



Effects of Exposure to Heat Stress During Late Gestation on the Daily Time Budget of Nulliparous Holstein Heifers

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Exposure of dairy cows to heat stress negatively affects welfare and performance during all phases of the lactation cycle. Detrimental effects include decreased milk and reproductive performance, reduced immune status and health, and altered natural behaviors. While we understand how mature cows respond to heat stress, the effects of late gestation heat stress on pregnant heifers is still unknown. Automated monitoring devices were used to document the behavioral activity of heifers during the pre- (final 60 d of gestation) and postpartum (first 60 d of lactation) periods. Twenty-five pregnant Holstein heifers were housed in a free-stall barn and enrolled to heat stress (HT; shade; $n = 13$) or cooling (CL; shade, soakers and fans; $n = 12$) treatments during the last 60 days of gestation. All animals were provided active cooling postpartum. Upon enrollment, heifers were fitted with a leg tag, which measured daily lying time, number of steps, and standing bouts, and a neck tag that measured eating and rumination times. Rectal temperatures (RT) and respiration rates (RR) were measured thrice weekly during the prepartum period. Relative to CL, HT heifers had elevated RT (38.8 vs. $38.7 \pm 0.04^\circ\text{C}$) and RR (59.6 vs. 44.4 ± 1.9 breaths/min) during the prepartum period. Heat-stressed heifers tended to spend more time eating (224 vs. 183 min/d) and less time ruminating (465 vs. 518 min/d) during the prepartum period compared to CL, but dry matter intake did not differ. During the postpartum period, HT heifers spent more time eating (209 vs. 180 min/d) during weeks 1–4 of lactation, but rumination time was similar. Lying time was reduced by 59 and 88 min per day during weeks –7 and –6 prepartum and 84 and 50 min per day during weeks 2 and 3 postpartum in HT heifers, relative to CL. The number of steps was greater for HT during the postpartum period, from weeks 2 to 9 (3019 vs. 2681 steps/d). Eating frequency was similar during pre- and postpartum periods, however, based on semi quantitative visualization of the smarttag reports, HT consumed larger meals at night during the pre- and postpartum periods compared with CL heifers. In summary, late-gestation exposure to heat stress affects the daily time budget of first lactation heifers during both the pre- and postpartum periods. Current insights of heat stress effects on behavioral responses of dairy heifers may contribute to the development of more effective management strategies to mitigate heat load.

Keywords: nulliparous heifers, heat stress, late gestation, behavior, cooling systems

INTRODUCTION

The effects of accumulated heat have been extensively studied in mature dairy cows as they have elevated internal heat loads caused by high milk production (Chebel et al., 2004), which is exacerbated by rising global temperatures (IPCC, 2014). To cope with heat stress, dairy cows display a wide variety of strategies to promote heat loss and to limit the extent of internal heat production. In lactating cows, these physiological responses include increased respiration rate, panting, sweating, shifts in metabolism, and reduced milk and reproductive performance (Toledo and Dahl, 2012; Tao and Dahl, 2013; Polsky and von Keyserlingk, 2017). Further, behavioral strategies in lactating animals encompass increased standing time (Nordlund et al., 2019), decreased activity and movement (West et al., 2003), reduced rumination time (Soriani et al., 2013), decreased dry matter intake (Wheelock et al., 2010) and modified drinking and eating behaviors. Although necessary for their survival, the physiological and behavioral coping strategies translate into production losses that represent a financial burden for the US dairy industry with economic losses estimated at \$1.2 billion in the absence of heat abatement technologies (Key and Sneeringer, 2014).

