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ORIGINAL PAPER

Heart rate, cardiac vagal tone, respiratory rate, and rectal temperature in dairy calves exposed to heat stress in a continental region

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Abstract

Changes in non-invasive stress biomarkers were studied in shaded ($n = 8$) and non-shaded ($n = 8$) Holstein bull calves exposed to extreme heat load conditions in a continental region. Ambient temperature and humidity data were recorded for the S and NS hutch and exercise pen environments. Temperature-humidity-index (THI) was also calculated. Respiratory rate, rectal temperature, heart rate, and the root mean square of successive differences between R–R intervals (RMSSD) were recorded as animal-based indicators during three periods: (1) day 1, *control day*, during which all calves were shaded for 24 h (shade removal from non-shaded calves at 2400 h); (2) day 2, *heat stress day*, with shade over shaded calves; and (3) days 3–5, *post-stress period*, with shade over shaded calves. On the heat stress day, the maximum temperatures were 44.3 and 46.7 °C for the non-shaded hutch and pen environments, respectively. The temperatures were with 6.2 and 6.9 °C ($P = 0.015$ and $P = 0.008$) and the THIs were with 5.9 and 4.2 units higher ($P = 0.020$ and $P = 0.032$) in the non-shaded than in the shaded environment for the hutch and exercise pen, respectively. Shaded calves had with 42.3 ± 3.2 breaths/min higher respiratory rate than non-shaded ones on the heat stress day at 1200 h ($P = 0.001$), which was moderated to 20.1 ± 2.4 breaths/min at 1600 h ($P = 0.023$). Significant differences in respiratory rate occurred earlier than in any other animal-based parameter between shaded and non-shaded calves on day 2. The only significant group difference in rectal temperature was found at 1200 h on day 2 when THI exceeded 91 units, with 0.59 °C higher values for non-shaded calves ($P = 0.045$). The heart rate on days 2 and 3 was higher for non-shaded calves than for shaded ones. Group differences were also significant at 0800 h (18.2 ± 1.2 beats/min, $P = 0.008$), 1200 h (22.3 ± 1.4 beats/min, $P = 0.003$), 1600 h (15.3 ± 0.8 beats/min, $P = 0.012$), and 2000 h (19.0 ± 1.1 beats/min, $P = 0.010$) on day 2. Following a rapid daytime reduction, RMSSD showed a nighttime overcompensation in non-shaded calves on day 3 (between 0000 and 0600 h) and day 4 (between 0000 and 0800 h), exceeding the levels recorded on day 1 (control) and the levels for shaded calves, thus suggesting a recovery of the autonomic nervous system from heat stress. Based on our results, shading effectively reduced heat stress as evidenced by heart rate and RMSSD in addition to traditional measures of heat stress. Respiratory rate and heart rates exhibited by non-shaded calves support that the well-being of pre-weaned calves can be impaired in continental regions during following a heat stress day without providing shade.

Keywords Calves · Temperature-humidity index · Heart rate · Respiratory rate · Heat stress · Shading

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Introduction

Because of the ongoing climate change and potential global warming, the interest in heat stress-related aspects of animal well-being has increased. Solar radiation is a major factor in heat stress and increases heat gain both directly and indirectly (Berman and Horovitz 2012). There are many ways to provide shade to reduce the thermal load of cattle (Turner et al. 1992); however, only few studies have evaluated the positive effects of shade on calf welfare during periods of elevated ambient temperatures (Spain and Spiers 1996; Coleman et al. 1996; Lima et al. 2013). In the USA, several design options for enclosed calf hutches are available with adjustable rear ventilation doors. However, the common housing system used worldwide is a polyethylene hutch with an opened exercise area, but without supplemental shade. Although this type of hutch design is successfully used in Hungary, direct solar radiation may cause heat stress for calves because of inappropriate shading.

