USING PRECISION LIVESTOCK FARMING (PLF) TECHNOLOGIES TO ASSESS THE IMPACT OF ENVIRONMENTAL STRESSORS ON ANIMAL WELFARE AND PRODUCTION EFFICIENCY ON MODERN DAIRY FARMS

A Thesis submitted by

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Abstract

In modern dairy farming systems, heat stress is still a significant challenge. Dairy cows will encounter sub-optimal welfare which can result in production decline, diseases and even mortality, especially for high-producing cows with lower heat tolerance. The frequency and magnitude of heat stress events or heat waves are predicted to keep increasing in coming decades associated with global warming. Therefore, greater attention is being paid to alleviating the effects of heat stress on dairy cows and livestock generally. Modelling and on-farm experiments have been undertaken in many countries to assess the influence of heat stress on livestock using modern computer technologies and other hi-tech tools. At the same time, mitigation approaches such as optimal shed structure, new cooling facilities, targeted feeding regimes, improved farm management and genetic selection have all been studied extensively. However, due to differences between farm conditions and varying heat tolerance of different breeds and coping ability, the results from different heat stress models provided a variety of thresholds for on-farm decision support. Therefore, determination of accurate heat stress thresholds to facilitate practical mitigation options are still difficult.

This study was initiated by summarizing the progresses achieved by previous studies on intensively kept dairy cows in relation to measuring, assessing and mitigating their heat stress. By taking comparative analysis of the published studies about thermal indices, animal responses and mitigation solutions, a range of recommendations were given for developing more accurate assessment and designing of more effective mitigation options. The review suggested that for achieving accurate and applicable thresholds of heat stress, it is necessary to establish monitoring systems embedded into routine farm management systems, which can be an add-on unit of current robotic milking system (RMS). The robust monitoring system would measure real-time data from the ambient environment, animal responses, as well as the operation pattern of mitigations. Furthermore, by facilitating big-data analysis techniques to be used on individual farms, (or for individual animal) it might be possible to implement self-calibration procedure for the assessment, thresholds and control algorithms responding to varied cow's production status, farm management factors and local climate changes.

The follow-up research presented in this thesis demonstrated the possibility of establishing more accurate heat stress threshold by taking advantage of the routinely collected datasets on robotic dairy farms and local weather stations. The dairy farm observed in this study situated in a subtropical climate region, held around 150 lactating cows and applied RMS with semi-free traffic. The farm management system recorded specific production, health and behaviour information of each individual animal over 5-year period (2013-2017), which was utilized for the analysis in this study. The historical climate conditions were measured by local weather station with dataset accessible on a government website, which provided the data of daily thermal parameters for this research. Furthermore, data-loggers were also positioned on farm from April 2016 to November 2017 to measure thermal parameters hourly.

By using the collected information, this study compared the performance of published thermal comfort indices (TCIs) as the indicators of cows' responses to heat stress. These TCIs included temperature humidity index (THI), black globe humidity index (BGHI), environmental stress index (ESI), equivalent temperature index (ETI), heat load index (HLI), respiration rate index (RR) and comprehensive climate index (CCI). The comparison also included the basic thermal parameters: dry bulb temperature (Tdb), relative humidity (RH), wet bulb temperature (Twb) and dew point temperature (Tdp). The strength of their correlation with daily milk yield (DMY) and milk temperature (MT) was tested statistically. The regression analysis using climate

dataset from local weather station and on-farm data-loggers were also compared to validate the accuracy of online data source. The statistical analysis found similar performance between TCIs and Tdb. It was also found that the inaccuracy of online data source, due to spatial variability between on-farm measurement and local weather station, could be neglected when modelling the association between TCIs and MT. A general threshold with significant decline of DMY was identified as THI>64 for cows with DMY around 31 kg/cow/day.
As Tdb can provide sufficient accuracy in the prediction of heat stress, the dynamic thresholds of daily minimum and mean temperature (Tmin and Tmean) were then established using individual information of 126 cows. The dataset was grouped according to the age, body weight (BW) and days in milk (DIM) of cows. Specific thresholds for different groups were identified using single broke-line regression between temperature and DMY or MT. Machine learning model was applied to transform these thresholds of different group into a decision tree of dynamic thresholds, which achieved overall 94% accuracy with the thresholds of Tmin, and 79% accuracy with the thresholds of Tmean. Moreover, for the whole herd, multiple broken-line regression was applied, which established four stages of heat stress including as thermal comfort stage (Tmin < 5 ^{o}C, Tmean < 9 ^{o}C), mild heat stress (Tmin: 5-6 ^{o}C, Tmean: 9-11 ^{o}C), effective heat stress (Tmin: 6-14 ^{o}C, Tmean: 11-16 ^{o}C) and critical heat stress (Tmin > 14 ^{o}C, Tmean > 16 ^{o}C) based on the change of DMY and MT.
To gain more understanding of the heat stress influence on animal behaviours in RMS, extra dependent variables were imported into new models involving rumination time (RT), time of milking (TM), miking frequency (MF), milking duration (MD), milking speed (MS), and milk yield per milking (MY). A new index – rumination efficiency index (REI) was created to evaluate the efficiency of rumination. According to the multiple broken-line regression, 5 minutes reduction of RT, 0.08 kg/cow/hour reduction of REI and 1% increase of low efficiency miking (LEM) were found to be associated with raising 1 ^{o}C of Tmean. It was also demonstrated that cows could not adjust their pattern of milking behaviour (e.g. visiting time pattern) coping with heat stress. Statistically, 86% of their milking event happened between 07:00 AM and 09:00 AM. However, REI and RMS performance can be improved by adjusting the pattern of milking behaviour such as milking interval (MI). The financial comparison between current pattern and adjusted pattern estimated that nearly $400 daily benefit could be gained.
In addition, this study also analysed the cumulative and lag effect of heat stress which were time-related. For the short-term effect, an intensity duration index (IDI) was defined by multiplying the mean temperature of the heat stress period with the duration of the period. Multiple levels of heat stress were then identified by IDI with different decline rate of DMY from -0.01 to -0.13 kg/cow/IDI. For long-term heat stress, the lag and cumulative effect was demonstrated by the negative correlation between the duration of heat stress during dry-off period and the production performance of the subsequent lactation period. The lag effect was found to be 3-4 days, while the cumulative effect could last for about 2 months. The regression between DMY and the average temperature of the period with heat stress during the 2 months before test day (HSmean) was found to perform stronger correlation (R^2 equals 0.73-0.77) than the regression between DMY and same day's temperature (R^2 equals 0.65-0.68).

Certification of thesis

This Thesis is the work of (Student name) Boyu Ji except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

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Statement of contribution

The following detail is the agreed share of contribution for candidate and co-authors in the presented publications in this thesis:

- **Article 1:** Ji, B., Banhazi, T., Perano, K., Ghahramani, A., Bowtell, L., Wang, C. & Li, B. (2018e). A review on measuring, assessing and mitigating heat stress of housed dairy cow with precision livestock farming techniques. Biosystems Engineering.

  The overall contribution of **Boyu Ji** was 70% to the conception and design, 80% to the analysis and interpretation and 80% to the drafting and production; **Thomas Banhazi** contributed 20% to the conception and design, 10% to the analysis and interpretation and 5% to the drafting and production; **Kristy Perano** contributed 5% to the analysis and interpretation and 5% to the drafting and production; **Afshin Ghahramani** contributed 5% to the analysis and interpretation and 5% to the drafting and production; **Les Bowtell** contributed 5% to the drafting and production; **Chaoyuan Wang** contributed 5% to the conception and design; **Baoming Li** contributed to the 5% to the conception and design.

- **Article 2:** Ji, B., Banhazi, T., Ghahramani, A., Les, B., Wang, C. & Li, B. (2018a). Modelling of heat stress in a robotic dairy farm. Part 1: Thermal comfort indices as the indicators of production loss. Biosystems Engineering.

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- **Article 4:** Ji, B., Banhazi, T., Ghahramani, A., Les, B., Wang, C. & Li, B. (2018c). Modelling of heat stress in a robotic dairy farm. Part 3: Animal behaviour and milking performance. Biosystems Engineering.

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Ji, B., Banhazi, T., Ghahramani, A., Bowtell, L, Wang, C. & Li, B. (2018a). Modelling of heat stress in a robotic dairy farm. Part 1: Thermal comfort indices as the indicators of production loss. Biosystems Engineering.

Ji, B., Banhazi, T., Ghahramani, A., Bowtell, L, Wang, C. & Li, B. (2018b). Modelling of heat stress in a robotic dairy farm. Part 2: Identify of the specific thresholds with production factors. Biosystems Engineering.

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Ji, B., Banhazi, T., Ghahramani, A., Bowtell, L, Wang, C. & Li, B. (2018d). Modelling of heat stress in a robotic dairy farm. Part 4: Lag and cumulative effect of heat stress. Biosystems Engineering.

Conference papers:

Ji, B., Banhazi, T., Wang, C., Li, B. (2017) A Review: The Influence and Mitigation of Ammonia and Dust. In 23-25th of October 2017 in Chongqing, China, 2017 International Symposium on Animal Environment and Welfare.

Ji, B., Banhazi, T., Wang, C., Li, B. (2017) PLF technologies: model development for solving heat stress problems on dairy farms. In 12-14th of September 2017 in Nantes, France, 8th European Conference on Precision Livestock Farming.

Ghahramani, A., Berndt, A., Cobon, D., Banhazi, T. & Ji, B. (2017). Climate change impact and adaptation of grazing systems in Australia and Brazil. In 54.º Meeting of the Brazilian Society of Animal Science (SBZ), At Foz do Iguaçu –Parana – Brazil.

Liu, Y., Wang, C., Ji, B., Liu, J., Ding, L., Li, B., & Shi, Z. (2016). Impacts of Surface Wind Speed and Deployment Duration on Measuring Accuracy of Closed Chamber. In 2016 Orlando, Florida July 17–July 20, 2016 ASABE Annual International Meeting (p. 1). American Society of Agricultural and Biological Engineers.

Wang, Y., Wang. C., Ji, B., Ding, L., Li, B., & Shi Z. (2016). Surface Wind Speeds and Simulated Rainfall on GHG Emission from Stockpiled Dairy Manure: a Laboratory Study. In 2016 Orlando, Florida July 17–July 20, 2016 ASABE Annual International Meeting (p. 1). American Society of Agricultural and Biological Engineers.

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Research involving human or animal subjects

The measurements and procedures used in this project (ID: 17REA007) were approved by the University of Southern Queensland's Animal Ethics Committee.

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List of abbreviations

Age	age of cow
AI	artificial insemination/ artificial intelligence
BGHI	black globe humidity index
BW	body weight
CC	fans and conductive cooling
CCI	comprehensive climate index
CR	conception rate
DIM	days in milk
DMI	dry matter intake
DMY	daily milk yield
ESI	environmental stress index
ETI	equivalent temperature index
FS	fans and misters
HLI	heat load index
HR	heart rate
IDI	Intensity duration index
KK	korral kool cooling facility
LD	Lactation duration
LEM	Proportion of robotic milking with low efficiency in daily robotic milking
LP	lying patterns
MD	Duration of robotic milking per visit
MF	Frequency of milking visit per day
MS	Milking speed of each robotic milking
MT	milk temperature
MY	milk yield
MY	Milk production of each robotic milking
Num	number of cows in herd
NumHSTmax	Duration of long-term heat stress with daily maximum temperature exceeding the threshold
NumHSTmin	Duration of long-term heat stress with daily minimum temperature exceeding the threshold
RH	relative humidity
RHmax	daily maximum relative humidity
Rhmin	daily minimum relative humidity
RR	respiration rate/ respiration rate index
RT	rectal temperature
SR	solar radiation
ST	skin temperature
SWR	sweat rate
T_allday	Average temperature between 06:00 AM and 18:00PM, which was measured by on-farm data-loggers
T_daytime	Average temperature between 06:00 AM and 18:00PM, which was measured by on-farm data-loggers
T_nighttime	Average temperature between 18:00PM and 06:00 AM+1, which was measured by on-farm data-loggers
Tbg	black globe temperature

TCI	thermal comfort index/ indices
Tdb	dry bulb temperature
Tdp	dew point temperature
THI	temperature humidity index
THRT	threshold temperature
THRT	threshold value
TI	thermal index/ indices
TM	The time of robotic milking
Tmax	daily maximum temperature
Tmax or min HSmean	Mean of maximum or minimum temperature during the days under heat stress
Tmax or min WCEmean	Daily maximum or minimum temperature adjusted by weighted cumulative effect
Tmean	daily mean temperature
Tmin	daily minimum temperature
Total Fat	The sum of daily fat content over the lactation period
Total MF	The sum of daily milking frequency over the lactation period
Total MP	The sum of daily milk production over the lactation period
Total Protein	The sum of daily protein content over the lactation period
Twb	wet bulb temperature
VT	vaginal temperature
WS	wind speed

Chapter 1. **General introduction**

1.1. Background and hypothesis

Farm managers are increasingly focused on enhancing animal comfort and welfare, which is necessary to maintain production efficiency of dairy cows (Phillips, 2018; Phillips, 2008). Heat stress is still one of the unavoidable challenges within modern dairy farming. For high-producing cows, the negative impacts of heat stress are exacerbated due to their lower heat tolerance and higher heat production. Measurements and assessments of heat stress have been undertaken for several decades, which developed several models to link thermal parameters and animal responses, such as heat load index (HLI) (Gaughan *et al.*, 2002) and comprehensive climate index (Mader *et al.*, 2010). Thresholds to categorize the levels of heat stress were also established from these models to provide decision support for farmers, such as Silva *et al.* (2007) and Hammami *et al.* (2013). General mitigations against heat stress include cooling facilities (Zimbelman, 2007), diet adjustment (Kanjanapruthipong *et al.*, 2015a) and genetic selection (Roland *et al.*, 2016). However, the results of assessment and mitigation to heat stress can be still varied between different breeds, climate and farm conditions. It still requires significant amount of time and labour cost for modification, when applying these approaches on specific farms. With the development of information technology, dynamic algorithm or artificial intelligence (AI) are expected to provide more applicable assessment or mitigation of heat stress than any constant equation or model, which could be self-modified according to specific farm condition. However, such studies are still rare in relation to heat stress.

Recently, various precision livestock farming (PLF) techniques are being applied by dairy farming systems (Banhazi *et al.*, 2012). Robotic milking system (RMS), as one major component of PLF, are widely adopted in Europe, Australia and America. The primary benefit of RMS is saving labour cost, which has been reported between 18% and 30% in comparison with conventional milking system (CMS) (Rodenburg, 2012). In addition to reduce labour cost, RMS is also able to collect high-frequency and long-term information of production and health condition for individual cow, which is accomplished by the sensors in milking robots (station) or wearable monitors (i.e. neck band or ear tag). This kind of information can possibly be utilized for routine detection of cows' health problems, such as lameness and mastitis (LeBlanc, 2016; Pastell and Madsen, 2008). In relation to heat stress, routinely measured parameters including milk temperature (MT) and rumination time (RT) are found to be sensitive indicators of animal responses (Chaudhari and Singh, 2015; Soriani *et al.*, 2013). Therefore, RMS can easily build a fundamental database for developing dynamic algorithm or artificial intelligent for solving heat stress problems. However, insufficient studies have been published in this area. Furthermore, as most measurements of heat stress were conducted in CMS, it is still unclear about the heat stress impacts on cows milked by RMS. Specific mitigations that can be taken in RMS (i.e. adjustment of robotic milking) also need further study.

1.2. Research Objectives

The objective of this study are:

- Review the progresses achieved in previous studies on measuring, assessing and mitigating heat stress of dairy cows.
- Compare the prediction performance of published thermal comfort indices (TCIs) by using data from field measurement and online database
- Identify accurate dynamic thresholds of heat stress via statistical and AI method
- Improve existed indices by adding new parameters
- Establish new indices for evaluating the impacts of heat stress
- Analyse the influence of heat stress in RMS and generate possible mitigations

1.3. Thesis outline

The thesis outline is provided separately for Chapter 2 to 6 as follows:

- *Chapter 2: A review on measuring, assessing and mitigating heat stress of housed dairy cow with precision livestock farming techniques*

  This chapter systemically summarized the development of technologies for measuring, assessing and mitigating heat stress. It was aimed to describe the gaps of current studies, as well as point out the potential directions for further studies including this study.

<u>*Chapter 3 to 6 report a series of analysis using 5-year dataset collected from RMS</u>

- *Chapter 3: Modelling of heat stress in a robotic dairy farm. Part 1: Thermal comfort indices as the indicators of production loss*

  This chapter compared the various published models assessing heat stress and demonstrated a simplified way of using thermal parameters in the assessment. This is a mathematical or statistical analysis and evaluation of the published studies reviewed in Chapter 2.

- *Chapter 4: Modelling of heat stress in a robotic dairy farm. Part 2: Identify of the specific thresholds with production factors*

  This chapter refined the thresholds of dry bulb temperature considering the specific production factors of cows (age, body weight and days in milk) that resulted in AI decision tree to support thresholds selection.

- *Chapter 5: Modelling of heat stress in a robotic dairy farm. Part 3: animal behaviour and milking performance*

  This chapter modelled the influence of heat stress on robotic milking performance and proposed a more suitable milking pattern for the robotic milking systems to enhance the milking efficiency of cows. A new rumination efficiency index (REI) was proposed to quantify the influence of heat stress on rumination time and milking behaviours.

- *Chapter 6: Modelling of heat stress in a robotic dairy farm. Part 4: Lag and cumulative effect of heat stress*

  This chapter developed several new indices of heat stress to quantify the duration, lag effect and cumulative effect. The intensity duration index (IDI) was developed for short term heat stress that can estimate the heat stress effects considering both of the temperature and duration. The heat stress mean temperature (HSmean) is established for long term heat stress, which can quantify the historical heat stress within a period of time. The new index demonstrated a good correlation with milk yield.

Chapter 2. A review on measuring, assessing and mitigating heat stress of housed dairy cow with precision livestock farming techniques

2.1. Abstract

Heat stress is a significant challenge for dairy farming systems. Dairy cows under heat stress will encounter sub-optimal welfare that can ultimately result in production loss for farmers. An increase in frequency and magnitude of heat stress events is predicted for coming decades. Thus there is a greater attention being paid on reducing the effects of heat stress on animals. In the past few decades, modelling and on-farm experiments have been used to assess the effect of heat stress on livestock. Mitigation solutions including optimal shed structure, ventilation systems, targeted feeding regimes, improved farm management and genetic selection have been explored widely on farms across the globe. However, under different farm conditions (e.g. with different mechanical or natural ventilation systems), the heat tolerance and coping ability of dairy cows can vary significantly. Until now, the results from different mathematical models have provided a variety of heat stress thresholds for on-farm use. In practice, it is still costly to determine an accurate heat stress threshold in order to design mitigation options. This review summarises the results of previous studies on the effects of heat stress on intensively kept dairy cows and the different approaches taken to address the issue. Here we undertook a comparative analysis of the published studies related to thermal indices, animal responses, (i.e. production loss and animal welfare), and mitigation approaches. A range of recommendations are made on conducting more accurate assessments and design of effective mitigation options. For future studies, there is a requirement to establish monitoring systems embedded into routine farm management systems. The robust monitoring system would need to acquire real-time data from the thermal environment (e.g. data from local weather stations), animal responses to heat stress (e.g. real-time respiration rate), and the operation pattern of mitigations (e.g. procedures and efficiencies). Furthermore, through big data analysis for each farm, self-recalibration needs to be automatically implemented for the assessment and control algorithms following the changes of cows' production status, farm management and local climate.

2.2. Introduction

Heat stress is defined as an event that affects animal's homeostasis and health due to physiologically harmful heat load (Gaughan *et al.*, 2012). The welfare and comfort of dairy cows are increasingly seen as moral and practical concerns, especially in developed countries (Silanikove, 2000; Phillips, 2008). Under heat stress conditions, the optimal welfare of dairy cows can be compromised via decreased feed intake, resting and rumination time (Grant, 2012). The sub-optimal animal comfort due to heat stress is the primary cause of production losses in the global dairy industry, especially for high-producing cows (e.g. -8.9 kg/cow/d under heat stress) (Biby, 2010). The general on-farms mitigation technique to combat heat stress for housed dairy cows is to control the thermal environment around animals (Mader *et al.*, 2007). However, the high cost associated with climate control systems cannot be economically justified in many cases (Zimbelman, 2007). The adjustment of diet (to reduce the negative effects of heat generation associated with increased metabolism) has been studied as a potential mitigation option, especially for cows at their early lactation stage (Kanjanapruthipong *et al.*, 2015b). There are nutritional strategies identified to cope with high ambient temperatures such as the use of a high energy diet to balance reduced feed intake and increased energy demand for thermoregulation, and use of protein with a low rumen degradability to balance increased N catabolism (Das *et al.*, 2016).

At the same time, the genetic selection of heat tolerant breeds has also been progressed with various level of success, such as the crossbreeding of Zebu-Hereford (Roland *et al.*, 2016). The selection of cow species and breeds suited to tolerate heat stress (at the expense of productive capacity) has been an important management strategy in hot and humid climates, with heat tolerant cows expected to become more widely used with projected global warming (Hoffmann, 2010). For example, in North Eastern Australia, the Belmont Red hybrid breed was developed to increase cow productivity through greater heat tolerance, parasite resistance and resilience to periodic severe under-nutrition (Rudder *et al.*, 1976; Coates *et al.*, 1987). Some modelling results have suggested that breeding for a greater tolerance to heat stress is unlikely to improve the livestock productivity (Moore and Ghahramani, 2014).

At present, various precision livestock farming (PLF) techniques are being developed for the benefit of modern livestock industries, such as robotic milking, precision feeding for individual animals, and farm management automation. Some of the PLF technologies have also been developed for detection of heat stress such as integrated monitoring for thermal environments and animal physiological responses (Pollard *et al.*, 2004; Schmidt *et al.*, 2004; Eigenberg *et al.*, 2008). In addition to hardware development, models and software development is also being undertaken to cooperate with the hardware (Black *et al.*, 2016; Willis *et al.*, 2016). A number of mathematical models (e.g. thermal comfort indices) have been developed using field observations to assess and predict the effects of heat stress on animals (Mader *et al.*, 2010; Gaughan *et al.*, 2008a). By using these models, a range of heat stress mitigation approaches such as auto-controlled sprinkling have been evaluated (Mader *et al.*, 2007). Moreover, the current literature provides varied heat-stress assessment for different on-farm conditions (e.g. different ventilation systems). In the field, selection and modification of the published assessment results, such as the thresholds, are always required with huge time and labour cost. Further development of modelling methods is still necessary to enable their application in the commercial farms and to overcome the bias between the results of different on-farm studies. This review aims to summarise the current knowledge of heat stress in dairy cows. The primary focus of this review will be on the published on-farm monitoring, thermal comfort indices, models, and the developed heat stress mitigation techniques for housed dairy cow. Moreover, the possible solutions by using PLF techniques for achieving more accurate modelling on heat stress assessment and mitigation will be discussed.

2.3. Mechanisms of thermal comfort

The dairy cow is homoeothermic animal that needs to keep her body temperature at a nearly constant level. As defined by DeShazer *et al.* (2009), the thermal neutral zone (TNZ) is the ambient temperature the animal requires to achieve the least thermal-regulatory effort. Within the TNZ range, vasoconstriction (minimum tissue conductance) and vasodilatation (maximum tissue conductance) regulate body temperature under the slightly colder or warmer ambient temperature. The general TNZ for mature beef and dairy cow is between 5 – 15 ^{o}C. However, it is obvious that cow with higher producing performance has a lower threshold temperature to enter heat stress. In addition, the threshold temperature between thermal noxious and extreme zones is still unclear. When the ambient temperature is out of the TNZ, lower/upper critical temperature (LCT/UCT) was identified as the thresholds forcing animal to cope via more thermal-regulatory efforts. Silanikove (2000) has defined the LCT as the ambient temperature decline to a level that a resting homeotherm has to increase the rate of heat production for maintaining thermal balance. The UCT was described as having three primary features: increased metabolic rate; increased evaporative heat loss, and minimal tissue thermal insulation. The author also categorised four stages of thermal well-being: the innocuous stage, the aversive stage, the noxious stage and the extreme stage. However, the threshold values of such thermal zones and stages can always change. Difference in breeds, farm conditions (e.g. building structure and facilities), management (e.g. feeding and milking frequency), and animal production levels can result in different values of TNZ, UCT and LCT (Johnson, 1987; Roenfeldt, 1998; Igono *et al.*, 1992; Wathes *et al.*, 1983; Nonnecke *et al.*, 2009; Spain and Spiers, 1996). With an average milk production of 30 kg/d/cow, Berman *et al.* (1985) reported that the UCT of a dairy cow is about 25 to 26 ^{o}C, while the LCT is about -37 to -16 ^{o}C as reported by Hamada (1971). Hahn (1999) reported specific temperature thresholds for cows in different production status, this information was refined by Silanikove (2000) to specify stages (Figure 2-1).

