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Effects of increasing air temperature on skin and respiration heat loss from dairy cows at different relative humidity and air velocity levels

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ABSTRACT

The focus of this study was to identify the effects of increasing ambient temperature (T) at different relative humidity (RH) and air velocity (AV) levels on heat loss from the skin surface and through respiration of dairy cows. Twenty Holstein dairy cows with an average parity of 2.0 ± 0.7 and body weight of 687 ± 46 kg participated in the study. Two climate-controlled respiration chambers were used. The experimental indoor climate was programmed to follow a diurnal pattern with ambient T at night being 9°C lower than during the day. Night ambient T was gradually increased from 7 to 21°C and day ambient T was increased from 16 to 30°C within an 8-d period, both with an incremental change of 2°C per day. A diurnal pattern for RH was created as well, with low values during the day and high values during the night (low: RH_l = 30–50%; medium: RH_m = 45–70%; and high: RH_h = 60–90%). The effects of AV were studied during daytime at 3 levels (no fan: AV_l = 0.1 m/s; fan at medium speed: AV_m = 1.0 m/s; and fan at high speed: AV_h = 1.5 m/s). The AV_m and AV_h were combined only with RH_m. In total, there were 5 treatments with 4 replicates (cows) for each. Effects of short and long exposure time to warm condition were evaluated by collecting data 2 times a day, in the morning (short: 1-h exposure time) and afternoon (long: 8-h exposure time). The cows were allowed to adapt to the experimental conditions during 3 d before the main 8-d experimental period. The cows had free access to feed and water. Sensible heat loss (SHL) and latent heat loss (LHL) from the skin surface were measured using a ventilated skin box placed on the belly of the cow. These heat losses from respiration were measured with a face mask covering the cow's nose and mouth. The results showed that skin SHL decreased with increasing ambient T and the decreasing rate was not affected by RH or AV. The average skin SHL, however, was higher under medium and high AV levels, whereas it was similar under different RH levels. The skin LHL increased with increasing ambient T. There was no effect of RH on the increasing rate of LHL with ambient T. A larger increasing rate of skin LHL with ambient T was observed at high AV level compared with the other levels. Both RH and AV had no significant effects on respiration SHL or LHL. The cows lost more skin sensible heat and total respiration heat under long exposure than short exposure. When ambient T was below 20°C the total LHL (skin + respiration) represented approx. 50% of total heat loss, whereas above 28°C the LHL accounted for more than 70% of the total heat loss. Respiration heat loss increased by 34 and 24% under short and long exposures when ambient T rose from 16 to 32°C.
Key words: dairy cow, ambient temperature, heat loss, heat stress

INTRODUCTION

Dairy cows are homeothermic animals and heat balance mechanism is important to sustain the body temperature (**T**). There are 2 modes of heat transfer between the animal and its environment, the sensible (nonevaporative) and the latent (or evaporative) heat loss. At certain ambient T, heat is mainly lost via the sensible way due to the difference between the skin surface T and the environmental T (objects and air). With increasing ambient T, there is a marked shift from sensible heat loss (**SHL**) to latent heat loss (**LHL**; Maia and Loureiro, 2005). In warmer conditions, increased respiration and sweating rate are 2 of the primary autonomic responses exhibited by animals (Gebremedhin et al., 2008). Dairy cows possess a very effective sweating capacity (Mount, 1979), in advanced

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bred for high productivity this sweating process is crucial to help the cows maintain heat balance. Maia et al. (2005) found that the respiratory heat loss in dairy cows increased linearly with the ambient T until 20°C, and then increased exponentially when the ambient T exceeded 25°C. Sweating facilitates evaporative heat loss from the skin surface, and under high ambient T this could account for 87.9% of the total LHL (Santos et al., 2017).

However, information on the absolute contribution of each single component of the total heat loss is scarce. How much heat is lost via the sensible route and latent route, and how much heat is lost through respiration and from the skin surface? The LHL from skin might be limited by the threshold of potential evaporation rate, which means not all produced sweat could be evaporated for heat dissipation. An understanding of the transition between SHL and LHL under different ambient conditions will help efficiently apply cooling systems. It is known that the evaporation rate of water could be limited by relative humidity (**RH**; Berman, 2009), therefore, information about the effects of increasing ambient T at different RH and air velocity (**AV**) levels on the adjustments of heat transfer routes is of significant importance. The objective of this study was to determine the effects of environmental conditions (air T, RH, and AV) and exposure time on LHL and SHL from the skin surface and through respiration of Holstein-Friesian dairy cows. Our hypothesis is that the level and proportion of SHL and LHL will change with increasing ambient T and this change is influenced by RH, AV, and exposure time (short or long).

MATERIALS AND METHODS

Animals and Feed

The experiment was conducted in 2021, in accordance with Dutch law and approved by the Institutional Animal Care and Use Committee of Wageningen University & Research (Wageningen, the Netherlands). Twenty Holstein-Friesian dairy cows were used with an average milk yield (±SD) of 30.0 ± 4.7 kg/d, 206 ± 39 DIM, BW of 687 ± 46 kg, and parity of 2.0 ± 0.7. Nineteen cows were at an average of 105 ± 38 d in pregnancy. Cows were grouped in 4 blocks of 5 cows based on parity and expected milk yield. Each cow within a block was randomly assigned to one of the 5 treatments as shown in Tables 1 and 2. The cows received ad libitum feed and water. Twice daily at 0500 and 1530 h, leftover feed was removed and fresh feed was added. The diet was formulated to meet or exceed the nutritional requirements of lactating cows according to the Dutch System (CVB, 2008), and the amount offered to each cow was adjusted daily to yield an excess (uneaten feed) of at least 5%.

Before the start of the experiment, a 7-d acclimatization for the cows was done in a facility approximately 2 km distanced from the climate-controlled respiration chamber (**CRC**). During the acclimatization period, the cows were housed in individual tiestalls, haltered, visited frequently by animal caretakers and received the experimental diet. After the acclimatization the cows were transferred to the CRC, there they started 3-d adaptation period during which, in addition to receiving feeding and milking visits, the cows were visited 2 times

Table 1. Five treatments each lasted for the 8-d period using climate-controlled respiration chambers (CRC)[1]

	Temperature,[3] °C		Relative humidity, %		Air velocity
Treatment[2]	2200–0700 h	1000–1900 h	2200–0700 h	1000–1900 h	0900–2100 h
RH_l × AV_l	7–21	16–30	50	30	Fan off
RH_m × AV_l	7–21	16–30	70	45	Fan off
RH_h × AV_l	7–21	16–30	90	60	Fan off
RH_m × AV_m	9–23	18–32	70	45	Fan on, speed 1
RH_m × AV_h	9–23	18–32	70	45	Fan on, speed 2

[1]2200–0700 h is nighttime, duration from 2200 h until next day at 0700 h; 1000–1900 h is daytime, duration from 1000 h until 1900 h at the same day; 0900–2100 h indicates air velocity treatment duration from 0900 h until 2100 h the same day, for first CRC. There was a 1-h delay for all the controlling parameters for second CRC.

[2]There were 5 treatments, representing different combinations of temperature, relative humidity (RH; low = RH_l, 30%; medium = RH_m, 45%; high = RH_h, 60%), and air velocity (AV; low = AV_l, 0.1 m/s; medium = AV_m, 1.0 m/s; high = AV_h, 1.5 m/s).

[3]7–21 (or 9–23) means the air temperature at d 1 was 7°C (or 9°C) and d 8 was 21°C (or 23°C) during nighttime; 16–30 (or 18–32) means the air temperature at d 1 was 16°C (or 18°C) and d 8 was 30°C (or 32°C) during daytime.

Table 2. Body weight, annual average milk yield, parity, DIM, and pregnant days of cows in 5 treatment groups (means ± SD)

	Treatment[1]				
Item	RH_l × AV_l	RH_m × AV_l	RH_h × AV_l	RH_m × AV_m	RH_m × AV_h
BW, kg	695 ± 54	671 ± 52	667 ± 41	721 ± 50	680 ± 29
Milk yield, kg/d	27.2 ± 7.2	30.8 ± 3.9	29.0 ± 6.9	32.0 ± 1.9	30.9 ± 2.0
Parity	2.3 ± 0.5	2.3 ± 0.5	2.5 ± 1.0	2.8 ± 1.0	2.5 ± 0.6
DIM	212 ± 35	192 ± 40	182 ± 54	227 ± 31	215 ± 35
Pregnant days	100 ± 27	116 ± 20[2]	85 ± 60	104 ± 30	120 ± 48

[1]There were 5 treatments, representing different combinations of relative humidity (RH; low = RH_l, 30%; medium = RH_m, 45%; high = RH_h, 60%), and air velocity (AV; low = AV_l, 0.1 m/s; medium = AV_m, 1.0 m/s; high = AV_h, 1.5 m/s).

[2]In treatment 2 there was 1 nonpregnant cow.

daily by a researcher. During each visit a simulation of data collection action was performed on the cow to learn about their individual temperaments and to allow the cow to get familiar with the actual data collection activity. In the CRC, cows could also see and hear other cows through transparent windows. Each cow was subjected to an 8-d experimental period in the CRC with a specific treatment consisting of combinations of T, RH, and AV.

