Domestic Animal Endocrinology 66 (2019) 43–47

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.journals.elsevier.com/domestic-animal-endocrinology

Effect of artificial shade on saliva cortisol concentrations of heat-stressed dairy calves

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ARTICLE INFO

Article history:
Received 2 June 2018
Received in revised form 31 August 2018
Accepted 15 September 2018

Keywords:
Heat stress
Dairy calves
Saliva cortisol
Artificial shading

ABSTRACT

Responses to heat stress have not been evaluated in dairy cattle by noninvasive techniques such as analysis of saliva cortisol concentrations. The aim of the present study was the assessment of saliva cortisol levels in Holstein bull calves with (n = 8) or without supplemental shade (n = 8) in response to acute heat stress. Measurements were carried out during a 5-d period [temperature, average/max (°C); day 1 (control, all calves shaded): 22.9/29.4, day 2 (heat stress day): 28.3/38.8, day 3: 26.2/33.5, day 4: 23.7/28.7, and day 5: 21.2/24.7]. The level of thermal stress was characterized with a temperature-humidity index (THI). Saliva cortisol levels did not differ between groups during the control day. On the heat stress day, saliva cortisol levels increased from 8:00 to 12:00 by 51% and 342% in shaded and nonshaded calves, respectively, and nonshaded calves showed higher cortisol concentrations at 12:00, 16:00, and 24:00. Saliva cortisol levels peaked at 12:00 on day 3 in both groups. On days 4 and 5, saliva cortisol did not show significant daytime elevations in either group; however, group differences remained significant until 20:00 on day 4. Based on our results, measurement of saliva cortisol concentrations is a promising approach to detect acute heat stress in dairy calves, which could be reduced by artificial shading.

1. Introduction

The occurrence of high ambient temperatures has extended from tropical areas into northern latitudes. Therefore, the well-being of livestock species, including dairy cattle, is often impaired during warm episodes of summer in the northern United States, Canada, and Europe. Preweaned calves that are kept in individual outdoor hutches are vulnerable to hot weather because their natural heat-dissipating behavior (eg, finding shade) is hindered [1]. Although calf hutches are widely used in Middle-European countries, extreme climatic conditions that cannot be compensated for by thermoregulatory mechanisms result in thermic stress that impairs calf welfare [2].

Homeorhetic regulators including mineralocorticoids and glucocorticoids are involved in the acclimatory responses to thermal stress in ruminants [3]; therefore, the concentrations of glucocorticoids (and their metabolites) are commonly used to detect stress in domestic animals [4]. Saliva samples can be easily taken at fixed time intervals before and after an imposed stress [5], and this is a minimally invasive method that assesses hypothalamic–pituitary–adrenal (HPA) axis reactivity in dairy cattle [6] as it correlates well with plasma cortisol with 0 min [7] or with a 10 min time lag [8,9]. Interestingly, responses of the HPA

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https://doi.org/10.1016/j.domaniend.2018.09.001

axis to heat exposure have been studied in adult dairy cattle [10] but not in calves.

The objective of this study was to characterize the changes in saliva cortisol concentrations of dairy calves exposed to heat stress. Considering previous reports showing significant effects of shading on the thermal environments of calves using artificial shade [11], we performed our experiment on shaded and nonshaded animals. We hypothesized that calves in shaded hutches would more successfully cope with a stressful thermal environment, and that this would be reflected in lower saliva cortisol levels compared to nonshaded calves.

2. Materials and methods

2.1. Animals and experimental design

The study was approved by the Pest County Government Office, Department of Animal Health (Permit Number: PE/EA/1973–6/2016). Measurements were carried out at a Hungarian dairy farm (N47°18′191″ E18°48′336″), during a 6-d period in the mo of August on the same animals and with the same experimental settings as described in our recent study [12]. We used preweaned Holstein bull calves (n = 16) at 1 mo of age with a mean body weight of 74 (±3.2) kg. Calves were housed in 1.65 × 1.20 m individual hutches with a 1.60 m^2 exercise pen in front of each hutch. Hutches were aligned in one row and oriented north to south to maximize the exposure to solar heating.

