, and 58.0 ± 0.5 DOA for GTT; 8.9 ± 0.4, 30.0 ± 0.4, and 58.1 ± 0.5 DOA for IC; and 9.1 ± 0.3, 30.7 ± 0.3, and 58.1 ± 0.4 DOA for AC. Animals were fasted overnight before the metabolic tests. A catheter (16 gauge × 7.5 cm, Extended Use MILACATH, MILA International, Inc., Erlanger, KY) was inserted into the jugular vein of each calf at least 1 h before the first metabolic test.

During GTT, 0.3 g/kg BW (Stanley et al., 2002; Yari et al., 2010) of glucose (dextrose 50%, wt/vol; Phoenix Scientific Inc., St. Joseph, MO) was infused into the jugular vein through the catheter followed by 10 mL of sterile saline solution to flush the catheter. Blood samples were drawn through the catheter, at –15, –5, and 0 min relative to the starting point of glucose infusion and 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120 min relative to the ending point of glucose infusion, into Vacutainer tubes containing sodium fluoride and potassium oxalate (Becton Dickinson). Samples were immediately placed in ice followed by centrifugation at 2,619 × g at 4 °C for 15 min to collect plasma. The catheter was flushed with sterile saline containing sodium heparin between samplings to avoid clotting and the first 2 mL of blood collected was discarded before each sampling.

The procedures and bleeding regimens for IC and AC were similar to GTT, except that 0.1 IU of insulin/kg of BW (100 IU/mL, human insulin, rDNA origin, Eli Lilly and Company,

Indianapolis, IN) was administrated during IC and 1.4 µg of epinephrine/kg of BW (1 mg/mL, International Medication Systems, Limited, South El Monte, CA) was infused during AC.

Insulin and Metabolite Analyses

Plasma concentrations of glucose (Autokit Glucose; Wako Chemicals USA, Inc., Richmond, VA), NEFA (HR Series NEFA-HR(2), Wako Chemicals USA, Inc., Richmond, VA), and BHBA (Autokit 3-HB, Wako Chemicals USA, Inc., Richmond, VA) were determined by colorimetric methods, and the inter- and intra-assays CV were 6.6 and 3.2%, 6.1 and 2.3%, and 7.8 and 7.8%, respectively. Insulin concentrations in the plasma were measured by radioimmunoassay (Malven et al., 1987), and the inter- and intra-assays CV were 10 and 7.1%, respectively.

Calculation and Statistical Analyses

The UNIVARIATE procedure of SAS 9.4 (SAS Institute, Cary, NC) was used to analyze the composition of pasteurized milk and the means ± SEM are reported. The dry period and gestation lengths of the dams and the heifer DOA at each metabolic test were subjected to ANOVA using the GLM procedure of SAS 9.4 and the LSM ± SEM are reported. Repeated measures data, such as starter intake, growth measures, hematocrit, and plasma concentrations of metabolites and insulin, were analyzed using the MIXED procedure of SAS 9.4. The statistical model included fixed effects of treatment, time and treatment by time with calf (treatment) as the random effect and the LSM ± SEM were reported.

For the metabolic tests analyses, the average concentration of a metabolite or insulin for the samples collected at -15, -5 and 0 min related to the infusion was used as a baseline value. The area under curve (**AUC**) between times was calculated based on the trapezoidal method, in which the metabolite or insulin concentration value was calculated by subtracting the baseline value from the actual value. The accumulated AUC of metabolites and insulin were calculated

from 5-30 min, 5-60 min and 5-120 min for all metabolic tests and were analyzed by the MIXED procedure of SAS 9.4. The statistical model included fixed effects of treatment, DOA and treatment by DOA with calf (treatment) as the random effect and the LSM ± SEM were reported. For each metabolic test within each test day, the plasma concentration of metabolites and insulin were analyzed using the MIXED procedure of SAS 9.4. The SAS model included fixed effects of treatment, min relative to infusion and treatment by min relative to infusion with calf (treatment) as the random effect, and the baseline value of metabolites or insulin was included in the SAS model as covariates. The LSM ± SEM were reported. Significance and tendency were declared when $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

RESULTS

Data of the dams

Cows involved in the current experiment were from a larger study (Ahmed and Dahl, unpublished). Briefly, the THI in the stall areas for dry cows were similar between treatments and averaged 77.7, however, HS cows had higher rectal temperature (39.28 vs. 38.95 °C, SEM = 0.02 °C) and respiration rate (66.7 vs. 49.1 breaths/min, SEM = 3.3 breaths/min) compared with CL cows. Both groups of cows had similar gestation length (HS: 276.3 d; CL: 276.1 d, SEM = 2.0 d), and dry period length (HS: 41.8 d; CL: 41.5 d, SEM = 0.9 d).

Growth and Intake

No differences ($P > 0.15$) were observed in birth weight, or BW, withers height, heart girth, and hip height from birth to 56 DOA between calves born to HS or CL cows (Table 1). However, there was a treatment by time interaction ($P < 0.01$) for BW from birth to 56 DOA, such that CL calves were heavier ($P < 0.01$) at 56 DOA compared with HS calves. As a result, the ADG was lower ($P < 0.05$) for HS calves from 29 to 56 DOA and from 0 to 56 DOA, but similar ($P = 0.24$)

from 0 to 28 DOA compared with CL calves. The amount of starter intake was considerable only after 4 wk of age (Figure 1). Calves born to HS cows consumed less (treatment by time interaction: $P < 0.01$) starter from 5 to 8 wk of age compared with those from CL cows.

Hematocrit, Insulin and Metabolite Concentrations

Compared with CL, calves born to HS cows tended ($P = 0.07$) to have a lower hematocrit from 1 to 56 DOA (Figure 2). No treatment effects ($P > 0.30$) were observed for plasma insulin or glucose concentrations from 1 to 56 DOA (Figure 3), but there was a tendency ($P = 0.07$) for treatment by time interaction for plasma glucose such that HS calves had lower glucose concentration at 14, 25 and 53 DOA compared with CL calves. Compared with CL, HS calves had similar plasma concentrations of NEFA and BHBA before 32 DOA, but elevated plasma NEFA and BHBA after 32 DOA (treatment by time interaction: $P < 0.05$; Figure 3).

