Heat Stress in Dairy Cows

Measuring and modelling the effects of environmental conditions on thermoregulatory responses

Mengting Zhou

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Propositions

1. Dairy farmers lack practical devices to detect the first signs of heat stress in their cows. (this thesis)
2. Evaporative cooling of air in barns for dairy cows is counterproductive in humid areas. (this thesis)
3. Measures to reduce the environmental impacts of nitrogen emission should focus on innovation of farming systems rather than reduction of livestock numbers. (other scientific field)
4. Animal-free experiments can serve scientific research sufficiently. (other scientific field)
5. Plant-based milk will replace cow milk in the near future. (social)
6. Discrimination of people based on geographic origin related to the Covid-19 pandemic is excusable. (social)

Propositions belonging to the thesis, entitled

Heat stress in dairy cows - Measuring and modelling the effects of environmental conditions on thermoregulatory responses

Mengting Zhou

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Measuring and modelling the effects of environmental conditions on thermoregulatory responses

Mengting Zhou

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CHAPTER 1.

General introduction

1.1 Heat Stress in dairy cows

Concern for animal welfare and comfort has increasingly grown in the past decades (Polsky and von Keyserlingk, 2017). Heat stress in dairy cows is an important and challenging issue as it affects health, welfare and productivity of dairy cows in modern, intensive farming systems. According to Kadzere et al. (2002), heat stress for dairy cows is related to all high temperature-related forces that induces responses from the sub-cellular to the whole animal level to help the cow avoid physiological dysfunction. The meaning and use of the term heat stress in this thesis is defined at the end of this section. The genetic selection for higher milk production per cow has resulted in the temperature, at which cows start experiencing heat stress, shifted to a lower point, as higher milk production leads to higher heat production (Ravagnolo et al., 2000). Climate change leading to an increase in the earth's surface temperature by 1.5°C between 2030 and 2052 has been predicted (IPCC, 2018), which could make the heat stress situation even worse for dairy cows, including all the accompanying negative effects on dairy cows' welfare, health, production and reproduction, and even causing increased mortality.

The cow is a ruminant and homeothermic domestic farm animal, which means its body core temperature should be maintained within a narrow range between 37.8 and 39.2°C (Yan et al., 2021b). When exposed to air temperatures that exceed its biological thermal comfort/neutral condition, a cow will respond with great efforts to maintain its thermal balance. To assess the thermal state of dairy cows, the general concept of thermo-regulation formulated by Mount (1979) in Figure 1-1 is generally used.

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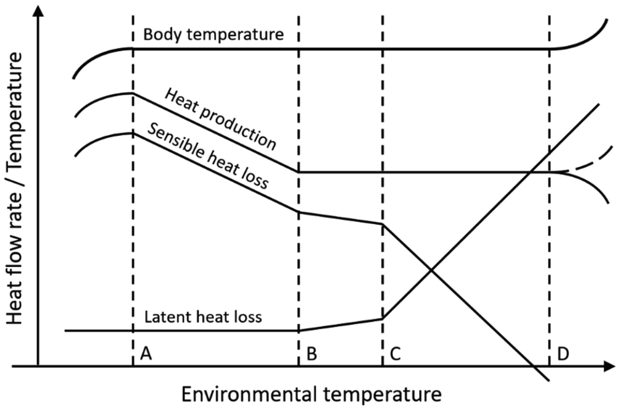

Figure 1-1. General concept of thermal-regulation in a homeothermic animal adapted from Mount (1979). A: temperature of summit metabolism and incipient hypothermia; B: lower critical temperature of comfort / thermoneutral zone; C: upper critical temperature of comfort zone; D: upper critical temperature of thermoneutral zone; BC: comfort zone (least thermoregulatory effort); BD: thermoneutral zone (minimal metabolism); AD: thermoregulatory range.

Within the temperature zone AD cows can keep their body core temperature constant. To the left beyond point A, when air temperature further decreases, the body enters the stage of hypothermia, while to the right beyond point D, when air temperature further increases, the body enters the stage of hyperthermia. The zone AD can be divided in to Zone AB and Zone BD. Within Zone AB, the body temperature is kept constant by regulation of heat production (mainly by shivering) when the sensible heat loss from the body increases with the decrease of the environmental temperature. Zone BD is defined as the thermoneutral zone in which the metabolic rate is minimum, constant, and independent of ambient temperature. The thermoneutral zone is demarcated by the lower and upper critical temperatures at points B and D, respectively. Zone BC is called the comfort zone, in which the animal feels most comfortable and the thermal balance can be easily maintained by adjustment of the body thermal resistance (Berman, 2004). The temperature range of the comfort zone varies in relation to body size, cattle breed, growth stage, feed and water intake (Kadzere et al., 2002). When the ambient temperature rises from C to D, cows endeavor to lose heat by increasing their respiration and sweating rate substantially. When exceeding the upper critical temperature D, in the short term the heat production increases, because the animal has to do a lot of effort (e.g. panting, sweating) to lose heat. If the high temperature continues for a longer period, the cow will reduce heat production by lowering feed intake. When the cow cannot balance heat loss with heat production during a prolonged period, the body temperature will rise and finally will reach fatal levels and the cow will succumb from heat stress (Norman et al., 2012).

The word ‘stress’ can be defined as the biological responses upon exposure to an adverse environmental condition (Selye, 1950). Current descriptions or definitions in literature for heat stress in dairy cows are mostly vague; we define ‘heat stress’ in our thesis as the state at which the cow is out of her thermal comfort zone (above point C in Figure 1-1) and adaptive mechanisms have to be activated to maintain the thermal balance.

1.2 Heat loss mechanisms

To maintain the thermal balance, the heat generated by the metabolism (maintenance, exercise, growth, lactation, gestation, feed digestion) must be equal to the heat lost to the environment (Fournel et al., 2017a). Cows dissipate heat by means of convection, radiation, conduction and evaporation (Wang et al., 2018). Figure 1-2 illustrates the heat transfer of a housed standing dairy cow with the ambient environment, which can be affected by the climatic factors including air temperature, relative humidity, and air velocity, and the temperature of objects in the environment (floor, roof, etc.). Up to a certain ambient temperature, heat is mainly lost as sensible heat (through convection, radiation and conduction). Each of these energy transfer processes is influenced by a particular temperature: for convective transfer it is the air temperature; for radiative transfer it is the mean radiant temperature of the surroundings; and for conductive transfer it is the temperature of the surface that is in contact with the skin of the animal (Mount, 1979). With increasing ambient temperature there is a marked shift from sensible to latent (evaporation of water) heat loss as illustrated in Figure 1-1 when environmental temperatures increase above point C (Maia et al., 2005a). Latent heat transfer takes place mainly at two sites of the animal: the respiratory tract and the skin surface. Under warm conditions, an increased respiration (water evaporation by the lungs) and sweating rate (water evaporation from the skin) are two of the primary autonomic responses exhibited by animals (Gebremedhin et al., 2008).

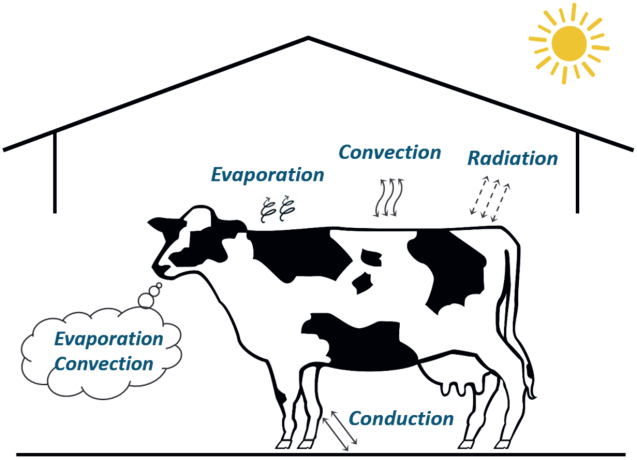

Figure 1-2. Illustration of the heat dissipation mechanism of a housed cow.

Maia et al. (2005b) found that the respiratory latent heat loss in dairy cows increased with increasing ambient temperature. A high aerial humidity may reduce the capacity for evaporative heat loss via respiratory water evaporation and lowers the evaporative cooling potential (Berman, 2006). Dairy cows possess a high sweating capacity (Mount, 1979), in advanced high producing breeds this sweating process is crucial to help cows to maintain their heat balance. According to Santos et al. (2017) latent heat loss by sweating could account for 88% of the total latent heat loss under high ambient temperature. However, the latent heat lost from the skin might be limited by the actual evaporation rate (Berman, 2009, Foroushani and Amon, 2022), which means not all produced sweat is evaporated for heat dissipation. In practice, to enhance convective heat loss during warm periods, ventilators in the barns are used to increase the air velocity around the cows. However, at higher ambient temperature, the benefit of high air velocity might diminish because of the low temperature difference between the air and the skin (Spiers et al., 2018). In such a situation higher air velocities are only effective by enhancing the evaporation rate when the potential evaporation rate of the environment is lower than the actual sweating rate of the cow. Therefore, a better understanding of the transition between sensible and latent heat loss under different ambient conditions will help to efficiently apply cooling systems.

1.3 Responses to the Thermal Environment

Within the thermoneutral zone, a homeothermic animal like a cow can balance heat production with heat dissipation (Mount, 1979). In the thermal comfort zone the energy needed for regulation of the heat dissipation is minimal (mainly vasomotion). When the ambient temperature rises above this thermal comfort zone, the cows must recruit extra responses (physiological and behavioral) in order to get rid of the produced heat. Numerous studies indicate that when exposed to high ambient temperatures dairy cows showed elevated respiration rate, skin temperature, heart rate and rectal temperature (Li et al., 2020, Pinto et al., 2020, Yan et al., 2021b). Since respiration is an efficient way to dissipate body (lung) heat under warm conditions, increased respiration rate is one of the first reactions to increase the evaporative heat loss to the environment (Silanikove, 2000). Meanwhile, skin temperature tends to further increase, caused by vasodilation - an enlargement of peripheral blood vessels under the skin surface - which facilitates heat transfer from the body core to the skin surface (McArthur, 1987). When the cow fails to dissipate all the produced heat, the body temperature will rise. It has been reported that milk production significantly decreases when rectal temperature is over 39°C and prolonged for more than 16 h, with 1.8 kg of milk yield declined for every 0.55°C increase in rectal temperature (West et al., 2003). Still, little research has been done on the ambient temperatures at which cows start to show symptoms of heat stress, as these temperatures depend on different factors, including relative humidity and air velocity levels.

1.4 Models to Predict Thermal Responses of Dairy Cows

In order to better understand the thermal processes and conditions of an animal and to appropriately apply heat mitigation strategies under hot climate conditions, it is of great importance to have a thermal balance model for dairy cows. The simulation of thermos-physiological responses requires the detailed modelling of two key components: a thermoregulation model of dairy cow and a heat dissipation model dealing with heat and mass transfer from the cow to its environment.

In previous decades several mathematical models have been developed for calculating the heat loss of dairy cows (McArthur, 1987, Ehrlemark and Sällvik, 1996). During the years these models and equations are extended from single equations to extensive ones resulting in a tremendous increase in complexity. The model from McGovern and Bruce (2000a) is a steady-state energy model that consisted of 153 elements describing the thermal environment, animal characteristics, as well as the distribution of heat transfer through body tissue, through the coat layer, heat transfer to the ambient air, heat loss from the respiratory tract, and the eventual rates of change in body temperature. This model was then adapted by Berman (2005) in order to make it suitable for Holstein dairy cows. Gebremedhin and Wu (2001) developed a mechanistic model which combined both heat and mass transfer to predict evaporative and convective heat losses for different levels of skin wetness and fur properties. This model provided insight in heat losses when applying cooling systems in the barn to avoid heat stress. Thompson et al. (2014) developed a dynamic heat exchange model, consisting of three state variables, and it was able to calculate changes in body core temperature in response to certain climate factors such as air temperature, vapor pressure, solar radiation and air velocity. The model was then improved by Li et al. (2021) by taking into account the conductive heat transfer between the cow and the ground when the animal lies down in pasture. However, for the existing models, as described above, the description of the physiological responses of cows was developed decades ago and are often lacking validation and knowledge has been developed to improve these models. Comprehensive interpretation of the physiological responses using recent data from modern cows are therefore needed to achieve more accurate predictions.

1.5 Knowledge Gaps

The following knowledge gaps have been identified, and will be addressed in the following chapters of this thesis:

(1) Little research has been done on the effect of increasing ambient temperatures in combination with different relative humidity and air velocity levels on the physiological responses in dairy cows under controlled environmental conditions. Up to date, the sequence of responses of dairy cows to various combinations of ambient conditions has not been identified; the critical temperatures, as mentioned in Figure 1-1 (points C and D), have not been determined for different relative humidity or air velocity levels.

(2) Information on the absolute contribution of each single component (sensible vs. latent; cutaneous vs. respiratory) of the total heat loss under various environmental conditions is scarce. How do cows adjust their mechanisms to dissipate heat via sensible and latent routes, and through respiration and from the skin surface under the effects of ambient temperature, relative humidity and air velocity?

(3) There is no adequate information about the total evaporative water loss from dairy cows for a long period (e.g. 24h). Current available data include various sources of errors: sweating rate measured on a small sample area represented for the total sweating rate, while sweating rates can differ among different skin regions; sweating rate measured during a short period (varying from 10 mins to 2 h) represented for the daily sweating rate, while there are cyclic sweating patterns; the weighing system was commonly used as a golden standard method to measure

sweating rate, while the gas exchange was neglected while this could account for a significant proportion of the weight change.

(4) The current thermoregulatory models are mostly developed based on the data from dairy cows in tropical areas and based on data from several decades ago. An updated dynamic model that can predict thermal physiological responses and thermal state of modern high productive dairy cows under various environmental conditions is missing. Besides, the knowledge of the efficacy and limitations of different cooling methods applied in different climate regions is lacking.

1.6 Objectives and Thesis Outline

The overall objectives of this thesis were to have better knowledge about the responses of dairy cows to various thermal environments and to have a tool that can accurately predict early signs of heat stress in dairy cows. To achieve these goals, an experiment was designed and conducted in climate-controlled respiration chambers to investigate how cows adjust their physiological responses from comfort zone (Figure 1-1: point B to C) to thermoneutral zone (Figure 1-1: point C to D), and up to a change of deep body temperature (Figure 1-1: above point D). A thermoregulatory model was then developed to predict the cow's physiological responses for assessment of its thermal status.

In ***Chapter 2***, the effects of increasing ambient temperature at different relative humidity and air velocity levels on the physiological and productive responses of dairy cows are investigated using climate-controlled respiration chambers. The inflection point temperatures at which a certain physiological response starts to change are determined for different conditions of RH and AV. In ***Chapter 3***, the sensible and latent heat losses from skin and through respiration of dairy cows are assessed and the effects of different environmental conditions (ambient temperature, relative humidity, air velocity) on these heat losses are determined. In ***Chapter 4***, the total evaporative water loss at different levels of temperature, relative humidity and air velocity is determined at daily level. The local cutaneous evaporative water loss measured by a ventilated skin box, as described in Chapter 3, is compared with the total cutaneous evaporative water loss determined with the water balance method. In ***Chapter 5***, a thermoregulatory model is presented to understand heat flows and physiological responses in dairy cows under different environmental conditions. The model can be used to timely and properly deal with expected weather conditions that potentially may cause heat stress. Based on model simulations effective cooling strategies can be advised to help the farmer to prevent heat stress in dairy cows under practical conditions. In the final ***Chapter 6***, the results and findings presented throughout the previous chapters are discussed in a wider context. Recommendations are made for future research.

2

CHAPTER 2.

Effects of increasing air temperature on physiological and productive responses of dairy cows at different relative humidity and air velocity levels

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Abstract

This study determined the effects of increasing the ambient temperature (T) at different relative humidity (RH) and air velocity (AV) levels on the physiological and productive responses of dairy cows. Twenty Holstein dairy cows were housed inside climate-controlled respiration chambers, in which the climate was programmed to follow a daily pattern of lower night and higher day temperatures with a 9°C difference, excluding effects from sun radiation. Within our 8-d data collection period, T was gradually increased from 7 to 21°C during the night (12 h) and 16 to 30°C during the day (12 h), with an incremental changes of 2°C per day for both nighttime and daytime temperatures. During each research period, RH and AV were kept constant at one of five treatment levels. A diurnal pattern for RH was created, with lower levels during the day and higher levels 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 the day at three levels: no fan (AV_l: 0.1m/s), fan at medium speed (AV_m: 1.0m/s), and fan at high speed (AV_h: 1.5m/s). Effects of short and long exposure time to increasing T were evaluated by collecting data two times a day: in the morning (short: 1 h (or less) - exposure time) and afternoon (long: 8 h - exposure time). The animals had free access to feed and water and both were ad lib. Respiration rate (RR), rectal temperature (RT), skin temperature (ST), DMI, water intake, milk yield and composition were measured. The inflection point temperatures (IPt) at which a certain variable started to change were determined for the different RH and AV levels and different exposure times. Results showed that IPt under long exposure time for RR (first indicator) varied between 18.9 and 25.5°C but was between 20.1 and 25.9°C for RT (a delayed indicator). The IPt for both RR and RT decreased with higher RH levels, while IPt increased with higher AV for RR but gave a minor change for RT. The ST was positively correlated with ambient T and ST was not affected by RH but significantly affected by AV. For RR, all IPt was lower under long exposure time than under short exposure time. The combination of higher RH levels and low AV levels negatively affected DMI. Water intake increased under all treatments except RH_l*AV_l. Treatment (RH_h*AV_l) negatively affected milk protein and fat yield while treatments (RH_m*AV_m and RH_m*AV_h) reduced milk fat yield. We concluded that RH and AV levels affected significantly the responses of RR, RT, ST and productive performance of high-producing Holstein cows. These responses already occurred at moderate ambient temperatures of 19 to 26°C.

Keywords: dairy cow, temperature, relative humidity, air velocity, heat stress, physiological response

2.1 Introduction

Once thought to be limited to (sub)tropical areas, the effects of high ambient temperatures on dairy cows have now become relevant in temperate climate areas due to rising global temperatures (Polsky and von Keyserlingk, 2017, Pinto et al., 2020). In addition, the intensive genetic selection of milk production has resulted in dairy cows that are more susceptible to heat stress than they were in the past (Ravagnolo et al., 2000), rendering the problem of heat stress during the summer increasingly prominent, along with all the accompanying negative effects on dairy cows' health (Kadzere et al., 2002, de Andrade Ferrazza et al., 2017), production (Hill and Wall, 2015) and reproduction (García-Ispierto et al., 2007, Schüller et al., 2014) as well as increased mortality risk (Vitali et al., 2009).

Dairy cows are particularly sensitive to high ambient temperatures (Kadzere et al., 2002). In the thermal comfort zone, cows can easily balance heat loss with heat production independent from ambient temperatures (Mount, 1979). However, when ambient temperatures rise above this (thermal comfort) zone, the cows must recruit extra physiological responses in order to get rid of the produced heat. The physiological responses could include increased respiration rate (RR), increased blood flow from the core body to the skin surface, increased sweating rate, increased water consumption, reduced rumination activities, increased heart rate, reduced heat production, and increased core body temperature (Burfeind et al., 2012, Hill and Wall, 2015, Galán et al., 2018, Amamou et al., 2019).

With regards to the response of increasing RR, Kadzere et al. (2002) reported that about 15% of accumulated metabolic heat is dissipated by the respiratory tract for evaporative heat loss under hot conditions. Heat stress occurs when a cow is exposed to temperatures that exceed its biological thermal comfort zone and she has to make a lot of effort - and may even fail to - dissipate enough heat to maintain her body thermal balance (Majkić et al., 2017). Li et al. (2020) reported that the rectal temperature (RT) of a high-producing cow started to increase when the ambient temperature was above 20.4°C. The models produced by McArthur (1987) showed that metabolic heat production began to decline above the temperature threshold of 23°C, in response to elevated body temperature. Recognizing the importance of ambient air humidity, the temperature-humidity index (THI) is frequently used to assess heat stress magnitude in dairy cows. For example, Pinto et al. (2020) determined the THI thresholds for RR, heart rate and RT of high-producing cows and suggested heat mitigation actions should be taken when THI rises above 65. Besides, the activation of the thermoregulatory mechanisms also varies with the duration of heat exposure (de Andrade Ferrazza et al., 2017, Pinto et al., 2020). Pinto et al. (2020) demonstrated an increase in the RR of the cows by prolonging time beyond a THI of 65.

Still, little research has been done involving the temperatures at which cows start to show symptoms of heat stress, as these temperatures depend on relative humidity (RH) and air velocity (AV) levels. In this paper we examined what order can be detected in the cows' responses to these changes, in order to determine whether the animals have a strategy or a certain method of coping. Other relevant questions remain: (1) under which ambient conditions will cows obviously react through physiological changes, and (2) could additional ventilation support the cow to remain within the thermal comfort zone? The objectives of this study were (1) to quantify the effects of increasing ambient temperature on physiological and productive parameters of Holstein dairy cows; (2) to determine the inflection point temperatures (IPt) for activation of adaptive mechanisms at different RH and AV levels; and (3) to assess the effect of different exposure times to increasing temperature conditions on physiological responses.

2.2 Materials and methods

The experiment was conducted in 2021 at the "Carus" animal research facilities of Wageningen University and Research (WUR), the Netherlands. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Wageningen University and were conducted under the Dutch Law on Animal Experiments (Project No. 2019.D-0032).

2.2.1 Animals and feed

Twenty Holstein Friesian dairy cows were used with an average milk yield (±SD) of 30.0±4.7 kg/d at 206±39 DIM, 687±46 kg of BW, and a parity of 2.0±0.7 lactations. Nineteen cows were pregnant and the average number of days they carried their calves was 105±38. Cows were grouped in four blocks of five cows based on parity and expected milk yield. Each cow within a block was randomly assigned to one of the five treatments. The BW, milk yield, parity, DIM and pregnant days of cows in different treatment groups are described in Table 2-1. The cows received ad libitum feed via a feed trough fixed in front of the cubicle and water via a drinking bowl.

All cows were subjected to the same feeding scheme. The cows were fed (Table 2-2) twice daily at 0500 and 1530, and the diet was formulated to meet or exceed the nutritional requirements of lactating Holstein cows according to the Dutch System (CVB, 2008). The amount of feed offered to each cow was adjusted daily to yield an excess (uneaten feed) of at least 5%.

Table 2-1. Body weight, annual average milk yield, parity, days in milk and pregnant days of cows in five treatment groups (means ± SD).

	Treatments				
Item	I (RH_l*AV_l)	II (RH_m*AV_l)	III (RH_h*AV_l)	IV (RH_m*AV_m)	V (RH_m*AV_h)
Body weight, kg	695±54	671±52	667±41	721±50	680±29
Milk yield, kg/day	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
Days in milk, day	212±35	192±40	182±54	227±31	215±35
Pregnant days, day	100±27	116±20[1]	85±60	104±30	120±48

[1]In treatment II there was one non-pregnant cow.

2.2.2 Acclimatization and adaptation period

To acclimate to the experimental conditions, cows were housed in tie-stalls 7 d prior to entering the climate-controlled respiration chambers (CRC), located approximately two kilometers from Carus. Like in CRC, the cows were then placed in individual tie-stalls, wore halters, were in frequent contact with animal caretakers and received the experimental diet.

Every single day for the first 3 d after entering the CRC, besides receiving feeding and milking visits, the cows were visited two times 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 chambers, cows could also see and hear other cows through transparent windows.

2.2.3 The Climate-controlled Respiration Chamber

In this study, two identical climate-controlled respiration chambers were used. Each chamber was split into two individual airtight compartments with thin walls equipped with transparent windows to allow audio and visual contact between two cows and thereby minimize the effects of social isolation on their behavior. Each compartment measured 12.8 m^2 and had a volume of 34.5 m^3 as described in detail by Gerrits and Labussière (2015). For each compartment the RH was monitored by one relative humidity sensor (Novasina Hygrodat100, Novasina AG, Lachen, Switzerland), and the air temperature (T) was monitored by five PT100 temperature sensors (Sensor Data BV, Rijswijk, the Netherlands) evenly distributed over the room at animal height as shown in Figure 2-1. For climate control the median value of all temperature sensors was used to rule out disproportional effects of potential deviating values. Experimental RH was achieved by means of a humidifier (ENS-4800-P, Stulz) and/or a dehumidifier (koeltechniek, Nijssen). The circulating air was heated or cooled depending on the deviation from set point temperatures. High air velocity (AV) was achieved using Professional Fans (500 mm diameter, model 8879, HBM Machines BV, the Netherlands) fixed on the ceiling of the chamber (at a height of 2.5 m above the floor) as described in Figure 2-1, in such a way that the wind was blowing on the axial body length of the cow from back to front. The chambers were artificially lit (390-440 lux) for 16 h per day (0500 to 2100 h for CRC 1 and 0600 to 2200 h for CRC 2) and during the night (2100 to 0500 in CRC 1 and 2200 – 0600 in CRC 2) the light was dimmed significantly (35-40 lux).

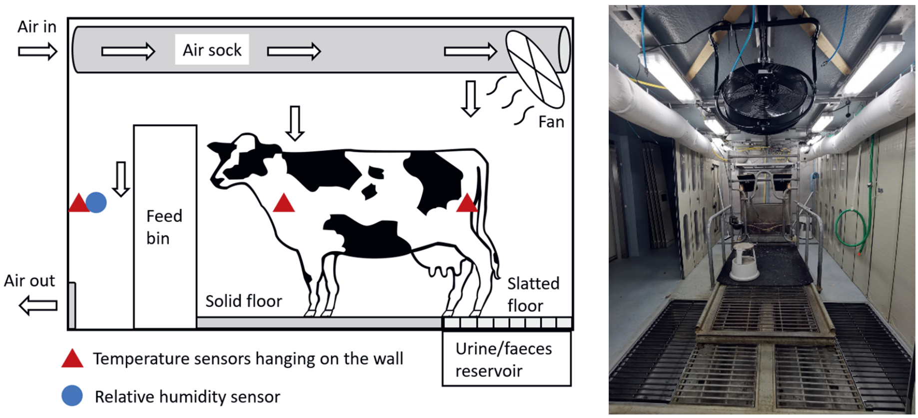

Figure 2-1. Schematic and overview photo of the climate-controlled respiration chamber. There are two temperature sensors hanging on each side wall (left and right), and one temperature sensor and one relative humidity sensor hanging on the wall in front of the cow. The material of solid floor is rubber 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 she could easily move forward/backward and lie down.

Table 2-2. Ingredients and chemical composition of the mixed diet fed to the cows in the experiment[1]

Item	Amount
Roughage ingredients (g/kg)	
Corn silage	623.2
Grass silage	376.8
Roughage chemical composition (% of DM)	
CP	9.0
NDF	42.4
ADF	23.5
Concentrate chemical composition (% of DM)	
CP	18.6
NDF	23.0
ADF	13.7

[1]Concentrate was fed at the rate of 6 kg/d. Roughage was adjusted to maintain ad libitum intake. Concentrate was ground to decrease the size of the particles, making it easier to mix it with the roughage.

2.2.4 Research Design

The diurnal patterns of the climatic condition were simulated from retrospective data obtained from the Dutch National Weather Service (KNMI, 2019), which is a typical diurnal pattern for Dutch weather during the summertime. The T and RH for day and night conditions were then applied in the treatments (Table 2-3, Figure 2-2).

Each cow was subjected to an 8-d research period in the CRC with a specific treatment consisting of combinations of T, RH and AV. Air temperatures inside the chambers were gradually increased from 7 to 21°C at night and 16 to 30°C during the day within 8 d (by steps of 2°C per day for both nighttime and daytime temperatures) as shown in Figure 2-2. The experimental treatments comprised three RH levels and three AV levels as described in Table 2-3. Three levels of RH during the day (d) and night (n) were: RH_l (low) 30% (d) and 50% (n); RH_m (medium): 45% (d) and 70% (n); and RH_h (high) 60% (d) and 90% (n). However, the capacity of the cooling system in the CRC (for the dehumidification of air) was not sufficient to reach the intended values of all treatment combinations. In particular, it proved not to be possible to achieve combinations of low air temperatures with low RH conditions during the first days of the research period. During the day (0900 to 2100 h), three AV levels were created: AV_l (low): fan off (0.1 m/s); AV_m (medium): fan speed level 1 (1.0 m/s); AV_h (high): fan speed level 2 (1.5 m/s). The fan was off during the night. For AV_m and AV_h the starting T was increased by 2°C, making the T range from 18 to 32°C during the day within 8 d. AV_m and AV_h were only combined with RH_m. The air 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. There were four replications per treatment.

Technically, the T and RH inside the CRC required a time span of three hours to adjust to new levels. The daytime T and RH were adjusted from 0700 to 1000 h, while the nighttime T and RH were adjusted from 1900 to 2200 h. The measurements for both chambers were conducted consecutively by the researchers, accounting for a 1-h time lag between the two chambers regarding changes in T, RH, AV, light setting, feeding and milking. The exposure times (the time points when data were collected) were defined as short when the cows were exposed to the new stable T levels within 1 h and the exposure time was defined as long when the cows were exposed to the new T levels for more than 8 h.