Exposure of heat stress during the dry period, a non-lactating phase between lactations, is associated with detriments to subsequent performance, health and welfare in dairy cows (Ouellet et al., 2020). Exposure to heat stress during the dry period represents losses estimated at \$800 million to the US dairy industry, when only effects measured at the dam level are accounted for (Ferreira et al., 2016). Recently, Laporta et al. (2020) reported that maternal heat stress during late gestation may also impact the phenotype at adulthood of the progeny as reflected by reduced lifespan of ~12 months and declines in milk yield estimated at 2.2, 2.3, and 6.5 kg/d during first, second, and third lactation respectively. Altogether, these losses have an estimated cost \$595 million when milk production losses, extra cost heifer rearing, and the cost of reduced productive life are included in calculations. To alleviate the negative effects of heat stress, heat abatement technologies such as shade, fans, soakers and misters are commonly used for lactating cows on US dairies (Spiers et al., 2018). These technologies are also becoming more frequently available for dry cows, especially in Southern US (Dado-Senn et al., 2019), but are rarely used in nulliparous heifer's management.

Late gestation nulliparous heifers undergo a period of extensive mammary gland development and rapid fetal growth while they are still growing. Yet, late gestation nulliparous heifers are often managed on pasture without or with minimal heat abatement infrastructure and are thus exposed to heat stress. Whereas, researchers (Thompson et al., 2012; Tao and Dahl, 2013; Fabris et al., 2019) have developed a comprehensive understanding of heat stress responses in adult lactating and dry cows, much remains to be known about the impact of heat stress on late gestation heifers. Further, irrespective of the thermal environment of the animals, information about the daily time budget of nulliparous late-gestation heifers is currently scarce. The hypothesis is that the exposure of pregnant

nulliparous Holstein heifers to hot conditions during late gestation induces behavior modifications that have lingering effects during lactation. Our objectives were to characterize natural behaviors of nulliparous Holstein heifers 60 d pre- and postpartum and examine the effects of late gestation heat stress on those behaviors.

Preliminary results of this study have been presented in an abstract form (Toledo et al., 2020).

MATERIALS AND METHODS

This study was conducted at the University of Florida Dairy Unit (Hague, FL) from June to November 2019. Animal handling and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Experimental Design, Treatments, and Management

Twenty-five nulliparous pregnant Holstein heifers blocked by body condition score on day of enrollment and predicted transmitting ability (PTA) for milk (Holstein Association USA and Zoetis Services LLC. Enlight, CLARIFIDE, Brattleboro, VT), were enrolled approximately 60 d before expected calving to one of two treatments: heat stress (HT, $n = 13$) or cooling (CL, $n = 12$). The present study represents a secondary objective to Davidson et al. (2020) During the *prepartum* period, all heifers were housed in the same sand-bedded free-stall barn. All heifers were housed in a sand-bedded freestall barn, with HT and CL treatments separated across 4 pens ($n = 6-7$ heifers per pen). Six weeks after first enrollment, heifers within the HT or CL treatment were switched to the neighboring pen to overcome potential pen effects. Heat stress heifers were provided with the shade of the barn only, while CL were provided with the shade of the barn in addition to water soakers over the feed line, and fans. Fans (J&D Manufacturing, Eau Claire, WI) ran continuously over the stalls and the water soakers (Rain Bird Manufacturing, Glendale, CA) turned on automatically for 1.5 min at 5-min intervals when ambient temperatures exceeded 21.1°C. After calving, all cows were housed in a shaded, sand-bedded, free-stall barn and were cooled by water soakers and fans as described above.

Before calving, all heifers were fed a close-up total mixed ration (TMR) as a group which was pushed up several times each day. After calving, all cows were fed a lactating cow TMR formulated to meet the nutrient requirements for lactating cows (NRC, 2001). Pre and Postpartum ration composition is described on Davidson et al. (2020). Free access to water was provided. After parturition, cows were milked twice daily at approximately 07:00 and 19:00 h according to the standard operating procedures of the University of Florida Dairy Unit.

Experimental Measurements

Ambient temperature and relative humidity of the barn were recorded every 15 min with Hobo Pro series temperature probes (Onset Computer Corporation, Pocasset, MA). Hobos were placed in each pen and measurements were recorded during

the whole experiment. The temperature-humidity index (THI) was calculated for each treatment group based on the equation recommended by Dikmen and Hansen (2009): $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$, T = ambient temperature ($^{\circ}C$) and RH = relative humidity (%).