Climate-Controlled Respiration Chamber

In this study, 2 identical CRC were used. Each chamber was split into 2 individual airtight compartments with thin walls equipped with transparent windows to allow audio and visual contact between 2 cows and thereby reduce the effects of social isolation on their behavior. Each compartment had a volume of 34.5 m^3 and dimension of length × width × height: 4.5 × 2.7 × 2.8 m, as described in detail by Gerrits and Labussière (2015). In each compartment the RH was monitored by one RH sensor (Novasina Hygrodat100, Novasina AG), and the ambient T was monitored by 5 PT100 temperature sensors (Sensor Data BV) evenly distributed over the room at animal height, as described in detail in Zhou et al. (2022). The different RH levels were achieved by means of a humidifier (ENS-4800-P, Stulz) or a dehumidifier (Koeltechniek, Nijssen) and the circulating air was heated or cooled depending on the deviation from set-point T, the control mechanism of which can be found in the book of Gerrits and Labussière (2015). Air velocity was achieved using a ventilator (Professional Fans; 500 mm diameter, model 8879, HBM Machines BV) that fixed on the ceiling of the chamber (2.5 m above the floor; Figure 1) so that the air flow moved through the axial body length of the

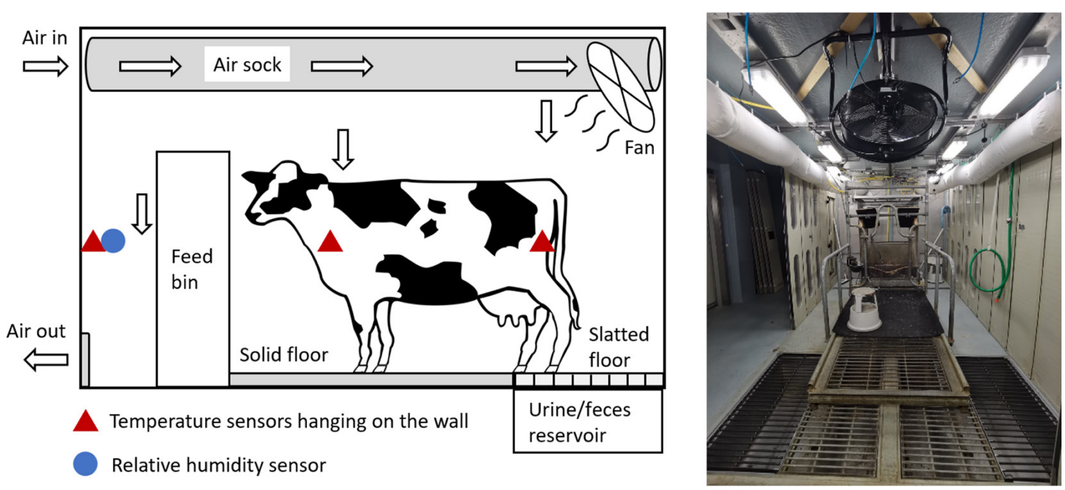

Figure 1. Schematic view and overview photo of the climate-controlled respiration chamber. There are 2 temperature sensors hanging on each side wall (left and right), and 1 temperature sensor and 1 relative humidity sensor hanging on the wall in front of the cow. The material of the solid floor is rubber mat, and the slatted floor is rubber-covered metal grills (Gerrits and Labussière, 2015). The cow inside the chamber was tied up loosely so that it could easily move forward or backward and lie down.

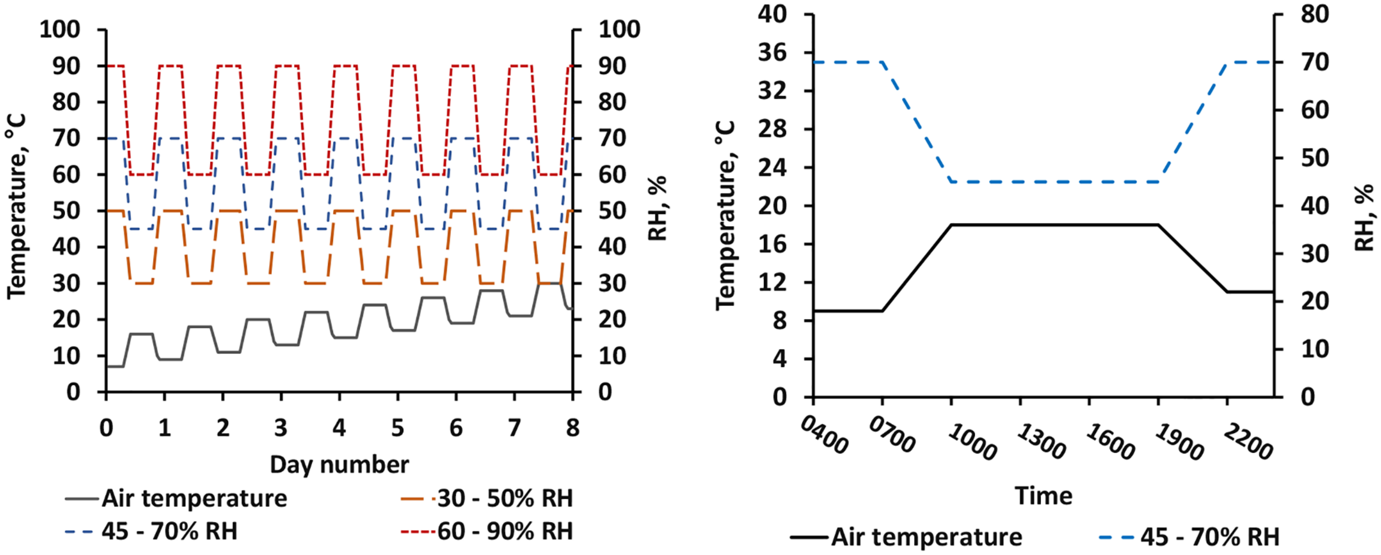

Figure 2. (a) Schematic temperature and relative humidity (RH) patterns during the 8 experimental days. Between 0700 and 1000 h, temperature and RH changed gradually to daytime levels and stayed constant until 1900 h. Between 1900 and 2200 h, temperature and RH gradually decreased to nighttime levels and stayed constant again until the next day at 0700 h. (b) An example of temperature and RH patterns of d 2 with 45 to 70% RH.

cow from back to front. The chambers were artificially lit for 16 h of daylight (390–440 lx, 0500–2100 h) and 8 h nightlight (35–40 lx, 2100–0500 h).

Experimental Design

The diurnal patterns of the climatic condition were mimicked based on the retrospective data obtained from the Dutch National Weather Service (KNMI, 2019), which is a typical diurnal pattern for Dutch weather during the summertime. The data of ambient T and RH for daytime (0700–1900 h) and nighttime (1900–0700 h) were then coupled into CRC and programmed for 5 different treatment groups (Table 1, Figure 2).

The ambient T, RH, and AV conditions for 3-d adaptation period in the CRC were set and controlled the same as the first day of the corresponding experimental period. The 8-d experimental period started right after the 3-d CRC adaptation. Ambient T inside the chambers was gradually increased at night and during the day (by steps of 2°C per day for both nighttime and daytime T) as shown in Figure 2. The experimental treatments comprised 3 RH levels and 3 AV levels as described in Table 1. Three levels of RH during the day and night were low (**RH_l**) 30% (day) and 50% (night); medium (**RH_m**), 45% (day) and 70% (night); and high (**RH_h**), 60% (day) and 90% (night). In daytime, AV was applied at low (**AV_l**, 0.1 m/s), medium (**AV_m**, 1.0 m/s), or high (**AV_h**, 1.5 m/s) levels. At nighttime, AV was kept at natural speed (AV_l). For AV_m and AV_h the ambient T was started at 2°C higher (from 18 to 32°C) than that with AV_l. Resulting from retrospective data (KNMI, 2019), in summertime RH during daytime ranged within the medium level. In addition, in compliance with saving the number of experimental animals (OIE, 2021), the AV_m and AV_h were only combined with the RH_m. More detailed description can be found in the previous study (Zhou et al., 2022). Because of the capacity of the CRC, the ambient T and RH required a time span of 3 h to adjust from one to a new level. As a result, the daytime condition was reached at 1000 h (set at 0700 h) and the nighttime condition was reached at 2200 h (set at 1900 h).

Data Collection

Practically, there was one set of apparatus and researchers for 2 CRC in each data collection, therefore, to achieve the same conditions in the 2 CRC in the same day, the ambient T, RH, AV, lighting, feeding, and milking was programmed to begin at 1 h later for the second CRC. The exposure time was defined as short when within 1 h the cows were exposed to the new ambient T and was defined as long when the cows were exposed to the new ambient T for more than 8 h.

CRC Condition. Ambient T and RH were continuously recorded automatically at 30-s intervals. Using the handheld anemometer (Testo 5–412–983, Testo SE & Co. KGaA), the AV at about 5 cm from the cow's body surface at 5 locations was measured twice daily at the same time as heat loss measurements, including neck, middle backbone, rump, and both lateral sides.

Heat Loss Data. Heat loss from skin surface was measured using a ventilated skin box (Figure 3a) similar as the one described by Gebremedhin et al. (2008). The ventilated box was designed with (1) a sampling box (inner dimensions of length × width × height: 200 × 99 × 32 mm) with 2 T and RH sensors (SHT85, Sensirion) mounted on both inlet and the outlet of the box; (2) an air suckling pump, which was connected at the outlet of the box; and (3) the box, which was fitted on the skin surface of the cow using 2 long belts that were wrapped around the middle trunk cylinder of the cow to ensure an airtight seal. The speed of air through the ventilated skin box was adjusted to be similar to the AV within the CRC. Data were automatically logged on a laptop at 1-s interval for 10 min for each cow. The data consisted of duplicate measurements of incoming and outgoing air T and RH, and of the airflow rate.