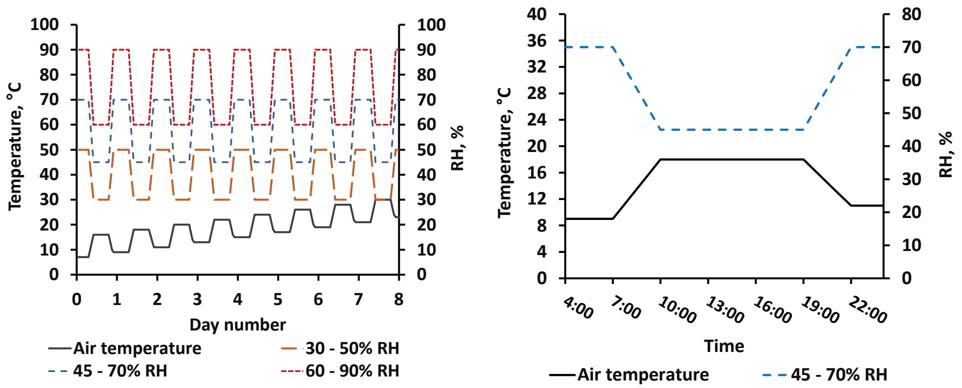

Figure 2-2. (a) Schematic temperature and relative humidity (RH) patterns during the 8-d research period. Between 0700 to 1000 h, the temperature and RH rose gradually to daytime levels and stayed constant until 1900 h. Between 1900 to 2200 h, the temperature and RH gradually decreased to nighttime levels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

Table 2-3. Temperature, relative humidity and air velocity treatment parameters used in climate-controlled respiration chambers.

Treatment	Temperature, °C		Relative humidity, %		Air velocity
	2100 - 0700 h[1]	1000 - 1900 h[2]	2100 - 0700 h	1000 - 1900 h	0900 - 2100 h[3]
I (RH_l*AV_l)	7 - 21[4]	16 - 30	50	30	Fan off
II (RH_m*AV_l)	7 - 21	16 - 30	70	45	Fan off
III (RH_h*AV_l)	7 - 21	16 - 30	90	60	Fan off
IV (RH_m*AV_m)	9 - 23	18 - 32	70	45	Fan on, speed 1
V (RH_m*AV_h)	9 - 23	18 - 32	70	45	Fan on, speed 2

[1]2100 - 0700 h stands for the duration of the night from 2100 h until next day 0700 h, for the first CRC.
[2]1000 - 1900 h stands for the duration of the day from 1000 h until 1900 h on the same day, for the first CRC.
[3]0900 - 2100 h stands for the duration of air velocity treatment from 0900 h until 2100 h on the same day, for the first CRC. There was a 1-h delay for all the controlling parameters for the second CRC.
[4]7 - 21 (or 9 - 23) marks the air temperature on d 1 as 7°C (or 9) and on d 8 as 21°C (or 23) at night, and the temperature was increased by 2°C for the following day within the period; 16 - 30 (or 18 - 32) marks the air temperature on d 1 as 16°C (or 18) and d 8 as 30°C (or 32) during the day, and the temperature was increased by 2°C for the following day within the period.

2.2.5 Data Collection

All the data collection procedures are described in Table 2-4. Throughout the 8-d research period, the T and RH of the chamber compartments were continuously and automatically recorded at 30-s intervals. Using a handheld anemometer (Testo 5-412-983, Testo SE & Co. KGaA), the actual AV was manually measured two times a day at five locations from a distance of about 5cm around the cows' body surface for 30 s each time: neck, middle trunk and rump, and both lateral sides. The dependent variables RR, skin temperature (ST), and RT were measured two times a day at 1000 and 1800 h. RR was measured by observing the movement of the flank quietly and the time needed for counting ten breaths was recorded with a stopwatch. ST was measured on four different parts of the body (heart, back, belly and rump) with a thermometer probe (Testo 0602 0393, Testo SE & Co. KGaA) and a handheld datalogger (Testo 435-4, Testo SE & Co. KGaA) by directly touching the surface of the skin under the hair. RT was measured by inserting a digital thermometer (VT 1831, Microlife AG) to the depth of approx. 3 cm into the rectum of the cow and the result was read once the beep sounded. During the measurement, the cows did not seem to respond noticeably to the measurements. Feed refusals were collected and weighed before the morning feeding and were analyzed for DM and chemical composition. The cows were milked inside the CRC twice daily at 0500 and 1530 h and the milk yield (MY) was recorded at each milking for each cow. Milk samples were collected at each milking for an analysis of fat, protein, and lactose composition by Veluwe IJsselstreek (Nunspeet, the Netherlands). Individual water intake was measured by reading the water meter (Unimag Cyble UT4 BH-A, Itron) two times daily just before milking.

Table 2-4. Data collection and measurement time in two Climate-controlled Respiration Chambers (CRC).

Items	Measurement device and method	Measurement time	
		CRC 1	CRC 2
Climatic parameters			
Air temperature	Air temperature is continuously measured by five temperature sensors.	Continuously, every 30 s.	Continuously, every 30 s.
Relative humidity	Relative humidity is continuously measured by a relative humidity sensor.	Continuously, every 30 s.	Continuously, every 30 s.
Air velocity	Measure five locations around the cows' body surface: neck, middle trunk and rump, and both lateral sides by using a handheld anemometer.	1000 h; 1800 h	1100 h; 1900 h
Animal parameters			
Respiration rate	Observe the movement of the flank quietly and the time needed for counting ten breaths; record using a stopwatch.	1000 h; 1800 h	1100 h; 1900 h
Rectal temperature	Insert a digital thermometer to the depth of approx. 3 cm into the rectum of the cow and read the result once the beep sounds.	1000 h; 1800 h	1100 h; 1900 h
Skin temperature	Measure four different areas by directly touching the skin surface under the hair (heart, back, belly and rump) using a thermometer probe.	1000 h; 1800 h	1100 h; 1900 h
DMI	Feed refusals are collected and weighed before the morning feeding, samples were collected and analyzed for DM content.	0500 h	0600 h
Milk yield	Milk yield is recorded at each milking; milk samples for fat, protein, and lactose analysis are collected in tubes at each milking and stored at 4°C until sent to the company for analysis.	0500 h; 1530 h	0600 h; 1630 h
Water intake	Water intake is measured by reading the water meter.	0500 h; 1530 h	0600 h; 1630 h

2.2.6 Statistical Analysis

The cow was considered as the experimental unit for all parameters. All statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC). One cow (receiving treatment IV: RH_m*AV_m) was excluded from the experiment because of mastitis.

Data were first analyzed to investigate the distribution, outliers and to determine which statistical model (linear or non-linear regression) would best fit the cows' responses to the treatments. In order to evaluate the effects of the treatment T, RH, AV on the response variables, data were first analyzed with a nonlinear model.

$$y_{ijk} = C_i + a_i \cdot z + cow_{ijk} + \varepsilon_{ijk}$$

where, y_{ijk} is the observed response variable; C_i is a constant over a range of temperature at each treatment level (i = 1...5); a_i is the regression coefficient for z and the interaction between z with the i-th treatment; z is the structural part that creates a broken-line regression of T with an inflection point (IPt); cow_{ijk} is the random effect of j-th cow for the k-th research day; and ε_{ijk} is the residual error.

$$z = (T > IPt) * (T - IPt), where\ (T - IPt)\ is\ defined\ as\ zero\ if\ (T \leq IPt)$$

The broken-line regression model was fit and IPt was determined for RR and RT by meeting fit statistics criterion, using SAS NLMixed procedure including cow random effects (Robbins et al., 2006). The starting values for a nonlinear model were first chosen by visually observing the data distribution and then were changed using the output from the model. The best fit model was determined by comparing Akaike information criterion (AIC) (smaller is better). The χ^2 test was used to test the significance of the model, and t-test was applied to pairwise compare the differences between treatments and between two exposure times (short and long).

If the model failed to converge, a linear regression model was used. The MIXED procedure was used to investigate the influence of increasing T for each treatment at different exposure times. Although multiple measurements per animal cannot be regarded as independent units of observations, repeated measures was 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 AIC values was selected. The linear regression model was as follows:

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

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 i-th treatment respectively; cow_{ijk} is the random effect of j-th cow for the k-th research day; and ε_{ijk} is the random residual error. The χ^2 test was used to test the significance of the model, and the adjusted Tukey t-test was applied using the PDIFF statement to pairwise compare the differences between treatments and between two exposure times (short and long).

For the production parameters, the average baselines for feed intake, drinking water intake, milk yield, protein yield and fat yield were calculated for the data from the first two research days. The MIXED procedure was applied to investigate the effect of increasing T on these parameters. Values are presented as least squares means with their standard deviation.

Model assumptions were evaluated for both the broken-line model and 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.

2.3 Results

2.3.1 Controlled-climate chambers and environmental conditions

The realized (measured) T and RH for the different RH treatments are shown in Figure 2-3. The daily cyclical temperatures were kept strictly constant according to set points with a deviation smaller than ±0.50°C. The RH_l failed to reach the set point (30 - 50%) within the first 5 d, but it got close from d 6 to 8. Similarly, RH_m could not reach the intended values (45 - 70%) within the first 3 d. AV was calculated by taking the average of each cow's five measurement points. AV_l ranged from 0.05 to 0.11 (mean 0.08 ± 0.01 m/s), AV_m ranged from 0.48 to 1.74 (mean 1.14 ± 0.30 m/s), and AV_h ranged from 0.72 to 1.98 (mean 1.35 ± 0.29 m/s).

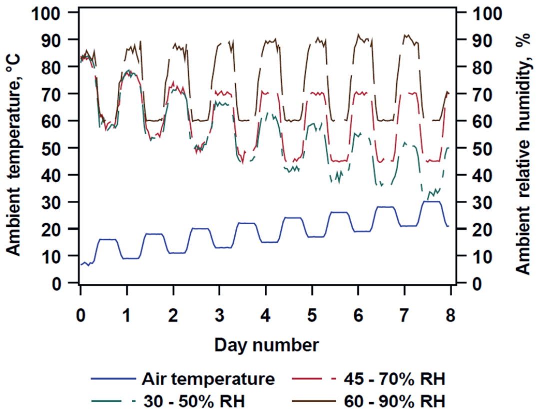

Figure 2-3. Average measured hourly temperature and relative humidity (RH) during the 8 d research period for three RH levels.

2.3.2 Physiological Responses to Treatments

From the model selection, the broken-line model fitted for RR and RT and the linear model fitted for ST. The results of the effects of different treatments and exposure times on the coefficients of the broken-line or linear model for different physiological parameters are given in Table 2-5.

The constants are reflecting the basal levels of RR at the lower ambient temperatures. They varied between 31.3 and 36.3 breath/min for short exposure and between 24.8 to 36.5 breath/min for long exposure. There were some differences between the constants of the different treatments, but these could not be directly linked to RH or AV levels. Besides, constant RR was different depending on exposure times in two treatments RH_h*AV_l and RH_m*AV_m (P = 0.066 and P = 0.002, respectively), the RR was lower in long exposure as compared to short exposure. Given the variation in basal RR between individual cows, milk yield was considered as a covariable for normal RR (data from first two research days) but no effect of milk yield was observed on basal RR. The IPt varied between 20.9 and 25.8°C for short exposure and between 18.9 and 25.5°C for long exposure. Generally, IPt increased with decreasing RH and increasing AV. Under the long exposure, IPt for RR decreased ($P < 0.05$) as RH increased: 25.5, 21.0 and 18.9°C for RH_l, RH_m and RH_h, respectively. The increased exposure time decreased IPt for RR for a combined treatment of RH_h*AV_l ($P < 0.05$) and RH_m*AV_h ($P < 0.05$), and there was a tendency of an effect of RH_m*AV_m ($P < 0.10$). Under AV_h, the IPt for RR was higher than the IPt at AV_l (21.0, 22.8°C for AV_l and AV_h, respectively; $P < 0.05$). The IPt for RR under AV_l or AV_m were not different. The slope (regression coefficient 'a' in broken-line model) varied between 4.1 to 9.4 breath/°C for short exposure and between 4.2 to 9.5 breath/°C for long exposure. For low RH the slope was much higher as compared to the other treatments ($P < 0.05$), which means that under low RH level, although the cows could maintain a basal RR for a wider ambient T range, they had to increase RR rapidly above IPt.

Rectal temperature was affected by increasing temperature in combination with different levels of RH and AV (Table 2-5). The RT during short exposure is not shown in Table 2-5 because it could not be fitted by a broken-line model. The average RT for short and long exposure times were 38.4±0.3 and 38.7±0.4, respectively ($P < 0.01$). For long exposure, constant RT remained within the range of 38.1 to 38.7°C for the five treatments. Under low AV conditions, the RT at high RH level was lower than the RT at other RH levels ($P < 0.05$). Treatment RH_m*AV_m had the lowest basal RT amongst the five treatments ($P < 0.05$). The IPt for RT varied between 20.1 to 25.9°C. Under low AV, the IPt for RT under RH_h was much lower ($P < 0.05$) compared with RH_l and RH_m (20.1°C vs. 25.3 and 25.9). The effects of AV on IPt for RR were not consistent: at a medium AV level, the IPt appeared smaller than at a high AV level ($P < 0.05$). The slope varied between 0.07 to 0.14 °C/°C. The effect of AV levels on the slope was evident:

the slope was significantly lower at medium or high AV levels than at the low AV level in combination with medium or high RH ($P < 0.05$).

Skin temperature increased linearly with increasing T ($P < 0.001$). For the five treatments, the intercept for ST varied between 21.8 to 29.4°C for short exposure and between 25.1 to 31.3°C for long exposure. The intercept for ST was larger in high RH than in RH_l and RH_m treatments with low AV under short exposure ($P < 0.05$), while under long exposure there was no difference in intercepts between the three RH levels with low AV. The intercept for ST was higher in the AV_h group than in the AV_m group under short exposure ($P < 0.05$), while under long exposure there was no difference. Generally, cows had lower ST intercepts at higher AV levels for both short and long exposure times. The slope (coefficients (a+b) in linear model) varied between 0.23 to 0.44 °C/°C for short exposure and between 0.18 to 0.36 °C/°C for long exposure. There was no interaction effect between RH with T on the increasing rate of ST with low AV level while there was an interaction ($P < 0.05$) of AV and T to an increasing rate of ST for both exposure times. The interaction of AV and T on ST were obvious, especially at AV_l compared with AV_m and AV_h (Figure 2-4 c_2). Under higher AV levels, the increase in ST slope was higher than that for AV_l. The average ST was higher ($P < 0.05$) for long compared with short exposure except for RH_m*AV_h, while under the short exposure the increase in ST per degree Celsius increase in T was more pronounced.

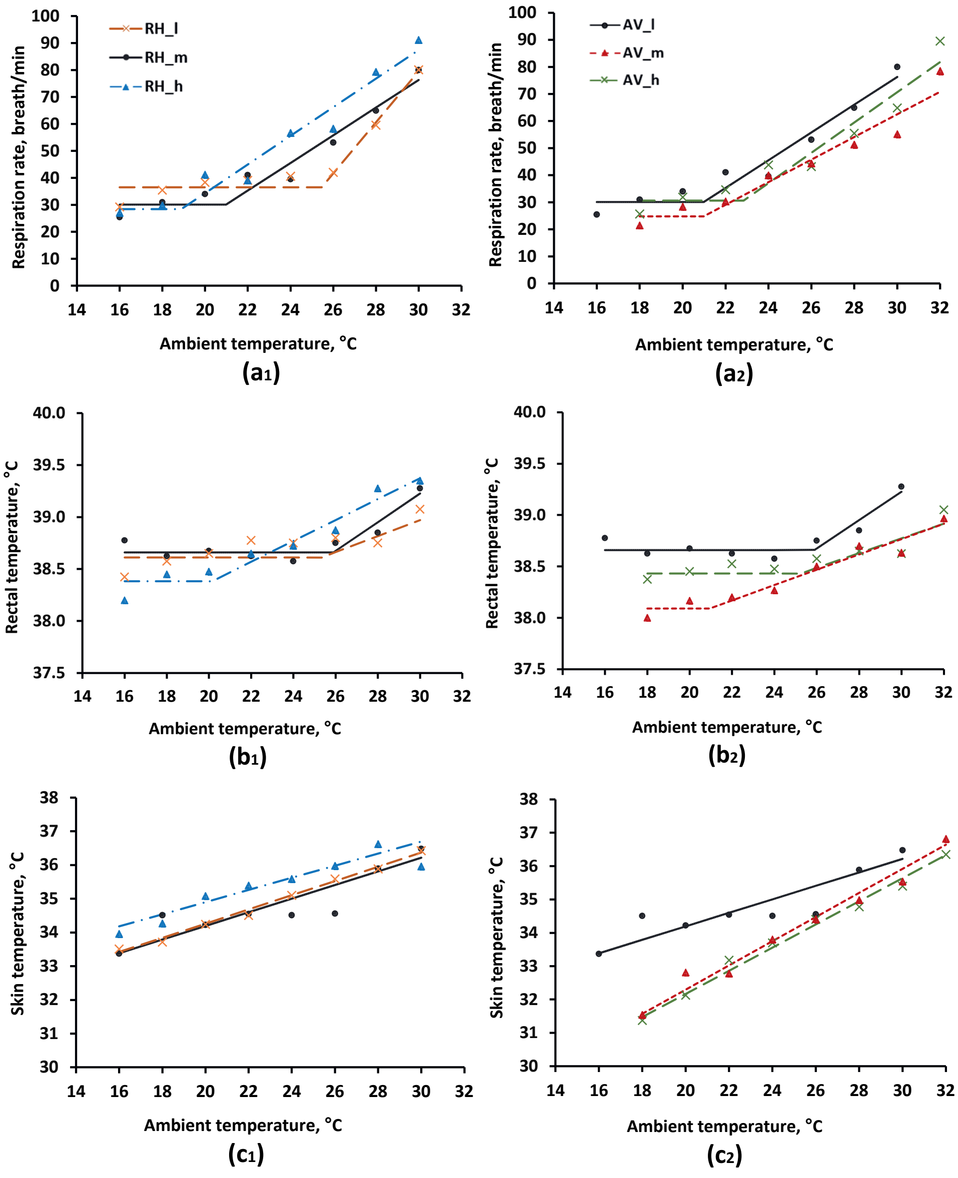

Figure 2-4. Relationships for the different treatments between ambient T and (a_1) respiration rate, (b_1) rectal temperature, (c_1) skin temperature under treatment I, II and III (RH_l, RH_m and RH_h: 30, 45 and 60%; AV low: 0.1 m/s); (a_2) respiration rate, (b_2) rectal temperature, (c_2) skin temperature under treatment II, IV and V (AV_l, AV_m and AV_h: 0.1, 1.0 and 1.5 m/s; RH_m: 45%).

Table 2-5. Coefficients (means ± SEM) from broke-line or linear regression with increasing temperature (T) on physiological parameters at different relative humidity (RH) and air velocity (AV).

Dependent variables	Exposure time[2]	Adjusted R^2	Regression model components	Treatments[6] I (RH_l*AV_l) N=4	II (RH_m*AV_l) N=4	III (RH_h*AV_l) N=4	IV (RH_m*AV_m) N=3	V (RH_m*AV_h) N=4
Respiration rate, breath/min	short	0.733	Constant[4]	36.0±2.35[a]	31.3±2.91[b]	36.3±5.01[ab]	33.6±2.26[ab]	32.7±2.35[b]
			IPt	25.8±0.50[a]	21.9±0.82[b]	20.9±0.69[c]	22.7±1.12[b]	24.2±0.84[d]
			Slope	9.4±2.28[a]	5.9±1.04[b]	6.4±0.64[b]	4.1±1.17[c]	5.3±1.23[bc]
	long	0.725	Constant	36.5±3.23[a]	30.1±3.70[b]	28.4±6.93[bc]	24.8±2.88[c]	30.6±3.69[b]
			IPt	25.5±0.42[a]	21.0±0.90[b]	18.9±1.04[c]	21.0±0.97[b]	22.8±0.88[d]
			Slope	9.5±1.34[a]	5.1±0.88[bc]	5.3±0.63[b]	4.2±0.54[c]	5.6±0.93[b]
P-value[1]			Constant	NS[5]	NS	0.066	0.002	NS
			IPt	NS	NS	0.011	0.051	0.039
			Slope	NS	NS	0.035	NS	NS
Rectal temperature, °C	Long[3]	0.581	Constant	38.6±0.13[a]	38.7±0.07[a]	38.4±0.08[b]	38.1±0.15[c]	38.4±0.10[b]
			IPt	25.3±0.92[a]	25.9±0.45[a]	20.1±1.2[b]	21.0±1.19[b]	25.3±1.32[a]
			Slope	0.08±0.03[ab]	0.14±0.05[c]	0.10±0.02[ac]	0.07±0.02[b]	0.07±0.02[b]
Skin temperature, °C	short	0.804	Intercept	27.4±1.34[a]	27.3±0.75[a]	29.4±1.10[b]	21.8±0.68[c]	23.8±0.82[d]
			Slope	0.28±0.06[a]	0.28±0.03[a]	0.23±0.05[a]	0.44±0.03[b]	0.38±0.03[c]
			b	NS	NS	NS	0.22±0.06[a]	0.16±0.06[a]
	long	0.811	Intercept	30.1±0.76[a]	30.4±0.83[a]	31.3±0.83[a]	25.1±0.92[b]	25.3±0.66[b]
			Slope	0.21±0.03[a]	0.19±0.04[a]	0.18±0.04[a]	0.36±0.04[b]	0.35±0.03[b]
			b	NS	NS	NS	0.19±0.05[a]	0.17±0.05[a]
P-value				0.014	0.005	0.022	0.047	NS

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

[1] *P*-value for statistical difference between two exposure times.

[2] Exposure time with 'short' means the cows stayed in the condition within one hour and with 'long' means the cows stayed in the condition for about 8 hours.

[3] The broken-line model could not be fitted for rectal temperature with short exposure time.

[4] Constant = basal values; IPt = inflection point temperature; b = regression coefficient for interaction between temperature and treatments.

[5] NS, $P \geq 0.10$.

[6] Treatment levels: RH_l: 30%; RH_m: 45%; RH_h: 60%; AV_l: 0.1m/s; AV_m: 1.0m/s; AV_h: 1.5m/s.

Table 2-6. Linear regression between DMI (kg/d), water intake (kg/d), milk yield (kg/milking), protein yield (kg/milking), fat yield (kg/milking) and ambient temperature.

Item			Treatment[5]				
			I (RH_l*AV_l) N=4	II (RH_m*AV_l) N=4	III (RH_h*AV_l) N=4	IV (RH_m*AV_m) N=3	V (RH_m*AV_h) N=4
DMI		Baseline[1]	17.9±0.53	20.5±0.37	18.8±0.29	21.1±0.64	18.3±0.39
		Slope	-0.003±0.065	-0.14±0.076	-0.14±0.045	-0.10±0.072	-0.023±0.065
		Slope *P*-value	NS[2]	0.079	<0.01	NS	NS
Water intake		Baseline	56.9±3.29	69.9±3.32	56.7±2.45	65.4±4.97	63.8±7.31
		Slope	0.38±0.58	1.61±0.71	0.63±0.26	2.24±1.04	1.25±0.31
		Slope *P*-value	NS	<0.05	<0.05	<0.05	<0.001
Milk yield	am[3]	Baseline	11.6±0.48	15.1±0.75	12.8±0.26	14.2±0.21	13.1±0.35
		Slope	0.018±0.046	0.041±0.053	0.039±0.049	-0.008±0.040	0.076±0.039
		Slope *P*-value	NS	NS	NS	NS	0.058
	pm[4]	Baseline	9.3±0.46	12.3±0.62	10.4±0.19	11.2±0.16	10.7±0.38
		Slope	0.021±0.063	-0.016±0.060	-0.020±0.038	0.025±0.035	-0.004±0.037
		Slope *P*-value	NS	NS	NS	NS	NS
Protein yield	am	Baseline	0.45±0.015	0.52±0.019	0.50±0.009	0.52±0.007	0.48±0.009
		Slope	-0.001±0.002	-0.001±0.002	-0.001±0.002	-0.002±0.001	0.001±0.001
		Slope *P*-value	NS	NS	NS	NS	NS
	pm	Baseline	0.35±0.015	0.42±0.016	0.39±0.006	0.40±0.006	0.37±0.011
		Slope	-0.000±0.002	-0.003±0.002	-0.003±0.001	-0.001±0.001	-0.002±0.001
		Slope *P*-value	NS	NS	<0.05	NS	NS
Fat yield	am	Baseline	0.60±0.013	0.70±0.030	0.68±0.025	0.75±0.010	0.62±0.030
		Slope	-0.003±0.003	-0.004±0.005	-0.003±0.004	-0.004±0.001	0.001±0.003
		Slope *P*-value	NS	NS	NS	<0.05	NS
	pm	Baseline	0.54±0.018	0.65±0.034	0.62±0.026	0.65±0.011	0.57±0.024
		Slope	-0.002±0.003	-0.005±0.004	-0.006±0.003	-0.003±0.002	-0.005±0.002
		Slope *P*-value	NS	NS	<0.05	0.055	0.066

[1]Baseline represents the values calculated from first two research days; Slope represents the regression coefficient for different variables with relationship of ambient temperature; Slope *P*-value shows the significance level that the slope differs from zero.
[2]NS, $P \geq 0.10$.
[3,4]am represents morning milking and pm represents afternoon milking.
[5]Treatment levels: RH_l: 30%; RH_m: 45%; RH_h: 60%; AV_l: 0.1m/s; AV_m: 1.0m/s; AV_h: 1.5m/s.

2.3.3 Productive Responses to Treatments

Treatment effects on DMI and water intake and milk yield are presented in Table 2-6. At the beginning of the research periods (the first two days), the basal DMI for cows in different treatments varied between 17.9 to 21.1 kg/d and the water intake varied between 56.7 to 69.9 kg/d. The DMI decreased 0.003 to 0.14 kg/d per °C increase across the five treatments. The most severe decrease in DMI was observed for treatment III (RH_h*AV_l) ($P < 0.01$). There was a tendency ($P = 0.079$) for decreased DMI under treatment II (RH_m*AV_l) as well. No difference was observed for the other three treatments. Water intake was positively related with increasing T ($P < 0.05$) for all treatments except for treatment I (RH_l*AV_l).

Increasing T had no effect on morning or afternoon milk yields for all the treatments (NS; $P >$ 0.10) except for treatment V (RH_m*AV_h) where the morning milk yield increased ($P =$ 0.058). However, under treatment III (RH_h*AV_l) the protein and fat yield for afternoon milking decreased with increasing T ($P <0.05$). Decreased fat yield was also a tendency under treatment IV and V (RH_m*AV_m, RH_m*AV_h) ($P = 0.055$ and 0.066, respectively), which showed that although the milk yield was not affected by increasing T the component yield could be affected.

2.4 Discussion

This study evaluated the effects of increasing temperatures at different RH and AV levels on thermoregulatory responses of Holstein Friesian dairy cows. The results of this study may contribute to the development of new strategies for heat stress mitigation that take into account the physiological adaptations of the animals.

2.4.1 Physiological Responses to Treatments

The increase in RR was the first reaction of cows under warm conditions attempting to maintain a constant body temperature by increasing evaporative heat loss from the respiratory-tract (Silanikove, 2000). Berman et al. (1985) found that RR in the lactating dairy cow started to rise when ambient temperatures surpassed 25°C. They suggested that respiratory evaporative heat loss is extremely important for maintenance of thermal stability in large cattle due to their large body size. In our study, under a combination of high RH and low AV, RR had increased slightly (IPt) just at 19°C. At lower RH levels the determined IPt was higher. The difference in IPt between low (30%) and high RH (60%) was approx. 5ºC for short (1 h) exposure and 6.5ºC for long (8 h) exposure, which was consistent with the results from other studies (Pinto et al., 2020). The IPt increased with increasing AV while the first step increase of IPt from low AV (0.1 m/s) to medium AV (1.0 m/s) seemed to be smaller than the second step increase from medium AV to high AV (1.5 m/s). According to Spiers et al. (2018), the benefit of fan cooling is highly dependent on T and because of the small difference between T and ST, the medium AV did not contribute much to dissipating the heat through convection. Additionally, the medium AV might not have been high enough to overcome the thermal resistance of the hair coat which resulted in a small difference in RR between two AV levels. Besides, if sweat can be fully evaporated without being limited by the ambient potential evaporation rate of the air at RH of 45% (Gash and Shuttleworth, 2007), the AV will not provide much help for the cutaneous evaporative heat loss. To assess heat stress magnitude, Gaughan et al. (2008) developed a heat load index based on panting responses incorporating air T, RH, black-globe temperature and AV; when air T and solar radiance were fixed, RH increased from 20% to 80%, and no chilling effects of AV increment (1 to 2 m/s) were found (Wang et al., 2018). In future research, a combination of higher RH levels (higher than 60%) with higher AV levels could be helpful for ascertaining the significant effect of AV, because at high RH, increasing AV could help to raise the potential evaporation rate and further increase cutaneous latent heat dissipation. Gebremedhin et al. (2008) reported that increased RH negatively affected the cutaneous latent heat loss in cows, which implies that RH conditions need to be monitored when implementing evaporative cooling using nebulizers (Berman, 2009). With a drop of RH levels from high (60%)

to low (30%), the IPt could be raised by approx. 5°C for short exposure and 6.5°C for long exposure, respectively. In Figure 2-5, the evaporative cooling process was simulated based on thermodynamics and psychrometrics (the temperature of the air could be reduced by transferring heat from air to evaporating water), the chamber's air temperature started at 30°C, and moisture was added at different RH levels (ASHRAE, 2009, Silva and Maia, 2011). The simulation showed that RH rose from 30% to 60% when moisture was added, implying that the chamber's air T could be lowered 6.8°C. From this simulation, in any environment where air T is high and there is no AV, applying evaporative spraying could cause an increase in RH, which might prevent the evaporation of sweat from the skin because of the damp air's insufficient evaporation potential. This implies that an evaporative cooling of the air in combination with a higher AV might not reduce the evaporation potential of the air, but further research is needed to confirm this hypothesis.