The effects of heat stress can be quantified by measuring physiological variables such as rectal temperature (RT), respiratory rate (RR), and hormone concentrations (Bernabucci et al. 2010). The few studies available on the calves' physiological responses to heat stress, focusing on management methods that are successful in alleviating thermal stress in dairy calves (Hill et al. 2011), have compared housing technologies for pre-weaned animals (Peña et al. 2016) or evaluated the effectiveness of supplementary shading of hutch-reared calves (Spain and Spiers 1996; Coleman et al. 1996). These studies have demonstrated that providing shade reduces the temperature inside hutches and lowers the calves' body temperature and RR. Nevertheless, it is not clear whether the increases in RT or RR are better indicators of heat stress than other non-invasive physiological parameters because they change with a shorter or longer delay (Lima et al. 2013).

Changes in the activity of the autonomic nervous system are one of the first phases of a stress response; however, studies concerning the assessment of immediate autonomic responses to heat challenge are lacking in cattle. In addition to the heart rate (HR), parameters of heart rate variability (HRV) have become generally accepted indicators of bovine stress because they reflect the cardiac vagal and sympathetic tone (Kovács et al. 2014). Interpretation of time-domain HRV as a measure of sympathetic activity remains a subject of debate; however, it is agreed that the root mean square of successive differences (RMSSD) between inter-beat intervals is the primary time-domain trait of HRV that reflects cardiac vagal tone (von Borell et al. 2007).

The present study evaluated changes in the RT, RR, HR, and RMSSD of dairy calves in response to extreme heat load. The possible stress-reducing effect of supplemental shading was also assessed. It was hypothesized that significant improvements in the HR and RMSSD would also be observed consistently with previous observations on RT and RR in shaded environments during heat stress.

Materials and methods

The study was approved by the Pest County Government Office, Department of Animal Health (Permit Number PE/EA/1973–6/2016). All procedures involving animals were approved by the Ethics Committee of the University of Veterinary Science.

Animals and experimental design

The experiment was carried out at a large-scale dairy farm in Hungary, which has a herd over 1000 lactating Holstein cows. The farm was visited between August 15 and 20, 2016 (temperature measured in shade during the investigation: average/min/max, 25.3/21.6/38.8 [°C], respectively). Pre-weaned Holstein bull calves ($n = 16$, age, 46.7 ± 1.8 days; age range, 44–49 days; body weight = 74.3 ± 1.6 kg; body weight range, 70.3–78.6 kg) were used. The calves were housed individually in mid-sized (1.65 × 1.20 m) plastic calf hutches (Calf-Tel ECO, Hampel Animal Care, WI, USA) with a 1.60-m^2 exercise pen. Hutches were aligned in the same row, oriented north to south, to maximize the exposure to solar heating and remained in the same location for the duration of the study.

The calves were assigned to shaded (S, $n = 8$) and non-shaded (NS, $n = 8$) groups. The 6-day experiment was split into four periods as follows: (1) day 0, 24-h habituation to the study environment between 0000 and 2400 h (preparation of the shading structure over the hutches of all calves was done between 0800 and 1400 h); (2) day 1, control, during which all calves were shaded for 24 h between 0000 and 2400 h (shade was removed from NS calves at 2400 h); (3) day 2, heat stress day, between 0000 and 2400 h; and (4) days 3–5, post-stress period, between day 3 0000 and day 5 2400 h. After shade removal from NS calves at day 1 2400 h, shade was provided only for S calves. The shading structure measured 32.5 × 3.4 m and covered all experimental hutches and exercise pens until day 1 2400 h. After removal of the shading structure from the NS calves, eight hutches were in direct sunlight 5 m from the shaded environment for the reminder of the study.

A raschel net was used as shading material, providing a rate of 85% shading, and was located 1.9 m above the ground. The bedding of the calves consisted of straw. For both groups, milk was provided at 0500 and 1600 h, whereas calf starter (Vitafort calf starter, Vitafort cPlc, Dabas, Hungary), alfalfa hay, and freshwater were available ad libitum.

