Aus dem Institut für Tier- und Umwelthygiene - ITU
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin
und dem
Leibniz-Institut für Agrartechnik und Bioökonomie - ATB
der Fachabteilung Technik in der Tierhaltung Potsdam

The influence of heat load in dairy cows housed in naturally ventilated barns in different climate zones

Inaugural-Dissertation
zur Erlangung des Grades eines
PhD of Biomedical Sciences
an der
Freien Universität Berlin

vorgelegt von
Severino Pinto
Tierarzt aus União da Vitória, Brasilien

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To the cows

“It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.” (Charles Darwin)

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List of Abbreviations

3xcool – Three cooling sessions per day

8xcool – Eight cooling sessions per day

AT – ambient temperature

bpm – breath/s per minute

NVB – naturally ventilated barn

RH – relative humidity

RR – respiration rate

THI – temperature-humidity index

1 INTRODUCTION

1.1 The impact of climate change on naturally ventilated barns (NVBs) and dairy production

As a consequence of long-term global warming, drier and hotter summers are becoming prevalent (WMO, 2018). These impacts are expected to worsen with ongoing climate change (Christensen et al., 2007). Thus, climate change may alter the main characteristics of livestock production systems (Gauly et al., 2013). Moreover, atmospheric conditions are major contributors to animal stress in warm and temperate climate conditions (Legates et al., 1991). Naturally ventilated barns (NVBs) have the advantage of being economical since natural ventilation does not require electrical energy to operate fans. Nevertheless, this housing system is particularly vulnerable to climate change because it depends on the immediate outside environment, which directly determines the microclimate conditions inside these barns (Hempel et al., 2018).

In Europe, the highly economically relevant dairy cattle sector is predominantly characterized by intensive milk production with high-yielding cows housed in naturally ventilated barns (Algers et al., 2009). Dairy cows under heat stress conditions experience significant issues, including reduced milk production, poor reproductive performance, poor health, and decreased animal welfare (Kendall et al., 2007, Gauly et al., 2013, Schutz et al., 2014). Due to the dairy cow's sensitivity to high temperatures, these negative effects are relevant for animals with high genetic merit (Kadzere et al., 2002). The responses to heat stress in dairy cows housed in loose housing systems are determined by a combination of environmental and animal-related factors. Some environmental parameters, such as temperature, relative humidity and air velocity, have been investigated to identify their effects on cow performance by establishing critical ambient temperatures for dairy cows (West, 2003). The most common environmental parameters include ambient temperature (AT) and relative humidity (RH) (Thom, 1959, NRC, 1971). The combined effect of both of these environmental conditions is presented as the temperature-humidity index (THI), which is calculated from an empirical formula that was first proposed for humans by Thom (1959).

1.2 Thermoregulation

Heat production and accumulation, combined with compromised cooling capability because of high temperature and humidity values in the surrounding environment, causes heat load in the cow (West, 2003, Polsky and von Keyserlingk, 2017). Consequently, heat stress in dairy cattle occurs when the capacity for heat dissipation exceeds the limit specified for normal activity and induces bodily adjustments to avoid physiological dysfunction (Kadzere et al., 2002, Dikmen and Hansen, 2009); this particularly affects cows with high milk yield which have already elevated internal heat loads, and their metabolic heat output is increased (Hahn, 1999, West, 2003). It is well documented that the optimal temperature range for dairy cows should be between -5 and 20 °C or for THI values to be below 68 or 72 (Armstrong, 1994, Hahn, 1999, Zimbelman and Collier, 2011). In an attempt to maintain their body temperatures, the animals adjust their behavioral and physiological responses to promote increased heat loss (Berman, 2005, Soriani et al., 2013).

1.3 Heat stress indicators

Responses of cows under heat stress conditions are homeostatic mechanisms that include increased water intake as well as the loss of body fluids due to sweating and panting (Silanikove, 2000, Soriani et al., 2013). Eventually, in response to heat stress, dairy cows increase salivation, reduce their heart rate and feed intake, and consequently decrease their milk production (Kadzere et al., 2002, Costa et al., 2015). Although effects such as reduced dry matter intake, rumination time, milk yield and/or fertility are indicative of heat stress, dairy cows present these signs relatively late. There is a specific lag time after exposure to hot conditions (Moallem et al., 2010, Soriani et al., 2013, Schueller et al., 2014). Based on these attributes, it is impossible to achieve immediate control of the cow’s thermal adaptation process and prevent a negative impact on the well-being and productivity of cows (Kadzere et al., 2002, Costa et al., 2015). In contrast, physiological parameters such as heart rate, body temperature, and respiration rate (RR) have been shown to be adequate and timely indicators of heat stress in dairy cows (Moallem et al., 2010, Costa et al., 2015, Galán et al., 2018).

1.4 Respiration rate as an important parameter of heat stress assessment

The advantage of noninvasive measurement methods to determine stress conditions in dairy cows is that they do not cause additional disturbances to the animals and thus there is no negative impact on the well-being and production level of the cows. In particular, RR is a sensitive indicator for assessing heat stress in dairy cows (Tucker et al., 2008) because when cattle are exposed to fluctuating ambient temperatures, the RR is consistently affected with little or no lag period. The physiological reference range of the RR in cattle varies from 24 to 36 breaths per minute (bpm) (Rosenberger, 1979), but it may range between 15 and 36 bpm (Jackson and Cockcroft, 2008). There is some evidence that RR is influenced by ambient temperature and relative humidity. The RR is highly efficient in regulating body temperature under heat stress (Berman, 2005, Bernabucci et al., 2010, Polsky and von Keyserlingk, 2017) through endogenous heat loss directly from the body core via the respiratory tract (Legates et al., 1991, Silanikove, 2000). Among the major physiological parameters reported in the literature, RR and panting have long been used as well-suited parameters for heat stress monitoring in cows (Gaughan et al., 2000, Mader et al., 2006). When correlating the RR with climate conditions, Mader et al. (2006) demonstrated that body temperature in feedlot cattle under hot conditions was regulated by an increase in RR. Further, the RR increases earlier than the rectal temperature in cows under hot conditions, and it is the earliest indicator of a cow's response to fluctuating air temperatures (Ferreira et al., 2006, Costa et al., 2015). This increment allows the cow to initiate heat dissipation before a significant increase in body temperature and before subsequent changes in normal body functions occur (Berman, 2005). In addition, Brown-Brandl et al. (2005) affirmed that the RR is a reliable physiological parameter under different weather conditions because it is easy to monitor without costly equipment.

1.5 Housing and animal management options for heat relief in heat-stressed dairy cows

In the context of climate change, the annual temperature in humid continental areas is expected to rise 2-4 °C by 2050, and heat mitigation systems will become increasingly popular (Fournel et al., 2017). Thus, management strategies to minimize the effects of heat stress have been emphasized in recent years. The effects of hot, humid conditions are thought to be mediated through an effect on cow body temperature (West, 2003). A broad spectrum of strategies can be used to mitigate the negative impact of heat stress on dairy production and profitability (Berman, 2006, Avendano-Reyes et al., 2010), but physical modification of the barn environment is the primary means.

In an attempt to mitigate the heat load in cows, alterations in housing and animal management strategies have been applied in hot environments (Polsky and von Keyserlingk, 2017); examples include the physical modification of the environment (e.g., shading, cooling), the genetic development of heat-tolerant breeds, and the improvement of nutritional management (West, 2003). However, there is limited information available regarding the efficiency of physical applications to abate thermal stress in cows under hot conditions. The typical methods can be divided into two groups: 1) modifying the environment to prevent reaching or limiting the time under the threshold temperature and 2) enhancing the heat exchange between cows and their environment. This heat exchange generally involves increasing heat loss from the body surface by mechanisms such as conduction (direct contact with a surface), convection (contact with a moving fluid), and evaporation (liquid-to-vapor phase change through the respiratory system and skin) (Fournel et al., 2017). In practice, this involves a combination of water and high wind speed, which increases the cooling rate by wetting cows or the air around them and thereby increase the convective heat transfer rate over the animals (West, 2003).

Approximately 30 years ago, sprinklers and forced ventilation were implemented in open shelters to alleviate heat stress in dairy cows (Berman et al., 1985). With the addition of water into the air with sprinklers and in combination with high wind speed from the fans, the evaporative cooling system has become an important method to improve heat dissipation and maintain homeostasis in dairy cows (Valtorta and Gallardo, 2004, Kendall et al., 2007, Ortiz et al., 2015). Above 35 °C, evaporative cooling becomes the only method for heat dissipation in dairy cows (Burgos et al., 2007). These methods enable high milk production in hot regions by improving heat release (Ortiz et al., 2015, Fournel et al., 2017). Several studies have demonstrated the effect of evaporative cooling, considering the effects of different frequencies of evaporative cooling on heat abatement. The methods varied among two (Valtorta and Gallardo, 2004), four (Avendano-Reyes et al., 2010), five (Flamenbaum et al., 1986), eight (Honig et al., 2012), and nine (Her et al., 1988) cooling sessions per day. These previous studies, however, evaluated animal parameters as the RR of cows only twice per day or once per week, which reduces the reliability of the heat stress assessment. In addition, these studies did not consider the immediate effect of body reactions on cooled animals, both when the cooling management was being administered and after the cooling session.

Therefore, a better understanding of the effect of evaporative cooling management to enhance heat abatement in dairy cows (from a global warming perspective) may be relevant. Specifically, an evaluation of the effect of different cooling frequencies per day on cows' responses under heat load conditions and the immediate effect of cooled animals with increased daily data collection is necessary.

1.6 Cow-related factors

Susceptibility to heat stress is cow specific and is influenced by various factors such as age, sex, genotype, performance, and body condition (Gaughan et al., 2000, Kadzere et al., 2002). However, in most studies on heat stress in cattle, cow-related factors have not been considered in the heat stress assessment and thereby certain aspects regarding individual animal responses may have been overlooked. In a study conducted with eight lactating dairy cows under different THI conditions, the average RR observed was 60 bpm under THI = 69.3 ± 0.5 and 87 bpm during exposure to heat stress with THI = 74.1 ± 0.3 (Ominski et al., 2002). Despite similar climatic conditions, several studies on dairy cows have reported very different RR data; for example, Costa et al. (2015) reported 38.6 ± 1.54 (mean ± SEM) bpm in the Girolando cattle breed, Kendall et al. (2007) reported 50 ± 2.4 (mean ± SEM) bpm in Holstein cows, and Chen et al. (2015) reported 88 ± 16.5 (mean ± SD) bpm in Holstein cows, demonstrating that dairy cows, both individually and as a group, are very sensitive to external influences as individual factors and different management systems and climate zones. Due to these individual responses, it is a challenge in dairy production to identify an accurate and applicable heat stress assessment method.

Body posture is a relevant factor to be considered in cows under heat load conditions. Lying cows may show heat stress earlier, even with a decreased temperature threshold (Berman, 2005). Some authors have documented that milk yield (Hahn, 1999) and days in milk (Sharma et al., 1983) are associated with heat metabolic increases and, consequently, heat load in dairy cows (Kadzere et al., 2002, West, 2003). Kadzere et al. (2002) noted that high-producing cows are more affected by elevated temperatures than low-producing cows under heat stress because the metabolizable energy used for milk production results in an increased body temperature. In addition, it has been repeatedly demonstrated that under heat stress conditions, the RRs of the cows increase (Legates et al., 1991, Eigenberg et al., 2000), and the cows spend more time in a standing position than lying down (Schutz et al., 2010).

The influence of coat color has also been investigated in recent years in dairy cows under heat stress conditions (Kendall et al., 2007, Tucker et al., 2008). Dark cows were more susceptible to solar radiation absorption but also demonstrated higher rates of heat loss than cows with light coats (Maia et al., 2005, Tucker et al., 2008). In contrast, when considering cooled cows, the coat color did not influence the RR (Kendall et al., 2007); however, cows with white-colored coats showed a preference to stay in unshaded areas relatively longer (Frazzi et al., 2000).

Although several researchers have studied the effects of heat stress in dairy cattle, these studies did not consider the individual responses of cows under heat stress conditions and cow-specific factors as potential variables in the data analysis.

1.7 Objectives

Based on the current knowledge, it has become clear that to reduce the uncertainty in heat stress assessments in dairy cows under different environmental conditions, measurements in cows should be carried out continuously, and cow-related factors should be considered in the analysis.

The overall objective of this study was to investigate the influence of heat load on the RR in lactating dairy cows housed in naturally ventilated barns under different climatic conditions and management systems.

To attain this objective, the study was divided into two experimental designs that resulted in two publications:

The objective of paper “A” was to evaluate the effects of barn climate conditions and cow-related factors, specifically, body posture (i.e., standing or lying) and daily milk yield and their association with the RR of lactating dairy cows in Germany. Specifically, the aims were to 1) determine the correlation between the RR and THI, 2) investigate the differences in the RRs between standing and lying cows, and 3) evaluate the influence of daily milk yield on the RR.

The results of this study were published in the Annals of Animal Science (impact factor: 1.018).

The data for paper “B” were collected in Israel. The objective of this study was to evaluate the effect of two different cooling frequencies on the RR of lactating dairy cows considering cow-related factors. Specifically, the hypothesis was that 1) cows with three cooling sessions per day would have a higher RR than cows cooled with eight sessions per day, 2) differences in the RR could also be observed among the phases before, during and after cooling, and 3) body posture, milk yield, days in milk and coat color influenced the RR.

The results of this study were published in Annals of Animal Science (impact factor: 1.018).

2 PUBLICATION "A"

Influence of barn climate, body postures and milk yield on the respiration rate of dairy cows

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INFLUENCE OF BARN CLIMATE, BODY POSTURES AND MILK YIELD ON THE RESPIRATION RATE OF DAIRY COWS*

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Abstract

The main objective of this study was to identify the influences of different climatic conditions and cow-related factors on the respiration rate (RR) of lactating dairy cows. Measurements were performed on 84 lactating Holstein Friesian dairy cows (first to eighth lactation) in Brandenburg, Germany. The RR was measured hourly or twice a day with up to three randomly chosen measurement days per week between 0700 h and 1500 h (GMT + 0100 h) by counting right thoraco-abdominal movements of the cows. Simultaneously with RR measurements, cow body postures (standing vs. lying) were documented. Cows' milk yield and days in milk were recorded daily. The ambient temperature and relative humidity of the barn were recorded every 5 min to calculate the current temperature-humidity index (THI). The data were analyzed for interactions between THI and cow-related factors (body postures and daily milk yield) on RR using a repeated measurement linear mixed model. There was a significant effect of the interaction between current THI category and body postures on RR. The RRs of cows in lying posture in the THI < 68, 68 ≤ THI < 72 and 72 ≤ THI < 80 categories (37, 46 and 53 breaths per minute (bpm), respectively) were greater than those of standing cows in the same THI categories (30, 38 and 45 bpm, respectively). For each additional kilogram of milk produced daily, an increase of 0.23±0.19 bpm in RR was observed. Including cow-related factors may help to prevent uncertainties of RR in heat stress predictions. In practical application, these factors should be included when predicting RR to evaluate heat stress on dairy farms.