Heat loss through respiration was measured using a face mask and a nose cup (Figure 3b and c). The face mask consisted of an inlet valve and an outlet valve. At the outlet, 3 airflow sensors (Mass Flow Meter SFM 3000, Sensirion) were mounted next to the valve, and these sensors measured the airflow rate from respira-

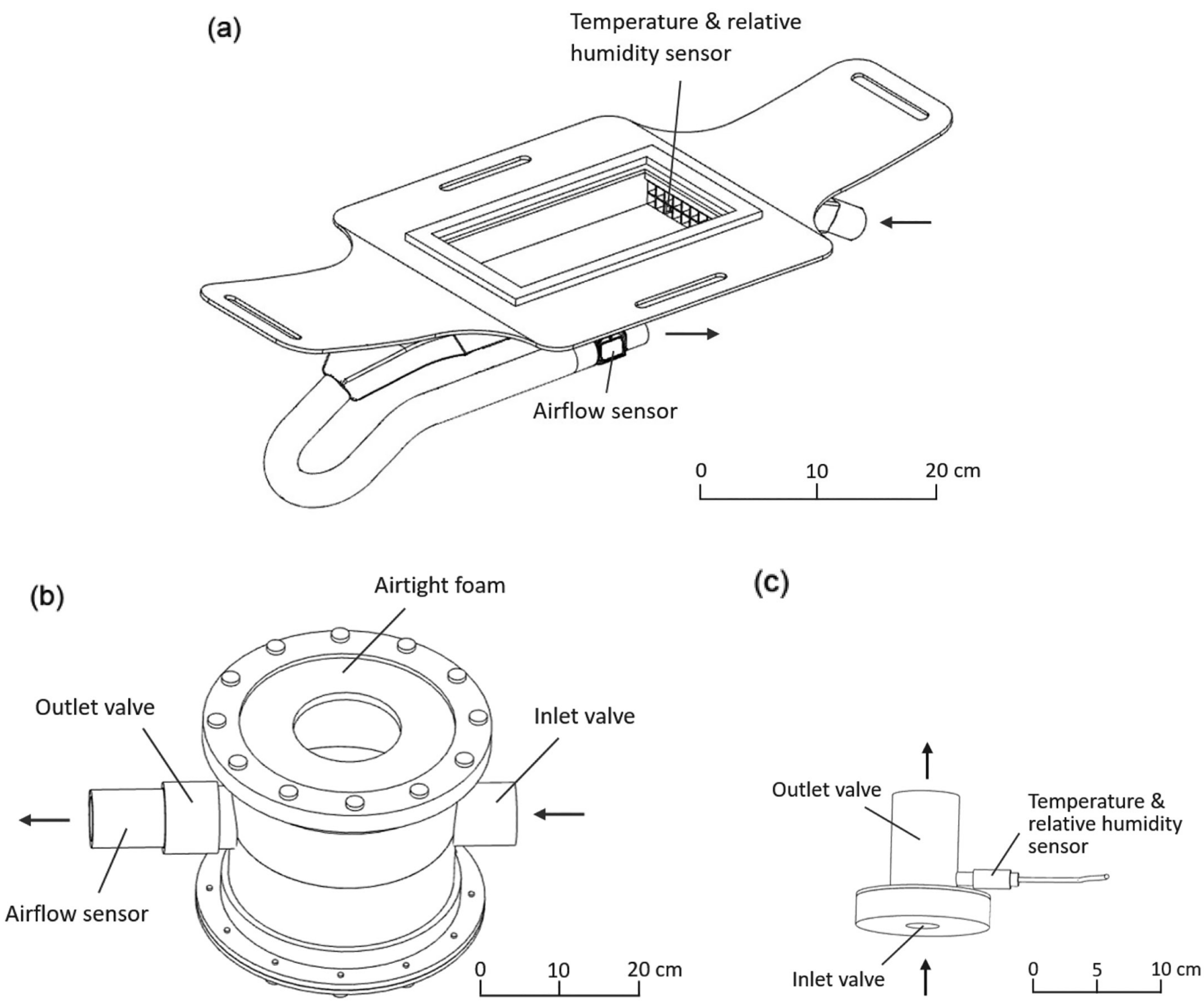

Figure 3. Apparatus for measuring heat loss from the skin surface and through respiration: (a) ventilated skin box with 2 temperature and relative humidity sensors at each side, and 1 airflow sensor at the outlet tube; (b) face mask with inlet and outlet valves at the sides, to ensure the exhaled air can only go through the airflow sensor; (c) nose cup made of insulation material so there is little heat loss to the environment.

tion. Data were collected at 0.1-s interval for 5 min on each cow. Data from the exhaled air was collected by fitting the nose cup over one of the cow's 2 nostrils. The nose cup consisted of 2 main components: an insulated, valved cylinder, and a T and RH sensor (Testo 06369735, Testo). The 2 valves fitted in the insulated cylinder, which closed during inhalation, allowed only exhaled air to enter; the exhaled air T and RH were then measured. The measuring time was on average 5 ± 2 min and depended on the speed the exhaled air T reached a stable state (oscillating ± 0.1°C). It was assumed that if only one nostril was sampled, the measurement setup at this nostril did not lead to a change in the flow resistance and that the measured values are representative for both nostrils.

Apparatus Calibration. After each single round, the ventilated skin box with sensors, the face mask and the nose cup were calibrated at the university air laboratory. The face mask was calibrated using an artificial reference cow (Wu et al., 2015), which consisted of an aluminum cylinder to provide a cow's tidal volume during respiration. The tidal volume of the artificial cow was determined by the cylinder's diameter and actuator's stroke length. The system then could be calibrated by measuring the airflow from the artificial reference cow with different known tidal volumes.

Data Processing and Calculation. The net heat loss or gain from the sampling area was calculated from the property differences of the incoming (in) and outgoing (out) air. The LHL from skin surface was estimated by the following equation:

$$LHL_s = \frac{Q_{e\_out} - Q_{e\_in}}{A_{sample}},$$

where LHL_S is the latent heat loss from skin surface (W/m^2); Q_e is the evaporative heat contained in the incoming or outgoing air (W), A_{sample} is the area of the sample (0.0198 m^2) of ventilated skin box.

Q_e was determined as follows:

$$Q_e = \lambda \cdot V \cdot \rho \cdot w,$$

where λ is the heat from water vaporization (J/g of water); V is airflow rate through the ventilated skin box (L/s); ρ is density of air (g/L); and w is the humidity ratio (kg of water/kg of dry air).

λ is dependent on the air T:

$$\lambda = -0.0001 \cdot T^2 - 2.3607 \cdot T + 2{,}503,$$

where T is the dry-bulb T (°C).

w can be calculated as follows:

$$w = \frac{0.6219 \cdot p}{(p_a - p)},$$

where p and p_a are water vapor pressure and air pressure (kPa), respectively. In this study 101.325 kPa was applied for air pressure. All the parameters used for calculating Q_e are based on T and RH measured at inlet or outlet and according to equations given by the ASHRAE Handbook (ASHRAE, 2009).

The SHL from the skin was estimated by the following:

$$SHL_s = \frac{Q_{s\_out} - Q_{s\_in}}{A_{sample}},$$

where SHL_s is SHL from the skin surface (W/m^2); Q_s is sensible heat contained in the incoming or outgoing air (W).

Q_s was determined as follows:

$$Q_s = h \cdot V \cdot \rho - Q_e,$$

where h is the enthalpy of the air mixed with vapor (J/g), calculated thus:

$$h = 1.006 \cdot T + w \cdot (\lambda + 1.86 \cdot T).$$

The LHL through respiration was estimated by the following:

$$LHL_r = \frac{Q_{e\_exhaled} - Q_{e\_inhaled}}{A_{body}},$$

where LHL_r is latent heat loss from respiration (W/m^2); Q_e is evaporative heat contained in the inhaled or exhaled air (W); A_{body} is the body surface area of each cow (m^2) and is a function of BW (Brody, 1945).

Q_e was determined as follows:

$$Q_e = \lambda \cdot \rho \cdot w \cdot V_{tidal} \cdot \frac{RR}{60},$$

where V_{tidal} is the tidal volume (L/breath), and RR is the respiration rate (breaths/min).

A_{body} was calculated according to Brody (1945) as follows:

$$A_{body} = 0.14 \cdot W^{0.57},$$

where W is the BW of the cow (kg).

The SHL through respiration was estimated by the following:

$$SHL_r = \frac{Q_{s\_exhaled} - Q_{s\_inhaled}}{A_{body}},$$

where SHL_r is the sensible heat loss from the respiratory tract (W/m^2); and Q_s is the sensible heat contained in the inhaled/exhaled air (W).

Q_s was determined as follows:

$$Q_s = \mathrm{h} \cdot \rho \cdot V_{tidal} \cdot \frac{RR}{60} - Q_e.$$

Statistical Analysis

All statistical analyses were performed in SAS 9.4 (SAS Institute Inc.). Data from one cow (RH_m × AV_m) was excluded from the analysis because of mastitis. Exploratory analyses were conducted to characterize the data distribution. The MIXED procedure was used to investigate the influence of increasing ambient T at 5 different combinations of RH and AV under different exposure times. Repeated measures were considered in the model including cow and experimental day as random effects. Different covariance structures were tested for each analysis, and the covariance structure with the smallest Akaike information criterion values was selected. The linear regression model was as follows:

$$y_{ijk} = \mu_i + (a + b_i) \cdot T + cow_{ij} + \varepsilon_{ijk},$$

where y_{ijk} is the observed response variables; μ_i is the intercept for each treatment level (i = 1, ..., 5); a and b_i are regression coefficients for T and the interaction between T with the ith treatment, respectively; cow_{ij} is the random effect of the jth cow in the ith treatment; and ε_{ijk} is the random residual error. The adjusted Tukey t-test was applied using the PDIFF statement to pairwise compare the differences between treatments and between 2 exposure times (short and long). Model assumptions were evaluated for both the linear model by examining the distribution of residuals (homogeneity of variance and normality) using the UNIVARIATE procedure. Significance was declared when $P \leq 0.05$ unless otherwise indicated.