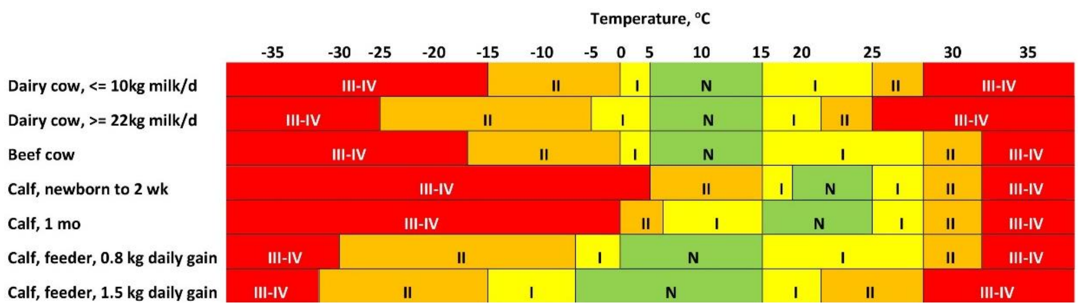

Figure 2-1 Critical ambient temperatures, thermal zones and stages for cow performance adapted from Hahn (1999) and Silanikove (2000), Zone-N: thermal neutral zone; Zone-I: thermal innocuous zone; Zone-II: thermal aversive zone; Zone-III: thermal noxious zone, and Zone-IV: thermal extreme zone.

By considering the internal heat balance of an animal, the heat input from nutrient and metabolic energy intake determines the capacity of heat loss and retained energy of an animal, as illustrated in Figure 2-2. The amount of heat production (heat loss) that is used to maintain body temperature will decrease the retained energy for production activities (DeShazer *et al.*, 2009; West, 2003; Kadzere *et al.*, 2002). It is commonly accepted that feeding a cow in an optimal thermal condition or TNZ is a way to increase production efficiency. Kadzere *et al.* (2002) concluded that the increased nutrient intake raises the milk production rate of dairy cows which also generate more metabolic heat production. The increased metabolic heat production maintains animal body temperature under cold conditions. However, it requires more heat dissipation to reduce body temperature under hot conditions.

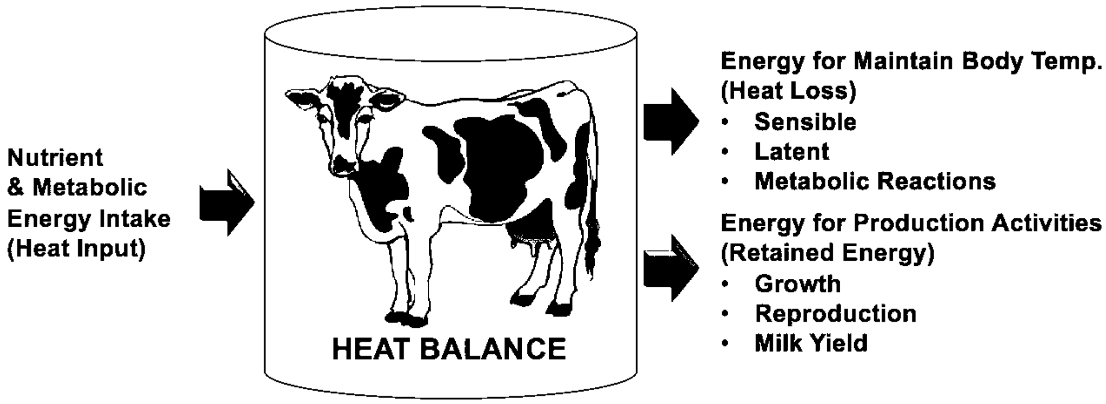

Figure 2-2 A schematic illustration of heat transfer within constant heat balance of dairy cow

The heat loss between ambient environment and the animal body can happen through sensible and latent heat exchange following thermodynamic principles. The sensible heat loss can occur via conduction, convection, and radiation, while the latent heat loss mainly happens as moisture evaporates. It should be noted that the decrease in sensible heat loss will always compensate for increased latent heat loss. The latent heat loss then usually follows the rule of psychometrics under standard pressure (101.325 kPa), as shown by DeShazer *et al.* (2009). Therefore, latent heat loss is typically minimised under high relative humidity conditions. It is also difficult to separate heat transfer by evaporation and convection, as wind flow will also enhance the moisture evaporation (Silanikove, 2000). Many physical factors can cause differences in thermal dynamic heat loss as summarised by DeShazer *et al.* (2009) (Table 2-1). Modelling of the heat balance has been the focus of several studies by simulating the heat transfer between ambient environments and animal body considering different animal activities (Turnpenny *et al.*, 2000a; Turnpenny *et al.*, 2000b).

Under principles of heat transfer, to some degree, animals can modify their behavioural and physiological activity patterns to cope with adverse thermal conditions (DeShazer *et al.*, 2009; West, 2003; Kadzere *et al.*, 2002). The basic animal responses to heat stress include increased respiration rate, sweating rate, lying vs standing patterns and animal preference to move to cooling areas, e.g. shelter. A series of nervous system responses and metabolic (biochemical) reactions occur simultaneously in order to maintain body temperature. Silanikove (2000) provided an overview of the physiological reactions of animals, including hormonal reactions, water balance metabolism, and nutrient supplementations. The biochemical reactions toward heat stress are not the primary focus of this review, as more integrated understanding of such physiological reactions have been summarized by other review articles (West, 2003; Rensis and Scaramuzzi, 2003; Zimbelman, 2007; Kadzere *et al.*, 2002; Biby, 2010; Roland *et al.*, 2016) In summary, the separation between heat transfer, behavioural responses, and biochemical reactions is only an artificial approach to simplify the complex system of thermal regulation. The separation of maintenance and production energy usage is also artificial (Kadzere *et al.*, 2002) to simplify the processes for assessments.

Table 2-1 Physical factors influencing energy transfer from the animal's body surface (DeShazer *et al.*, 2009 as adpated from ; Hahn, 1976).

	Modes of Heat Transfer			
Factors	Convection	Conduction	Radiation	Evaporation
Animal characteristics				
Configuration of animal	X	X[a]	X[b]	X[c]
Surface temperature of animal	X	X	X	X[d]
Emissivity of animal's surface			X	
Environmental characteristics				
Surrounding surface temperature		X	X	
Air temperature	X			
Air velocity	X			X
Air vapor pressure				X
Surrounding shape factor for radiation			X	
Emissivity of surrounding surface			X	
Thermal resistance of contact surface		X		
Heat capacity of contact material		X		

[a] For standing animals, conductive heat transfer is negligible; for animals lying, the area of animal surface in contact with the floor or supporting structure, conductive heat transfer is a factor.
[b] Area of the animal exposed to the radiation source or sink.
[c] Wetted area of the animal surfaces, including the respiratory passages.
[d] Temperature of the animal surface is an indirect factor because vapor pressure is a function of temperature.

2.4. Measurement of heat stress–indicators in the field studies

As presented in Table 2-2, this section introduces the key indicators in the studies of heat stress assessment. The measurement of heat stress indicators would require real-time data acquired in relation to environmental and animal responses. With the development of PLF techniques, robust monitoring systems are available for detecting most of these indicators. However, some of the indicators (e.g. rectal temperature) still require manual detection which can be time and labour intensive procedure.

Table 2-2 Instruments applied in field studies to evaluate the severity of heat stress

Type of parameters		Devices	Manufacture	Range	Uncertainty	References
Thermal parameters	Temperature & humidity	Tinytag Plus 2 logger	Gemini loggers Ltd, Chichester, UK	-25 – 80 °C 0 – 100 % RH	±1% full scale °C ±3% full scale RH	Schuller *et al.* (2014); Banhazi *et al.* (2008a); Banhazi *et al.* (2008c); Banhazi *et al.* (2008b)
		HMP45 data loggers	Vaisala, Helsinki, Finland	-40 – 60 °C 0 – 100 % RH	±1% full scale °C ±3% full scale RH	Tucker *et al.* (2008)
		HOBO Pro data loggers	Onset Computer Corporation, Pocasset, MA., USA	-40 – 70 °C 0 – 100 % RH	±0.5% full scale °C ±3.5% full scale RH	Mader *et al.*, 2007; Uzal Seyfi, 2013; Tucker *et al.*, 2008; Scharf *et al.*, 2012; Seyfi, 2013; Ortiz *et al.*, 2015
	Wind speed	Hall effect anemometer	NRG Systems, Hinesburg, VT, USA	1 – 96 m/s	±0.1 m/s	Tucker *et al.* (2008)
		Hot-wire anemometers	Alnor Instruments (Shoreview, Minn)	0-20 m/s	±5% full scale	Banhazi *et al.* (2008c)
	Solar radiation	Pyranometers Licor Li200x	Campbell Scientific Inc. Logan, UT, USA	400-1100 nm of daylight spectrum	±3-5% full scale	Tucker *et al.* (2008)
	Integrated measurement	Automated weather stations, Vantage PRO weather recorder	Davis Instruments, Hayward, CA., USA	-76 – 54 °C 0 – 100 % RH 0-1800 W/m^2 1-809 m/s	±1.5 °C ±3% full scale RH ±5% full scale W/m^2 1 m/s	Eigenberg *et al.* (2005a)
		110-WS-18 Portable weather station	Novalynx Corp., Grass Valley, CA., USA	-40 –604 °C 0 – 100 % RH 0.4 – 1.1 microns spectral 1-57 m/s	±1.5 °C ±3% full scale RH ±5% full scale W/m^2 ± 1mph	Legrand *et al.* (2011)
Animal responses	Sweating rate	Evapo-meter	Delfin Technologies Ltd., Kuopio, Finland	0-200 g/m^2 h	±8-10% full scale	Rungruang *et al.* (2014)

	Respiration rate	RR Automatic dataloggers	U.S. Meat Animal Research Central., USA	Not applicable	±2-3 BPM	Eigenberg *et al.* (2005a)
	Heart rate	Equine T56H transmitter	Polar Electro, Inc. Bethpage, NY, USA	Not applicable	Not applicable	Kumar and Hancke (2015)
	Rectal temperature	Digital thermometer, GLA-M500	Agricultural Electronics, San Luis Obispo, CA., USA	Unknown	Unknown	Church *et al.* (2014)
		Stainless steel probe YSI	1700 Brannum Lane, Yellow Springs, OH., USA	0 – 60 ºC	±0.2 ºC	Brown-Brandl *et al.* (2003)
	Skin temperature	Infrared thermometer Model RAYST80XB	Raytek Corporation, Santa Curz, CA, USA	-32 –760 ºC	<±1% full scale ºC	Scharf *et al.* (2012)
		Agema 489 thermal camera	FLIR Systems, Boston, USA	-15 –50 ºC	±2 ºC	Banhazi *et al.* (2009b)
	Vaginal temperature	Vaginal controlled drug	CIDR™, InterAg, Hamilton, New Zealand	Unknown	Unknown	Schütz *et al.* (2009)
		Thermochron sensor	Thermochron, SL, KN Laboratories, Osaka, Japan	-30 – 85 ºC	±0.5 ºC	Nabenishi *et al.* (2011)
	Lying patterns	HOBO Pendant G	Onset Computer Corporation, Pocasset, MA., USA	±29.4 m/s²	±2-3% full scale	Wang *et al.* (2016)
		ICEQube/ICETag	IceRobotics Ltd, Bankhead Rd, South Queensferry, British	Not applicable	Not applicable	(Maltz *et al.*, 2011)
Production data		Variables including the body weight, feed intake, milk yield, and conception rate are frequently recorded on-farms using a number of PLF devices in dairy and other livestock industries.				Banhazi and Banhazi (2015); Banhazi *et al.* (2015); Banhazi *et al.* (2011)

Thermal parameters

Thermal parameters are the key factors to calculate heat transfer. These parameters primarily include temperature, humidity, wind speed and solar radiation. As shown in Table 2-2, there are a wide range of commercial sensors in the market that can be used for monitoring these parameters. These devices have been used to monitor microclimates in dairy farming systems, thermal environment of the area, as well as the internal condition of bedding material (Mader *et al.*, 2007; Uzal Seyfi, 2013; Tucker *et al.*, 2008; Scharf *et al.*, 2012; Seyfi, 2013; Ortiz *et al.*, 2015a).

Temperature and humidity are the basic parameters to determine the thermal comfort. The temperature gradient through the animal body and ambient temperature leads to the sensible heat loss of the animal. The required parameters to calculate heat transfer are dry bulb temperature (Tdb), wet bulb temperature (Twb), dew point temperature (Tdp) and relative humidity (RH). Currently, temperature and humidity can be measured using commercial devices such as the Tinytag Plus 2 logger from Hasting Data Loggers, NWS, AUS(Schuller *et al.*, 2014; Banhazi *et al.*, 2008a; Banhazi *et al.*, 2008c; Banhazi *et al.*, 2008b). A similar sensor fitted with data logger (Hygrochron, KN Laboratories, Osaka) was used to detect these variables, as indicated by Nabenishi *et al.* (2011). HMP45, a humidity and temperature sensor (Vaisala, Helsinki, Finland) was applied in field measurement (Tucker *et al.*, 2008). HOBO Pro temperature and humidity sensor (Onset Computer Corporation, Pocasset, MA) has been widely applied in on-farm studies.

Wind speed (WS) or air velocity can influence the efficiency of convection and evaporation heat transfer. Under a natural ventilation system, the detection can only obtain mean value of air velocity for an approximate area. In a ventilated airspaces, the efficiency of fans and inlets/outlets can determine air velocity and air distribution within buildings. Therefore, airspeed is usually monitored around exhaust fans. Anemometers are widely applied in studies such as #40 Hall effect anemometer provided by NRG Systems, Hinesburg, VT, USA (Tucker *et al.*, 2008) or Hot-wire anemometer from Alnor Instruments, Shoreview, Minn (Banhazi *et al.*, 2008c; Banhazi, 2013). The sensitivity of the currently available instruments is still insufficient for low-speed air movement detection, although hot-wired anemometers can detect airspeeds around 0.1 m/s. There are a number of manual/portable anemometers available to measure low speed air movements, but their application is costly, associated with relatively large errors, and requires an optimized sampling plan (Van Overbeke *et al.*, 2015).

Solar radiation (SR) is the primary factor in heat transfer through radiation, especially at open lots. The evaluation of shelter efficiency is usually accomplished by comparing solar radiation with or without shading constructions (Kendall *et al.*, 2006). Tucker *et al.* (2008) have applied Pyranometers such as Licor Li200x Pyranometer (Campbell Scientific Inc. Logan, UT, the USA) for a direct measurement of solar radiation. Other devices such as Ruakura Meteorological Stations (AgResearch, New Zealand), have been used to measure the solar radiation as well as the hours of sunshine (Kendall *et al.*, 2006).

Black globe temperature (Tbg) is a parameter of the thermal environment that represents the effects of ambient temperature, wind cooling and solar radiation on cows (Li *et al.*, 2009). Tbg was measured using a matte black copper ball (12.5-15 cm diameter) with a temperature sensor inside it (Lee, 1953). Currently, integrated monitoring stations (weather stations) are commercially available to measure several thermal indicators within one device as listed in Table 2-2.

The measurement of thermal parameters can be performed at three levels; (1) regional, (2) building (herd), and (3) animal (individual) levels. At present, undertaking measurements at a single animal level are still difficult due to behaviour issues and varied postures of animals. Compared to measurements undertaken at building level, the heat transfer can be influenced by the varied behaviours of individual animals, such as standing or lying in different areas of the building. Therefore, further research is needed to develop reliable measurement methods for the direct monitoring of the thermal parameters around a single animal. The forecasting or estimation of thermal stress is still based on the data collected from regional and/or building levels.

Animal Responses

Physiological, behavioural, and immunological responses are the primary coping responses to the sub-optimal thermal environment. These responses can change from normal to impaired levels corresponding to the level and duration of adverse thermal conditions (Hahn, 1999). The ways animals cope with heat stress are described as acclimatisation (endocrine, cellular and metabolic responses to several stressors), acclimation (refers to a single stressor) and adaptation (a permanent change in the genotype) (Roland *et al.*, 2016). In this case, short-term acute events and long-term chronic challenges can cause varied responses of animals and finally influence the production performance (such as milk production). The TNZ of cow can shift during acclimatisation. Moreover, such responses could also be impacted by many other factors, such as mitigation strategies and genotype of the animal.

Sweating or sweating rate (SWR) is one of the obvious responses to high ambient temperature among the several physiological and behavioural responses. Animals use sweating as an effective cooling system through evaporative heat transfer. The sweating rate is highly dependent on the blood flow rate and sweat glands in unit skin area (Blazquez *et al.*, 1994). The skin moisture loss is technically difficult to measure and highly variable results have been obtained in the range between 77 and 279 g moisture/ m^2/h (Robertshaw and Vercoe, 1980; Blazquez *et al.*, 1994). Consequently, the detection of SWR is also very difficult. However, devices such as the evapo-meter (Delfin Technologies Ltd., Kuopio, Finland) were applied by Rungruang *et al.* (2014) to measure the sweating rate on dairy cow skin, clipped and shaved with a razor, and top of the hair coat (unclipped and unshaved) shoulder areas. Similar study was also conducted to measure the skin wetness of pig using infrared camera, which found the angle of camera, time lag after spraying and the size of animal could cause influence on the accuracy of the result (Banhazi *et al.*, 2009b).

Respiration rate (RR) or panting is an early warning indicator of entering heat stress. The animal body can increase respiration rate to dissipate excess heat. Silanikove (2000) has reported that panting is a way to cool the brain under heat stress (Silanikove, 2000). Lanham *et al.* (1986) found the RR could significantly decline after drinking water, which reduces internal body temperature via conductive cooling of the water. The measurement of RR is usually performed using visual detection. Monitoring of the panting frequency can be performed by human observation or via scanning or video recordings during constant time intervals (Scharf *et al.*, 2012). Panting score is usually given as breath per minute (Gaughan *et al.*, 2008a). U.S. Meat Animal Research Central (USMARC) has developed automatic monitoring devices that fixes such automated data logging system on the animal body (Eigenberg *et al.*, 2000). These devices have been applied in research (Brown-Brandl *et al.*, 2005; Eigenberg *et al.*, 2005a) to model the relationship between RR and heat stress.

The overall evaporation heat transfer via sweating and panting is defined as respiratory and cutaneous water (RCW) losses. Silanikove *et al.* (1997) reported the RCW loss of a 600 kg lactating cow could be as high as 13 kg/day. A significant increase in respiration rate can threaten animal health through respiratory alkalosis (Benjamin, 1981). Studies also reported air pollutants e.g. ammonia could possibly compromise respiration by impacting the body buffers, which have exacerbated the heat stress on animals (Costa *et al.*, 2003).

Heart rate (HR) can be measured and used to estimate the heat loss and retained energy (illustrated in Figure 2) in ruminants (Brosh, 2007; Green, 2011). The current HR monitor designs are based on electrocardiograph analysis, and these units transmit the data remotely, which minimizes the presence of humans close to the animals. Devices such as implantable very high frequency systems (VHF) combined with PSL-iEGG2 sensor (Alphonce *et al.*, 2017) or other integrated system with T56H transmitter (Kumar and Hancke, 2015) are also available commercially(Brosh, 2007).

Rectal temperature (RT) is an indicator of core body temperature which is highly related to the production performance (Zimbelman *et al.*, 2009; Johnson *et al.*, 1963). RT stays nearly constant in TNZ while tend to increase with increasing ambient temperature (Zimbelman *et al.*, 2009). Lemerle and Goddard (1986) found greater RR could prevent an increase in rectal temperature. Church *et al.* (2014) investigated the relationship between cow eye temperature and rectal temperature. Their study reported a strong positive correlation between eye and rectal temperatures. This positive correlation indicated that heat stress might be predicted reliably via detecting eye temperature, despite the fact that eye temperatures were consistently lower than rectal temperatures. However, significant impact from environmental factors (e.g. wind speed) was reported, which needs to be well-managed to ensure reproducible and meaningful readings from the monitoring devices. There are several commercially available devices for measuring rectal temperature, such as digital thermometers GLA-M500 (Agricultural Electronics, San Luis Obispo, CA) (Church *et al.*, 2014) and GLA 525/550 (Hi-Performance Digital Thermometer, San Luis Obispo, CA) (Wheelock *et al.*, 2010). Another version, a GLMA M700 (San Luis Obispo, CA) has been applied by Zimbelman (2007). Brown-Brandl *et al.* (2003) used the YSI (1700 Brannum Lane, Yellow Springs, OH 45387) stainless steel probe inserted to a depth of approximately 20 cm and recorded temperature with one minute intervals on Pace Scientific Inc. Pocket Logger (6407 Idlewild Rd., Suite 2.214, Charlotte, NC 28212). Bouraoui *et al.*

(2002) measured the rectal temperature using a veterinary digital thermometer (Jorgen Kruuse A/S, China, Model: MT 1681) inserted nearly 60 mm into the rectum for 60 seconds.

Skin temperature (ST) was also investigated by some researchers using an infrared thermometer (Model RAYST80XB, Raytek Corporation, Santa Curz, CA, USA) (Scharf *et al.*, 2012; Legrand *et al.*, 2011) and thermocouples (5SC-TT-T-30-36, Omega Engineering Inc., Stamford, CT) to measure skin temperatures on shaved areas of the animals' shoulders. An infrared gun (Raynger MX^{TM} model RayMX4pU Raytek C, Santa Cruz, CA) has been utilised to measure the skin temperature of the rump and loin of the animal from the right and left aside (Zimbelman, 2007). Similarly in piggery studies, to detect skin temperature, an Agema 489 thermal camera (FLIR Systems, Boston, USA) was applied by Banhazi *et al.* (2009b), and the compute image analysis was done by applying VideoPro32 Colour Image Analysis System software, version 2.17 (Leading Edge Pty. Ltd., Adelaide, Australia).

Using current technology, most of the measurements of SWR, RR, RT, and ST still require manual detection i.e. touching and inserting or scanning the animal body with portable monitors. These manual detections methods limit the frequency of data acquisition (i.e. real-time vs. hourly measurements). Human observation can cause errors and inconsistencies, adding uncertainty to collected data. Moreover, there is still an uncertainty if the interactions with animals during the manual detection can add extra stress to animals, which may influence animal responses to heat stress (Brosh, 2007).

Vaginal temperature (VT) is another method of measuring core body temperature, which can be conducted automatically with a sensor placed in the abdominal cavity. Such measurement can be performed by telemetry systems consisting of an implantable transmitter and a CorTemp^{TM} data logger with 30 s data logging interval (HQ, Inc. 9th Street Drive, West Palmetto, FL., USA.) (Eigenberg *et al.*, 2005b). Another method is using vaginal controlled drug release insert (CIDR^{TM}, InterAg, Hamilton, New Zealand) fitted with microcontroller Minilog-TX data logger (Vemco Ltd., Shad Bay, Nova Scotia, Canada) that has been applied in measurements of internal body temperatures (Schütz *et al.*, 2009; Kendall *et al.*, 2006; Tucker *et al.*, 2008). During these studies, temperature loggers were inserted into the vaginal cavity. Similar instrument, such as a thermal sensor (Thermochron, SL, KN Laboratories, Osaka, Japan) attached to an intravaginal drug release device (PRID, Ceve Sante Animale, Libourne, France) was used by Nabenishi *et al.* (2011).