Key words: dairy cow, heat stress, temperature-humidity index, cow-related factors, naturally ventilated barn

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Barn climate conditions are major contributors to animal stress in warm and temperate climate zones (Legates et al., 1991). Heat stress in cows occurs when the capacity for heat dissipation exceeds the range specified for normal activity and induces body adjustments to avoid physiological dysfunction (Kadzere et al., 2002). Therefore, heat stress in dairy cattle is considered as an important cause for reduced production and animal welfare (West, 2003; Herbut et al., 2015). A combination of ambient temperature and relative humidity as in the THI (temperature-humidity index) formula is commonly used to estimate the effects of barn climate conditions on heat load of cows (Heinicke et al., 2018; Herbut and Angrecka, 2018 a). The heat stress threshold for dairy cows varies with a THI between 68 and 72 (Armstrong, 1994; Bryant et al., 2007; Zimbelman and Collier, 2011). In order to ensure homeostasis and facilitate the release of excess metabolic heat into the environment, cattle adjust their behavior and physiological reactions (Berman, 2005; Soriani et al., 2013). Heat stress can be evidenced by its effects on the performance of dairy cows, but these signs only become apparent late after the onset of heat stress (Moallem et al., 2010; Schueller et al., 2014). In contrast, the physiological parameter respiration rate (RR) has been shown to be a reliable and early indicator of heat stress in dairy cows (Kabuga, 1992; Gaughan et al., 2000; Chen et al., 2015). In addition, measuring RR has the advantage of being a non-invasive method that can determine stress in dairy cows without causing additional disturbance to the animals (Aharoni et al., 2005).

The RR of dairy cattle under thermo-neutral conditions ranges from 15 to 36 breaths per minute (bpm) (Rosenberger, 1990; Jackson and Cockcroft, 2008) and is influenced by THI and cow-related factors as body postures and milk yield (Kadzere et al., 2002; Berman, 2003). In a previous study of eight lactating Holstein dairy cows, the average observed RR was 60 bpm under THI = 69.3±0.5 and 87 bpm during exposure to heat stress (THI = 74.1±0.3) (Ominski et al., 2002). In addition to THI, various factors can influence the susceptibility of dairy cows to heat stress, such as sex, breed, body postures, lactation phase and milk production as well as shading and lack of shade (Gaughan et al., 2000; Berman, 2005). Despite being performed under similar THI range of 69 to 72, several studies of dairy cows have reported very different RR data, for example, 38.6±1.54 (mean±SEM) bpm in Costa et al. (2015 a), 50±2.4 (mean±SEM) bpm in Kendall et al. (2007), and 88±16.5 (mean±SD) bpm in Chen et al. (2015). Although studies of heat stress in dairy cows have demonstrated the impact of heat load on RR, body postures and milk yield have been considered as potential influential variables in previous data analyses.

Body posture is important as recumbent animals may show heat stress at lower temperatures than do standing animals (Berman, 2005; Herbut and Angrecka, 2018 b), and cows prefer to ruminate in a lying posture (Acatincăi et al., 2010). In addition, high yielding cows have a higher risk to suffer from heat stress in elevated temperatures (Kadzere et al., 2002; Gauly et al., 2013).

Based on current knowledge (Gaughan et al., 2000; Kadzere et al., 2002; Berman, 2005), the precise assessment of heat stress in dairy cows under varying THI requires the inclusion of cow-related factors such as body postures and milk yield into the assessment. In our study, we tested the hypotheses that: cows under high THI conditions show higher RRs; lying cows show higher RRs than standing cows; and cows with higher milk yields have higher RRs under different THI. Therefore, the objective of the present study was to evaluate the effects of barn climate conditions and cow-related factors, specifically, body postures (i.e., standing or lying) and daily milk yield, on the RR of lactating dairy cows.

Material and methods

Animals, housing and management

The study was conducted on the research dairy farm of the Agricultural Research and Education Center for Animal Breeding and Husbandry "Gross Kreutz" in Brandenburg, Germany (coordinates: 52°23'47.4"N, 12°46'02.8"E). The climate of this region is continental.

The data were collected during two time periods from June to August 2015 (hot period) and from January to April 2016 (cold period). The measurement days were chosen randomly based on expected weather conditions to cover a wide range of different situations. The experimental barn was designed for a capacity of 51 cows. The experimental animals were all lactating Holstein Friesian dairy cows from the first to eighth lactation. The group was a high-yielding group, and cows that dropped below 30 kg milk per day usually left the group within a few days. During the experimental period, the health status of the cows was constantly evaluated by a veterinarian who selected only healthy cows for the measurements. The cows were milked 3 times a day by an automatic milking system (AMS, Lely Astronaut A4, Maassluis, the Netherlands). The average daily milk yield was 41.08±6.72 kg per cow and the minimum yield observed in this high-yielding group was 25 kg per cow. The days in milk (DIM) ranged from 7 to 337 (mean±SD: 118.3±67.1) during the study. Once a week, the body condition score (BCS) of the cows in the experiment was assessed, and the mode of the scores was 2.75 on a 1 to 5 point scale with 0.25 increments.

The cows were fed a totally mixed ration twice a day. Additional concentrate was fed in the AMS based on individual DIM and milk yield. The animals were housed in a naturally ventilated barn, as already used by Heinicke et al. (2018) and by Hempel et al. (2018), aligned in an NE-SW orientation with a floor area of 686 m^2 (13.7 m^2 per cow). The feeding alley was 27.7 m long (animal feeding place ratio of 1:1). The cows had access to 51 lying cubicles with a mixture of straw and lime as bedding material, 34 of which were arranged in a double row and 17 in a single row. An automatic scraper removed manure from the concrete walking alleys approximately once per hour. The waiting area in front of the AMS had a slatted floor.

Animal measurements

The RR was observed visually by counting right thoracoabdominal movements for thirty seconds and multiplying the value by two (i.e., breaths per minute, bpm) at a distance of approximately 15 m between the animal and the observer, which is a method adapted from Kabuga (1992). In a pilot test, three was determined as the appropriate number of observers to reduce the variation in RR measurements between observers, so three observers were used for RR measurements during the experimental period. In the pilot test, the maximum difference found between two observers was 6 bpm, and the average relative difference between observers based on this maximum was 25.4% (95% confidence limits: 21.2 to 30.2%) or approximately 1.5 bpm as an absolute value.

Two RR datasets were collected. The first dataset was collected from a group of 30 multiparous cows per day, whose RR was measured two times per day (i.e., between 0700 h and 1000 h and between 1100 h and 1400 h; GMT + 0100 h). For the second dataset, the RR of 15 primiparous and multiparous cows per day was observed hourly (i.e., from 0700 h to 1500 h; GMT + 0100 h). At the beginning of both time periods the cows were randomly selected from the herd and always the same cows were used per experimental day. Between the measurement days, some cows were replaced by others due to management decisions (e.g., health status, milk yield, dry period stage). Therefore, a total of 84 cows were included in the analysis over the whole time period. The cows were categorized into three lactation number groups and two DIM subgroups as follows: first and second lactation with subgroups in DIM < 100, in DIM ≥ 100 and cows in the group of third or greater lactation without further subdivision into DIM groups. According the lactation number and DIM, there were some cows participating in both dataset collections. The time of the data collection was chosen to comprise a representative range from low to high ambient temperatures during the day period. Both datasets were collected during both time periods in 2015 and 2016, with up to three measurement days per week. This approach was used to account for variability between cows and within cows. Thirteen cows were present during both measurement periods. Cow body postures (i.e., standing vs. lying) was documented during the RR measurements. Relevant cow data (i.e., milk yield and DIM) were noted from the herd management system (Herde 5.9, DSP-Agrosoft GmbH, Ketzin, Germany).

Environmental measurements

The ambient temperature (AT) and relative humidity (RH) of the air in the barn were recorded every 5 min with eight data loggers (EasyLog USB 2+, Lascar Electronics Inc., Whiteparish, UK) positioned 3.4 m above the floor at eight locations inside the building according to the methodology published by Hempel et al. (2018). Mean AT and mean RH of all loggers were calculated at each time point. The temperature-humidity index was calculated according to NRC (1971) as follows:

$$THI = (1.8 \times T°C + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T°C - 26)$$

where:

$T°C$ is the dry bulb temperature (in °C) and RH is the relative humidity (in %). The categories of *THI* for heat stress in dairy cattle were assigned according to Zimbelman and Collier (2011), adapted from Armstrong (1994), as follows: $THI < 68$ as no stress; $68 \leq THI < 72$ as the stress threshold; $72 \leq THI < 80$ as mild stress; $80 \leq THI < 90$ as moderate stress, and $THI \geq 90$ as severe stress.

Statistical analysis

The data from both datasets of RR collections were pooled for statistical analysis. Initially, the RR values were linked to the THI values from the start of every five-minute interval for the analysis. A regression analysis of RR as a function of THI was performed separately for standing and lying cows. An exponential function with base "e" was fitted to the observed RRs. To allow comparisons with the stress levels found in the literature, THI was classified as described above in *Environmental measurements*. A linear mixed model with repeated measurements for each cow was used to test the influences of the environmental and cow-related factors (body postures, daily milk yield) on RR. The model assumed normally distributed residuals with homogeneous variance, and these prerequisites were checked visually after fitting the model. The lactation number category was not included in the model, because the measurements during the trial periods were not carried out with the same cow in different lactations. The fixed factors in the model were THI category (THI < 68 no stress; 68 ≤ THI < 72 stress threshold; 72 ≤ THI < 80 mild stress; 80 ≤ THI < 90 moderate stress, and THI ≥ 90 severe stress) and body postures (standing vs. lying). The interaction between body postures and THI category was also included. The co-variables were daily milk yield and the interaction of daily milk yield with THI category. The random cow effect considered a cow-specific intercept, as well as an interaction between body postures and THI category. The model was as follows:

$$Y_{ijkl} = \mu + p_i + thi_j + (p \times thi)_{ij} + td_m + a \cdot dmy + b_j \cdot dmy + cow_{ki} + p_{ik} + thi_{jk} + (p \times thi)_{ijk} + e_{ij}$$

where:

Y_{ijkl} is the observed RR on the *k*th cow during the *l*th measurement in postures i and in temperature-humidity index category j on the *m*th test-day, μ is the mean RR, p_i is the cow's body postures, thi_{ijl} is the temperature-humidity index category, td_m is the fixed test day effect, *dmy* is the daily milk yield with the regression coefficient α for the general slope and the regression coefficient b_j for the interactions with the THI category, cow_{kl} is the random cow effect with p_{ik} and thi_{jk} as cow-specific postures and THI category effects, and e_{ijkl} is the residual.

A variance component covariance structure was used for random effects and repeated measurements. Factor influences were tested at a significance level of 0.05. The differences between the factor levels of the significant factors were post hoc tested by t-tests in multiple pairwise comparisons. The P-values of those multiple comparisons were adjusted by a simulation of the true 95%-quantile of the contrasts, maintaining a global significance level of 0.05. Model viability was checked by a visual examination of the residuals (homogeneity of variance and normality). All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Environmental parameters

Overall, the mean values for AT, RH, and THI during the measurements (from June 2015 to April 2016) were 17.72±8.83°C (mean±SD), 74.02±18.39%, and 61.82±13.52, respectively. Table 1 shows the monthly barn climate conditions during the experimental period of the present study.

Table 1. Barn climate characteristics during the experimental period (June 2015 to April 2016)

	Temperature (°C)			Relative humidity (%)			Temperature-humidity index		
	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max
2015									
June	22.4±3.8	14.1	29.4	55.3±12.9	32.4	87.1	68.4±4.5	57.5	76.1
July	21.4±4.4	13.6	31.3	63.8±14.5	34.4	93.5	67.7±5.5	56.6	79.3
August	23.5±5.1	16.0	34.5	70.9±17.1	36.2	95.5	70.9±6.5	60.7	85.1
2016									
January	4.7±4.9	–8.3	11.9	94.9±5.2	77.6	99.8	40.8±8.9	17.8	53.6
February	4.5±2.7	–0.9	10.2	92.0±8.2	72.7	100.0	40.7±5.2	30.6	51.5
March	6.4±2.0	–0.3	11.1	91.9±7.9	60.9	100.0	44.1±3.9	31.8	53.2
April	12.1±3.2	5.9	20.5	76.2±11.6	54.5	95.1	54.2±5.2	43.5	67.0

Animal-related parameters

The dataset of 2922 animal observations (i.e. THI < 68: 1536; 68 ≤ THI < 72: 342; 72 ≤ THI < 80: 784 and 80 ≤ THI < 90: 260) from a total of 84 cows with between two and nine observations per day each during the whole experimental period of 54 days was included in the model. Table 2 shows the mean RR for lying and standing cows in different THI categories including the effect on RR among THI categories. The RR differed significantly between all THI categories and body postures (lying vs. standing), except for between the postures in the 80 ≤ THI < 90 category. In the categories with THI values less than 80, lying cows showed higher RRs than standing cows. There were significant interactions between THI category and body postures (P<0.01). In both standing and lying cows the RR increased with increasing THI value (Table 2).

There was a significant influence of milk yield of cows on RR in different THI categories (P=0.0056). Figure 2 shows the regressions and confidence limits of the effect of body postures (standing vs. lying), daily milk yield of cows in kg produced per day and in different THI categories. The average increase in RR was 0.23±0.19 (mean±standard error) per additional kg of milk produced beyond a 25 kg daily milk yield (Figure 2). Cows with a high milk yield of 60 kg per day tended to present higher RR (9 bpm) than cows with a low milk yield of 25 kg per day.