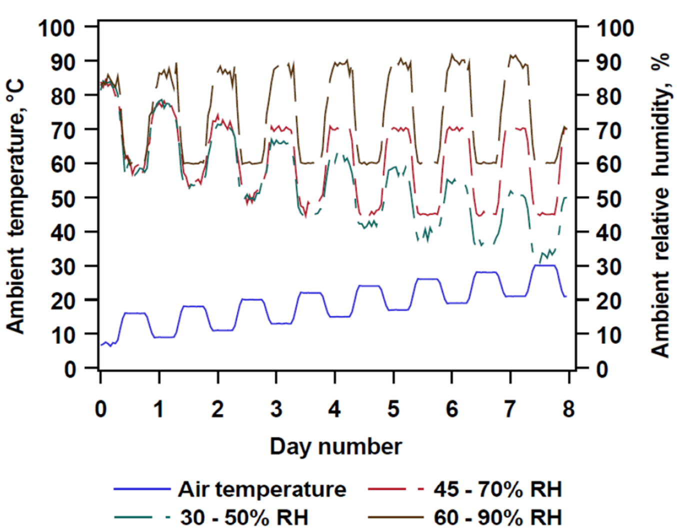

Figure 4. Average measured hourly temperature and relative humidity (RH) during 8-d experimental period.

RESULTS

Climate-Controlled Respiration Chambers Conditions

The microclimate conditions inside the CRC were reported in a previous study (Zhou et al., 2022). Briefly, the daily cyclical T were kept strictly constant according to set points with a deviation smaller than ± 0.50°C. The lowest RH (RH_l; 30%) and RH_m failed to reach the set points at the beginning but got closer later, as shown in Figure 4. The AV around the cow body surface was calculated by taking the average of 5 measurement points resulting in achievable AV at 3 sets of AV_l, 0.08 ± 0.01 m/s; AV_m, 1.14 ± 0.30 m/s; and AV_h, 1.35 ± 0.29 m/s. Average AV achieved at position of the ventilated skin box was AV_l, 0.09 ± 0.03 m/s; AV_m, 0.82 ± 0.27 m/s; and AV_h, 1.05 ± 0.39 m/s.

Heat Loss from Skin Surface

The responses of the SHL and LHL from skin under different treatments and exposure times are given in Figure 5. Average SHL from skin surface, combining all values at ambient T within 18 to 30°C, was similar at different RH levels (P > 0.05), whereas skin SHL increased with increasing AV (P < 0.05). The skin SHL under long exposure was higher (P < 0.05) than under short exposure; only for the condition with medium AV level (1.0 m/s) this difference was not significant (see Figure 5a and b). Average LHL from the skin surface

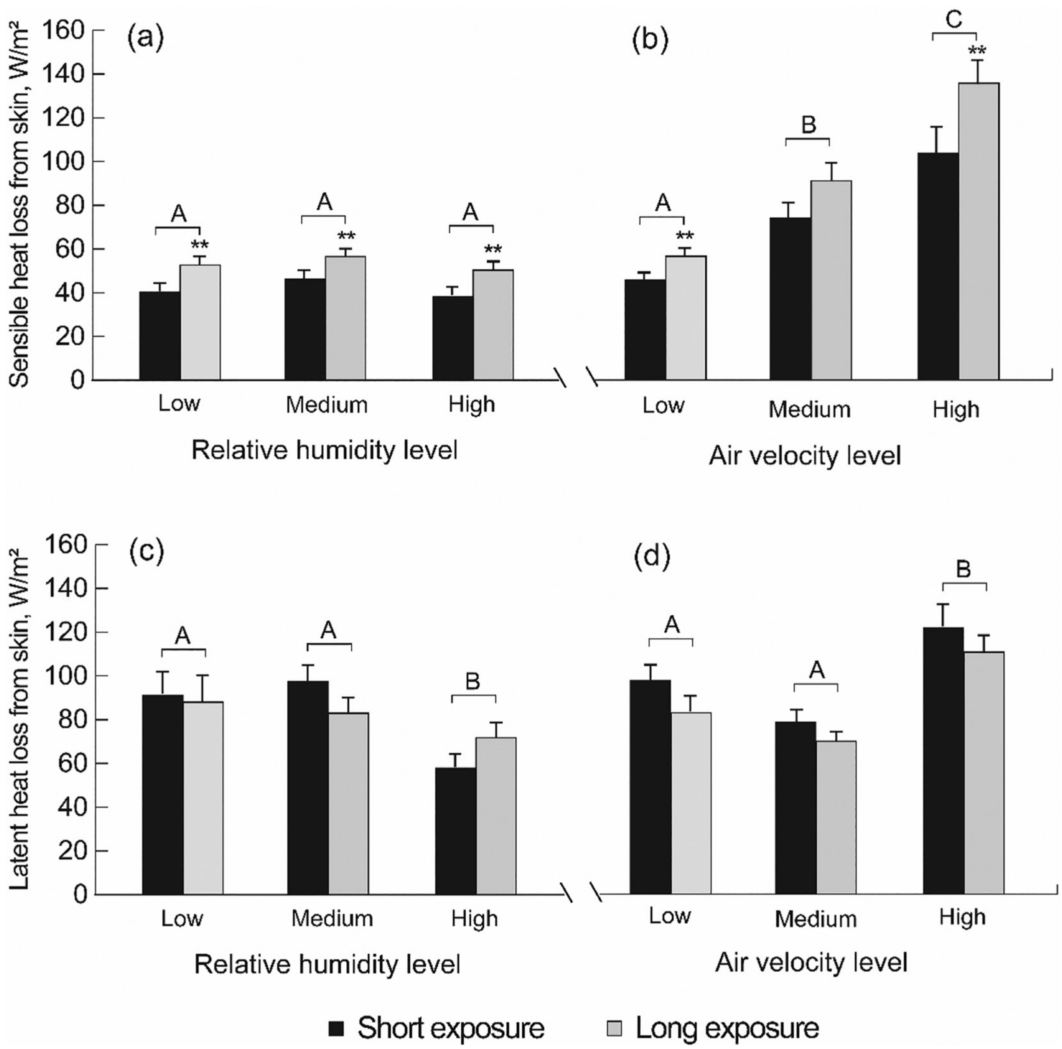

Figure 5. Mean sensible heat loss from skin (a and b, W/m^2) and mean latent heat loss from skin (c and d, W/m^2) within the ambient temperature range of 18 to 30°C at short and long exposure times for different relative humidity (RH) levels (low, medium, and high) and air velocity (AV) levels (low, medium, and high). The different letters in the same figure indicate a significant difference (Tukey-Kramer, $P < 0.05$) between treatments. Asterisks indicate a significant difference between 2 exposure times (*$P < 0.10$, **$P < 0.05$, ***$P < 0.01$). Error bars represent SEM. The RH effects were studied at low AV, and AV effects were studied at medium RH; the medium RH levels (a, c) are the same treatment as the low AV levels (b, d).

was lower at high RH level than at low/medium RH levels (Figure 5c). Skin LHL showed no difference ($P > 0.05$) between low and medium AV levels, with both being lower ($P < 0.05$) than at high AV level. There was no significant difference ($P > 0.05$) for skin LHL between short and long exposure times for all treatments.

The skin SHL decreased linearly with increasing ambient T (Figure 6). The decreasing rate of skin SHL with increasing ambient T varied between −2.95 to −6.28 W m^{-2} °C^{-1} for short exposure and between −2.97 to −6.78 W m^{-2} °C^{-1} for long exposure. There was no significant interaction effect of RH or AV on the decreasing rate of skin SHL for both exposure times ($P > 0.05$).

The skin LHL increased linearly with increasing ambient T (Figure 7). This increasing rate varied between 2.74 to 13.83 W m^{-2} °C^{-1} for short exposure and between 4.72 to 11.54 W m^{-2} °C^{-1} for long exposure. There was no significant interaction effect of RH on the increasing rate for both exposure times. For AV, however, cows under high AV level had a larger increasing rate of skin LHL than cows under low and medium AV levels ($P < 0.05$).

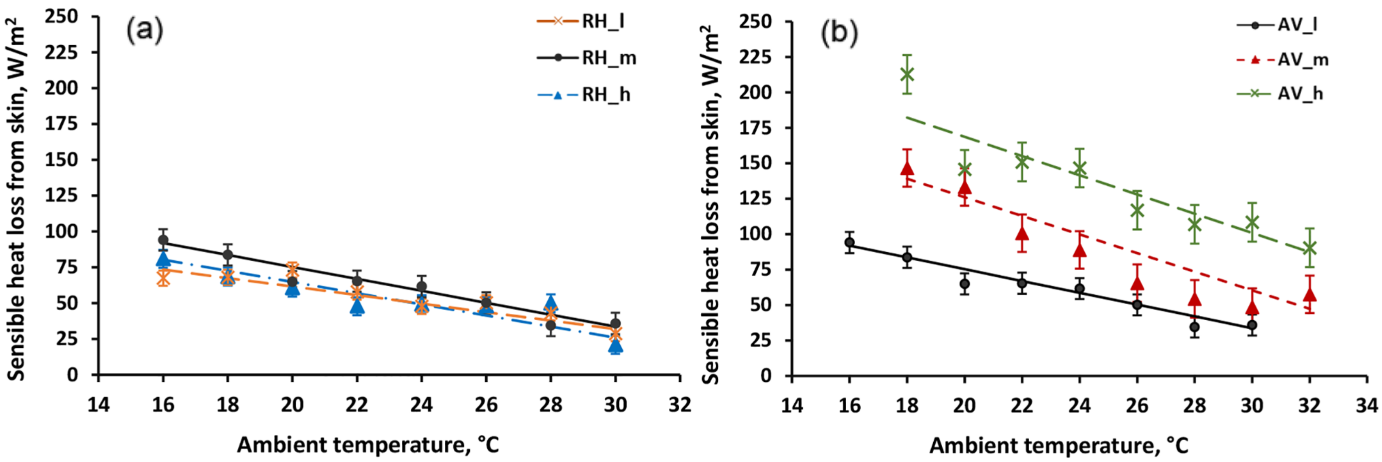

Figure 6. Sensible heat loss from the skin surface under long exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with low (RH_1; 30%), medium (RH_m; 45%), and high (RH_h; 60%) relative humidity levels, and (b) at the same relative humidity level (45%) with low (AV_1; 0.1 m/s), medium (AV_m; 1.0 m/s), and high (AV_h; 1.5 m/s) air velocity levels. Error bars represent SEM.