Automated monitoring devices (Nedap, Netherlands) were used to document the behavioral activity of heifers during the pre- (final 60 d of gestation) and postpartum (first 60 d of lactation) periods. Upon enrollment, all heifers were equipped with a “Smarttag Leg” (434 MHz) and a “Smarttag Neck” (FER4). Behavior recordings started 1 week after enrollment to allow animals to acclimate to the experimental pens. The leg tag measured daily lying time, number of steps, and standing bouts, while the neck tag quantified eating and rumination times. Additionally, frequency of eating and meal size were analyzed by semi quantitative visual observation of the daily reports provided by the smarttags reports. The “Nedap Smarttags” utilize a G-sensor, which uses acceleration as a measure of movement in a particular direction, based on a three-dimensional accelerometer. The tags distinguish forward and backward motions, left and right and up and down movements. The NEDAP smarttags have been previously validated to assess behavioral activity level in dairy cattle (Van Erp-Van der Kooij et al., 2016).

Rectal temperature (RT, $^{\circ}C$) was measured thrice weekly at 14:00 h during the 60-d prepartum period with a digital thermometer (GLA Agricultural Electronics, GLA M900, accuracy $\pm 0.1^{\circ}C$, San Luis Obispo, CA). Respiration rates (RR, breaths/min) were recorded thrice weekly at 14:00 h during the same period by counting flank movements for 1 min. Additionally, during the prepartum period, daily dry matter intake (DMI) was measured per pen by subtracting the weigh-back left the next morning from the previous days' amount of feed delivered. After calving, body weight (BW, kg) was measured weekly, and milk yields were retrieved from AfiFarm Dairy Herd Management Software (AfiMilk Ltd., Kibbutz, Afikim, Israel) and recorded until 105 days in milk.

Statistical Analysis

All statistical analyses were performed in SAS (version 9.4, SAS Institute, Inc.). Data were tested for covariance (Levene's test) and normality of distribution was tested by evaluating Shapiro-Wilk statistics using the univariate procedure. Raw data was transformed [$\log_{10}(x)$ or square root] when it was deemed appropriate to meet the homogeneity of variance criteria. The THI and DMI were averaged per day and analyzed by pen using generalized linear mixed models using the PROC MIXED procedure of SAS with the main effects of pen (1–4), day (repeated measure), and their interaction. All of the other variables were submitted to repeated-measures variance analysis using a mixed model (PROC MIXED procedure). The model included the fixed effect of treatment, time (i.e., week) and the interaction between treatment and time, and the cow within treatment was used as a random effect. Behavioral measurements recorded pre-calving (7–1 week before calving), during the last week before calving (6–1 d before calving), and during the 10 d after calving and post-calving (2–9 weeks after calving) were analyzed separately. All statistical comparisons were performed

by Tukey-Kramer testing. Significance was set at $p \leq 0.05$ while tendencies were declared at $0.10 \leq P > 0.05$.

RESULTS

Temperature-Humidity Index, Physiological Responses, and Performance

Temperature-humidity index measurements recorded at the experiment pen level were similar between CL and HT treatments (77.3 vs. 77.3 ± 0.2 ; $P = 0.99$) throughout the entire experiment, indicating that both groups of heifers were exposed to similar thermal environmental conditions. Relative to HT, CL heifers had $0.1^{\circ}C$ lower RT (38.7 vs. $38.8 \pm 0.04^{\circ}C$; $P = 0.02$). and lower respiration rates (44.4 vs. 59.6 ± 1.9 breaths/min $P < 0.001$), indicating that the heat abatement was achieved. Furthermore, during the prepartum period, daily DMI (8.0 vs. 8.3 ± 1.0 kg; $P = 0.76$) and BW (598.9 vs. 609.2 ± 6.6 kg; $P = 0.13$) were similar between treatments. Cooled heifers had higher milk yield (35.8 vs. 31.9 ± 1.4 kg/d), when compared with HT heifers.