Table 2. Least-square means of respiration rate (RR in bpm) for interactions of different cow postures and temperature-humidity index (THI) categories (n = 84 cows)

THI category	Respiration rate Standing posture		Respiration rate Lying posture		P-value
	mean	SEM[2]	mean	SEM[2]	
THI < 68	30.0	0.78	36.9	0.85	<0.01
68 ≤ THI < 72	37.9	0.99	46.1	1.37	<0.01
72 ≤ THI < 80	44.6	0.88	52.7	1.07	<0.01
80 ≤ THI < 90	75.4	1.39	68.9	2.14	0.07
P-value	<0.01		<0.01		

[2]Standard error of the mean (SEM).
Multiple custom pairwise comparison was performed (α=0.05).
P-values in the last column indicate respiration rate differences according to body postures.
P-values in the last row indicate respiration rate differences according to THI categories.

RR increased with increasing THI category (P<0.001) regardless of body postures. Figure 1 shows data of individual cow RR in bpm in different body postures (standing vs. lying) depending on the THI category. High variability of RR among cows was observed under identical THI conditions. The variability increased with increasing THI.

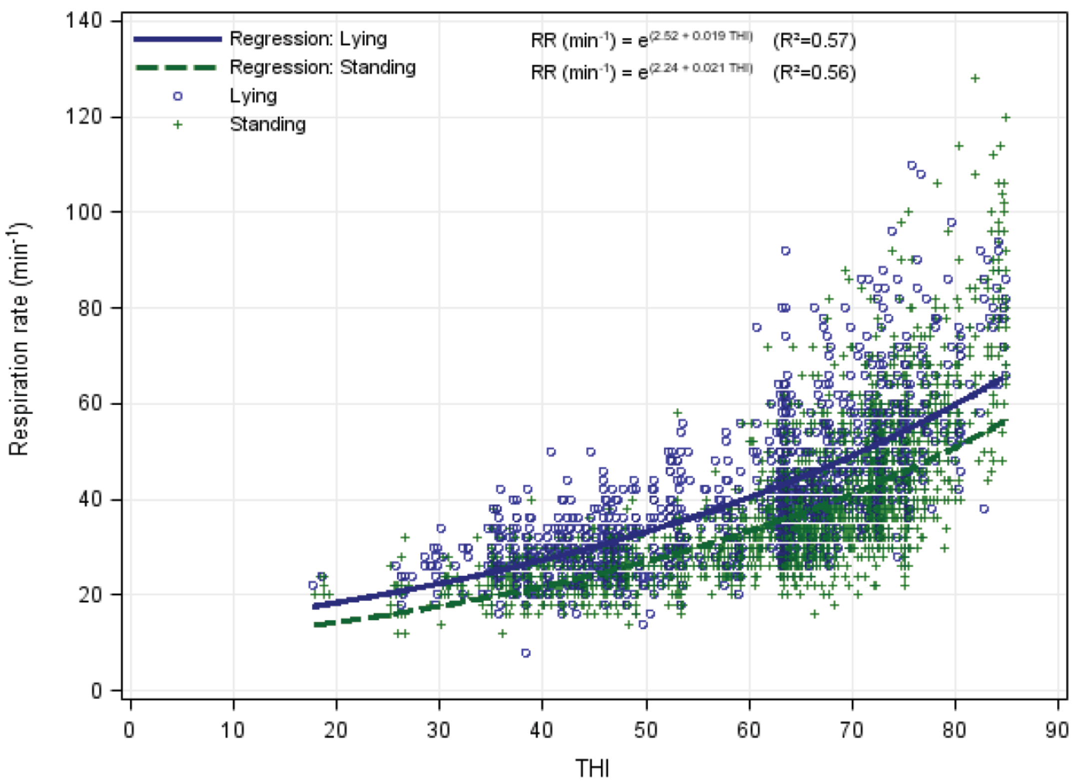

Figure 1. Individual cow respiration rate per minute with regard to body postures: standing (+) and lying (○). The dotted line denotes the regression analysis for the standing posture, and the solid line denotes the regression analysis for the lying posture

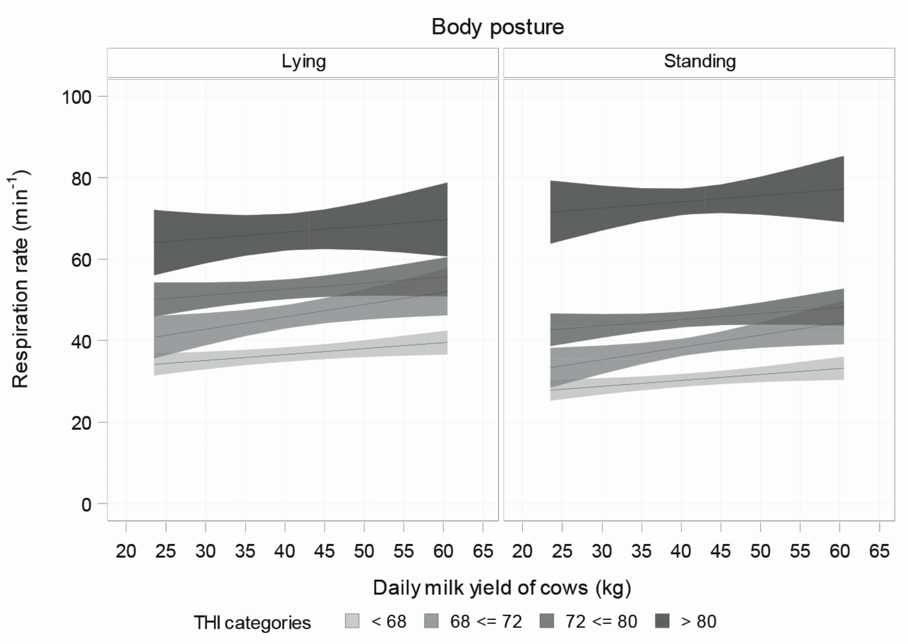

Figure 2. Respiration rate (RR) in breaths per minute (bpm) of cows in different body postures (standing vs. lying), including daily milk yield of cows and in different THI categories (THI < 68; 68 ≤ THI < 72; 72 ≤ THI < 80 and 80 ≤ THI < 90). Regressions (lines) as well as the 95% confidence limits (shaded bands) for mean RR as a function of milk yield are shown

Discussion

Effects of THI on physiological parameters such as RR have been documented before (Kabuga, 1992; Brown-Brandl et al., 2005; Chen et al., 2015) and were confirmed by our study. In the present study, the data showed a RR of 30±8.51 (mean±SD) bpm for standing cows and a RR of 37±11.1 bpm for lying cows at THI below 68. Jackson and Cockcroft (2008) reported that the physiological RR of cows ranges from 15 to 36 bpm, but like in other previous studies no differentiation was made between standing and lying posture. However, the results of our study differ from results of Garner et al. (2017), who considered THI between 55 and 61 as thermoneutral conditions for high-yielding dairy cows (mean daily milk yield: 23 kg/cow) and observed RRs between 43 and 56 bpm. We supposed that the RR do not differ solely due to the THI conditions to which the cows were subjected in this study. Various factors such as body postures and milk yield may influence the respiration rate of cows. Some works confirm that the individual cow factors can influence the physiological parameters and susceptibility of dairy cows to heat stress (Gaughan et al., 2000; Berman, 2005), however, those cow factors were not included in the analysis of Jackson and Cockcroft (2008) and Garner et al. (2017). Further studies (using the same THI formula) in which THI < 62 (recently freshened cows; Spiers et al., 2004), and THI < 75 (non-lactating multiparous cows; Ferrazza et al., 2017) were defined as no heat stress conditions observed different RR results as 59 bpm and 39 bpm, respectively. Therefore, there seemed to be an effect of lactation period and milk yield on the RR of cows, even under thermoneutral conditions.

The mean RR in the 68 ≤ THI < 72 category increased by 21.9% (9 bpm units) compared to that in cows under THI < 68 conditions. The THI of 68 is considered as the heat stress threshold of dairy cows (Bryant et al., 2007; Zimbelman and Collier, 2011). When THI increased to ≥ 80 in our study, RR increased by 39 bpm over those observed under THI < 68 conditions. Influences of heat stress on RR were also observed in a study with eight steers carried out by Brown-Brandl et al. (2005), in which RR differed by 15 bpm between baseline and heat stress levels. The more significant increase in our study may be explained by the use of high-yielding dairy cows instead of steers. In another study conducted with eight lactating cows (producing 37.4 kg of milk per day), RR increased from 60 bpm (THI = 69) to 87 bpm (THI = 80; Ominski et al., 2002).

Based on THI conditions of 68 ≤ THI < 72, an average bpm of 42±11.9 (mean±SD) was observed in our study. This RR value is comparable to the value (37 bpm) reported in a previous study but with a mean THI of 74 (Ferrazza et al., 2017). Previous studies described large variation among cows under similar environmental conditions. The RR varies among different studies in THI conditions ≤ 73, with reported RR values of 54±2.4 (mean±SEM) bpm (Kendall et al., 2007), 60±1.9 bpm (Ominski et al., 2002) and 67±3.7 bpm (Brown-Brandl et al., 2005; Costa et al., 2015 b). Our study confirms a high variability in RR (28 to 38 bpm) among cows under conditions of THI < 68. In dairy cows under an average THI condition of 74, Ferrazza et al. (2017) observed a RR variation between 26 and 61 bpm. It is plausible to assume that the observed variability in RR is indicative of differences in heat stress adaption among cows (Kendall et al., 2007). Hence, the cows are not adapted to hot conditions, the cows tend to react individually and might reduce the milk production and animal welfare (Herbut and Angrecka, 2013).

Measurement locations also played a role in our study: although our environmental data were collected by eight loggers positioned in different locations inside the building at 3.4 m height, THI varied by up to±2 units among locations within the barn, which is in agreement with a previous study (Hempel et al., 2018). Microclimates can be observed in different areas within a given barn (Herbut et al., 2015). Our study had a distinctively greater spatial resolution of THI measurements due to the higher number of loggers inside the barn, a real environmental situation could not be observed. However, Hempel et al. (2018) affirmed that positioning of the loggers between 3.4 and 4 m height is suitable for a representative presentation of the barn environment because below this range (i.e., in animal occupied zone), deviations in relative humidity of the environmental data can be observed. In addition, in most studies investigating heat stress in the field, ambient temperature and humidity measurements were obtained either from nearby weather stations or on site at one or two locations (Schueller et al., 2014).

Several factors must be considered when investigating the relationship between THI and RR. Previous studies reported influences of the body postures on the wind convection of cows. Lying cows show a decrease of 42% of the body surface area in heat dissipation compared to standing cows (Frazzi et al., 2000; Wang et al., 2018). To our knowledge, this is the first study reporting that body postures influenced RR under different THI conditions. The influences of individual cow factors such as milk yield, body condition and behavior on the physiological reactions of cows has been described in previous studies (Tucker et al., 2008; Schutz et al., 2010; Gauly et al., 2013). However, these previous studies did not focus on the influences of cow-related factors such as body postures on the RR of heat-stressed dairy cows. Our results show that the RR values of lying cows were significantly greater than those of standing cows under the same THI conditions. Cows in a lying posture showed 7±0.51, 8±1.32, and 8±0.88 (mean±SEM) bpm more than did standing cows under no stress, at the stress threshold, and in mild heat stress, respectively. The importance of body postures suggests that lying cows may develop heat stress earlier and at a lower temperature threshold than do standing cows (Berman, 2005). The straw bedding used in the present study might increase the heat load in lying cows; hence standing cows are more exposed to airflow and increase the wind convection (Wang et al., 2018). In THI conditions > 74, cows avoided to rest on the straw bedding during the day (Angrecka and Herbut, 2017). In addition, some authors have suggested that the body contours of cows change when they lie down, causing the rumen to compress the diaphragm and thereby reducing lung capacity and respiration effectiveness (Santos and Overton, 2001; Tucker et al., 2008; Reece and Rowe, 2017). This phenomenon was observed in our results even under low or absent heat stress conditions.

In conditions of THI ≥ 80, the cows showed no significant differences in RR due to body postures. We observed that when cows were already under high heat stress conditions, body postures was not a load factor for RR response, with an average RR of 72 bpm. The prevalence of RRs of approximately 70 to 80 bpm suggests that these RRs provide heat stress relief for the cows (Stevens, 1981; Berman, 2005). High RR values are associated with long periods of standing to release heat efficiently by wind convection and avoid the breathing discomfort of lying down (Frazzi et al., 2000; Berman, 2005; Wang et al., 2018). Soriani et al. (2013) observed a negative relationship between rumination time and respiration rate in lactating cows under heat stress conditions, although rumination time in the lying down postures enhances production as well as cow comfort (Acatincăi et al., 2010; Herbut and Angrecka, 2018 a).

Our results demonstrated significant differences in RR in bpm with respect to daily milk yield in addition to body postures. The RR increased with increasing daily milk yield. The respiration rates of cows with a milk yield of 60 kg per day were 9 bpm higher than those of cows with a 25 kg milk yield per day. High-yielding cows are likely to be more affected by THI increases, because of the metabolizable energy used for milk production (Hahn, 1999; Kadzere et al., 2002), where high-producing cows have significantly more heat to dissipate than low-producing cows (West, 2003; Herbut et al., 2015). Published studies considering RR reactions in relation to milk yield in dairy cows are not common. In a recent study with lactating cows conducted by Santos et al. (2017), the authors did not observe changes in RR with regard to milk yield level, although, the cows included in their study had an average milk yield of 20 kg in comparison with 41 kg in the present study. Furthermore, Dikmen and Hansen (2009), who conducted a study about rectal temperature in lactating cows, did not identify a relationship between milk yield and rectal temperature.

In conclusion, the present study provides quantified evidence that the respiration rate (RR) in dairy cows increases with THI. The effects of body postures and milk yield on RR under different THI conditions were determined. Cows in a lying posture showed higher RRs than standing cows in "no stress" (THI < 68), "stress threshold" (68 ≤ THI < 72) and "mild stress" (72 ≤ THI < 80) THI categories. The RRs in high-producing cows (> 25 kg milk per day) increased for each additional kilogram of milk produced. The consideration of cow-related factors (body postures and milk yield) can reduce the uncertainty in the correlations between RR data and heat stress assessments. Further research is necessary to verify whether body postures and milk yield influence the RR of lactating dairy cows under hot climate conditions and under different management strategies for heat stress relief (e.g., cooling). Our results support the use of RR as an early heat stress indicator. Determining the differences between cows within the same THI category with greater precision will require the development of an RR sensor or a corresponding learning algorithm for individual animals.