Between 8:00 and 14:00 on day 0, a shading structure was prepared over the hutches and pens of all calves. A sun shade net with shade rate of 80% was used as shading material and was located 1.9 m above the ground. The following 10 h served as a habituation period for the calves to get used to the novel environment. On day 0, the temperature ranged from 18.3°C to 27.5°C in the hutch environment. Day 1 served as the control period, and all hutches remained shaded throughout the day. At 24:00, the shading net was removed from 8 hutches that remained exposed to direct sunlight throughout the experiment ("nonshaded" group, n = 8) and were compared to the 8 hutches that remained shaded ("shaded" group, n = 8). On day 2 ("heat stress day"), the maximal temperatures were 37.5°C and 43.7°C in the shaded and nonshaded calf environments, respectively. A "post-stress period" (days 3–5) was characterized with decreasing heat load with maximal temperatures of 30.3 and 33.5°C (day 3), 26.5 and 28.1°C (day 4) and 24.3 and 24.5°C (day 5) for the shaded and nonshaded environments, respectively. Both shaded and nonshaded calves were fed the same diet throughout the experiment. Milk was provided at 5:00, whereas calf starter, alfalfa hay, and fresh water were available ad libitum.

2.2. Assessment of the thermal environment

Thermal environment around the animals was assessed by recording the ambient temperature and humidity at 10-min intervals. Data loggers were fitted inside the hutches (VOLTCRAFT DL-181THP, Conrad Electronic SE, Hirschau, Germany) and in the outside pen areas (Testo 175 H1, Testo Inc, Sparta). Temperature and humidity values averaged for the 4 h before the saliva samplings were used for further analysis. The temperature-humidity index (THI) was used to characterize the microclimatic environment: $(0.35 \times T_{db} + 0.65 \times T_{wb}) \times 1.8 + 32$, where T_{db} = dry bulb temperature and T_{wb} = wet bulb temperature [13].

2.3. Saliva cortisol

Using a synthetic swab (Salivette cortisol, Sarstedt, Nümbrecht-Rommelsdorf, Germany), saliva samples were taken between day 1 at 0:00 and day 5 at 24:00 h in 4-h intervals. The swabs were placed loosely onto the tongue of the calf until they were well soaked with saliva. The swabs were then inserted into Salivette polypropylene tubes, which were placed on ice immediately after sampling and stored at 4°C until centrifugation at 1,000 × *g* for 10 min. At least 1.5 mL saliva per sample was obtained and frozen at −20°C until analysis.

Cortisol concentrations were measured by a direct radioimmunoassay method without extraction using 1,2,6,7-3H-cortisol (TRK 407; Radiochemical Center, Amersham, UK) and a highly specific polyclonal antibody raised against cortisol-21-HS-BSA in rabbits [12]. The cross reactivity of the assay was as follows: cortisol, 100%; corticosterone, 19%; prednisolone, 9.5%; deoxycortisol, 6.4%; 17α-OH progesterone, 5.7%; progesterone, 2.6%; and any of 22 other steroids, 0.54% to 0.0001%. The assay standards (cortisol FW 362.5; Sigma Chemical Company, St. Louis, MO) were prepared in cortisol-free plasma (range: 2,000 fmol to 31.25 fmol per tube). The antibody-bound and free fractions were separated by cold dextran-coated charcoal suspension after an 18 to 24 h incubation period. Radioactivity was measured with a TriCarb liquid scintillation counter (Perkin Elmer, Inc, DownersGrove, IL). The sensitivity of this assay system was 11.37 fmol/tube. Within the concentration range of 2.0 to 100.0 nmol/mL, the intra- and interassay coefficients of variation varied between 3% and 8%, and 5% to 10%, respectively. Samples with a cortisol concentration higher than 100.0 nmol/L were reassayed after dilution [14].

2.4. Statistical evaluation

All statistical analyses were performed in the R–3.3.1 statistical environment and language [15]. Daily variations in mean values of THI calculated for 4-h periods in the hutch and pen environments were compared using two-way factorial ANOVA with repeated measures followed by the Tukey-Kramer post hoc test or the Student's *t*-test. Explanatory variables involved environment (shaded vs nonshaded) and 4-h recording periods (ie, 0:00–4:00, 4:00–8:00, 8:00–12:00, 12:00–16:00, 16:00–20:00, and 20:00–24:00).

Saliva cortisol concentrations obtained for shaded and nonshaded calves were compared using linear mixed models, with the group and time of sampling (ie, 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00) as fixed factors. All models included calf as a random factor and included all possible interactions between main factors. Comparison of shaded and nonshaded groups was made with the Fisher-

type z-transformation-based z-test in all models. A P-value < 0.05 was considered significant. All results are expressed as mean plus SEM values.