Measurements and analyses

Meteorological data, RR, and RT Six hutches, three from each group (the first, the fourth, and the eighth in a row), were outfitted with VOLTCRAFT DL-181THP devices (Conrad Electronic SE, Hirschau, Germany) placed in the back of the hutch to measure the ambient temperature and relative humidity within the hutches (Fig. 1).

Six Testo 175 H1 devices (Testo Inc., Sparta, USA) were fitted to 2-m poles at 1 m above the ground, to measure the ambient temperature and relative humidity above the exercise pens of the same hutches. A 2-h recording frequency was chosen for temperature and humidity between day 1 0000 h (first recording) and day 5 2400 h (last recording). The THI was calculated using the equation by Bianca (1962): $THI = (0.35 \times T_{db} + 0.65 \times T_{wb}) \times 1.8 + 32$, where T_{db} is the dry bulb temperature, T_{wb} is the wet bulb temperature, and RH is the relative humidity. This THI was recommended by Bohmanova et al. (2007) for "low-relative-humidity" (continental) climatic regions.

The RR (breaths/min) was recorded by counting the movements of the abdominal muscles in the flanks during respiration (Spain and Spiers 1996) while the calves were in a lying posture. This was done simultaneously by two independent investigators between day 1 0000 h (first observation) and day 5 2400 h (last observation), with a 4-h sampling frequency (see Supplementary File 1). The RT was measured immediately after RR observation with a 10-s digital thermometer (Digi-Vet *SC 12*; Jørgen Kruuse A/S, Langeskov, Denmark) that was inserted 8 cm into the rectum.

Posture of the calves Posture (lying vs. standing) was recorded with the HOBO Pendant G data logger (Onset Computer Corporation, Bourne, MA, USA) validated for dairy calves (Bonk et al. 2013). As advised by Bonk et al. (2013), the logger was attached to one of the hind legs of the calves. Following the recommendation of (Ledgerwood et al. 2010), a 30-s sampling interval was chosen for the detection of lying bouts. Data were downloaded to the Onset HOBOware Software (Onset Computer Corporation, Bourne, MA, USA) and exported to Microsoft Excel. The Onset Pendant G data logger recorded the g force on the x, y, and z axes on a scale of − 1 to 1. Information from the x axis was used to categorize logger readings as lying (< 0.75) and standing (≥ 0.75) posture. This cutoff value was determined based on preliminary visual observation from video recordings.

Heart rate and RMSSD Heart rate was recorded continuously between day 1 0000 h and day 5 2400 h using a mobile recording system that included an electrode belt, a HR sensor, and a Polar V800 Equine HR receiver (POLAR, Kempele, Finland). Devices were fitted to animals on day 0, and electrodes were positioned over the cardiac area, which was previously shaved (Stewart et al. 2010). The electrode sites were covered with ultrasound transmission gel (Aquaultra Blue, MedGel Medical, Barcelona, Spain). Because of the limited storage capacity of the HR receivers, data were downloaded every 24 h. This procedure lasted approximately 5 min per animal and was performed after morning feeding (around 0500 h). During all times of data downloading, the electrodes were covered with extra gel. Devices were removed from the calves after day 5 2400 h, and data were transmitted to the Polar FlowSych program (POLAR, Kempele, Finland).

Heart rate and RMSSD were quantified using equal lengths of 5-min samples selected from the inter-beat interval signal stream. The RMSSD is used to estimate short-term beat-to-beat variations representing the vagal activity of the autonomic nervous system in dairy calves (Stewart et al. 2010). Four sample per animal was used for analysis per 2 h (see Supplementary File 2), when calves were in undisturbed lying posture. A total of 985 valid 5-min inter-beat interval samples were used for HRV analysis, 487 from S (means ± SD, 60.9 ± 2.1; range, 56–66 per animal), and 498 from NS calves (means ± SD, 62.3 ± 2.5; range, 58–70 per animal). The analysis was performed with the Kubios HRV software (version 2.2; Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland).