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Effect of two cooling frequencies on respiration rate in lactating dairy cows under hot and humid climate conditions

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EFFECT OF TWO COOLING FREQUENCIES ON RESPIRATION RATE IN LACTATING DAIRY COWS UNDER HOT AND HUMID CLIMATE CONDITIONS*

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Abstract

The aim of this study was to evaluate the effects of evaporative cooling at two different frequencies per day on the respiration rate (RR) of lactating dairy cows, considering cow-related factors. Twenty multiparous Israeli Holstein dairy cows housed in a naturally ventilated cowshed were divided randomly into two treatment groups. The cows of both groups were exposed to 3 or 8 cooling sessions per day (3xcool vs. 8xcool, respectively). The RR was observed hourly, with a maximum of 12 measurements per day. Body posture (standing vs. lying) was simultaneously documented. Milk yield was recorded daily. Coat color was determined from a digital photograph. The RR of standing and lying cows was lower in the 8xcool group (60.2 and 51.6 breaths per min (bpm), respectively) than in the 3xcool group (73.1 and 65.6 bpm, respectively). For each increment of five kilograms of milk produced, RR increased by one bpm, and the RR of cows in early days in milk (DIM) was 12.3 bpm higher than that of cows in late DIM. In conclusion, eight cooling sessions per day instead of three lead to a RR abatement in heat-stressed cows under hot conditions, and cow-related factors directly impact the RR during heat stress assessment.

Key words: heat stress, evaporative cooling, cow-related factors, precision livestock farming (PLF), animal welfare

Heat stress in dairy cows is considered an important problem hindering production, reproductive performance, and animal welfare (Kendall et al., 2007). These

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negative effects are relevant for dairy cows with high genetic merit, which are considered to be more sensitive to heat stress (Kadzere et al., 2002). Cows with high milk yield performances produce more body heat as a result of metabolic processes (Hahn, 1999; West, 2003). Respiration rate (RR) is one possibility to regulate the body temperature under heat load (Bernabucci et al., 2010; Polsky and von Keyserlingk, 2017) through endogenous heat loss via the respiratory tract (Legates et al., 1991). Therefore, RR is a sensitive indicator to assess heat load in dairy cows (Tucker et al., 2008; Galán et al., 2018).When cattle are exposed to fluctuating ambient temperatures, the RR is consistently affected with little or no lag period. RR is a simple parameter to monitor without costly equipment (Brown-Brandl et al., 2005).

The typical summer season in the coastal plain of Israel is characterized by stable hot and humid weather with minor fluctuations (Moallem et al., 2010). In the last 30 years, evaporative cooling (forced ventilation and sprinklers) was implemented in Israel to alleviate heat stress in dairy cows in open cowsheds (Berman et al., 1985). Evaporative cooling provides a short term cooling effect (Valtorta and Gallardo, 2004; Kendall et al., 2007). Above 35°C, evaporative cooling becomes the only method for heat dissipation in dairy cows to maintain homeostasis (Burgos et al., 2007). These methods enable high milk production in hot regions by improving heat release (Ortiz et al., 2015; Fournel et al., 2017).

Susceptibility of cows to heat stress is individual and influenced by various factors related to the cow (Gaughan et al., 2000; Kadzere et al., 2002). Body posture is a relevant factor to be considered in cows under heat stress conditions. Lying cows may show heat stress earlier, even with a lower temperature threshold (Berman, 2005; Herbut and Angrecka, 2018). In a recent study, we observed a higher RR in lying cows than in standing cows at a temperature-humidity index (THI) below 80 (Pinto et al., 2019).

Some authors documented that milk yield (Hahn, 1999) and days in milk (Sharma et al., 1983) are associated with heat metabolic increase and, consequently, heat load in dairy cows (Kadzere et al., 2002; West, 2003). The influence of coat color, length and density of hair have also been investigated in recent years in dairy cows under heat stress conditions (Maia et al., 2005; Kendall et al., 2007; Tucker et al., 2008). Dark cows were more susceptible to absorbing solar radiation but also demonstrated higher rates of heat loss than cows with light coats (Maia et al., 2005; Tucker et al., 2008), because white coat cows presented longer hairs (15.13 ± 0.16 mm) and a higher density (1296 ± 21 hairs/cm²) than the cows with black coats (12.97 ± 0.16 mm and 921 ± 21 hairs/cm², respectively; Maia et al., 2005). In contrast, when considering cooled cows, the coat color did not influence the RR (Kendall et al., 2007).

Several studies have demonstrated the effect of evaporative cooling, considering different frequencies of heat abatement. The frequency varied from two (Valtorta and Gallardo, 2004) to four (Avendano-Reyes et al., 2010), five (Flamenbaum et al., 1986), eight (Honig et al., 2012) and nine (Her et al., 1988) cooling sessions per day. However, these studies evaluated RR of cow solely twice per day or once per week which increase the uncertainty of heat load assessment. In addition, previous studies did not consider the immediate effect on cooled animals, both when the cooling management was being applied and after the cooling session. Therefore, the objective of this study was to evaluate the effect of two different cooling frequencies on the RR of lactating dairy cows, considering cow-related factors. Specifically, we hypothesized that cows with three cooling sessions per day would have a higher RR than cows cooled eight times per day. Differences in RR could also be observed among the phases before, during and after cooling. Furthermore, we hypothesized that body posture, milk yield, days in milk (DIM) and coat color would influence the RR.

Material and methods

Animals, housing and management

The study was conducted on the research dairy farm of the Agricultural Research Organization, Volcani Center in Rishon Letsiyon, Israel. The experiment was carried out during summer, on 25 measurement days from July to August 2016. A total of 20 lactating Israeli Holstein dairy cows from a group of 30 cows in the barn, second to seventh lactation were included in the trial. The cows were housed all together (both treatment groups) in one single naturally ventilated cowshed. The cowshed floor was a dry manure (elsewhere known as "compost barn") aligned in a NW-SE orientation (31°59'34.3N 34°48'59.1E). The cowshed was equipped with three high-volume, low-speed ceiling fans (730 cm in diameter; capacity: 722,000 m^3 of air/h), which worked continuously day and night. The cowshed was divided by light mobile fences. Both groups of cows were exposed to exactly the same conditions and the same farm handling and housing conditions, the only different parameter was the desired experimental parameter, the cooling frequency. The cows were assigned randomly to two different cooling frequencies per day i.e., with three cooling sessions per day (3xcool; n = 10) and with eight cooling sessions per day (8xcool; n = 10).

The cooling sessions were implemented in the waiting yard of the milking parlor, which is located about 20 m from the cowshed, the path from the cowshed to the cooling yard was 70 m. The cooling area had a well-drained concrete floor and had dimensions of 12 × 9 m (108 m^2), with approximately 3.6 m^2 cow^{-1}. The cooling area was equipped with three large side fans (2 m in diameter; capacity: 120,000 m^3 of air/h each) to produce airflow perpendicular to the cows body surface (10.6 m/s air velocity nearby of the fan). A total of 30 sprinklers (720 L/h) were fixed 2.8 m above the ground (approximately 1.4 m above the cows) over the whole area of the cooling yard. Each cooling session was 45 min long and consisted of nine cycles in which the cows received one-minute showers followed by four minutes of ventilation. The 3xcool group received cooling before each milking time (i.e., 0415, 1215 and 1915 h) three times per day. The 8xcool group received eight cooling sessions per day at 0100, 0415, 0930, 1215, 1445, 1700, 1915 and 2200 h, respectively. The 2nd, 4th and 7th cooling sessions in the 8xcool group were followed by milking. For further cooling sessions, the 8xcool group was brought out five additional times between milking, while the 3xcool remained inside the cowshed without cooling.

All the cows were fed a total mixed ration and were milked three times daily at 0505, 1305 and 2005 h in a double herringbone parlor with 13 places each side.

The average daily milk yield of the herd was 44.23 ± 7.70 kg (mean ± SD) per cow. The days in milk (DIM) were on average 128 ± 64.9 on the first experimental day. Days in milk were classified according to the lactation period (early: DIM ≤ 100; middle: DIM > 100 and ≤ 200 and late: ≥ 201 DIM). Milk yield per day and DIM were provided by the management software of the cowshed. Once a week, the health status (i.e., body temperature, heart rate and behavior) and body condition score of the cows were measured.

The coat color of every cow was determined from two digital photographs from both body sides of each cow using image analysis software (ImageJ version 1.51, Wayne Rasband NIH, Bethesda, MD, USA) as previously described by Kendall et al. (2007) and Tucker et al. (2008). Cows were categorized as having dark (mean ± SD: 85 ± 7.6% black hair), mixed (59 ± 8% black hair) or light (19 ± 4.8% black hair) coat color.

The trials comply with the supervision of the ARO Animal Care Committee (approval number 685/16 IL). The animals were humanely treated during their day-to-day care by the farm staff and during the study.

Environmental measurements

Ambient temperature (AT) and relative humidity (RH) of the air were recorded every 5 min with nine data loggers (EasyLog USB 2+, Lascar Electronics Inc., Whiteparish, England) positioned inside the buildings (seven in the cowshed and two in the cooling yard) 3 meters above the floor. A total of 109,793 climate datapoints were recorded during the measurement period. The temperature-humidity index (THI) was calculated according to NRC (1971) as follows:

$$THI = (1.8 \times T_{db} + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26)$$

where:

T_{db} is the dry bulb temperature (in °C),

and *RH* is the relative humidity (in %).

The sprinkler water temperature was measured directly on the water outlet nozzle twice daily with a digital thermometer (Fisher Inc., Pittsburgh, PA, USA). The average sprinkler water temperature was 25.5 ± 1.7°C (mean ± SD; range: 22.3 to 27.6°C).

Animal observations

The respiration rate (RR) of the cows was observed hourly in one of three time segments (i.e., 1500 to 0200 h, 0900 to 1700 h, or 0600 to 1400 h), yielding 9 to 12 measurements per cow per day. According to Kabuga (1992), the RR was observed visually by counting right thoracoabdominal movements for thirty seconds and multiplying the value by two (documented as breaths per minute, bpm). The cows were randomly observed within the group. Body posture (standing and lying) was documented. When the cows moved to the cooling yard or back to the cowshed, a 15-min adaptation phase was provided before RR counting started. Observation time relative to cooling was classified as precooling (last observation in the cowshed before cooling), during cooling (all observations in the cooling yard) and post cooling (first observation after return to the cowshed).

Statistical analysis

All data collected during the experimental period were used for analysis. The analysis included a total of 4,686 RR observations in 20 cows. A linear mixed model with repeated measurements per cow to test the effect of treatment group (3xcool vs. 8xcool) on analyses of RR was performed. Fixed factors in the models as group and cow-related factors, such as body posture (standing vs. lying), daily milk yield, DIM class (early, middle and late) and coat color (dark, mixed and light), were included. All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The model used was as follows:

$$Yijklm = \mu + GRP_i + COL_j + (POS \times LOC)_k + DIM_l + a \cdot MY + C_{im} + \varepsilon_{ijklm}$$

where:

Y is the dependent variable of the natural logarithm of the RR,

μ is the general mean,

GRP is the effect of the *i*th treatment group (3xcool, 8xcool),

COL is the effect of the *j*th coat color,

POS is the effect of the *k*th cow posture (standing or lying),

LOC is location of the animals during the measurements (cooling yard or cowshed,

POS × *LOC* is the effect of the *k*th combination of cow posture and cow location,

DIM is the effect of the *l*th lactation period (early: DIM ≤ 100;

middle: DIM > 100 and ≤ 200 and late: ≥ 201 DIM),

a is the regression coefficient for milk yield (*MY*),

C is the random effect of the *m*th cow in treatment group *i*,

ε is the random residual.

A variance component covariance structure was used for random effects and repeated measurements. Factor influences were tested at a significance level of 0.05. The differences between the significant factors were post hoc tested by t-tests in multiple pairwise comparisons. The P-values of those multiple comparisons were adjusted by a simulation of the true 95%-quantile of the contrasts, maintaining a global significance level of 0.05. Model viability was checked by a visual examination of the residuals (homogeneity of variance and normality). Interactions of groups in the precooling, during cooling and post cooling phases of RR reaction were tested. The cooling effect (RR differences between cooling and precooling phases) and post cooling effect (RR differences between cooling and post cooling phases) were calculated. A linear regression analysis of the cooling effect and post cooling effect data was performed, and the effect of treatment group was considered.

Results

Environmental conditions

The average THI calculated was 78.3 ± 3.26 (mean ± SD). The average THI was 76.0 ± 1.22 at 0000 h and 82.3 ± 1.11 at 1200 h. The daily averages of ambient temperature and THI during the experimental period are shown in Figure 1.

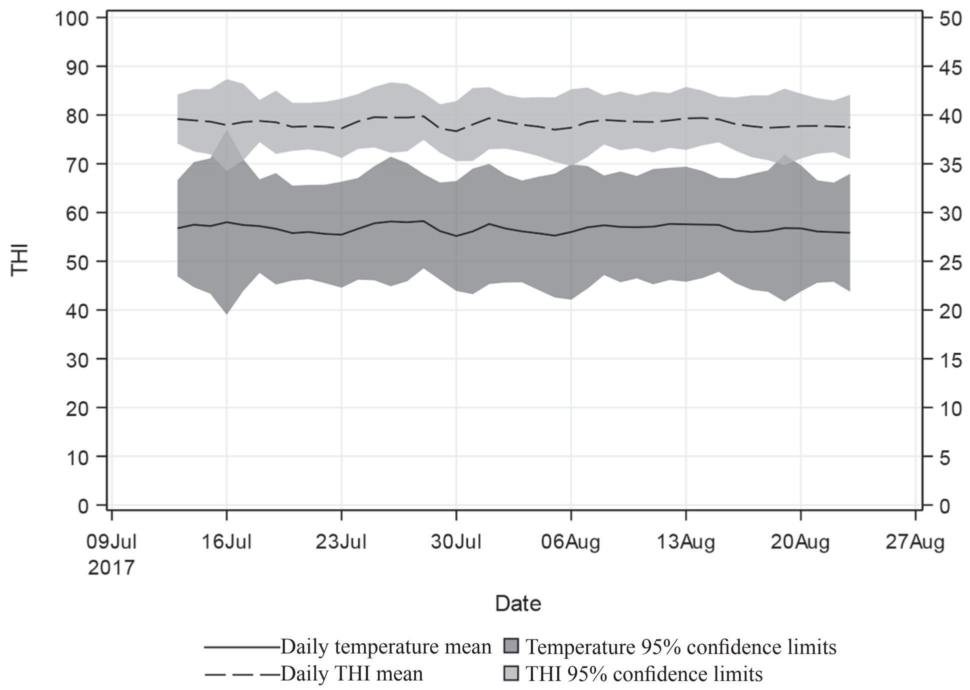

Figure 1. Daily average ambient temperature of the barn and temperature-humidity index (THI) during the experimental period (25 measurement days) from July to August 2016

Influence of cooling frequency on respiration rate

The RR differed between the 3xcool and 8xcool groups depending on cow body posture (standing and lying) and location (cowshed vs. cooling yard, P<0.001). During the cooling time, cows remained in a standing posture. However, no differences in RR during the cooling period were observed between the 3xcool and 8xcool groups (Table 1).