Metabolic tests

Relative to those born to CL cows, HS calves had lower ($P < 0.05$) glucose AUC_{60} and AUC_{120} after GTT (Table 2, Figure 4). However, no treatment effects were observed for insulin AUC to GTT (Table 2). During IC, although no differences ($P > 0.15$) were observed for glucose AUC (Table 3), HS calves had higher ($P < 0.05$) insulin AUC compared with CL (Table 3, Figure 5) and a tendency ($P = 0.07$) for treatment by time interaction for AUC_{30}, such that HS calves had a higher ($P < 0.01$) AUC_{30} at 8 DOA compared with CL. Additionally, HS calves had a higher ($P = 0.04$) NEFA AUC_{60} compared with CL (Table 3, Figure 6). Maternal heat stress had no impact ($P > 0.10$) on the calves glucose or NEFA responses to AC (Table 4).

DISCUSSION

Similar to previous studies using the same experimental model (Tao et al., 2012b; Thompson et al., 2014), in the current experiment the cooling system was effective in abatement of heat

stress and reduced the heat strain of cows during the dry period, as indicated by the lower rectal temperature and respiration rate of CL cows compared with HS cows. These differences in physiological indicators of heat stress suggest that the experimental model produced a maternal uterine environment consistent with hyperthermia and therefore an effective HS treatment was achieved.

Unexpectedly, there was no difference in calf birth weight between treatments, which is in contradiction with previous reports (do Amaral et al., 2011; Tao et al., 2011; Monteiro et al., 2014). Tao et al. (2012a) suggest that the lower birth weight associated with late gestation maternal heat stress is due to a combination of shorter gestation length, direct impact of fetal hyperthermia and fetal growth retardation by maternal heat stress induced impairment of placental function. In this regard, in the current experiment, similar gestation length between HS and CL dams provided fetuses from both groups with similar time to grow. Also, similar calf birth weight between treatments suggests that the extent of changes in the uterine thermo-environment and placental function by maternal heat stress was not sufficient to reduce the somatic growth of the fetus, or the fetus metabolically adapted to the undesirable uterine environment, or both. Indeed, in the current study, the difference in hematocrit of calves born to HS and CL cows suggests that the in utero environment was altered by maternal heat stress. Exposure of the ewes to heat stress causes uterine hypoxia (Regnault et al., 2007) due to impaired placental function, and causes a reduction in fetal metabolic heat production (Laburn et al., 2002) in order to cope with the fetal hyperthermia. Thus, the increased hematocrit of HS calves relative to CL calves during the preweaning period may indicate a carryover effect from in utero hypoxia and reduction in fetal metabolic rate caused by maternal heat stress. This

hypothesis has never been confirmed in a bovine model, but suggests that fetal development was altered by the maternal heat stress, which in turn influenced calf postnatal performance.

Different from previous research (Tao et al., 2012a; Monteiro et al., 2014), in which both groups of calves had similar growth rate from birth to weaning, in the current study, heifers born to HS dry cows had lower starter intake and, as a consequence, lower ADG and BW at 56 DOA compared with those from CL dry cows. Reasons for the discrepancy between studies are unknown, but the data in this specific study indicate that maternal heat stress exerts carryover effects on calf postnatal growth and, perhaps, on nutrient absorption and utilization. Compared with those from CL cows, heifers from HS cows had greater plasma concentrations of NEFA and unexpected and surprisingly higher BHBA concentrations after 32 DOA, without a change in plasma insulin. The higher NEFA and BHBA concentrations of calves born to HS compared with CL dams coincide with the difference in starter intake between treatments, but are in contradiction with the traditional view that a calf with lower starter intake has less ruminal BHBA production, and hence a lower plasma BHBA concentration (Quigley and Bernard, 1992). These unusual plasma metabolite profiles may suggest that calves from HS dams absorb and utilize less free fatty acid and ketones as energy sources compared with those from CL dams; in other words, to support the higher growth rate, CL calves may have taken more NEFA and BHBA from blood to provide energy compared with HS. Additionally, these data may indicate that maternal heat stress alters the calf's preference of energy sources, such that calves from HS cows prefer to use glucose rather than fatty acid or ketones compared with those from CL cows, especially after the percentage of energy provided by starter intake increased. Consistent with that idea, both groups of calves had a similar growth rate during the first 28 DOA days of age, when most of the ingested energy was from milk lactose and lipids. Although no overall

treatment effect was observed, the tendency for occasional lower plasma glucose concentration during the pre-weaning period of HS calves relative to CL also suggests a higher preference for glucose utilization. During lactation, the HS dairy cow develops a preference to use glucose as a fuel rather than partitioning it toward milk synthesis (Wheelock et al., 2010). Thus, it seems that maternal heat stress during late gestation results in a similar phenomenon in the fetus, which carries over into postnatal life. Moreover, the time of blood sample collection could account for some of the difference in blood metabolites observed in the present study. Quigley and Bernard (1992) reported that plasma concentration of BHBA is lower in blood samples collected before feeding compared with 2 h after feeding, and the difference between treatments (milk vs. solid feeding) is more pronounced at 2 h after feeding. Thus, the bleeding schedule (before morning feeding) of the current study may also mask some of the potential effects of the different starter intake between HS and CL calves on plasma BHBA concentration.