Lying patterns (LP) is the main behaviour response of animals to cope with heat stress. The change in frequency, duration, and position of lying behaviours usually corresponds with the different level of heat stress (Anderson *et al.*, 2013). It is known that standing can help cow to increase the available surface of evaporation or convective cooling from ventilation, while lying can increase the available surface of conductive cooling from bedding material. (Anderson *et al.*, 2013; Smith *et al.*, 2016; Provolo and Riva, 2009). Core body temperature (CBT) will intensively affect the lying behaviour of a cow as reviewed by Allen *et al.* (2013). The measurement of lying behaviour can be visually conducted by observing the cows (Tucker *et al.*, 2008), but this methods is labour intensive and thus expensive. Automatic approaches include video recording system such as Aycan Alarm systems, from Security Joint Stock Company, Samsun, Turkey, (Uzal Seyfi, 2013) and sensor devices such as HOBO Pendant G, from Onset Computer Corp (Wang *et al.*, 2016).

Several behavioural indices have been developed to assess the comfort of cow, such as cow comfort index (CCI) (Cook *et al.*, 2005), free-stall use index (SUI) (Overton *et al.*, 2002), and cow stress index (CSI) (Mattachini *et al.*, 2011). These indices calculate the ratio of cows to specific behaviour (e.g. lying vs. standing sows per building or farm). The seasonal variation of these indices is correlated to thermal indices and Uzal Seyfi (2013) specifically suggested an accurate time range for daily behaviour observation. Instead of continuous observation, Uzal Seyfi (2013) recommended performing observations during 08:00 AM to 15:00 PM and 19:00 PM to 24:00 PM in the autumn and summer, 12:00 PM to 16:00 PM in the winter and 10:00 AM to 12:00 PM and 20:00 PM to 22:00 PM in the spring. It was also found that the building structure e.g. insulated or uninsulated building can affect the value of these behaviour indices even under the same thermal conditions (Provolo and Riva, 2009). Stocking density was also demonstrated to have an influence on these indices, the 129% stocking density can reduce ratio of SUI ≥ 85%, compared with 82% and 100% stocking density (Wang *et al.*, 2016).

Production Performances

The measurement of production performances is a routine task for farmers and farm managers. Several research projects, management systems, and report standards have been developed and applied by agricultural institutes for farmers (Ghavi Hossein-Zadeh *et al.*, 2013; Bryant *et al.*, 2007; Carabano *et al.*, 2016a). The measured variables are mainly the body weight, feed intake, milk yield, and conception rate recorded in databases for farm or regional management. This section reviews the primary indicators affected by heat stress: feed intake, milk production, and fertility.

Feed intake or dry matter intake (DMI) decreases to reduce diet-induced thermogenesis (DeShazer *et al.*, 2009). It is reported that a heat stressed dairy cow could have negative energy balance (NEBAL), when feed intake was not meeting energy demands (energy input in Figure 2) of maintenance (heat loss) and lactation (retained energy) (Allen *et al.*, 2013). Moreover, under heat stress, the reduction of feed intake also exacerbates the degree of NEBAL and decreases the milk production (Rhoads et al., 2009). Measurement of DMI can be performed by automated feeding systems such as Insentec feed bins (Rough- age Intake Control system, Insentec B.V) (Wang *et al.*, 2016).

Milk yield (MY) or milk production is the primary concern during heat stress. The majority of studies on the impact of heat stress on dairy cows have used MY as the indicator of production performance (e.g. investigating correlation between MY and rectal temperature) (Table 2-4). Some research has described that reduced milk production might result in reduced metabolic heat production as well (West, 2003). Key indicators of production performance related to heat stress, such as the quantity and quality of milk production (protein, fat, casein, lactose and total milk solids) can be automatically reported by milk analysers incorporated into the milking stations, such as FT120, from Foss Elctric, Hillerod, Denmark (Kendall *et al.*, 2006). This theoretically enables producers to assess the impact of heat stress on farms. Furthermore, factors including interval and duration in milk station for individual animals are also available for analysis of the animals' milking behaviour.

Conception rate (CR) or productivity is another indicator of the production performance which can be affected by the heat stress. Conception rate is defined as the success rate of artificial insemination, or the inverse of the number of services per conception (Ghavi Hossein-Zadeh *et al.*, 2013). Rensis and Scaramuzzi (2003) reported that heat stress in summer can cause nearly 20-30% reduction in conception rate compared with the thermal conditions in winter time. Moreover, they pointed out that the effect of heat stress was lasting. The effect of summer heat stress could even cause negative after-effects during the autumn. It was also reported that increased uterine temperature can be measured as another indicator of heat stress which is linked to decreased conception rate (Biby, 2010). In modern dairy farms, information about production performance is always recorded by farmers as part of their management systems. There are systems developed to monitor animal reproduction by collecting data such as breeding dates and pregnancy detection e.g. HerdeW version 5.5, from Software Projecktierungs und Handels GmbH, Aschara, Germany (Schuller *et al.*, 2014). Traditionally, detection of the pregnancy is performed manually by rectal palpation (Morton *et al.*, 2007a).

In summary, the measurement of the heat stress indicators is the first step for assessing the heat stress impact on farms. However, no uniform measurements systems are available for recording these key variables on farms. Thus, different results may be generated by farmers and researchers depending on the type of devices and operating procedures used.

2.5. Assessment of heat stress – the development of thermal indices

As illustrated in Table 1, several factors can affect heat transfer and heat balance within intensive livestock systems. Thermal indices (TI) are developed to model the linkage between such factors and the indicators of animal responses to assess the heat stress impact on animals. This section will summarise the published thermal TIs (Table 2-3), simulated thresholds of indices (Table 2-4), and results from field measurement (Table 2-5). The initial studies on heat stress were focused on heat tolerance and adaptability of animal through indicators of animal body condition such as RT and RR (Rhoad, 1944; Bianca, 1963; Benezra, 1954). Many studies have used the temperature humidity index (THI) as an indicator of heat stress (Sevi *et al.*, 2001). As shown in Table 2-3, the original THI was established as a discomfort index (THI-1) using Tdb and Twb for evaluating human comfort (Thom, 1959). The adapted form of THI equation (THI-4) was used to model the rectal temperature of bull calves and different coefficients of Tdb and Twb (Bianca, 1962). THI-5 and 6 were studied on Heifer cows (MY: 15.5 kg/d) in a climate chamber (Berry *et al.*, 1964; Yousef, 1985). The values calculated by these two indices (THI-5,6) were used as the basis for a livestock weather safety index (LCI, 1970). THI-7 was used to report a strong relation between thermal condition, milk production and animal comfort (Johnson, 1965). In the past 50 years, a range of equations were developed to estimate THI, as described in Table 2-4.

Assuming uniform responses from all animals on a farm to a thermal stressor is unrealistic due to differences between animals such as age, genotype, and production level, thermal variance of ventilation and solar radiation (Berman, 2005). As an example of the impact from different production levels on thermal tolerance, Zimbelman *et al.* (2009) found the threshold of THI-6 has declined to 68 compared with the previous stress value 72, as the milk production level increased from about 15 kg/d to around 30 kg/d after the initial THI equation was established. Gorniak *et al.* (2014) found the value might be even as low as 60, denoting a reduction in feed intake and milk production for the temperate climate in Germany. Similar threshold (THI = 62) was also found by Hammami *et al.* (2013) from an experiment in Luxembourg. Due to these limitations for using THI equation, the reported thresholds were only suitable to the condition where it was developed. The modification to THI equations has been conducted in much published research (Mader *et al.*, 2006; Buffington *et al.*, 1981; Yousef, 1985; Baeta *et al.*, 1987; Gaughan *et al.*, 2002; Berman, 2005; Eigenberg *et al.*, 2005a; Gaughan *et al.*, 2008a). These studies attempt to include more related factors to the model such as wind speed and solar radiation, as well as the specific biological differences due to breed type, coat colour, and health status. Tbg was used to replace dry bulb temperature in the general THI equation which developed the black globe humidity index (BGHI) (Buffington *et al.*, 1981). The equivalent temperature index (ETI) was built by analysing the relationship between milk production and the heat-loss rates (Baeta *et al.*, 1987). By considering wind speed, this index demonstrated the importance of ventilation cooling in hot and wet condition. By incorporating the effect of wind speed and solar radiation into THI-6, Mader *et al.* (2006) provided an improved regression (higher R^2) with panting score to estimate the discomfort of heat stress of feedlot cattle. Brown-Brandl *et al.* (2005) used further environmental parameters to develop the respiration rate index (RR) at a feedlot for different breeds. Their research demonstrated the importance of considering heat tolerance with or without shading, especially for animals under THI>78. Gaughan *et al.* (2002) developed the heat load index (HLI) for feedlot beef cattle. Modelling was based on panting observations in four Australian commercial feedlots. The management factor, shaded or unshaded, was taken into account. They used Tbg, RH, and WS to mathematically describe the microclimate condition of the animals. The study finally determined thresholds value (starting net gain of body heat) for different body and environmental conditions of animals. The index was modified with another study including 13 feedlots farm and used as the basis for a risk assessment program on the web server (Gaughan *et al.*, 2008a). Based on the HLI equations (HLI-1,2), the comprehensive climate index (CCI) was developed to assess both cold and heat stress (Mader *et al.*, 2010). They used the CCI index to estimate an apparent temperature which represented ambient temperature that the animals would feel. CCI included varied adjustment equations for the main equation which accounted for the different weight of thermal factors. However, the latest indices are primarily modelled on feedlot cows fed in open areas. The effort to understand the differences between beef cows and dairy cows as well as the variability between intensive and extensive farming systems is still necessary. Berman (2005) introduced an index, the threshold temperature (THRT) by simulating the thermal balance of Holstein dairy cows (Berman, 2005). The index was used to estimate the air temperature threshold when dairy cows need heat relief. The simulation of using the THRT in practical estimations used a thermal balance model consisting of 153 elements such as the effects of milk production, hair coat depth, ambient temperature, humidity, wind speed and exposed body surface (posture) (McGovern and Bruce, 2000). Based on these varied effects, the study introduced multiple equations for different production levels to calculate the air temperature when respiratory heat loss is occurring. In practical, on-farm heat stress studies (Table 2-5), not all of these developed indices were selected to be included in the predicting equations. Currently, the equation of THI-6 was used more extensively in field studies (Table 2-5). There is a limitation for application of TIs, in part due to the limitation of the available instrument, the difficulty of measurement, and weight of thermal factors in different equations.

The assessment of cow heat stress generally has reported the threshold values of heat stress (such as the breakpoint of decline in production performance), the interaction of time effect (such as the time lag of heat stress), and the coefficient between thermal indices and animal responses (such as reduction in milk yield per day per unit increase in THI). The maximum and minimum THI values were used to quantify the intensity of heat stress (Herbut and Angrecka, 2012).The maximum THI value was usually measured at mid or early afternoon which indicated the highest heat stress suffered by dairy cows. Inversely, the minimum THI value was measured for quantifying the night time cooling efficiency. For example, the threshold of mortality was reported based on maximum THI > 80 and minimum THI > 70 (Vitali *et al.*, 2009). The research found that the coefficient between mortality and THI had an increase from -0.542 to 232.75 deaths per 1000 cattle after exceeding the threshold point (THI maximum > 80). Several threshold values were developed based on varied thermal indices as shown in Table 2-4. The coefficient between animal production and heat stress level is one of the primary interests in related studies listed in Table 2-5 (e.g. Hammami et al. (2013)).

Different studies have related the significant effect from heat stress to different exposure time (e.g. two weeks (Berry *et al.*, 1964), 24-48 hours (Spiers *et al.*, 2004), and 2 days (West *et al.*, 2003)). García-Ispierto *et al.* (2007) reported the impact of heat stress on the conception rate to be highly dependent on timing of the stress and the artificial insemination service. Their results indicated that prediction of impact by the heat stress using THI equation should focus on the three days before and one day after the artificial insemination (AI). They also reported that the heat stress predicted by a THI equation could decrease the ratio of pregnancy and foetal loss during 21-28 days of gestation. To provide effective mitigation, farmers were required to monitor at least five weeks before and one week after service (Morton *et al.*, 2007a). It can be concluded that application of the short-term values of THI (e.g. THI during stress time) has no value in predicting animal responses to heat stress. Studies that determine THI values for a longer-time period (e.g. fortnightly) have a greater value; however, the long-time results (e.g. thresholds) cannot be applied in real-time mitigation (e.g. ventilation control). A long term assessment (e.g. two weeks with exposure to high THI) cannot prevent the milk yield loss under heat stress. Zimbelman (2007) recommended that the assessment following the heat stress event should not exceed 48 hours. Therefore, farmers can provide timely mitigation measures to avoid milk yield losses in a two week time window after start of the heat stress.

The finding of 20 publications on heat stress research studies as summarised in Table 2-5 were focused on cow mobs with a varied number of animals from <20 to >10000 with time durations in range between few weeks and several years. They had selected cows with a body condition score from 2 to 6. The range of daily milk yield level was between 15 to 32 kg/d/cow. Calculated as a coefficient with unit increasing of TI, the physiological responses of animal were quantified in the range between -0.11 and -0.23 hour/TI of lying time, and +0.09 to +0.54 BPM/TI of RR. The core body temperatures (VT or RT) were quantified in a range of +0.01 to +0.293 ^{o}C/TI. The production performance was quantified in a range of -0.13 to -0.254 kg/TI of DMI, -0.125 to -1.48 kg/TI of MY, and -0.5 to -1.1 %/TI of conception rate. Several factors such as varied regional climate condition or animal body conditions caused the variance of these results. However, there is still insufficient understanding of the most effective way to deal with these factors. Several studies have aimed to determine the reasons for such differences by evaluating the performance of published TIs. Bohmanova *et al.* (2007) compared the performance of seven THI indices (THI 1 to 7) for predicting milk production loss using data collected from 61 herds in America. The author indicated that the weight of humidity in THI equation is the primary reason for the inconsistency under different climates. Silva *et al.* (2007) evaluated the performance of equations of THI-5, BGHI, ETI, ESI, HLI-1, and RR on their regression with animal physiological responses. The author recommended ETI and HLI equations for evaluating tropical heat stress conditions. However, in the study of Kendall *et al.* (2006), HLI was reported to be less useful compared to THI to account for the vaginal temperature. Another evaluation of these equations by Hammami *et al.* (2013) for Holstein breeds recommended that THI-8 was the best choice for preliminary heat stress forecast in Luxembourg. This was related to the robust nature of the equation and taking into account the wind speed and solar radiation. Another evaluation of the thermal indices was conducted by comparing their Pearson correlation coefficient between indices' values and physiological responses (Li *et al.*, 2009). The study used data collected from 5 different states in America and University of Arizona Parker Agriculture Research Complex in the same year 2008. The correlation between indices' values and animal responses (skin temperature, rectal temperature, and sweating rate) did not gain high correlations (< 0.4) when the data set was pooled. However, the results collected from Parker Agriculture Research Complex showed reasonably good correlation between respiration rate and THI-adj, BGHI and HLI. Dikmen and Hansen (2009) have even pointed out that a direct application of the ambient temperature (Tdb) as the predictor of rectal temperature might have a similar performance as THI equations in sub-tropical regions.

Table 2-4 is presenting calculated threshold values for different thermal indices under multiple climate conditions from cold-dry to hot-wet. In this table, the microclimate condition was simulated for wind speeds in range between 0 and 7 m/s, while the solar radiation was varied between 475 and 1112.5 W/m^2. In Table 2-4, threshold values of these thermal indices are categorised as normal, alert, urgent and emergency as different levels of heat stress. It is likely that under the same climate conditions, different thermal indices would result in different assessment. For instance, in Table 2-4, the thermal condition with Tdb equal to 27^{o}C and RH equal to 68% will be considered as Danger level with THI-7, while it will be assessed as Normal level with THI-2. As discussed above, the difference can be related to the varied contribution of the parameters in the equations. To improve the accuracy of the equation by adding enough parameters to account for different climate or farm conditions, national or international studies are necessary (e.g. Gaughan *et al.* (2008b)). The proposed outcome can be a guideline for farmers to select a suitable method to estimate TI for their specific farm conditions and animal breed. However, the time resource and the labour cost can be an important driver in their selection for evaluation method.

In summary, the development of thermal indices has been undertaken for several decades to provide a modelling tool in order to assess the likely impact of heat stress. The recent version of TI equations (RR or HLI) permitted the provision of an early warning function for heat stress. In general, they have applied panting score or respiration rate as a sensitive indicator of animal responses to heat stress. Temperature, humidity, wind speed and solar radiation are the most required environmental factors considered in the recent equations (RR or HLI). With the improvement in technology for measurements, studies can now be conducted on animals in different regions/countries and studies can be conducted for longer periods. Animal related variables such as breed, coat colour, body condition and production status have also been considered in model development in previous studies (Gaughan *et al.*, 2008b; Gaughan *et al.*, 2002; Carabano *et al.*, 2016b). All these improvements have resulted in development of equations to deal with a greater complexity of calculation and to be applicable to practical conditions.

Table 2-3 Summary of TI equations

Equation	Farm, Facility & Time arrangement	Number of Animals	Breed & Production Level	Indicator of Responses	Reference
THI-1 = 1.8 * [0.4 * (Tdb+Twb)] +47 THI-2 = 1.8 * (0.15Tdb + 0.85Twb) + 32 THI-3 = 0.72Tdb + 0.72Twb + 40.6 ESI = 0.63Tdb - 0.03RH + 0.002SR+ 0.0054Tdb * RH – 0.073(0.1+SR)-1 【HUMAN】	N/A	N/A	N/A	N/A	Thom (1959); Moran et al. (2001)
THI-4 = 1.8 * (0.35Tdb + 0.65Twb) + 32	Climate chamber	4	Ayrshire bull calves 【FEEDLOT CATTLE】	RT	Bianca (1962)
THI-5 = Tdb + 0.36 Tdp + 41.5 THI-6 = 0.8Tdb + (Tdb - 14.4) * RH + 46.4	Climate chamber	56	Heifers MY: 15.5 kg/d	Unknown	Berry et al. (1964); Yousef (1985)
THI-7 = 1.8 * (0.55Tdb + 0.2Twb) + 49.5	Unknown	Unknown	Holstein	MY	Johnson (1965)
THI-8 = 4.51 + THI-6 – 1.991WS + 0.0068SR	Pens with varied shelter (14:00-17:00, 82-83 days)	72 (exp.1) 96 (exp.2)	Angus Angus crossbred 【FEEDLOT CATTLE】	RR	Mader et al. (2006)
BGHI = Tbg + 0.36Tdp + 41.5	Open pasture with shad structure (24 hour, 4 non-consecutive days)	30	Mostly Holsteins	RT, RR, MY	Buffington et al. (1981) Yousef (1985)
ETI = 27.88 – 0.456Tdb + 0.012Tdb2 – 0.491RH + 0.001RH2 + 1.151WS – 0.126WS2 + 0.020Tdb * RH - 0.046Tdb * WS (T:16-40℃, RH:40-90%, WS: 0.5-6.5 m/s)	Unknown	Unkown	Lactating dairy cow	MY	Baeta et al. (1987)
HLI-1 = 33.2 + 0.2RH + 1.2Tbg – (0.82WS)0.1 – log(0.4WS2 + 0.001)	4 feedlots (2 months)	2187	【FEEDLOT CATTLE】	RR, Behaviors	Gaughan et al. (2002)
RR = 5.1Tdb + 0.58RH – 1.7WS + 0.039SR – 105.7	Pends with shade structure (4 months)	96	Angus, Hereford, Pinzgauer, Red Poll	RR	Eigenberg et al. (2005b)

			【FEEDLOT CATTLE】		
THRT = 33.7 + 7.9WS – 3.8WS2 -3.3WVP + 1.6WS*WVP (T:20-45, RH: 0.8-3.9kPa, WS: 0.2-2 m/s)	Data simulation for indoor farming	N/A	Holstein (MY:45kg/d)	Thermal Balance Outputs	Berman (2005)
HLI-2 = 8.62 + 0.38RH + 1.55Tbg – 0.5WS + e2.4-WS (Tbg>25) HLI-2 = 10.66 + 0.28RH + 1.3Tbg – WS (Tbg<25) [e = 2.71828]	13 feedlots Varied shade specifications (>1 year)	>10000	B.Taurus, B. Idicus Waygu Crossbreed 【FEEDLOT CATTLE】	RR, Tympanic temperature,	Gaughan et al. (2008a)

Table 2-4 Threshold values based on thermal indices equations

Tdb (ºC)	RH (%)	WS (m/s)	SR (W/m^2)	THI-1	THI-2	THI-3	THI-4	THI-5	THI-6	THI-7	THI-8	BGHI	ETI	HLI-1	RR	HLI-2
20.0	52.5	7.0	475.0	72	59	65	61	65	65	74	59	72	18	65	28	67
21.0	54.8	6.5	517.5	73	61	67	63	67	67	76	62	74	20	67	37	70
22.0	57.0	6.0	560.0	75	63	68	65	68	68	77	65	75	21	69	46	72
23.0	59.3	5.5	602.5	76	65	70	67	70	70	79	68	77	23	70	55	75
24.0	61.5	5.0	645.0	78	67	71	69	71	72	80	70	79	25	72	64	78
25.0	63.8	4.5	687.5	79	69	73	71	73	73	81	73	80	27	74	73	81
26.0	66.0	4.0	730.0	81	72	75	73	74	75	83	76	82	29	76	82	84
27.0	68.3	3.5	772.5	83	74	76	75	76	77	84	79	84	31	77	91	87
28.0	70.5	3.0	815.0	84	76	78	77	77	78	86	82	86	34	79	99	90
29.0	72.8	2.5	857.5	86	78	80	80	79	80	87	86	87	36	81	108	94
30.0	75.0	2.0	900.0	88	80	81	82	81	82	89	89	89	39	83	117	97
31.0	77.3	1.5	942.5	89	83	83	84	82	84	90	92	91	42	85	126	101
32.0	79.5	1.0	985.0	91	85	84	86	84	86	92	95	92	44	87	135	105
33.0	81.8	0.5	1027.5	93	87	86	88	85	88	93	99	94	47	89	144	111
34.0	84.0	0.0	1070.0	94	89	88	90	87	90	95	102	96	50	94	153	118
35.0	86.3	0.0	1112.5	96	92	89	93	88	92	96	104	97	53	95	161	

*Thresholds for THI and BGHI: Normal:<74; Alert:74-79; Danger:79-84; Emergency:>84; (Eigenberg *et al.*, 2005b)

*Thresholds for ETI: Normal:<30; Alert:30-34; Danger:34-38; Emergency:>38; (Silva *et al.*, 2007)

*Thresholds for HLI-1: Normal:<89; Alert:89-92; Danger:92-95; Emergency:>95; (Silva *et al.*, 2007)
*Thresholds for HLI-2: Normal:<80; Alert:80-88; Danger:88-92; Emergency:>92; (Hammami *et al.*, 2013)
*Thresholds for RR: Normal:<90; Alert:90-110; Danger:110-130; Emergency:>130;(Eigenberg *et al.*, 2005b)
*The legend of color and levels: Normal, Alert, Danger, Emergency

Table 2-5 Summary of studies using thermal indices

Responses	Thermal Index	Animal NUM	Duration	Genotype	Production and body status	Environmental condition	Coefficient with thermal index value	Reference
LP	THI-6 HLI-2	24	2 months	Holstein Friesian	601 kg body weight 2.5-3.75 (1-10) body condition score Non-lactating	Tdb: 18.2 – 31.4 ℃ RH: 19 – 64% WS: 0 – 2.8 m/s SR: 169 – 308 W/m2 THI: 62 – 77.7 HLI: 54-81	-0.16 to -0.23 hour lying time /24 hour/ THI -0.11 to -0.16 hour lying time /24 hour/ HLI	Legrand et al. (2011)
RR	THI-5 BGHI ETI ESI HLI-1 RR	413	1 year	Holstein Jersey	15 kg/cow/day milk yield	Tdb: 25 – 35 ℃ RH: AVG 65 % WS: AVG 9.6 m/s	+0.099 bpm respiration rate / THI +0.155 bpm respiration rate / BGHI +0.520 bpm respiration rate / ETI +0.464 bpm respiration rate / ESI +0.542 bpm respiration rate / HLI-1 +0.344 bpm respiration rate / RR	Silva et al. (2007)
RT or VT	THI-6 HLI-2	40	20 days	Holstein Friesian	514 kg body weight 3-4 (1-10) body condition score	Tdb: 7.7 – 27.8 ℃ THI: 46.4 – 74.1 HLI: 41.8 – 81	+0.01℃ vaginal temperature / THI	Kendall et al. (2006)
	THI-5 HLI-2	27	18 days	Holstein Friesian	193 lactation days 510 kg body weight 4-5 (1-10) body condition score	Tdb: 1 – 24℃ RH: 32 – 94% Tbg: 0 – 33	+1.9% of internal body temperature / THI +0.6% of internal body temperature / HLI	Schütz et al. (2009)