Table 1. Least-square means of the respiration rate (mean ± SE) of standing and lying cows in the 3xcool and 8xcool groups in different places

	Cowshed			Cooling yard		
	3xcool	8xcool	P-value	3xcool	8xcool	P-value
Body posture						
Standing	73.1±0.64	60.2±0.72	< 0.001	47.1±0.66	43.9±0.37	NS
Lying	65.6±0.65	51.6±0.57	< 0.001	–	–	–

NS: No significant effect.
Significant test level P<0.05.

Respiration rate of cows on precooling, cooling and post cooling phases

The average RR was 77.9 ± 0.59 bpm, 43.6 ± 0.51 bpm and 56.2 ± 0.54 bpm during the precooling, cooling and post cooling phases, respectively. The RRs of each group in different cooling times and different phases of cooling are shown in Table 2.

Table 2. Respiration rate of cows (mean ± SE) in different measurement phases arranged by group and cooling number

Group	Cooling number	Cooling time	Precooling	Cooling	Post cooling
3xcool	1	0415-0500	75.1 ± 2.22	37.3 ± 1.14	39.0 ± 0.58
	2	1215-1300	92.6 ± 1.15	49.0 ± 0.84	60.4 ± 1.21
	3	1915-2000	90.8 ± 2.15	42.6 ± 1.29	57.1 ± 1.53
8xcool	1	0100-0145	71.2 ± 1.64	38.4 ± 0.79	45.6 ± 1.01
	2	0415-0500	62.6 ± 2.16	36.6 ± 0.92	40.2 ± 0.99
	3	0930-1015	74.6 ± 1.25	42.8 ± 0.65	56.4 ± 1.17
	4	1215-1300	73.8 ± 1.22	44.9 ± 0.82	64.6 ± 1.32
	5	1445-1530	85.0 ± 1.61	49.2 ± 1.19	71.4 ± 2.25
	6	1700-1745	73.5 ± 2.58	44.2 ± 1.69	53.2 ± 1.54
	7	1915-2000	73.2 ± 2.29	40.3 ± 0.90	61.0 ± 1.45
	8	2200-2245	61.0 ± 1.45	39.5 ± 1.03	42.3 ± 1.25
P-value			< 0.001	0.6586	0.6571

P-values indicate differences between 3xcool and 8xcool at a significant test level of $P<0.05$.

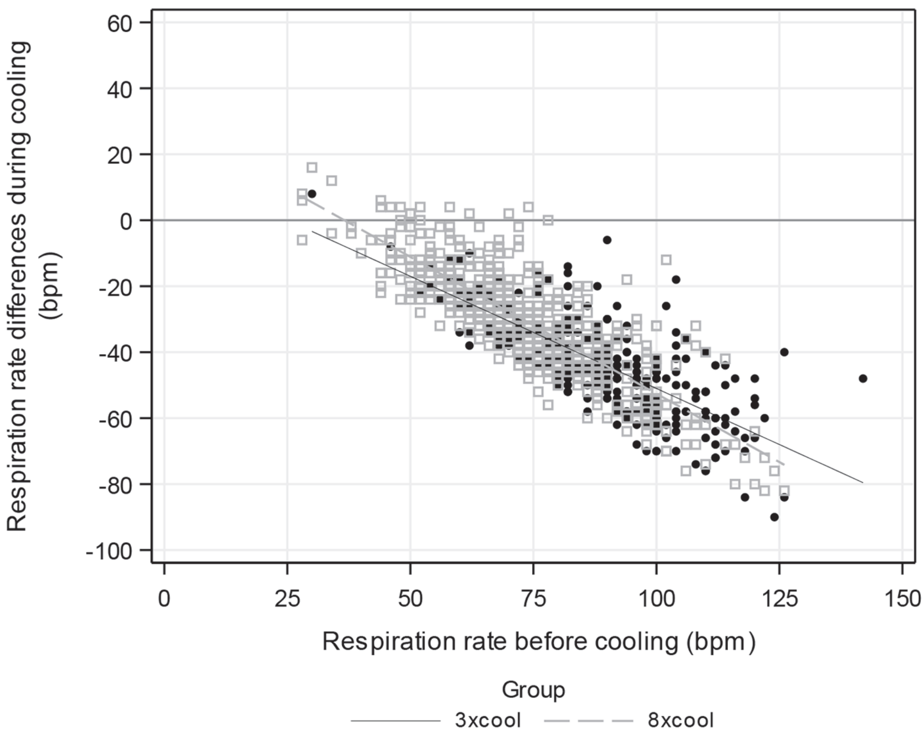

Figure 2. Differences in respiration rates (RR) between the precooling and cooling phases of 3xcool (black dots) and 8xcool (white squares) cows depending on the precooling RR

During the precooling phase, the RR of 3xcool cows (89.1 ± 0.85 bpm, mean ± SE) was higher than the RR of 8xcool cows (73.1 ± 0.56). There was no significant difference in RR between the 3xcool and 8xcool groups during the cooling (45.6 ± 0.67 and 42.8 ± 0.37, respectively) and post cooling phases (55.9 ± 0.94 and 56.3 ± 0.65, respectively).

Cows with high RR values during the precooling phase showed a large RR decrease during the cooling phase in both groups (P<0.001), but no differences in the cooling effect between the 3xcool and 8xcool groups (P=0.1008) were observed (Figure 2). Cows of both groups with RRs over 50 bpm during precooling showed a stronger RR decrease than did those with RRs under 50 bpm during the precooling phase.

In the analysis of the post cooling effect, cows with a high RR during cooling showed a small RR increase in the post cooling phase, and no differences between the 3xcool and 8xcool groups were observed (P = 0.5595, Figure 3).

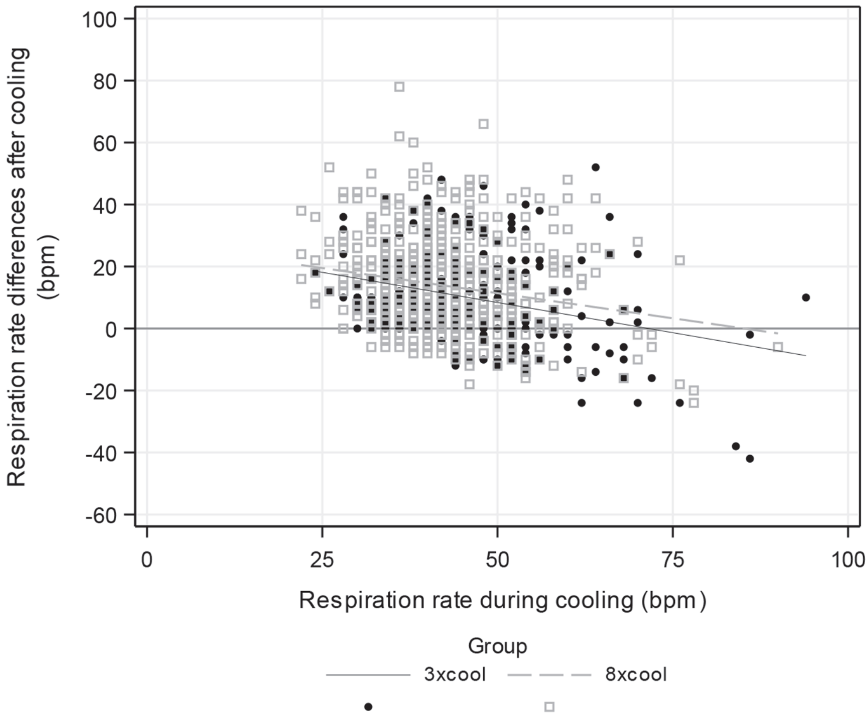

Figure 3. Differences in the respiration rate between the post cooling and cooling phases of 3xcool (black dots) and 8xcool (white squares) cows depending on the RR during the cooling phase

Effects of cow-related factors on respiration rate

The body posture showed significant influences on the RR of cows (P<0.001). The RRs of standing cows were 8 and 9 bpm higher than those of lying cows in the 3xcool and 8xcool groups, respectively (Table 1).

An increase in milk yield directly influenced the RR. With each additional kg of milk produced per day under hot conditions, the RR increased by 0.20 bpm (P<0.001).

Cows in the early DIM period showed higher RR values (71.3 ± 0.98 bpm, mean ± SE) than cows in the middle DIM (64.7 ± 0.55 bpm) and cows in the late DIM (59.0 ± 1.23 bpm) (P<0.001).

There was no effect of coat color on the RR between the 3xcool and 8xcool groups (P=0.6213). The average RR of cows with dark, mixed and light coat color was 63.1 ± 0.50, 68.6 ± 0.51 and 64.1 ± 0.72, respectively.

Discussion

Environmental conditions

In the present study, the experimental barn was located in the coastal plain of Israel. The climate of this region is predominantly semi arid with a high relative humidity, and the study was conducted in what is typically the hottest season in Israel. The cows were constantly under moderate to severe heat stress with an average THI of 78. Our study corroborates the conditions described by Honig et al. (2012), who calculated a mean THI of 78 in the same location. At no time during the experimental period was the THI within the thermoneutral zone of cattle (55 ≤ THI ≤ 61) according to Garner et al. (2017).

Relation between environmental conditions and respiration rate

The average RR (62.6 bpm) of the cows inside the cowshed differed from that reported by Valtorta and Gallardo (2004) and by Kendall et al. (2007), who observed an average RR of 54 bpm and 24 bpm, respectively, with both studies using evaporative cooling as the heat relief method. The THI of our study was higher than that in those studies. For example, in the study by Valtorta and Gallardo (2004), the average THI was 71, and Kendall et al. (2007) observed a THI ranging between 56 and 73 units, while we accepted a tendency of cows to show a high RR in the present study. This RR finding is also considered above the stress threshold between 50 and 60 bpm defined by Berman et al. (1985). Even when we compared our results with those of a similar study carried out by Honig et al. (2012) under the same climate conditions and using eight cooling sessions per day, the RR (50 bpm) was lower than our findings in the present study.

Influence of cooling sessions on respiration rate

The results of the present study showed that 8xcool decreased the RR of dairy cows inside the cowshed, which is in accordance with the results obtained by Avendano-Reyes et al. (2010) and Honig et al. (2012). Avendano-Reyes et al. (2010), who compared different cooling management systems with three observations per week, observed that cows with two cooling sessions per day have a higher RR (9 bpm in the morning and 5 bpm in the afternoon) than do cows cooled in four sessions per day. Considering two different cooling frequencies, Honig et al. (2012) also observed a higher RR (6 bpm in the morning and 33 bpm in the afternoon) in cows cooled in five sessions per day compared with cows cooled in eight sessions per day. In the present study, a higher RR (14 bpm, mean of different day times) was found in 3xcool cows than in 8xcool cows. However, Honig et al. (2012) counted the RR twice per week (in the morning and afternoon), and our measurements were carried out hourly, as performed by Gaughan et al. (2008).

The cooling was efficacious in reducing the RR of standing cows by approximately 36% (26 bpm) in the 3xcool cows and by approximately 26% (16 bpm) in the 8xcool cows during the cooling phase compared with the RR in the precooling phase. The higher RR decrease in 3xcool cows was due to the higher RR values in the precooling phase of this group than in the 8xcool group, with a baseline of 47.1 ± 0.66 bpm in 3xcool cows and 43.9 ± 0.37 bpm in 8xcool cows during the cooling phase. A similar RR baseline of approximately 47 bpm during cooling was also observed in a previous study with steers (Gaughan et al., 2008). Other studies have demonstrated a reduction in RR due to cooling, where the cows with an average baseline RR of 88 bpm before cooling showed a reduction of 13 bpm after 48 min of cooling (Chen et al., 2015). Additionally, with 2 cooling sessions/day, a reduction of 23 bpm after cooling application was described (Valtorta and Gallardo, 2004). In both studies, the RR of the cows was observed before and after the cooling process; hence, in our study, an additional measurement during the cooling took place.

Although no differences were observed in the RR between the 3xcool and 8xcool groups in the post cooling phase, the RR increment of the 8xcool cows was less than that of the 3xcool cows until the following cooling time, as also observed by Honig et al. (2012), who compared eight and five cooling sessions in dairy cows. The increase in the RR in the post cooling phase in both groups seemed to be a response to the high climate conditions that the animals were subjected to inside the barn, as also observed by Chen et al. (2015) after the cooling period. The RR increased from 8 to 40 bpm two hours after cooling, related to the THI increase (Gaughan et al., 2008). Some authors have suggested that heat stressed cows may keep RR and body temperature below baseline during the cooling period and until 30 min post cooling (Flamenbaum et al., 1986; Chen et al., 2015), which is comparable to our study, where the RR of cows was determined an average of 53 ± 0.12 min (mean ± SE) after the cooling phase, and the values were always above the baseline.

The positive effect of cooling on heat-stress relief in cattle is already clearly demonstrated in the literature (West, 2003; Ortiz et al., 2015; Fournel et al., 2017). In addition to this favorable effect of cooling on cows in the present study, we observed that 8xcool cows experienced heat accumulation approximately two hours later than did 3xcool cows. Whereas the 3xcool cows reached a higher RR (92.6 bpm) at 1215 h, the 8xcool cows reached a higher RR (85.0 bpm) before the session at 1445 h. In those time sessions, the 3xcool cows showed a 44 bpm abatement during cooling compared to the 36 bpm reduction among the 8xcool cows. This result demonstrates a strong effect of cooling in cows with more cooling sessions per day, which prevents heat accumulation even during the hottest period of the day at 1200 h (THI = 82.3). These results are supported by other studies that observed an improvement in body temperature (Flamenbaum et al., 1986) and RR (Tresoldi et al., 2018) abatement with the increase in cooling exposure.