Coupled with similar glucose responses, the slower insulin clearance of HS calves after insulin infusion indicate a relatively high level of insulin resistance in peripheral tissues, such as muscle and adipose tissue, and thus limited insulin-mediated glucose entry into the peripheral tissues, compared with CL. Additionally, the slower insulin clearance to IC observed in HS calves relative to CL tended to be more pronounced at an earlier age, such as at 8 DOA, which is consistent with the observation of Tao and Dahl (2013) that calves born to HS dry cows have higher plasma insulin concentration after colostrum ingestion, at 1 d after birth, compared with those from CL cows. In contrast, Tao et al. (2014) utilized a similar experimental model as in the current experiment, but observed that weaned calves born to HS dry cows had more rapid glucose but similar insulin clearance after IC compared with those from CL cows. However, different from Tao and Dahl (2013) and the current experiment, in which only heifer calves were

used, there was a small and unbalanced number of bulls and heifers within each treatment in the experiment conducted by Tao et al. (2014). As reviewed by Kapoor et al. (2006), male and female offspring respond very differently to prenatal stress across different species. Therefore, the discrepancy between studies may indicate that bulls and heifers respond to late gestation maternal heat stress differently, especially regarding the insulin action on peripheral tissues. The physiological and molecular mechanisms of enhanced insulin resistance in the offspring after maternal heat stress are unknown. In weaned dairy bull calves, hyperthermia induced by heat stress had no impact on insulin and glucose responses to an IC (O'Brien et al., 2010), indicating that heat stress has no impact on peripheral insulin resistance in growing cattle. However, for the rapidly growing fetus, the altered uterine environment coupled with fetal hyperthermia due to maternal heat stress may have different influences on peripheral insulin action. The more pronounced difference in insulin clearance after IC between treatments at an early age is intriguing and may suggest that calves from HS cows gradually adapt to the extra-uterine environment and the tissue insulin resistance induced by maternal heat stress is diminished as animals grow during postnatal development.

In contrast to the effects on insulin, compared with those from CL cows, heifers born to HS dry cows had a more rapid glucose clearance after glucose infusion with similar insulin responses, which is consistent with the previous observation after weaning (Tao et al., 2014) and suggests a higher capacity for glucose disposal, but similar pancreatic insulin release to glucose stimulation in heifers from HS cows relative to CL during the preweaning period. Glucose tolerance after a GTT is determined by a combination of the insulin response after glucose infusion, insulin sensitivity in peripheral tissues, and the non-insulin dependent glucose disposal due to a mass action (Kahn et al., 1994). With the similar insulin responses after GTT and higher

level of insulin resistance observed in IC, the data in the current study indicate that late gestation maternal heat stress improves the offspring's postnatal insulin-independent glucose absorption and basal glucose uptake (Kahn et al., 1994), which partly supports the hypothesis that heifers from HS cows have a preference to use glucose as a fuel compared with those from CL cows, as discussed above. Thus, these data suggest an enhanced expression of the non-insulin dependent glucose transporter (**GLUT**) in calves from HS dams relative to CL. Among the facilitated GLUT that are insulin independent, GLUT1 is the ubiquitous form expressed in cells and tissues and responsible for basal glucose uptake (Hocquette and Abe, 2000; Zhao and Keating, 2007). In contrast to GLUT4, whose expression in muscle and adipose tissue decreases in the calf from birth to 1 yr of age, the protein expression of GLUT1 in peripheral tissues is constant (Abe et al., 2001). Thus, the maternal thermal insult may alter the GLUT1 expression of the fetus and that altered expression profile may persist into postnatal life, at least during the preweaning period. However, the design of the current experiment limits the ability to distinguish the possible different expression of the GLUT on the tissue level, but that question deserves further exploration.

Fatty acid metabolism of the fetus can be altered by in utero environment, and that in turn influences postnatal adipose tissue deposition (Sarr et al., 2012). In the ewe, maternal heat stress during early to mid-gestation results in decreased expression of β_2-AR mRNA and protein in perirenal adipose tissue and reduces the NEFA release after an AC in the lamb at 21 DOA, suggesting a compromise of adipose tissue mobilization caused by maternal heat stress during early to mid-pregnancy (Chen et al., 2010). In contrast, in the current experiment, both groups of calves had similar glucose and NEFA responses to AC, which indicates that maternal heat stress during late gestation in cattle has no impact on adrenaline mediated fat mobilization and

glycogenolysis in the liver and muscle of the female offspring, or at least during the preweaning period. The reason for the discrepancy between studies is unknown, but may be due to the species differences, the time frame and extent of maternal heat stress applied, or a combination of those effects. In addition to epinephrine, fatty acid metabolism is controlled by insulin, which serves as an anti-lipolytic factor and enhances lipogenesis (Hayirli, 2006). In the present study, the similar NEFA response to the IC before the nadir of the NEFA concentration (~ 30 min after IC), suggests that there was no difference between heifers from HS and CL cows regarding insulin action on lipolysis or lipogenesis, whereas the higher response of heifers born to HS cows compared with CL after NEFA nadir may indicate a stronger negative feedback on lipolysis by glucagon and epinephrine after insulin infusion. With the similar NEFA response after AC between HS and CL calves, it is unlikely that the adipose tissue responsiveness to epinephrine plays a role in the observed NEFA response during IC. However, the possibility of a more pronounced epinephrine release after IC in HS calves compared with CL cannot be excluded. Similar to epinephrine, the release of glucagon is increased after a drop in blood glucose concentration to stimulate gluconeogenesis and glycogenolysis and restore the euglycemia, and it also stimulates an acute lipolysis (Vaughan and Steinberg, 1963). Thus, increased glucagon release and/or enhanced adipose tissue responsiveness to glucagon may partly explain the stronger NEFA responses to AC in heifers from HS cows compared with those from CL, but such hypothesis has never been evaluated. Nevertheless, the data indicate that maternal heat stress during the late gestation alters heifer's fatty acid metabolism in their early life, which, however, is insulin and epinephrine independent.