3. Results

3.1. Thermal environment

No differences were found in THI between the hutch and pen environments during the experiment. Changes in THI measured in the hutch environment during the experimental period are presented in Figure 1. Mean THI increased from 77.3 ± 0.2 to 78.1 ± 0.2 and from 80.7 ± 0.3 to 82.5 ± 0.3 units when comparing the 8:00 to 12:00 and 12:00 to 16:00 periods on the heat stress day ($P = 0.002$ and $P < 0.001$) and decreased to 67.3 ± 0.1 and 68.1 ± 0.2 units between 20:00 and 24:00 in the shaded and nonshaded hutch environments, respectively ($P < 0.001$ for both cases). Between 12:00 and 16:00 on day 3, mean THI was 77.6 ± 0.2 and 81.2 ± 0.3, which decreased to 66.2 ± 0.1 and 66.7 ± 0.2 units between 20:00 and 24:00 in the shaded and nonshaded hutch environments, respectively ($P = 0.002$ and $P < 0.001$). On days 4 and 5, mean THI did not exceed 72 units during the daytime in shaded or nonshaded environments, and differences in mean THI were nonsignificant between consecutive 4-h periods on these days.

On the heat stress day, shading had a significant effect on THI between 8:00 to 12:00 and 12:00 to 16:00 the hutch environment ($P < 0.001$ for both periods). Differences between the shaded and nonshaded pen environments were moderate on day 3 (between 8:00–12:00: $P = 0.050$, between 12:00–16:00: $P = 0.023$). On days 1, 4, and 5, no differences in THI were found between shaded and nonshaded environments.

3.2. Saliva cortisol

Generally, saliva cortisol levels showed a similar pattern in shaded and nonshaded calves throughout the 5-d experiment (Fig. 2). During day 1 (control), mean saliva cortisol concentrations varied between 6.5 and 10.2 ng/g and between 6.7 and 9.3 ng/g in shaded and nonshaded calves, respectively, with minor individual and no group differences. On days 2, 3, and 4, significant daytime rises and nighttime falls were observed for both groups. On day 2, saliva cortisol levels were balanced in both groups until 8:00 and then increased suddenly by 51% versus 342% for

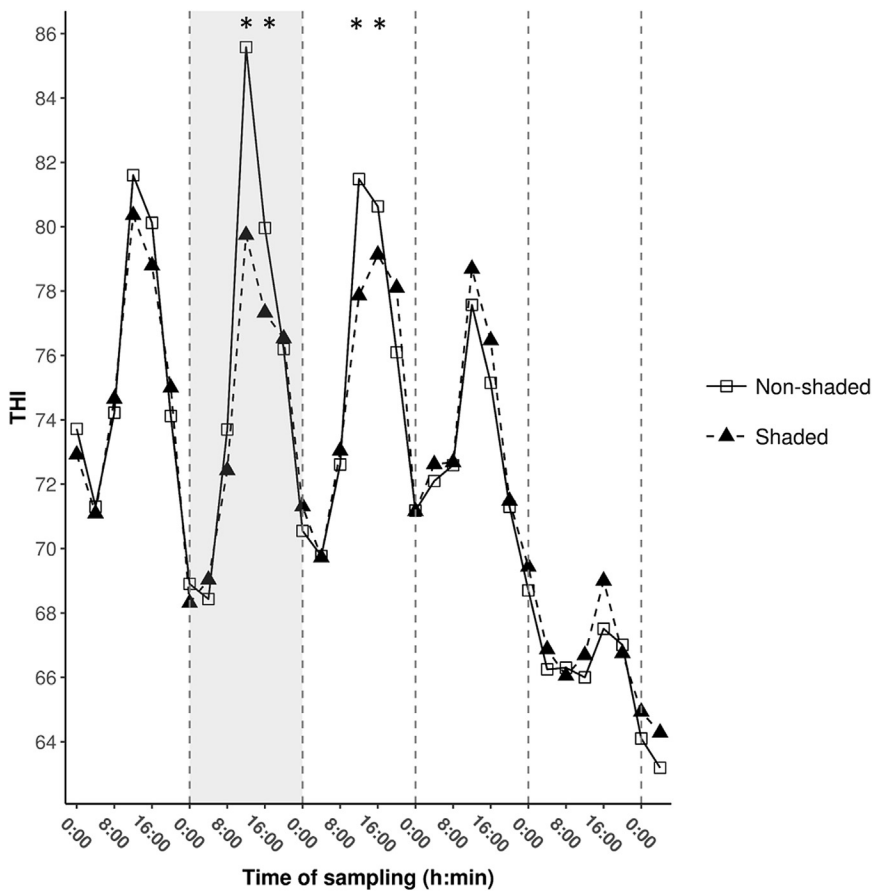

Fig. 1. Changes in the temperature-humidity index (THI) measured in the shaded (▲) and nonshaded (□) hutch environments during the 5-d experiment. During day 1 (control), both environments were shaded. The gray area between the first and the second dashed vertical lines represents the "heat stress day." Significant differences between groups: $^*P < 0.05$.