Heat Loss Through Respiration

The cows lost more heat through respiration (SHL and LHL) under long exposure than short exposure for all treatments (Figure 8; $P < 0.10$). The regression lines for respiration SHL or LHL showed no significant difference across different RH or AV levels ($P > 0.05$). Therefore, subsequent analysis used the combined results from 5 treatments.

With the increase of ambient T, respiration SHL linearly decreased and respiration LHL linearly increased (Figure 9). The respiration SHL at the ambient T of 16°C was 9.8 W/m^2 and as ambient T increased to 32°C, it decreased to 5.3 W/m^2 under short exposure and this decrease was from 12.3 to 6.2 W/m^2 under long exposure. With increasing ambient T, respiration LHL increased from 33.8 to 53.1 W/m^2 under short exposure and from 42.4 to 61.7 W/m^2 under long exposure. When ambient T increased from 16 to 32°C, the percentage of increase in total respiration heat loss was 34 and 24% for short and long exposure times. The decreasing rate of SHL under short and long exposure time were 0.38 and 0.28 W m^{-2} °C^{-1}, respectively, whereas the increasing rate of LHL was the same (1.21 W m^{-2} °C^{-1}) for short and long exposure times.

The duration of exposure to the experimental conditions affected exhaled air T (Figure 10a; $P < 0.05$).

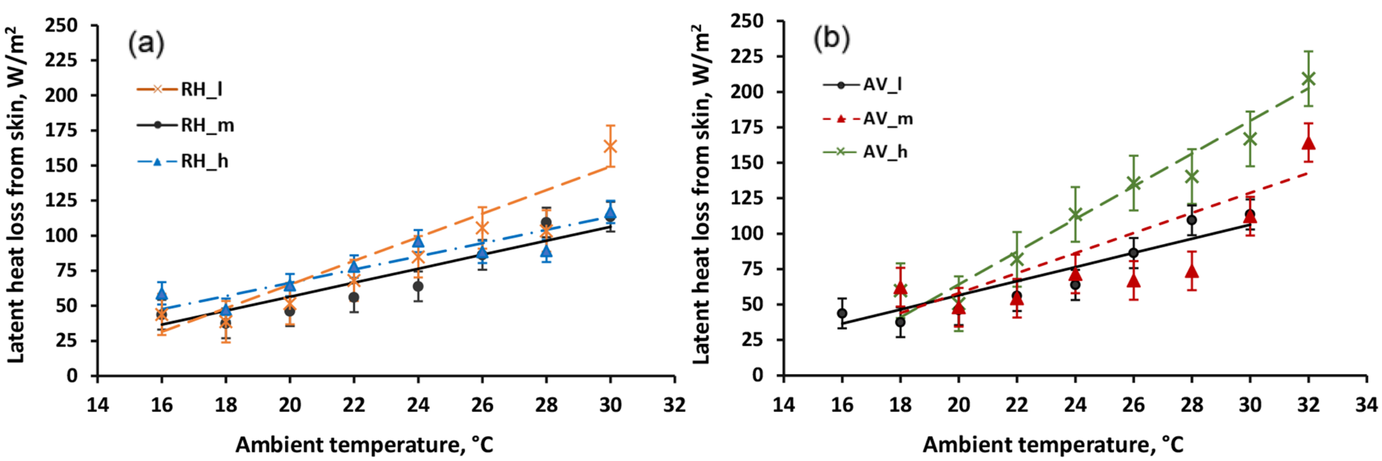

Figure 7. Latent heat loss from the skin surface under long exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with low (RH_l; 30%), medium (RH_m; 45%), and high (RH_h; 60%) relative humidity, and (b) at the same relative humidity level (45%) with low (AV_1; 0.1 m/s), medium (AV_m; 1.0 m/s), and high (AV_h; 1.5 m/s) air velocity levels. Error bars represent SEM.

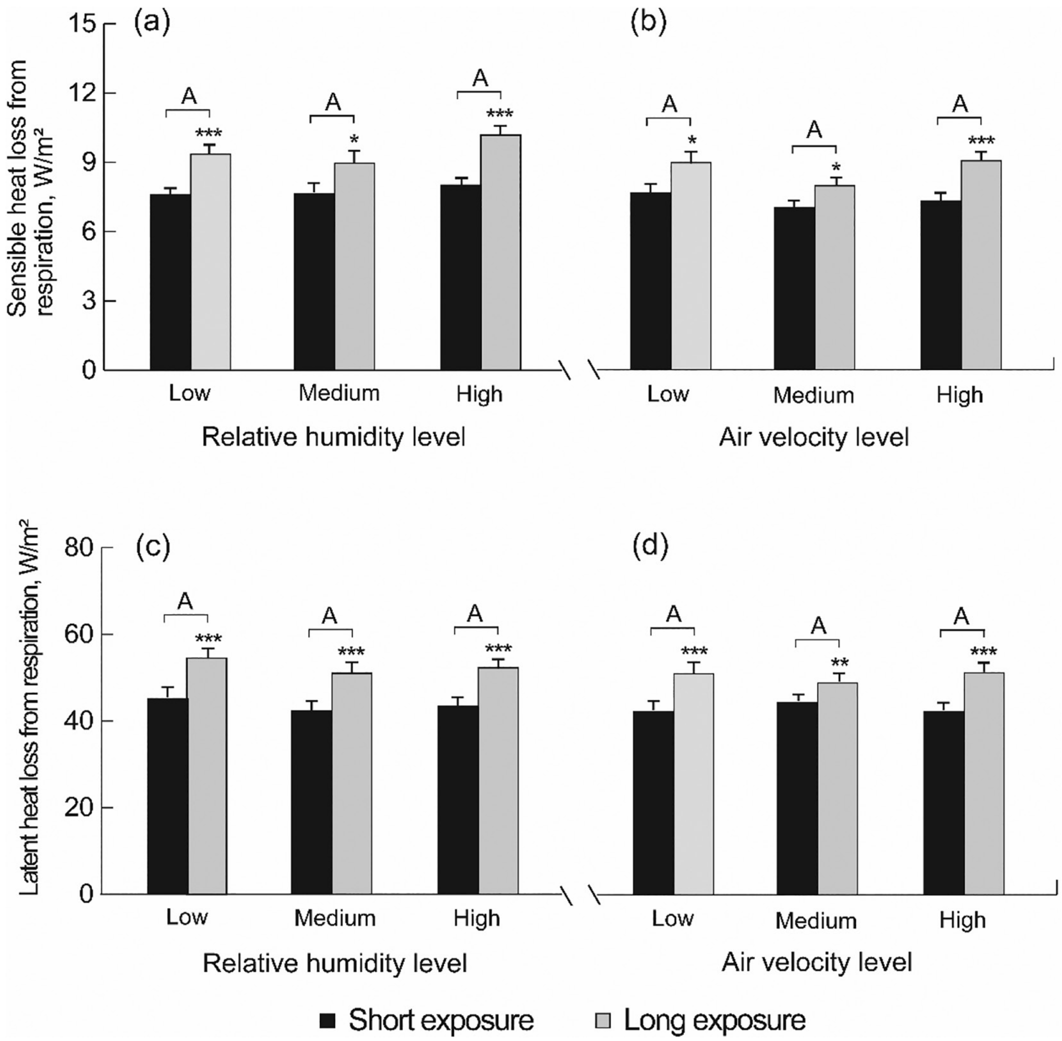

Figure 8. Mean sensible heat loss from respiration (a and b, W/m^2) and mean latent heat loss from respiration (c and d, W/m^2) within the ambient temperature range of 18 to 30°C at short and long exposure times with different relative humidity (RH) levels (low, medium, and high) and air velocity (AV) levels (low, medium, and high). The different letters in the same variable indicate a significant difference (Tukey-Kramer, $P < 0.05$) between treatments. Asterisks indicate a significant difference between 2 exposure times ($*P < 0.10$, $**P < 0.05$, $***P < 0.01$). Error bars represent SEM. The RH effects were studied at low AV, and AV effects were studied at medium RH; the medium RH levels (a, c) are the same treatment as the low AV levels (b, d).

When ambient T increased from 16 to 32°C, the exhaled air T increased by 1.6°C (from 35.0 to 36.6°C under short exposure and from 35.4 to 37.0°C under long exposure; Figure 10a). The exhaled air T had the same increasing rate for short and long exposure times of 0.097°C per 1°C increase in ambient T. Exposure time effected respiratory volume (L/m), and respiratory volume at short exposure (ranging from 147 to 253 L/min) was lower than at long exposure (ranging from 187 to 301 L/min) as illustrated in Figure 10b. The respiratory volume increased on average by 6.8 L/min per 1°C increase in ambient T under both short and long exposure times.