Behavioral Activity

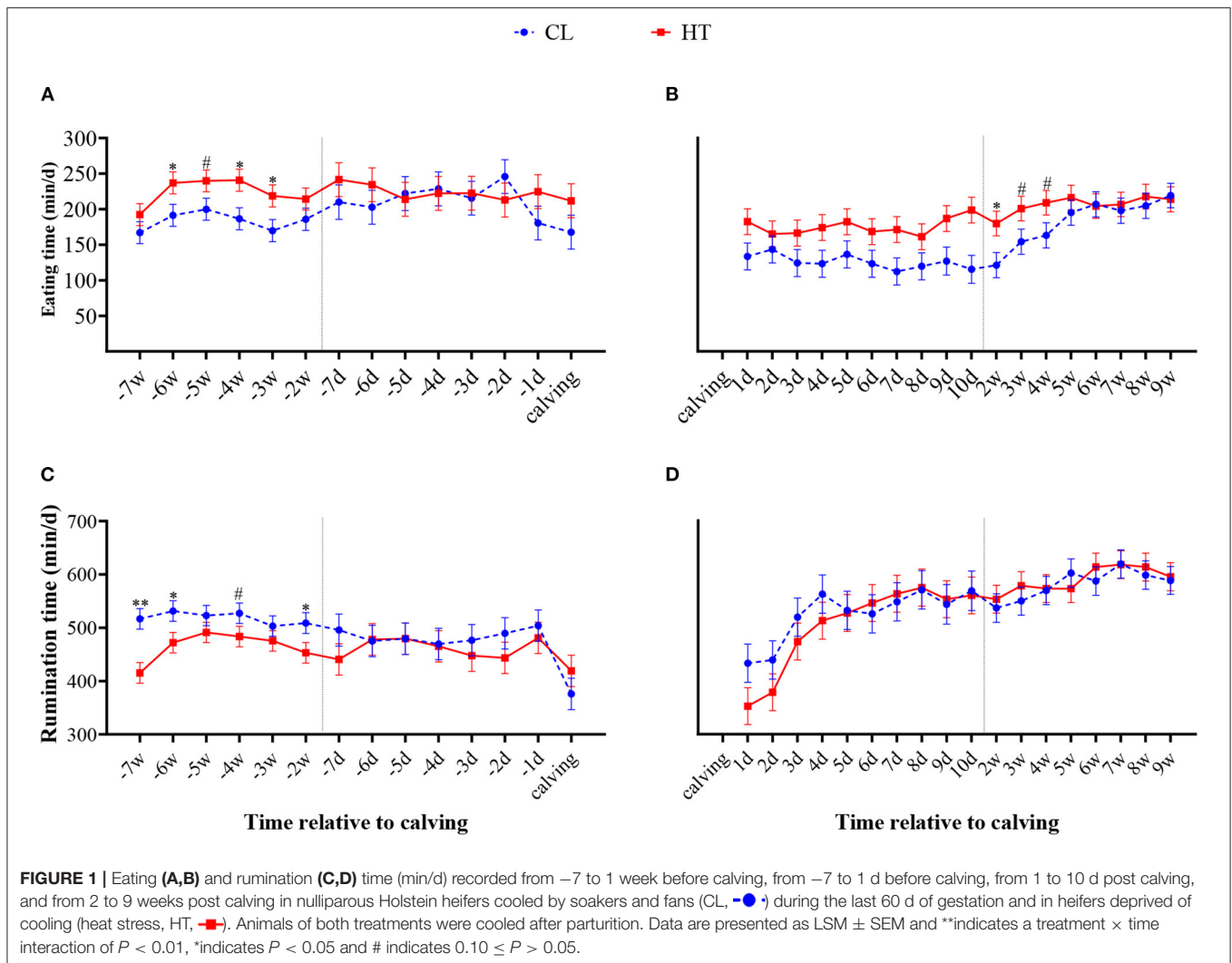
Heat stressed heifers tended to spend more time eating (224 vs. 183 ± 15 min/d; $P = 0.07$) and spent less time ruminating (465 vs. 518 ± 18 min/d; $P = 0.05$) during the prepartum period (from -7 to -2 weeks before calving) compared to CL heifers (Figures 1A,C). Further, a treatment by time (i.e., week) interaction was observed during the last 6 d before calving for eating time, with HT animals spending more time eating than CL animals (Figure 1A).