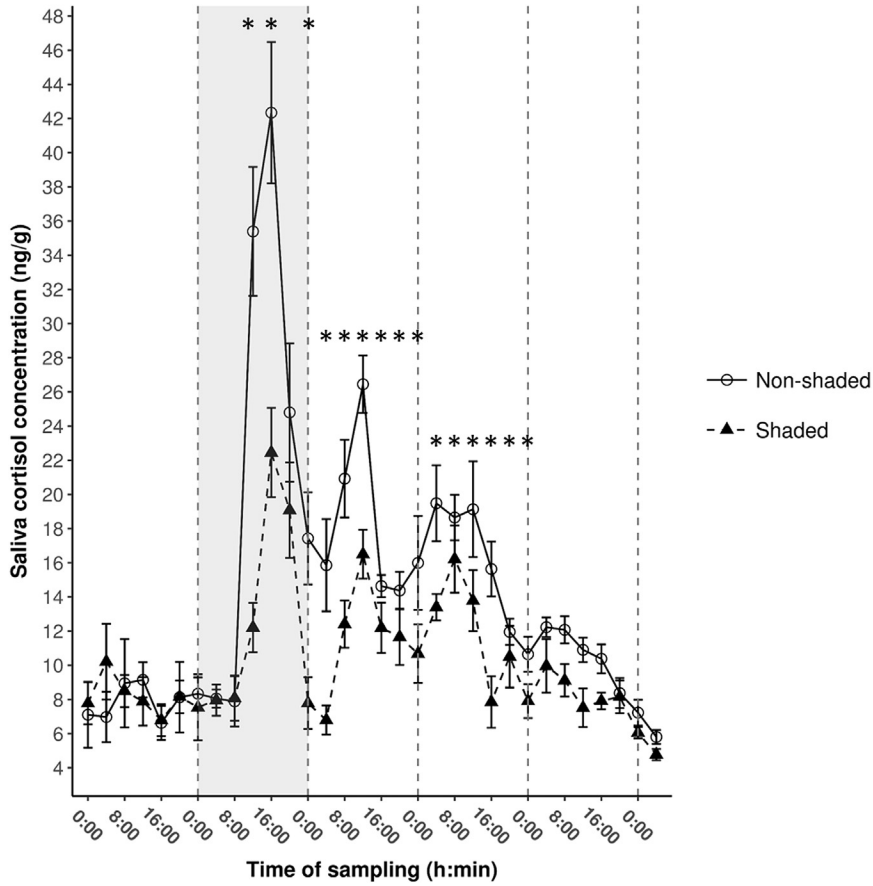

Fig. 2. Changes in saliva cortisol concentrations of shaded (▲, n = 8) and nonshaded (○, n = 8) dairy calves during the 5-d experiment. Data are presented as means ±SEM. During day 1 (control), all calves were shaded. The gray area between the first and the second dashed vertical lines represents the "heat stress day." Significant differences between groups: *$P < 0.05$.

shaded versus nonshaded calves, respectively, at 12:00 ($P = 0.586$ and $P < 0.001$) to reach peak levels at 16:00 in both groups (Fig. 2). Nonshaded calves showed higher cortisol concentrations at 12:00, 16:00, and 24:00 ($P < 0.001$, $P < 0.001$ and $P = 0.046$) than shaded ones, with significant interactions between group and time of sampling ($F_{5,70} = 8.00$, $P < 0.001$). After 16:00 on day 2, saliva cortisol gradually declined in both shaded ($P = 0.886$) and nonshaded calves ($P < 0.001$) to reach its lowest values (6.9 ± 0.7 and 16.0 ± 2.3 ng/g, respectively) at 4:00 on day 3. Like the significant rise (from 12:00 to 16:00, $P = 0.025$), this decline (from 20:00 to 24:00, $P = 0.009$) occurred with a 4-h delay in shaded calves compared to nonshaded calves (Fig. 2).