It has been extensively studied that maternal HS during the dry period impairs calf immunity (Tao et al., 2012; Monteiro et al., 2014) during the pre-weaning period and lowers heifer survival

later in the life (Monteiro et al., 2013). Further, heifers born to dry period HS cows produce less milk during their first lactation compared with those from CL dams (Monteiro et al., 2013), however, the physiological mechanism is still unknown and could result from the altered metabolism. In other livestock species, various maternal insults (Arnott et al., 2012; Antolic et al., 2015), including heat stress (Chen et al., 2010; Boddicker et al., 2014), alter neonatal metabolism and future performance. Yet, such research in dairy cattle is surprisingly scarce. In the current study, the possible increase in usage of glucose in the heifers born to HS cows compared with CL is intriguing and indicates that late gestation maternal heat stress influences calf glucose metabolism throughout the entire pre-weaning period. Abe et al. (2001) reported that GLUT1 protein expression in the peripheral tissues of calves persists during postnatal life up to 1 yr of age, suggesting that the increased glucose uptake associated with late gestation maternal heat stress may persist into adulthood and, in turn, influence heifer body growth and composition and, perhaps, milk synthesis by limiting the milk lactose biosynthesis during lactation. Further studies need to be conducted to examine the impact of maternal heat stress on calf metabolism beyond the preweaning period.

CONCLUSIONS

Maternal heat stress during the dry period reduces heifer calf grain intake and growth during the preweaning period. Combined with altered basal metabolites profiles, the results from the metabolic tests suggest that heifers born to dry period HS cows have a higher insulin-independent glucose disposal and altered fatty acid metabolism during the postnatal period compared with those from CL cows.

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REFERENCES

Abe, H., Y. Kawakita, K. Hodate, and M. Saito. 2001. Postnatal development of glucose transporter proteins in bovine skeletal muscle and adipose tissue. J. Vet. Med. Sci. 63:1071-1075.

Antolic, A., X. Feng, C. E. Wood, E. M. Richards, and M. Keller-Wood. 2015. Increased maternal nighttime cortisol concentrations in late gestation alter glucose and insulin in the neonatal lamb. Physiol Rep. 3:e12548-e12561.

Arnott, G., D. Roberts, J. A. Rooke, S. P. Turner, A. B. Lawrence, and K. M. D. Rutherford. 2012. BOARD INVITED REVIEW: The importance of the gestation period for welfare of calves: Maternal stressors and difficult births. J. Anim. Sci. 90:5021-5034.

Boddicker, R. L., J. T. Seibert, J. S. Johnson, S. C. Pearce, J. T. Selsby, N. K. Gabler, M. C. Lucy, T. J. Safranski, R. P. Rhoads, L. H. Baumgard, and J. W. Ross. 2014. 2014. Gestational heat stress alters postnatal offspring body composition indices and metabolic parameters in pigs. PLoS ONE. 9:e110859.

Chen, X., A. L. Fahy, A. S. Green, M. J. Anderson, R. P. Rhoads, and S. W. Limesand. 2010. β2-Adrenergic receptor desensitization in perirenal adipose tissue in fetuses and lambs with placental insufficiency-induced intrauterine growth restriction. J. Physiol. 588:3539-3549.

Dikmen, S., E. Alava, E. Pontes, J. M. Fear, B. Y. Dikmen, T. A. Olson, and P. J. Hansen. 2008. Differences in thermoregulatory ability between slick-haired and wild-type lactating Holstein cows in response to acute heat stress. J. Dairy Sci. 91:3395-3402.

do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. Bubolz, and G. E. Dahl. 2009. Heat-stress abatement during the dry period: Does cooling improve transition into lactation? J. Dairy Sci. 92:5988-5999.

do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. Bubolz, and G. E. Dahl. 2010. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. Domest. Anim. Endocrinol. 38:38-45.

do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. Bubolz, and G. E. Dahl. 2011. 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. 94:86-96.

Hayirli, A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet. Res. Commun. 30:749-774.

Hocquette, J-F., and H. Abe. 2000. Facilitative glucose transporters in livestock species. Reprod. Nutr. Dev. 40:517-533.

Kahn, S. E., R. L. Prigeon, D. K. McCulloch, E. J. Boyko, R. N. Bergman, M. W. Schwartz, J. L. Neifing, W. Kenneth Ward, J. C. Beard, J. P. Palmer, and Jr. Daniel Porte. 1994. The contribution of insulin-dependent and insulin-independent glucose uptake to intravenous glucose tolerance in healthy human subjects. Diabetes. 43:587-592.

Kapoor, A., E. Dunn, A. Kostaki, M. H. Andrews, and S. G. Matthews. 2006. Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. J. Physiol. (Lond.). 572:31-44.

Laburn, H. P., A. Faurie, K. Goelst, and D. Mitchell. 2002. Effects on fetal and maternal body temperatures of exposure of pregnant ewes to heat, cold, and exercise. J. Appl. Physiol. 92:802-808.

Malven, P. V., H. H. Head, R. J. Collier, and F. C. Buonomo. 1987. Periparturient changes in secretion and mammary uptake of insulin and in concentrations of insulin and insulin-like growth factors in milk of dairy cows. J. Dairy Sci. 70:2254-2265.

Monteiro, A. P. A., S. Tao, I. M. Thompson, and G. E. Dahl. 2014. 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. 97:6426-6439.

Monteiro, A. P. A., S. Tao, I. M. Thompson, and G. E. Dahl. 2013. Effect of heat stress in utero on calf performance and health through the first lactation. J. Anim. Sci. 91(Suppl.2):184. (Abstr.)

O'Brien, M. D., R. P. Rhoads, S. R. Sanders, G. C. Duff, and L. H. Baumgard. 2010. Metabolic adaptations to heat stress in growing cattle. Domest. Anim. Endocrinol. 38:86-94.

Quigley, J. D., and J. K. Bernard. 1992. Effects of nutrient source and time of feeding on changes in blood metabolites in young calves. J. Anim. Sci. 70:1543-1549.

Regnault, T. R. H., B. de Vrijer, H. L. Galan, R. B. Wilkening, F. C. Battaglia, and G. Meschia. 2007. Development and mechanisms of fetal hypoxia in severe fetal growth restriction. Placenta. 28:714-723.