A benchmark for cooling frequencies in dairy cows is not concretely defined in the literature. Flamenbaum et al. (1986) concluded that five cooling sessions per day were sufficient to maintain low body temperatures in high-producing dairy cows. However, positive effects were also observed in body temperature and RR reduction of dairy cows with eight (Honig et al., 2012) and nine (Her et al., 1988) cooling sessions per day. We agree that eight cooling times per day improved the RR and heat stress abatement in dairy cows, with an average RR reduction of 14 bpm in 8xcool cows compared to 3xcool cows in the present study.

Effects of cow-related factors on respiration rate

Body posture influenced the RR values in both groups. The RR of standing cows was on average 7 and 8 bpm higher than the RR of lying cows in the 3xcool and 8xcool groups, respectively. Previous studies also reported that body posture influences cows under heat stress conditions. The authors reported that heat stressed cows spend more time during the day standing to improve wind convection and hence increase body temperature dissipation (Frazzi et al., 2000; Tucker et al., 2008; Angrecka and Herbut, 2017). Although the lying posture in dairy cows is a good indicator of cow comfort (Acatincăi et al., 2010; Herbut and Angrecka, 2018), lying cows decrease approximately 42% of the body surface area exposed to air (Wang et al., 2018), and may show early heat stress conditions, even with a lower temperature threshold (Berman, 2005). According to our previous study, where the RR of cows in a lying posture was 7 bpm higher than that of standing cows under a THI below 80, the results of the present study differed. Standing and lying cows in the previous study showed no differences in RR when the THI was above 80. We presume that the cows in the present study increased the RR in the standing posture to improve the effectiveness of heat relief by breathing and wind convection due to the hot environmental conditions over an extended period. Additionally, regarding the rate of each body posture (standing or lying) during the RR count, no differences between the 3xcool and 8xcool groups were observed. Hypothetically, the 8xcool cows were expected to stand more often than the 3xcool cows because they received more cooling sessions per day in a standing posture. Nonetheless, among all observations, 3xcool cows spent 30% of their time in the standing posture, compared to 29% for the 8xcool cows. We therefore presumed that the high standing posture rate in 3xcool cows was observed because they used their free time to improve heat dissipation, while the 8xcool cows used that free time for resting, as mentioned in other studies (Berman, 2006; Honig et al., 2012). In contrast to other studies that evaluated the influence of environmental temperature on milk yield losses (Ravagnolo et al., 2000; Moallem et al., 2010), we aimed to identify the influence of milk yield and DIM on the RR reaction in heat-stressed cows. The RR increased one bpm over the average per five-kilogram of milk produced per day under heat stress conditions. We also determined that the RR of early lactation cows was 17% higher than that of cows in the late lactation period (DIM $\geq$ 201 days). With these two cow factors, we assumed that cows with high milk production per day increased the RR in conditions of heat stress. A large energy demand for milk production during early to mid-lactation and associated cows under high THI conditions tend to increase the metabolic heat output (Hahn, 1999; Kadzere et al., 2002). High-producing cows have more heat to dissipate during the first 60 days of lactation (Sharma et al., 1983; West, 2003), which requires a particular management of cooling procedures to relieve the heat.

Our study demonstrated that coat color does not need to be considered during heat stress assessments as a cow-related factor in cows housed in a cowshed. In a study comparing cooled and non-cooled cows in pastoral systems, there were no differences in the average RR associated with coat color category, even in non-cooled cows, which were constantly exposed to solar radiation (Kendall et al., 2007).

This study would be more powerful with an additional control group without cooling throughout the day, as published in other studies (Her et al., 1988; Valtorta and Gallardo, 2004; Chen et al., 2015 and 2016). During the summer in hot countries, however, failure to employ minimal cooling for high production cows directly impacts animal welfare and is not practicable in commercial barns (Honig et al., 2012). In addition, the ability to reduce heat load in cattle while reducing water consumption is an important issue for future studies in dairy production.

Under hot climate conditions, heat stressed cows lowered the RR by 14 bpm when applied eight cooling sessions per day instead of three cooling sessions. The continuous measurements reduce the uncertainty of the heat stress assessment in dairy cows. The 8xcool cows exhibited heat accumulation two hours later (85.0 bpm at 1445 h) than 3xcool cows (92.6 bpm at 1215 h). The RR of standing cows inside the cowshed was 8 bpm higher than that of lying cows. For each additional five kilograms of milk produced, the respiration rate increased by one bpm. Additionally, cows in the early lactation period (DIM $\leq$ 100) tended to have a 17% higher RR than cows in the late lactation period. Our results suggest that eight cooling times per day improve the RR abatement in heat-stressed dairy cows under hot conditions. Further research is warranted to examine practices in water and energy consumption, labor expenditure, animal management and welfare that are suitable for cooling efficiency improvement.

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4 DISCUSSION

Heat stress is a significant issue in dairy production, especially in dairy cattle with high genetic merit (Kadzere et al., 2002, West, 2003). Several factors influence the individual cow's susceptibility to the heat load (Gaughan et al., 2000). The individual responses of dairy cows are challenging, and it is difficult to assess a universal and reliable heat stress threshold. Considering the above, precision livestock farming aims to reduce the uncertainty in heat stress assessments in dairy cows. Avoiding heat load is highly relevant for the well-being of cows and profitability of dairy production, and environmental and animal-related factors need to be considered in animal husbandry chain appraisals. Hence, the overall objective of this study was to investigate the influence of heat load on the respiration rate in lactating dairy cows housed in naturally ventilated barns under different climatic conditions and management systems. To achieve the aim of the study, experiments in two contrasting climate zones under different management systems were carried out, which resulted in two publications. Publication "A", which was carried out in Germany during 2015 and 2016, evaluated the effects of barn climate conditions and cow-related factors, specifically, body posture (i.e., standing or lying) and daily milk yield the RR of lactating dairy cows. The experiments carried out in Israel during summer 2017 resulted in publication "B", which evaluated the effect of two different cooling frequencies on the RR of lactating dairy cows considering cow-related factors such as body posture (i.e., standing and lying), daily milk yield and coat color.

4.1 Effects of environmental conditions on the respiration rate in different climate zones

In the present study, it was observed that the THI varied between the climate zones (i.e., Germany and Israel) in the same season. During the experimental period in summer 2015 and 2016 in Germany, an average THI of 68.3 ± 6.14 (mean ± SD) was observed, which is considered a heat-stress threshold for dairy cows (Bryant et al., 2007, Zimbelman and Collier, 2011). It is well documented that the optimal temperature range for dairy cows should be between -0.5 and 20 °C and that the THI threshold should be between 68 and 72 (Armstrong, 1994, Hahn, 1999, Bryant et al., 2007, Zimbelman and Collier, 2011), or even lower with a THI of 55 ≤ THI ≤ 61 in the thermoneutral zone (Garner et al., 2017). Climate conditions in Germany are predominantly continental, and for this region, there is a marked temperature increase during the summer season, especially in July and August, with THIs > 60 (Brugemann et al., 2012). The assessment of the THI showed that during summer, the cows in Germany were regularly exposed to heat stress conditions during the day with an average THI of 70.2 ± 6.19 (mean ± SD) and that they could stabilize during the night when the THI was lower (66.4 ± 5.48). This stabilization period during the night is essential for the cows to be able to tolerate relatively high daytime air temperatures (West, 2003) because the cows are not adapted to these hot conditions (Angrecka and Herbut, 2017). However, that same condition was not observed in Israel during the experimental period in 2017. First, climatic conditions during the study demonstrated an average THI of 78.3 ± 3.26 (mean ± SD), which was much higher than that observed in the study in Germany. Moreover, the cows in Israel would not have had the potential for heat relief during the night without a cooling system since the average THI observed during the day was 82.3 ± 1.11 and during the night was 76.0 ± 1.22. The climate of this region is predominantly semiarid, and the present study was conducted in a coastal plain during the summer, which is typically the hottest season in Israel with high relative humidity (Honig et al., 2012). In addition, there was no time during the experimental period in Israel where the THI was in the range of 55 ≤ THI ≤ 61, which is considered the thermoneutral zone for cattle according to Garner et al. (2017).

An increase in the THI results in changes to the physiological parameters of cows (Kabuga, 1992, Brown-Brandl et al., 2005, Chen et al., 2015). The RR is the most common physiological parameter used for early heat stress detection in cattle (Galán et al., 2018). Notably, most heat stress research has been carried out using RR observations twice a day or twice a week. In the present study, the data were collected hourly, as described in Gaughan et al. (2008). This method was considered likely to produce more reliable results. In the results of paper “A”, the RR increase correlated with the THI increase, and this was also observed in the second study (paper “B”). These results were in accordance with the literature, which showed a direct effect on the RR increment with high THI conditions (Kendall et al., 2007). The RR response of the cows in Germany were expressed with a large standard deviation throughout the daytime due to the high environmental differences between day and night, which was not observed in Israel. In this location, the Israeli cows remained under constant heat stress during both the day and the night, though the bpm decreased during the time when the cooling method was applied. Comparisons of the RRs between the two locations in the present study cannot be considered statistically significant due to the marked differences in the experimental animals and management systems of at each farm. However, considering the same THI conditions inside the barn (between 72 ≤ THI < 80), an average RR of 48.7 bpm for the cows in Germany and 62.6 bpm for the cows in Israel was observed, regardless of the animal-related factors. The reference range of the RR in cattle varies from 15 to 36 bpm (Rosenberger, 1979, Jackson and Cockcroft, 2008), and the heat stress threshold is defined with an RR between 50 and 60 bpm in dairy cows (Berman et al., 1985).

Though the THI has been used in several studies as an environmental parameter for heat stress assessment in cattle, this approach has its limitations because it reflects the average conditions in the facility and not the microenvironment of the individual cow (Burgos et al., 2007). Microclimates can be observed in different areas within a given barn (Herbut et al., 2015). In most studies investigating heat stress in the field, ambient temperature and relative humidity are obtained either from nearby weather stations or on site at one or two locations (Schueller et al., 2014). Sensors for environmental data are usually positioned in different locations inside the building with more than one logger. In a study undertaken in a naturally ventilated barn by our research group, the THI varied by up to ± 2 units among loggers and locations within the barn (Hempel et al., 2018). The authors carried out their experiment in the same barn in Germany as that in the present replicable study, and the study demonstrated reputed spatial heterogeneity of THI measurements. Despite the limitations of using a single THI to predict heat load conditions, the association of this index with animal parameters is an important heat stress indicator in dairy cows.

4.2 Effects of cooling on dairy production under heat stress conditions

In the context of climate change and the prospect of an annual temperature rise of 2-4°C in humid continental areas by 2050, heat mitigation systems are becoming increasingly important (Fournel et al., 2017). The heat mitigation system proposes to improve animal welfare in intensive livestock farming and improve the productivity of the system. Cooled cows increase milk production by approximately 0.79 kg milk per day under summer season conditions, which is when daily ambient temperatures tend to rise (Armstrong, 1994).

The strategy of heat abatement differed between the two experiments in the present study. During the experiment in Germany, the barn was equipped with two fans with the aim of increasing the airflow. These were manually turned on when the ambient temperature reached approximately 20 C. This cooling method may be less efficient than evaporative cooling systems; however, this system does have lower maintenance costs associated with livestock production in continental countries. Systematically, the installation of fans during hot days leads to a reduction in the relative humidity inside the barn. A reduction in the relative humidity produces appropriate conditions for body heat relief in cows through sweat evaporation (West, 2003). If the cows are housed in a barn equipped with fans during the hottest period of the summer, their body temperature and RR show a significant reduction, and hence an increase in milk yield can also be observed (Frazzi et al., 2000, West, 2003).

The positive effect of evaporative cooling on heat-stress relief in dairy cattle has been clearly demonstrated previously (West, 2003, Ortiz et al., 2015, Fournel et al., 2017). When we carried out a comparison between two different cooling frequencies per day (3xcool vs. 8xcool) in the study in Israel, we observed a reduction in the RR in heat-stressed cows that received eight cooling sessions per day. During the experimental period, the cows were observed hourly to obtain an accurate assessment of heat mitigation during the measurements. Cows with eight cooling sessions per day showed RRs with approximately 14 bpm less than those in cows with only three cooling sessions per day. Several previous studies have investigated the influence of relatively more cooling sessions per day on the welfare conditions of lactating dairy cows (Avendano-Reyes et al., 2010, Honig et al., 2012). These results demonstrated that the RR decreased when the cows received more cooling sessions per day. However, these earlier studies evaluated the RR in cows fewer times per day than the present study; therefore, the reliability of our results is increased because of the increased number of observations. In addition to differences in the RRs between both cooling frequency treatments, this study also confirmed the positive effect of evaporative cooling on mitigating the heat load of cows through RR responses. Cows in the 3xcool group demonstrated an RR decrease of approximately 36% (26 bpm), and cows in the 8xcool group cows showed a decrease of approximately 26% (16 bpm) during the cooling phase when the RR was compared with the RR in the precooling phase. These cows, however, showed no differences in the RR baseline during the cooling phase, with 47.1 ± 0.66 bpm in the 3xcool group and 43.9 ± 0.37 bpm in the 8xcool group. A similar RR baseline of approximately 47 bpm during cooling was also observed in a previous study with steers (Gaughan et al., 2008).

The work in Israel demonstrated that cows with more cooling sessions per day tended to have a decreased RR after cooling. Consequently, their heat accumulation was delayed by approximately two hours. Whereas the 3xcool cows reached a high RR (92.6 bpm) at 1215 h, the 8xcool cows reached a high RR (85.0 bpm) before the cooling session at 1445 h. The present study showed a favorable effect of evaporative cooling on cows housed in hot conditions. These effects were also observed in several studies in the literature as improvements in body temperature (Flamenbaum et al., 1986), RR and heat relief in dairy cows (Honig et al., 2012, Tresoldi et al., 2018). A benchmark for cooling frequencies in dairy cows is not concretely defined in the literature. Flamenbaum et al. (1986) concluded that five cooling sessions per day were sufficient to maintain low body temperatures in high-producing dairy cows, while Her et al. (1988) observed a significant reduction in body temperature with nine cooling sessions per day. Additionally, Honig et al. (2012) observed positive effects on body temperature and RR reduction with eight cooling sessions per day.