Saliva cortisol levels peaked at 12:00 on day 3 in both groups. During the afternoon, cortisol concentrations decreased to the half of the maximum in nonshaded calves at 16:00 (48.3%, $P = 0.039$), whereas in shaded calves, this decline was more moderate (26.1%, $P = 0.675$). On day 4, saliva cortisol showed similar changes on day 3 in both groups, with a slight increase at 4:00 that was followed by a nonsignificant daytime elevation ($P = 0.752$ and $P = 0.685$, respectively) and a decrease from 12:00 and 16:00 in both shaded and nonshaded calves ($P = 0.075$ and $P = 0.008$). Group differences remained significant throughout day 3 until 24:00 on day 4 ($P < 0.001$ for all samples). During day 5, levels of saliva cortisol ranged between 7.2 and 9.4 ng/g and between 8.0 and 11.9 ng/g, respectively, in shaded and nonshaded calves, without significant group differences (Fig. 2).

4. Discussion

Several authors have reported the effects of in utero exposure to heat stress on blood cortisol in calves during the preweaning period [16,17]; however, this is the first study to investigate HPA responses to a hot environment in dairy calves. An important message of the present study is that supplemental shading of calves can reduce the amount of cortisol secretion. Saliva cortisol levels indicated a higher stress load in calves in hutches exposed to direct solar radiation that persisted for about 48 to 56 h after the initial rise in THI (from 12:00 on the heat stress day).

All calves were exposed to an extreme heat load in the present study on days 2 and 3, with significant THI differences between shaded and nonshaded environments during the daytime. Changes in saliva cortisol concentrations followed the changes in THI with higher levels during the daytime, except for day 5. On the heat stress day, the levels

of saliva cortisol measured at 16:00 were 200% and 500% higher, respectively, in shaded and nonshaded calves compared to those measured on day 1 at 16:00. On days 3 and 4, saliva cortisol showed a similar pattern to day 2 but with lower daytime elevations. Alvarez and Johnson [18] also reported an increase in glucocorticoid levels by 62% in the second hour of heat exposure in cows, reaching a peak (120%) after 4 h. Our results also suggest that the highest cortisol levels occur within 2 to 4 h after exposure to the highest heat load (between 12:00 and 16:00); however, the relatively low frequency of sampling did not allow the exact determination of peak levels in this study. We attempted to reduce the number of contacts with the calves in this study to prevent experimentally induced effects on cortisol levels.

In the present study, during day 5, the levels of saliva cortisol returned to the levels obtained for the control day in both groups. A similar turnover phenomenon for cortisol levels was reported by Christison and Johnson [19] in adult cows exposed to chronic, mild heat stress 1 to 2 d following the onset of the thermal stress. In our study, the extreme heat load persisted during day 2 and was followed by a slightly decreased yet high daytime heat exposure on day 3, which could also be considered as chronic. However, in our study, significant nighttime falls in THI (Fig. 1) might have supported nighttime HPA recoveries of calves from heat stress, followed by lower daytime cortisol release. Therefore, our results might reflect the adaptation of calves to the stressful environment.

Based on our results, the assessment of saliva cortisol levels allows a possible approach to the characterization of the stress response of dairy calves to thermal challenge. Higher stress level in calves kept in nonshaded hutches underscores the importance of solving future challenges in maintaining thermal well-being of dairy calves in hot continental environments.

Acknowledgments

Levente Kovács was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, Budapest, Hungary [BO/00040/16/4]; the OTKA Postdoctoral Scholarship of the National Research, Development and Innovation Office, Budapest, Hungary [NKFIH-6,493–1/2016]; the New National Excellence Program 'Bolyai plus' Project of the Ministry of Human Capacities [ÚNKP-18-4-SZIE-3] and the Research Center of Excellence project of the National Research, Development and Innovation Office, Budapest, Hungary [1,476–4/2016/FEKUT]. Viktor Jurkovich was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, Budapest, Hungary [BO/29/16/4].

Conflicts of interest: There are no conflicts of interest for any of the authors.

Authors' contributions: Plan of the experiment was performed by Levente Kovács and Viktor Jurkovich. The experiment was performed by Levente Kovács, Luca Fruzsina Kézér, Viktor Jurkovich. Article writing was performed by Levente Kovács. Statistical analysis was performed by Ferenc Ruff and Mikolt Bakony. Supervision was performed by Ottó Szenci.

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