Statistical evaluation

All statistical analyses were performed using the PAST Software Package (Hammer et al. 2001). Data were tested for constant variance (Levene's test), and the Shapiro-Wilk test was used for testing the equality of error variances. The shading effect on the hutch and pen microclimate was

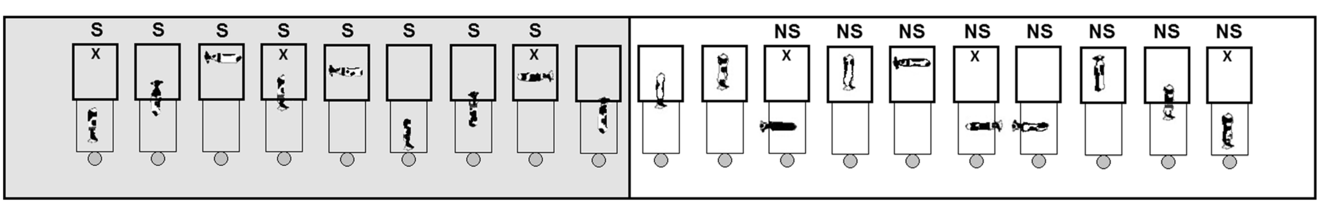

Fig. 1 The spatial arrangement of the hutches for shaded (S) and non-shaded (NS) calves. A raschel net located 1.9 m above the ground was used as shading material, with shading rated at 85%. The shading material covered both the hutch and pen environments. "X" represents hutches fitted with temperature-humidity devices in the hutch and the exercise pen environments. During day 1 (control), all calves were shaded

calculated using two-way factorial ANOVA followed by the Student's *t* test. The effect of shading on RR, RT, HR, and RMSSD was tested using two-way factorial ANOVA. In this analysis, all measured animal-based data were used, irrespectively of whether the animals were in the hutch or in the pen. Explanatory variable was the environment (shaded vs. non-shaded). The Tukey-Kramer post hoc test or the Student's *t* test was used for pairwise comparisons between groups. The level of $P < 0.05$ was considered as significant in cases of both environmental and physiological variables.

Results and discussion

Temperature and THI

Changes in ambient temperature and THI recorded for the hutch environment are shown in Fig. 2a, b. Temperature and THI showed gradual daytime increments between 0600 and 1600 h and falls after 1600 h during the 5-d period for both S and NS environments (Fig. 2a, b). On the heat stress day, extremely elevated temperature (44.3 °C) and THI values (92.6 units) were recorded at 1600 h for the NS hutch environment. As shown in Fig. 2a, b, maximal temperature and THI decreased from day 2 to day 5 for both S and NS environments. Because of an expected cold wave that arrived at 2000 h on day 4, the temperature and THI decreased markedly on day 5, with moderate fluctuations during the last 24 h of the study.

During the heat stress day, the maximal temperature values were 6.2 and 6.9 °C higher in the NS than in the S environments measured in the hutch and in the exercise pen, respectively ($P = 0.015$ and $P = 0.008$). Maximal THI values were also higher in the NS than the S environment by 5.9 and 4.2 units measured in the hutch and pen environments, respectively ($P = 0.020$ and $P = 0.032$). Indeed, previous findings demonstrated that, in a southern climate, a shade cloth similar to ours (80%) and positioned 1.2 m above the ground reduced ambient temperature measured at 1500 h in commercial calf hutches only by 1.7 °C (Coleman et al. 1996). When focusing on nighttime THI on the heat stress day, slightly higher values were observed for the S hutch and pen environments than for NS ones at 2000 and 2200 h (Fig. 2b); however, differences were non-significant ($P = 0.225$ and $P = 0.450$). This was because of the presence of the shade cloth.

Differences in the temperature and THI between the S and NS environments occurred earlier in the hutch (1200 h, $P = 0.012$) than in the pen (1600 h, $P = 0.008$) on the heat stress day; on day 3, the opposite was observed. These observations indicate that shading the hutch is recommended during warm daytime episodes when calves are more likely to rest in the hutch rather than in the pen.