Prepartum differences in eating time lingered during the postpartum period. In the first 10 d postpartum, HT cows spent more time eating relative to their CL counterparts (179 vs. 130 ± 13 min/d; $P = 0.02$; Figure 1B). In addition, a treatment by time interaction was measured from 2 to 9 weeks postpartum as HT cows spent more time eating from week 2 to 4 of lactation, but no difference in rumination time was observed ($P = 0.81$) between treatments during either of the postpartum periods analyzed (Figure 1D).

Lying time was reduced in the HT ($P = 0.01$) by 59 and 88 min/d during weeks -7 and -6 prepartum. However, lying time was similar between treatments during the last 7 d before calving ($P = 0.97$). Further, treatment by time (i.e., week) interactions were detected during the first 10 d postpartum and from 2 to 9 weeks in lactation ($P = 0.009$ and $P = 0.001$, respectively; Figures 2A,B).

The number of steps was similar between treatments during both prepartum periods ($P = 0.98$ and $P = 0.82$), and in the first 10 d following parturition ($P = 0.24$; Figure 2C). However, the number of steps tended to be greater for HT cows during the postpartum period, from weeks 2 to 9 ($3,010 \pm 115$ vs. $2,681 \pm 17$ steps/d; $P = 0.06$) relative to CL cows (Figure 2D). Throughout the entire experiment (60 d prepartum and 60 d postpartum), no difference was observed between treatments in the daily number of standing bouts (all $P > 0.1$; Figures 2E,F).

Eating, rumination and lying times recorded during both pre- (-7 to -2 weeks and last 7 d before calving) and postpartum (up to 10 d and 2–9 weeks in lactation) periods are summarized



in **Tables 1, 2**. Further, daily eating, rumination, and lying times measured in late-gestation dry cows and lactating cows retrieved from other studies are also presented in **Tables 1, 2** to serve as a basis for comparison.