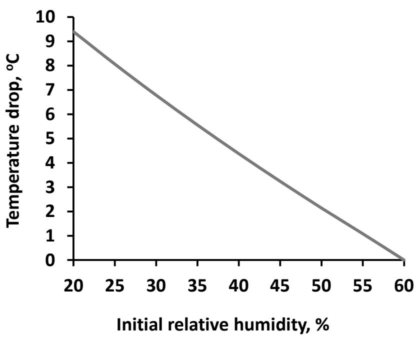

Figure 2-5. Relationships between the drop of the temperature of ambient air (having an initial temperature of 30°C) and the initial relative humidity using evaporative cooling to reach 60% relative humidity (ASHRAE, 2009).

Limited research has been reported on the effect of exposure time on physiological responses. Our study was the first one to investigate the exposure time effect and we found that IPt for RR was decreased under long exposure time. In our study the ambient conditions were controlled such that we could clearly see the effect of exposure time on RR. Pinto et al. (2020), however, performing a study in a conventional barn during the summer period, found it difficult to say whether the increased RR (2.9 breath/min increase after the critical threshold was exceeded) was due to the increase of heat load magnitude or whether it was because of increasing exposure duration.

Temperature, °C	Relative Humidity, %						
	30	35	40	45	50	55	60
16	60	60	60	60	60	60	60
17	61	61	61	61	62	62	62
18	62	62	63	63	63	63	63
19	63	64	64	64	64	64	65
20	64	65	65	65	65	66	66
21	66	66	66	66	67	67	67
22	67	67	67	68	68	68	69
23	68	68	69	69	69	70	70
24	69	69	70	70	71	71	72
25	70	70	71	71	72	72	73
26	71	72	72	73	73	74	74
27	72	73	73	74	75	75	76
28	73	74	75	75	76	77	77
29	74	75	76	77	77	78	79
30	76	76	77	78	79	79	80
31	77	77	78	79	80	81	81
32	78	79	79	80	81	82	83

Figure 2-6. Temperature Humidity Index calculated according to NRC (1971). The colors indicate the severity levels of the heat stress (Zimbelman et al., 2009): green (≤ 67) is no heat stress; yellow (68 - 71) is mild heat stress; orange (72 - 79) is moderate heat stress; red (≥ 80) is severe heat stress. The THI values in three white blocks were temperature and relative humidity conditions in our experiment.

When the cow fails to dissipate heat, the RT will rise. Brown-Brandl et al. (2003) continuously recorded RR and detected a delayed increase of RT compared to RR. The relationship between RT and T has been studied by many researchers. A recent study by Li et al. (2020) stated that the RT started to rise at T of 20.4°C without mentioning the conditions of RH or AV, the IPt of which was only comparable to that under treatment RH of 60% with low AV in this study. Pinto et al. (2020) and Yan et al. (2021b) reported that the critical THI threshold for RT was 70, which was in line with our results from treatment I (RH_l*AV_l and 25°C) (Figure 2-6). In our study, we found that the average RT from short exposure was lower than that from long exposure. According to McGovern and Bruce (2000b), the heat increment that was not dissipated by all cooling methods would be stored in the core body, causing the body temperature to rise. In agreement, we could present that the effects of the exposure time on RT is an important factor when assessing the heat tolerance of cows under high ambient T, especially under high RH without the intervention of AV. This observation indicated that during warm days with high RH, evaporative cooling could not be effective. However, when conducting the experiment in commercial farms, Mullick (1960) found that a high RH had a tendency to lower the RT, which could be due to the fluctuation of air T and RH. When the ambient RH was raised from 30 to 60% by evaporative cooling, the T could be decreased from 30 to 23.2°C (Figure 2-5) and there was no positive effect for the RT. The most commonly practiced heat stress intervention on conventional farms, at least in the Netherlands, is to provide high air speed around cows using either head-level or ceiling-level fans. The AV in the practice is comparable with the AV_m in this study and RH levels in the barns are generally around 45 to 60% (RH_m in our design) (André et al., 2011). Presumably, given the mentioned RH, the AV_m in the practice could be effective enough for the cows to maintain the RT at normal range. In addition, the difference in individual cows' characteristics, such as body condition (fatness) or genetics, can influence their responses to heat stress (Gaughan et al., 2000, Berman, 2005). However, those cow factors were not included in our analysis. In addition, the way we installed fans in the CRC (Figure 2-1) was different from how it is usually done on farms. We measured AV at five points and found the highest AV around the rump and the lowest AV around the lateral sides, which might explain the limited effects of higher AV on IPt for RT. Besides, one cow was excluded from treatment IV because of mastitis, which led to lower estimation accuracy for the IPt for RT. The importance of exposure time when identifying heat stress responses has been recognized by other researchers (Kaufman et al., 2018, Peng et al., 2019). One example that should be considered when planning cooling schemes is that once T exceeds the IPt, the shorter exposure time is the more stable RT that the cow can maintain.

The cows' ST increases with an increase in the ambient T if there is no cooling facility. We observed that when providing high AV, the cows' ST was much lower than under low AV, especially when the ambient T was low. When there was less need for heat transfer (under low ambient T) there was no extra blood vessel dilation (McGovern and Bruce, 2000b, Silanikove, 2000). According to Collier et al. (2006), who did not provide RH or AV information, as ST rose above 35°C, the cows gradually began to store heat as indicated by increased RT. We could agree with the authors as the linear lines in Figure 2-4 b1 and c1 presented asymptotic patterns except at treatment RH_m*AV_m, which could be due to individual variation in different cows. It was difficult to compare the results with previous studies without sufficient information on the RH and AV conditions. As said, except for treatment RH_m*AV_m, under the intervention of AV (Figure 2-4 b2 and c2) the behavior of the linear lines for ST and RT were different. The starting ST in treatment II was already approximately 3°C higher than the other two treatments (Figure 2-4 c2), consequently the basal RT in treatment II was obviously higher than the other two treatments (Figure 2-4 b2). This confirmed that the different responses in individual cows were significant. The starting ST in the high RH treatment (Figure 2-4 c1) was already higher compared to other groups, and whether this high starting ST led to the lowest IPt for RR and RT (Figure 2-4 a1 and b1) was an interesting finding. In theory, convective heat transfer occurs between T gradients; in this case, as ST was already high, the capacity to transport surface heat to the ambient air was limited. Therefore, it led to early increases in both RR and RT.

Although the Holstein Friesian cows in this study were not at the peak of their production level (were entering late lactation), results still showed that the cows responded physiologically to temperatures even a bit lower than 20°C. The first response to increased T was an increase in RR, whereas RT did not increase until T was above 20°C. Yan et al. (2021b) reported that THI threshold at which RT and RR began to increase in China was lower for early-lactation cows compared to late-lactation cows. This meant that for high-producing cows near peak production, the IPt for RR and RT could be even lower. In addition, this study only mimicked a gradual rise in temperature over the 8-d period rather than a heat wave exposure over several days – which is common in the sub-tropics and tropics (Pinto et al., 2019). The adverse effect of heat can be underestimated if animals do not have a recovery period of lower temperatures during the night (Gaughan et al., 2008).

2.4.2 Productive Responses to Treatments

One of the primary measurements of productivity in farm animals in general and in particular in dairy cows is DMI (Spiers et al., 2018). According to Mount (1979), DMI decreases with high ambient temperature in order to decrease the animal's heat production, which compensates for the lowered heat dissipation. In this study, the cows maintained their DMI when they were under low RH or in the case of high RH combined with high AV. We also reported that effects of medium and high RH without fan on DMI were equally significant, which was comparable with other studies (Hill and Wall, 2017, Herbut et al., 2021). West et al. (2003) found a decrease in daily DMI of 0.51 kg for each unit increase in THI between 73 and 82, whereas Hill and Wall (2017) reported that the decrease rate of DMI was 0.03 kg for every increased unit of THI. Our results showed that under medium RH, increasing AV had a positive effect on DMI.

Water is one of the most important nutrients for the dairy cow (West, 2003). In our study, despite some water spilling at the beginning when the cows were adapting to the CRC, there was a very obvious increase in water intake with increasing T. The cow is bred to maintain a milk yield consisting of approx. 87% water, and under heat stress there is evaporative heat dissipation through respiration and sweating. Water intake can/should be increased to compensate for this. In our study, treatment group IV (45% RH and 1.0 m/s AV) had the greatest increase in water intake, whereas treatment I (30% RH and 0.1 m/s AV) had the lowest. The reason for this could be: 1) cows in treatment I randomly had the lowest milk yield or 2) there was not much evaporative heat dissipation. According to West (2003), water intake increased by 1.2 kg per °C increase in ambient temperature, which is within the range of increase we observed in our study.

There were no obvious changes in milk yield with increasing T across the five treatments. This is interesting because Zimbelman et al. (2009), Gauly et al. (2013) and Hill and Wall (2015) all reported that milk yield began to decline at an average THI of 68 and the decreasing rate could be 2.2 kg/day. A THI of 68 is similar to a combination of 45% RH at 22°C or 30% RH at 24°C in this study (Figure 2-6). However, Linvill and Pardue (1992) found that milk yield only started to decrease after 4 d with a THI above 74. Given the complex design with a diurnal pattern of T/RH/AV in this study, several explanations could be given for this contradiction: 1) the cows were able to recover overnight in the cooler temperatures: according to Igono and Johnson (1990), milk yield only declined when the cow's rectal temperature exceeded 39°C for more than 16 h which was never reached in our study because of the lower T during the night; 2) the total daily yield of nutritional components of the milk declined but we could not directly see this aspect from milk yield only; 3) the reduced milk yield would appear later as a delayed response to heat stress (Linvill and Pardue, 1992, Polsky and von Keyserlingk, 2017), which we were unable to observe due to the current research set up and time span.

The statistical analysis of DMI and milk yield showed that at low RH (30%) the cows were able to sustain DMI and milk, protein and fat yield across the range of T used in the trial (16 to 30°C). AV did not show a positive effect on milk components. As mentioned before, once the gradient between ST and T became asymptotic, the function of high AV was weaker in terms of dissipating heat when sweat evaporation is not limited, despite many studies reporting a positive effect of fan cooling on milk production (Calegari et al., 2014, Sunagawa et al., 2015, Wu et al., 2016). This finding is again of great interest for the industry, given most available cooling systems focus on increasing AV with or without nebulizing water (Avendaño-Reyes et al., 2010, Fournel et al., 2017b, Spiers et al., 2018). However, in our study we did not investigate whether higher AV might have an effect combined with high RH level. In any case, it is important for dairy farmers to consider the RH inside the barn when designing evaporative cooling systems.

This study was simulated in CRC to avoid confounding effects, which allowed us to study the relationship between physiological responses and ambient temperature and duration of high temperatures. Although the cows were housed in an unnatural situation, they could still respond behaviorally, for example by decreasing their lying time or increasing their water intake. However, cows in this study were not free to walk around or play with others like on a real farm, which would have certain effects on the physiological responses to heat load. It is important to remember that this study offers information about cows housed indoors, whereas cows at pasture also need deal with radiation from the sun. When there is no shade, the IPt could be significantly lower. Inside a barn with a group of cows, cows have more behavioral options to react to changing indoor climates. In rising temperatures, however, cows will tend to move away from the heat radiating from other cows, which would render the interaction effects between cows rather low in heat stress situations. However, we recommend validating these results found under semi-lab conditions in practical circumstances.

2.5 Conclusion

Above IPt, significant changes can be observed in dairy cows' thermal physiological responses. Increased RR was the first indicator showing that the cow was reacting to high ambient temperature. The IPt for RR increased with decreasing RH and with increasing AV. The decrease in IPt for RR from low to high RH almost compensated for the decrease in T with an evaporative cooling of the ambient air. Rectal temperature increased above an ambient temperature ranging from 20.1 to 25.9°C. The increase in RT was an indicator that ambient temperature was above the upper limit of the thermal neutral zone. The IPt for RT was lowest when the highest RH level was combined with the lowest AV level. Generally, the effects of

AV on RR and RT are relatively small at medium RH levels. This might be different at higher RH levels, when the evaporation potential of the ambient air is limited. Higher AV levels lowered ST, but this difference became smaller with increasing T. The effects of exposure time (1 or 8 h) on RR, RT and ST of increased T were significant. This means that cows respond with physiological changes at lower ambient T if forced to remain in hot conditions for a long time.

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3

CHAPTER 3.

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°C 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 only combined with RH_m. Totally there were five treatments with four replicates (cows) for each. Effects of short and long exposure time to warm condition were evaluated by collecting data two 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 prior to 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 while 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, while 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.

Keywords: dairy cow, ambient temperature, heat loss, heat stress

3.1 Introduction

Dairy cows are homoeothermic animals and heat balance mechanism is important to sustain the body temperature. There are two modes of heat transfer between the animal and its environment, the sensible (non-evaporative) and the latent (or evaporative) heat loss. At certain ambient temperature (T), heat is mainly lost via the sensible way due to the difference between the skin surface temperature and the environmental temperature (objects and air). With increasing ambient T there is a marked shift from sensible to latent heat loss (Maia and Loureiro, 2005). In warmer conditions, increased respiration and sweating rate are two of the primary autonomic responses exhibited by animals (Gebremedhin et al., 2008). Dairy cows possess a very effective sweating capacity (Mount, 1979), in advanced bred for high productivity this sweating process is crucial to help the cows maintain heat balance. Maia et al. (2005b) 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 latent heat loss (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 latent heat lost (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 sensible heat loss (SHL) and LHL under different ambient conditions will help efficiently apply cooling systems. It is known that the vaporization rate of water could be limited by relative humidity (Berman, 2009), therefore, information about the effects of increasing ambient T at different relative humidity (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 temperature, relative humidity, and air velocity) and exposure time on latent and sensible heat loss from the skin surface and through respiration of Holstein-Friesian dairy cows. Our hypothesis is that the level and proportion of sensible and latent heat loss will change with increasing ambient T and this change is influenced by RH, AV and exposure time (short or long).

3.2 Materials and Methods

3.2.1 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, 687 ± 46 kg BW, and parity of 2.0 ± 0.7. Nineteen cows were at an average of 105 ± 38 d in pregnancy. Cows were grouped in four blocks of five cows based on parity and expected milk yield. Each cow within a block was randomly assigned to one of the five treatments as shown in Table 3-1 and 3-2. 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%.

Prior to the start of the experiment, a 7-d acclimatization for the cows was done in a facility approximately two kilometers distanced from the climate-controlled respiration chamber (CRC). During the acclimatization period, the cows were housed in individual tie-stalls, 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 besides receiving feeding and milking visits, the cows were visited two times 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.

Table 3-1. Five treatments each lasted for the 8-d period using Climate-controlled Respiration Chambers (CRC).

Treatment[1]	Temperature, °C		Relative humidity, %		Air velocity
	2200 - 0700 h[2]	1000 - 1900 h	2200 - 0700 h	1000 - 1900 h	0900 - 2100 h
RH_l*AV_l	7 - 21[3]	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]There were five treatments, representing different temperature, relative humidity and air velocity combinations.
[2]2200 - 0700 h stands night time duration from 2200 h until next day 0700 h; 1000 - 1900 h stands daytime duration from 1000 h until 1900 h at the same day; 0900 - 2100 h stands air velocity treatment duration from 0900 h until 2100 h at the same day, for first CRC. There was one hour delay for all the controlling parameters for second CRC.
[3]7 - 21 means the air temperature at Day 1 as 7°C and Day 8 as 21°C during night time; 16 - 30 (or 18 - 32) means the air temperature in Day 1 as 16°C (or 18) and Day 8 as 30°C (or 32) during daytime.

Table 3-2. Body weight, annual average milk yield, parity, days in milk and pregnant days of cows in five treatment groups (means ± SD).

	Treatments				
Item	RH_l*AV_l	RH_m*AV_l	RH_h*AV_l	RH_m*AV_m	RH_m*AV_h
Body weight, kg	695±54	671±52	667±41	721±50	680±29
Milk yield, kg/day	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
Days in milk, day	212±35	192±40	182±54	227±31	215±35
Pregnant days, day	100±27	116±20[1]	85±60	104±30	120±48

[1]In treatment II there was one non-pregnant cow.

3.2.2 The Climate-controlled Respiration Chamber

In this study, two identical climate-controlled respiration chambers were used. Each chamber was split into two individual airtight compartments with thin walls equipped with transparent windows to allow audio and visual contact between two 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 relative humidity sensor (Novasina Hygrodat100, Novasina AG), and the ambient temperature (T) was monitored by five PT100 temperature sensors (Sensor Data BV) evenly distributed over the room at animal height as described in detail in Zhou et al. (2022a). 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 temperatures, 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 3-1) so that the air flow moved through the axial body length of the cow from back to front. The chambers were artificially lit for 16 h daylight (390 - 440 lux, 0500 to 2100 h) and 8 h nightlight (35 - 40 lux, 2100 to 0500 h).

3.2.3 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 climate control and programed for five different treatment groups (Table 3-1, Figure 3-2).

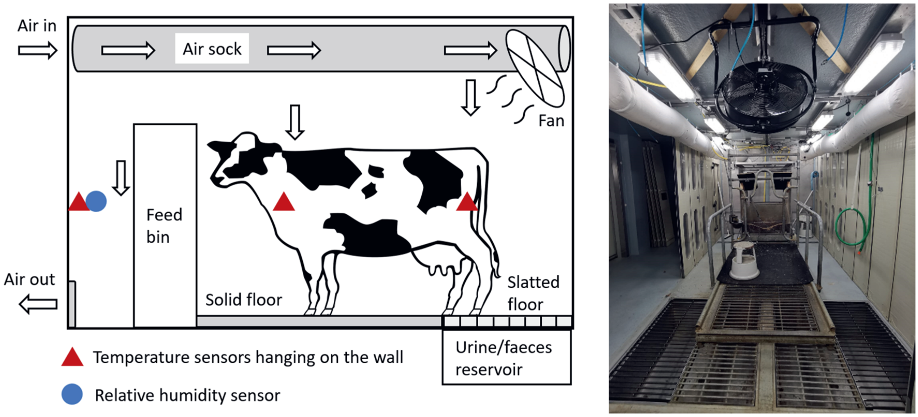

Figure 3-1. Schematic view and overview photo of the climate-controlled respiration chamber. There are two temperature sensors hanging on each side wall (left and right), and one temperature sensor and one relative humidity sensor hanging on the wall in front of the cow. The material of solid floor is 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 she could easily move forward/backward and lie down.

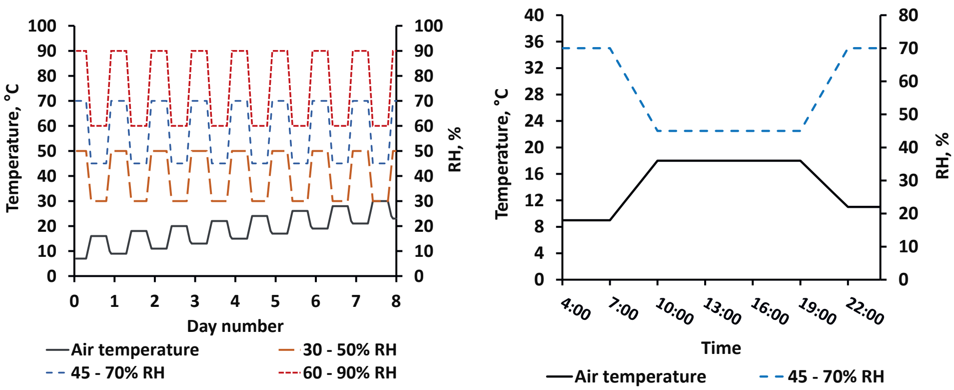

Figure 3-2. (a) Schematic temperature and relative humidity (RH) patterns during the 8 experimental days. Between 0700 to 1000 h temperature and RH changed gradually into daytime levels and stayed constant until 1900 h. Between 1900 to 2200 h, temperature and RH gradually decreased into nighttime levels and stayed constant again until next day 0700 h; (b) An example of temperature and RH patterns of Day 2 with 45 - 70% relative humidity.

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 temperatures) as shown in Figure 3-2. The experimental treatments comprised three RH levels and three AV levels as described in Table 3-1. At nighttime, AV was kept at natural speed (AV_l 0.1 m/s). In daytime, three AV levels were applied either with AV_l (low): 0.1 m/s; or AV_m (medium): 1.0 m/s; or AV_h (high): 1.5 m/s. 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 medium level. In addition, in compliance with saving number of experimental animals (2021 ©OIE - Terrestrial Animal Health Code), the AV_m and AV_h were only combined with RH_m. More detailed description can be found in the previous study (Zhou et al., 2022a). Because of the capacity of the CRC, the ambient T and RH required a time span of three hours 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).

3.2.4 Data Collection

Practically, there was one set of apparatus and researchers for two CRC in each data collection, therefore, to achieve the same conditions in the two CRC in the same day, the ambient T, RH, AV, lighting, feeding, milking was programed 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.

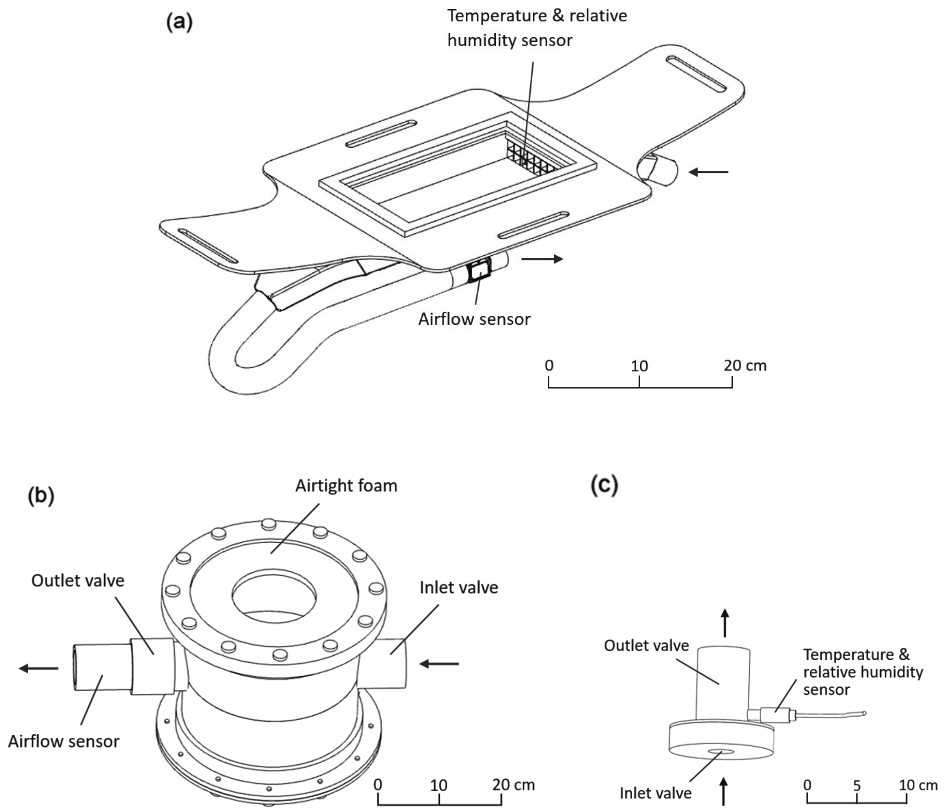

Figure 3-3. Apparatus for measuring heat loss from the skin surface and through respiration. (a): ventilated skin box, with two temperature and relative humidity sensors at each side, and one airflow sensor at the outlet tube; (b): face mask-there is an inlet and outlet valve at two sides respectively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

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 five locations: neck, middle backbone and rump, and both lateral sides was measured.

Heat loss data. Heat loss from skin surface was measured using a ventilated skin box (Figure 3-3a) 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 two temperature 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; 3) the box which was fitted on the skin surface of the cow using two long belts which 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 was 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 temperature and RH, and of the airflow rate.

Heat loss through respiration was measured using a face mask and a nose cup (Figure 3-3b and 3-3c). The face mask consisted of an inlet valve and an outlet valve. At the outlet, three airflow sensors (Mass Flow Meter SFM 3000, Sensirion) were mounted next to the valve, and these sensors measured the airflow rate from respiration. Data was 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 two nostrils. The nose cup consisted of two main components: an insulated, valved cylinder and a temperature & RH sensor (Testo 06369735, Testo). The two valves fitted in the insulated cylinder, which closed during inhalation, allowed only exhaled air to enter; the exhaled air temperature and RH were then measured. The measuring time was on average 5 ± 2 minutes and depended on the speed the exhaled air temperature 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 and outgoing air. The latent heat loss from skin surface was estimated by:

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

where, LHL_s is the latent heat loss from skin surface (W/m^2); Q_e is the evaporative heat contained in the incoming/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 per kg dry air).

λ is dependent on the air temperature:

$$\lambda = -0.0001 \cdot T^2 - 2.3607 \cdot T + 2503$$

where, T is the dry-bulb temperature (°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/outlet and according to equations given by the ASHRAE (2009).

The sensible heat loss from the skin was estimated by:

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

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

Q_s was determined as follows:

$$Q_s = \mathrm{h} \cdot V \cdot \rho - Q_e$$

where h is the enthalpy of the air mixed with vapor (J/g).

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

The latent heat loss through respiration was estimated by:

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

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

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

3

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

where, W is the body weight of the cow (kg).

The sensible heat loss through respiration was estimated by:

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

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:

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

3.2.5 Statistical Analysis

All statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC). 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 five different combinations of RH and AV under different exposure times. Repeated measures was 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 AIC 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 i-th treatment respectively; cow_{ij} is the random effect of the j-th cow in the i-th 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 two 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 ≤ 0.05 unless otherwise indicated.

3.3 Results

3.3.1 Climate-controlled respiration chambers conditions

The microclimate conditions inside the CRC were reported in a previous study (Zhou et al., 2022a). Briefly, the daily cyclical temperatures were kept strictly constant according to set points with a deviation smaller than ± 0.50°C. The RH_l and RH_m failed to reach the set points at the beginning but got closer later, as shown in Figure 3-4. The AV around the cow body surface was calculated by taking the average of five measurement points resulting in achievable AV at three set: 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.

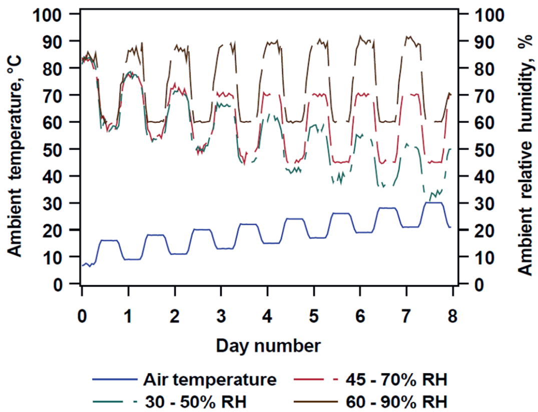

Figure 3-4. Average measured hourly temperature and relative humidity (RH) during 8-d experimental period.

3.3.2 Heat loss from skin surface

The responses of the sensible and latent heat loss from skin under different treatments and exposure times are given in Figure 3-5. Average sensible heat loss (SHL) from skin surface, combining all values at ambient T within 18 to 30°C, was similar at different RH levels ($P > 0.05$), while 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 3-5a and 3-5b). Average latent heat loss (LHL) from the skin surface was lower at high RH level than at low/medium RH levels (Figure 3-5c). 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 3-6). 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 3-7). 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 ($P < 0.05$).