Sarr, O., K. Yang, and T. R. H. Regnault. 2012. In utero programming of later adiposity: The role of fetal growth restriction. J. Pregnancy. 2012:134758.

Stanley, C. C., C. C. Williams, B. F. Jenny, J. M. Fernandez, H. G. Bateman, II, W. A. Nipper, J. C. Lovejoy, D. T. Gantt, and G. E. Goodier. 2002. Effects of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. J. Dairy Sci. 85:2335-2343.

Tao, S., J. W. Bubolz, B. C. do Amaral, I. M. Thompson, M. J. Hayen, S. E. Johnson, and G. E. Dahl. 2011. Effect of heat stress during the dry period on mammary gland development. J. Dairy Sci. 94:5976-5986.

Tao, S., J. W. Bubolz, B. C. do Amaral, I. M. Thompson, M. J. Hayen, S. E. Johnson, and G. E. Dahl. 2011. Effect of heat stress during the dry period on mammary gland development. J. Dairy Sci. 94:5976-5986.

Tao, S., and G. E. Dahl. 2013. Invited Review: Heat stress impacts during the dry period on dry cows and their calves. J. Dairy Sci. 96:4079-4093.

Tao, S., A. P. A. Monteiro, M. J. Hayen and G. E. Dahl. 2014. Short Communication: Maternal heat stress during the dry period alters whole body insulin response of calves postnatally. J. Dairy Sci. 97:897-901.

Tao, S., A. P. Monteiro, I. M. Thompson, M. J. Hayen, and G. E. Dahl. 2012a. Effect of late gestation maternal heat stress on growth and immune function of dairy calves. J. Dairy Sci. 95:7128-7136.

Tao, S., I. M. Thompson, A. P. A. Monteiro, M. J. Hayen, L. J. Young, and G. E. Dahl. 2012b. Effect of cooling heat-stressed dairy cows during the dry period on insulin response. J. Dairy Sci. 95:5035-5046.

Thompson, I. M., S. Tao, A. P. Monteiro, K. C. Jeong, and G. E. Dahl. 2014. Effect of cooling during the dry period on immune response after Streptococcus uberis intramammary infection challenge of dairy cows. J. Dairy Sci. 97:7426-7436.

Vaughan, M., and D. Steinberg. 1963. Effect of hormones on lipolysis and esterification of free fatty acids during incubation of adipose tissue in vitro. J. Lipid Res. 4:193-199.

Wheelock, J. B., R. P. Rhoads, M. J. VanBaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644-655.

Yari, M., A. Nikkhah, M. Alikhani, M. Khorvash, H. Rahmani, and G. R. Ghorbani. 2010. Physiological calf responses to increased chromium supply in summer. J. Dairy Sci. 93:4111-4120.

Yates, D. T., A. S. Green, and S. W. Limesand. 2011. Catecholamines mediate multiple fetal adaptations during placental insufficiency that contribute to intrauterine growth restriction: Lessons from hyperthermic sheep. J Pregnancy. 2011:1-9.

Zhao, F-Q., and A. F. Keating. 2007. Expression and regulation of glucose transporters in the bovine mammary gland. J. Dairy Sci. 90(E. Suppl.):E76-E86.

Table 1. Birth weight, grain intake, and growth performance from birth to 56 days of age of calves born to dams exposed to either heat stress (n = 10) or cooling (n = 10) during the dry period

Variable	Heat stress	Cooling	SEM	*P*-value
Birth weight, kg	35.7	36.3	1.5	0.79
Grain DMI, kg/d	0.34	0.53	0.07	0.05
Body weight, kg	48.3	52.0	2.0	0.19
Withers height, cm	78.5	79.1	0.9	0.64
Heart girth, cm	83.7	85.6	1.1	0.24
Hip height, cm	82.0	81.8	1.0	0.90
ADG, kg/d				
0-28 d	0.43	0.49	0.04	0.24
29-56 d	0.49	0.78	0.07	0.02
0-56 d	0.46	0.63	0.04	0.01

Table 2. Insulin and glucose responses to glucose tolerance tests of calves born to dams exposed to either heat stress (HS, n = 9) or cooling (CL, n = 10) during the dry period

	8[1]		29		57			*P*-value		
	HS	CL	HS	CL	HS	CL	SEM	TRT[2]	Day	TRT×Day
Insulin AUC[3] (ng×min/mL)										
30 min	47	36	13	17	23	24	14	0.14	< 0.01	0.99
60 min	62	47	24	27	33	38	17	0.15	< 0.01	0.96
120 min	46	44	35	36	32	51	18	0.81	0.15	0.20
Glucose AUC (mg×min/dL)										
30 min	1344	1456	1675	1721	1899	1922	66	0.36	< 0.01	0.74
60 min	1285	2010	2391	2724	2619	3247	187	< 0.01	< 0.01	0.44
120 min	766	1398	2261	2430	2364	3777	410	0.03	< 0.01	0.36

[1]Days of age.
[2]TRT = treatment.
[3]AUC = area under the curve

Table 3. Insulin, glucose and NEFA responses to insulin challenges of calves born to dams exposed to either heat stress (HS, n = 9) or cooling (CL, n = 10) during the dry period