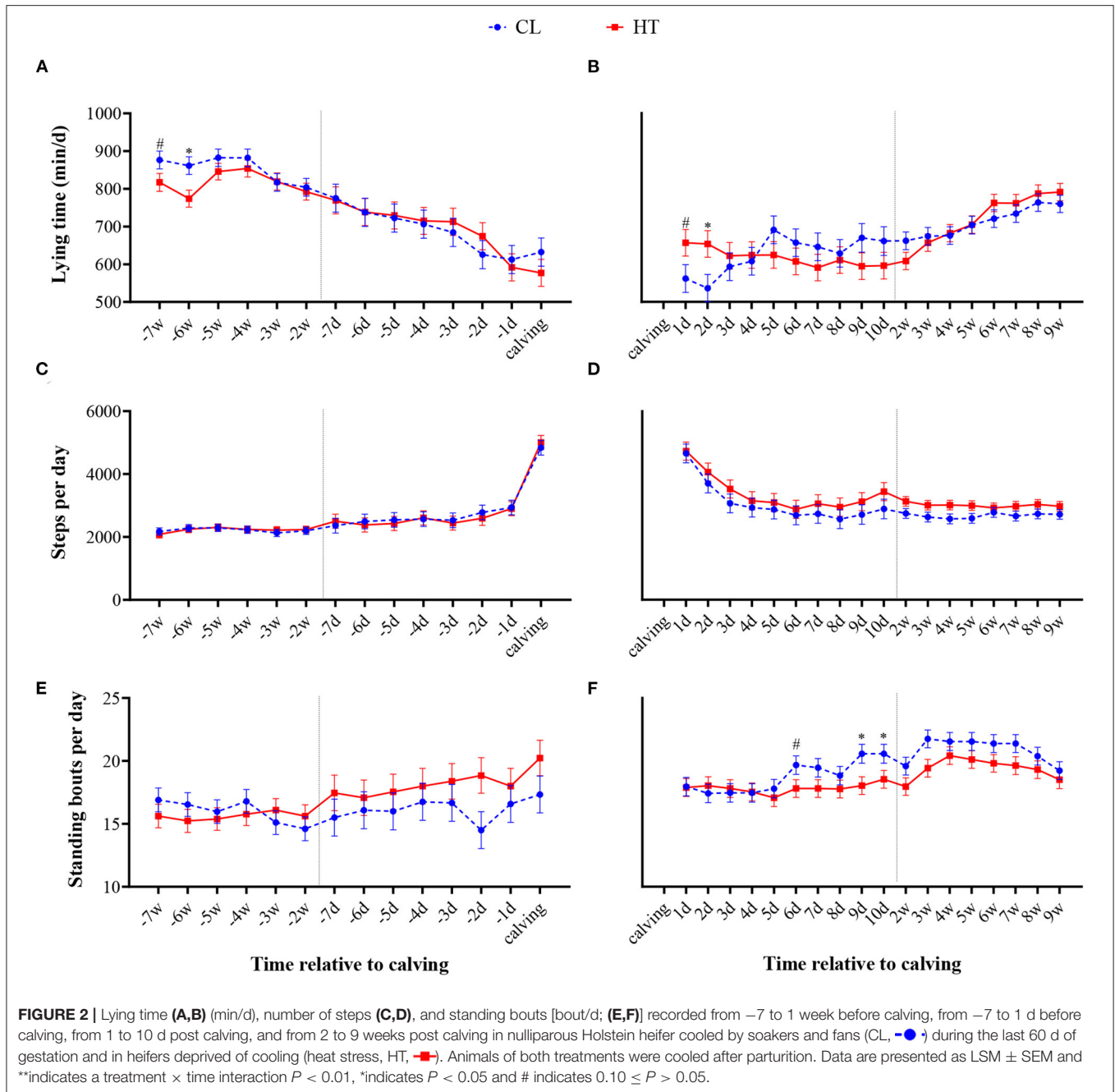
Data collected by visual observation of the smarttags reports was analyzed and revealed that eating frequency of HT and CL heifers was similar during pre- and postpartum ($P < 0.45$ and $P < 0.67$, respectively), however, HT had larger meals at night during both the pre- and postpartum periods ($P < 0.01$ and $P = 0.04$, respectively) compared to CL heifers.

DISCUSSION

During the past decade, it has been well documented that multiparous dairy cows exposed to heat stress during the dry period negatively impacts performance. A series of controlled experiments indicate that heat stress during late gestation compromises mammary gland development during the dry period (Tao et al., 2011), decreases subsequent lactation milk production

(do Amaral et al., 2009; Tao et al., 2011; Thompson et al., 2012), negatively affects early postpartum immune response (do Amaral et al., 2009, 2011; Thompson et al., 2012), metabolic function (do Amaral et al., 2009), occurrence of postpartum diseases, and reproductive performance (Toledo and Dahl, 2012). However, there has been little work on adaptations to sustained acute or chronic heat stress in late-gestation nulliparous heifers. Further, specific effects of heat stress on heifer behavior in late gestation has not been studied.

Lactating dairy cows are considered to experience no heat stress when THI is <72 , mild stress when THI is between 73 and 77, significant stress when THI is between 78 and 88, severe stress when THI is between 89 and 99, and possible death when THI is >99 (Toledo and Thatcher, 2022). In the present study, THI measurements in the stall areas indicated that all heifers were exposed to similar thermal environmental conditions of heat stress during late gestation. Similar to previous studies (Tao et al., 2011; Thompson et al., 2012; Fabris et al., 2017) where mature cows were exposed to heat stress during late gestation, in the present study heifers provided with active cooling devices



had decreased RR and RT compared to heifers without access to those, confirming that the cooling system of fans and soakers was effective in alleviating heat stress in CL heifers.