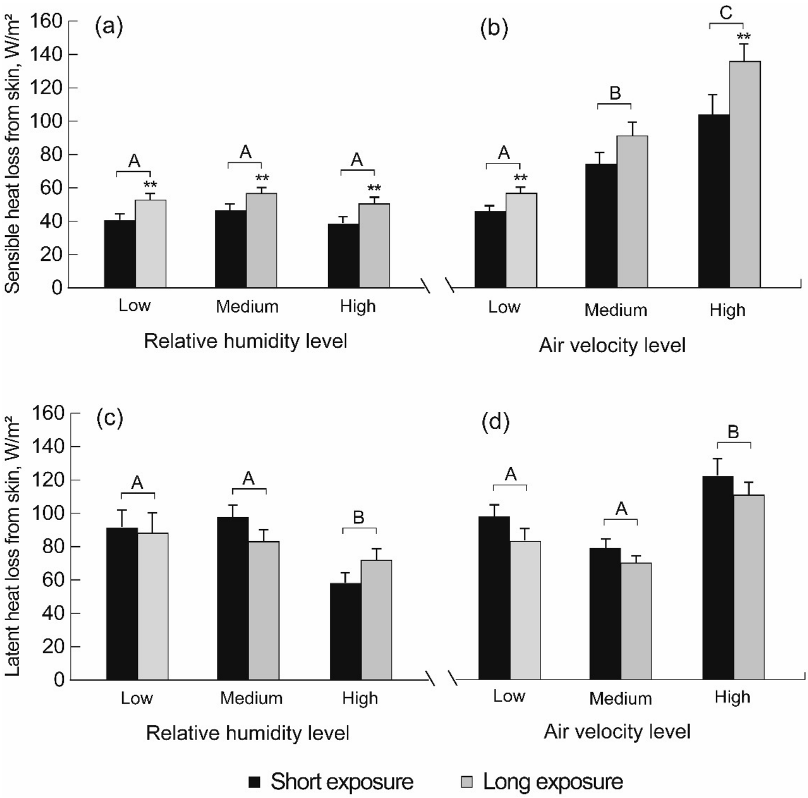

Figure 3-5. Mean sensible heat loss from skin (figure a and b, W/m^2) and mean latent heat loss from skin (figure c and d, W/m^2) within the ambient temperature range of 18 to 30°C at short and long exposure times for different relative humidity (RH) levels (low, medium and high) and air velocity (AV) levels (low, medium and high). The different letters in the same figure indicate a significant difference (Tukey-Kramer, $P < 0.05$) between treatments. Stars indicate a significant difference between two exposure times (*$P < 0.10$, **$P < 0.05$, ***$P < 0.01$). Error bars represent SEM. Note: RH effects were studied at low AV and AV effects were studied at medium RH; the medium RH level in figure a and c are the same treatment as the low AV level in figure b and d.

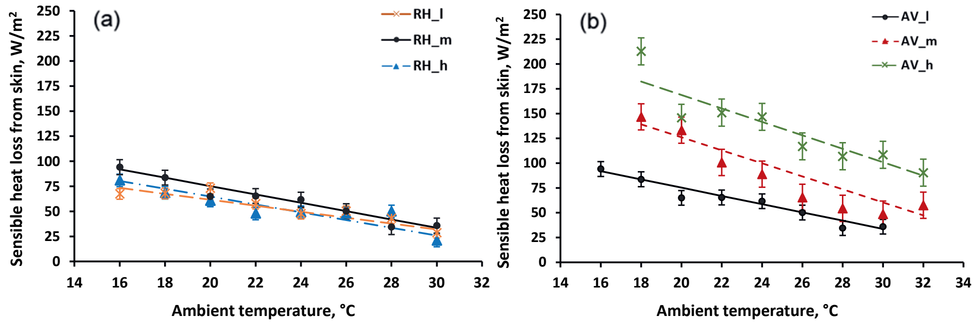

Figure 3-6. Sensible heat loss from the skin surface under long exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with three relative humidity levels (RH_l, RH_m and RH_h: 30, 45 and 60%), and (b) at the same relative humidity level (45%) with three air velocity levels (AV_l, AV_m and AV_h: 0.1, 1.0 and 1.5 m/s).

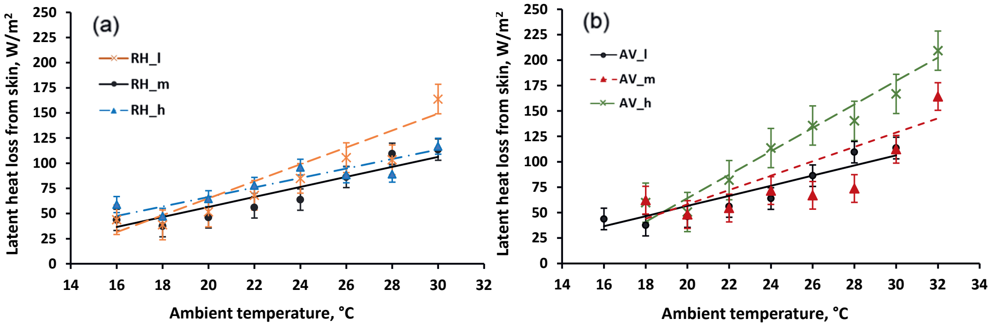

Figure 3-7. Latent heat loss from the skin surface under long exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with three relative humidity levels (RH_l, RH_m and RH_h: 30, 45 and 60%), and (b) at the same relative humidity level (45%) with three air velocity levels (AV_l, AV_m and AV_h: 0.1, 1.0 and 1.5 m/s).

3.3.3 Heat loss through respiration

The cows lost more heat through respiration (SHL and LHL) under long exposure than short exposure for all treatments (Figure 3-8; $P < 0.10$). 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 five treatments.

With the increase of ambient T, respiration SHL linearly decreased and respiration LHL linearly increased (Figure 3-9). 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, while 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 temperature (Figure 3-10a; $P < 0.05$). When ambient T increased from 16 to 32°C, the exhaled air temperature 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 3-10a). The exhaled air temperature had the same increasing rate for short and long exposure times of 0.097°C per 1°C increase in ambient T. Exposure time impacted on 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 3-10b. 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.

3.3.4 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 3-11). Total heat loss from the skin showed a dominant share (70 to 80%) of the whole heat loss while 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, while SHL through respiration only showed a minor contribution of 1.7 to 6.5% of the whole heat loss. As ambient T rose above 20°C, skin SHL subsided and skin LHL took charge.

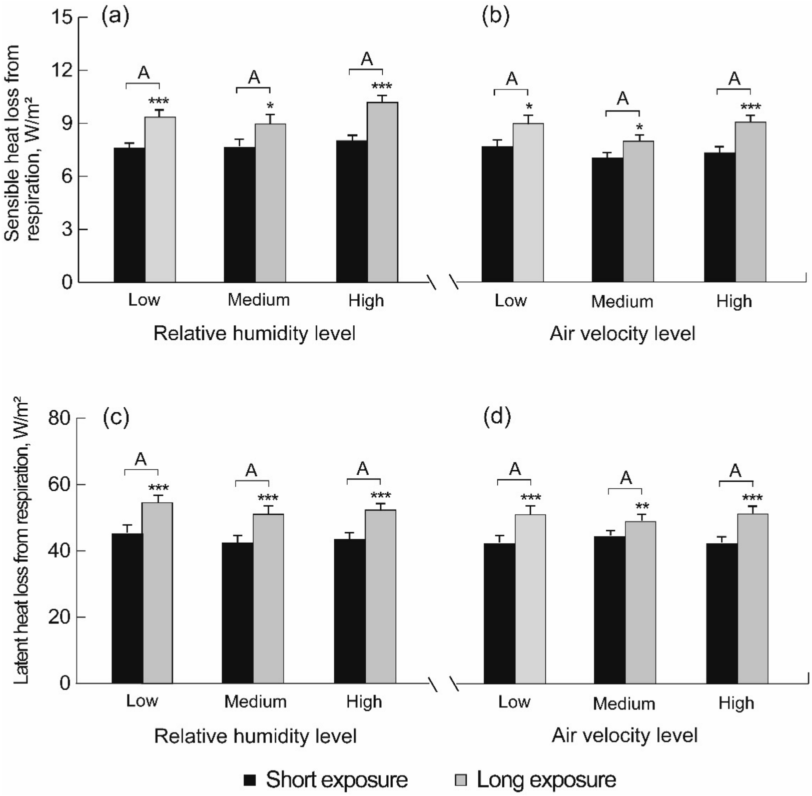

Figure 3-8. Mean sensible heat loss from respiration (figure a and b, W/m^2) and mean latent heat loss from respiration (figure c and d, W/m^2) within the ambient temperature range of 18 to 30°C at short and long exposure times with different relative humidity (RH) levels (low, medium and high) and air 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. Stars indicate a significant difference between two exposure times (*$P < 0.10$, **$P < 0.05$, ***$P < 0.01$). Error bars represent SEM. Note: RH effects were studied at low AV and AV effects were studied at medium RH; the medium RH level in figure a and c are the same treatment as the low AV level in figure b and d.

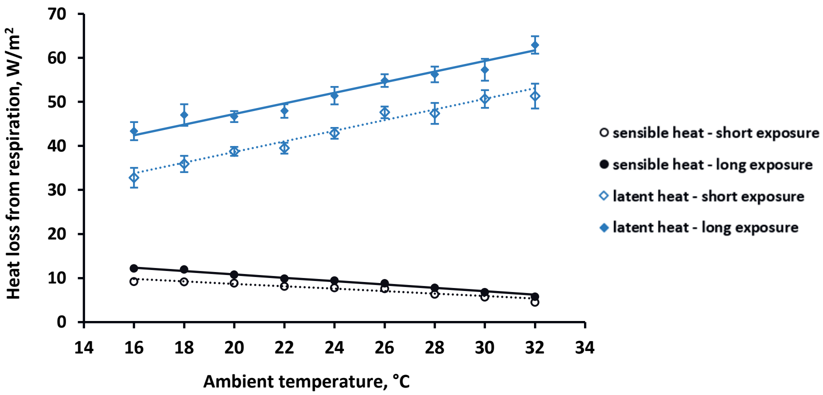

Figure 3-9. Sensible (SHL_r; short exposure: $SHL_r = 14.22 - 0.28 \cdot T_a$; long exposure: $SHL_r = 18.46 - 0.38 \cdot T_a$) and latent ($SHL_r$; short exposure: $LHL_r = 14.46 + 1.21 \cdot T_a$; long exposure: $LHL_r = 23.08 + 1.21 \cdot T_a$) heat loss from respiration in relation to ambient temperature at two exposure times. Only lines are shown that showed a significant difference.

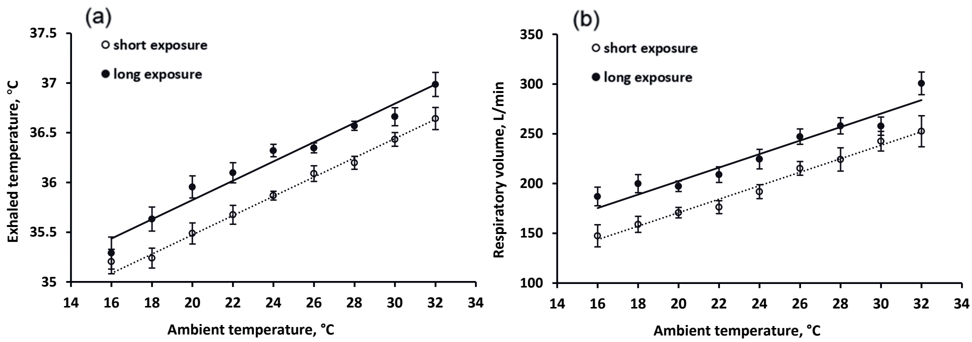

Figure 3-10. (a) Exhaled air temperature (ExT, °C); short exposure: $ExT = 33.54 + 0.097 \cdot T_a$; long exposure: $ExT = 33.89 + 0.097 \cdot T_a$) and (b) Respiratory volume (ResV, L/min; short exposure: $ResV = 34.94 + 6.79 \cdot T_a$; long exposure: $ResV = 66.74 + 6.79 \cdot T_a$) in relation to ambient temperature at two exposure times.

R^2=0.61

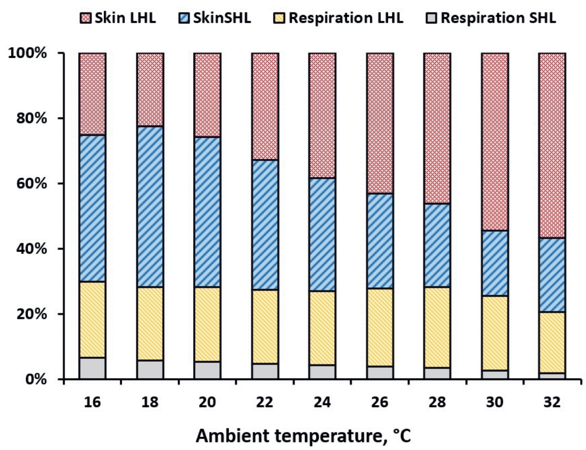

Figure 3-11. Relative heat loss by skin latent, skin sensible, respiration latent and respiration sensible of dairy cows at different ambient temperatures. LHL means latent heat loss and SHL means sensible heat loss.

3.4 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.

3.4.1 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 temperature 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 is in agreement 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 approx. double the value at low AV (0.1 m/s) in our study. Hence, factors that could influence the temperature 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) as compared to 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/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 temperature and hence in skin SHL. Zhou et al. (2022a) found skin temperature (averaged from four different skin parts) was significantly higher at 60% RH than that at 30 and 45% RH given the same ambient T, causing a larger temperature difference between skin surface and air at 60% RH, and hence giving a larger skin SHL. Possible reason could be that skin temperature in previous study was an average skin temperature measured on four different parts while 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 temperature at long exposure time (Zhou et al., 2022a).

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 - 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, humid, and solar load at 1 m/s AV on cows' sweating rate 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 is in agreement 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. Besides, cows reduced their metabolic heat production due to decreased milk yield at high ambient T (Zhou et al., 2022a) thus requiring less skin LHL under high RH condition compared to 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 temperature 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 3-7b) despite the cows having similar skin temperatures (Zhou et al., 2022a). 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 temperature on the sweating rate; in other words, cows at low AV had a high skin temperature, and thus a high sweating rate, while cows at medium AV had a low skin temperature 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 air velocity would not help to dissipate heat if the RH and ambient T are both too high – as seen in sub-tropical 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. In order 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. Besides, 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 five 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. (2009a) 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 5 h period. In our study we measured skin LHL for 10 minutes, whereby the results from the latter 5 minutes 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.

3.4.2 Heat loss through respiration

Respiration SHL accounted for a fairly small percentage of the total heat loss via respiration and it, in 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. (2005b) (5.5 to 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., 2022a). The values of respiration SHL and LHL differed from other studies (Maia et al., 2005b, 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 temperature and respiratory volume. Inhaled air temperature rapidly approaches the body temperature, 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 temperature and water content, while 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 temperature measured by the nose cup in our study was much higher than that from other studies (Donald, 1981, Maia et al., 2005b), especially under low ambient T conditions. The measurement approach here is very important, since in the other studies the exhaled air could easily be mixed with ambient air. Maia et al. (2005b) measured exhaled air temperature 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 temperature directly by placing a small thermometer in the nostril of a cow. In addition, the thermometer needs time to respond while the exhaled air could quickly spread out in the environment before the thermometer could catch the real temperature. Therefore, the nose cup we used in this study seems to be more reliable for measuring an accurate exhaled air temperature. This could explain why the exhaled air temperature was underestimated by previous studies, especially at low ambient T. To illustrate, exhaled air temperature from Donald (1981) was approx. 25°C at ambient T of 16°C, while the lowest exhaled air temperature measured in our study at 16°C was 34.3°C. The exhaled air temperature was higher under long exposure, probably as a result of a higher body temperature of cows exposed for a longer time to high ambient T conditions (Zhou et al., 2022a).

A face mask was used to measure the respiration rate and tidal volume in this study. Despite the fact that there were three 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. (2005b). This is the reason why we studied respiratory volume in L/min (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 impact 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 temperature 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 temperature gradient between ambient T and exhaled air temperature. 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. Besides 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.

3.5 Conclusion

The latent heat loss 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 approx. 70 - 80% of the total heat loss. Skin sensible heat loss decreased while skin latent heat loss increased with increasing ambient T. Both sensible and latent heat losses from skin were positively impacted by AV. Heat loss from respiration accounted for 20 - 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 h vs. 8 h). It is recommended to study the interaction effect between RH and AV on heat loss.

Acknowledgments

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Appendix

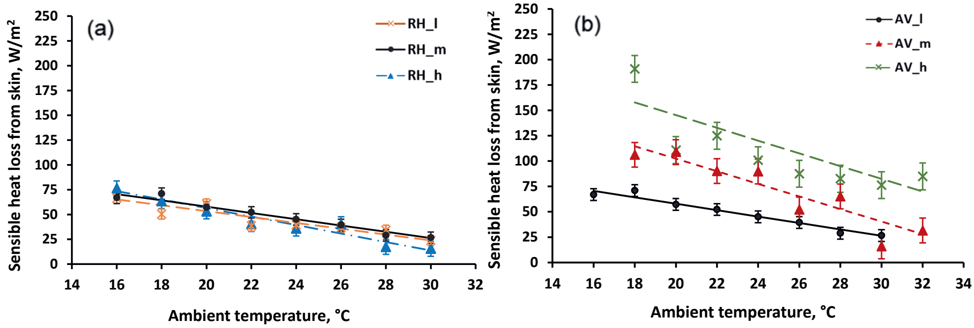

Figure 3-1. Sensible heat loss from the skin surface under short exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with three relative humidity levels (RH_l, RH_m and RH_h: 30, 45 and 60%), and (b) at the same relative humidity level (45%) with three air velocity levels (AV_l, AV_m and AV_h: 0.1, 1.0 and 1.5 m/s).

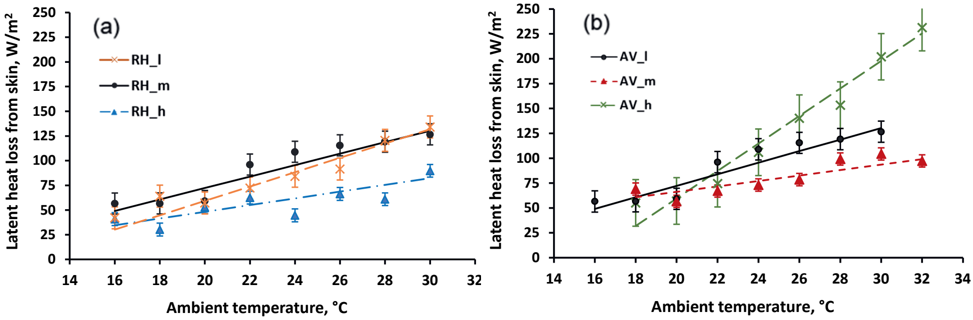

Figure 3-2. Latent heat loss from the skin surface under short exposure in relation to ambient temperature (a) at the same air velocity levels (0.1 m/s) with three relative humidity levels (RH_l, RH_m and RH_h: 30, 45 and 60%), and (b) at the same relative humidity level (45%) with three air velocity levels (AV_l, AV_m and AV_h: 0.1, 1.0 and 1.5 m/s).

Table 3-1. Coefficients from linear regression with increasing temperature (T) on heat loss from skin at different relative humidity (RH) and air velocity (AV).

Dependent variables	Exposure time[2]	Regression model components	Treatments[4]						
			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 (treatments)[5]
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)[1]			<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[3]	NS	NS	NS	NS		

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

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

[2]Exposure time with 'short' means the cows stayed in the condition for 1 h and with 'long' means the cows stayed in the condition for 8 h.

[3]NS, $P \geq 0.10$.

[4]Treatment levels: RH_l: 30%; RH_m: 45%; RH_h: 60%; AV_l: 0.1m/s; AV_m: 1.0m/s; AV_h: 1.5m/s.

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

3

Table 3-2. Coefficients from linear regression with increasing ambient temperature (T) on heat loss from respiration at different relative humidity (RH) and air velocity (AV).

Dependent variables	Exposure time[2]	Regression model components	Treatments[4]						
			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 (treatments)[5]
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[3]	<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)[1]			<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] *P*-value (exposure time) for statistical difference between two exposure times.

[2] Exposure time with 'short' means the cows stayed in the condition for 1 h and with 'long' means the cows stayed in the condition for 8 h.

[3] NS, $P \geq 0.10$.

[4] Treatment levels: RH_l: 30%; RH_m: 45%; RH_h: 60%; AV_l: 0.1m/s; AV_m: 1.0m/s; AV_h: 1.5m/s.

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

4

CHAPTER 4.

Evaporative water loss from dairy cows in climate-controlled respiration chambers

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Abstract

The effects of ambient temperature (T) on total evaporative water loss from dairy cows at different relative humidity (RH) and air velocity (AV) levels were studied. 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°C to 30°C within an 8-d period, both with an incremental change of 2°C per day. The effect of three RH levels with a diurnal pattern were studied, as well, with low values during the day (D) and high values during the night (N): low (D: 30%; N: 50%), medium (D: 45%; N: 70%), and high (D: 60%; N: 90%). The effects of AV were studied during daytime at 3 levels: no fan (0.1 m/s), fan at medium speed (1.0 m/s), and fan at high speed (1.5 m/s). The medium and high AV were only combined with medium RH. In total there were five treatments with four replicates for each. The animals had free access to feed and water. Based on the water balance principle inside the respiration chambers, the total evaporative water loss from dairy cows at daily level was quantified by measuring the mass of water in the incoming and outgoing air, condensed water, added water from humidifier, evaporative water from wet floor/drinking bowl/manure reservoir/water bucket. Water evaporation from a sample skin area was measured with a ventilated skin box and water evaporation through respiration with a face mask. The results showed that RH/AV levels had no significant effect on total evaporative water loss, while the interaction effect between RH/AV with ambient T was significant ($P = 0.0024$). Cows at high RH had a tendency for a lower increasing rate of evaporative water loss compared to cows at low RH (0.61 vs. 0.79 kg/d per 1°C increase of ambient T; $P = 0.065$). Cows at medium and high AV levels had a higher increasing rate than cows at low AV (0.91 and 0.95 vs. 0.71 kg/d per 1°C increase of ambient T; $P < 0.05$). The increase of evaporative heat loss from dairy cows was mainly due to the increase of evaporation (of sweat) from the skin. The skin water evaporation determined with the water balance method (minus evaporation from respiration) and the ventilated skin box method showed no significant difference (P = 0.387). The implication of this study is that cows at high ambient T mainly depend on evaporative cooling from the skin. The ventilated skin box method, measuring only a small part of the skin during a short period during the day, can be a convenient and accurate way to determine the total cutaneous evaporative water loss from cows.

Keywords: dairy cow, total evaporative water loss, cutaneous evaporative heat loss

4.1 Introduction

Evaporative heat loss is the main thermoregulatory mechanism of cows under high air temperature conditions because sensible heat loss is limited by the small temperature gradient between body surface and warm environment (Taneja, 1958, Gebremedhin and Wu, 2001). Thus, cow's ability to endure hot environments is dependent on the amount of heat it can dissipate via evaporation, either by sweat from the skin (Gebremedhin et al., 2008) or through the respiratory system (da Silva et al., 2012). The most reliable technique at present to determine the total evaporative water loss is to observe the body weight change within a certain duration in response to a thermoregulatory sweating/respiration stimulus (Finch et al., 1982, Holmes, 1985, Cheuvront and Kenefick, 2017, Castro et al., 2021), which requires costly high-precision weighing scales and careful work. Therefore, most available data in recent literature on moisture evaporated from skin in dairy cows are primarily from studies conducting measurements on a small area of the skin surface, using paper discs, ventilated capsules or hand-held electronic calorimeters (Berman, 1957, Hillman et al., 2001, Maia and Loureiro, 2005, Gebremedhin et al., 2008, de Souza et al., 2018).

However, local measurements of the sweating rate can have limitations, because of a small sample area, a short measurement time, and measurements taken on a shaved skin or under still air conditions. Berman (1957), Gebremedhin et al. (2010) found that there was a significant difference in sweating rate between black and white hair coats and Gebremedhin et al. (2010) found that cows sweat in a cyclic pattern. It is well known that the entire body surface of an animal is involved in the thermal exchanges with the environment (Turnpenny et al., 2000). In this case, estimating total cutaneous evaporative water loss using the local sweating rate might have a large inaccuracy, which would be even worse when multiplying the body surface area estimated from empirical equations (Berman, 2003). Although measuring the evaporative water loss from the whole body surface of dairy cows under different conditions is a challenge, it is of great interest and can be helpful for validating the former mentioned measurement methods and for a better estimation of the contribution of the latent heat loss from the skin to total heat loss, which is important information when applying cooling systems.

The objectives of this study were (1) to determine how dairy cows adjust their total evaporative water loss at different temperatures, relative humidity and air velocity, and (2) to investigate if the local cutaneous evaporative water loss measured by ventilated skin box was in agreement with the total cutaneous evaporative water loss derived by the water balance method. We hypothesized that total evaporative water loss at increasing ambient temperatures is affected by different relative humidity and air velocity levels, and cutaneous evaporative water loss measured with the ventilated skin box on a small area of the skin surface might reveal large discrepancies when compared with the total cutaneous evaporative water loss derived from the water balance method.

4.2 Materials and Methods

4.2.1 Experimental Design

The experiment was conducted in 2021, approved by the Institutional Animal Care and Use Committee of Wageningen University & Research (Wageningen, The Netherlands) and in accordance with Dutch law (Project No. 2019.D-0032). Twenty Holstein-Friesian dairy cows were used with an average milk yield (±SD) of 30.0 ± 4.7 kg/d, 206 ± 39 DIM, 687 ± 46 kg BW, and parity of 2.0 ± 0.7. Nineteen cows were pregnant at an average of 105 ± 38 d. Cows were grouped in four blocks of five cows based on parity and expected milk yield. Each cow within a block was randomly assigned to one of the five treatments.

Cows were subjected to a 3-d adaptation period and a subsequent 8-d experimental period. The ambient T, RH and AV conditions for 3-d adaptation period in the climate-controlled respiration chambers (CRC) were set and controlled the same as the first day of the corresponding experimental period. 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 temperatures) as shown in Figure 4-1. Three levels of RH during the day (d) and night (n) were: RH_l (low) 30% (d) and 50% (n); RH_m (medium): 45% (d) and 70% (n); and RH_h (high) 60% (d) and 90% (n). At nighttime, AV was kept at natural speed (AV_l 0.1 m/s). In daytime, three AV levels were applied either with AV_l (low): 0.1 m/s; or AV_m (medium): 1.0 m/s; or AV_h (high): 1.5 m/s. 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. More detailed description can be found in the previous study (Zhou et al., 2022a).

4.2.2 The Climate-controlled Respiration Chamber

In this study, two identical climate-controlled respiration chambers were used. Each chamber was split into two individual airtight compartments with thin walls equipped with transparent windows to allow audio and visual contact between two 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 relative humidity sensor (Novasina Hygrodat100, Novasina AG), and the ambient temperature (T) was monitored by five PT100 temperature sensors (Sensor Data BV) evenly distributed over the room at animal height as described in detail in Zhou et al. (2022a). 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 temperatures, the control mechanism of which can be found in the book of Gerrits and Labussière (2015). The settings for air velocity were 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 4-2) so that the air flow moved through the axial body length of the cow from back to front. The chambers were artificially lit for 16 h daylight (390 - 440 lux, 0500 to 2100 h) and 8 h nightlight (35 - 40 lux, 2100 to 0500 h).

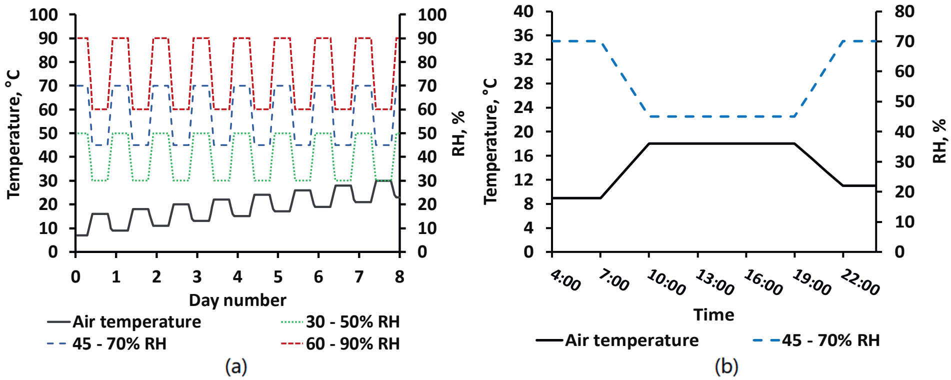

Figure 4-1. (a) Schematic temperature and relative humidity (RH) patterns during the 8 experimental days. Between 0700 to 1000 h temperature and RH changed gradually into day levels and stayed constant until 1900 h. Between 1900 to 2200 h, temperature and RH gradually decreased into night levels and stayed constant again until next day 0700 h; (b) An example of temperature and RH patterns of Day 2 with 45 - 70% relative humidity.