						SR: 0 – 1171 W/m2	(basic internal body temperature 38.4 °C)	
	THI-6 HLI-2	36	20 days	Holstein Friesian	158 lactation days 499 kg body weight 3.5-6 (1-10) body condition score 20.8 kg/cow/day milk yield	Tdb: 8.5 – 28.6 °C RH: 9 – 47% WS: 0 – 31.7 m/s SR: 0 – 1309 W/m2 THI: 47.8 – 77.7 HLI: 45.6 – 102.2	+0.07°C vaginal temperature / HLI No relation to THI	Tucker et al. (2008)
	THI-7	10-48 (8 separate studies)	1 month	Holstein	90 – 140 lactation days	Tdb:8 – 40 °C RH: 8 – 40 % THI: 60 – 80	+0.06°C rectal temperature / THI	Zimbelman (2007)
	THI-5 BGHI ETI ESI HLI-1 RR	413	1 year	Holstein Jersey	15 kg/cow/day milk yield	Tdb: 25 – 35 °C RH: AVG 65 % WS: AVG 9.6 m/s	-0.053°C rectal temperature / THI (NS) +0.054°C rectal temperature / BGHI (NS) +0.293°C rectal temperature / ETI +0.209°C rectal temperature / ESI +0.286°C rectal temperature / HLI-1 +0.114°C rectal temperature / RR	Silva et al. (2007)
DMI	THI-7	10-48 (8 separate studies)	1 month	Holstein	90 – 140 lactation days	Tdb:8 – 40 °C RH: 8 – 40 % THI: 60 – 80	-0.13 kg/cow/day dry matter intake / THI	Zimbelman (2007)
	THI-6	14	2 month	Holstein Friesian	19-20 kg/cow/day milk yield	Tdb:14.7 – 38.9 °C RH: 18.5 – 82.4% THI: 68 – 78	-0.18 kg/cow/day dry matter intake / THI	Bouraoui et al. (2002)
	THI-3	Unknown	2 season winter summer	Jersey Sindhi	Unknown	THI: 74 – 86	-0.254 kg/cow/day dry matter intake / THI	Henry et al. (2014)

MY	THI-3	145	15 years	Holdeo (Holstein Friesian * Deoni)	Unknown	Tdb: 22 – 38℃ RH: 25 – 54% WS: 6.2 m/s	-18.22 kg of seasonal lactation milk yield / THI -142.59 days of lactation / THI	Mote et al. (2016)
	THI-6 HLI-2	40	20 days	Holstein Friesian	514 kg body weight 3-4 (1-10) body condition score	Tdb: 7.7 – 27.8 ℃ THI: 46.4 – 74.1 HLI: 41.8 – 81	-0.2 kg/cow/day milk yield / THI -0.125 kg/cow/day milk yield / HLI	Kendall et al. (2006)
	THI-1 to 7	94362	Unknown	Unknown	28-30 kg/cow/day milk yield 166-174 lactation days	Tdb: 1 – 38 ℃ RH: 11 – 99% THI: 31 – 92	-0.22 to -0.57 kg/cow/day milk yield / THI	Bohmanova et al. (2007)
	THI-6	14	2 month	Holstein Friesian	19-20 kg/cow/day milk yield	Tdb:14.7 – 38.9 ℃ RH: 18.5 – 82.4% THI: 68 – 78	-0.4 kg/cow/day milk yield / THI	Bouraoui et al. (2002)
	THI-6	174	3 months	Holstein Friesian	Group 1: 12 kg/cow/day milk yield Group 2: 21 kg/cow/day milk yield Group 3: 32 kg/cow/day milk yield	Tdb:15.5 – 33 ℃ RH: 35 – 96 % THI: 60 – 80	Group 1: -0.18 kg/cow/day milk yield / THI Group 2: -0.28 kg/cow/day milk yield / THI Group 3: -0.36 kg/cow/day milk yield / THI	Herbut and Angrecka (2012)
	THI-6	1173	9 years	Holstein	Unknown	Tdb: -13.6 – 37.5 ℃ RH: 7 – 100 % THI: 34.2 – 87.5	-1.48 kg/cow/day milk yield / THI	Ghavi Hossein-Zadeh et al. (2013)
	THI-3	>10000	16 years	Deoni	246 lit/cow/month milk yield	Tdb: 21 – 39 ℃ RH: 22 – 51 %	-0.347 lit/cow/month milk yield / THI	Thorat et al. (2016)
	THI-6 THI-8 HLI-2 ETI ESI CCI	23963	11 years	Holstein	5 – 330 lactation days 23-24 kg/cow/day milk yield	Tdb: 0 – 18℃ RH: 72 – 91% SR: 36 – 164 W/m2 WS: 2 m/s	-0.164 kg/cow/day milk yield / THI-6 -0.152 kg/cow/day milk yield / THI-8 -0.123 kg/cow/day milk yield / HLI-2	Hammami et al. (2013)

							-0.146 kg/cow/day milk yield / ETI -0.109 kg/cow/day milk yield / ESI -0.154 kg/cow/day milk yield / CCI	
	THI-6	191012	6 years	Holstein	5 – 305 lactation days Multiparous	THI: 50-85	-0.91-1.27 kg/cow/day milk yield / THI	Bernabucci et al. (2014)
	THI-6	6813	10 years	Unknown	15.5-17.5 kg/cow/day milk yield	THI: 46 – 79	-0.2 kg/cow/day milk yield / THI	Bouraoui (2009)
CR	THI-6	1150	2.5 years	Holstein	10345 kg annual milk production/cow	Tdb: 2.3 – 29.8 °C RH: 49-96%	-0.02 odds ratio / THI -0.63% conception rate / THI -0.50% conception rate / hours with THI > 73	Schuller et al. (2014)
	THI-6	11302	3 years	Holstein Friesian	613.5 kg body weight 25-29 kg/cow/day milk yield	Tdb: 12.2 – 32.1 °C RH: 9 – 47% THI: 55.8 – 79.9	-0.55% conception rate / THI Critical point: THI>69 1 day before AI	Nabenishi et al. (2011)
	THI-5	8155	2 years	Holstein Friesian	26 year around calving	THI: 66 – 78	-1.11% conception rate / THI with THI > 70	Morton *et al.* (2007a)
	THI-6	1735	3 years	Holstein	10499-10266 kg annual milk production/cow	Tdb: 5.6 – 31.8 °C RH: 72 – 94 % THI: 42.9 – 80.7	-0.309% conception rate / THI mean -0.318% conception rate / THI max	García-Ispierto et al. (2007)
	THI-6	6813	10 years	Unknown	15.5-17.5 kg/cow/day milk yield	THI: 46 – 79	-0.926% conception rate / THI	Bouraoui (2009)

2.6. Mitigation of heat stress – the way of cooling

To enhance cow comfort and minimize production loss under adverse thermal conditions during summer time, environmental management is a necessity (Grant, 2012). The mitigation strategies for housed cows following physical or thermodynamic principles (conduction, convection, radiation and evaporation cooling) will be reviewed in this section. In practical terms, other approaches (not included in this review) such as fixed time artificial insemination (Rensis and Scaramuzzi, 2003), nutrition/feeding (Kanjanapruthipong *et al.*, 2015b; Wheelock *et al.*, 2010; Rungruang *et al.*, 2014; Miron *et al.*, 2010; Miron *et al.*, 2008) and genetic improvements (Hansen, 2007) are also important to provide combined benefits with thermal cooling facilities, but will not be included in this review.

Roof and Shading

The effect of solar radiation load as a contributor to heat stress was researched by Pollard *et al.* (2004). Shading constructions should generally be made available on dairy farms, especially on feedlot dairies. The purpose is to minimize the solar load (radiation heat transfer) in the afternoon and maximize the efficiency of conductive or convection cooling (Sparke *et al.*, 2001). The parameters of the material, size, and orientation have been studied for nearly 5 decades (Givens, 1965; Hahn *et al.*, 1962; Bond *et al.*, 1967). An animal radiation heat balance (Rbal) was derived by Berman and Horovitz (2012). The author defines radiation from animal surrounding sources as the underside of the roof providing the shade, the cool and shaded ground, the hot unshaded ground surrounding the shadow created by the shade, the indirect diffuse radiation from a cool sky, and the radiation from the sun-exposed ground surrounding the shade. The primary effect factors of Rbal were determined as the intensity of radiation sources, roof and shaded surface dimensions, and animal density. The author also concluded that the increase of the shaded area (shaded ground) per cow would only provide a small effect, as the surface of the cow's body only had a small radiation heat gain from the shaded ground. Shoshani and Hetzroni (2013) reported some new barn construction designs: the sliding roof, shuttered roof, open ridge roof and pagoda (capped-gable) roof. The author mentioned varied building specific dimensions such as orientation, width, height and slope of the roof should be adjusted for different local conditions. Wind and sunshine direction are the two key factors for these dimensions. For the efficiency of shading mitigation, Kendall *et al.* (2006) reported the influence from the shading facility could only gain limited mitigation compared with enough night cooling for the cow under temperate summer conditions. It was also found that although shading protects animals from highest solar radiation, the different treatments of shading (non-shading, 25%, 50% and 90% shading) had no difference on lying behaviour and only 0.2 ^{o}C reduction (37.9 ^{o}C – 25% shading vs. 37.7 ^{o}C – 90% shading) of minimum body temperature (Tucker *et al.*, 2008). Studies also found that the colour of the cow's body had a significant influence on the body temperature changes caused by solar radiation, as well as the preference of the shading type. Similar results were also demonstrated by Schütz *et al.* (2009).

Bed Cooling

Cows lose heat through conduction when lying down, if the surface in contact with the cows is lower than body temperature. Cummins (1998) used different bedding materials for the dairy cow and found cows preferred ground limestone. The naturally occurring conductive cooling when cows lie on sand bedding was studied by Radoń *et al.* (2014), who developed a transient heat exchange computational model that predicted that cows would lose about 400 W (or 339 W/m^2) when lying on sand bedding for typical summer afternoon conditions for Bratislava, Slovakia (steady-state flux was lower). This model assumed that stalls were not over-crowded and, consequently, the sand bedding was able to cool off between lying bouts. Cows may also choose to lie down in cooler areas. Research performed on a free-stall dairy barn (with cubicles in 4 lines, head to head) by Seyfi (2013) found that the cows preferred to use the courtyard for resting during all seasons instead of the high-cost stalls. The cows preferred the dry and clean area with a soft floor and high air speed. Therefore, an engineer should pay more attention to the design of the courtyard area. Adequate shade areas and good drainage could be the important factors for a comfort courtyard area. Cows may also sacrifice comfort and hygiene to seek out conductive cooling when heat stressed. Herbut and Angrecka (2018) studied cows behaviour under heat stress and reported that as the daily average THI increased from 62.6 to 73.2, cows decreased the amount of time lying in stalls from 9.2 to 6.7 h/d and increased the amount of time lying in manure alleys from 0.5 to 1.4 h/d, apparently as a way to cool down. However, cows lying in a manure alley to cool off is uncomfortable and will lead to poor hygiene.

Some studies have investigated enhancing natural conductive cooling by actively chilling water then using the chilled water to cool the bedding for the cows. Perano *et al.* (2015b) developed and evaluated a conductive cooling system that circulated chilled water through modified cow's waterbeds. The conductive cooling system was evaluated with both 4.5 and 10 °C circulating water and using about 1 cm of sawdust bedding on top of the waterbed. The study reported that the cooled cows maintained nearly 5% higher milk yield, 14% higher dry matter intake and 18 bpm lower respiration rate compared to control cows when all the cows were challenged with heat stress. Perano *et al.* (2015b) also demonstrated that for waterbeds, an active heat sink is needed since installing waterbeds on top of concrete and relying on passive conductive cooling from the waterbed to the concrete surface did not have any measurable effect. Gebremedhin *et al.* (2016) used computational fluid dynamics to model the system studied by Perano *et al.* (2015b) and predicted that when a cow was lying down on a waterbed cooled with 4.6 °C water, the cow would conduct 430 W/m^2 to the waterbed. In another study, Ortiz *et al.* (2015a) tested a conductive cooling system comprised of a flat-plate heat exchanger chilled with 7 °C water and covered with 25 cm of bedding. The conductive cooling system was tested with both dried manure and sand bedding at different climate-controlled conditions (hot and dry, hot and humid, and thermos neutral). The study reported an increase in milk yield from 29.6 kg/cow/d to 31.0 kg/cow/d for cooled cows for the sand bedding under hot and humid conditions treatment. On the contrary, for the dried manure bedding under hot and humid conditions, the study reported a reduction in milk yield from 29.1 kg/cow/d to 27.6 kg/cow/d for cooled cows. There was no change in milk production for cooled cows for other treatments, nor was there any change in feed intake or respiration rate for any of the treatments. The Ortiz *et al.* (2015a) study measured a maximum steady-state flux of 28.6 W/m^2 in their system due to the insulating effect of the 25-cm bedding, which is likely why the Ortiz *et al.* (2015a) system measured less benefit to the cows then the Perano *et al.* (2015b) study.

Ventilation and Evaporative Cooling

The most direct way for cooling the cows is using nature ventilation. Marciniak (2014) compared the cooling efficiency between the barn with flat roof exhaust ducts and air supply from side curtain and the barn with roof ridge and air inlets. The former one gains more comfortable thermal conditions for the cows. However, the author also pointed out that natural ventilation can only provide limited mitigation to heat stress. Fans and air mixers are strongly recommended for the severe heat stress period. The basic rule of effective evaporation cooling is the sufficient moisture holding capacity of ventilation air. Sprinkler systems wet the cow with large water droplets, and consequently, don't rely on good water holding capacity in the air whereas mister systems and fogger systems rely on small water droplets evaporating quickly. Thus, sprinkler systems are better suited for high humidity conditions than mister systems (Collier *et al.*, 2006). Sprinkler systems wet the cow to provide direct evaporative cooling to the cow, but the cooling effect is only indirectly measured by THI. However, fogger or mister systems evaporative cool the air to aid in cooling the cow; consequently, the cooling benefit to the cow is indirect but the cooling is quantified by the reduction in THI. One drawback to evaporative cooling is the increase in moisture in the environment, which can lead to health problems such as mastitis and lameness, especially if the rest area is wetted (Nienaber and Hahn, 2007). Typically sprinkler systems are used in areas where cows are standing (Martin *et al.*, 2012) to avoid wetting the bedding.

A novel fan-sprinkler configuration for free stall cooling was studied by Hillman *et al.* (2005). The study obtained a 0.3 ℃ reduction of vaginal temperature when comparing the fan-sprinkler cooling to general fan cooling. Smith *et al.* (2007) evaluated and reported another option of the free-stall building which applies low profile cross ventilation (LPCV) to provide temperature control for cows during all seasons. The system can maintain the dairy cow in her TNZ and with stable core body temperature. However, the efficiency of such systems applying under hot-humid conditions was not as good as applying under hot-dry condition. The efficiency of other commercial fan/mist systems were also compared by several studies (Oetting *et al.*, 2002; Spurlin *et al.*, 2002; Collier *et al.*, 2003). Roland *et al.* (2016) reviewed 10 on-farm studies applying different ventilation systems. The author concluded the respiratory diseases had a higher occurrence in mechanically ventilated buildings compared with ones with natural ventilation. Such negative influences could be more harmful to calves. A study was also conducted on linking the operation of evaporation cooling facility (sprinkler) with THI threshold (Mader *et al.*, 2007). In the experiment, the water sprinkler was controlled based on data collected from a weather station. When the THI threshold (based on THI-6) was higher than 68 at 09:00 AM, the electronic solenoid would start water flow of the sprinkler. An electronic timer was also applied to control the duration of sprinkling as 20 min/ 1.5 hours between 10:00 AM to 17:00 PM. Another sprinkling strategy without automatic control was used for comparison. Sprinkling was operated once per day at 10:00-12:00 AM or 14:00-16:00 PM based on whether the THI value obtained from local weather report was higher than 77. The automatically controlled sprinkling gained significant reduction in the animal panting score compared with the once per day sprinkling, which indicates that the sprinkling was helping to directly cool the cows. Mader *et al.* (2007) also concluded that cows that have acclimatised to sprinkled microclimate would gain heat stress when the sprinkling stopped after a hot summer. That means their tolerance to heat stress might be weakened. Cook *et al.* (2007b) also reported that cows may take several weeks to fully acclimate to heat-stress conditions, so care must be taken to not abruptly stop cooling cows.

Some studies paid attention to improving the automatic control strategies, such as Daskalov *et al.* (2006) and Soldatos *et al.* (2005). These improved control strategies (nonlinear robust control) applied non-linear modelling and varying breakpoints. Compared with the traditional control strategies which applied fixed breakpoints, the new one can control multiple variables simultaneously without affecting each other. However, these strategies only rely on thermal variables which do not consider animal responses in the calculation. Another improvement was made by Qi and Deng (2009). The developed new strategy upgraded previous single-input single output (SISO) system to multi-input-multi-output (MIMO) system. The upgrading applied linear modelling for controlling temperature and humidity simultaneously. Related to water cooling, Legrand *et al.* (2011) reported that cows would make considerable use of a shower to reduce heat load. However, high variance existed between individuals. The author also tested the behavioural response to water cooling of cows. The results indicated a significant relationship between behaviours with heat stress, but no difference between water cooling and the control group.

It is important to note the effect of night cooling as the primary nature cooling approach. The importance of night cooling was emphasised by several researchers such as Scott *et al.* (1983). Igono *et al.* (1992) reported that enough night cooling could enhance the heat tolerance of dairy cows and result in less MY loss even with high ambient temperature. Moreover, cooling the animal during her dry period can also gain improvement on subsequent lactation (Do Amaral *et al.*, 2009). Moreover, providing fresh drinking water can also dissipate heat stress for dairy cows. Pereyra *et al.* (2010) found the absorbed energy at 31 ^{o}C could be lower than 18 ^{o}C with enough water intake. However, the water temperature did not affect the amount of water intake.

Comparison of Cooling Systems

Grant (2012) reported a summary on the benefit of cooling facilities in several studies which were adapted and supplemented in Figure 2-3. Evaporative cooling systems tended to have the most cooling benefit. Seven studies examined the effects of evaporative cooling systems (fans with sprinklers, misters, and/or foggers) and found that the increase in milk production ranged from 0.6 to 7.7 kg/d/cow and averaged 4.1

kg/d/cow. Conductive cooling with waterbeds improved milk production by 2.1 kg/d/cow. Cooling systems with fans only, shade only, or conductive cooling with a flat-plate heat exchanger were less effective and only improved milk production by 0.4 to 0.8 kg/d/cow. However, the water usage for sprinkler cooling system can be a huge cost for farmers, while the efficiency might be reduced by hot-humid conditions (Smith *et al.*, 2007). In arid regions water may be a limited resource, and maximum water usage may be regulated by government authorities (Martin *et al.*, 2012). Sprinkler systems may require from 3,650 to 15,500 L/cow for a 120-d cooling season (Frazzi *et al.*, 2000; Meyer *et al.*, 2002; Perano *et al.*, 2015a). Moreover, the mitigation of heat stress gained more benefit on milk production for cows during early period of lactation (7.7 kg/cow/d vs. 5 kg/cow/d with same mitigation strategy)(Do Amaral *et al.*, 2008; Tao *et al.*, 2011).

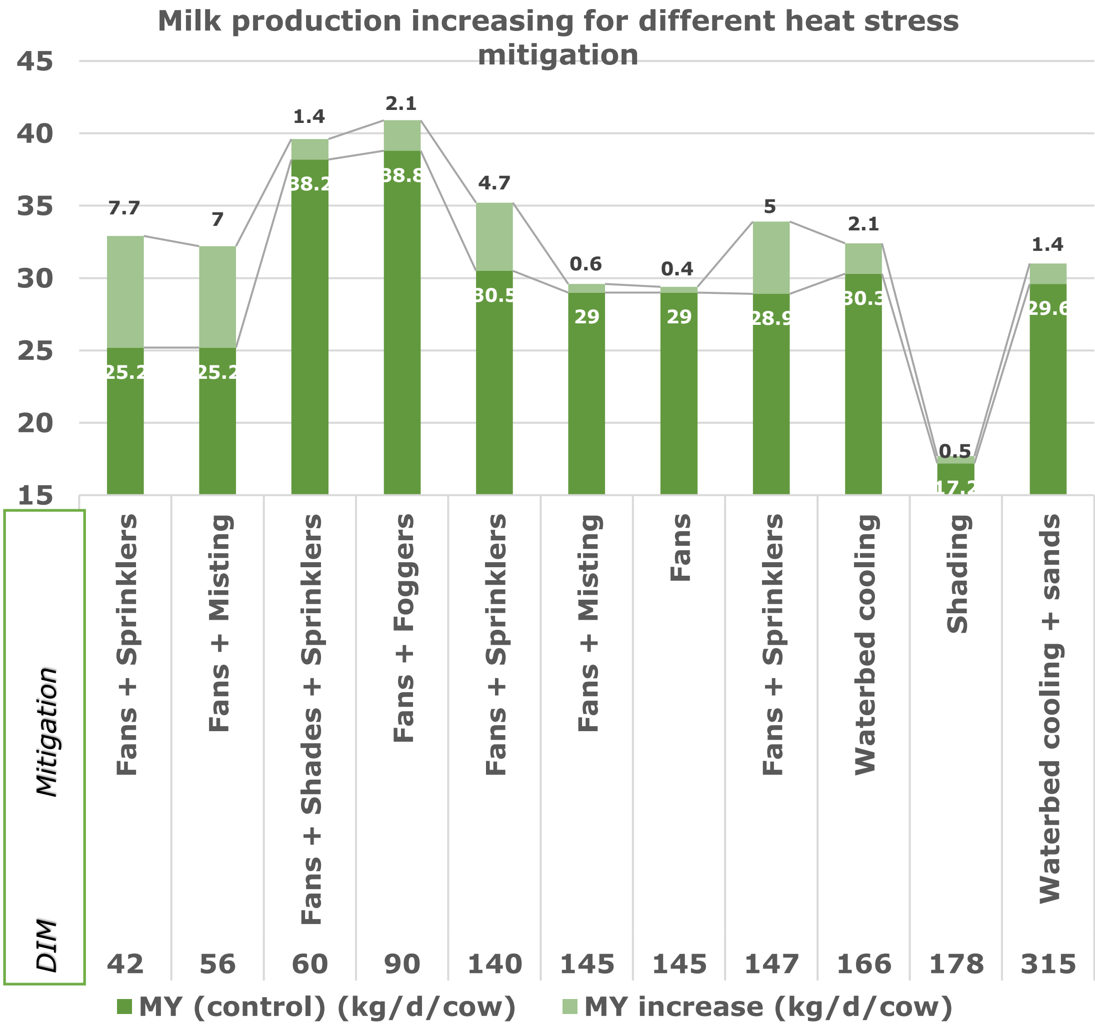

Figure 2-3 Effect of thermodynamic cooling strategies adapted from Grant (2012), data cited from (Avendano-Reyes *et al.*, 2006; Urdaz *et al.*, 2006; Do Amaral *et al.*, 2008; Do Amaral *et al.*, 2009; Adin *et al.*, 2009; Tao *et al.*, 2011; Ortiz *et al.*, 2015b; Frazzi *et al.*, 2000; Kendall *et al.*, 2006)

Economic Returns from Cooling Systems

Several studies have concluded that fans and sprinklers are a good investment for cow cooling. Dhuyvetter *et al.* (2000) used data from two different high-producing farms in Kansas, US (51.9 to 52.6 kg/day for one farm and 39.4 to 40.0 kg/day for the second farm) and demonstrated that there was a 3 – 6% increase in cows' milk production under heat stress when the cooling system included fans over the feedline and just over the stalls in addition to sprinkler over the feedline and estimated economic paybacks of \$50 to \$63 per cow per lactation (higher producing farm) and \$47 to \$60 per cow per year (lower producing farm). They estimated up to 6X more payback for cooling 2nd and subsequent lactation cows vs. 1st lactation cows.