Behavioral activity is influenced by factors such as health (Dittrich et al., 2019), management (Grant and Albright, 2001), time relative to calving (Ouellet et al., 2016) and environmental conditions (Nordlund et al., 2019). Further, changes in the behavioral activity of farm animals are widely used as welfare and comfort indicators (Haley et al., 2000; Muller and Schrader, 2003) and to investigate animal production parameters (Phillips and Rind, 2001). Herein, to isolate the effects

of heat stress on behavior, measurements recorded prepartum (weeks -7 to -1), during the last week prepartum (6-1 d before calving), during the 10 d following parturition and postpartum (weeks 2-9) were analyzed separately. To the best of our knowledge, the present experiment is the first of its kind to evaluate the effects of late-gestation heat stress or heat stress abatement on pre- and postpartum behavior in nulliparous heifers in free-stall housing conditions. In addition, we compared the daily time budget of nulliparous heifers to what is reported for adult late-gestation and lactating animals in the literature.

TABLE 1 | Eating, rumination, and lying times (min/d) automatically measured in late-gestation nulliparous heifers and late-gestation dry cows housed in different conditions.

Behavior/Treatment ^d	Late-gestation Nulliparous Heifers ^a		Calving week Nulliparous Heifers ^b		Late-gestation cows ^c		References
	CL/TN	HT	CL/TN	HT	CL/TN	HT	
Eating, min/d	183	224	209	223	166	147	(Karimi et al., 2015)
					205	–	(Schirmann et al., 2013)
Rumination, min/d	518	465	471	456	655	–	(Ouellet et al., 2016)
					283	243	(Karimi et al., 2015)
Lying, min/d	854	817	687	689	962	–	(Jensen et al., 2005)
					1,050	966	(Karimi et al., 2015)
					768	–	(Ouellet et al., 2016)

^aBehaviors automatically recorded from 7 to 2 weeks before calving in the present study.

^bBehaviors automatically recorded during the last 7 d before calving in the present study.

^cBehaviors automatically recorded during the 3 weeks before calving or last 7 d before calving retrieved in different studies.

^dCL/TN, animals exposed to active cooling by fans and soakers or housed in thermoneutral conditions; HT, animals deprived of cooling or exposed to high temperature-humidity index.

TABLE 2 | Eating, rumination, and lying times (min/d) automatically measured in postpartum nulliparous heifers and lactating cows.

Behavior/Treatments ^d	Postpartum nulliparous heifers ^a		Calving week nulliparous heifers ^b		Lactating cows ^c		References
	CL/TN	HT	CL/TN	HT	CL/TN	HT	
Eating, min/d	130	179	180	209	224	–	(King et al., 2016)
Rumination, min/d	511	496	588	593	340–410	–	(Pahl et al., 2015)
					535–545	493–520	(Müschner-Siemens et al., 2020)
					660–720	–	(Cook, 2004)
Lying, min/d	637	604	666	638	600	480	(Cook et al., 2007)
					540	360	(Nordlund et al., 2019)

^aBehaviors automatically recorded from 0 to 10 d postpartum in the present study.

^bBehaviors automatically recorded from 2 to 9 weeks postpartum in the present study.

^cBehaviors automatically recorded during in lactating multiparous cows.

^dCL/TN, animals exposed to active cooling by fans and soakers or housed in thermoneutral conditions during the last 60 d of gestation; HT, animals deprived of cooling or exposed to high temperature-humidity index during last 60 d of gestation.

We observed changes in eating behavior, nulliparous HT pregnant heifers tended to spend more time eating during the *prepartum* period relative to their cooled counterparts. This difference lingered after calving as an interaction between treatment and time was observed from calving to 10 d after calving and from 2 to 9 weeks after calving. During the last 3 weeks of gestation, Karimi et al. (2015) visually assessed that heat stressed dry cows tended to spend less time eating relative to cooled animals. However, the authors reported no differences between treatments in eating time per DMI. The range of *prepartum* eating time reported in the present study (HT = 223–224 min/d; CL = 183–209 min/d) is higher than the values reported by Karimi et al. (2015) for adult animals (HT = 147.4 min/d; CL = 166.2 min/d). This is surprising given that heifers enrolled in our study had a lower DMI (~8 kg) relative to mature animals from the Karimi et al. (2015) study (~14 kg). This difference may be associated to different techniques used to assess behavioral changes (visual vs. automated device) and by the physiological stage of the animals. In addition, we observed a shift in eating patterns as heat stressed heifers consumed larger meals at night during the pre- and postpartum periods relative to CL heifers. Such changes were also previously reported in mature dairy cows (West, 1999; Petrer et al., 2006).