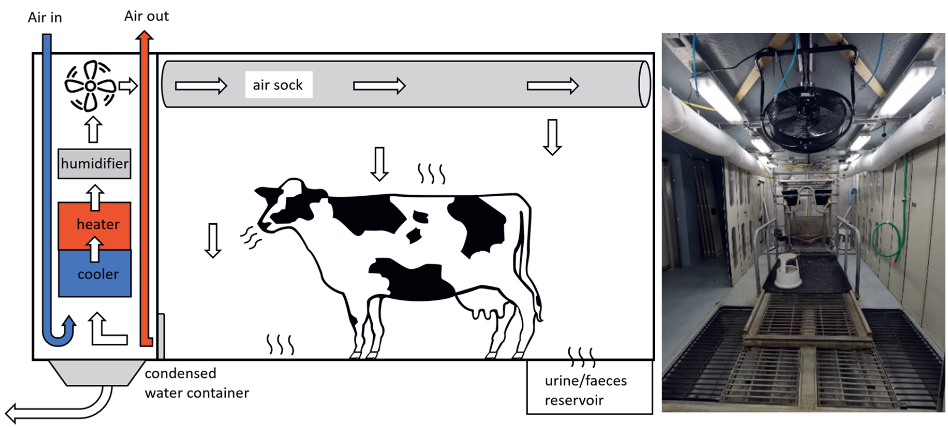

Figure 4-2. Schematic sideview and overview photo taken from the back of the climate-controlled respiration chamber (Gerrits and Labussière, 2015).

4.2.3 Data Collection

CRC condition. Throughout the 8-d experimental period, the T and RH of the chamber compartments were continuously recorded at 30-sec intervals automatically. Using a handheld anemometer (Testo 5-412-983, Testo SE & Co. KGaA), actual AV was manually measured two times a day at 5 locations with about 5cm distance around the cows' body surface for 30 sec each: neck, middle trunk and rump, and both lateral sides.

Metabolic heat production. Throughout the 8-d experimental period, CO_2 and CH_4 production and O_2 consumption were measured at 12-min intervals and automatically recorded, as described by Gerrits and Labussière (2015). Based on these data, metabolic heat production was calculated (McLean, 1972).

Evaporative water loss. In order to quantify the total evaporative water loss from the cow during the experimental period, a complete water balance inside the CRC (Figure 4-2) was calculated as follows for each cow:

$$Evaporative\ water\ loss = (A + B) - (C + D + E + F + G + H)$$

In this equation, evaporative water loss is the total evaporative water loss of a cow, i.e. water evaporated from the skin surface and from the respiratory track, kg/d. Variable A is the mass of water in the outgoing air, calculated daily by measuring the volume and humidity of the outgoing air. Variable B is the amount of water that condensed in the heat exchangers. Water was collected in a tank outside the chamber, which was weighed and recorded daily. Variable C is the mass of water in the incoming air, calculated daily by measuring the volume and humidity of the incoming air. Variable D is the amount of water evaporated from a bucket with a known surface area (0.16 m^2), which was measured and recorded continuously at 5-s intervals. The evaporated water was then divided by the surface area of the bucket to calculate evaporation rate in kg m^{-2} for different periods, thereby used to calculate variables E, F and G.

Variable E is the volume of water evaporated from wet solid floor. The wet area on the solid floor was determined three times a day (at the same measurement times of local cutaneous evaporative water loss) by estimating the proportion of the solid floor wetted with drinking water and urine during different periods using the method from Huynh et al. (2007). Variable F and G are the amount of water evaporated from the manure reservoir and drinking water bowl, respectively. Variables E, F and G were calculated by multiplying the surface area of the wet floor, reservoir and bowl with the water evaporation rate per m^2. Variable H is the volume of water that was added to maintain the setup humidity in the CRC. The volume of water sprayed into the air by a humidifier was measured daily. To calculate the energy used for evaporation the enthalpy of vaporization at 35°C was used: 2417.9 kJ heat per 1 kg of water. The total cutaneous evaporative water loss (kg/d) was calculated by subtracting respiratory water loss from total evaporative water loss and this was converted to the unit of g m^{-2} h^{-1} by applying the body surface area ($0.14W^{0.57}$) estimated according to Brody (1945).

Three times daily (0600, 1000 and 1800 h), local cutaneous evaporative water loss from a sampling area at belly was measured using a ventilated skin box and evaporative water loss through respiration was measured using a face mask and a nose cup. More detailed description can be found in (Zhou et al., 2022b). To determine evaporative water loss at daily level, evaporative water loss, during the day condition (1000 to 1900 h) was calculated using the mean of the values measured at 1000 and 1900 h, during the temperature increasing condition (0700 to 1000 h) evaporative water loss was calculated using the mean of the values measured at 0600 and 1000 h, during the temperature decreasing condition (1900 to 2200 h) evaporative water loss was calculated using the mean of two values measured at 1900 and 2200 h, and during the night condition (2200 to 0600 h) evaporative water loss was calculated using the value measured at 0600 h.

4.2.4 Statistical Analysis

Statistical procedures were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC). All data were screened to confirm the normality and homoscedasticity of variances. Using these data, we tested the effects of ambient temperature for all the dependent variables on mixed models (PROC MIXED). Each model used variance components as the covariance structure, with fixed effects for ambient temperature, treatment (combination of relative humidity and air velocity), and their interaction, and the baseline milk yield (average milk yield from first 2 d) as covariate. Cow was included as a random effect.

Cutaneous evaporative water loss rates derived by the water balance method were analyzed using least squares method by fitting generalized linear models (PROC GLM), including the fixed effects of two methods, ambient temperature, and interaction of both. The root mean square deviation (RMSD), the mean bias (MB), and the coefficient of determination (R^2) were employed to determine the deviation of the cutaneous evaporative water loss rate derived by the local measurement using ventilated skin box from water balance method. The differences between two methods were quantified by RMSD as follow:

4

$$RMSD = \sqrt{\sum (x_i - y_i)^2 / n}$$

where x_i and y_i are cutaneous evaporative water loss rates derived by ventilated skin box and water balance method respectively; n is the number of measurements.

The MB is the average bias, calculated as:

$$MB = \sum (x_i - y_i) / n$$

The R^2 is calculated as:

$$R^2 = 1 - \frac{\sum (x_i - y_i)^2}{\sum (x_i - \bar{x})^2}$$

where $\bar{x}$ the mean of x_i (i = 1,2, ..., n).

4.3 Results

In Table 4-1, descriptive statistics are presented for each element used for calculating the water balance under different treatments.

Table 4-1. The mean (±SE) of different elements for calculating the total evaporative water loss (EWL, kg/d) from dairy cows under different treatments.

Treatments[1]	Air water Out-In[2]	Condense water[3]	Humidifier water[4]	EWL from wet floor[5]	EWL else[6]	EWL from cow[7]
RH_l*AV_l	5.03±0.21	20.8±0.97	0	0.622±0.074	3.68±0.23	21.5±0.82
RH_m*AV_l	7.15±0.58	20.1±0.52	0	0.960±0.077	3.15±0.14	23.1±0.72
RH_h*AV_l	10.1±0.82	13.7±0.64	2.78±0.441	0.284±0.059	2.21±0.13	18.5±0.67
RH_m*AV_m	7.36±0.56	22.2±0.94	0	1.10±0.21	4.72±0.20	23.7±1.1
RH_m*AV_h	7.90±0.53	21.9±0.65	0	0.731±0.13	4.56±0.18	24.5±0.84

[1]Treatments during the day and night: RH_l: 30 - 50%; RH_m: 45 - 70%; RH_h: 60 - 90%; AV_l: 0.1 - 0.1m/s; AV_m: 1.0 - 0.1m/s; AV_h: 1.5 - 0.1m/s. RH means relative humidity and AV means air velocity.
[2]Air water Out-In: the difference between the amount of water in the outgoing air and incoming air, kg/d.
[3]Condense water: the amount of water that was condensed in the heat exchangers, kg/d.
[4]Humidifier water: the amount of water that was sprayed into the air by a humidifier to maintain the setup humidity, kg/d.
[5]EWL from wet floor: the amount of water evaporated from wet solid floor, kg/d.
[6]EWL else: the amount of water evaporated from drinking bowl, manure reservoir and water bucket, kg/d.
[7]EWL from cow: the amount of total evaporative water loss from skin surface and respiration from dairy cows, kg/d.

4.3.1 Effect of ambient temperature, relative humidity and air velocity

Total evaporative water loss from cows (respiration and sweating) generally increased with increasing ambient T for individual cows as shown in Figure 4-3, and the difference between individual cows was substantial. When evaluating the effect of RH or AV, the baseline milk yield (average milk yield from first 2 d) was included in the statistical model as a covariate. Milk yield was positively related with evaporative water loss (P = 0.052). The RH/AV had no significant effect on evaporative water loss, while the interaction effect between RH/AV with ambient T was significant (P = 0.0024). Specifically, at low AV level, cows at high RH had a tendency for a lower increasing rate (0.61 kg/d per 1°C increase of ambient T) of evaporative water loss compared to cows at low RH (0.79 kg/d per 1°C increase of ambient T; $P = 0.065$). At medium RH, cows at medium and high AV levels had higher increasing rate (0.91 and 0.95 kg/d per 1°C increase of ambient T, respectively) than cows at low AV (0.71 kg/d per 1°C increase of ambient T; $P < 0.05$). Figure 4-4 shows the partitioning of metabolic heat production, in which total evaporative heat loss increased and sensible heat loss (the difference between total heat production and latent heat loss, assuming that the cows were in heat balance) decreased with increasing ambient T. Figure 4-4 also shows that the increase of evaporative heat loss from dairy cows was mainly due to the increase of evaporation (of sweat) from the skin. The respiratory evaporative heat loss measured with the face mask increased only slightly with increasing ambient T (Zhou et al., 2022b).

4.3.2 Comparison of cutaneous evaporative water loss rate measured by two methods

There was no significant difference on rate of cutaneous evaporative water loss between two employed methods ($P = 0.387$). The RMSD and MB of the rates of cutaneous evaporative water loss obtained by water balance method and ventilated skin box were 39.1 and 0.44 g m^{-2} h^{-1}, equaling 42% and 0.5% of the total cutaneous water loss obtained by the first method, respectively. The values measured by ventilated skin box were slightly higher than those calculated using the water balance method. The R^2 was equal to 0.87 (Figure 4-5) by using the correlation-regression approach (intercept = 0), which means 87% of the variance of the rates of cutaneous evaporative water loss calculated using the water balance method can be explained by the values measured by ventilated skin box.

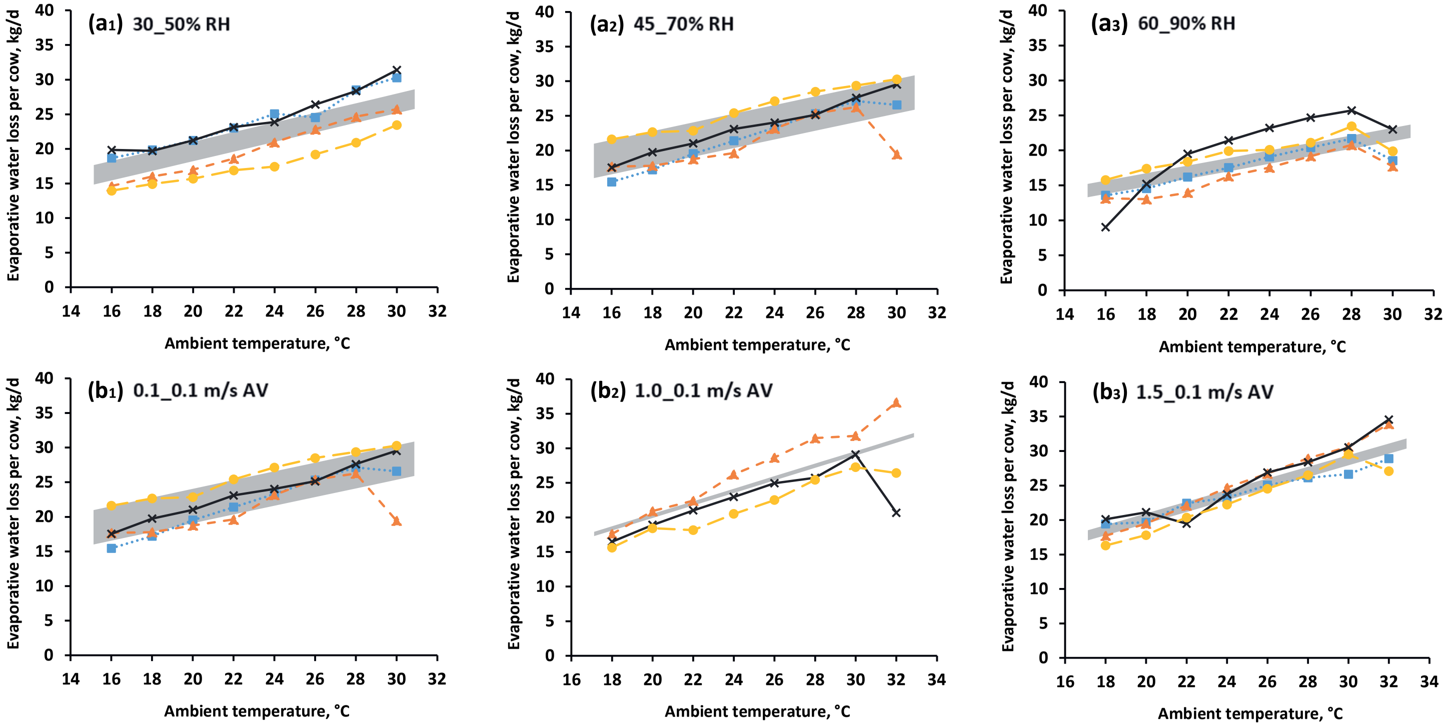

Figure 4-3. Evaporative water loss with increasing daytime ambient temperature for individual cows (four cows per graph) at different relative humidity (RH) levels with fixed air velocity (AV) level [(a1): 30% daytime - 50% nighttime, (a2): 45% daytime - 70% nighttime, and (a3): 60% daytime - 90% nighttime at 0.1 m/s daytime - 0.1 m/s nighttime] and different AV levels with fixed RH level [(b1): 0.1 m/s daytime - 0.1 m/s nighttime, (b2): 1.0 m/s daytime - 0.1 m/s nighttime, and (b3): 1.5 m/s daytime - 0.1 m/s nighttime at 45% daytime - 70% nighttime]. Figure (a2) is the same as (b1). Grey blocks represent the statistical relationship between evaporative water loss with ambient temperature at the range of milk yield from four cows.

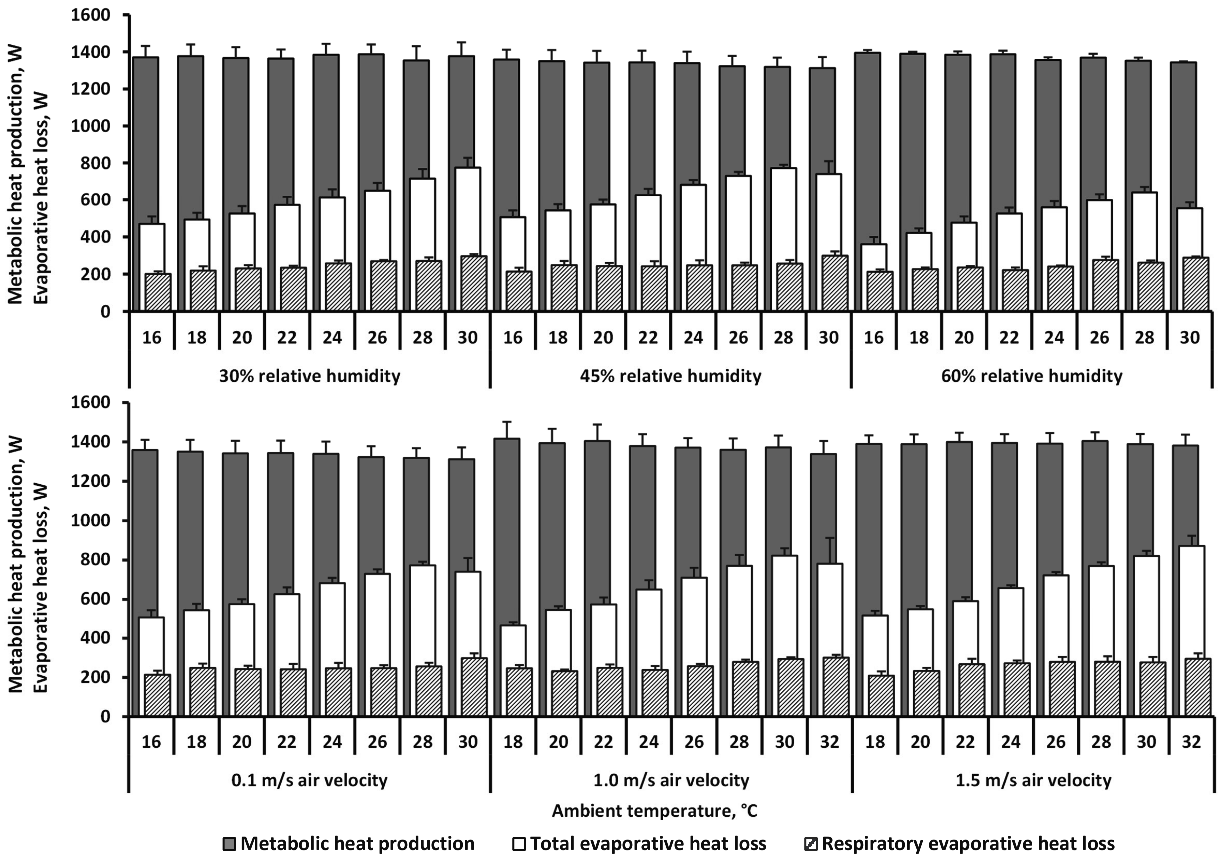

Figure 4-4. Relationship between the mean (±SD) of metabolic heat production (W), total evaporative heat loss (W) and respiratory heat loss (W) with increasing ambient temperature at different relative humidity and air velocity levels.

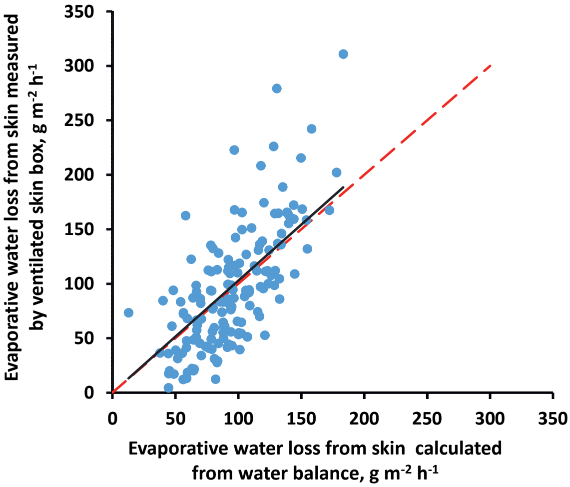

Figure 4-5. Fitted regression line (intercept = 0) of the evaporative water loss from the skin measured by ventilated skin box against the evaporative water loss from the skin calculated from water balance. Identity line (slope =1 and intercept = 0) is in red.

4.4 Discussion

Quantification of the total evaporative water loss is of interest to study the different routes of heat losses of dairy cows. This information also gives insight into the effect of different cooling systems on additional heat losses. To the best of our knowledge, this is the first study to determine the total evaporative water loss rate from dairy cows at daily level, which can avoid some sources of errors associated with different sweating rates between different skin regions, and cyclic sweating patterns (Berman, 1957, Gebremedhin et al., 2008, Liang et al., 2009b, de Souza et al., 2018). With the design of the current experiment, we were able to estimate the total evaporative water loss from cows, as well as separate it between skin evaporation and respiratory evaporation.

4.4.1 Effect of ambient temperature, relative humidity and air velocity

Total evaporative water loss rate increased as environmental temperature rose. This is a consequence of the decreased temperature gradient between the skin surface and surrounding air, causing a decrease in sensible heat loss which needs to be compensated by an increase of the latent heat loss. We included baseline milk yield as covariate when analyzing the total evaporative water loss because cows producing more milk would normally have a higher metabolic heat production (Ravagnolo et al., 2000) and thereby a need for a higher total heat loss.

Castro et al. (2021) used a weighing system as a gold standard method to quantify the total evaporative water loss by observing the acute changes in body mass. This method, however, has limitations as it neglects gas losses from the body (Cheuvront and Kenefick, 2017), which is a larger error source in cows because of rumination activity compared to human beings. The range of cutaneous evaporative water loss rate measured by this weighing system over a period of 2 h was 54 to 375 g m^{-2} h^{-1} within ambient air temperatures around 26 to 34°C (Castro et al., 2021). In our study, the range was between 12.8 to 183.4 g m^{-2} h^{-1}, which was the daily means of cutaneous water loss under higher ambient temperatures during the day (16 to 32°C) and lower ambient temperatures during the night (7 to 23°C). The mass loss by gas exchange (the difference between the production of CO_2 and CH_4 and the consumption of O_2) was calculated in this study to vary from 28.5 to 41.1 g m^{-2} h^{-1}. The calculated gas loss weight could account for 8 to 76% of the cutaneous water loss measured by Castro et al. (2021), causing considerable errors when this gold standard method is used as reference value to compare different measurement methods especially under cool conditions.

In this study, we found that cows had more or less equal heat production and evaporative heat loss at low and medium RH levels. This could explain the inflection point temperatures at which rectal temperature started to increase at low and medium RH levels were similar (25.3 and 25.9°C, respectively) (Zhou et al., 2022a). While at high RH level, the inflection point temperature was a lot lower (20.1°C), which was likely due to the higher metabolic heat production as well as the lower evaporative heat loss. To be specific, at high ambient T and high RH, evaporating sweat to ambient air became more difficult (Berman, 2009) as shown in Figure 4-4. As a result, skin temperatures at high RH level were higher than those at low or medium RH levels, which caused more sensible heat dissipation to the ambient air. We found cows had higher evaporative heat loss at medium and high AV levels than at low AV level, while the inflection point temperature for rectal temperature at medium level was lower than others (Zhou et al., 2022a). The physiological responses, rectal temperature and evaporative heat loss, could happen in a parallel sense; so cows were getting into heat stress giving a higher rectal temperature and a higher evaporative heat dissipation (da Silva and Maia, 2013).

Interestingly, the increase of evaporative heat loss from dairy cows under warm conditions was mainly due to the increase of evaporation (of sweat) from the skin. The increase of respiration rate as a response to increasing ambient temperatures seems to be mainly meant to compensate for the reduced temperature gradient between inhaled and exhaled air. Therefore, evaporative skin cooling is of utmost importance when cows are exposed to heat load, although this effect was partly caused by the setup of the experiment that the RH was independent of the increasing T while in reality RH often decreases with increasing T.

4.4.2 Comparison of cutaneous evaporative water loss rate measured by two methods

The bias of the local cutaneous evaporative water loss rate obtained by employing the ventilated skin box with the total rate determined by the water balance method was quite small (0.5%). The random deviation was larger (42%), but that could not only be accounted for by errors in the ventilated skin box method. The rather good overall agreement is an interesting result because there are several error sources when applying the ventilated skin box to measure cutaneous evaporative water loss as mentioned before: 1) the sweating rate varies between different locations (de Souza et al., 2018); 2) there are cyclic sweat secretion patterns, causing variations in time (Gebremedhin et al., 2010); and 3) the air flow rate through the ventilated box is different from the air flow surrounding the cow trunk (McLean, 1963). The results from

Castro et al. (2021) showed there was a significant difference (P = 0.0398) between the cutaneous evaporative water loss measured by three different methods (weighing system, ventilated capsule and colorimetric paper discs). Although they mentioned that the water loss determined by the weighing system was the gold standard method (Finch et al., 1982), we found, as mentioned before, that the gas exchanges, which was not included in their calculation (Castro et al., 2021), could account for a considerable amount of the weight change of a cow. As a result, the comparison between different methods became difficult.

In this study, the cutaneous evaporative water loss at daily level measured by ventilated skin box was calculated from the weighted means of three measurements at different times during daytime. Whether this estimation would cause a big error for representing the daily cutaneous evaporative water loss is unknown. The values measured by the ventilated skin box show good agreement with those measured by the water balance method despite the fact that the cows were evaluated at daily level with high ambient temperatures during the day and lower ambient temperatures during the night. Whether this finding can be applied to other conditions requires further investigation.

4.5 Conclusion

1) Total evaporative water loss increased as ambient temperature rose. The RH/AV levels had no significant effect on total evaporative water loss, while the interaction effect between RH/AV with ambient T was significant. The increase of evaporative heat loss from dairy cows was mainly due to the increase of evaporation from the skin.

2) A comparison of data measured by ventilated skin box with parallel data derived by water balance method revealed no significant differences in cutaneous evaporative water loss.

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CHAPTER 5.

Development and evaluation of a thermoregulatory model for predicting thermal responses of dairy cows

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Abstract

This study developed a three-node mechanistic model to simulate processes of Holstein dairy cows physiological regulation and heat dissipation under various environmental conditions based on bio-physical laws. This thermal balance model calculated the heat flow through three main nodes at the body core, skin and coat of a dairy cow. Heat production by the animal and heat flow between the animal and the environment, including convection, long-wave radiation, skin evaporation and respiration, were calculated. Sub models of physiological regulation, including tissue resistance, respiration and sweating rate, under different conditions were specifically improved and developed based on old version from previous literature using the training dataset from an experiment conducted recently in climate-controlled respiration chambers, providing an improved modelling of the thermo-physiological process of dairy cows. The model requires information of climate and animal characteristics as inputs, and outputs body core, skin and coat temperatures. This thermal balance model was evaluated through the testing experimental dataset. The root mean squared errors of prediction for body core and skin temperatures were 0.3 and 1.2°C, respectively. This model was able to calculate dynamic changes in body core heat storage and the body and skin temperature variations during day and night, giving higher values of body core and skin temperatures when cows were exposed for a longer time-period to warm conditions. A simulation study was conducted based on a Holstein dairy cow with 600 kg of body weight and 30 kg of daily milk yield with increasing ambient temperature at different relative humidity and air velocity levels. The predicted effects of ambient temperature, relative humidity and air velocity on the physiological responses of dairy cow from the simulation were generally in line with the experimental results. The model is reliable to predict the thermal status of dairy cows under various climate conditions, and to predict the benefits of different cooling methods and their limitations.

Keywords: dairy cow, heat stress, mechanistic model, thermoregulation

Nomenclature	
A_s	Skin surface area, m^2
c_{pa}	Specific heat capacity of air, kJ kg^{-1} $°C^{-1}$
c_{pb}	Specific heat capacity of the body core, kJ kg^{-1} $°C^{-1}$
c_{ps}	Specific heat capacity of the skin, kJ kg^{-1} $°C^{-1}$
D	Diameter of cow's body core, m
D_p	Days of pregnancy, day
H_a	Enthalpy of the ambient air, J kg^{-1}
H_{ex}	Enthalpy of the exhaled air, J kg^{-1}
HP	Metabolic heat production rate, W
k_a	Thermal conductivity of the air, W m^{-1} K^{-1}
m	The slope of the saturation vapor pressure curve, Pa $°C^{-1}$
M	Body mass of the cow, kg
M_b	Mass of the body core segment, kg
M_s	Mass of the skin segment, kg
Nu	Nusselt number
q''_{b_s}	Conductive heat flux between body core and skin, W m^{-2}
q''_{cv}	Convective heat flux at coat, W m^{-2}
q''_{evap}	Evaporative heat flux at skin, W m^{-2}
q''_{lw}	Long-wave radiative heat flux at coat, W m^{-2}
q''_{po}	Potential evaporative rate, W m^{-2}
q''_{resp}	Heat loss through respiration, W m^{-2}
q''_{s_c}	Conductive heat flux between skin and coat, W m^{-2}
q''_{store}	Heat storage in body core, W m^{-2}
q''_{sw}	Evaporative heat flux when all the produced sweat evaporates, W m^{-2}
RH	Relative humidity, %
RR	Respiration rate, breath/min
r_t	Tissue resistance, m^2 °C W^{-1}
r_c	Overall thermal resistance of the hair coat, m^2 °C W^{-1}
SW	Sweating rate, g m^{-2} h^{-1}
T_a	Ambient air temperature, °C
T_b	Body core temperature, °C
T_c	Coat temperature, °C

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T_{ex}	Exhaled air temperature, °C
T_r	Radiant temperature of environment, °C
T_s	Skin surface temperature, °C
v_a	Air velocity, m/s
V_t	Tidal volume, m^3 $breath^{-1}$
VPD	Vapor pressure deficit, Pa
Y_m	Milk yield, kg/d
ρ_a	Density of the ambient air, kg m^{-3}
ρ_{ex}	Density of the exhaled air, kg m^{-3}
ρ_s	Density of the sweat, kg m^{-3}
λ	Latent heat of vaporization of sweating, J g^{-1}
γ	Psychrometric constant, 66 Pa $°C^{-1}$
ε_c	The emissivity of cow coat for long-wave radiation, 0.95
σ	Stefan-Boltzmann constant, 5.67×10^{-8} W m^{-2} K^{-4}

5.1 Introduction

When a dairy cow is exposed to temperatures that exceed her thermal comfort zone, she has to do a lot of effort or even cannot dissipate enough heat to maintain her body thermal balance and she can get in a state called heat stress (Kadzere et al., 2002). The thermoregulatory mechanisms of dairy cows for losing heat consist of three main routes; sensible heat loss from the coat surface to the environment, evaporative heat loss from the skin surface and respiratory heat loss. If the ambient temperature increases, sensible heat loss from the coat surface reduces and this is compensated by an increase of the evaporative skin heat loss and respiratory heat loss and eventually, when heat production cannot be balanced by heat loss, the remaining heat is stored in the body resulting in an increased body temperature (Mount, 1979). A higher body temperature will result in a higher sensible heat loss by the increase in temperature difference between the skin surface and the environment. However, an increase in body temperature will negatively affect health and welfare of dairy cows. A mechanistic model that could simulate the heat balance accurately under different thermal conditions is missing and would be very beneficial to predict and to prevent heat stress in dairy cows.