Both Heat Loss Modes: From Skin and Through Respiration

As ambient T increased, the division of heat loss from skin surface and through respiration changed accordingly (Figure 11). Total heat loss from the skin showed a dominant share (70–80%) of the whole heat loss, whereas heat loss through respiration accounted for 20 to 30%. Total LHL accounted for 49 to 76% of total heat loss and it increased with increasing ambient T. Total SHL accounted for 24 to 51% and it decreased with increasing ambient T, whereas SHL through respiration only showed a minor contribution of 1.7 to 6.5%

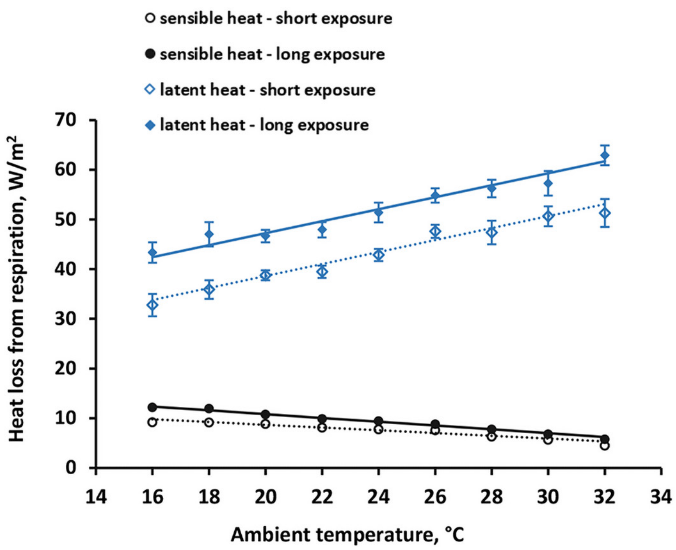

Figure 9. Sensible heat loss (SHL_r; short exposure: $SHL_r = 14.22 - 0.28 \cdot T_a$; long exposure: $SHL_r = 18.46 - 0.38 \cdot T_a$) and latent heat loss ($LHL_r$; short exposure: $LHL_r = 14.46 + 1.21 \cdot T_a$; long exposure: $LHL_r = 23.08 + 1.21 \cdot T_a$) from respiration in relation to ambient temperature (T_a) at 2 exposure times. Error bars represent SEM.

of the whole heat loss. As ambient T rose above 20°C, skin SHL subsided and skin LHL took charge.

DISCUSSION

In this study, data collection time was designed in such a way that the confounding effect of feeding time (0500 and 1530 h) was largely avoided.

Heat Loss from Skin Surface

In homeothermy animals, dairy cows included, the skin surface is an important anatomical organ for the body to exchange heat with the ambient environment. Heat transfer (including both loss and gain) via the skin surface can happen via convection, radiation, conduction, and evaporation. Under this experimental design, the modes of short-wave radiation from solar and conduction (cows' surface contacting to cooler surface by behavior e.g., cooling mattress) were excluded. Generally, under heat stress conditions and in sunny weather, cows stay inside the barn or try to stay in the shade. When there is no shade, heat stress could be a lot more severe than the conditions studied in this experiment.

The T difference between the skin surface and the ambient air plays a significant role in the loss of sensible heat from the skin surface. Up to a certain ambient T, cows mainly lose heat via the sensible way (Mount, 1979; Maia and Loureiro, 2005). The negative relationship between skin SHL and ambient T agrees with previous studies (Mount, 1979; Maia and Loureiro, 2005; Thompson et al., 2014). In the study of Maia and Loureiro (2005) the decreasing rate was 7.35 W m^{-2} °C^{-1} at an AV range between 0.1 to 5 m/s, which was approximately double the value at low AV (0.1 m/s) in our study. Hence, factors that could influence the T difference, such as RH and AV, need to be considered. The skin SHL increased with increasing AV; our study showed that the amount of skin SHL could be twice as high at high AV (2.0 m/s) compared with what it was at low AV (0.1 m/s). The large effect of AV on skin SHL was confirmed by Spiers et al. (2018), who found that the skin SHL remained similar both without fans at 23.8°C and with fans at 33.2°C. It was also found in our study that with increasing ambient T the skin SHL decreased faster at high AV level compared with low or medium AV levels, indicating the reduced benefit of higher air velocities under warm conditions. No effect of RH was found on skin SHL in this study. One might expect that under high ambient T, the RH level may play a role in skin T and hence in skin SHL. Zhou et al. (2022) found skin T (averaged from 4 different skin parts) was significantly higher at 60% RH than that at 30 and 45% RH given the same ambient T, causing a larger T difference between skin surface and air at 60% RH, and hence giving a larger skin SHL. Possible reason could be that skin T in previous study was an average skin T measured on 4 different parts, whereas skin SHL in this study was only measured on a small area of the belly (at the location where the ventilated box was placed). When exposure time was long, the cows had a higher skin SHL than under short exposure. This effect is probably caused by the higher skin T at long exposure time (Zhou et al., 2022).

From a biological point of view, an asymptote relationship is expected for skin LHL, because there is always a minimum amount of water evaporation from the skin (Kadzere et al., 2002). However, due to our experimental setup, there were not enough points in the lower ambient T range to estimate this asymptote, and according to Johnson and Vanjonack (1976), the evaporative heat loss began to increase markedly between 16.6 and 18.3°C. Within the ambient T range of our measurements, the linear relationship showed the best fit. Under warm conditions the cow has to increase its skin LHL to compensate for the lower SHL and thereby maintain a thermal equilibrium (Gebremedhin et al., 2008). We found that at low RH (30%), cows had a higher skin LHL than at higher RH levels. Under higher RH conditions, the sweat cannot be fully evaporated, because the sweating rate is higher than the potential (maximum) evaporation rate (Berman, 2009). Gebremedhin et al. (2010) studied the effects of hot, hu-

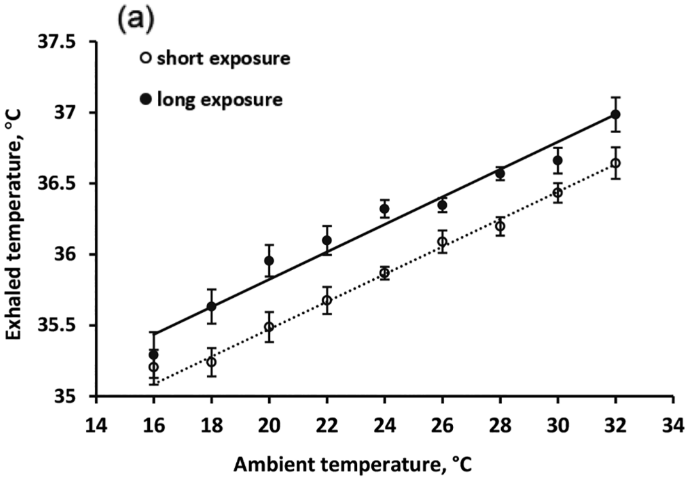

Figure 10. (a) Exhaled air temperature (ExT, °C; short exposure: $ExT = 33.54 + 0.097 \cdot T_a$; long exposure: $ExT = 33.89 + 0.097 \cdot T_a$) and (b) respiratory volume (ResV, L/min; short exposure: $ResV = 34.94 + 6.79 \cdot T_a$; long exposure: $ResV = 66.74 + 6.79 \cdot T_a$) in relation to ambient temperature (T_a) at 2 exposure times. Error bars represent SEM.

mid, and solar load at 1 m/s AV on cow sweating rates and reported that sweating rates were higher in the hot and dry condition (THI 79.6, 35.1°C, 23.1% RH) than in the warm and humid condition (THI 79.6, 29.1°C, 69.2% RH). When RH is higher, the moisture gradient between the skin surface and ambient air is reduced, consequently reducing the efficacy of evaporative cooling. This agrees with our findings. Although cows might have similar sweating rates at different RH levels, a higher RH will lower the partial vapor pressure difference, and consequently, according to the fundamentals of thermodynamics (Berman, 2006), evaporation will be lower. In addition, cows reduced their metabolic heat

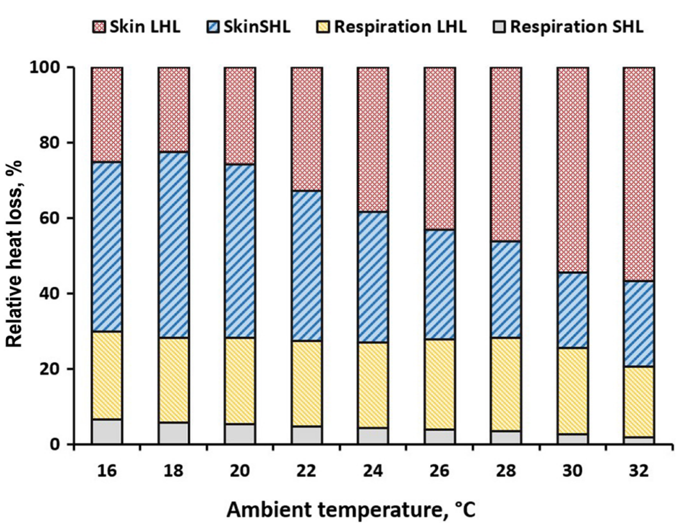

Figure 11. Relative heat loss by skin LHL, skin SHL, respiration LHL, and respiration SHL of dairy cows at different ambient temperatures. LHL = latent heat loss; SHL = sensible heat loss.