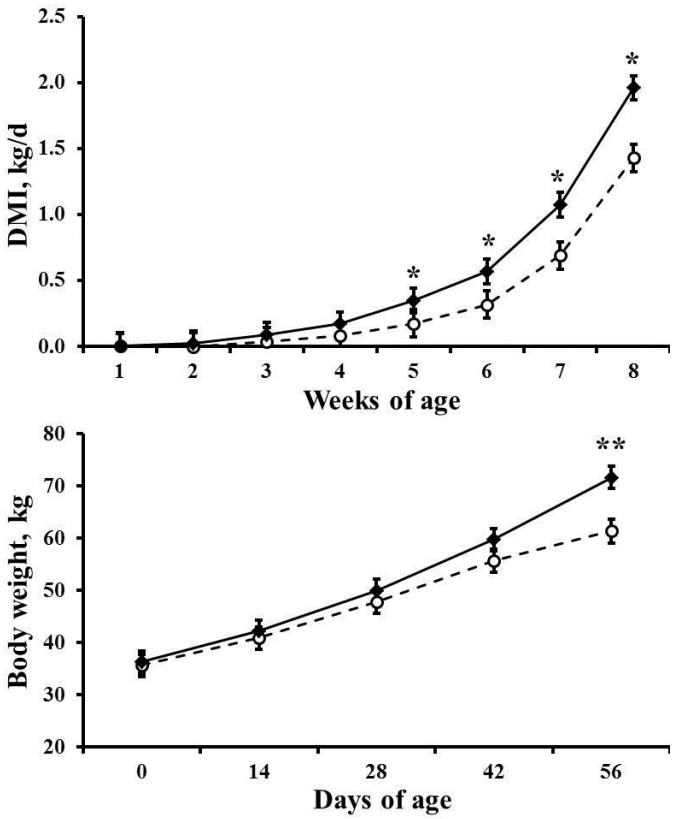
	8[1]		29		57			*P*-value		
	HS	CL	HS	CL	HS	CL	SEM	TRT[2]	Day	TRT×Day
Insulin AUC[3] (ng×min/mL)										
30 min	83	58	54	51	54	47	5	0.01	< 0.01	0.07
60 min	101	72	73	60	67	55	8	0.01	< 0.01	0.51
120 min	110	81	89	61	75	55	15	0.04	0.13	0.96
Glucose AUC (mg×min/dL)										
30 min	-681	-686	-717	-733	-658	-526	65	0.61	0.07	0.35
60 min	-1869	2075	-2186	-2259	-1766	-1681	162	0.73	< 0.01	0.48
120 min	-3186	-3621	-4319	-4270	-2757	-3140	290	0.46	< 0.01	0.42
NEFA AUC (µEq×min/dL)										
30 min	-4655	-5398	-5142	-5051	-6439	-6059	1110	0.93	0.49	0.86
60 min	4329	-4599	-153	-2282	2427	-6356	3209	0.04	0.84	0.45
120 min	23987	4464	27885	25264	26862	8179	9161	0.13	0.30	0.52

[1]Days of age.
[2]TRT = treatment.
[3]AUC = area under the curve

Table 4. Glucose and NEFA responses to adrenaline challenges of calves born to dams exposed to either heat stress (HS, n = 9) or cooling (CL, n = 10) during the dry period

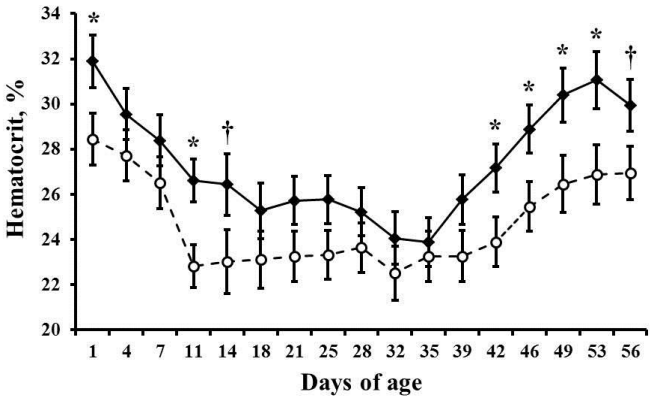
	8[1]		29		57			*P*-value		
	HS	CL	HS	CL	HS	CL	SEM	TRT[2]	Day	TRT×Day
Glucose AUC[3] (mg×min/dL)										
30 min	122	180	320	346	321	365	52	0.34	< 0.01	0.95
60 min	110	197	422	418	453	516	100	0.58	< 0.01	0.88
120 min	-9	-8	236	38	283	375	192	0.83	0.23	0.74
NEFA AUC (µEq×min/dL)										
30 min	1392	692	1599	1529	2386	1718	656	0.38	0.32	0.86
60 min	829	-772	1639	-741	1011	1304	1658	0.40	0.80	0.74
120 min	2043	-1084	4445	-2137	513	-424	3933	0.42	0.94	0.68