The simulation of thermo-physiological responses requires the detailed modelling of two key components: a physiological-regulation model of dairy cows and a heat dissipation model dealing with heat and mass transfer from the core to the environment. In previous decades several mathematical models have been developed for calculating the heat loss from dairy cows (McArthur, 1987, Ehrlemark and Sällvik, 1996). During the years these models and equations are extended from single equations to extensive ones resulting in a tremendous increase in complexity. The model of McGovern and Bruce (2000b) is a steady-state energy model for cows that consisted of 153 elements describing the thermal environment, animal characteristics, as well as the distribution of heat transfer through the body tissue, through the coat layer, the heat transfer to the ambient air, the heat loss from the respiratory tract, and the eventual rates of change in body temperature. This model was adapted by Berman (2005) in order to make it suitable for Holstein dairy cows. Gebremedhin and Wu (2001) developed a mechanistic model which combined both heat and mass transfer to predict evaporative and convective heat losses for different levels of skin wetness and fur properties. This model provided insight in heat losses when applying cooling processes in the barn to avoid heat stress. Thompson et al. (2014) developed a dynamic heat exchange model, consisting of three state variables, and it was able to calculate changes in body core temperature in response to climate factors such as air temperature, vapor pressure, solar radiation and air velocity. The model was then improved by Li et al. (2021) by taking into account the conductive heat transfer between the cow and the floor when the animal lies down. However, there is still a lot of uncertainty in different input parameters of the model (e.g. estimation of physiological responses). For these existing models, as described above, the description of the physiological responses of cows was developed decades ago and lacking validation or improvement. Comprehensive interpretation of the physiological responses using recent data from modern cows are therefore needed to achieve more accurate thermoregulation modelling.

Therefore, the objective of this study was to have a high-quality mechanistic dynamic calculation model which could be used for accessing the thermal status of dairy cows including their physiological responses for early detection and prevention of heat stress. To achieve this goal, we developed a dynamic thermoregulatory model that could predict the temperature of body core, skin and coat of dairy cows by integrating and improving the equations from previous studies. The model was evaluated by comparing the predicted values with the measured results from climate-controlled respiration chambers. The predictive performances of the model under different environmental conditions, in the higher temperature range, and under short/long exposures to high temperatures were investigated. Furthermore, some simulations were done to show the effects of combinations of ambient temperature (T), relative humidity (RH) and air velocity (AV) on body core and skin temperature.

5.2 Materials and methods

5.2.1 Model development

5.2.1.1 Heat balance equations

The two-node model by Gagge et al. (1972) is commonly used for evaluation of human body thermal responses under transient environmental conditions. This model structure is applied in our study, allowing for the prediction of dynamic physiological responses of dairy cows under changing environmental conditions. The cow body is represented by two concentric cylinders for the core and skin layers (Figure 5-1). A uniform layer of hair coat covers the skin layer over the whole body. Conductive heat loss from the skin to the ground is neglected in our model.

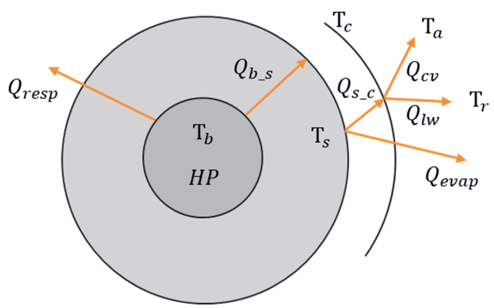

Figure 5-1. Schematic representation of the thermal balance model. T_b: body core temperature (°C); T_s: skin temperature (°C); T_c: coat temperature (°C); T_a: air temperature (°C); T_r: radiant temperature of surrounding objects (°C); HP: metabolic heat production (W); Q_{resp}: heat loss through respiration (W); Q_{b_s}: conductive heat flow between body core and skin (W); Q_{evap}: evaporative heat loss through sweating from the skin surface (W); Q_{s_c}: conductive heat flow between skin and coat (W); Q_{cv}: convective heat flow from coat surface (W); Q_{lw}: long-wave radiative heat flow from coat surface (W). Conductive heat loss is not taken in to account.

The model is based on the energy balance equations at body core and skin nodes. At the body core, the heat is produced by metabolism (HP), and the heat is lost via respiration and conduction to skin. The rest of the core energy is stored and causes the core temperature to rise. Therefore, the energy balance equation at the body core node is:

$$d(M_b c_{pb} T_b)/dt = HP - A_s\,(q''_{resp} + q''_{b\_s}) \tag{1}$$

The energy balance equation at the skin node is expressed as:

$$d(M_s c_{ps} T_s)/dt = A_s\,(q''_{b\_s} - q''_{s\_c} - q''_{evap}) \tag{2}$$

The energy balance of the coat layer assumes that the heat stored in the coat is small and is neglected:

$$0 = q''_{s\_c} - q''_{cv} - q''_{lw} \tag{3}$$

The skin surface area (A_s, m^2) of dairy cows is estimated from body mass (M, kg) (Brody, 1945):

$$A_s = 0.14M^{0.57} \tag{4}$$

The mass of the skin segment (M_s, kg) is calculated according to Smith and Baldwin (1974) and the mass of the body core segment (M_b, kg) is calculated by the difference of the total mass and skin mass:

$$M_s = 1.11M^{0.51} \tag{5}$$

$$M_b = M - M_s \tag{6}$$

Coat mass is very small and is ignored. The heat capacity of the body core segment and skin segment is assumed to be the same as 3472 J kg^{-1} $°C^{-1}$ as reported by Gebremedhin et al. (2016).

5.2.1.2 Metabolic heat production

The daily heat production (HP, W) is calculated according to the equation from CIGR (2002) with correction of air temperature:

$$HP = 5.6 \times M^{0.75} + 22 \times Y_m + 1.6 \times 10^{-5} \times D_p + 4 \times (20 - T_a) \tag{7}$$

where M is the cow body mass, kg; Y_m is milk yield, kg/d; D_p is pregnant days.

However, along with the genetic development and nutritional improvement, cows produce currently more milk (Loker et al., 2012), which means they have a greater dry matter intake and produce more heat and thus are more likely to get into heat stress (Zimbelman et al., 2009). The CIGR model was developed based on data from more than three decades ago, therefore it is necessary to evaluate whether this model is still suitable for predicting the heat production of modern dairy cows precisely. To estimate the heat production, experimental data from climate-controlled respiration chambers on lactating cows was used and the detailed description can be found in Section 2.2.

5.2.1.3 Heat transfer from body core to skin

The heat flux transferred from the body core to the skin surface ($q''_{b\_s}, W\ m^{-2}$) is calculated as:

$$q''_{b\_s} = (T_b - T_s)/r_t \tag{8}$$

where r_t is tissue resistance, m^2 °C W^{-1}.

Physiological control of blood flow rate allows the cow to change its tissue resistance and thus regulate the conductive heat from core body to skin layer. As ambient temperature increases, skin temperature rises resulting in a reduced temperature gradient between body core and the skin surface ($T_b - T_s$). Thereby the tissue resistance must be reduced for metabolic heat to be transferred to the skin surface, otherwise the heat would be stored in the core causing body core temperature to increase. There are typically two values of tissue resistance, corresponding to vasoconstriction (maximum) and vasodilation (minimum) respectively. However, little information is available about the variation of the tissue resistance of dairy cows evoked by the change of the ambient conditions.

In this study, the tissue resistance was determined from the heat flow from body core to the skin and the temperature gradient between the body core and the skin. This heat flow is the metabolic heat production minus respiratory heat loss, which leaves the core directly through the respiration tract. It was usual to measure tissue resistance when body core temperature is constant (no heat storage). The most popular model predicting tissue resistance applied in present thermal-regulatory models (Li et al., 2021, Yan et al., 2021a) was from McArthur (1987), who calculated tissue resistance using the data of Blaxter and Wainman (1964) by assuming a value of 10 W m^{-2} for the respiratory heat loss. This would lead to inaccurate estimation of tissue resistance because respiratory heat loss increased with increasing ambient temperature (Maia et al., 2005b, Zhou et al., 2022b). In this experiment, there was a net increase in body core temperature and therefore the heat storage in the body core was considered as well when calculating the tissue resistance. The calculation of tissue resistance was done as follows:

$$r_t = (T_b - T_s)/(\frac{HP}{A_s} - q''_{resp} - q''_{store}) \quad (9)$$

To determine heat storage in the body core, the body core temperature differences between the morning and afternoon measurements were calculated and then the conductive heat transfer from body core to skin was calculated by subtracting the respiratory heat loss and heat storage from the metabolic heat production. The maximum tissue resistance is reached under cold conditions when a cow has to reduce heat dissipation through vasoconstriction to increase the tissue resistance, and the minimum tissue resistance is reached under hot conditions when a cow cannot dissipate more heat through vasodilation. According McGovern and Bruce (2000), r_t remained almost constant (minimum) at about 0.0156 °C m^2 W^{-1} when T_s exceeded a threshold value of about 35.5°C.

5.2.1.4 Heat transfer from skin to the environment

The heat flux transferred from skin to coat (q''_{s_c}, $W\ m^{-2}$) is calculated as:

$$q''_{s\_c} = (T_s - T_c)/r_c \quad (10)$$

where r_c is the overall thermal resistance of the hair coat, m^2 °C W^{-1}, calculated according to McArthur and Monteith (1980).

Evaporative heat release from skin surface.

The evaporative heat loss through sweating from skin surface (q''_{evap} , $W\ m^{-2}$) is determined by taking the minimum value of latent heat loss from the skin when all the produced sweat evaporates (q''_{sw}, $W\ m^{-2}$), and the potential evaporative rate (q''_{po}, $W\ m^{-2}$) determined by the environmental conditions (Gash and Shuttleworth, 2007):

$$q''_{evap} = \min\,(q''_{sw}, q''_{po}) \tag{11}$$

$$q''_{sw} = \lambda \times SW/3600 \tag{12}$$

$$q''_{po} = \frac{\frac{\gamma}{1000} \times 6.43(1 + 0.536 \times v_a)\left(\frac{VPD}{1000}\right)}{\left(\frac{\lambda}{1000}\right)\left(\frac{m+\gamma}{1000}\right)} \frac{\rho_s \times \lambda}{86400} \tag{13}$$

$$m = \frac{5336}{T_a^2} e^{21.07-\left(\frac{5336}{T_a}\right)} \tag{14}$$

where λ is the latent heat of vaporization of sweating, $J\ g^{-1}$; SW is the sweating rate, $g\ m^{-2}\ h^{-1}$; γ is the psychrometric constant, 66 Pa/°C; v_a is the air velocity, m/s; VPD is the vapor pressure deficit, computed by taking the difference between the saturation vapor pressure and the actual vapor pressure at the skin layer, Pa; m is the slope of the saturation vapor pressure curve, Pa/°C; ρ_s is the density of sweat, which is equal to the density of water.

At high temperatures, evaporative heat loss from the skin is the primary mode of heat loss in dairy cows (Gebremedhin and Wu, 2001). Sweating rate was preliminarily plotted against both body core temperature and skin temperature in this study because only body and skin temperature were assumed to be the mechanistic drivers in dairy cows (Thompson et al., 2011).

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Skin temperature appeared to be more closely related to sweating rate and thus in this study an exponential function of sweating rate on skin temperature was fitted.

Sensible heat exchange between coat surface and environment.

The convective heat transfer (q''_{cv} , $W\ m^{-2}$) across the coat is determined by the temperature gradient between coat surface and the ambient air:

$$q''_{cv} = k_a \text{ Nu } (T_c - T_a)/\text{D} \tag{15}$$

where k_a is the thermal conductivity of the air, W m^{-1} K^{-1}; D is the body core diameter of the simulated animal, m; Nu is the Nusselt number (Gebremedhin and Wu, 2001).

The long-wave radiation is emitted as thermal radiation (q''_{lw} , $W\ m^{-2}$) from the surroundings to the dairy cow, but also from the cow to its surroundings, depending on the temperature of the cow and the temperature of the surroundings.

$$q''_{lw} = \sigma\varepsilon_c((T_c + 273.15)^4 - (T_r + 273.15)^4) \tag{16}$$

Where σ is Stefan-Boltzmann constant, W m^{-2} K^{-4}; $\mathcal{E}_c$ is the emissivity of the cows coat.

5.2.1.5 Heat transfer through respiration

The heat flux from the body core to ambient air through respiration (q''_{resp} , $W\ m^{-2}$) is calculated as:

$$q''_{resp} = V_t \text{ RR } (\rho_{ex}H_{ex} - \rho_a H_a)/60A_s \tag{17}$$

where V_t is tidal volume, m^3 $breath^{-1}$; RR is respiration rate, breath/min; H_{ex} and H_a are enthalpy of the exhaled air and ambient air respectively, J kg^{-1}; ρ_{ex} and ρ_a are specific density of the exhaled air and ambient air respectively, kg m^{-3}. The calculation of above elements (the enthalpy and density of air at different temperature) can be found in ASHRAE (2009).

The respiration rate (RR, breath/min) is always correlated with environmental variables including air T, RH and AV (Maia et al., 2005b, Li et al., 2020). According to Zhou et al.

(2022a), RR was nearly constant when the ambient temperature was low and the inflection point temperature at which RR started to increase varied for different RH and AV levels and different exposure times. Therefore, it is difficult to predict RR precisely with environmental variables. Body core/skin temperature plays a vital role in controlling the thermal regulators. A preliminary assessment of the data showed that RR was highly correlated with skin temperature. Therefore, in this study an exponential relationship between RR and skin temperature was fitted.

Inhaled air temperature rapidly approaches the body temperature, 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 temperature and water content, while it remains saturated with water vapor (Walker et al., 1962). Therefore, in this study, the exhaled air temperature was fitted as a function of both body core temperature and skin temperature.

5.2.2 Experimental data

The experimental data was from the study by Zhou et al. (2022a) conducted in the climate-controlled respiration chambers at Wageningen University with 20 lactating Holstein-Friesian dairy cows (mean ± SD parity 2.0 ± 0.7, days in milk 206 ± 39, daily milk yield 30.0 ± 4.7 kg, and body weight 687 ± 46 kg). Air T and RH were recorded every 30-s intervals and AV was recorded three times daily the same as the animal-related measurements. Heat production was computed based on the animal's consumed oxygen and produced carbon dioxide and methane which were recorded every 15-min intervals (Gerrits and Labussière, 2015). Rectal temperature, skin temperature, coat temperature, respiration rate, sensible and latent heat losses from skin surface and through respiration were collected three times a day at 0600, 1000 and 1800. The measurement methods were described in detail in Zhou et al. (2022b). The study was originally conducted to determine the effects of environmental conditions on different responses of cows. There were five treatments (four cows each) combining different RH and AV levels (Figure 5-

2). Data from one cow was not used because of mastitis. In totally, there were 456 animal-related measurements. From each treatment, one cow was randomly selected for testing the model (comprising 26% of the measurements), and the remaining cows were used for training the model (comprising 74% of the measurements). This selecting method was applied in order to avoid uneven data because of different treatments.

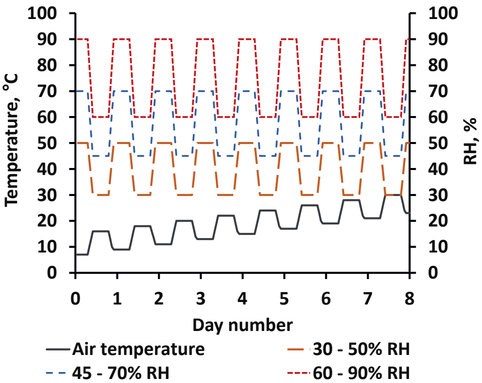

Figure 5-2. Schematic patterns of the set-point air temperature and relative humidity (RH) during the 8-d research period. Between 0700 to 1000 h, the temperature and RH rose gradually to daytime levels and stayed constant until 1900 h. Between 1900 to 2200 h, the temperature and RH gradually decreased to nighttime levels and stayed constant again until the next day 0700 h. Daytime temperature increased from 16 to 30°C, nighttime temperature increased from 9 to 21°C.

5.2.3 Model evaluation

The testing dataset (n = 120) of cows under five treatments with 8 temperature levels combining 5 RH/AV levels was used to evaluate the model performance. Each treatment contained one cow with 3 measurements at different times over 8-d experimental periods. The root mean square error (RMSE) reflects the deviation of predicted variables from observed variables. The mean bias (MB) was used to determine the systematic bias.

$$\mathrm{RMSE} = \sqrt{\frac{\sum_1^n (O-P)^2}{n}} \tag{18}$$

$$\mathrm{MB} = \frac{\sum_1^n (O - P)}{n} \tag{19}$$

Where O is the observed value, P is the predicted value and n is the number of the observations.

5.2.4 Model simulation

A dynamic simulation example was conducted based on one cow from one treatment combining ambient T ranging from 16 to 30°C with a RH of 60% during the day and ranging from 7 to 21°C with a RH of 90% during the night at an AV of approx. 0.2 m/s (fan off). The temperatures of body core, skin and coat were simulated during the 8-d experimental period as well as the different heat fluxes. The measurement points during the experiment were qualitatively compared with the predicted values.

In addition, the effects of RH and AV on the physiological responses of dairy cows were studied by conducting another simulation based on a representative dairy cow with 600 kg BW, 30 kg milk yield and 100 days of pregnancy. Four levels of RH (from 30 to 60% in 10% steps) at 0.2 m/s AV and three levels of AV (0.2, 1.0 and 2.0 m/s) at 60% RH, combining with increasing ambient T (from 14 to 32°C in 2°C steps), were set as environment input.

5.3 Results

5.3.1 Model development

Figure 5-3 shows the performance of the CIGR equation predicting metabolic heat productions of dairy cows by comparing them with measured heat production data in the experiment. It gave sufficient accurate results of heat production estimation for modern dairy cows with MB and RMSE of 19 and 81 W, respectively (1% and 6% of the mean observed heat production). Therefore, the CIGR equation was accepted for the present model.

Figure 5-4 shows the calculated tissue resistance by Eq. 9. When the skin temperature increased from 27 to 37°C, the tissue resistance decreased almost linearly with skin temperature according to the relation:

$$r_t = -0.005\ T_s + 0.195 \tag{20}$$

The adjusted $R^2 = 0.91$ for the training dataset indicated that 91% of the variance of tissue resistance was accounted by skin temperature.

An exponential function of sweating rate on skin temperature was fitted with adjusted $R^2 = 0.20$:

$$SW = 0.312\ e^{0.173T_s} \tag{21}$$

The sweating rate model was also compared with other two models available in recent literature using skin temperature as predictor (Figure 5-5).

An exponential relationship between RR and skin temperature was found with adjusted $R^2 = 0.60$ (Figure 5-6):

$$\mathrm{RR} = 1.5 \times 10^{-5}\ e^{0.41T_s} + 21 \tag{22}$$

An increase in RR is followed by a decrease in tidal volume (adjusted $R^2 = 0.68$) (V_t, m^3/breath) (Figure 5-7).

$$V_t = 0.0591\ RR^{-0.674} \tag{23}$$

The exhaled air temperature (T_{ex}, °C) could be predicted as a function of both body core temperature and skin temperature with adjusted $R^2 = 0.63$ (Figure 5-8):

$$T_{ex} = 0.50T_b + 0.21T_s + 9.54 \tag{24}$$

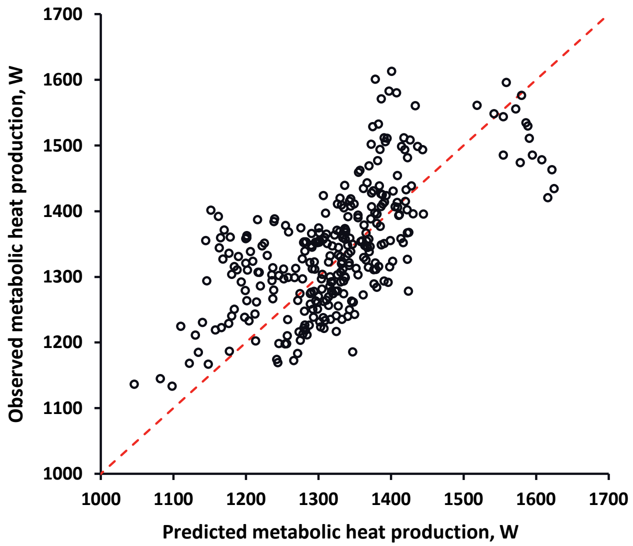

Figure 5-3. Predicted metabolic heat production (with Eq. 7) of 19 cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 304) with 2 measurements per day (twice milking) against observed metabolic heat production. The dashed line represents a perfect prediction.

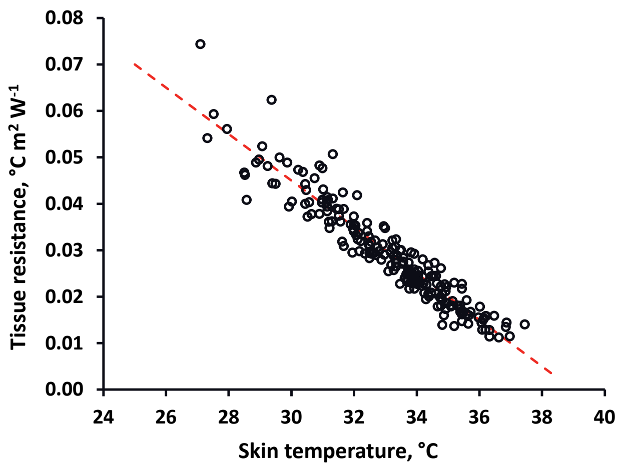

Figure 5-4. Estimated tissue resistance of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 336) plotted against skin temperature using the training dataset. The dashed line represents the fitted regression line (Eq. 20).

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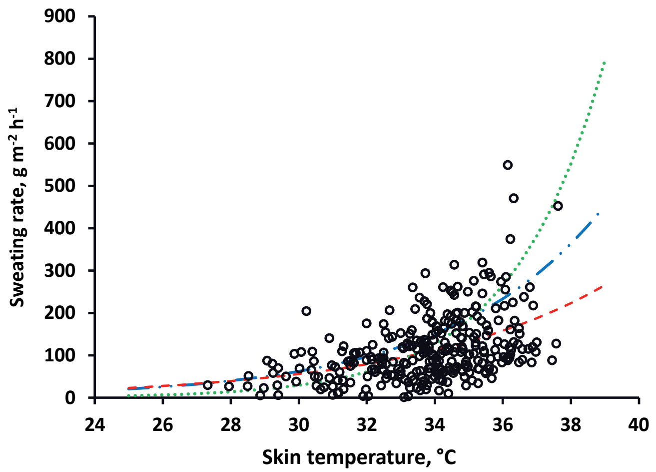

Figure 5-5. Measured sweating rate of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 336) plotted against skin temperature using the training dataset. The red dash line represents the fitted regression line (Eq. 21). The blue and green lines represent the sweating rate models for Bos taurus from Thompson et al. (2011) ($SW = 0.085e^{0.22T_s}$) and from Maia et al. (2008) ($SW = 91.97e^{(T_s-33.11)/2.73}$), respectively.

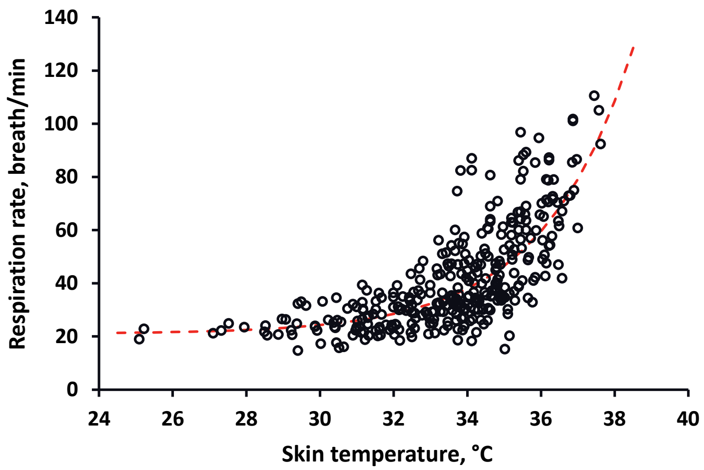

Figure 5-6. Measured respiration rate of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 336) plotted against skin temperature using the training dataset. The red dashed line represents the fitted regression line (Eq. 22).

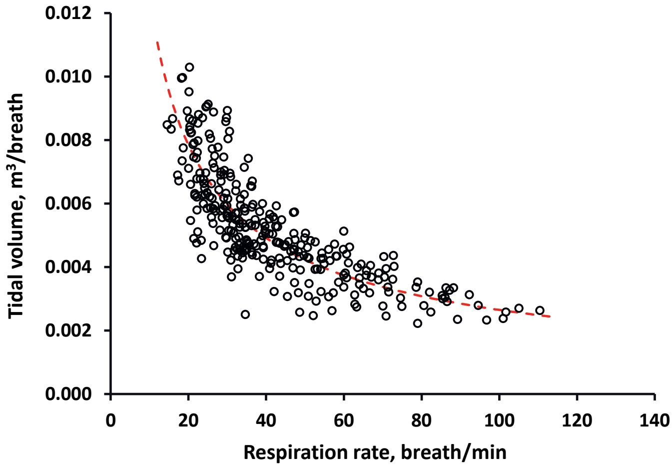

Figure 5-7. Measured tidal volume of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 336) plotted against their respiration rate using the training dataset. The dashed line represents the fitted regression line (Eq. 23).

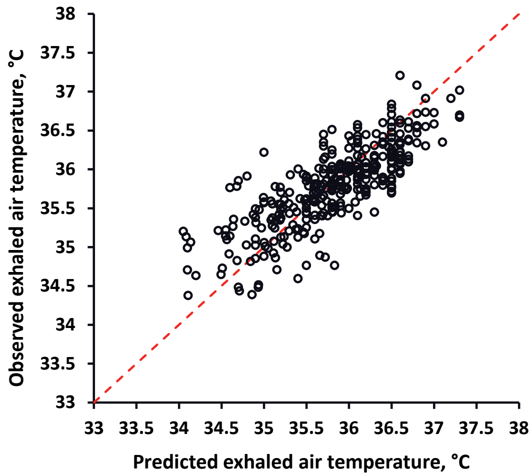

Figure 5-8. Predicted exhaled air temperature (Eq. 24) against observed exhaled air temperature of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 336) using the training dataset. The dashed line represents the perfect prediction.

5.3.2 Model evaluation

The results of the model evaluation for the physiological variables are shown in Table 5-1. For the testing dataset, the mean of the predicted body core temperature was 0.11°C higher compared to the observed temperature. The RMSE was 0.30°C. The means of the predicted skin and coat temperature were 0.59 and 0.63°C higher compared to the observed temperatures,

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respectively. The RMSE was 1.2°C for both skin and coat temperatures. Figure 5-9 suggests that the model was likely to overestimate the body core temperature after longer exposure to warm conditions and the skin temperature could be predicted with higher accuracy when the ambient temperature was getting higher. The respiration rate and sweating rate were predicted based on the skin temperature (Figure 5-10). The model overestimated respiration rate with 1.2 breath/min. The sweating rate was overestimated at low values and underestimated at high values of observations.

Table 5-1. Results of the statistical analysis for comparing the predicted and measured physiological responses of cows (N = 120, training dataset containing 5 cows under five treatments with 8 temperature levels combining 5 RH/AV level with 3 measurements per day).

	Body temperature, °C	Skin temperature, °C	Coat temperature, °C	Respiration rate, breath/min	Sweating rate, $g\ m^{-2}\ h^{-1}$
Predicted mean[1]	38.5±0.3	33.9±2.2	33.4±2.3	43±16	130±40
Observed mean[2]	38.4±0.3	33.3±1.9	32.9±2.4	42±20	113±79
MB[3]	-0.11	-0.59	-0.63	-1.2	-8.3
RMSE[4]	0.30	1.2	1.2	11	59
Relative RMSE[5]	0.8%	3.7%	3.6%	27%	52%

[1]Predicted mean ±SD is the mean ±SD of the predictions.
[2]Observed mean ±SD is the mean ±SD of the observations.
[3]MB is the mean bias of the predictions.
[4]RMSE is the root mean squared error of the predictions.
[5]Relative RMSE is the relative RMSE of the mean of observations.