Physiological variables

Generally, the RR, RT, and HR showed a similar pattern throughout the entire experiment, with daytime rises and nighttime falls, whereas RMSSD displayed an opposite

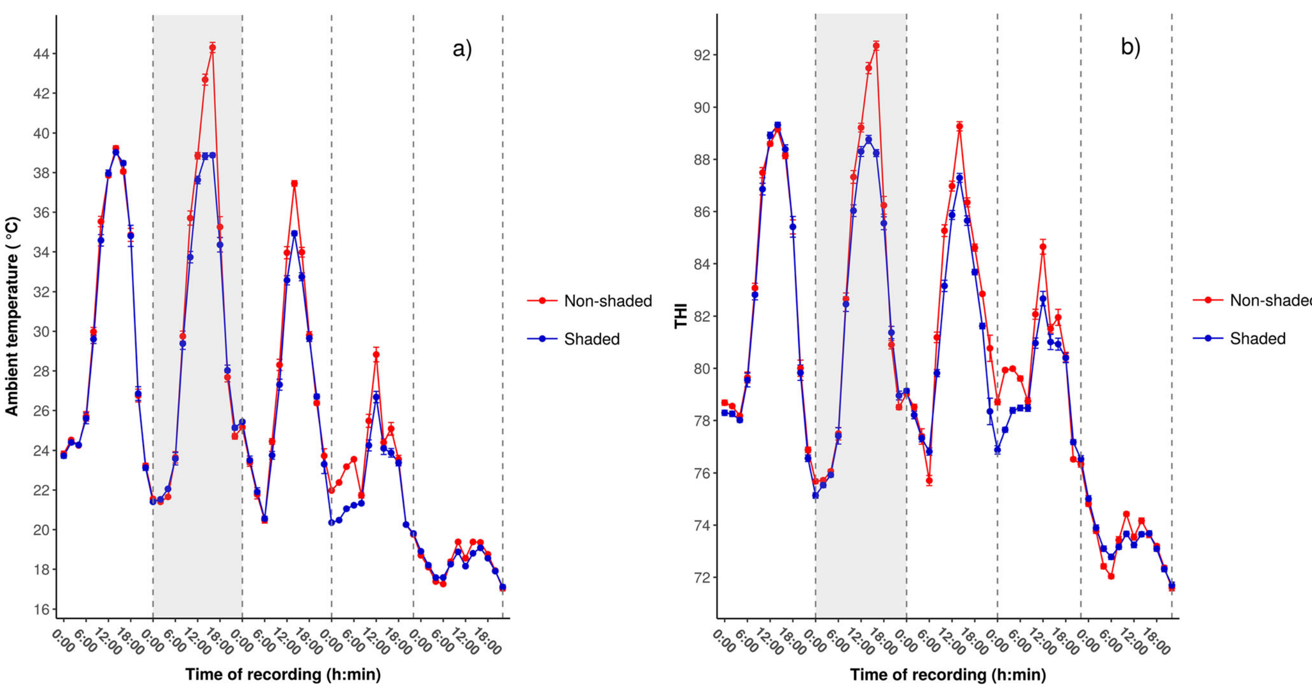

Fig. 2 Changes in ambient temperature (**a**) and temperature-humidity index (THI) (**b**) in the shaded and non-shaded hutch environments during the 5-day study period. The gray area between the first and the second dashed vertical lines represents day 2, the "heat stress day"

pattern, with decreased daytime and increased nighttime values for both S and NS calves (Fig. 3a–d).