production due to decreased milk yield at high ambient T (Zhou et al., 2022) thus requiring less skin LHL under high RH condition compared with other conditions. Our results are consistent with previous studies which demonstrated that cutaneous evaporation was reduced under high levels of RH (McLean, 1963; Maia and Loureiro, 2005; Gebremedhin et al., 2008). Most available heat loss models estimated skin LHL using skin T as the only independent variable (Gatenby, 1986; Thompson et al., 2011; Nelson and Janni, 2016). However, we found that cows dissipate more skin LHL at high AV (1.5 m/s) than at medium AV (1.0 m/s) above 20°C of ambient T (Figure 7b) despite the cows having similar skin T (Zhou et al., 2022). This means that cows were able to increase their sweating rate under warm conditions once water was removed from the skin surface. Our observations also appear to confirm the finding on human beings from Adams et al. (1992) and Nadel and Stolwijk (1973) that an increased sweating rate occurs when the skin surface is dry. It could be because when water is readily evaporated from the skin, such as usually occurs when the AV is high, the osmotic gradient is maintained and water can be more actively drawn from inside toward the skin surface at any level of sweating drive (Peiss et al., 1956). Interestingly, no significant difference was found on skin LHL between low and medium AV groups in this study. The reason could be that the effect of medium AV was not big enough to compensate for the effect of low skin T on the sweating rate; in other words, cows at low AV had a high skin T, and thus a high sweating rate, whereas cows at medium AV had a low skin P-value (exposure time) for statistical difference between 2 exposure times but higher AV, and thus, a high sweating rate as well.

It is recommended to look further into the effect of AV on the sweating mechanism.

Under commercial barn conditions, monitoring environmental conditions such as ambient T, RH and AV are important to determine interventions for reducing heat stress in dairy cows. Evaporative cooling of ambient air can also be subjected to some limitations, because high RH would reduce the skin LHL, especially in humid climates (Berman, 2009). In this situation, only higher AV would not help to dissipate heat if the RH and ambient T are both too high, as seen in subtropical regions. Skin cooling with sprinklers in combination with forced ventilation is a preferable solution for skin evaporative heat dissipation when sweating rate is lower than the potential evaporation rate, especially in dry climates (Chen et al., 2020).

According to da Silva et al. (2012), different parts of the skin surface of a cow have different sweating rate levels. In this research we only measured LHL on a small sampling area, which could not represent the LHL from the entire body surface. To check this, a total evaporative heat loss calculation is of interest, similar as was done on pigs by Huynh et al. (2007), in which the authors calculated the total water balance based on the incoming and outgoing air of CRC. In addition, it is not realistic to keep the AV inside the ventilated skin box the same as the AV of surrounding cows. We measured the AV at 5 points and found the highest AV around the rump and the lowest AV around the lateral sides; thus, we had a generally higher AV inside the ventilated skin box than the real AV flowing above the belly. Consequently, this could have altered the skin SHL and LHL. Gebremedhin et al. (2010) and Liang et al. (2009) observed that cows sweat in a cyclic manner; there is a filling phase and a secretory phase in a cow's sweating process. They reported that the sweating rate varied over time under the same environmental conditions during a 5-h period. In our study we measured skin LHL for 10 min, whereby the results from the latter 5 min were used in the analyses. During the first 5 min the cows were adapting to the ventilated skin box. The sweating cycle probably depends on the activity, feeding, milking cycles of the cows, and because these were all similar for the cows in our study, it may be assumed that our cows were more or less at the same phase of the sweating cycle. However, the measurement times in our study might not be representative for the average sweating rate for the whole day.

Heat Loss Through Respiration

Respiration SHL accounted for a fairly small percentage of the total heat loss via respiration and it, in an absolute sense, decreased little with increasing ambient T. The absolute amount of respiration SHL (12.3 to 6.2 W/m^2) was double the value reported in the study by Maia et al. (2005; 5.5–2.4 W/m^2) within the ambient T range of 16 to 32°C. Respiration LHL increased with increasing ambient T. Both the respiration SHL and LHL were higher under long exposure, most probably caused by the higher respiration rate and rectal T after long exposure (Zhou et al., 2022). The values of respiration SHL and LHL differed from other studies (Maia et al., 2005; Santos et al., 2017): especially under cool conditions, the respiration LHL was much higher in this study. This could be explained by our methods for measuring exhaled air T and respiratory volume. Inhaled air T rapidly approaches the body T, which is reached by the time it gets to the lungs and becomes saturated with water vapor. When the air passes back outwards it exchanges some heat with the upper respiratory tract; this will lower the T and water content, whereas it remains saturated with water vapor (Walker et al., 1962). In this study, the RH of exhaled air was 100% for all measurements, which is in line with some classic studies on the human respiratory tract (Cole, 1953; Walker et al., 1962). The exhaled air T measured by the nose cup in our study was much higher than that from other studies (Donald, 1981; Maia et al., 2005), especially under low ambient T conditions. The measurement approach here is very important, because in the other studies the exhaled air could easily be mixed with ambient air. Maia et al. (2005) measured exhaled air T by placing a thermometer in the outlet valve of a face mask, where the measured air has already become a mixture of exhaled air and ambient air. A similar method was used by da Silva et al. (2012), who measured exhaled air T directly by placing a small thermometer in the nostril of a cow. In addition, the thermometer needs time to respond, whereas the exhaled air could quickly spread out in the environment before the thermometer could catch the real T. Therefore, the nose cup we used in this study seems to be more reliable for measuring an accurate exhaled air T. This could explain why the exhaled air T was underestimated by previous studies, especially at low ambient T. To illustrate, exhaled air T from Donald (1981) was approximately 25°C at ambient T of 16°C, whereas the lowest exhaled air T measured in our study at 16°C was 34.3°C. The exhaled air T was higher under long exposure, probably as a result of a higher body T of cows exposed for a longer time to high ambient T conditions (Zhou et al., 2022).

A face mask was used to measure the respiration rate and tidal volume in this study. Despite the fact that there were 3 adaptation days for cows to get used to the mask, there was still some influence of the mask on the respiration behavior. Probably because of the resistance of the valves inside the mask, we noticed that the respiration rate measured by the mask was lower than counted from flank movements during the period without the mask. We noticed that this lower respiration rate when putting up the mask was accompanied by deeper breathing of the cow, probably to overcome the resistance caused by the mask. This negative relationship between respiration rate and tidal volume was also found by Maia et al. (2005). This is the reason why we studied respiratory volume in liters per minute (respiration rate times tidal volume) rather than tidal volume alone, assuming respiratory volume was less influenced by the face mask.

In this study, we did not see obvious effects of RH or AV on respiration heat loss. According to Berman (2006), rising RH could reduce the water loss from respiration but the maximal effect of RH was reached at about 40% and higher RH did not further reduce the respiratory water loss. The lowest experimental RH level in our study was 30%, and the effect of different RH levels on respiration LHL was very small. Actually, the total respiration heat loss did not increase as fast as was estimated in previous studies (da Silva et al., 2012; Santos et al., 2017) because of the lower measured exhaled air T by these authors at the lower ambient T range as discussed before. The increase of respiration rate or respiratory volume was mostly to offset the decreasing T gradient between ambient T and exhaled air T. Under high ambient T conditions, skin LHL accounted for about 75% of the total LHL and the rest was accounted for by respiration LHL. The increasing skin LHL reduced the need for a very high respiration rate at high ambient T, thus reducing possible problems caused by respiratory alkalosis (da Silva et al., 2012).

Taken as a whole, results of this study show that SHL decreases with increasing ambient T and this is compensated with increasing LHL. Forced ventilation should be strong enough (AV >1.0 m/s at 45% RH) to improve the evaporation of sweat as well as to trigger the transport of sweat from subcutaneous sweat gland to the skin surface when ambient T is high. Evaporative cooling from the evaporation of sweat is limited by the amount of sweat produced, by a high RH and by a low AV. To improve skin LHL it is advised to combine forced ventilation with the wetting of the animal's skin surface when the sweating rate is low. The enhanced AV flowing over the skin surface makes the potential evaporative rate high enough and the LHL from skin surface less dependent on the RH of the ambient air. In addition, former mentioned important results from this study, better understanding of the modes of different heat loss routes under the effects of environmental conditions (ambient T, RH, and AV) of Holstein cows is also of central importance for further development of existing mechanistic heat balance models. Such models can serve for efficient implementation of heat stress alleviation methods.

CONCLUSIONS

The LHL accounted for approximately 50% of the total heat loss and the rest was lost as sensible heat when ambient T was below 20°C. Under warm conditions, when ambient T rose above 28°C, evaporation became the main route of heat loss, accounting for approximately 70 to 80% of the total heat loss. Skin SHL decreased, whereas skin LHL increased with increasing ambient T. Both SHL and LHL from skin were positively affected by AV. Heat loss from respiration accounted for 20 to 30% of the total heat loss, and it increased by 34 and 24% under short and long exposures when ambient T rose from 16 to 32°C. Cows lost more sensible heat from skin surface and total heat through respiration when they were exposed to warm conditions for a longer time (1 vs. 8 h). It is recommended to study the interaction effect between RH and AV on heat loss.