5.3.3 Model simulation

An example of the simulated and measured temperature of body core, skin and coat over 8-d experimental period is shown in Figure 5-11. The predicted body core temperature kept stable at around 38.2°C for the first 5 d until the air temperature increased to 26°C at 1000h on the 6th d. After 8h exposure to the warm condition, the body core temperature rose to 38.8°C, while the skin temperature rose to 36.2°C and the coat temperature followed a similar pattern. Alongside the decreasing air temperature, the body core, skin and coat temperatures decreased to 38.5, 35.3 and 34.8°C, respectively, at 0600 on the 7th d. Again with ambient temperature increasing from 21 to 30°C at 0700 h on the 8th d, the body core temperature rose from 39.2 to 39.9°C at 1000 and 1800h respectively, while the skin and coat temperatures rose from 36.7 to 37.3°C and 36.5 to 37.0°C, respectively. The observed temperature of body core, skin and coat followed the predicted dynamic patterns generally very well, with some large deviating points.

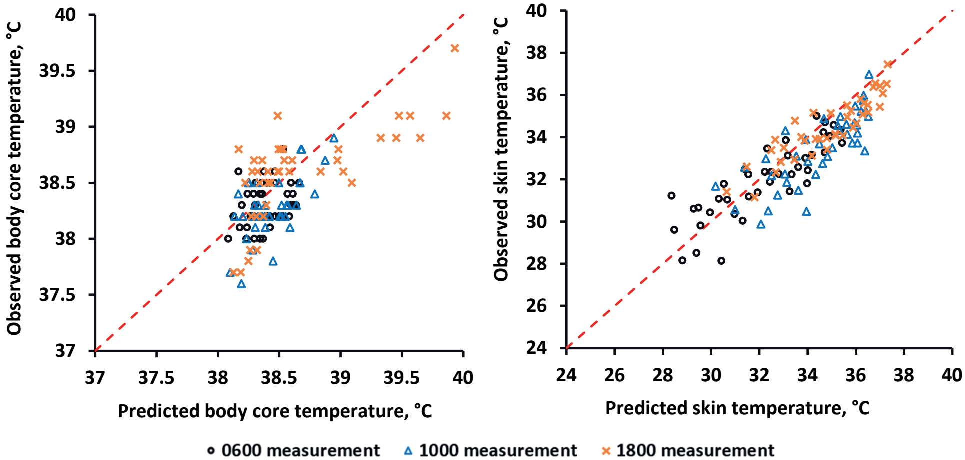

Figure 5-9. Observed body core temperature (left) and skin temperature (right) of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 120) against predicted body core temperature and skin temperature, respectively, based on the thermal balance model. The red dash lines represent a perfect prediction and different types of dots represent different measurement times.

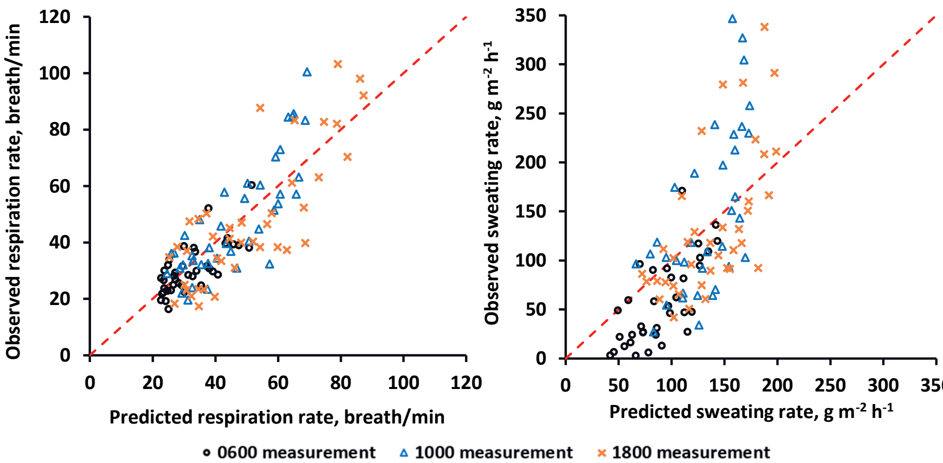

Figure 5-10. Observed respiration rate (left) and sweating rate (right) of cows under five treatments with 8 temperature levels combining 5 RH/AV levels (N = 120) against predicted respiration rate and sweating rate, respectively, based on the thermal balance model. The red dash lines represent a perfect prediction and different types of dots represent different measurement times.

Figure 5-12 shows the predicted and observed heat flux over 8-d experimental period. Sensible heat from coat surface was the major way to dissipate heat under cool conditions, especially during the night. Evaporation from the skin surface became important starting from 6^{th} d, as evaporative heat loss exceeded sensible heat loss during the daytime when the ambient temperature was 26°C. Heat dissipation through respiration followed a relatively stable pattern with a slight increase as ambient temperature increased. The predicted heat fluxes generally had a large deviation from the observed heat fluxes.

In Figure 5-13 the simulated responses of body core temperature to increasing ambient T are shown. At the four levels of RH, the body core temperature was relatively constant until 22°C. Above this ambient temperature at 50 and 60% RH body core temperature increased obviously at 24°C while at the other two RH levels the rise was relatively small. The AV showed a bigger effect on the inflection point temperature. At low AV the cow started to increase body core temperature at 24°C while this inflection point temperature is postponed to 28°C at 2.0 m/s AV. The effect of AV on the body core temperature became smaller with increasing ambient T (the lines get closer at higher ambient T).

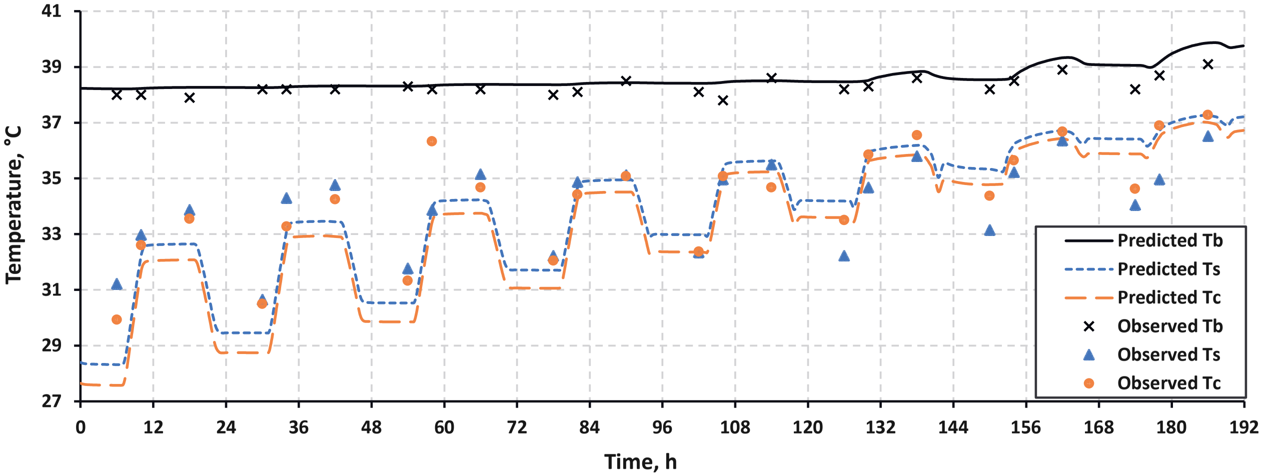

Figure 5-11. Observed (one cow with treatment RH = 60%, AV= 0.1 m/s and increasing air temperature) and predicted (thermal balance model) results of body core temperature (T_b), skin temperature (T_s) and coat temperature (T_c) during an 8-d experimental period.

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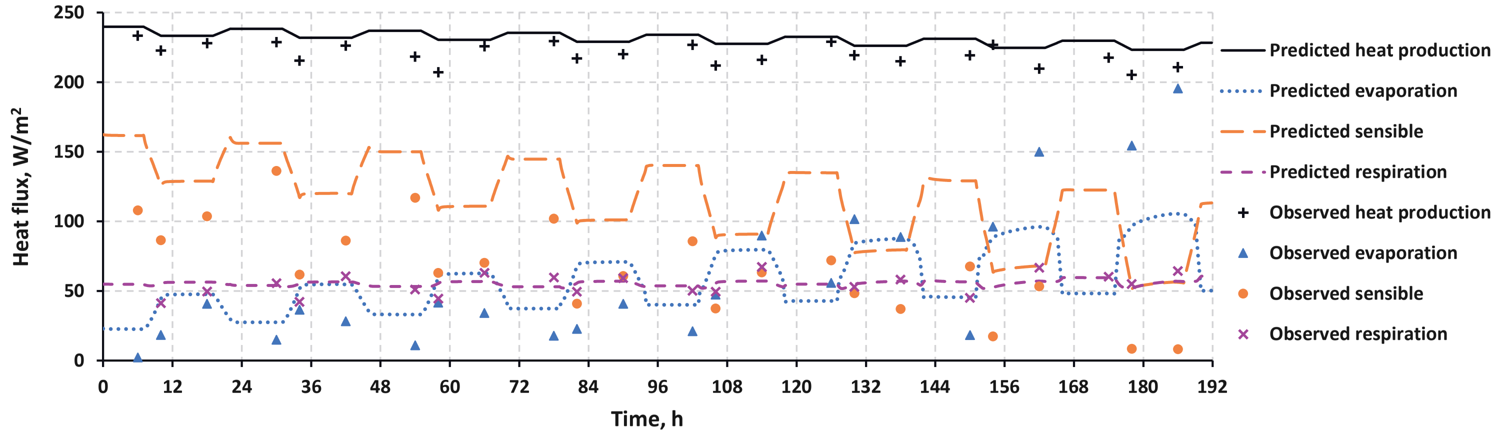

Figure 5-12. Observed (one cow with treatment RH = 60%, AV= 0.1 m/s and increasing air temperature) and predicted results of heat production, evaporative heat loss through sweating from skin surface, sensible (convective and long-wave radiative) heat from coat surface, and heat loss through respiration varying with time during an 8-d experimental period.

5.4 Discussion

5.4.1 Model evaluation

The prediction of body core and skin temperatures (see Table 5-1) was generally satisfactory within the experimental range of ambient T, RH and AV levels. The RMSE of body core temperature from this model was much smaller than that from the model of Li et al. (2021): 0.30°C vs. 1.16 and 0.40°C (two datasets). We found a relatively larger discrepancy between predicted and observed body core temperatures at short exposure (1-h) compared with long exposure (8-h) to high ambient temperature conditions (Figure 5-9). The reason could be that the sweating rate equation applied in our model underestimated the sweating rate under hot conditions (Figure 5-10), which resulted in less evaporative heat loss, causing larger heat storage in the body core after long time exposure to high ambient temperature and thus a higher predicted body core temperature. Li et al. (2021) explained that they had a much higher body core temperature (min = 39.0°C and max = 42.1°C) than normal due to the load of solar radiation. However, their lowest input ambient temperature was below 20°C, so cows could deal with it even with the load of solar radiation. In addition, there was no literature available presenting heat-stressed dairy cows with 42.1°C rectal temperature since in practical situations the highest rectal temperature was below 41.5°C even with THI above 85 (Yan et al., 2021b). A rise of 1°C or less in body core temperature is enough to reduce performance (McDowell et al., 1976), which makes body temperature a sensitive indicator of the physiological response of the cow to heat stress. The possible reason could be that the tissue resistance model that Li et al. (2021) applied underestimated the conductive heat transfer from body core to skin layer. The skin temperature also plays very dominant role in controlling thermal regulation. Yan et al. (2021a) constructed ten thermal models to predict skin temperature of dairy cows, and the model showing the best prediction accuracy (RMSE of 0.65°C) was found when the input data of measured body core temperature was included. However, the two models with a similar structure as our model showed lager deviations with RMSE of 2.2 and 3.4°C compared to 1.2°C from this study. In addition, our model performed also better for predicting skin temperature of cows subjected to heat stress, which was in line with the results from Yan et al. (2021a). The equations 20 - 24 of our model were derived from an experiment conducted in climate-controlled chambers. In these chambers ambient T, RH and AV were kept constant for a longer period. On practical farms cows are more subjected to a dynamic environment. This may affect the way cows are reacting on the environment. Absolute body core/skin temperatures are affected physiologically by many factors (Jessen, 2012, Singh et al., 2013) and it is very difficult to accurately predict absolute temperatures for individual cows in real life. The physiological regulation sub models, including tissue resistance, sweating rate, respiration rate, tidal volume and exhaled air temperature were newly-developed or improved based on old equations using the most recent data from modern high-production cows. There were big variations between/within individual cows as shown in Figure 5-4 to 5-8. As mentioned in the very beginning, this thermoregulatory model was developed to predict thermal status for early detection of heat stress. The thermal responses from cows at herd level can be predicted with high accuracy from our model, while the individual variations were difficult to be identified. However, it is generally also not so important to exactly predict individual cow's skin and body temperature, as cooling systems are always implemented for a group of cows and cows should be able to choose between a more or less cooled place (e.g. fans or sprinklers).

In our study, the simulation patterns of body core, skin and coat temperatures were in good agreement with observations (Figure 5-11). Accurate estimations of body core and skin temperature can be used as suitable indicators to show the thermal status of cows (Becker et al., 2020). However, large discrepancies in heat fluxes of sensible and evaporative heat loss from skin were observed comparing to the experimental data (Figure 5-12). The predicted sensible heat loss was much higher than the measured ones while the predicted evaporative heat loss was higher for the first 5 days and was lower for the next 3 days than the measured ones. The sweating rate was only determined by the skin temperature, which made the sensitivity of the sweating rate on the skin temperature genuinely high. According to Jessen (2012) the thermoregulatory effector mechanisms of dairy cows could be activated or inhibited by changes in skin temperature, which was in accordance to the meta-analysis done on sweating rate by Thompson et al. (2011) including twelve studies. However, according to da Silva et al. (2012) different body parts of the cow show significant variation in latent heat loss. The respective means of observed evaporative heat flux were in decreasing order: neck, flank and hindquarters. For the validation data, only latent heat loss from the belly was used, which could be a lack of accuracy when representing the latent heat loss from the whole body. Besides, sweating rates showed considerable variation between cows (Gebremedhin et al., 2010), which was in line with our experimental data (Figure 5-5). This indicated that it was of great difficulty to predict the evaporative heat loss from the skin for individual cows with high accuracy. In the literature, most sweating rate data was determined by calculating the produced sweat from the measured evaporated water (Gatenby, 1986, Gebremedhin et al., 2008, Maia et al., 2008). This would lead to underestimation of the sweating rate when the evaporation of sweat was limited by the environmental conditions (e.g. high ambient T combined with high RH and low AV), which would result in a prediction error in the thermal balance model. When comparing our sweating rate model to the other two models (Figure 5-5), the exponential equation of the model from Maia et al. (2008) increased very sharply at high skin temperatures, resulting in large over predictions in sweating rate. The model of Thompson et al. (2011) fitted better to our training dataset than the model of Maia et al. (2008), but it overestimated the sweating rate under low skin temperature conditions. It was more suitable for cows in tropical areas, who are adapted to hot climates and could sweat more than cows in temperate areas at the same skin temperature (Finch, 1985).

The long-wave radiative heat transfer is depending on the temperature difference between the cows coat surface and the radiant temperature of the surroundings. The surroundings or elements in the barn that interact with the cow could normally be divided in five different components: ceiling, walls, floor, barn equipment and neighboring cows. In our experimental study the temperatures of the surrounding elements were all the same because of the equal temperature in the whole climate chambers during a long period, while in practice this will vary. The mean radiant temperature used in the current heat balance model for validation was equal to the ambient air temperature. Therefore it was difficult to evaluate the radiative heat loss very well with our experimental data because of the little variation. Actually, there are very few studies available to evaluate the radiative heat loss since it is hard to measure accurately. Hillman et al. (2001) used an infrared pyrometer to measure radiant temperature of the surrounding and then calculated the radiative heat loss with fundamental equations (Eq. 16). They reported that the long-wave radiative heat contributed to less than 10% of the total heat loss, which was a little lower than our simulation results (10 - 20%) under similar environmental conditions. The percentage of the cows surface interacting with each different type of surrounding should be taken into account as input for the model as well as the radiant temperatures and emissivity of each type of surrounding, whereas these are not easy to be measured in practical situations.

Our model assumed the internal heat transfer as a pure conduction process and employed a uniform heat transfer coefficient of tissues (tissue resistance) to calculate the heat transfer rate from the body core to the skin surface by conduction. The tissue resistance in this study was related to skin temperature as shown in Figure 5-4. In their thermoregulatory model, Thompson et al. (2014) applied the tissue resistance equation from Finch (1985), which was a function of body core temperature only. However, when the ambient temperature increases, the skin temperature also increases while the body core temperature keeps constant under cool conditions or increases slower than skin temperature (Zhou et al., 2022a), which lowers the temperature difference between the body core temperature and skin temperature, resulting in a decreasing heat transfer from the body core to the skin surface. In this way the heat transfer from the body core to the skin surface would slightly decline with an increasing ambient temperature, which is inconsistent with reality (the heat transfer from the body core to skin surface should be constant in the thermal comfort zone).

5.4.2 Model application

As proposed in the beginning, this thermoregulatory model was developed to provide effective and efficient management suggestions for mitigating heat stress on farms. Zhou et al. (2022a) reported that the inflection point ambient T for rectal temperature to rise was about 25°C at 30 and 45% RH at low AV (without fan), which was in line with our simulation output (approx. 24°C at 30 and 40% RH). The inflection point ambient T for rectal temperature was only 20°C at 60% RH (Zhou et al., 2022a), which was a bit lower than the simulated prediction in this study (22°C at 60% RH, Figure 5-13). Although little difference was predicted between the RH levels on the inflection point temperature at which body core temperature started to rise (varying between 22 and 24°C), the rising rate of the body core temperature was much larger at higher RH levels. This indicates that with increasing ambient T, it is more difficult for cows at high RH to dissipate heat from the body than cows at low RH. If evaporative cooling was applied to cool down the environment temperature from 30 to 24°C, the RH would, at the same time, increase from 30 to 60% (Zhou et al., 2022a). In this circumstance, the body core temperature could be reduced from 40.0°C (30°C ambient T with 30% RH) to 39.0°C (24°C ambient T with 60% RH) as shown in Figure 5-13. If a fan can be applied to create higher airspeeds (2.0 m/s), cows could have a larger thermoneutral temperature range until 28°C even at 60% RH. The large effect of AV was also confirmed by Spiers et al. (2018), who found that the skin sensible heat loss remained similar both without fans at 23.8°C and with fans at 33.2°C. The simulations suggest that evaporative cooling (lowering ambient T by increasing RH) can be effective in relieving heat stress at low RH. In more humid climates the benefit of evaporative cooling is weak because there is little space to reduce the air T by adding water to the air (Berman, 2009), but it can be compensated by applying fans to increase convective heat loss and potential evaporation rate. However, as the ambient T continues to increase, the benefit of high AV become smaller because of a smaller temperature difference between coat surface and ambient air (Zhou et al., 2022b). Therefore, in tropical regions sprinklers are commonly applied by delivering coarser droplets to wet cows (Tresoldi et al., 2019) and not so much for adiabatic cooling of air as done with nozzles spraying mist. The cooling effects of sprinklers can be enhanced when combined with increased airspeed because the evaporation of water droplets is less limited by RH.

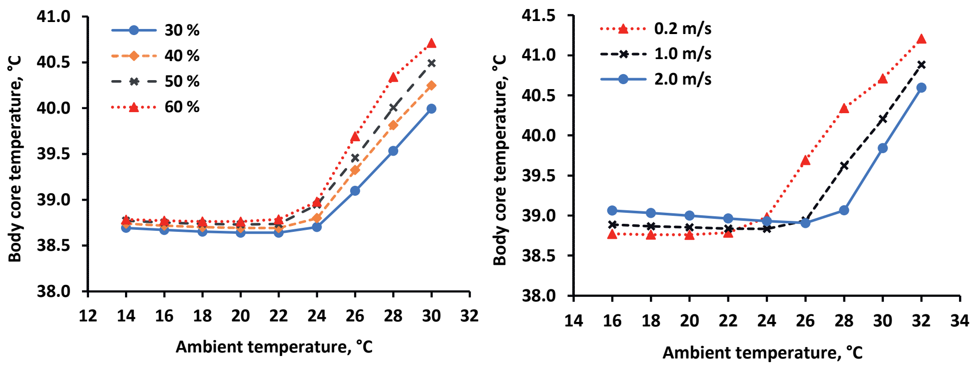

Figure 5-13. Predicted body core temperature by the thermal balance model against the ambient temperature at different relative humidity levels exposed to 0.2 m/s air velocity (left) and different air velocity levels exposed to 60% relative humidity (right).

Overall, the proposed model, including improved description of internal (tissue resistance), sweating and respiration heat transfer process based recent data from high-producing Holstein dairy cows, predicted the body core, skin and coat temperatures with satisfactory accuracy under various combinations of environmental conditions. Using the three-node model, it will be effective and reliable to evaluate the effect of the thermal environments on the thermal status of dairy cows under hot conditions, as well as to provide guiding information for heat stress mitigation.

5.5 Conclusion

In this study, we constructed a thermoregulatory model to predict the dynamic change of body and skin temperatures under various conditions. The prediction performance was evaluated and the conclusions are:

(1) The model was able to predict the body core and skin temperatures accurately (0.30 and 1.2°C of RMSE, respectively).

(2) The model was able to calculate dynamic changes in heat storage and the body core and skin temperature variations during day and night, giving higher predicted values of body core and skin temperatures than measured when cows were exposed for a longer time-period to warm conditions.

(3) The predicted effects of ambient temperature, relative humidity and air velocity on the physiological responses of dairy cows were in line with the experimental results. The model can be applied to predict early signs of heat stress in practice as well as to provide information to use the cooling methods effectively.

(4) In future work, conductive heat loss between skin and floor should also be included in the model and the thermal properties of the barn wall/roof/floor materials should be taken into account to for better prediction of radiative heat transfer. Besides, it is of great importance to conduct an experiment using different cooling systems to validate our model predictions for the effects of cooling methods.

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6

CHAPTER 6.

General discussion

6.1 Introduction

Preventing heat stress is one of the main challenges within modern dairy farming as it negatively affects animals' comfort, health, welfare and productivity. Successful development of genetic techniques in dairy industry has enhanced productivity of milk from 7 kg/d in 1940 to 25 kg/d in 1995 (Kadzere et al., 2002) and to over 30 kg/d nowadays, causing modern cows being more susceptible to heat stress due to higher heat production and thus lower heat tolerance (Becker et al., 2020, Yan et al., 2021b). In addition, long-term rise of global temperature could aggravate the heat stress problems for dairy cows. Early prediction and identification of thermal stress in cows is crucial to timely take the necessary actions in daily farm management .

The main objectives of this thesis were to have better knowledge about the responses of dairy cows to various thermal environments and to have a tool that can accurately predict early signs of heat stress in dairy cows. To achieve these goals, an experiment was designed and conducted in climate-controlled respiration chambers to investigate how cows physiologically respond to heat stress conditions and a thermoregulatory model was developed to assess the thermal status of dairy cows under various environmental conditions. This thesis was subdivided as follows: quantification of the physiological and productive responses (Chapter 2) and skin and respiration heat loss (Chapter 3) of dairy cows to increased ambient temperatures (T) at different relative humidity (RH) and air velocity (AV) levels, a comparative analysis on two methods for measuring evaporative water loss in dairy cows (Chapter 4), and development, validation and evaluation of a dynamic mechanistic model that predicts the thermoregulatory responses of indoor dairy cows to various environmental conditions (Chapter 5).

In Chapter 2, the effects of increasing ambient T on physiological and productive responses of dairy cows were quantified and the inflection point temperatures (IPt) for the activation of adaptive mechanisms were determined at different RH and AV levels. An experiment was conducted with twenty Holstein dairy cows in climate-controlled respiration chambers, including five treatments combining different T/RH/AV. We observed that the respiration rate was the first indicator that showed the cow was reacting to increasing T, and IPt for respiration rate increased with decreasing RH (varying between 18.9 and 25.5°C under short exposure) and increasing AV (varying between 21.0 and 22.8°C under short exposure). Longer exposure time to treatments resulted in physiological responses occurring earlier at lower ambient T.

In Chapter 3, a more in depth study was done to determine the effects of increasing ambient temperatures on sensible and latent heat loss from the skin and through respiration at different RH and AV levels. The results showed that the latent heat loss accounted for 50% of the total heat loss when ambient T was below 20°C and this increased to 70 - 80% when T rose above 28°C. The majority of heat was lost from the skin while respiration heat loss accounted for 20 - 30% of the total heat loss. Skin sensible heat loss rate was positively affected by AV. The skin latent heat loss rate was higher at low RH and high AV. Cows lost more sensible heat from skin surface and total heat through respiration when they were exposed to warm conditions for a longer time (8 h vs. 1 h).

In Chapter 4 it was determined how dairy cows adjusted their total evaporative water loss at different ambient T, and at different RH and AV levels. Two methods to determine the evaporative water loss from the skin were compared, the method with the ventilated skin box and the water balance method. Total evaporative water loss increased as ambient temperature rose, and it was affected by both interactions between RH with T and AV with T (the increasing rate of evaporative water loss varied between 0.61 and 95 kg/d per 1°C increase of ambient T). The two methods to determine cutaneous evaporative water showed similar results.

In Chapter 5, we developed a dynamic thermoregulatory model for predicting the temperature of body core, skin and coat of dairy cows by integrating and improving the equations from literature. The model was evaluated by the experimental dataset obtained from the studies described in Chapters 2 and 3. The results of the evaluation showed that the root mean squared errors of prediction for body core and skin temperatures were 0.3 and 1.2°C, respectively. The predicted effects of ambient T, RH and AV on the physiological responses of dairy cow from the simulations were in line with the experimental results.

In this final chapter we discuss our main findings, as outlined above, in a broader context. Firstly, the new findings about the thermoregulatory responses, including physiological, productive and heat loss changes, of dairy cows to increasing ambient T at various RH and AV levels are summarized. Secondly, based on the experimental results and the developed thermal balance model, further simulations to predict the effects of current available cooling approaches under different climate conditions were conducted. Then some suggestions are given to the usage of the current commonly used thermal index. Finally, the limitations of the thesis are discussed and some ideas for future research are proposed.

6.2 When does heat stress occur?

The currently available indicators to evaluate the thermal status of dairy cows vary in their applicability and practicality. Indicators that provide information on the thermal environments, physiological conditions and production of the animals are commonly selected like temperature-humidity index, rectal temperature and milk production. The animal experiment we conducted in climate-controlled respiration chambers allowed for the measurements on environmental factors (air temperature, relative humidity and air velocity), physiological responses (respiration rate, skin temperature, coat temperature and rectal temperature), production performance (milk yield, milk composition) as well as heat losses (sensible and latent heat from skin and respiration). When studying heat stress in dairy cows, the concept of thermal-comfort and thermoneutral zone from Mount (1979) is used. In this thesis, we define the inflection point temperatures for respiration rate and rectal temperature as the upper critical temperatures of thermal-comfort and thermoneutral zone, respectively.

6.2.1 Responses to the thermal environment

The increase in respiration rate is the first reaction of cows which can be directly and easily observed by farmers, to increasing ambient T (Chapter 2). The IPt we determined can be as early as 19°C at high RH levels. Actually, the control of blood flow (vasodilation) is already taking place before respiration rate starts to rise (Nelson. and Janni., 2016). In contrast to other regulatory responses (such as increasing respiration rate or sweating rate), vasodilation does not require much energy or water loss (Romanovsky, 2014). Vasodilatation causes tissue resistance to decrease as ambient T rises so that the blood can take more heat from body core to skin surface. In this study, the tissue resistance was determined based on the heat flow from body core to the skin and the temperature gradient between the body core and the skin. It was related to skin temperature because the regulation of vasomotion needs the thermal signal from the skin surface (Romanovsky, 2014). In this context, skin temperature is considered to play an important role in thermoregulation and as one of the main determinant factors to assess thermal comfort of dairy cows. An increase of body core temperature (rectal temperature in this thesis) is the key indicator that tells whether or not the cow fails to dissipate sufficient heat by the primary regulation of vasodilation, respiration and sweating. An increase of the body core temperature is also a sign that the cow is out of the thermoneutral zone and enters heat stress status. The IPt determined for rectal temperature is depending on RH and AV and varied from 20.1 to 25.9°C.

In order to find out the reasons why the cow behaved differently under different environmental conditions from the perspective of heat exchange mechanisms, we measured the sensible and latent heat loss from the skin and through respiration. With increasing ambient T, the temperature gradient between skin / exhaled air and surrounding is getting smaller, resulting in less sensible heat loss via skin / respiration. Consequently, cows physiological response is an elevation of the skin temperature and the respiration rate, to compensate the reduced sensible heat dissipation. Meanwhile, the sweating rate is increased to increase latent heat loss and in this way the cow can keep its thermal balance. Under low (< 20°C) ambient T conditions, thermoregulation in cows is very little affected by RH since they mainly lose heat via the sensible way, which is more related to ambient T. The effect of RH shows up when sweating rate starts to increase because latent heat loss from evaporation of sweat will be limited by the environmental potential evaporation rate and the ability to add respiratory water to the ambient air decreases due to high RH levels. The effect of AV on sensible heat loss is significant under low ambient T conditions and getting weaker with rising ambient T. This can be explained by the smaller T difference between skin and environment.