St-Pierre *et al.* (2003) modelled economic impacts of heat stress and potential economic benefits of cooling via fans, fans + sprinklers, or the fogger system Korral Kool. Their model for dairy cows considered calves (0 to 1 yr), yearling heifers (1 to 2 yrs), and dairy cows separately. Their model accounted for economic impacts from DMI, MY, Preg Rate, Days Open, Cull Rate, and Death Loss as functions of maximum THI for the day according to climate data and the THI threshold of 70. The study did not distinguish between primiparous cows and multiparous cows. The models for dairy replacements accounted for DMI loss, Gain loss, and Death Loss as a function of the daily max THI and the respective THI thresholds (72 for yearlings and 70 for calves). Estimated reduction in milk production per cow per year ranging from 68 kg/cow/yr to 2072 kg/cow/yr for the US state with the least heat stress (Wyoming) to the most heat stress (Lousiana). They estimated annual losses due to heat stress of up to \$383 (Texas 345,000 cows) and \$337 (Florida, 155,000 cows) per cow per year without abatement. With abatement Florida (High = \$50.131 million/ 155,000 cows = \$323.43 per cow per year), Texas (Intensive = \$129.680/ 345,000 cows = \$375.88 per cow per year) implies that the "optimum cooling" is only profiting \$7 per cow in Texas and \$14 in Florida.

Based on literature values, Ferreira *et al.* (2016)concluded that milk yield for multiparous cows could be reduced by as much as 5 kg per day for the entire lactations if cows were heat stressed during the dry period based on other studies documenting milk production losses of 1.2 kg/d to 7.5 kg/d in the subsequent lactations due cows being heat stressed while dry. Ferreira *et al.* (2016) did not include other economic effects of heat stress in their economic analysis, but other studies have documented the effects of heat stress during the dry period to be decreased fertility in cows (Wiersma and Armstrong, 1989), decreased immunity and fertility (Do Amaral *et al.*, 2011; Thompson and Dahl, 2012) and reduction in birth and growth rates of calves from cows that were heat-stressed during their dry period. Ferreira *et al.* (2016) considered a heat stress day to be a day with average THI over 68. Reduced profit per cow per year could be \$68 (Wisconsin), \$101 (California), to \$233 (Florida) if cows make 5 kg/d less in their subsequent lactation after being heat stressed. Similarly, Adin et al., (2009) measured an increase of 5.3% in milk yield in subsequent lactations due to evaporative cooling during the dry period in a study performed in Israel. Adin et al., (2009) also reported an average decrease in body temperature of 0.2 ^{o}C, and a decrease in respiration rate from 66.7 to 39.7 bpm. The cooling system used was 10 min of wetting cows using individual sprinklers at 120 L/h followed by 30 min of fans.

Perano *et al.* (2017) performed an economic comparison of cooling systems using fans only, fans + misters, Korral Kool, or conductive cooling with fans. The study assumed that cows were in a climate where THI reached 80 to 85 for at least 2 hr/day, which is classified by Moretti *et al.* (2017) as "danger" level for lactating dairy cows but considered moderate heat stress by other authors (Renaudeau *et al.*, 2012). Perano *et al.* (2017) used the change in core body temperature due to the different cooling systems to predict the change in milk yield based on the relationship between core body temperature and milk yield of a heat-stressed cow established by Spiers *et al.* (2004) and Perano *et al.* (2015a) and assumed that the milk profit was $0.33/kg as Ferreira *et al.* (2016) estimated. Literature values for expected reduction in core body temperature of the four systems came from the following sources: conductive cooling (Perano *et al.*, 2015a); Korral Kool (Tarazón-Herrera *et al.*, 1999); fans + misters (Chanchai *et al.*, 2010); and fans only (St-Pierre *et al.*, 2003). Cost to run the cooling systems was based on assuming a 10-yr life for the system and calculating the investment cost for the system as well as the operational costs of electricity and water (if applicable). Other less easily quantified traits such benefits to long term health of the cow or sustained milk production after the heat stress is gone were not included in the analysis. The results showed that the fans + misters system had the most economic benefit (Figure 2-4).

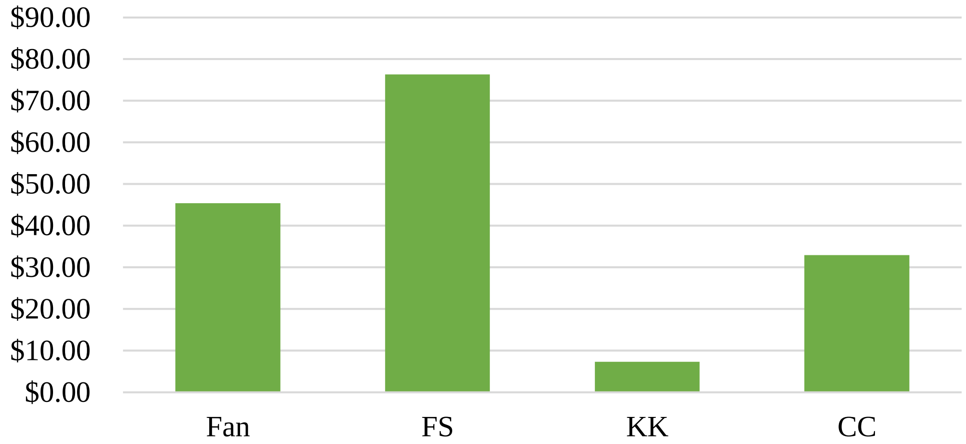

Figure 2-4 Comparison of economic returns with different cooling systems adapted from Perano *et al.* (2017).Fan – fans only, FS – fans + misters, KK – Korral Kool, CC – fans + conductive cooling.

2.7. Discussion – the factors causing variation

The variability between different studies conducted under different conditions can create unexpected uncertainty, which is always a challenge. Studies have identified the key factors causing the variation, and provided quantified solutions to minimize the variation. However, the results from these studies are still limited in relation to bringing them into a full application, e.g. providing replicable adjustment of the TI calculations for different farms. Below, a range of animal related factors including breeds and genetic, difference between laboratory and field studies, animal status and responses, as well as the accuracy of instrument will be discussed.

Breed and Genetic

At present, different genetic breeds have been selected for general dairy farming system. These breeds would include Ayrshire, Brown Swiss, Busa, Canadienne, Dairy Shorthorn, Dutch Belted, Estonian Red, Fleckvieh, Friesian, Girolando, Guernsey, Holstein, Illawarra, Irish, Moiled, Jersey, Kerry, Lineback, Meuse Rhine Issel, Milking Devon, Montbéliarde, Normande, Norwegian Red, Randall, and Sahiwal. The genetic of B.- Indicus- and Zebu-type cow has shown a better heat tolerance than B.-Taurus type cow (Kadzere *et al.*, 2002). A conventional crossbreeding between B.-Taurus and B.-Idicus cow would result in an improved thermal tolerance, but lower milk yield performance (Biby, 2010). Sharma et al. (1983) found that milk production of Jersey cows is less affected by the heat stress compared to the Holsteins. The assessment of the USA Holstein breed (172,411 sires and 10.5 milk cows) showed that high heat stress tolerance can be transmitted from a cow to her daughters, which can enable the daughters to achieve higher pregnancy rate, longer productive life and lower milk production (Bohmanova *et al.*, 2005). The gene imposing (such as stock hair gene) and embryo technique have been used to improve the heat stress tolerance of Holstein cows (Olson *et al.*, 2003; Dikmen *et al.*, 2008; Hansen, 2007). However, further research is still required to identify potential benefits from genetic improvements. Bohlouli *et al.* (2013) performed a regional study on Iranian Holstein dairy cows which collected data from nearly 10000 dairy cow during five years' test-day milk production. They found that the response to heat stress had a heritable component that could vary with the number of lactation days. Carabano *et al.* (2016b) reported that the comfort zones (expressed as THI thresholds) with maximum production performance were different across climatic and production conditions. This difference is because of an animal in warm climate conditions can suffer higher heat stress as their THI threshold for upper comfort zone is 6-7 times higher in comparison with temperate climate conditions. However, their production performance in the comfort zone was lower. It was also reported by Bryant *et al.* (2007) that the threshold value of THI to reduce production performances (milk-solids) varied within different breeds. The threshold for the Holstein Friesian (HF) breed was 68 followed by 75 and 69 for Jersey (J) and crossbred (HF*J), correspondingly. Bohmanova *et al.* (2005) found the milk yield reduction ranged from -0.48 to 0.38 kg per THI (THI>72) corresponding to different transmitting ability series (PTAs) in a national genetic evaluation in U.S. Based on the studies summarized in Table 4, the genotype information of the herd is still limited. Although the two most popular breeds in the study were Holstein and Friesian cows, specific genetic information was usually ignored.

Laboratory versus Field Studies

Roland *et al.* (2016) concluded in the review paper that studies dealing with thermal regulation and thermal stress had been performed on different numbers of cows. These include studies on a small number of animals, specific climatic region or observations from a single herd, farm or research station. However, there is a lack of statistical analysis between different studies testing same parameters on different number of animals or regions. Legates *et al.* (1991) found the respiration rate and rectal temperature of dairy cow could be higher in a chamber experiment than field measurement. However, for beef cows, the opposite results were found (Eigenberg *et al.*, 2000). In this case, rumen temperature was recommended by (Scharf *et al.*, 2012) to overcome the difference between laboratory and field measurements. Other factors such as stocking density can also affect the animal responses (Wang *et al.*, 2016), which will not usually be considered in laboratory studies. Moreover, field studies can focus on a variety of regions (dairy herds or farm) in a country (Gantner *et al.*, 2011) or multiple countries (Gaughan *et al.*, 2008a), which may include several climate conditions simultaneously. In contrast, laboratory studies can only simulate one kind of climate condition at one time. The influence of farm management is also typically ignored in laboratory studies. Garcia-Ispierto *et al.* (2007b) reported the influence from AI technicians to fertility performance and concluded the odds ratio could vary from 0.49 to 0.65. The author also emphasised the influence of farm management (such as milking frequency) which could change the performance of daily milk yield and fertility (milking on the insemination day) (Al-Katanani *et al.*, 1999).

Animal Status and Lag of Response

Even under same farm management and unique heat conditions, the animal responses such as respiration rate and rectal temperature could be changed diurnally. The changes can be caused by a metabolic shift in a day (Brown-Brandl *et al.*, 2003). Dairy cows under different production status also have varied tolerance to heat stress. Gantner *et al.* (2011) conducted research on dairy cows in different lactation status (heifers to 5^{th} lactation) and concluded the most significant milk yield reduction was found with heifers under heat stress. However, Bernabucci *et al.* (2014) found the influence was more severe in multiparous cows compared with the first lactation. Also, iteration of days in milk, seasonal and regional effects were found by Huang *et al.* (2008), which could cause a varied effect on conception rate. Research also reported that the responses of an animal to the thermal environment has lag which correlates to the body size of the animal (Hahn *et al.*, 1999).The sensitive responses such as RR and SW are still difficult to be real-time measured automatically and accurately (Scharf *et al.*, 2012). However, for developing early warning system towards heat stress, the improvement of devices for monitoring these parameters is still important. The potential for field implementation of related studies is notable (Eigenberg *et al.*, 2008).

Instrument and Measurement

It has been reported that, in the environmental measurement, temperature and humidity conditions are not identical to the entire animal house (Herbut and Angrecka, 2012). Specific shape and construction of the building can cause differences between TIs' values in particular occupied zones. The new indices which take account of solar radiation and wind speed can partially reduce the differences in temperature and humidity measurement. However, the variance on solar radiation and wind speed measurement can still cause differences.

2.8. Solutions in Precision Livestock Farming

Monitoring animal behaviour responses might not be a good solution for measuring heat stress without robust instruments. The reason is that using behaviour patterns to adjust ambient temperature is laborious and inaccuracy. Silanikove (2000) summarised that the behaviour measurement might occur in the absence of a productive response, especially in the short-term. In recent days, with the development of computer vision techniques and attachable behaviour detectors, monitoring animal behaviour is more feasible, accurate and user-friendly for farmers (Eigenberg *et al.*, 2000). Sensors which were applied for laboratory studies are usually commercially available with large potential of field implementation (Maltz *et al.*, 2011). The variance and lag of heat stress measurements are expected to be solved by some early warning techniques supported by a regional (even global) database sharing consistent standards. The early warning and regional database approaches have already been achieved by some researchers, such as the applications developed by Eigenberg *et al.* (2007). However, further improvements and better applications are still required. Nienaber and Hahn (2007) indicated that such a system should include sensors and cameras linked to computer network and programming. The purpose is to finally provide farmers with a decision support system which can function in the environment and on the animal. Optimal treatment to an individual animal level can be taken in the face of thermal uncomforting. In a review paper for heat wave problems and global warming, Kuczynski *et al.* (2011) emphasised the importance of superior environment control systems which could allow individual animals to find their optimal conditions. Better adjustment possibilities for individual animals could be provided within these systems compared with the one that only offers a unique operation to the whole environment. From this view of point, PLF has a wealth of possibility for further improvements. As emphasised by Banhazi *et al.* (2009a), the important objective of modelling animal, environment and building control systems is to reduce the burden of the human farmer accurately.

To minimise the variance between heat stress studies, current efforts are being made to develop measurement and modelling on heat stress covering sufficient geographical area (national and global range) following widely agreed standards such as (Gaughan *et al.*, 2008a). Web-based early warning forecast systems were developed based on these wide range studies as listed in Table 6. The forecast systems obtain weather data from the nearest weather station around the farm and predict the heat stress level for the coming 1-5 days. The interval of prediction updating defaults as 24 hours which can also be changed by users for a shorter period. These kinds of studies and practical outcomes are necessary for providing regional threshold standards, guidebooks for farmers and early warning alerts. However, the economic and labour costs could be huge. The data of these projects would need to be updated periodically (2-5 years) as the climate changes (Segnalini *et al.*, 2013; Segnalini *et al.*, 2011) and genetic evolution (West, 2003) can always cause inconsistency in the results. Moreover, for practically using the website, Gaughan *et al.* (2012) concluded the forecasting from the website could not specify the effect from building a structure (such as wind speed in the building) for the different farm. It also reported that the different ventilation system, and floor type can cause varied heat transmission between internal and external environment (Seedorf *et al.*, 1998). These variances will always cause inaccurate forecasting of heat stress for specific farm conditions.

Table 2-6 Web-based weather forecast sites for livestock, adapted from Gaughan *et al.* (2012)

Country	Species	Web Address	Online Service
AUS	Dairy cows	www.coolcows.com.au	• Action generator • Cost benefit calculator • Weather forecaster
AUS	Feedlot beef	http://chlt.katestone.com.au/public-forecasts-2/?site=152	• Daily heat load index forecast
USA	Feedlot beef	https://www.ars.usda.gov/plains-area/clay-center-ne/marc/docs/heat-stress/indexregion/	• 7 days heat stress forecast • List of actions for heat stress mitigation
USA	Dairy/beef cattle	http://www.mesonet.org/index.php/agriculture/monitor	• Daily weather forecast • Calculation of cattle comfort index
USA	Livestock	http://weather.uky.edu/mrf_lsi.htm	• 10 days heat stress forecast • Hourly livestock heat stress forecast • Heat stress index calculator
USA	Livestock	http://www3.abe.iastate.edu/livestock/heat_stress.asp	• Heat stress forecast and calculator (currently inaccessible)

Instead of modelling the heat stress within huge geographical region, the dynamic and flexible modelling of heat stress for specific farm is another potential approach by using the PLF technologies. As reviewed in previous section, most of the devices for measuring microclimate on-farm are commercially available. The production data is routinely recorded by the farm management system. Moreover, the physiological and behaviour parameters (e.g. milking frequency, rumination time and milk temperature) can be measured even for individual cow with high frequency (daily or hourly) in robotic milking system. These advantages of PLF technology reduce the labour and time cost for data collection, and provide a big database for modelling. However, the usage of these database for heat stress modelling is still limited. To develop a dynamic on-farm heat stress modelling system, the primary steps may include: 1) develop the new TCI with the best performance in the regression with the parameters recorded in the on-farm database (e.g. regression between Tdb and RT); 2) determine the accurate thresholds for different levels of heat stress using the new TCI and establish the heat stress profile for the individual cow, which can be used for further heat stress mitigation; 3) link the heat stress models with the ventilation facilities (e.g. to control the fans operations) and farm management system (e.g. to change the milking frequency and interval), which enables dynamic adjustment of heat stress mitigation; and 4) convert the statistical models into artificial intelligent algorithms, which allows the models to be self-calibrated with key factors (e.g. the days in milk).

2.9. Conclusions

Solving heat stress is the primary challenge of current dairy farming. Although much progress has been achieved in the research area, limited practical improvements have been provided to real-farming. This review has focused on summarising the progress achieved in current heat stress studies. The published thermal comfort indices were compared, as well as their applications in field studies. The performances of current indices have a huge variance affected by several factors, such as the difference between laboratory and field studies. Similar variance also exists in mitigation solutions. Fans and sprinklers are reported as the most effective cooling facilities for heat stress. Nevertheless, the energy cost and reduced performance under hot-humid conditions are still the major concern in a real application. To overcome such variabilities of performance; current studies tend to involve enough number of farms or regions in one study. Information will be collected specifically from each farm and stored in the database. Several signs of progress (web-based weather forecast sites) were gained from such global studies. However, the upgrading of these databases is always necessary to correspond to climate and genetic changes. As well as focusing on widely applicable solutions, more attention can be paid to a single farm. The potential solution in this way may be achieved depending on PLF techniques. The system could dynamically calibrate the control algorithm (such as THI). The calibration could be automatically conducted based on the continual on-farm monitoring. Robotic sensors and cooling facilities would be connected to and controlled by the system. The historical database of the single farm can be built up for providing accurate mitigation strategies for either the individual animal or the whole herd.

Chapter 3. Modelling of heat stress in a robotic dairy farm. Part 1: Thermal comfort indices as the indicators of production loss

3.1. Abstract

Thermal comfort indices (TCI) were developed to assess heat stress and model the relationship between thermal parameters and animal responses. The published models mainly include temperature humidity index (THI), black globe humidity index (BGHI), environmental stress index (ESI), equivalent temperature index (ETI), heat load index (HLI), respiration rate index (RR) and comprehensive climate index (CCI). Most of these models applied dry bulb temperature (Tdb), wet bulb temperature (Twb), dew point temperature (Tdp), relative humidity (RH), wind speed (WS) and solar radiation (SR) as their thermal parameters, while the animal responses can vary depending on the proposed usage of the model such as predicting the production loss by defining the daily milk yield (DMY) as animal response. The performance of these TCIS can be varied when dealing with different climate condition, animal breed and farm management systems. This study was conducted to compare these published TCIs by testing the strength of their correlation with DMY and milk temperature (MT) on a robotic farm situated in a subtropical climate region. The comparison also included the regression between basic thermal parameters (Tdb, RH, Twb and Tdp) and animal responses (DMY and MT). Moreover, two datasets of thermal parameters measured on-farm and at local weather stations were also compared to demonstrate the feasibility of using on-line database for modelling of heat stress on farms in the future. The statistical analysis found using Tdb can provide similar performance of assessing heat stress as other TCIs. The spatial variability between on-farm measurement and local weather station can be neglected when modelling between TCIs and MT. The threshold with significant decline of DMY was reported as THI >64 for cows with average DMY 31 kg/cow/day. The daily minimum TCIs were found to be highly correlated with production loss indicating that night-time cooling was important for preventing production losses. The potential of implementing a simplified assessment of heat stress using to on-line dataset (automatic weather stations) was demonstrated by this study.

3.2. Introduction

Managing heat stress is one of the main challenges for dairy farmers. Heat stress is defined as the specific heat load caused by thermal conditions affecting animal's homeostasis and health (Gaughan et al., 2012). As a result, the welfare and comfort of cows can be compromised via decreased feed intake, interrupted resting and rumination time (Grant, 2012; Phillips, 2018; Phillips, 2008). The sub-optimal welfare and comfort of cows can ultimately reduce the production performance of the animals (Biby, 2010). Furthermore, heat stress can result in increased mortality and financial losses for dairy farmers (Bernabucci *et al.*, 2014). Assessment and evaluation of the heat stress have been undertaken for several decades in both laboratory and field studies (Roland et al., 2016) more references are needed. The basic assessment of thermal comfort was based on detecting the body temperature of livestock. However, for on-farm mitigation, measuring body temperature, e.g. rectal temperature (RT) has been difficult to obtain and labour intensive. As an effective alternative, modelling the relationship between the ambient thermal condition and animal body temperature or other health-related indicators e.g. respiration rate (RR) has provided a practical solution. Such models were introduced as animal thermal comfort indices (TCIs), of which the initial one was adopted from human discomfort index (Thom, 1959).

According to the theory of sensitive and latent heat transfer, the primary indicators of ambient thermal condition were characterised as dry bulb temperature (Tdb) and relative humidity (RH), which were also calculated as wet bulb temperature (Twb) (Thom, 1959) or dew point temperature (Tdp) in some studies (Berry et al., 1964; Yousef, 1985). In last decades, there has been a continues development and modifications of the approaches for modelling TCIs, with a focus on adjusting the weight of Tdb and RH to apply the models across different climate conditions and animal breeds (LCI, 1970). Moreover, wind speed (WS) and solar radiation (SR) were added into the modelling when the measurement implemented by using new sensors and technologies (Mader et al., 2006). Thresholds and coefficients between animals' responses and TCI values were usually reported as the assessment result of heat stress (Silva *et al.*, 2007). Even though a number of TCIs were developed and modified, there have been few models that were developed based on cows' responses to heat stress (Baeta *et al.*, 1987; Johnson, 1965). In addition, with the increased production performance of dairy cow genotype selection in past years, the heat tolerance of these high-producing cows is decreased compared to the older breed (Zimbelman *et al.*, 2009). Thus, the developed TCI models by using low-producing cows may produce an inaccurate assessment for current breeds (Ji *et al.*, Submitted-d).

Of the published thresholds, thermal comfort zones were identified to include three key parameters: thermal neutral zone (TNZ), lower and upper critical temperature (LCT/UCT) (DeShazer *et al.*, 2009). More specified zones were categorized from thermal innocuous zone to thermal extreme zone (Silanikove, 2000). Ji *et al.* (Submitted-d) reported the inconsistency of applying the TCIs to identify thermal zones, even with uniform thermal parameters (Tdb, RH, WS and SR). In a practical dairy farm management, the applications of the developed TCIs and the thresholds for heat stress mitigation are still limited. This is caused by the difficulty for continuous, synchronous and reliable measurements of thermal conditions and cows' responses, as well as the requirement to modify the published models for the local environment (Ji *et al.*, Submitted-d)

With the development of robotic/automatic farming systems (AMS), available information on cows' responses is readily available. Modern dairy farming systems maybe enhanced using TCIs and their thresholds in real farm heat stress mitigation. Moreover, for the measurement of the on-farm thermal condition, the information from local weather station (e.g. government weather forecast http://www.bom.gov.au) is commercially available which could reduce the cost of implementing on-farm measurement. The modelling of heat stress based on on-line dataset requires fewer equipment on the farm, and enable the forecasting of heat stress for farmers. However, the comparison between models using on-line dataset and on-farm measurement is required to demonstrate the acceptable variability of thermal parameters between these two approaches.

In this study, three objectives will be accomplished. The first is to compare the published TCIs based on their performance of predicting production loss. The second is comparing the application of the data collected from the on-line database (local weather station) and on-farm measurement. The last one is to refine the thresholds and coefficient value of the selected TCI for heat stress assessment of dairy cow. The overall aim of this study is to provide a simplified and reliable TCI for heat stress assessment in practical farm management.

3.3. Materials and Methods

Farm, housing and animal

A dairy farm located in Gatton, QLD, AUS was selected in this study for data collection. The farm applied AMS with three milking robotics (LELY Astronaut, Lely Industries NV, Maassluis, the Netherlands) installed in the milking station (position shown in Figure 1). After training the animals to cooperate with AMS, the herd was managed with free traffic, which allowed the cows to freely move between feeding and resting area, as well as semi-free access to the milking station. The herd management system (LELY T4C, Lely Industries NV, Maassluis, the Netherlands) limited the maximum visit times per day of each cow by electronically closing/opening the gates of the milking stations. The visit control was dependent on the production status and other health factors of an individual animal that identified by their ear tag when they moved close to the receiver attached to the gate. The farm applied solid manure as the bedding material in the resting area. Replacement of the bedding material was performed periodically.