The RR peaked at 1200 h throughout the day for both groups (Fig. 3a), with the minimum between 0000 and 0800 h in the morning. In agreement with earlier findings on dairy calves during summer (Spain and Spiers 1996; Lima et al. 2013), higher RR were observed in NS calves than in S calves during the afternoon, which was significant on the heat stress day at 0800, 1200, and 1600 h, and for day 3 at 1200 and 1600 h and for day 4 at 1200 h (Fig. 3a). The S calves had lower RR than NS calves at 1500 h in a humid subtropical climate, on average by 10 breaths/min (Spain and Spiers 1996). Respiratory rates of NS calves were with 42.3 ± 3.2 breaths/min higher compared to N calves at 1200 h on the

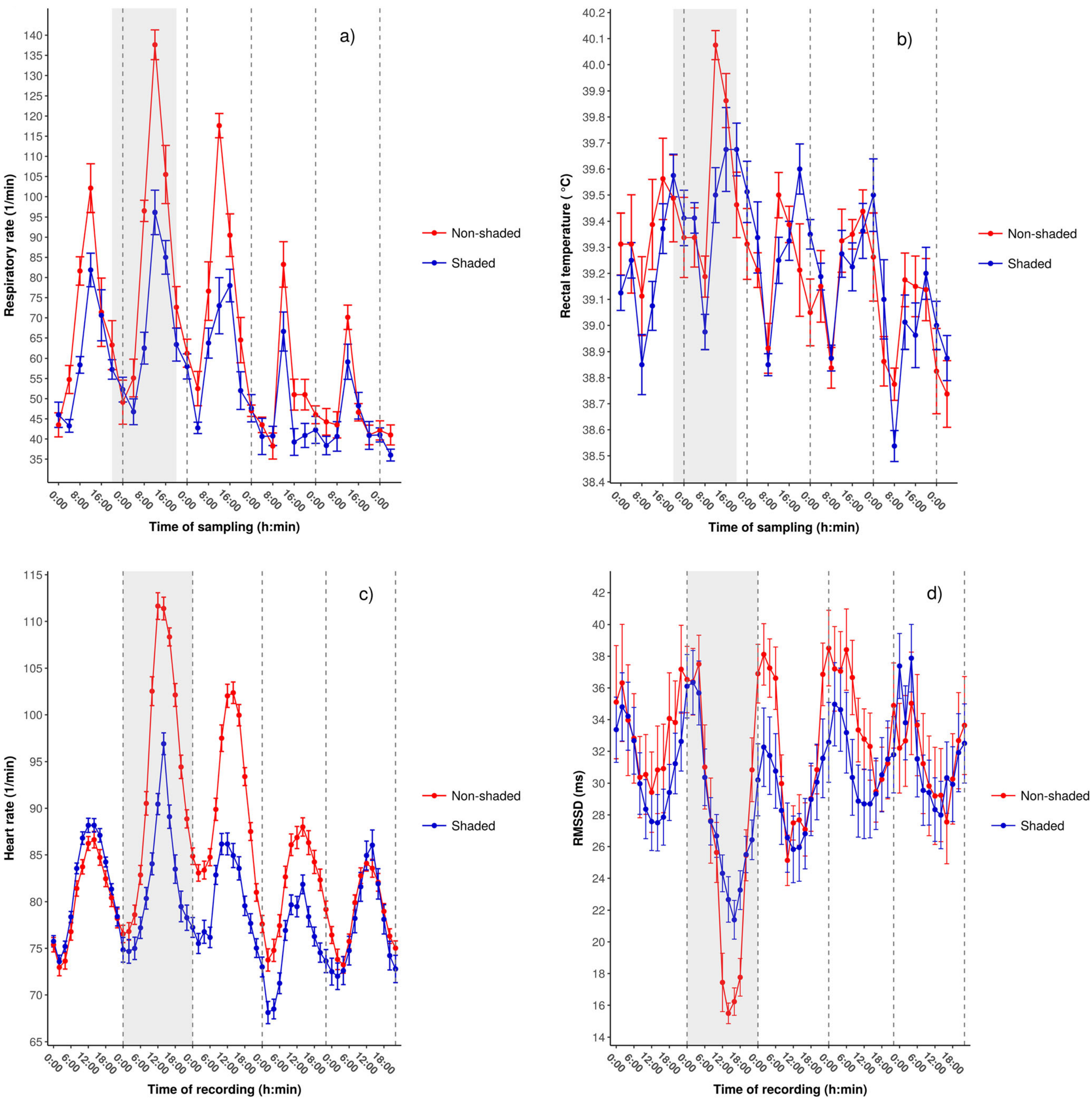

Fig. 3 Changes in respiratory rate (**a**), rectal temperature (**b**), heart rate (**c**), and the root mean square of successive differences in R-R intervals (RMSSD) (**d**) of shaded ($n = 8$) and non-shaded ($n = 8$) dairy calves during the 5-day study period. Group means ± SEM are based on data recorded with 4-h (respiration rate, rectal temperature) and 2-h (heart rate, RMSSD) sampling intervals. The gray area between the first and the second dashed vertical lines represents day 2 ("heat stress day"). Differences between shaded and non-shaded groups are marked by $^*P < 0.05$

heat stress day ($P = 0.001$), which were moderated to 20.1 ± 2.4 breaths/min at 1600 h ($P = 0.023$). It should be noted that in our study, extremely elevated temperatures persisted in the NS environment during the heat stress day with a maximum of 44.3 °C for the hutch and 46.7 °C for the pen environments. The earliest differences between the S and NS calves occurred in RR on day 2 (Fig. 3a), suggesting that during the early phase of heat stress, calves increase first their respiratory effort to cope with the hot environment and maintain homeothermy.

Rectal temperature exceeded maximum 0.4 °C the upper threshold of the normal core body temperature of 1-month old dairy calves that is 39.2 °C (Piccione et al. 2003) during the 5-day experiment, except on the heat stress day when maximal RT exceeded this value by 0.48 and 0.88 °C in the S and NS calves at 1200 and 1600 h, respectively (Fig. 3b). Maximal values were observed for RT also at 1200 h on days 2 and 3 for the NS calves, while RT peaks shifted with 4–8 h towards the evening or the night for the S calves (Fig. 3b). Minimal values were measured at 0800 