[1]Days of age.
[2]TRT = treatment.
[3]AUC = area under the curve



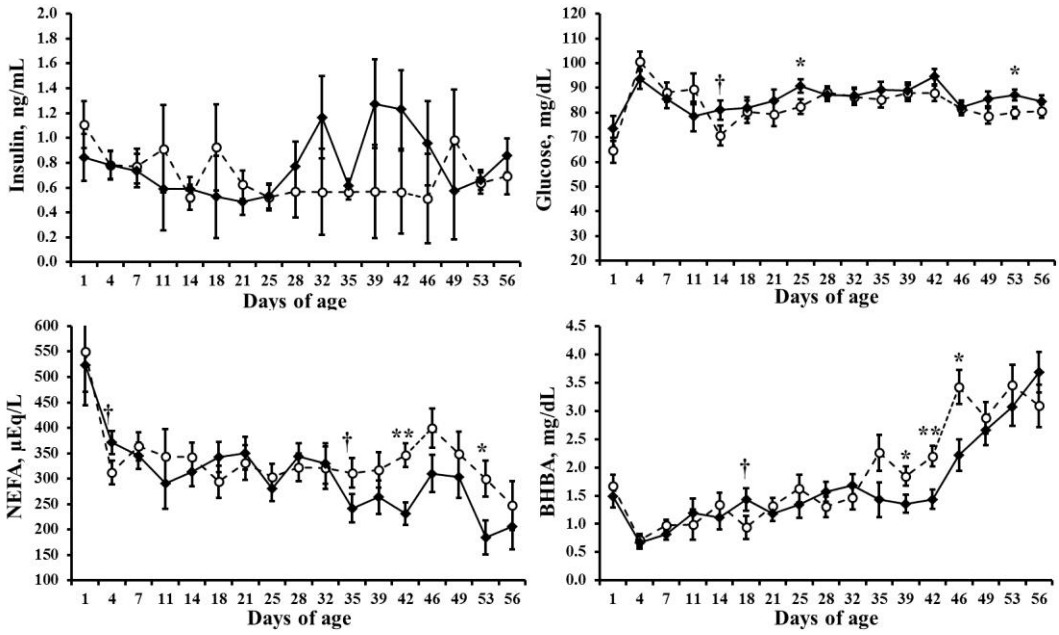
Monteiro Figure 1

Figure 1. The calf starter DMI and BW of calves born to dams exposed to either heat stress (n = 10) or cooling (n = 10) during the dry period. Solid diamond (♦) and open circles (○) represent cooling and heat stress, respectively. For starter DMI, there was a treatment effect ($P = 0.05$) and a treatment by time interaction ($P = 0.01$). For BW, there was a treatment by time interaction ($P < 0.01$), but no treatment effect ($P = 0.19$). $^{**}P \leq 0.01$.



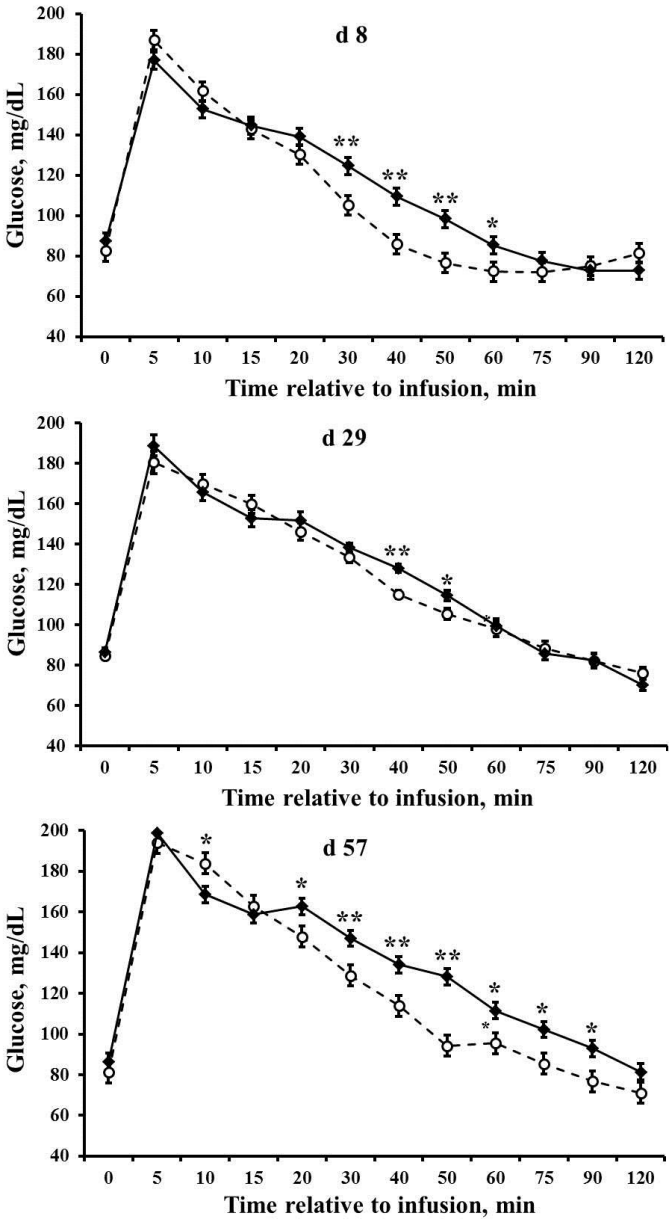
Monteiro Figure 2

Figure 2. Hematocrit of calves born to dams exposed to either heat stress (n = 10) or cooling (n =10) during the dry period. Solid diamond (♦) and open circles (○) represent cooling and heat stress, respectively. There was a tendency for treatment effect ($P = 0.07$) and a treatment by time interaction ($P = 0.04$). *$P \leq 0.05$, †$P \leq 0.10$.