From April 2016 to November 2017, average 160 Holstein lactation cows were held in the farm, with an average of 180 days in milk (DIM). The average daily milk yield (DMY) during this period was 29.5 kg/cow/day, and the average body weight (BW) was 791 kg/cow. The age of cows was ranged from 2 to 11 years.

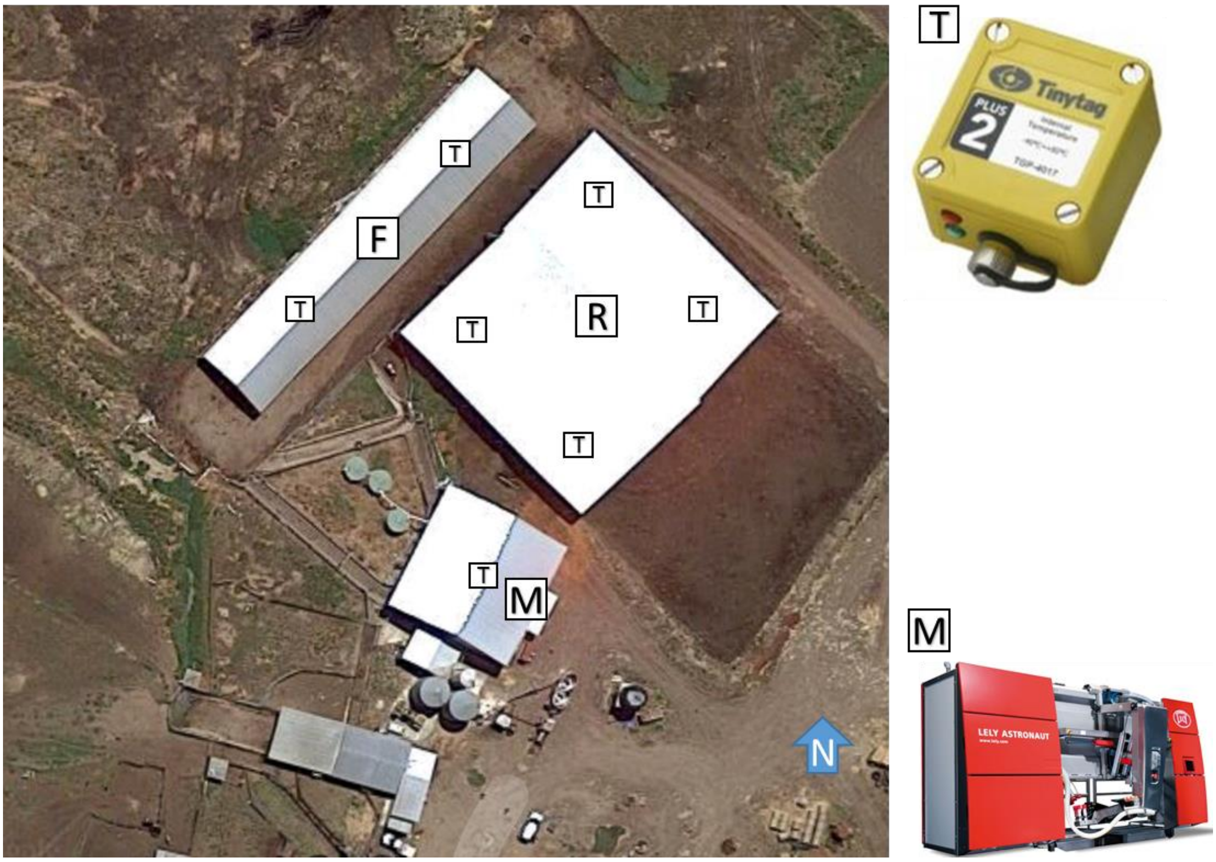

Figure 3-1 Farm layout and position of sensors. The letter "T" indicates the positions of TinyTag2 Plus data loggers; letter "R" indicates the position of resting (lying) area of cows; letter "F" indicates the feeding area, and letter "M" indicates the milking station with AMS. Adapted form google map (https://www.google.com.au/maps/@-27.5045056,152.4202448,143m/data=!3m1!1e3)

Environment measurement

The on-farm measurement of Tdb and RH was conducted by using Tinytag 2 Plus Data Logger from Hasting Data Loggers, NWS, AUS (Schuller *et al.*, 2014; Banhazi *et al.*, 2008a; Banhazi *et al.*, 2008b; Banhazi *et al.*, 2008c). The measurement scale of the temperature sensors was -45°C to +75°C, with an accuracy of ±0.5°C at 25°C in the datasheet. The humidity sensors provided a measurement scale of 0% to 100%, with an accuracy of ±3% at 25°C. All the sensors were calibrated by the manufacturer before delivered to the researcher. Different numbers of sensors were placed in different areas as shown in Figure 1. The data logging interval was 30 minutes. Periodically data collection and relaunching of the data logger was performed from April 2016 to November 2017.

The measurement of regional climate was done by the weather station in the University of Queensland, Gatton, AUS, which is nearly 8 km away from the farm. The climate variables of the measurement involved Tdb, RH, WS and SR. The dataset was provided by the Bureau of Meteorology, Australian Government (http://www.bom.gov.au) with free access.

Production measurement

The AMS machine measured the production performance of individual cows during each milking. The data of daily milk production (DMY, kg/cow/day) and the average daily milk temperature (MT) was collected from April 2016 to November 2017 and used to calculate the production performance of the herd.

Data processing

The data collected from on-farm data loggers, a local weather station, and the robotic farming system was filtered by removing outliers (Mead, 2017). The values of daily maximum, minimum and mean were calculated using the data downloaded from data loggers on the farm. The diurnal and seasonal patterns of Tdb and RH were formulated based on this dataset to provide a basic description of the changes in thermal condition on the farm during different seasons. The comparison of each month's data was taken by using Duncan's multiple range tests applying 95% significant level. Although climate data was measured hourly in the weather station, the government website only provided the record of Tdb, RH, WS and SR at 9 am and 3 pm during the daily measurement, as well as the daily maximum and minimum value. The daily mean value of weather station was calculated based on the average of these four values.

The data processed from on-farm measurement, as well as the local weather station (maximum, minimum and mean value), was utilized for calculating the value of the published TCI equations from 1959 to 2010 in Table 3-1. The basic TCIs (THIs) were developed using Tdb and RH in the calculation for the different thermal condition. Among equations, $THI_{1\text{ to }3}$ was developed for human discomfort index(Thom, 1959), as well as the equation of ESI (Moran *et al.*, 2001). THI_4 was developed by modelling the rectal temperature of bull calves with the thermal condition (Bianca, 1962). $THI_{5\text{ to }7}$ and ETI were established on low-producing dairy cows (DMY: 15.5 kg/cow/d) in a climate chamber (LCI, 1970; Johnson, 1965; Baeta *et al.*, 1987). WS and SR were added in the new TCIs to improve the performance of the modelling. THI_8 was developed by Mader *et al.* (2006) to adjust the equation of THI_6. Black globe temperature (Tbg) was an integrated index to evaluate heat load from solar radiation. It was used to established BGHI (Buffington *et al.*, 1981) and $HLI_{1\text{ and }2}$ (Gaughan *et al.*, 2002; Gaughan *et al.*, 2008b). The equation of RR was developed by modelling on the respiration rate and thermal condition (Brown-Brandl *et al.*, 2005). The latest version of TCI was CCI, which was designed to quantify both heat and cold stress of animal (Mader *et al.*, 2010). The equation was formulated as a sum of three adjustment factors for RH, WS and SR. The value of basic TCIs (equations 1-7) of each day was calculated by inputting the data of Tdb and RH (daily maximum, minimum and mean) from both on-farm measurement and local weather station. As WS and SR were not measured on-farm, the calculation of new TCIs (equations 8-14) was only based on the data from local weather station.

Table 3-1 TCIs from 1959 to 2010

Num. of Eq.	Equation	Reference
(1)	$THI_1 = 1.8 \times [0.4 \times (T_{db} + T_{wb})] + 47$	Thom (1959)
(2)	$THI_2 = 1.8 \times (0.15 \times T_{db} + 0.85 \times T_{wb}) + 32$	
(3)	$THI_3 = 1.8 \times [0.4 \times (T_{db} + T_{wb})] + 40.6$	
(4)	$THI_4 = 1.8 \times (0.35 \times T_{db} + 0.65 \times T_{wb}) + 32$	Bianca (1962)
(5)	$THI_5 = T_{db} + 0.36 \times T_{wp} + 41.5$	LCI (1970) Johnson (1965)
(6)	$THI_6 = 0.8 \times T_{db} + (T_{db} - 14.4) \times RH + 46.4$	
(7)	$THI_7 = 1.8 \times (0.55 \times T_{db} + 0.2 \times T_{wb}) + 49.5$	
(8)	$THI_8 = 4.51 + THI_6 - 1.991 \times WS + 0.0068 \times SR$	Mader *et al.* (2006)
(9)	$BGHI = \ T_{bg} + 0.36T_{dp} + 41.5$ $Where: T_{bg} = (1.33\ T_{db}) - (2.65T_{db}^{0.5}) + 3.21\log(SR + 1) + 3.5\ ;$	Buffington *et al.* (1981)
(10)	$ETI = 27.88 - 0.456T_{db} + 0.012T_{db}^2 - 0.49RH + 0.001RH^2 + 1.151WS - 0.126WS^2 + 0.02T_{db}RH - 0.046T\_dbWS$	Baeta *et al.* (1987)
(11)	$ESI = 0.63T_{db} - 0.03RH + 0.002SR + 0.0054T_{db}RH - 0.073(0.1 + SR)^{-1}$	Moran *et al.* (2001)
(12)	$HLI_1 = 33.2 + 0.2 \times RH + 1.2 \times T_{bg} - (0.82 \times WS)^{0.1} - \log(0.4 \times WS^2 + 0.001)$	Gaughan *et al.* (2002); Gaughan *et al.* (2008b)
(13)	$HLI_2 = \begin{cases} 10.66 + 0.28 \times RH + 1.3 \times T_{bg} - WS\ (T_{bg} \leq 25) \\ 8.62 + 0.38 \times RH + 1.55 \times T_{bg} - 0.5 \times WS + e^{2.4-WS}(T_{bg} > 25) \end{cases}$	
(14)	$RR = 5.1 \times T_{db} + 0.58 \times RH - 1.7 \times WS + 0.039 \times SR - 105.7;$	Brown-Brandl *et al.* (2005)
(15)	$CCI = RH_{adj} + WS_{adj} + SR_{adj}$ $Where: RH_{adj} = e^{[(0.00182 \times RH) + (1.8 \times 10^{-5} \times T_{db} \times RH)]} \times [(0.000054 \times T_{db}^2) + (0.00192 \times T_{db}) - 0.0246] \times (RH - 30);$ $WS_{adj} = \left(\frac{-6.56}{e^{\left[\frac{1}{(2.26 \times WS + 0.23)^{0.45}}\right] \times [2.9 + 1.14 \times 10^{-6} \times WS^{2.5} - \log_{0.3}(2.26 \times WS + 0.33)^{-2}]}} \right) - 0.0056 \times WS^2 + 3.33;$ $SR_{adj} = (0.0075SR) - (0.00002SRT_{db}) + (0.00005T_{db}^2 SR^{0.5}) + 0.1T_{db} - 2.$	Mader *et al.* (2010)

Statistics

As the research was conducted on a single herd and farm, influence from different genotype and farming conditions e.g. building structures or facilities were exclude from the statistical analysis. To determine the best TCI for assessing heat stress, the single linear regression between calculated TCIs and responses of cows was taken as follows:

$$y = a + bx + \varepsilon,$$

Where y is the dependent variable of animal responses including DMY (kg/d/cow) and MT (°C); x is the independent variable indicating thermal condition; a is the intercept; b is the coefficient of the thermal indicator; ε is the random residual term. In addition to the TCIs, for comparison, Tdb and RH measured in farm and local weather station were considered as two direct indicators of thermal conditions. The selection of best indicator was based on the comparison between the regression results. The value of R squared was used to evaluate the performance of regressions. The value of correlation coefficients was applied to compare the sensitivity of the indicator.

The selected indicator was then applied to estimate the critical threshold values of heat stress. The threshold values were developed based on the significant changes in correlation coefficients by using the model of broken-line regression as follows

$$y = \begin{cases} c + \varepsilon, & x \leq THR \\ a + bx + \varepsilon, & x > THR \end{cases}$$

Where, c is a constant value when animals' performance is not affected by the thermal condition; when the value of indicator (x) is higher than the threshold (THR), the relationship returns to single linear regression, as described above. Beyond the critical threshold, thresholds for two more levels of heat stress (moderate and extreme) were also estimated by comparing the changes of coefficient values (decline of DMY and raise of MT) within a different range of thermal conditions.

The thermal comfort of the animal was determined using the fitted lines of broken-line regression (Kadzere et al., 2002). If DMY was related to the heat stress, the segments with positive or none impact (slope ≥ 0) considered as the Normal level of thermal conditions without heat stress. For MT prediction, the segments with negative or none impact (slope ≤ 0) are the Normal level. Beyond the Normal level, the levels of heat stress (Alert and Urgent) are categorized based on the significant increase/decrease of the impact (slope). For further comparison of the performance of different indices, the consistency of utilizing the thresholds of different indices to identify heat stress was evaluated. The percentage of the days with same identification results of heat stress was used to determine the consistency between each two pair of indices.

All data processing and statistical analysis were done using R 3.4.3. (R Development Core Team, 2017). The single linear regression was done by using the basic function of "lm"(Chambers, 1992), and the broken-line regression was performed based on the "lm.br" package (Adams, 2017).

3.4. Results and Discussion

Basic description

Table 3-2 presents minimum, maximum, mean and standard deviation of data collected in this study. The daily mean Tdb measured on the farm during the study period was between 9 and 31 °C, while the data from local weather station ranged from 9 to 36 °C. The range of daily RH recorded on-farm measurement i.e. 36-98% was smaller than the range based on local weather station i.e. 11-95%.

The average monthly thermal data over observation period collected by local weather station are shown in Figure 3-2. The high temperature of the hot season (Dec-Feb) ranged from 20 to 35 ^{o}C with a daily average temperature of 28 ^{o}C (Figure 3-2a). The average daily Tdb of the cold season (Jun-Aug) was about 15^{o}C. During these three months, the difference between daily maximum and minimum Tdb increased from approximately 12 to 20 ^{o}C. The RH was lower in November and February, which were the starting and ending of hot season (Figure 3-2b). However, RH suddenly increased after the hot season to the highest level of the year in March. WS and SR followed similar monthly patterns as the temperatures (Figure 3-2c). It can be seen in Figure 3-2[d], that the pattern of MT and DMY had a reverse pattern. From cold to hot season, a 2^{o}C increasing of MT was associated with more than 10 kg decline in DMY. In addition, a significant decrease in DMY also occoured during the mid of cold season (July), which probably was an indication of cold stress. However, the impact of cold stress (-1kg DMY) was much less than heat stress, while no impact on MT was observed.

The monthly pattern of wind direction is displayed in Figure 3-3 below. The main direction of the steady strong wind in a hot season was usually blowing from West to East, which brought the dry air from the inland desert to the coast. In the cold season, the wind direction was opposite (from East to West), which brought the humid air from ocean to the inland.

The correlation between daily Tdb and RH measured on the farm and local weather station is listed in Table 3-3. Significant correlations ($P<0.05$) were demonstrated between each variable from the two measurements. The R^2 values (0.86-0.93) of Tdb correlations showed acceptable agreement between the measurements on the farm and local weather station. However, the R^2 value of RH was less than 0.25. Particularly, with minimum RH, the value was only 0.02. Moreover, for both datasets from farm measurement and local weather station, no significant correlation was found between daily Tdb and RH, except the daily minimum Tdb and daily minimum RH (R^2 = 0.36, $P<0.05$). The non-significant correlation indicated a limited interaction between these two variables.

Table 3-2 The weather and animal-related variables measured at the study site.

Category	Variable	Units	Min	Max	Mean	SE.
Farm measurement	T_min	°C	2.00	24.00	14.27	5.31
	T_max	°C	9.00	38.00	27.15	4.65
	T_mean	°C	9.00	31.00	19.85	4.39
	RH_min	%	1.00	96.00	33.10	18.30
	RH_max	%	67.00	105.00	84.61	11.51
	RH_mean	%	36.00	98.00	61.18	12.93
Local weather station (web data)	T_min	°C	-1.00	27.30	12.49	5.69
	T_max	°C	15.00	45.70	27.45	5.15
	T_mean	°C	9.40	35.88	21.53	4.81
	RH_min	%	8.00	95.00	42.22	17.54
	RH_max	%	10.00	98.00	64.33	14.64
	RH_mean	%	11.00	95.00	53.27	14.75
	WS	m/s	1.30	13.70	5.61	1.99
	SR	w/m²	17.36	363.42	205.44	72.59
Animal status	Num	cows	118.00	196.00	156.41	24.27
	DIM	day	122.00	273.00	181.90	33.42
	BW	kg	711.71	996.14	910.48	49.14
	Age	year	1.10	11.10	3.86	1.91
Animal responses	MT	°C	38.02	41.38	39.13	0.64
	DMY	kg/cow/day	18.60	39.40	31.39	3.90

Table 3-3 Correlation between thermal variables measured on the farm and local weather station

Correlation	Intercept	Coefficient	Sign.a	R^2	SE.
T_min	3.22	0.87	***	0.93	1.34
T_max	1.65	0.90	***	0.86	1.47
T_mean	-0.93	0.95	***	0.90	1.29
RH_min	27.58	0.13	*	0.02	18.16
RH_max	63.40	0.32	***	0.22	10.22
RH_mean	49.58	0.22	***	0.08	12.45

a – significant level: “NS” – insignificant; “*” – P<0.05; “**” – P<0.01; “***” – P<0.001.

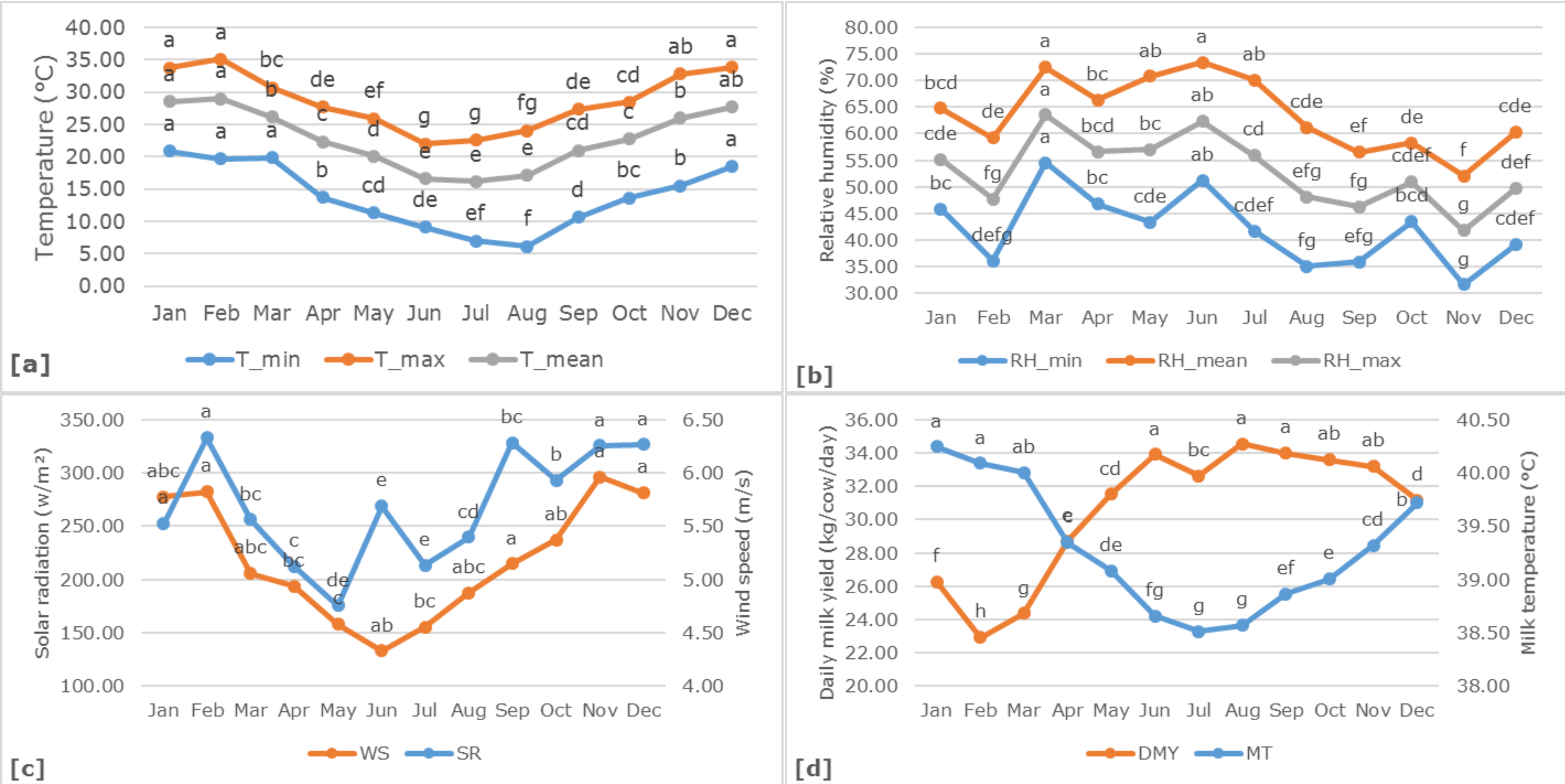

Figure 3-2 Monthly patterns of variables. The sub-figures [a] to [d] illustrate the monthly pattern of temperature, relative humidity, wind speed, solar radiation, milk temperature and daily milk yield. The result of Duncan's multiple range test is noted with letters. The significant level is chosen as 0.05.

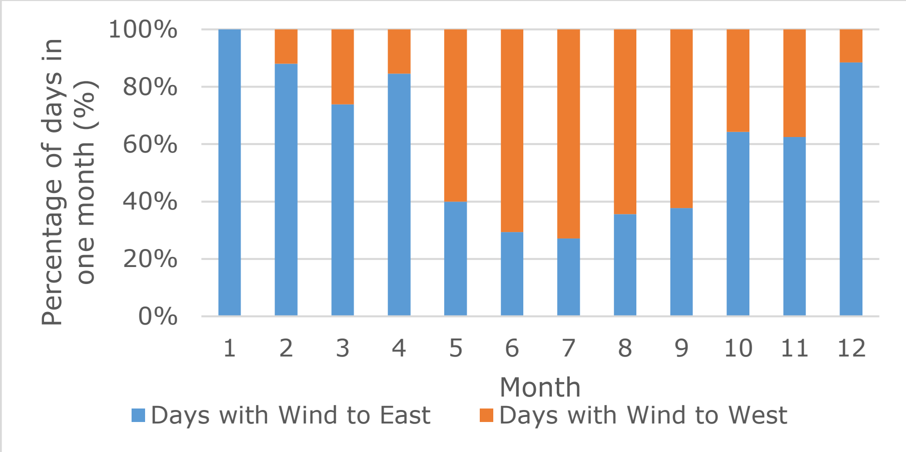

Figure 3-3 Monthly pattern of wind direction. The distribution of wind direction is explained as the number of days with the direction towards East or West in each month.

Comparison of the indices

The performances of different TCIs in Equation 2-7 for prediction of heat stress were evaluated by assessing correlation coefficient between TCI and DMY/MT. Table 3-4 shows the performance of the main thermal variables including daily minimum, maximum, and mean value of Tdb, RH, Tdp and Twb. Overall, prediction of heat stress by using MT achieved a higher value of R^2 than the prediction of DMY. All correlation was significant ($P<0.05$), except the ones with minimum RH and Tdp measured on the farm. The prediction using Tdb measured on the farm and local weather station had a similar performance. However, the Tdb measured on local weather station had a higher impact on DMY and MT. For example, for the prediction of DMY by using maximum Tdb, the coefficient values were -0.25 and -0.35 kg/Tdb for the data measured on farm and weather station, respectively. Moreover, the predictions based on maximum Tdb obtained lower R^2 compared with the minimum and mean values. To predict MT, the mean value of Tdb resulted in better performance than the maximum and minimum values. Most of the prediction using RH resulted in low performance ($R^2<0.10$), however, the maximum RH measured on farm showed better results ($R^2 = 0.15$ and 0.32, for DMY and MT). In addition, the prediction applying Tdp and Twb obtained similar performance as using Tdb. For the prediction of DMY, the best prediction was achieved by using minimum Tdb on the farm ($R^2 = 0.42$). For the prediction of MT, the highest R^2, as well as the sensitivity, was achieved by using Twb.