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APPENDIX

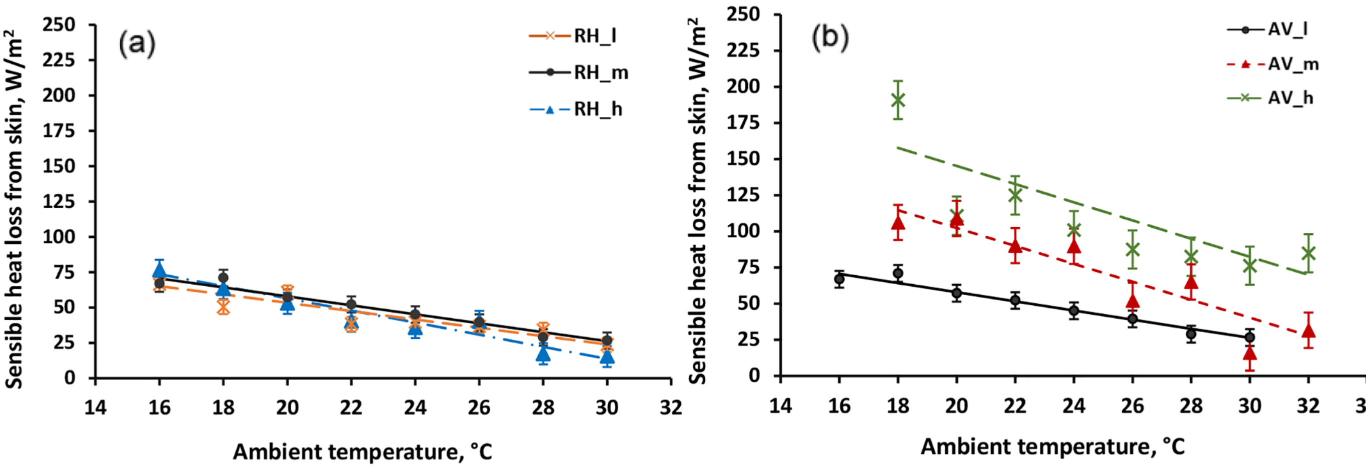

Figure A1. Sensible heat loss from the skin surface under short exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with low (RH_l; 30%), medium (RH_m; 45%), and high (RH_h; 60%) relative humidity, and (b) at the same relative humidity level (45%) with low (AV_1; 0.1 m/s), medium (AV_m; 1.0 m/s), and high (AV_h; 1.5 m/s) air velocity levels. Error bars represent SEM.

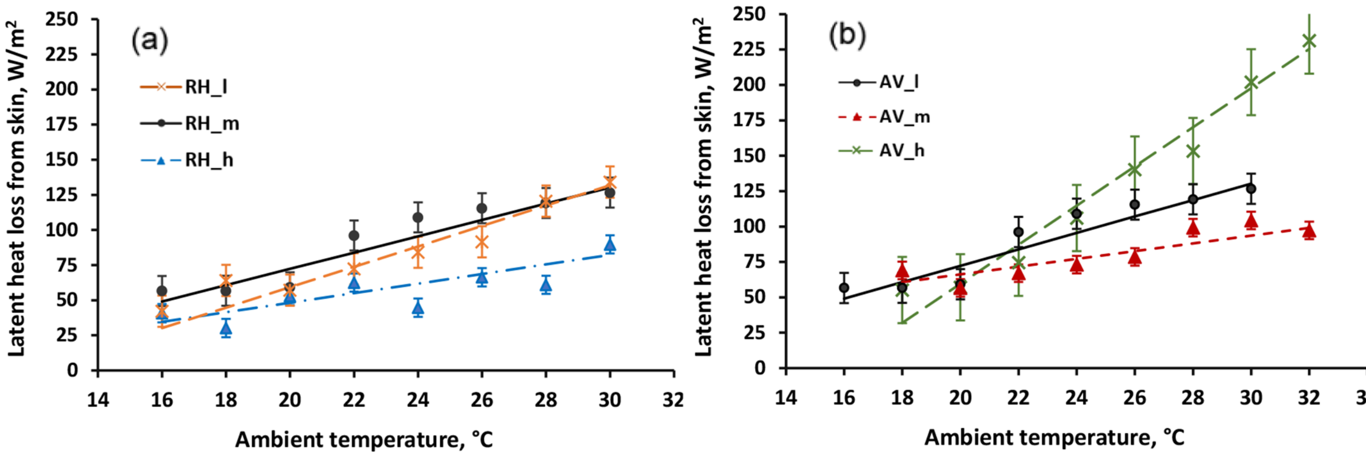

Figure A2. Latent heat loss from the skin surface under short exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with low (RH_l; 30%), medium (RH_m; 45%), and high (RH_h; 60%) relative humidity, and (b) at the same relative humidity level (45%) with low (AV_1; 0.1 m/s), medium (AV_m; 1.0 m/s), and high (AV_h; 1.5 m/s) air velocity levels. Error bars represent SEM.

Table A1. Coefficients from linear regression with increasing temperature on heat loss from skin at different relative humidity (RH) and air velocity (AV)

Dependent variable	Exposure time[1]	Regression model components	Treatment[2] RH_l × AV_l n = 4	RH_m × AV_l n = 4	RH_h × AV_l n = 4	RH_m × AV_m n = 3	RH_m ×AV_h n = 4	SE	P-value (treatment)[3]
Sensible heat loss from skin, W/m^2	Short	Intercept	112.2^{a}	121.0^{a}	142.2^{ab}	226.2^{b}	270.8^{b}	32.0	0.042
		Slope	−2.95	−3.16	−4.28	−6.20	−6.28	1.34	0.35
	Long	Intercept	121.0^{a}	158.6^{a}	142.5^{a}	257.0^{b}	304.1^{b}	27.8	0.0033
		Slope	-2.97^{a}	-4.17^{ab}	-3.89^{ab}	-6.56^{b}	-6.78^{b}	1.16	0.11
P-value (exposure time)[4]			<0.05	<0.05	<0.05	NS	<0.05		
Latent heat loss from skin, W/m^2	Short	Intercept	-86.0^{a}	-43.8^{a}	-19.9^{a}	11.4^{a}	-216.9^{b}	43.7	0.070
		Slope	7.26^{a}	5.80^{a}	3.41^{a}	2.74^{a}	13.83^{b}	1.82	0.0013
	Long	Intercept	−103.0	−43.3	−27.9	−83.2	−166.7	55.7	0.54
		Slope	8.41^{a}	4.99^{a}	4.72^{a}	7.07^{a}	11.54^{b}	2.34	0.25
P-value (exposure time)			NS[5]	NS	NS	NS	NS		

[a,b]Values within a row with different superscripts differ, $P < 0.05$.

[1]Short exposure time means the cows stayed in the condition for 1 h; long exposure time means the cows stayed in the condition for 8 h.

[2]Treatment levels: RH_l = low RH, 30%; RH_m = medium, 45%; RH_h = high, 60%; AV_l = low AV, 0.1 m/s; AV_m = medium, 1.0 m/s; AV_h = high, 1.5 m/s.

[3]P-value (treatments) for statistical difference among 5 treatments for intercepts or slopes.

[4]P-value (exposure time) for statistical difference between 2 exposure times.

[5]NS = not significant: $P \geq 0.10$.

Table A2. Coefficients from linear regression with increasing ambient temperature on heat loss from respiration at different relative humidity (RH) and air velocity (AV)

			Treatment[2]						
Dependent variable	Exposure time[1]	Regression model component	RH_l × AV_l n = 4	RH_m × AV_l n = 4	RH_h × AV_l n = 4	RH_m × AV_m n = 3	RH_m × AV_h n = 4	SE	*P*-value (treatment)[3]
Exhaled air temperature, °C	Short	Intercept	33.39	34.28	33.53	33.60	32.42	0.39	0.052
		Slope	0.10	0.071	0.10	0.091	0.13	0.016	0.081
	Long	Intercept	33.83	34.66	33.87	33.78	33.10	0.41	0.19
		Slope	0.10	0.07	0.09	0.10	0.12	0.018	0.33
P-value (exposure time)			<0.05	<0.01	NS[4]	<0.05	<0.05		
Respiratory volume, L/min	Short	Intercept	22.92	59.69	−18.57	4.72	−1.29	50.11	0.77
		Slope	7.18	6.10	9.27	7.78	7.87	2.00	0.82
	Long	Intercept	84.78	99.66	103.36	−38.44	21.35	46.74	0.34
		Slope	6.00	5.38	5.71	10.32	8.50	1.95	0.40
P-value (exposure time)[5]			<0.01	<0.05	<0.05	NS	<0.05		
Sensible heat loss from respiration, W/m^2	Short	Intercept	13.39	13.32	12.75	16.64	13.01	1.77	0.74
		Slope	−0.25	−0.23	−0.20	−0.38	−0.25	0.071	0.62
	Long	Intercept	18.11	18.84	18.19	15.76	16.88	1.92	0.88
		Slope	−0.37	−0.40	−0.33	−0.31	−0.34	0.081	0.95
P-value (exposure time)			<0.01	<0.05	<0.01	<0.10	<0.01		
Latent heat loss from respiration, W/m^2	Short	Intercept	3.64	6.35	13.75	17.65	7.60	10.08	0.89
		Slope	1.75	1.66	1.18	1.05	1.40	0.41	0.71
	Long	Intercept	16.42	28.80	34.50	4.31	14.05	10.73	0.45
		Slope	1.59	1.00	0.73	1.78	1.53	0.45	0.46
P-value (exposure time)			<0.01	<0.01	<0.01	<0.05	<0.01		

[1]Short exposure time means the cows stayed in the condition for 1 h; long exposure time means the cows stayed in the condition for 8 h.

[2]Treatment levels: RH_l = low RH, 30%; RH_m = medium, 45%; RH_h = high, 60%; AV_l = low AV, 0.1 m/s; AV_m = medium, 1.0 m/s; AV_h = high, 1.5 m/s.

[3]*P*-value (treatments) for statistical difference among 5 treatments for intercepts or slopes.

[4]NS = not significant: $P \geq 0.10$.

[5]*P*-value (exposure time) for statistical difference between 2 exposure times.