Although cooling systems show benefits for dairy production and animal welfare, important external factors must be considered before the implementation of this system. Sprinkler-fan systems and evaporative cooling pads may require a significant amount of water and electricity, a specific design for optimum operation and regular maintenance to ensure they work effectively (Fournel et al., 2017). With advances in evaporative cooling technology, two primary concerns arise with the use of sprinkler systems. First, depending on the herd size, a large volume of water is needed for cooling. Therefore, these systems generate equally large amounts of wastewater that must be managed. Such high quantities of water may become economically and environmentally unsustainable in the near future, especially for dairy farms that are located in places where the environmental temperatures continue to rise and the fresh water supply is limited (Polsky and von Keyserlingk, 2017). Second, despite sprinklers greatly reducing the RR, their use also results in increased cow avoidance behaviors such as changing the head position and lowering the head to avoid the sprinklers (Schutz et al., 2011, Chen et al., 2016), an effect that was also observed in the Israel experiment in the present study.

4.3 Effects of cow-related factors on heat stress assessment in dairy cows

The present research included cow-related factors with the aim of enhancing the reliability of the data obtained during heat stress assessment in dairy cows. According to several authors, such as Gaughan et al. (2000) and Kadzere et al. (2002), specific cow-related factors such as age, sex, genotype, performance and body condition directly affect the predisposition of the cattle's susceptibility to heat stress. These studies describe a large variation in RR responses among cows under similar heat load conditions. These variations could be reduced by considering cow-related factors in the heat stress measurements in dairy cows instead of considering only environmental and animal physiological parameters.

The results of this PhD work demonstrate the importance of considering cow-related factors in the analysis of heat stress assessments in dairy cows. With these results, it was observed, for example, that body posture has a significant influence on the RR of lactating dairy cows. In the first paper, where the animals were under threshold to mild heat stress conditions (THI $\leq$ 80), the RR in lying cows was 8 bpm higher than that in standing cows. To my knowledge, this is the first study reporting that body posture influences the RR under different THI conditions. The results obtained in the experiments in Germany were in accordance with Berman (2005), who suggested that lying cows may develop heat stress earlier than standing cows, even with a decreased temperature threshold. Wang et al. (2018) described a 42% decrease in body surface area in lying cows compared to standing cows, with the former posture hindering heat dissipation. Although a lying posture in dairy cows is a good indicator of cow comfort (Acatincăi et al., 2010), several studies reported that heat stressed cows spent more time in a standing posture to improve the wind convection, hence increasing body temperature dissipation (Frazzi et al., 2000, Berman, 2005, Tucker et al., 2008). In particular, the straw bedding used in our study might increase the heat load in lying cows, as observed by Angrecka and Herbut (2017). The authors observed avoidance of the straw bedding by resting cows during the day, with a THI > 74. Some authors have also suggested that the body contours of cows cause rumen compression in the diaphragm when the cows are lying down, thereby reducing the lung capacity and respiration effectiveness (Tucker et al., 2008, Reece and Rowe, 2017). Different results were observed in the study carried out in Israel. Since the cows constantly experienced an average THI of 78.3 ± 3.26 (mean ± SD), the body posture showed significant influences on the RR of cows. Unlike the findings in Germany, the standing cows in Israel had an RR that was approximately 9 bpm higher than that in lying cows. I presume this high RR in standing cows is intended to improve the effectiveness of heat relief through respiration and wind convection.

In a recent study of lactating cows conducted by Santos et al. (2017), the authors observed no changes in the RR with regard to milk yield. However, the cows included in their study produced an average of 20 kg milk per day, unlike the present study in which the cows produced an average greater than 40 kg milk per day. Cows with high milk production yields are prone to heat stress due to their genetic merit and high metabolism (Kadzere et al., 2002). This can also affect physiological responses under a heat load environment. In the present research, the cows demonstrated an increase in the RR of one bpm per five-kilograms of additional milk produced per day. In particular, in the Israeli study, lactating cows in early lactation showed 17% more bpm than cows in the late lactation period (≥ 201 days in milk). According to Hahn (1999) and Kadzere et al. (2002), as a result of the large energy demand for milk production during early- to mid-lactation and high THI conditions, the cows tend to increase their metabolic heat output. High-producing cows have more heat to dissipate during the first 60 days of lactation (Sharma et al., 1983, West, 2003). These conditions demand particular cooling procedures for heat relief in dairy cows.

With the aim of evaluating the effect of an additional cow-related factor on the RR of cows under heat stress conditions, we included the coat color of cows as a cofactor in the analysis. The coat color effect was considered only in the experiment in Israel, where the management system exposed the cows to solar radiation along the unshaded route to the cooling yard and/or milk parlor. This same management strategy was not needed in Germany because the cows were housed indoors for the whole experimental period. The study in Israel demonstrated that coat color had no influence on the RR of heat-stressed cows and confirmed that it is not necessary to include coat color as a cow-related factor in the analysis when the cows are not constantly exposed to solar radiation. In a previous study in which cooled and noncooled cows were compared in pastoral systems, the authors observed no differences in the average RR between diverse coat color categories, even in noncooled cows with constant exposure to solar radiation (Kendall et al., 2007).

Further research is necessary to verify whether other cow-related factors, such as lactation number, body weight, body condition score, and pregnancy status, influence the RR of lactating dairy cows under heat stress conditions as well as under different management strategies for heat stress relief (e.g., cooling).

Overall, with the global decrease in the number of dairy farms and consequent herd size increases, the demand for technologies to improve dairy production management is a challenge. These challenges can be successfully addressed by deploying precision dairy farming innovations that aim to increase cow well-being and the efficiency of the processes, which also prevent productivity losses in the system. In addition, the threat of climate change has made heat stress the focus of dairy husbandry research. Therefore, the improvement of data collection per individual on-farm animal may reduce uncertainty in the assessment of health and welfare conditions; early heat stress detection will continually improve as a dairy production strategy.

5 CONCLUSION

The present study provided quantitative evidence that cows housed in naturally ventilated barns under heat load conditions demonstrated marked increases in the RRs regardless of climate zone. Cooling strategies in hot climate conditions reduce the heat load in dairy cows through evaporative cooling. Heat-stressed cows with eight cooling sessions per day decreased their RR by 14 bpm in comparison with cows with only three cooling sessions per day. Furthermore, cows cooled eight times per day had a heat accumulation delay of approximately two hours. Additionally, cow-related factors such as body posture, milk yield and days in milk influenced the RR in dairy cows under different heat stress conditions. When the THI reached 80 in the continental climate zone, the RR of lying cows was approximately 8 bpm more than that of standing cows. However, in the hot climate zone, where the average THI was constantly under 78, the standing cows had a RR 9 bpm more than that of lying cows. Milk production also influenced the RR in cows. For each five-kilogram of additional milk produced per day, the cows tended to increase their RR by one bpm under heat stress conditions. Furthermore, early-lactation cows had an RR 17% higher than that of cows in the late-lactation period. The present study pointed out that hourly RR measurements improve the data quality and may reduce the uncertainty of the results of heat stress assessment in dairy cows. In future research on evaporative cooling, the efficiency of this system with regard to water and energy consumption, labor expenditure and animal management and welfare needs to be investigated and optimized fully. In addition, other cow-related factors, such as lactation number, body weight, body condition score, pregnancy status, etc., should be evaluated in further research to verify their influences on the physiological responses of lactating dairy cows under heat load conditions and different cooling strategies.

6 SUMMARY

The influence of heat load in dairy cows housed in naturally ventilated barns in different climate zones

Due to their sensitivity to high temperatures, heat stress effects are prevalent in high-yielding dairy cows. To reduce uncertainty in the influence of heat stress, detailed representative and individual animal measurements should be considered. Therefore, the overall objective of this study was to investigate the influence of heat load on the respiration rate (RR) of lactating dairy cows housed in naturally ventilated barns under different climatic conditions and management systems.

In the first part of the study, the RR of lactating dairy cows in Germany was investigated hourly, with the aim of evaluating the effects of the temperature-humidity index (THI) on the cows' responses considering the differences between standing and lying cows and daily milk yield as covariables in the analysis. The consideration of cow-related factors provided evidence for reducing the uncertainty in the heat stress assessment of dairy cows. In detail, with THI below 80, the RR in lying cows tended to be 8 bpm higher than that in standing cows, and the RR in high-yielding cows increased one bpm for each additional kilogram of milk produced per day.

Although the first paper showed evidence of cow-related factors influencing RR in cows housed in naturally ventilated barns in a moderate climate, it remained unclear whether the same effect would be observed in high-yielding dairy cows housed under hot climate and high humidity conditions but supplied with cooling management systems for body stabilization. Therefore, in a second part of the study, the aim was to investigate the RR responses of high-yielding dairy cows cooled at two different frequencies per day (3 cooling sessions vs. 8 cooling sessions) and analyze the differences in the RR according to body posture (standing vs. lying), daily milk yield and coat color (black, mixed and white). As noted in the first part of the study, hourly RR measurements were also applied to the second study, which was conducted in Israel during the summer season. The results of this study demonstrated that heat-stressed cows receiving eight cooling sessions per day showed an average RR 14 bpm less than that of cows that received three cooling sessions. Additionally, the cows cooled eight times per day demonstrated a heat accumulation delay of approximately two hours in their RR response. Unlike the experiment in Germany, the Israeli experiment, which was conducted in a hot and humid climatic region, showed a RR 9 bpm higher in standing cows than in lying cows. Daily milk yield also appeared to be an

influencing factor on the RR, whereby the cows tended to increase their RR by one bpm per every five kilograms of additional milk produced per day. In addition, the RR (in bpm) in early-lactation cows was 17% higher than that in late-lactation cows.

Overall, this study demonstrated that hourly RR measurements improve the reliability of heat stress assessments in dairy cows. Future research should consider the efficiency of evaporative cooling in dairy husbandry considering energy consumption, labor expenditure, animal management and welfare. In addition, other cow-related factors should be evaluated in further research to verify influences on the physiological responses of lactating dairy cows under heat stress conditions and in different cooling strategies.

7 ZUSAMMENFASSUNG

Einfluss von Wärmebelastung auf Milchkühe in frei gelüfteten Ställen in verschiedenen Klimazonen

Aufgrund der Empfindlichkeit von Kühen gegenüber hoher Temperaturen sind Wärmebelastungseffekte bei Hochleistungsmilchkühen relevant. Um die Unsicherheit der Einflüsse dieser Auswirkungen zu verringern, sollten detailliertere repräsentative Messungen am Tier in Betracht gezogen werden. Deshalb war das übergeordnete Ziel dieser Arbeit, den Einfluss der Wärmebelastung auf die Atmungsfrequenz (RR) bei laktierenden Milchkühen in frei gelüfteten Ställen in verschiedenen Klimazonen und Managementsystemen zu untersuchen.

In der ersten Studie wurde die RR stündlich bei laktierenden Milchkühen in Deutschland mit dem Ziel untersucht, die Auswirkungen des Temperatur-Luftfeuchtigkeits-Index (THI) auf Reaktion der Kühe unter Berücksichtigung der Unterschiede zwischen stehenden und liegenden Kühen und der täglichen Milchleistung als Co-Variablen in der Analyse zu bewerten. Die Berücksichtigung kuhindividueller Faktoren in der Auswertung liefert Hinweise, dass sich dadurch die Unsicherheit der Beurteilung der Wärmebelastung von Milchkühen verringert. Bei einem THI unter 80 war die RR bei liegenden Kühen 8 Atemzüge pro Minute höher als bei stehenden Kühen. Bei Kühen mit hoher Milchleistung erhöhte sich die RR um einen Atemzug pro Minute für jedes zusätzliche Kilogramm Milch, das pro Tag erzeugt wurde.

Die erste Veröffentlichung hat gezeigt, dass kuhindividuelle Faktoren die RR bei Kühen in frei gelüfteten Ställen in einem gemäßigten Klima beeinflussen. Ob derselbe Effekt jedoch auch bei Milchkühen mit hoher Milchleistung bei heißem Klima und hoher Luftfeuchtigkeit, und zusätzlichem Kühlungssysteme, welche die Verdunstung verbessern und damit der Erfrischung, beobachtet werden kann, blieb unklar.

Daher bestand das Ziel der zweiten Studie zum einen darin, die RR-Reaktionen von Hochleistungsmilchkühen zu untersuchen, bei denen zwei unterschiedliche Kühlfrequenzen pro Tag (3 Abkühlungen pro Tag vs. 8 Abkühlungen pro Tag) angewandt wurden, und zum anderen darin, die Unterschiede in der RR hinsichtlich der Körperhaltung (stehend vs. liegend), der täglichen Milchleistung und der Fellfarbe (schwarz, gemischt und weiß) zu betrachten. Wie bereits in der ersten Studie, wurden auch in der zweiten Studie stündliche Atemfrequenzmessungen an den Kühen während des Sommers durchgeführt, dieses Mal in

Israel. Die Ergebnisse dieser Studie zeigten, dass bei wärmebelasteten Kühen, die achtmal statt dreimal eine Kühlung pro Tag erhielten, die RR durchschnittlich 14 Atemzüge pro Minute geringer war.

Zusätzlich zeigten die Kühe, die achtmal am Tag gekühlt wurden, in ihren RR-Reaktionen eine Wärmestauverzögerung von etwa zwei Stunden. Im Gegensatz zu der Untersuchung, die in Deutschland durchgeführt wurde, zeigte die Untersuchung in Israel unter heißen und feuchten Klimabedingungen, dass die RR bei stehenden Kühen im Vergleich zu liegenden Kühen neun Atemzüge pro Minute höher war. Die tägliche Milchleistung wirkte sich zusätzlich als Einflussfaktor auf die RR aus, wobei die Kühe tendenziell ihre RR um einen Atemzug pro Minute für je fünf Kilogramm mehr der durchschnittlichen Milchproduktion pro Tag erhöhten. Darüber hinaus war die RR bei frisch laktierenden Kühen um 17% höher als bei spätlaktierenden Kühen.

Insgesamt zeigten diese zwei Studien, dass stündliche Atemfrequenzmessungen die Zuverlässigkeit von Wärmebelastungsbewertungen bei Milchkühen verbessern. Zukünftige Hitzestressuntersuchungen müssen die Effizienz der Verdunstungskühlung in der Milchviehhaltung an Hand von Energieverbrauch, Arbeitsaufwand, Tiermanagement und Tierwohl berücksichtigen. Zusätzlich könnten andere kuhindividuelle Faktoren in weiteren Forschungsarbeiten ausgewertet werden, um den Einfluss von Wärmebelastungsbedingungen und verschiedenen Kühlungsstrategien auf die physiologischen Reaktionen von laktierenden Milchkühen zu überprüfen.