The comparison of the predictions using basic TCIs in equations 1 to 7 including daily minimum, maximum and mean value of THI_1 to THI_7 are presented in Table 3-5. All indices showed better performance for predicting MT than DMY. The predictions using the maximum value of the index resulted in lower R^2 value than using minimum or mean values. The overall prediction based on the THIs from local weather station resulted in better performance than the THIs on the farm. The prediction using THI_1 and THI_3 had the same performance, as they applied the same weight of Tdb and Twb in the equation. For the prediction of DMY, the best performance ($R^2 = 0.40$) was achieved by using the minimum value of THI_6 on the farm, however, the R^2 was 0.02 less than the R^2 using the minimum value of Tdb on the farm. For the prediction of MT, all of the mean THI measured by local weather station had a similar value of R^2 (0.83-0.86), which were better than using other THIs.

The comparisons by using TCIs (THI_8, BGHI, ETI, ESI, HLI_1, HLI_2, RR and CCI) for the prediction of heat stress is presented in Table 3-6. The best model performance achieved by these predictions had similar correlation values as the prediction using THI_1 to THI_7 (Table 3-

6). The prediction of MT using THI_8 and ETI had lower R^2 value than using other advanced TCIs. The use of maximum value usually had less R^2 than using the minimum and mean value in the prediction of MT.

Table 3-4 Simple correlation coefficient between DMY, MT and basic thermal variables (Tdb, RH, Tdp and Twb)

Variables			Coefficient	R2	SE.	Sign.[a]	Coefficient	R2	SE.	Sign.
Tdb	Farm[b]	Min	-0.37	0.42	2.27	***	0.09	0.72	0.30	***
		Max	-0.25	0.11	2.80	***	0.09	0.44	0.42	***
		Mean	-0.44	0.36	2.38	***	0.12	0.79	0.26	***
	Web[c]	Min	-0.41	0.36	3.11	***	0.10	0.71	0.35	***
		Max	-0.35	0.22	3.45	***	0.10	0.60	0.41	***
		Mean	-0.46	0.33	3.20	***	0.12	0.78	0.30	***
RH	Farm	Min	-0.01	0.00	2.97	NS	0.00	0.00	0.56	NS
		Max	-0.10	0.15	2.75	***	0.03	0.32	0.46	***
		Mean	-0.06	0.06	2.88	***	0.01	0.06	0.55	***
	Web	Min	-0.03	0.02	3.87	***	0.01	0.02	0.64	***
		Max	-0.03	0.02	3.87	***	0.01	0.02	0.64	***
		Mean	-0.03	0.02	3.87	***	0.01	0.02	0.64	***
Tdp	Farm	Min	-0.03	0.01	2.95	NS	0.00	0.01	0.56	NS
		Max	-0.30	0.22	2.62	***	0.10	0.66	0.33	***
		Mean	-0.30	0.31	2.47	***	0.08	0.55	0.38	***
	Web	Min	-0.26	0.26	3.36	***	0.06	0.46	0.47	***
		Max	-0.37	0.28	3.32	***	0.09	0.62	0.39	***
		Mean	-0.37	0.30	3.26	***	0.09	0.62	0.39	***
Twb	Farm	Min	-0.27	0.21	2.65	***	0.06	0.31	0.47	***
		Max	-0.30	0.20	2.67	***	0.10	0.64	0.34	***
		Mean	-0.40	0.35	2.39	***	0.11	0.68	0.32	***
	Web	Min	-0.41	0.33	3.18	***	0.09	0.62	0.39	***
		Max	-0.51	0.32	3.22	***	0.13	0.77	0.31	***
		Mean	-0.56	0.38	3.06	***	0.14	0.83	0.26	***

a – significant level: "NS" – insignificant; "*" – $P<0.05$; "**" – $P<0.01$; "***" – $P<0.001$

b – the data measured on the farm.

c – the data collected from government website, which is measured by local weather station

Table 3-5 Simple correlation coefficient between DMY, MT and basic TCIs (THI_1 to THI_6). Values of THI1-7 were calculated using Equations 1-8

Variables			Coefficient	R2	SE.	Sign.[a]	Coefficient	R2	SE.	Sign.
THI_1	Farm[b]	Min	-0.25	0.34	2.41	***	0.06	0.56	0.38	***
		Max	-0.20	0.16	2.73	***	0.07	0.56	0.38	***
		Mean	-0.30	0.37	2.36	***	0.08	0.76	0.28	***
	Web[c]	Min	-0.29	0.36	3.13	***	0.07	0.68	0.36	***
		Max	-0.32	0.28	3.30	***	0.08	0.73	0.34	***
		Mean	-0.37	0.38	3.08	***	0.09	0.86	0.24	***
THI_2	Farm	Min	-0.17	0.25	2.58	***	0.04	0.38	0.44	***
		Max	-0.17	0.19	2.68	***	0.06	0.62	0.35	***
		Mean	-0.23	0.36	2.38	***	0.06	0.71	0.30	***
	Web	Min	-0.23	0.34	3.16	***	0.05	0.64	0.38	***
		Max	-0.28	0.31	3.23	***	0.07	0.77	0.31	***
		Mean	-0.31	0.39	3.05	***	0.08	0.85	0.25	***
THI_3	Farm	Min	-0.25	0.34	2.41	***	0.06	0.56	0.38	***
		Max	-0.20	0.16	2.73	***	0.07	0.56	0.38	***
		Mean	-0.30	0.37	2.36	***	0.08	0.76	0.28	***

	Web	Min	-0.29	0.36	3.13	***	0.07	0.68	0.36	***
		Max	-0.32	0.28	3.30	***	0.08	0.73	0.34	***
		Mean	-0.37	0.38	3.08	***	0.09	0.86	0.24	***
THI_4	Farm	Min	-0.19	0.30	2.48	***	0.05	0.48	0.40	***
		Max	-0.16	0.17	2.71	***	0.06	0.58	0.36	***
		Mean	-0.24	0.36	2.37	***	0.06	0.74	0.29	***
	Web	Min	-0.23	0.35	3.14	***	0.05	0.67	0.37	***
		Max	-0.27	0.30	3.27	***	0.07	0.75	0.32	***
		Mean	-0.31	0.38	3.06	***	0.08	0.86	0.24	***
THI_5	Farm	Min	-0.20	0.26	2.56	***	0.05	0.39	0.44	***
		Max	-0.10	0.07	2.87	***	0.03	0.20	0.50	***
		Mean	-0.31	0.37	2.36	***	0.08	0.76	0.28	***
	Web	Min	-0.28	0.35	3.14	***	0.06	0.67	0.37	***
		Max	-0.31	0.28	3.32	***	0.08	0.72	0.34	***
		Mean	-0.37	0.37	3.09	***	0.09	0.86	0.24	***
THI_6	Farm	Min	-0.32	0.40	2.31	***	0.08	0.68	0.32	***
		Max	-0.18	0.17	2.71	***	0.06	0.56	0.37	***
		Mean	-0.31	0.37	2.36	***	0.08	0.78	0.27	***
	Web	Min	-0.32	0.37	3.10	***	0.07	0.71	0.35	***
		Max	-0.33	0.29	3.28	***	0.09	0.73	0.33	***
		Mean	-0.38	0.38	3.08	***	0.09	0.84	0.26	***
THI_7	Farm	Min	-0.28	0.39	2.33	***	0.07	0.65	0.33	***
		Max	-0.20	0.14	2.76	***	0.07	0.50	0.40	***
		Mean	-0.33	0.37	2.36	***	0.09	0.78	0.26	***
	Web	Min	-0.31	0.36	3.12	***	0.07	0.70	0.35	***
		Max	-0.30	0.25	3.37	***	0.08	0.67	0.37	***
		Mean	-0.38	0.36	3.13	***	0.09	0.83	0.26	***

a – significant level: “NS” – insignificant; “*” – $P<0.05$; “**” – $P<0.01$; “***” – $P<0.001$.
b – data measured on farm.
c – data collected from government website, which is measured by local weather station

Table 3-6 Simple correlation coefficient between DMY, MT and advanced TCIs (THI_8, BGHI, ETI, ESI, HLI_1, HLI_2, RR and CCI)

Variables		**Coefficient**	**R2**	**SE.**	**Sign.**[a]	**Coefficient**	**R2**	**SE.**	**Sign.**
THI_8	Min	-0.29	0.35	3.14	***	0.07	0.72	0.34	***
	Max	-0.18	0.16	3.57	***	0.04	0.35	0.52	***
	Mean	-0.28	0.29	3.29	***	0.07	0.69	0.36	***
BGHI	Min	-0.31	0.39	3.05	***	0.07	0.74	0.33	***
	Max	-0.25	0.25	3.38	***	0.07	0.66	0.38	***
	Mean	-0.32	0.35	3.15	***	0.08	0.81	0.28	***
ETI	Min	-0.48	0.27	3.33	***	0.11	0.51	0.45	***
	Max	-0.08	0.05	3.80	***	0.02	0.07	0.62	***
	Mean	-0.43	0.30	3.25	***	0.11	0.65	0.38	***
ESI	Min	-0.44	0.36	3.11	***	0.10	0.70	0.35	***
	Max	-0.48	0.30	3.26	***	0.12	0.75	0.32	***
	Mean	-0.55	0.38	3.06	***	0.14	0.86	0.24	***
HLI_1	Min	-0.31	0.37	3.10	***	0.07	0.69	0.36	***
	Max	-0.29	0.28	3.31	***	0.08	0.72	0.34	***
	Mean	-0.34	0.36	3.12	***	0.09	0.84	0.26	***
HLI_2	Min	-0.26	0.33	3.18	***	0.06	0.63	0.39	***
	Max	-0.23	0.29	3.27	***	0.06	0.72	0.34	***
	Mean	-0.24	0.34	3.16	***	0.06	0.79	0.29	***
RR	Min	-0.07	0.35	3.15	***	0.02	0.69	0.36	***
	Max	-0.08	0.28	3.32	***	0.02	0.70	0.35	***
	Mean	-0.09	0.36	3.12	***	0.02	0.85	0.25	***
CCI	Min	-0.25	0.38	3.08	***	0.06	0.69	0.36	***
	Max	-0.22	0.33	3.18	***	0.05	0.74	0.33	***
	Mean	-0.27	0.41	3.00	***	0.06	0.84	0.26	***

a – significant level: “NS” – insignificant; “*” – P<0.05; “**” – P<0.01; “***” – P<0.001.

Identify the thermal comfort levels

By using lines fitted to the broken-line regression, normal, alert, and urgent levels of heat stress determined as shown in Figure 3-4 to 3-9. Most of these regression had R^2 values higher than 0.85, with exception of the prediction of DMY using minimum THI of on-farm measurement (R^2=0.74) and minimum CCI of local weather station (R^2=0.78).

For example, in Figure 3-4, the minimum Tdb on a farm with Normal level was lower than 8.46 ^oC, with positive impact (slope = 0.20 kg/^oC). When minimum Tdb was higher than 8.46 ^oC, the impact became negative (slope = -0.25 kg/^oC), which indicated an Alert level of heat stress. Then, the Urgent level of heat stress happened with minimum Tdb higher than 18.00 ^oC, and the impact could be -1.41 kg/^oC, which was nearly 6 times production loss compared with the Alert heat stress. However, not all of the regression identified all three levels with two thresholds. Some of the regressions could only classify two levels, which were the Alert and Urgent level of heat stress, such as the regression between DMY and mean Tdb on a farm in Figure 3-4.

In Figure 3-4, the range of minimum Tdb (from 8.46 to 18.00 ^oC) on the farm under Alert level was smaller than the range of this variable on local weather station (from 6.83 to 24.80 ^oC). Under Alert and Urgent heat stress, the negative impact of minimum Tdb on local weather station was larger than twice of the impact of the data measured on the farm (slope = -0.55 vs. -0.25 kg/^oC for Alert level, slope = -2.83 vs. -1.41 kg/^oC for the Urgent level). The impact of mean Tdb was relatively lower than the impact of minimum Tdb, especially under Urgent heat stress (-1.57 vs. -2.83 kg/^oC, for mean and minimum Tdb measured by local weather station, respectively). In addition, the threshold of entering Urgent level using mean Tdb on the farm (21.87 ^oC) is nearly 10 ^oC lower than the threshold using the data from local weather station (32.18 ^oC).

In Figure 3-5, the regression between MT and Tdb showed similar impact under different levels of heat stress. The range of minimum Tdb on-farm under Alert level (from 6.43 to 18.57 ^oC) was higher than the range of minimum Tdb on local weather station (from 2.06 to 16.02 ^oC). The thresholds of mean Tdb entering Urgent level were similar for the data measured on the farm and local weather station (20.59 vs. 20.29 ^oC, respectively).

In Figure 3-6, similar thresholds of mean THI entering Alert level were found with the data collected on farm and local weather station (63.74 vs. 63.67, respectively). However, the impact of heat stress under Alert level was higher when using the data from local weather station (slope = -0.52 kg/THI, compared with -0.37 kg/THI on the farm). The threshold of mean THI entering Urgent level using the data on the farm was lower than the threshold for local weather station (67.81 vs. 80.84, respectively). However, under Urgent heat stress, the impact (slope = -4.12 kg/THI) of mean THI using the data from local weather station was nearly 6 times of the impact based on the data from farm measurement (slope = -0.68kg/THI).

In Figure 3-7, the data measured from the farm and local weather station had similar thresholds of minimum THI (47.45 vs. 49.13 for Alert level, 64.56 vs. 62.51 for the Urgent level). However, the impact of minimum THI under Urgent heat stress that applied farm data was more than twice of the impact based on the data from local weather station (slope = 0.39 vs. 0.17 ^oC/THI). For the data measured on the farm and local weather station, similar threshold and impact of the Urgent heat stress were identified by mean THI (67.02 vs. 71.43, slope = 0.13 vs. 0.15 ^oC/THI, respectively).

In Figure 3-8 and 3-9, the minimum and mean CCI was unable to identify Normal level for the prediction of either DMY or MT. The impact of minimum CCI towards DMY decreased (from -0.33 to -0.04 kg/CCI) when the minimum CCI was higher than 22, which indicated inaccuracy of the equation. The impact of ESI for each level of heat stress was higher than the impact of BGHI.

Based on the broken line regression illustrated in Figure 3-4 to 3-9, and described above. The thresholds of different indices for Normal, Alert and Urgent level are summarized and listed in Table 3-7. The results of consistencies are listed in Table 3-8.

In Table 8, the mean of THI, BGHI and ESI provided high consistency of identifying heat stress based on the decline of DMY, which were all higher than 90%. The using of minimum Tdb, mean THI, BGHI and ESI from local weather station could provide more than 80% consistency of the identification. When just identify the Normal and Alert level, the minimum Tdb measured on the farm and local weather station had 93% consistency, which was the same value of the R^2 in their correlation (Table 3-3). In Table 3-8, the consistency between Tdb and THI increased when using the increase of MT to identify heat stress. However, the consistency between THI, BGHI and ESI decreased. Most of the indices had more than 90% consistency of the two levels identification, except the consistency between minimum THI and other indices. Moreover, the correlation between THIs using the dataset from on-farm measurement and local weather station was tested and shown in Table 3-9. The R^2 of correlations were higher using the mean value of the dataset than using the maximum and minimum value. The R^2 of THI_2, THI_4 and THI_5 had values lower than 0.90 when using the maximum and minimum value.

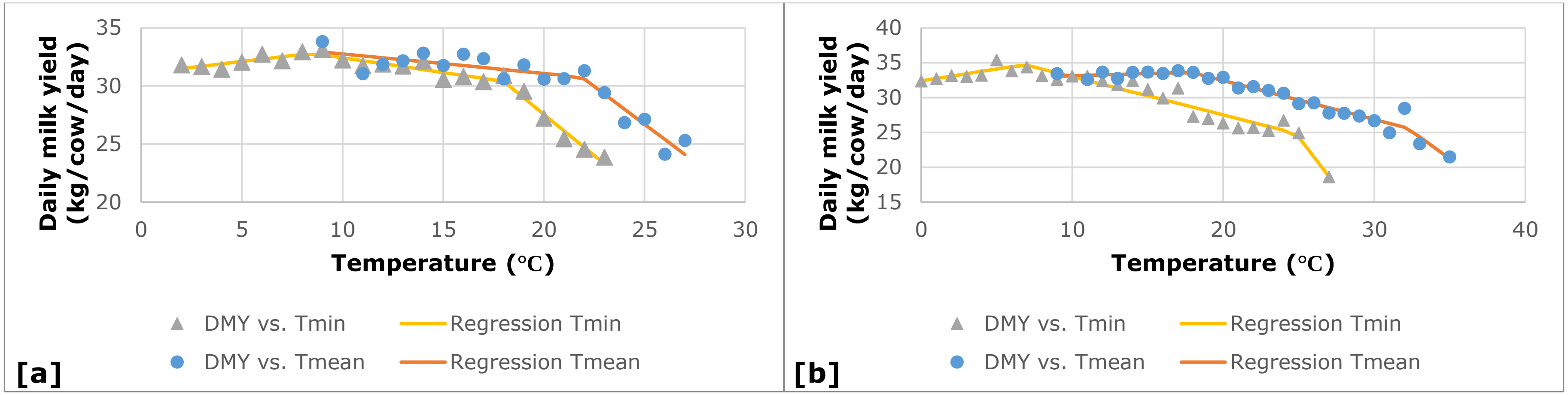

Figure 3-4 Broken-line regression between DMY and Tdb. The sub-figure [a] is for the data measured on the farm, and [b] is for the data collected from local weather station

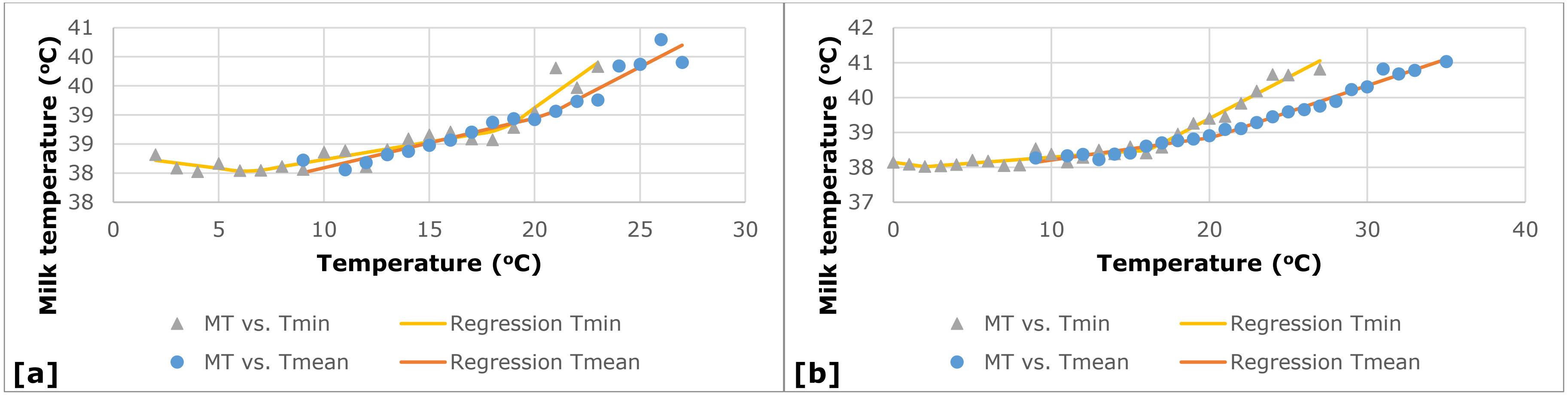

Figure 3-5 Broken-line regression between MT and Tdb. The sub-figure [a] is for the data measured on the farm, and [b] is for the data collected from local weather station

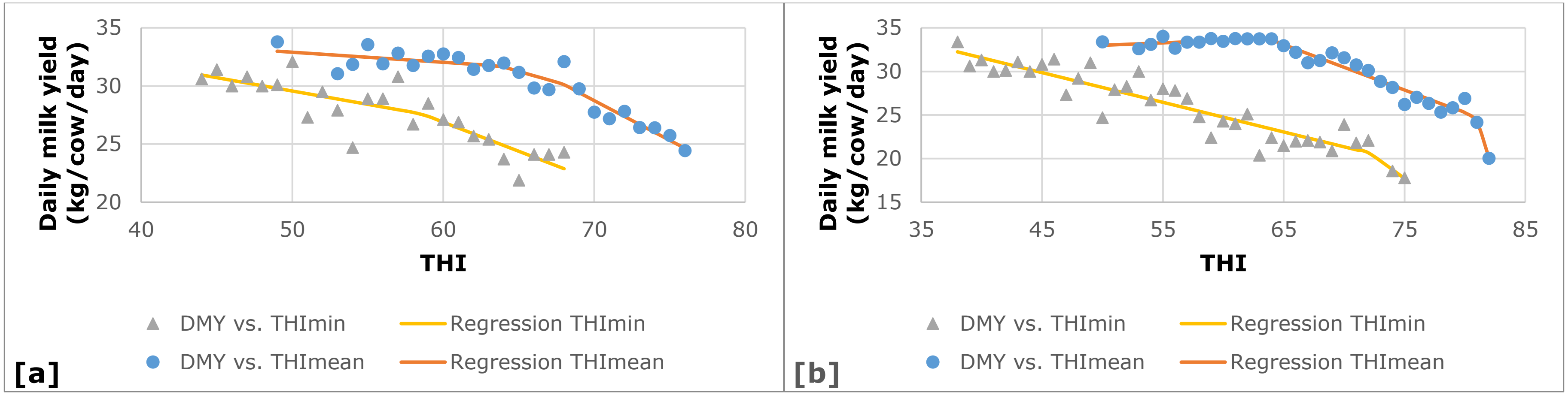

Figure 3-6 Broken-line regression between DMY and THI. The sub-figure [a] is for the data measured on the farm, and [b] is for the data collected from local weather station

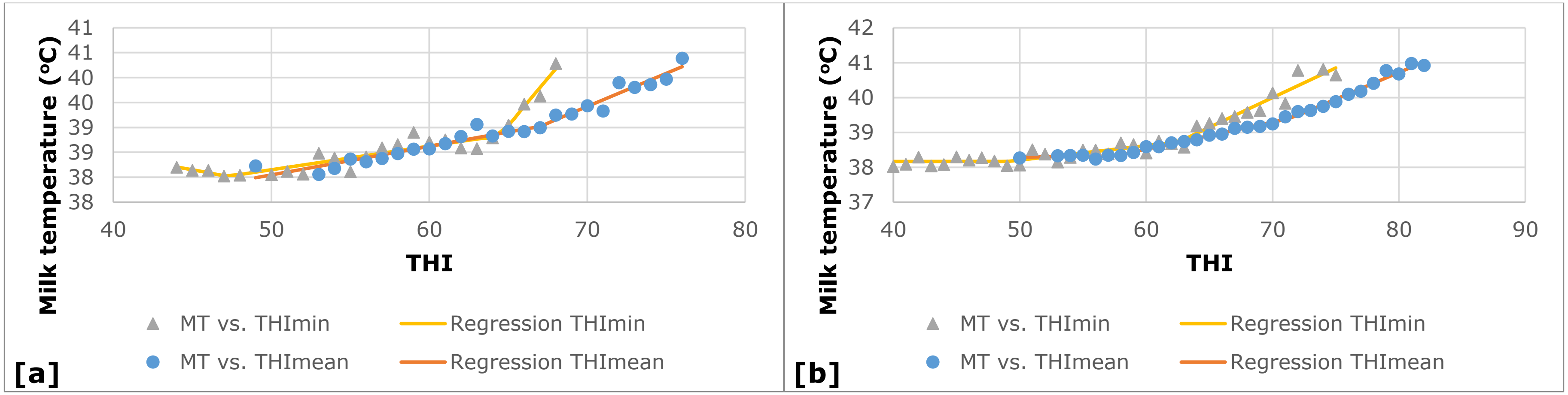

Figure 3-7 Broken-line regression between MT and THI. The sub-figure [a] is for the data measured on the farm, and [b] is for the data collected from local weather station