6.2.2 Effects of relative humidity and air velocity

The results of physiological responses (Chapter 2) were generally in agreement with the results of heat loss responses (Chapter 3 and 4). Figure 6-1 shows the temperature of different layers (body core, exhaled air, skin and coat) of the cows, averaged from four cows in each treatment, changing with increasing ambient T at different RH and AV levels. Apparently, the temperature gradients between rectum with skin (conductive heat transfer from body core to skin), between exhaled air with ambient air (heat loss through respiration), and between coat with ambient air (sensible heat loss from coat to ambient air) are all getting smaller with increasing ambient T. The negative relationship between sensible heat loss from coat surface and ambient T (Chapter 3) can be inferred from the temperature gradient. Besides, it was found in our study (Chapter 3) that at lower ambient T the sensible heat loss was much higher at high AV level (1.5 m/s) than at lower AV levels (0.1 and 1.0 m/s), but this difference decreased with increasing T compared with low/medium AV (0.1 or 1.0 m/s) levels, which can be interpreted from Figure 6-1: the positive effect of medium AV on convective heat loss can only compensate the reduced sensible heat loss (convective and radiative) due to the smaller temperature gradient between coat and ambient air; the help of high AV in enhancing the convective heat loss is getting less when the

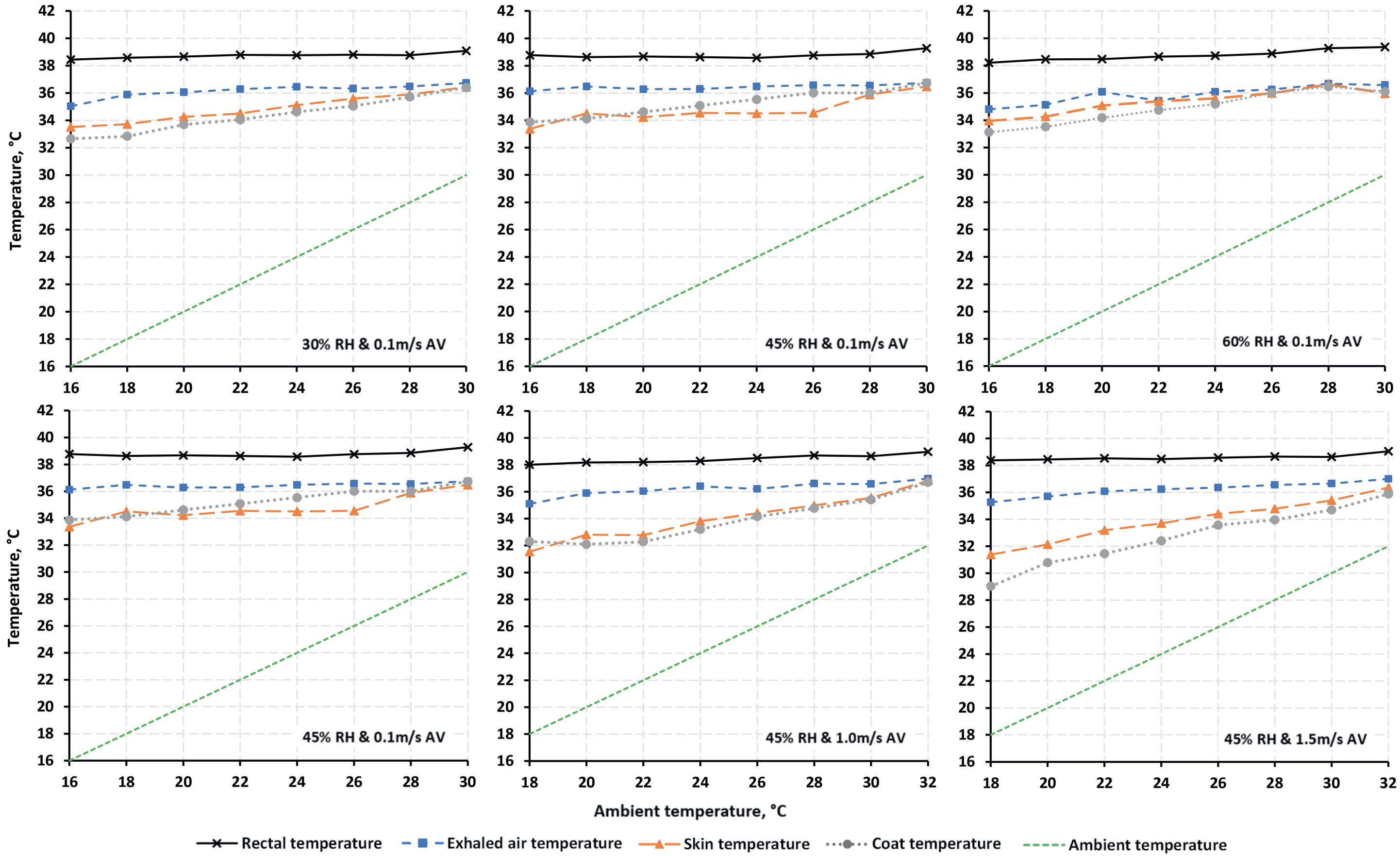

Figure 6-1. Temperatures at different layers (body core, exhaled air, skin and coat) of the cows changing with increasing ambient T at different RH and AV levels.

temperature gradient decreases with ambient T. At the same time, sweating rate increased to increase latent heat loss. We found that at low RH (30%), cows had a higher latent heat loss than at higher RH levels. It seems that under higher RH conditions, the sweat cannot be fully evaporated, because the sweating rate is higher than the potential (maximum) evaporation.

The exhaled air temperature measured in our study (from 35.0 to 36.6°C under short exposure and from 35.4 to 37.0°C under long exposure) was much higher than that from other studies (from 25 to 34°C within the same ambient T range of our study) (Donald, 1981, Maia et al., 2005b). This is an important finding concerning the measurement of respiratory heat loss. Until now it generally was believed that elevated respiration rate was meant to enhance the respiratory heat loss, while in this thesis, we concluded that the elevated respiration rate was mainly required to compensate for the reduced temperature gradient between exhaled and ambient air. Based on this finding, we improved the thermoregulatory model with respect to sub modelling of respiratory heat loss.

There were no obvious changes in milk yield with increasing ambient T within the temperature range of our study. However, decreased protein and fat yield was observed under high RH conditions. The cow is naturally programed to maintain the milk yield consisting of a large amount of (approx. 87%) water (Chandan, 2011), and we found there was a very significant increase in water intake with increasing ambient T. Besides the loss in respiration and sweating, the water can be largely used for milk since it costs less energy compared to protein and fat and it is the most important milk component for the survival of the calf (Drackley, 2008). In addition, the cows were able to recover overnight during the cooler temperature period, as given the design of our experiment. A reduction of milk yield could also appear later as a delayed response to heat stress (Linvill and Pardue, 1992, Polsky and von Keyserlingk, 2017), which we were unable to observe due to the current research set up and time span.

6.3 Cooling Interventions and their limitations

In the context of global warming, cooling systems will become more and more popular especially in temperate climate regions like the Netherlands because most dairy barns are naturally ventilated, and cows are directly exposed to outside climatic conditions and vulnerable to climate change (Heinicke et al., 2018). Therefore, a better understanding of the efficacy of different cooling options applied for different climatic conditions to reduce heat stress of modern high-producing dairy cows is essential from sustainability perspective. There are two ways to help cow mitigate heat stress: 1) to enhance the heat loss from cows and 2) to modify the environment by lowering the ambient temperature.

6.3.1 Enhance heat loss from cows

Air velocity is an important factor in the relief of heat stress, as it affects both convective and evaporative heat losses as discussed before. The predicted effects of application of fans are quantified based on the output from our thermoregulatory model (Chapter 5). Figure 6-2a shows how the body core temperature changes with increasing ambient temperature at different air velocity levels. There is a significant effect of AV level on the IPt at which body core temperature starts to increase (varying from 22 to 26°C). In other words, if a fan is applied to create a high AV (2.0 m/s), the thermoneutral temperature range is extended and the start of "moderate" heat stress is postponed. However, when the ambient T exceeds 28°C, the benefit of high AV become weaker as the body temperatures are getting closer (the difference decreasing from 1.2°C at 28°C ambient T to 0.6°C at 32 ambient T°C, Figure 6-2a). Under this situation, sprinklers are commonly applied combined with fans to promote latent heat loss by generating droplets to wet the cow's skin. In Figure 6-2b the predicted effect of sprinklers under hot and humid condition (34°C ambient T and 60% RH) is shown for three levels of skin wetness (0, 25 and 50%) and three levels of air velocity (0.2, 1.0 and 2.0 m/s). From this figure we can see 1) that without sprinkling (wetness area = 0) the effect on body core temperature for different AV levels is small (varying from 41.3 to 41.7°C); 2) when the cow is wetted with a sprinkler, body core temperature is significantly decreasing with increasing wetness area, and the rate of decrease is influenced by the air velocity; 3) when the skin surface is 25% wetted, the increase of air velocity from 0.2 to 1.0 m/s and from 1.0 to 2.0 m/s plays the similar role in decreasing the body core temperature, while when the wetness area is increased to 50%, the positive effect of 2.0 m/s air velocity is largely reduced compared to 1.0 m/s air velocity. This seems mainly because the body core temperature has already been reduced to normal levels by wetting 50% skin area of the cows combined with 2 m/s air velocity. Then less effect can be expected from additional wetting or higher AV. Based on these findings, we can deduce that depending on the exact environmental situation (T & RH), a smart combination of wetting and air velocity is needed for effective and cost-efficient cooling of the cows. Further complex simulations, combining different cooling methods, can be conducted for various climatic conditions when needed for practical managements.

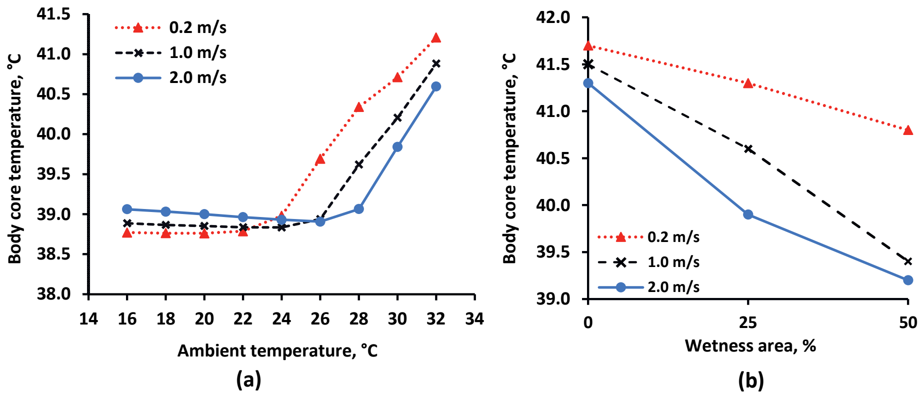

Figure 6-2. (a) Predicted body core temperature by the thermal balance model against the ambient temperature at different air velocity levels exposed to 60% relative humidity condition. (b) Predicted body core temperature by the thermal balance model against the percentage of wetness skin area at different air velocity levels exposed to 34°C ambient temperature and 60% relative humidity.

6.3.2 Modify the environment

Evaporative cooling systems use the energy from the air to evaporate water from atomizing nozzles or cooling pads. The evaporation of water into warm air lowers the air temperature and at the same time increases the relative humidity. Knowing the relationships between ambient conditions and the conditions created by evaporative cooling would make it possible to estimate the potential for heat stress relief by evaporative cooling in different environments. In Figure 6-3a, the evaporative cooling process is simulated based on thermodynamics and psychrometrics (ASHRAE, 2009): the air temperature starts at 30°C, and moisture is added at different RH levels to reach 70% or 90% RH. The predicted performance of evaporative cooling under hot conditions (30°C) is shown in Figure 6-3b: 1) when the RH is increased to 70%, the body core temperature is reduced more when the initial RH is lower; 2) if the RH is continued to increase until 90%, the results show that the predicted body core temperature is even higher than that under the initial conditions (45 and 60%) without evaporative cooling. The simulations tell that evaporative cooling can be counterproductive if it is applied under humid conditions because the high RH can largely suppress the latent heat loss by sweating and respiration while the positive effect of a lowered air temperature cannot compensate this decline. Nevertheless, the application of evaporative cooling can still be effective in relieving heat stress when high AV can be added to enhance both convective (large temperature gradient between skin and ambient) and latent (increased potential evaporation rate) heat loss from skin.

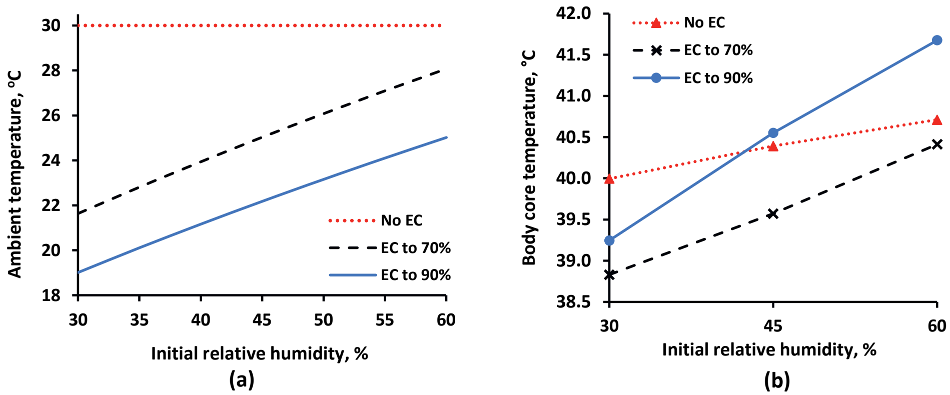

Figure 6-3. (a) Predicted final ambient temperature (having an initial temperature of 30°C) against the initial relative humidity using evaporative cooling. (b) Predicted body core temperature by the thermal balance model against the initial relative humidity at different final relative humidity levels using evaporative cooling when the initial ambient temperature is 30°C. No EC means no application of evaporative cooling. EC to 70% and EC to 90% mean applying evaporative cooling to increase relative humidity to 70% and to 90%, respectively.

6.3.3 Suggestions on application of THI

The temperature-humidity index (THI) was developed as a means of estimating the level of heat stress imposed on cows by taking the combined effect of air T and RH into account (NRC, 1971) and is one the most widely used indicators world-wide for cooling interventions implemented on practical farms at present (Wang et al., 2018). However, the thermal index was developed based on an experiment conducted over 60 years ago on a total of 56 cows producing 15.5 kg of milk/d on average at the University of Missouri where the climate was hot and humid during summertime. The THI could over- or under-estimate the heat-stress level imposed on modern cows as THI gives exactly the same value for all cows. Yan et al. (2021b) found that the rectal T of high-producing cows (47.9 kg/d) was 0.26 - 0.38°C higher than that of low-producing cows (15.6 kg/d) under the same heat stress conditions ($67 < THI < 86$), which confirms that the THI will underestimate the heat stress level for modern high-producing cows. In addition, recent studies of dairy cows demonstrate considerate variability in THI thresholds regarding milk losses (varying from 60 to 72) and physiological responses (varying from 65 to 72) (Zimbelman et al., 2009, Pinto et al., 2020). We found the IPt for respiration rate was 26°C at 30% RH while it was 19°C at 60% RH (Chapter 2), corresponding to THI of 71 and 65, respectively. This means the THI underestimates the heat stress level at climatic conditions which combine relatively cool air T and high RH. At the moment cooling systems are generally started to be used according to the THI thresholds (Zimbelman et al., 2009). However, cows under various climatic conditions, giving similar values of THI, require different cooling strategies as we predicted in the previous sections. For instance, when we have a look at two typical climatic conditions with a THI of 80: (a) 30% RH and 34°C, and (b) 60% RH and 30°C. The feasible and effective cooling strategy can be deduced to be (a) evaporative cooling of the air, because the ambient T can be decreased dramatically by increasing the RH; (b) wetting the cow combined with high AV, because at 30°C the benefit of high AV has already become very weak and wetting the cow can enhance the latent heat loss from skin. Therefore, in order to avoid misapplication of cooling interventions, it is crucial to consider the climatic factors independently and it would be of great benefit to have a model, like the one presented in Chapter 5, to evaluate the effects of various cooling approaches before implementation and to apply these systems in a smart and effective way.

6.4 Future Research

Solving heat stress is one of the primary challenges facing current dairy farming world-wide. Although progress has been made in this research area within this thesis, such as quantification of effects of various environmental factors on responses of cows, development of a reliable model for thermal status prediction, development of advanced measurement devices, and prediction of benefits of different cooling methods and their limitations, there are still several related areas with further research interest, which are:

1. The animal experiment was conducted in climate-controlled respiration chambers to avoid confounding effects, which allowed us to study the relationship between physiological responses and ambient temperature and duration of high temperatures. However, cows in this

study were not free to walk around or play with others like on a real farm, which would have certain effects on the physiological responses to heat load. We recommend validating these results found under semi-lab conditions in practical circumstances. In addition, the potential delayed response of fat-and protein-corrected milk yield needs further study.

2. The skin ventilated box used in this experiment could only measure the latent heat loss from the skin, whereas we converted this latent heat loss to sweating rate to use it in our thermoregulatory model assuming the sweat was fully evaporated. It would be more accurate if we could design and develop a device measuring sweating rate accurately.

3. In our thermoregulatory model, we did not consider the conductive heat transfer between skin surface with the floor. It would be helpful to include the conductive heat transfer sub model to help predicting the effect of using cooling mattresses for cows.

4. It is of great importance to conduct an experiment using different cooling systems to validate our model predictions for the effects and limitations of cooling methods.

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Summary

Concern for animal welfare and comfort has increasingly grown in the past decades. Heat stress in dairy cows is an important and challenging issue on health, welfare and productivity in modern intensive farming systems, and negative effects of heat stress are likely to increase due to global warming. The cow is a homeothermic animal, which means that her body core temperature should be maintained within a narrow range. When exposed to ambient temperatures that exceed her biological thermal comfort zone, the cow will directly respond to maintain thermal balance. Heat stress in this thesis is therefore defined as the state at which the cow is out of her thermal comfort zone. Typical responses of cows under heat stress are increased respiration and sweating rate, and lowered feed intake and milk yield. The responses of cows to the same ambient temperature might be different in combination with various relative humidity and air velocity levels, but detailed quantitative information from the literature is lacking. In this thesis, we aimed to gain better knowledge about the responses of dairy cows to various thermal environments and to have a tool that can accurately predict early signs of heat stress in dairy cows.

A general introduction of this thesis is presented in **Chapter 1**. Firstly, the current issue of heat stress in dairy cows is introduced and the theoretical concept of Mount (1979) how cows respond to keep thermal balance of the body is described. Behavioral and physiological responses in the comfort zone, thermal neutral zone and above are described. Secondly, the studies on actual physiological and thermoregulatory responses of cows under various environmental conditions are reviewed as well as quantitative models predicting the thermal status of cows. Thirdly, the gaps existing in the current knowledge which limit our ability to improve the efficiency for detection and prevention of heat stress are identified. Finally, the main objective of this thesis is described as to have better knowledge about the responses of dairy cows to various thermal environments and to have a tool that can accurately predict early signs of heat stress in dairy cows.

Given the main objectives of this thesis, an experiment was designed and conducted in climate-controlled respiration chambers (results reported in **Chapter 2, 3 and 4**). Twenty Holstein Friesian dairy cows were randomly assigned to five treatments combining different levels of air temperature (T), relative humidity (RH) and air velocity (AV). The climate was programmed to follow a daily pattern of lower night and higher day T with a 9°C difference within 8-d experimental period. The ambient T was gradually increased from 7 to 21°C during the night (12 h) and 16 to 30°C during the day (12 h), with an incremental change of 2°C per day for both nighttime and daytime T. During each experimental period, RH and AV were kept constant for the 5 treatments (RH and AV combinations). A diurnal pattern for RH was created, with lower levels during the day and higher levels during the night: low (30–50%), medium (45–70%), and high (60–90%). The effects of 3 AV levels were studied: no fan (0.1 m/s), fan at medium speed (1.0 m/s), and fan at high speed (1.5 m/s). In **Chapter 2**, we determined the inflection point temperatures (IPt), at which cows start to respond to keep its thermal balance, at different RH and AV levels. Respiration rate, skin temperature and rectal temperature were measured twice a day. Increased respiration rate was the first indicator showing that the cow was responding to a high ambient T. Results showed that the effect of RH on IPt for respiration rate was significant. At high RH level (60%) cows responded with an increased respiration rate approx. 5°C earlier than at low RH level (30%) (18.9 vs. 25.5°C). The increase in rectal temperature was an indicator that ambient temperature was above the upper limit of the thermal neutral zone, and IPt for increased rectal temperature varied between 20.1 to 25.9°C under different RH and AV levels. Higher AV levels lowered skin temperature (31.6 vs 33.8°C at ambient T of 18°C at high and low AV levels), but the skin temperature difference at different AV levels became smaller with increasing ambient T (35.8 vs 36.1°C at ambient T of 30°C at high and low AV levels). The effects of exposure time (1 h vs. 8 h) on physiological responses to increased ambient T were significant, which means that cows responded with additional physiological changes at lower ambient T if forced to remain in hot conditions for a longer time.

In order to better understand the adaptive physiological mechanisms of cows to different thermal environments, we studied the heat dissipation from skin and through respiration (**Chapter 3**). Sensible and latent heat loss from the skin surface were measured using a ventilated skin box placed on the belly of the cow. Sensible and latent heat losses from respiration were measured with a face mask covering the cow's nose and mouth. Heat loss from the skin showed a dominant share (70 - 80%) of the total heat loss, whereas heat loss through respiration accounted for 20 to 30%. Skin sensible heat loss decreased (the decreasing rate of varied between -2.95 and -6.28 W m^{-2} °C^{-1}) while skin latent heat loss increased (the increasing rate varied between 2.74 and 13.83 W m^{-2} °C^{-1}) with the same extent increasing ambient T. Both, sensible and latent heat loss from the skin was positively impacted by AV. Sensible and latent heat loss through respiration were higher after long exposure (5.3 vs. 6.2 W/m^2 sensible and 53.1 vs. 61.7 W/m^2 latent heat loss under short and long exposures at ambient T of 32°C, respectively), most probably caused by the higher respiration rate and rectal temperature after long exposure as we found in **Chapter 2**.

In **Chapter 4**, we further investigated the latent heat loss from cows by making a complete water balance of the climate-controlled respiration chambers. The total evaporative water loss from dairy cows at daily level was quantified by measuring the mass of water in the incoming and outgoing air, the condensed water, the added water from the humidifier, the evaporative water from the wet floor/drinking bowl/manure reservoir/water bucket. The results showed that at high RH level and increasing ambient T cows tended to increase their evaporative water loss less compared to cows at low RH level. Furthermore, we found that the increase of latent heat loss was mainly due to an increase of evaporation (of sweat) from the skin. The results from the balance method were compared with the latent heat loss measured with the ventilated skin box (**Chapter 3)** and these methods showed no significant difference (P = 0.387). The bias of the local cutaneous evaporative water loss rate obtained by employing the ventilated skin box with the total rate determined by the water balance method was quite small (0.5%). However, the random deviation was larger (42%), but that could not only be accounted for by errors in the ventilated skin box method. These results mean that the ventilated skin box method, measuring only a small part of the skin during a short period during the day, can be a convenient way to determine the skin latent heat loss from cows, but with certain acceptable error.

In **Chapter 5**, a thermoregulatory model was developed to understand the responses and heat flows in dairy cows under various environmental (heat stress) conditions. Sub models of physiological regulation, including tissue resistance, respiration and sweating rate, under different ambient conditions were developed (based on literature) and specifically improved using the data from the experiment (**Chapter 2, 3 and 4**). This resulted in an improved model of the thermo-physiological responses of dairy cows to high ambient temperatures. This model was able to calculate dynamic changes in body core heat storage and the body and skin temperature variations during day and night with sufficient accuracy, with the root mean squared errors of prediction for body core and skin temperatures of 0.3 and 1.2°C, respectively. An additional benefit of the model is that it can also be used to predict the effects of different cooling methods on heat stress mitigation and their limitations.

A general discussion of this thesis is presented in **Chapter 6**. Firstly, the new findings about the thermoregulatory responses of dairy cows to increasing ambient T at various RH and AV levels are summarized. Secondly, based on the experimental results and the developed model, some simulations to predict the effects of current available cooling approaches under different climatic areas were conducted. We recommended a smart combination of wetting and air velocity was needed for effective and cost-efficient cooling of the cows depending on the exact environmental situation (T & RH). And the simulation of evaporative cooling for ambient air

showed that it can be counterproductive if it is applied under humid conditions because the high RH can largely suppress the latent heat loss by sweating and respiration while the positive effect of a lowered air temperature cannot compensate this decline. Thirdly, the limitations of the thesis are discussed and some ideas for future research are proposed. Experiment was conducted in climate-controlled respiration chambers and cows in this study were not free to walk around or play with others like on a real farm, and we recommend to validate the results in practical circumstances. Besides, we did not consider the conductive heat transfer between skin surface with the floor in our thermal balance model. It would be helpful to include it for future assessment of the effects of cooling mattresses.

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About the Author

Mengting Zhou was born in Ningbo, China on July 22nd, 1993. After graduating from high school in 2011 she started studying Vehicle Engineering at China Agricultural University and graduated in 2015. She received two master degrees within two years, one in Applied Mechatronics with a thesis entitled "Simultaneous localization and mapping (SLAM) based on LiDAR - Create map with pure LiDAR" from Harper Adams University (UK), and the other in Agricultural Engineering with a thesis entitled "Simulation analysis of reverse reconstructed D-bale knotter and structure optimization of rope releasing rod" from China Agricultural University in 2017. After that, she started her PhD study in the Farm Technology Group of Wageningen University & Research (Netherlands), funded by the Sino-Dutch Dairy Development Centre and China Scholarship Council. During her PhD studies, she specialized in livestock environment and welfare, and worked on measuring and modelling the effects of environmental conditions on thermoregulatory responses from dairy cows. In 2022, she had a short-term internship focusing on relationship between tidal volume and exhaling pressure from dairy cows, in Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Germany. Now Mengting has finished her PhD thesis and is ready to continue the scientific journey with passion.

Contact: zhoumt0720@gmail.com

List of Publications

Zhou M., Aarnink A.J.A., Huynh T.T.T., Van Dixhoorn I.D.E., and Groot Koerkamp P.W.G. 2022. Effects of increasing air temperature on physiological and productive responses of dairy cows at different relative humidity and air velocity levels. *Journal of Dairy Science*, 105(2), 1701-1716. *Editor's Choice*.

Zhou M., Huynh T.T.T., Groot Koerkamp P.W.G., Van Dixhoorn I.D.E., Amon T., and Aarnink A.J.A. 2022. Effects of increasing air temperature on skin and respiration heat loss from dairy cows at different relative humidity and air velocity levels. *Journal of Dairy Science*, 105(8), 7061-7078. *Editor's Choice*.

Zhou M., Groot Koerkamp P.W.G., Huynh T.T.T., and Aarnink A.J.A. 2022. Evaporative water loss from dairy cows in climate-controlled respiration chambers. (Under review)

Zhou M., Groot Koerkamp P.W.G., Huynh T.T.T., and Aarnink A.J.A. 2022. Development and evaluation of a thermoregulatory model for predicting thermal responses of dairy cows. *Biosystems Engineering.* (Accepted)

Talmón D., **Zhou M.**, Carriquiry M., Aarnink A.J.A., and Gerrits W.J.J. 2022. Effect of animal activity and air temperature on heat production, heart rate and oxygen pulse in lactating Holstein cows. *Journal of Dairy Science*. (Accepted)

Training and supervision plan

Training and Supervision		Graduate School WIAS
Name PhD candidate	Mengting Zhou	
Group	Farm Technology	
Promotor	Prof. Dr. Ir. P.W.G. Groot Koerkamp	
Co-promotor	Dr. Ir. A.J.A. Aarnink	
	Dr. T.T.T. Huynh	

Education and Training	Year
The Basic Package (2 ECTS[1])	
WIAS Introduction Day	2017
Research Integrity & Ethics in Animal Sciences	2017
Disciplinary Competences (17 ECTS)	
Research Proposal	2018
A PhD Course on CFD & Ventilation in Arhus University	2018
WIAS/PE&RC Advanced Statistics Course Design of Experiments	2018
MSc course Livestock Technology FTE30306	2018
Basic Statistics	2021
Professional Competences (4 ECTS)	
Information Literacy PhD including Endnote Introduction	2017
Presenting with Impact	2018
Project and Time Management	2018
WIAS course Techniques for Scientific Writing and Presenting	2018
Societal Relevance (2 ECTS)	
Societal impact of your research	2018
Presentation Skills (2 ECTS)	
WIAS Annual Conference, Wageningen, Netherlands, Poster	2021
ISAEW, Chongqing, China, Oral	2021
Teaching competences (5 ECTS)	
Supervising 1 MSc thesis	2021
Supervising 1 BSc thesis	2021
Supervising 1 MSc thesis	2022
Total = 32 ECTS	

[1]One ECTS credits equals a study load of approximately 28 hours.

Colophon

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