8 RESUMO

Efeito do estresse pelo calor em vacas de leite confinadas em estábulos naturalmente ventilados em diferentes zonas climáticas

Devido à sensibilidade a altas temperaturas, o estresse pelo calor é um fator relevante em vacas de alta produção de leite. Com o intuito de melhorar a confiabilidade dos dados sobre o efeito do estresse pelo calor em vacas de produção de leite, medições mais representativas e detalhadas dos animais devem ser consideradas. Desse modo, o objetivo do presente estudo foi avaliar os efeitos do estresse pelo calor na frequência respiratória (RR) de vacas leiteiras confinadas em estábulos naturalmente ventilados em diferentes condições climáticas e sistemas de manejo.

No primeiro artigo do presente trabalho foi avaliado na Alemanha os efeitos do Índice de Temperatura-Umidade (THI) na RR coletada de hora em hora de vacas leiteiras confinadas em um estábulo free-stall naturalmente ventilado, considerando como co-variáveis nas análises estatísticas, fatores individuais relacionados a vaca sendo: a posição da vaca (Deitada em decúbito lateral ou em estação) e a produção de leite diária de cada animal. A inclusão de fatores individuais relacionados a vaca possibilita uma melhor avaliação do estresse pelo calor nos animais. Detalhadamente, os resultados do referente estudo demonstraram que vacas em posição deitada sob condições de THI inferior à 80 tendem a apresentar oito respirações por minuto (bpm) à mais do que vacas em estação. Além disso, na mesma condição ambiental as vacas com maior produção de leite diária demonstraram aumento de um bpm para cada quilograma de leite produzido acima da média diária do rebanho.

Apesar de o primeiro artigo ter mostrado evidências de que os fatores individuais das vacas influenciaram a RR de vacas leiteiras confinadas numa região de clima continental, o mesmo efeito permanecia desconhecido em vacas de alta produção leiteira, estabuladas em uma região de costa em clima semiárido, porém, com condições de altas temperaturas e umidade relativa do ar e com um sistema de manejo de resfriamento diário dos animais. Logo, o objetivo do segundo artigo foi avaliar a RR de vacas de alta produção de leite confinadas e submetidas a um sistema de manejo de resfriamento diário, considerando fatores individuais relacionados à vaca sendo: a posição da vaca (Deitada em decúbito lateral ou em estação), produção de leite diária e coloração da pelagem (predominante preta, mista, predominante branca). O estudo foi realizado durante a estação de verão em um estábulo modelo compost barn naturalmente ventilado e localizado em uma região de

costa de Israel. A RR dos animais foi coletada de hora em hora e os mesmos foram divididos em dois grupos, submetidos à duas frequências diferentes de resfriamento, sendo o primeiro grupo com três vezes diárias e o segundo grupo com oito vezes, ambos os grupos recebendo a cada ciclo de resfriamento 45 minutos de duração. O grupo de vacas que recebeu um total de oito vezes o resfriamento por dia apresentou RR de 14 bpm menor do que vacas que receberam somente três vezes diárias, sendo observado também um retardo de até duas horas após o ciclo de resfriamento para a vaca atingir um limite máximo de RR. Diferentemente dos resultados obtidos na Alemanha, as vacas pesquisadas em Israel quando em posição de estação apresentaram uma RR de nove bpm maior em comparação às vacas deitadas. A produção de leite diária demonstrou efeito significativo na RR das vacas em Israel, demonstrando aumento de um bpm para cada cinco quilogramas de leite produzido acima da média do rebanho. Sendo demonstrado também que vacas paridas recentemente tenderam a apresentar uma RR em bpm (17%) maior que vacas em uma fase de lactação mais avançada.

No presente trabalho foi demonstrado primeiramente que a avaliação de hora em hora da RR aumenta a confiabilidade de avaliação do estresse pelo calor em vacas leiteiras. Em trabalhos futuros, a eficiência de sistemas de arrefecimento do calor por evaporação na bovinocultura leiteira como, consumo de energia, mão de obra, manejo dos animais e bem-estar animal devem ser considerados. Além disso, fatores individuais relacionados à vaca devem ser incluídos nas análises de parâmetros fisiológicos das vacas em lactação em condições de estresse pelo calor e em diferentes sistemas de manejo de resfriamento.

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10 PUBLICATIONS

10.1 Research articles

Galán, E.; Llonch, P.; Vilagra, A.; Levit, H.; **Pinto, S.**; del Prado, A. (2018): A systematic review of non-productive animal-based indicators of heat stress resilience in dairy cattle. Plos One. https://doi.org/13:e0206520.

Hempel, S.; Menz, C.; **Pinto, S.**; Galán, E.; Janke, D.; Estellés, F.; Müschner-Siemens, T.; Wang, X.; Heinicke, J.; Zhang, G.; Amon, B.; del Prado, A.; Amon, T. (2019): Heat stress risk in European dairy cattle husbandry under different climate change scenarios - uncertainties and potential impacts. Earth System Dynamics Discussions. 2019: 1-38. https://doi.org/10.5194/esd-2019-15

Pinto, S.; Hoffmann, G.; Ammon, C.; Amon, B.; Heuwieser, W.; Halachmi, I.; Banhazi, T. Amon, T. (2019): Influence of barn climate, body postures and milk yield on the respiration rate of dairy cows. Annals of Animal Science. 19: 469-481. https://doi.org/10.2478/aoas-2019-0006

Pinto, S.; Hoffmann, G.; Ammon, C.; Heuwieser, W.; Levit, H; Halachmi, I.; Amon, T. (2019): Effect of two cooling frequencies on respiration rate in lactating dairy cows under hot and humid climate conditions. Annals of Animal Science. 19: 1-14. https://doi.org/10.2478/aoas-2019-0026

10.2. Oral presentations

The presenters are underlined

Ammon, C.; Amon, B.; Amon, T.; Demba, S.; Englisch, A.; Heinicke, J.; Hempel, S.; Hoffmann, G.; Janke, D.; König, M.; **Pinto, S.**; Reinhardt, A.; Roth, C.; Schröter, K.; Siemens, T.; Stollberg, U.: *Untersuchungen zur Bewertung von Tierwohl und Umweltwirkungen in frei gelüfteten Rinderställen. 13. Informationsveranstaltung "Umweltverträgliche Landwirtschaft"* (Investigations for the animal welfare assessment and environmental impacts in naturally ventilated cattle barn. 13. Information event "Sustainability Agriculture"), Berlin, 27.04.2017-28.04.2017.

Amon, T.; Qianying, Y.; Heinicke, J.; **Pinto, S.**; Hoffmann, G.; Hempel, S.; Müschner-Siemens, T.; Janke, D.; Ammon, C.; Amon, B.: Optimisation of environment-animal-welfare interactions in high-performance dairy cows housed in naturally ventilated barns. In Proceedings: International Symposium on Animal Environment and Welfare 2019, Chongqing, China, 21.10.2019 – 24.10.2019.

Goldshtein, S.; Levit, H.; **Pinto, S.**; Halachmi, I.; Parmet, Y.: Animal response (rumen-temperature) based cattle cooling method ease heat stress. 68th Annual Meeting of the European Federation of Animal Science, Tallinn, Estonia, 28.08.2017-01.09.2017.

Heinicke, J.; Hempel, S.; **Pinto, S.**; Ammon, C.; Amon, T.; Englisch, A.; Hoffmann, G.: Wirkung von Hitzestress auf Verhaltens- und Vitalitätsparameter von Milchkühen. Effect of heat stress on behaviour and vitality parameters of dairy cows. 13th Conference Construction, Engineering and Environment in Livestock Farming, Stuttgart-Hohenheim, 18.09.2017-20.09.2017.

Hempel, S.; König, M.; Janke, D.; **Pinto, S.**; Siemens, T.; Goldstein, S.; Heinicke, J.; Levit, H.; Hoffmann, G.; Halachmi, I.; Zhang, G.; Ammon, C.; Amon, B.; Amon, T.: Climate and animal monitoring for adapted smart dairy barns. 3rd European Climate Change Adaptation Conference (ECCA), Glasgow, Great Britain, 05.06.2017-09.06.2017.

Hempel, S.; König, M.; Janke, D.; **Pinto, S.**; Siemens, T.; Heinicke, J.; Ammon, C.; Amon, B.; Amon, T.: Microclimate monitoring as basis for smart cattle barns. 68th Annual Meeting of the European Federation of Animal Science, Tallinn, Estonia, 28.08.2017-01.09.2017.

Levit, H.; Goldshtein, S.; **Pinto, S.**; Elazary, AK.; Bloch, V.; Ben Meir, Y.; Gershon, E.; Miron, J.; Halachmi, I.: Real-time animal response to climate changes. Fifth and Final Dairy Care Conference, Thessaloniki, 19.03.2018 – 20.03.2018.

Levit, H.; **Pinto, S.**; Amon, T.; Goldshtein, S.; Gershon, E.; Elazary, AK.; Bloch, V.; Ben Meir, Y.; Fortnik, Y.; Jacoby, S.; Arnin, A.; Miron, J.; Halachmi, I.: Cooling regime timing set by bolus sensor mitigated heat stress in dairy cows. In Proceedings: ECPLF 2019, Cork, Ireland, 26.08.2019 – 29.08.2019.

Pinto, S.: Influence of barn climate and cow-related factors on the respiration frequency of dairy cows in hot weather. Colloquium at PhD day in ATB, Potsdam, Germany, 16.10.2018

Pinto, S.: Natur Abzocke: Massentierhaltung und die Folgen. Grenzenlos Modul III: Praxiseinsatz an berufsbildenden Schulen, Potsdam, Germany, 29.11.2017.

Pinto, S.: Influence of barn climate and cow-related factors on the respiration rate of dairy cows under different climatic conditions. Colloquium in Volcani Center, Rishol Letzion, Israel. 17.08.2016.

Pinto, S.: Influence of heat stress on vital parameters and milk yield of dairy cows housed in naturally ventilated barns in different climate zones. Colloquium in ATB Potsdam, Germany, 17.05.2016.

Pinto, S.; Hempel, S.; Siemens, T.; Heinicke, J.; Hoffmann, G.; Ammon, C.; Amon, T.: Influence of barn climate on physiological parameters and behavior of dairy cows considering cow-related factors. OptiBarn Open Meeting - Dairy Cattle and Climate Change, Valencia, Spain, 18.01.2017.

Pinto, S.; Hoffmann, G.; Ammon, C.; Amon, T.: Effect of body posture on respiration rate, heart rate and rectal temperature of dairy cows under thermoneutral conditions. In Proceedings: ECPLF 2019, Cork, Ireland, 26.08.2019 – 29.08.2019.

Pinto, S.; Levit, H.; Müschner-Siemens, T.; Hoffmann, G.; Ammon, C.; Halachmi, I.; Heuwieser, W.; Amon, T.: Influence of evaporative cooling on respiration rate of lactating cows under hot climate conditions. In Proceedings: EurAgEng 2018 Conference, Wageningen, Netherland, 08.07.2018 – 12.07.2018.

Pinto, S.; Pauw, W. ; Ammon, C. ; Heinicke, J. ; Heuwieser, W. ; Amon, T. . Effect of lactation number on the respiratory rate of dairy cows on hot days. In: 67th Annual Meeting of the European Federation of Animal Science, 2016, Belfast. Book of Abstracts of the 67th Annual Meeting of the European Federation of Animal Science. Wageningen: Wageningen Academic Publishers, 2016. v. 22. p. 484-484.

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Pinto, S.; Siemens, T.; Hoffmann, G.; Hempel, S.; Ammon, C.; Heinicke, J.; Heuwieser, W.; Amon, T.: Influence of environmental climate conditions on respiration rate and behavior of lactating dairy cows. 10. Doktorandensymposium & DRS Präsentationsseminar “Biomedical Sciences” (10th Doctoral symposium and DRS seminar presentations “Biomedical Sciences”), Berlin, 22.09.2017.

Siemens, T.; Amon, T.; **Pinto, S.**; Heinicke, J.; Hempel, S.; Hoffmann, G.; Ammon, C.; Halachmi, I.; Janke, D.: Influence of environmental climate conditions on animal welfare criteria of lactating dairy cows. 68th Annual Meeting of the European Federation of Animal Science, Tallinn, Estonia, 28.08.2017-01.09.2017.

Siemens, T.; Hoffmann, G.; Hempel, S.; Ammon, C.; Heinicke, J.; **Pinto, S.**; Amon, T.; Amon, B.: Wirkung von Hitzestress auf das Wiederkauverhalten von Milchkühen. Effect of heat stress on rumination activity in lactating dairy cows. 13th Conference Construction, Engineering and Environment in Livestock Farming, Stuttgart-Hohenheim, 18.09.2017-20.09.2017.

Siemens, T.; **Pinto, S.**; Heinicke, J.; Hempel, S.; Hoffmann, G.; Ammon, C.; Amon, T.: Influence of environmental climate conditions on animal welfare criteria of lactating dairy cows. MACSUR Scientific Conference 2017, Berlin, Germany, 22.05.2017-24.05.2017.

10.3 Poster presentations at conferences

Pinto, S.; Pauw, W. ; Englisch, A. ; Ammon, C. ; Heinicke, J. ; Heuwieser, W. ; Amon, T. . Influence of cow-related factors on respiration rate of dairy cows under hot climate conditions. In: Precision Dairy Farming 2016, Leeuwarden. Precision Dairy Farming2016.

Pinto, S.; Siemens, T.; Hoffmann, G.; Ammon, C.; Heinicke, J.; Heuwieser, W.; Amon, T.: Influence of environmental conditions on respiration rate of dairy cows. N2 Science Communication Conference, Berlin, 06.11.2017-08.11.2017.

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12 DECLARATION OF INDEPENDENCE

I declare that I conducted all of the studies described herein, and the manuscripts were produced independently. I confirm that I have used only the specified resources and tools to complete this thesis. My personal contributions to the research projects presented under this cumulative doctoral thesis are summarized in the following table.

Contribution	Research project 1/ Publication 1	Research project 2/ Publication 2
Study design	+++[1]	+++
Data collection	+++	+++
Data analyses	++	++
Manuscript writing	+++	+++
Manuscript editing	+++	+++

[1]Score: + ≤ 50%; ++ = 50-70%; +++ ≥70%

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