h on all experimental days for both groups, which were paralleled by the decreased heat load during the early morning hours. Previous studies on RR and RT in heat stressed dairy calves recorded these variables only at 0700 and 1500 h (Spain and Spiers 1996) or at 0900 and 1500 h (Lima et al. 2013); therefore, comparison of our results on RR and RT fluctuations with the author's findings is not possible. The only group difference in RT was observed at 1200 h on the heat stress day (Fig. 3b) when THI exceeded 91 units, with 0.59 °C higher values for NS calves compared to S ones ($P = 0.045$). Consistent with our results, some studies reported no (Spain and Spiers 1996) or only minor differences (Coleman et al. 1996) between RT in S and NS calves during the morning or the afternoon hours. Although none of the differences were significant, slightly higher nighttime (between 2200 and 0400 h) RT was found for S calves than for NS calves throughout the experimental period.

Our study provided essential information on the cardiac adaptation of dairy calves exposed to extreme heat load in shaded and non-shaded thermal environments. To the best of our knowledge, there is no study that evaluated HR and HRV in heat-stressed calves. One of our major findings was that physiological responsiveness to extremely elevated temperatures can be characterized in dairy calves by the assessment of HR and RMSSD. Changes in these parameters reflected impaired cardiac function during the heat stress day in both groups (Fig. 3c, d). Heart rate gradually increased during daytime reaching its maximum between 1200 and 1400 h, and the lower peaks were observed at 0200 h for both S and NS calves throughout the experiment, except for day 5 where minimums were observed at 0400 h (Fig. 3c). Heart rate remained increased even at 1600 h when RR showed a rapid decrease from its maximum value (Fig. 3a, c). A similar phenomenon was previously reported by Bianca (1958), who found that during the phase in which breathing became slower and deeper, HR did not decrease in young calves. The prolonged high HR during the afternoon could be the result of the increased activity and heat production of the respiratory muscles during this forced "second-phase breathing." Recently, we observed a circadian rhythm of HR in summer in non-lactating cows, with peaks between 1600 and 1700 h (Kovács et al. 2016). Our present results might reflect a similar phenomenon.