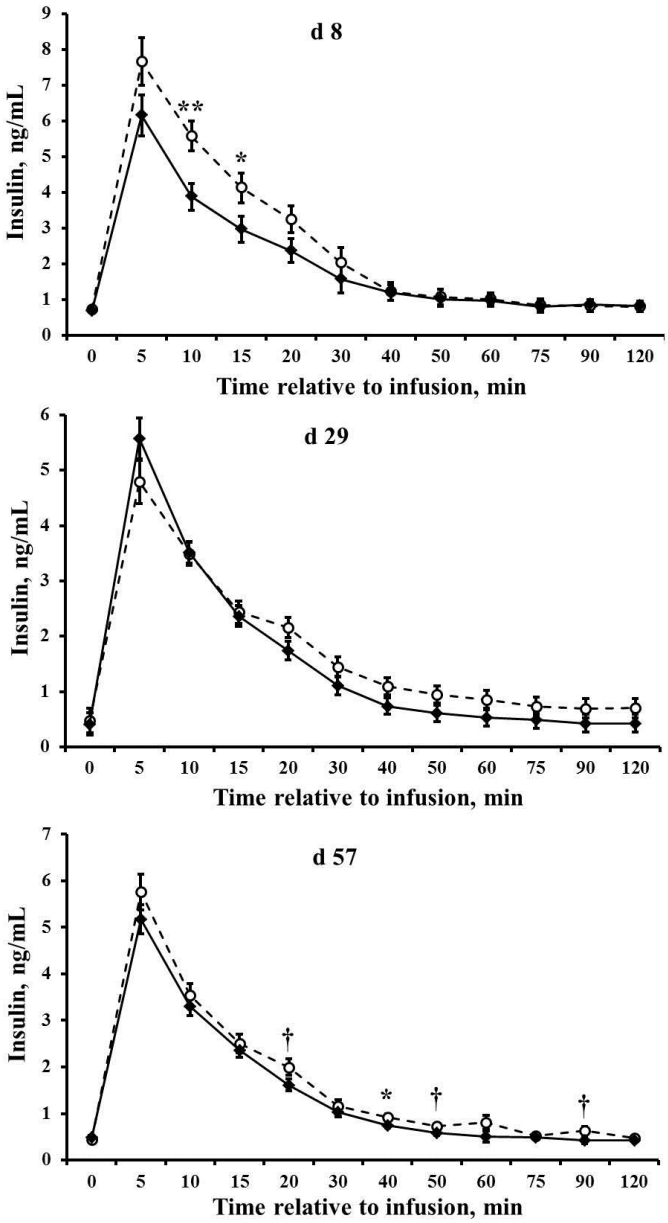
Monteiro Figure 3

Figure 3. The plasma concentrations of insulin, glucose, NEFA and BHBA of calves born to dams exposed to either heat stress (n = 10) or cooling (n =10) during the dry period. Solid diamond (♦) and open circles (○) represent cooling and heat stress, respectively. For plasma insulin concentration, there were no effects of treatment (P = 0.96) nor treatment by time interaction (P = 0.65). For plasma glucose concentration, there was no treatment effect (P = 0.38), but a tendency for treatment by time interaction (P = 0.07). For plasma NEFA and BHBA concentrations, there was no treatment effects (P = 0.18 and P = 0.39, respectively), but a treatment by time interaction (P = 0.02 and P = 0.04, respectively). **$P \leq 0.01$, *$P \leq 0.05$, †$P \leq$ 0.10.



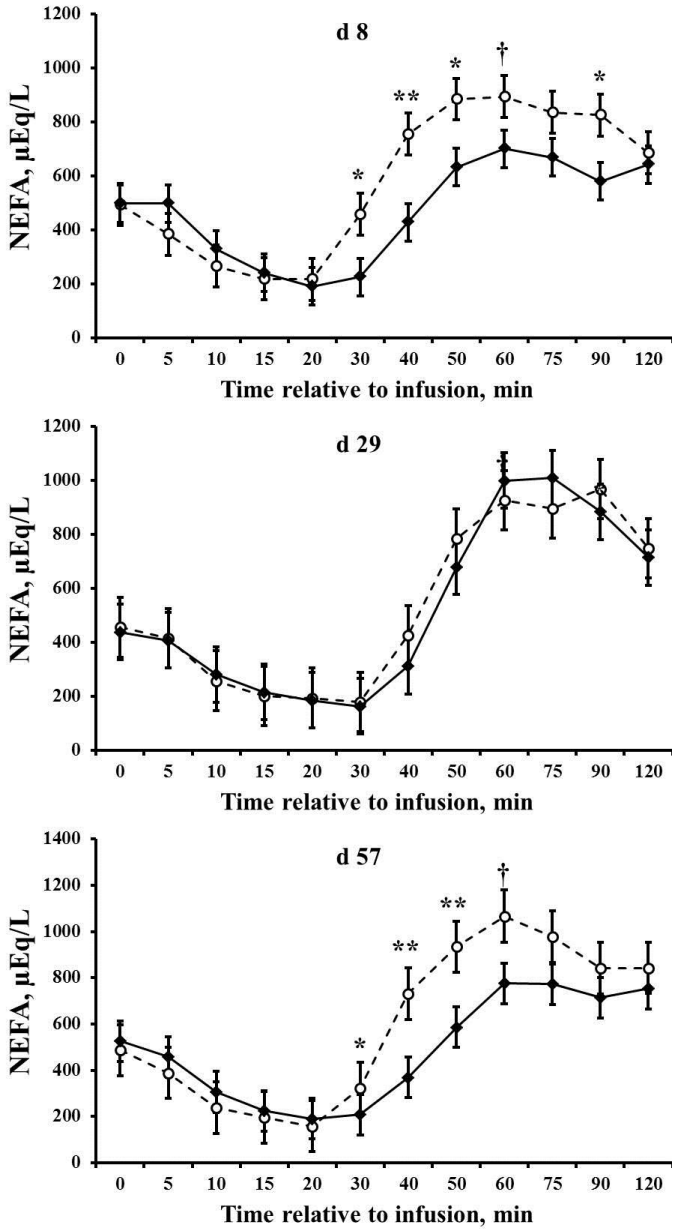
Monteiro Figure 4

Figure 4. Glucose responses to glucose tolerance test of calves born to dams exposed to either heat stress (n = 9) or cooling (n =10) during the dry period, at 8, 29 and 57 days of age. Solid diamonds (♦) and open circles (○) represent cooling and heat stress, respectively. Effects of treatment ($P = 0.16$, $P = 0.42$ and $P = 0.02$ at 8, 29 and 57 days of age, respectively), minute ($P < 0.01$) and treatment by minute interaction ($P < 0.01$). $^{**}P < 0.01$, $^{*}P < 0.05$.



Monteiro Figure 5

Figure 5. Insulin responses to insulin challenge of calves born to dams exposed to either heat stress (n = 9) or cooling (n =10) during the dry period, at 8, 29 and 57 days of age (DOA). Solid diamond (♦) and open circles (○) represent cooling and heat stress, respectively. Effects of treatment ($P = 0.16$, $P = 0.05$ and $P = 0.15$ at 8, 29 and 57 DOA, respectively), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.04$, $P = 0.33$ and $P = 0.06$ at 8, 29 and 57 DOA, respectively). $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.10$.



Monteiro Figure 6

Figure 6. NEFA responses to insulin challenge of calves born to dams exposed to either heat stress (n = 9) or cooling (n = 10) during the dry period at 8, 29 and 57 days of age (DOA). Solid diamond (♦) and open circles (○) represent cooling and heat stress, respectively. Effects of treatment ($P = 0.12$, $P = 0.87$ and $P = 0.15$ at 8, 29 and 57 DOA, respectively), minute ($P < 0.01$) and treatment by minute interaction ($P = 0.03$, $P = 0.99$ and $P = 0.03$ at 8, 29 and 57 DOA, respectively). $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.10$.