Prepartum rumination time was also altered by heat stress. Pregnant nulliparous heifers exposed to heat stress in late gestation tended to spend less time ruminating compared with their cooled herd mates. Although rumination time is mainly affected by diet characteristics, others studies also reported a negative association between heat stress and rumination time in primiparous and multiparous lactating cows (Kazdere et al., 2002; Soriani et al., 2013), and in late-gestation dry cows (Karimi et al., 2015). In mature animals, it was postulated that depressing the rumination time might delay passage of the rumen digesta, which would reduce the ruminal capacity for additional food and thereby play a pivotal role in the negative effect of heat stress on DMI and, consequently, on milk production (Warren et al., 1974; Church, 1988; Moallem et al., 2010). However, this was not observed in nulliparous late-gestation heifers, as DMI was similar between treatments. We postulate that the reduction in rumination is a coping strategy attempting to limit internal heat production. The range of *prepartum* rumination time reported for nulliparous heifers in this study (457 to 518 min/d) corresponds to the range reported for primiparous and multiparous high-producing cows during lactation (Kononoff et al., 2003; Yang and Beauchemin, 2006; Soriani et al., 2013) and in the last days before calving

(Ouellet et al., 2016), but it was higher than the time reported in late-gestation adult cows by Karimi et al. (2015). The differences between the two studies could be related to the technique used to record rumination time. Moreover, Soriani et al. (2013) observed that rumination occurred mostly at night and that cows under heat stress reduced their daytime rumination. However, the system used to measure rumination time in our study did not record the daytime and nighttime patterns. Interestingly, differences in rumination time between groups did not linger after calving, and rumination time was similar to what has been reported previously in mature cows (Kononoff et al., 2003; Yang and Beauchemin, 2006; Soriani et al., 2013).

Mean daily lying time was reduced by 59 and 88 min/d during the first 2 weeks of the *prepartum* period and by 83 and 50 min/d during weeks 2 and 3 *postpartum* in nulliparous heifers that were exposed to HT during the last 60 d of gestation. A significant reduction in lying time, thus a converse increase in standing time, was previously reported as a behavioral adaptation to heat stress in primiparous and multiparous lactating animals to promote heat loss to the environment (Overton et al., 2002; Cook et al., 2007; Nordlund et al., 2019). In the current study, lying time of heifers of both treatments were within typical resting period for mature cows (Jensen et al., 2005) and is typical of that found for lactating adult animals housed in freestalls (Cook, 2004) and in late-gestation cows housed in individual stalls (Karimi et al., 2015; Ouellet et al., 2016).

Interestingly, a significant interaction was observed between treatment and time for the number of standing bouts during the *prepartum* period (−7 to −2 weeks before calving). However, the number of standing bouts was not impacted by treatment in the two experimental periods in our study. Therefore, a shift in the number of standing bouts does not seem to be a behavioral strategy to cope with heat stress in nulliparous heifers. Ultimately, we observed an increase in the daily number of steps in *prepartum* HT heifers from 2 to 9 weeks post-calving, even though all heifers were cooled during the *postpartum* period. This difference may be related to the rotation of cows between pens as lactation advanced, herein, the distance between the freestall pens

and milking parlor, thereby not directly impacted by the experimental treatments.

CONCLUSIONS

Heat stress during the last 60 d of gestation altered the behavior of nulliparous heifers and had lingering behavioral effects after parturition. Heat-stressed heifers adapted their behavior by increasing eating time and meal size at night and by reducing rumination and lying to minimize internal heat production and to optimize heat loss to the environment during the *prepartum* period. Implementing cooling systems for late-gestation nulliparous heifers seems to be beneficial for welfare and performance.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at the University of Florida.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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