Calves exhibited a progressive decrease in RMSSD during daytime in both groups with lower peaks between 1200 and 1600 h. The minimum RMSSD in the NS and S calves was lower by 14.3 and 6.1 ms, respectively ($P = 0.005$ and $P = 0.025$), on the heat stress day than on the control day (Fig. 3d). The high THI values in the present study (above 88 units for both groups between 1200 and 1600 h) may explain the decreased vagal activity and increased HR in all calves during the afternoon of the heat stress day. Decrease in cardiac vagal tone characterizes the first-phase stress response in farm animals exposed to environmental stressors (von Borell et al. 2007).

On days 2 and 3, NS calves exhibited higher HR than S calves between 8000 and 2000 h (Fig. 3a). Group differences were also significant at 0800 h (18.2 ± 1.2 beats/min, $P = 0.008$), 1200 h (22.3 ± 1.4 beats/min, $P = 0.003$), 1600 h (15.3 ± 0.8 beats/min, $P = 0.012$), and 2000 h (19.0 ± 1.1 beats/min, $P = 0.010$). Interestingly, a previous study found no effect of climatic conditions on HR recorded either at 0700, 1200, 1600, or 1800 h in climate-controlled chambers (daily temperatures ranging from 29.4 to 40.0 °C) in dairy calves (O'Brien et al. 2010).

Significantly lower RMSSD was observed on the heat stress day in NS calves compared to S ones at 1200, 1400, and 1600 h ($P = 0.012$, $P = 0.04$, $P = 0.010$). A drastic daytime decrease on the heat stress day and a moderate daytime decrease on day 3 was followed by a nighttime overcompensation of RMSSD between 0000 and 0600 h in NS calves with higher mean values than shown by S calves (Fig. 3d); however, there were no significant group differences. Supplementing the decreased nighttime HRs, the increased nighttime RMSSD reflects increased vagal activity between 2200 and 0600 h from day 2 to day 3 and from day 3 to day 4, suggesting a slow recovery of the autonomic nervous system from heat stress. Interestingly, in contrast to RMSSD, the nighttime HRs of the NS calves did not differ from those of the S calves on days 2 and 3. It seems that the RMSSD changes mirror an effective coping mechanism of the NS calves and support the notion that nighttime recovery is essential in coping with excessive heat loads (Scott et al. 1983; Hahn and Mader 1997).

It should be noted that since group differences in all physiological parameters decreased on day 4 and diminished on day 5 being within the normal ranges on both days, no chronic physiological consequences of the extreme heat load could be assumed in the present study.

Conclusions

A shade positioned 1.9 m above the ground could be an efficient method in reducing surrounding heat load for both the hutch and pen environments in a continental region as shown by ambient temperatures and THI as well. Shading effectively reduced heat stress in pre-weaned Holstein bull calves as evidenced by HR and RMSSD in addition to traditional measures of heat stress. Since changes in RR, HR, and RMSSD occurred earlier than RT, these parameters should be considered as more immediate indices of heat stress than RT in preweaned calves. Despite the marked group differences on the heat stress day in RMSSD, in contrast to RR and HR, shading had no effect on vagal tone during the reminder of the study period. Therefore, to expand the understanding on the autonomic adaptation to heat stress in calves, further investigations are still warranted involving a larger sample size.

Besides providing insights on the acute physiological responses to heat stress of dairy calves, our results have also practical implications. Shading of individually kept young calves in not a common method in large-scale dairies; however, based on the present results, it should be recommended during warm episodes in summer, especially in those climatic regions where heat stress represents a concern. It might support not only well-being but also production.

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