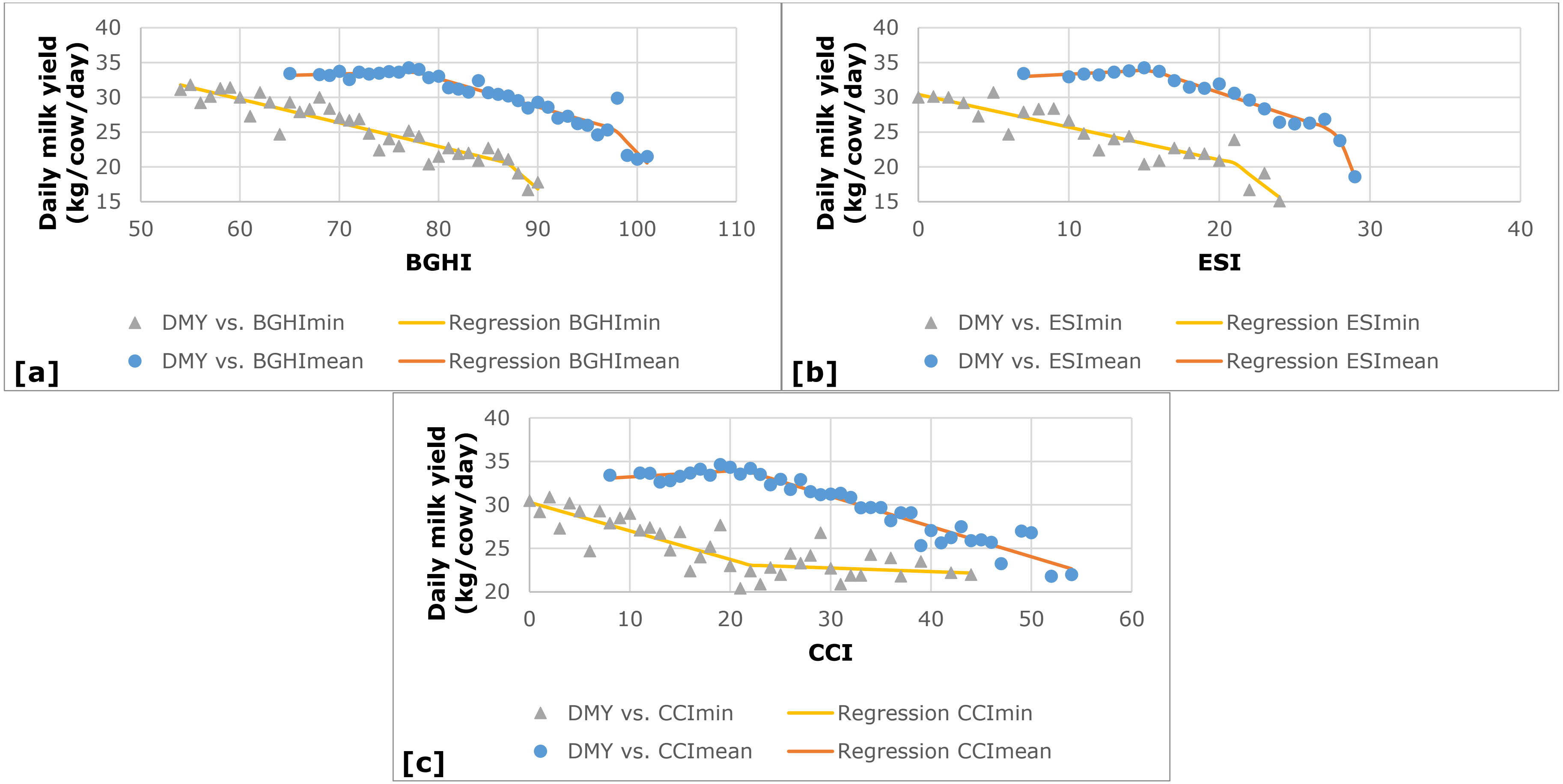

Figure 3-8 Broken-line regression between DMY and advanced TCIs. The sub-figure [a] is for BGHI, [b] is for ESI, and [c] is for CCI.

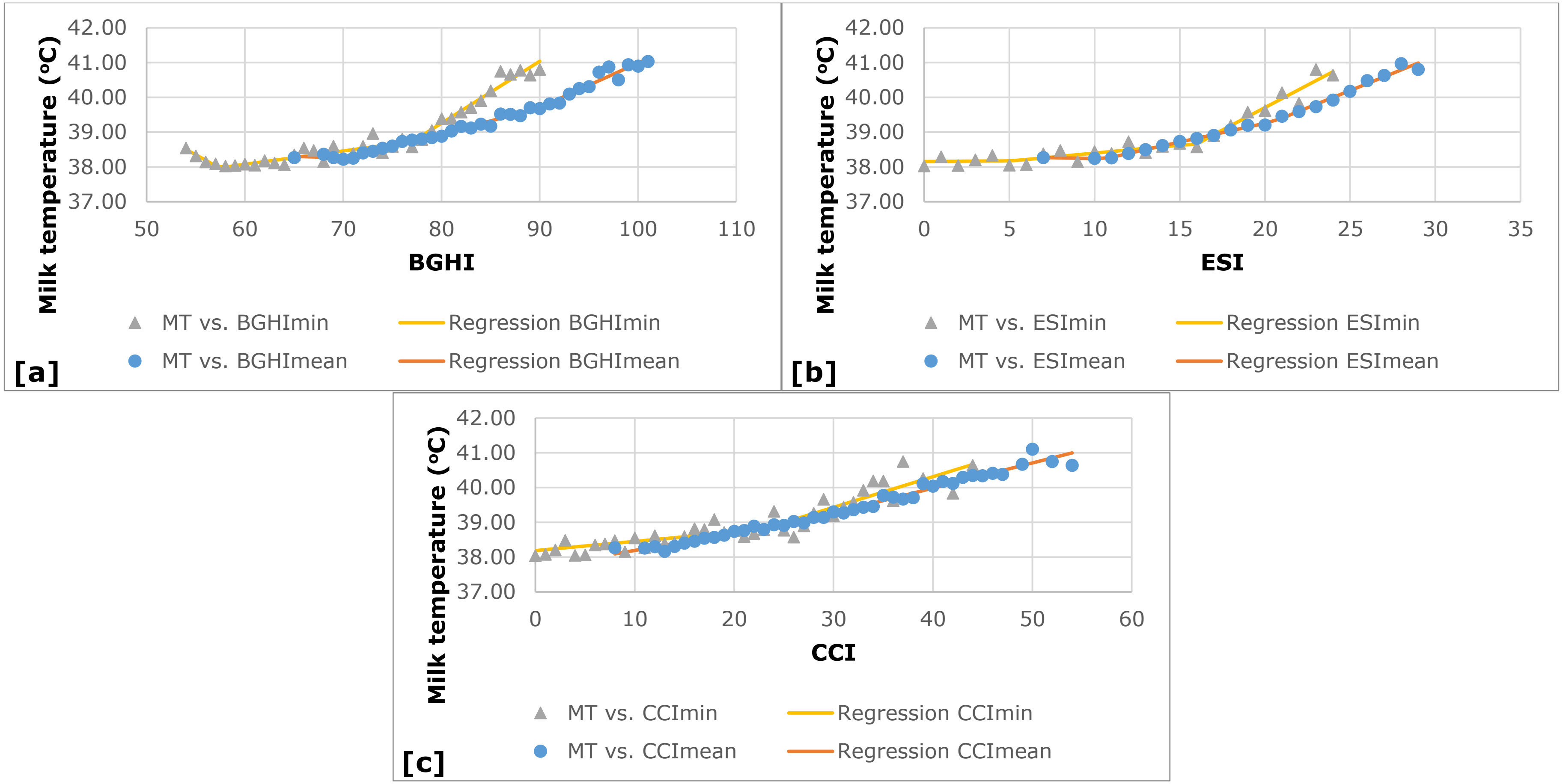

Figure 3-9 Broken-line regression between MT and advanced TCIs. The sub-figure [a] is for BGHI, [b] is for ESI, and [c] is for CCI

Table 3-7 Threshold values for alerting heat stress based on significant changes in DMY and MT

Variables		vs. DMY				vs. MT		
		Normal	Alert	Urgent		Normal	Alert	Urgent
Farm	Tmin	<9	9 to 18	>18	Tmin	<6	6 to 19	>19
	THImean	<64	64 to 68	>68	THImin	<47	47 to 65	>65
Web	Tmin	<7	7 to 25	>25	Tmin	<2	2 to 16	>16
	THImean	<64	64 to 81	>81	THImin	<49	49 to 63	>63
	BGHImean	<78	78 to 98	>98	BGHImean	<70	70 to 90	>90
	ESImean	<15	15 to 28	>28	ESImean	<11	11 to 21	>21

Table 3-8 Consistency in using the thresholds to identify different levels of heat stress.
(The % of the days during observation identified with the same level of heat stress using paired TCIs)

Consistency (%)		Farm		Web			
		Tmin	THImean	Tmin	THImean	BGHImean	ESImean
Identify 3 levels of heat stress according to the decline of DMY[a]							
Farm	Tmin	100.00	60.00	73.00	59.00	63.00	67.00
	THImean	-	100.00	45.00	61.00	58.00	49.00
Web	Tmin	-	-	100.00	80.00	81.00	87.00
	THImean	-	-	-	100.00	95.00	90.00
	BGHImean	-	-	-	-	100.00	91.00
	ESImean	-	-	-	-	-	100.00
Identify 2 levels of heat stress according to the decline of DMY[b]							
Farm	Tmin	100.00	69.00	93.00	79.00	83.00	87.00
	THImean	-	100.00	70.00	86.00	84.00	74.00
Web	Tmin	-	-	100.00	80.00	82.00	87.00
	THImean	-	-	-	100.00	96.00	90.00
	BGHImean	-	-	-	-	100.00	92.00
	ESImean	-	-	-	-	-	100.00
Identify 3 levels of heat stress according to the increase of MT[a]							
Farm	Tmin	100.00	69.00	93.00	79.00	83.00	87.00
	THImean	-	100.00	70.00	86.00	84.00	74.00
Web	Tmin	-	-	100.00	80.00	82.00	87.00
	THImean	-	-	-	100.00	96.00	90.00
	BGHImean	-	-	-	-	100.00	92.00
	ESImean	-	-	-	-	-	100.00
Identify 2 levels of heat stress according to the increase of MT[b]							
Farm	Tmin	100.00	96.00	97.00	79.00	92.00	94.00
	THImin	-	100.00	98.00	75.00	97.00	96.00
Web	Tmin	-	-	100.00	81.00	97.00	97.00
	THImin	-	-	-	100.00	79.00	79.00
	BGHImean	-	-	-	-	100.00	99.00
	ESImean	-	-	-	-	-	100.00

a – the consistency of identifying three levels of heat stress (normal, alert and urgent)
b – the consistency of identifying two levels of heat stress (normal, alert)

Table 3-9 R^2 of correlation between THIs based on the measurement on the farm and local weather station

Cor	Min	Max	Mean
THI_1	0.90	0.92	0.95
THI_2	0.78	0.85	0.90
THI_3	0.90	0.92	0.95
THI_4	0.85	0.89	0.93
THI_5	0.77	0.59	0.94
THI_6	0.95	0.92	0.96
THI_7	0.96	0.91	0.96

Discussion of key issues

The dairy farm in this study located in Gatton, Queensland, Australia, which had a humid subtropical climate. The basic climate scenario included hot and humid summers, as well as mild and sunny winters. Due to the position of Gatton, which was away from the coast but at low elevation in the Brisbane Valley, the temperatures in summer were among the hottest in south-east Queensland. Thus droughts and heat waves can be problematic for agricultural productivity. Based on the measurement of thermal environment in this study, the overall climate of the year was warm and humid. However, the relative humidity in a hot season was lower than the one in cold season. Moreover, the measured dry bulb temperature had limited correlation with the relative humidity, which was different from other studies measured in the subtropical area (Dikmen and Hansen, 2009). This partially indicated the influence of Monsoon in the region of southern Queensland. The pattern of Monsoon was plotted in Figure 3-4 with a clear reverse of wind direction. The rainfall and air moisture could be influenced, thus the microclimate on the farm could not be explained simply by the basic psychometric theory, and more researches might be required for this kind of climate condition.

The temperature measured at the farm and local weather station showed acceptable agreement. The measured relative humidity at the farm had limited agreement with the local weather station. Therefore, the TCIs which took more weight of relative humidity in the equation could lead to lower consistency, such as THI_2, THI_4 and THI_5 in Table 3-9. The previous studies have demonstrated the application of downscaling large-scale climate information to small-scale had did not necessary influence the precision of estimating plant phenology (Maak and von Storch, 1997; Matulla *et al.*, 2003), as well as the prediction of heat stress for livestock farming (Milani *et al.*, 2015). This study demonstrated the consistency of using TCIs measured on farm and local weather station to predict heat stress. In Table 3-8, the consistency between Tdb on farm and BGHI and ESI on local weather station were higher than 80% to predict the decline of DMY. For MT prediction, the consistency was higher than 90%. Scharf *et al.* (2012) compared the application of respiration rate and rumen temperature to identify heat stress of cattle under laboratory and field studies. The rumen temperature was recommended as the consistent variable between environments. In this study, MT was more consistent between farm and local weather station, compared with DMY. The prediction of heat stress using the correlation between thermal data and MT could result in a more robust outcome, regardless of the spatial variability.

Bohmanova *et al.* (2007) compared THI 1-7 in two regions (south-east and south-west of United States) and concluded the ratio of wet and dry bulb temperature (Twb/Tdb) could affect the performance of THIs under humid or arid condition. The study found THI_6 with the lowest ratio obtained the highest performance for the arid climate, whereas THI_2 with the highest ratio performed the best for the humid condition. In this study, no significant improvement was found by using THI_2 to predict heat stress compared with THI_6. This might be caused by the limited correlation between the Tdb and RH in this study. However, the improvement of prediction performance (the increase of R^2) as reported by Bohmanova *et al.* (2007) is less than 0.01. It was hard to conclude that adjusting the weight of relative humidity (or ratio between Twb and Tdb) in THI equation could lead to significant improvement of the prediction.

For the prediction of heat stress, the comparison of using basic and advanced TCIs had been performed by many studies such as Kendall *et al.* (2006), Silva *et al.* (2007), Li *et al.* (2009), Hammami *et al.* (2013) and Milani *et al.* (2015). However, there is still no unique conclusion based on these studies, as they were undertaken in different climate conditions. The HLI was recommended by Silva *et al.* (2007) for the prediction of heat stress in tropical climate areas, whereas Kendall *et al.* (2006) reported the insignificant relationship between HLI and DMY based on the data from oceanic climate areas. In this study, by comparing the best performance of prediction using basic thermal indicators, basic TCIs and advanced TCIs, Tdb can provide the same performance as other TICs, with $R^2 \approx 0.40$ for DMY and $R^2 \approx 0.85$ for MT. The consistency test presented in Table 3-8 also proved the agreement (>80) between Tdb, THI, BGHI and ESI to identify the days with different levels of heat stress. This is similar to the conclusion from Dikmen and Hansen (2009), which was based on the correlation with rectal temperature for the cows in a subtropical region. It indicated the possibility to simplify the assessment of heat stress by only using Tdb as the major TCI.

With different dependent variables in the models, the evaluation of heat stress could be varied, even under uniform thermal condition. The BGHI was not correlated with rectal temperature but significantly correlated with respiration rate as reported by Silva *et al.* (2007). Li *et al.* (2009) found the correlation between TCIs and respiration rate was usually higher than the correlation between TCIs and skin temperature or sweating rate. In this study, the correlation between TCIs and DMY was relatively lower than the correlation between TCIs and MT. This is similar to the results from West *et al.* (2003), which demonstrated the relatively low correlation between TCIs and DMY or dry matter intake. West *et al.* (2003) also found the correlation between TCIs and MT afternoon (PM) was higher than the correlation before noon (AM). In this study, the correlation between most of the maximum TCIs and MT was lower than the correlation between a minimum or mean TCIs and MT. The maximum TCIs usually indicated the most serious heat stress in the afternoon, however, it was different from the overall thermal condition afternoon (PM) as applied by West *et al.* (2003). The same difference existed between minimum TCIs and the thermal condition before noon (AM). Therefore, it was difficult to compare this kind of result between these two studies. However, West *et al.* (2003) selected the minimum and mean ambient temperature as the variables with the greatest influence on MT. This partially proved one finding of the present study that the animal responses were more influenced by the overall daily heat stress (mean TCIs) and nighttime cooling (minimum TCIs). A similar influence was also found with DMY, where the effect of minimum and mean TCIs was always higher than the maximum TCIs. Kendall *et al.* (2006) also reported that enough nighttime cooling could prevent the decline of DMY even for non-shaded cows staying with maximum THI and HLI.

Three levels of heat stress (Normal, Alert, and Urgent) were identified with different TCIs and thresholds in this study. For the mean THI, when the value exceeded 64, heat stress happened with a significant decline of DMY. The value was lower than 69 as reported by Bouraoui *et al.* (2002), as the production level of the present herd (31 kg/cow/d) was higher than the reported herd (<20 kg/cow/d). For the production level of 35 kg/cow/d, Zimbelman *et al.* (2009) found that the critical value of average THI could be 68. However, the decrease of DMY was 2.2kg/THI with THI exceeding 68 as reported by Zimbelman *et al.* (2009), while in the present study the decrease was only 0.4-0.5 kg/THI. With the same level of DMY decline, the threshold of mean THI found in this study was close to the one reported by Hammami *et al.* (2013), which was 62 with 0.36 kg/THI. However, the production level of the herd in that study was around 23 kg/cow/d. The database of Hammami *et al.* (2013) only included the test result of first-lactation cows, which could be the reason for the low heat stress tolerance. Even with same statistic approach, different studies applied a different range of animal responses (e.g. decline of DMY) to identify the levels of heat stress and the thresholds, it is still necessary to establish a standard to quantify the influence of heat stress.

Moreover, as shown in Table 3-6, most of TCIs had a lower value of threshold when identifying the heat stress based on the increase of MT. This demonstrated a higher sensitivity toward heat stress compared with DMY. Studies also demonstrated the correlation between milk temperature, ambient temperature, internal body temperature and animal health (Chaudhari and Singh, 2015; Pohl *et al.*, 2014). By applying a threshold of MT to warn heat stress, farmers could be made aware of the problem earlier when compared to monitoring DMY reduction.

3.5. Conclusions

The prediction of heat stress using Tdb can provide similar good performance as other TCIs. By using the regression between TCIs and MT, the impact of spatial variability between on-farm measurement and local weather station can be reduced when assessing heat stress. The identified thresholds for different level of heat stress at the research site suggested that heat stress can be alerted with daily mean THI of greater than 64 for the cow with 31kg/cow/day milk production. However, quantification of the effect of heat stress (e.g. decline of DMY) under different levels of heat stress is still difficult. The heat stress with high minimum TCIs maybe more detrimental to cattle than heat stress with the maximum TCIs, as the night time cooling is essential for the animal to prevent the decline of production performance. The potential of simplified assessment of heat stress according to on-line dataset was demonstrated by this study.

Chapter 4. **Modelling of heat stress in a robotic dairy farm. Part 2: Identify of the specific thresholds with production factors**

4.1. Abstract

Thresholds of heat stress were identified by determining the temperature or the value of thermal comfort indices with significant change of animal responses (e.g. decline of daily milk yield). However, the published thresholds could lead to inaccuracy when dealing with specific climate conditions, animal breeds and production factors. Thus, dynamic thresholds might be able to provide better assessment of heat stress with self-calibration capabilities. In this study, a large dataset of individual age, body weight (BW), days in milk (DIM), daily milk yield (DMY) and milk temperature (MT) of 126 lactating Holstein cows was collected from a robotic dairy farm over 5 years. The ambient dry bulb temperature was collected from local weather station and processed as daily minimum and mean temperature (Tmin and Tmean). The raw dataset of individual cow was grouped according to their age, BW and DIM. Specific thresholds of dry bulb temperature for different group of cows were identified using the single broken-line regression between temperature and DMY/MT. The specific thresholds for different group of cows were imported into a machine learning model to develop decision tree of dynamic thresholds. Thresholds for the whole herd were also identified by using multiple broken-line regression considering temperature, age, BW and DIM as independent variables. Four stages of heat stress were established as thermal comfort stage (Tmin < 5 ^oC, Tmean < 9 ^oC), mild heat stress (Tmin: 5-6 ^oC, Tmean: 9-11 ^oC), effective heat stress (Tmin: 6-14 ^oC, Tmean: 11-16 ^oC) and critical heat stress (Tmin > 14 ^oC, Tmean > 16 ^oC) based on the change of DMY and MT. Dynamic thresholds of critical heat stress were established for Tmin (0, 10 and 12 ^oC) and Tmean (5, 7, 13, 14, 15, and 16 ^oC). The model of dynamic thresholds using decision tree had overall 94% accuracy with the thresholds of Tmin, and 79% accuracy with the thresholds of Tmean. The importance of cooling the cows during early lactation period was demonstrated by the model, as lower threshold was determined with DIM below 40 days.

4.2. Introduction

Homoeothermic animals including the dairy cows need to keep their body temperature at an appropriate constant level. The internal heat balance is known as a basic mechanism in controlling the animals' body temperature. The heat produced during metabolism (heat input) would be consumed for maintaining body temperature e.g. sweating and production activities e.g. milking (heat output) (DeShazer *et al.*, 2009; West, 2003; Kadzere *et al.*, 2002). The heat stress can disturb this balance as the animal would be required to spend more energy to maintain body temperature leaving less energy for production activities, which leads to production loss. It was found that the increased nutrient intake will result in a greater heat input in high-producing dairy cows as they can generate more metabolic heat, consequently, these animals are less tolerant to heat stress (Kadzere *et al.*, 2002). If the feed intake (heat input) of dairy cows do not meet the energetic demands of maintenance and lactation (heat output), negative energy balance (NEBAL) could occur (Allen *et al.*, 2013). In addition to production loss, the NEBAL effect can damage the animal's health and finally cause death. Vitali *et al.*, (2009) reported that severe heat stresses can cause up to 23.3% of herd mortality.

Effective mitigation of heat stress is still a significant challenge for dairy farming, and design of adequate mitigation requires accurate assessment of heat stress effect. The assessment of heat stress is generally based on modelling between thermal environments and animal responses. Several thermal comfort indices (TCIs) are developed for the assessment under different climate condition or for different animal breeds (LCI, 1970; Buffington *et al.*, 1981; Mader *et al.*, 2006; Brown-Brandl *et al.*, 2005; Gaughan *et al.*, 2008b). Studies on dairy cows were also conducted to link the results of these models with other indicators of production performance, such as feed intake (Zimbelman, 2007), milk production (Bouraoui *et al.*, 2002) or conception rate (Nabenishi *et al.*, 2011). However, the TCIs' applications require measurement on the animal parameters which were only applicable in laboratory studies (Berry et al., 1964; Yousef, 1985) or in labour intensive field studies (Gaughan *et al.*, 2002; Gaughan *et al.*, 2008b). The difference between the TCIs, animal breeds, climate conditions and farming facilities could always lead to variability in the results.

Developing reliable models that can be easily parametrised could be the right approach. These are simplified models with parameters that can be easily and reliably can be parametrised is an effective solution to enhance the applicability of heat stress assessment. Previously has been reported that simply using ambient temperature (dry bulb temperature) as the predictor of rectal temperature can provide similar performance as TCI equations in sub-tropical regions (Dikmen and Hansen, 2009) and this was also demonstrated in Part I of current study (Ji *et al.*, Submitted-a) Moreover, the results based on small number of animals under laboratory conditions (Yousef, 1985) or a large number of animals over several regions (Gaughan *et al.*, 2008b) have little relevance for heat stress problems experienced on specific farms. For example, most of the identified thresholds for alerting heat stress are too general to consider differences between animal physiological status, production factors or age (Silva *et al.*, 2007), which would lead to more effective mitigation of heat stress.

In an Automatic/Robotic Milking System (AMS), measurements associated with the production and physiological status of animals (such as body weight, feed intake and production level etc.) are performed automatically and routinely when the cow visits the milking robot. Of the collected information, milk temperature (MT) can possibly be applied as an indicator of animals' response to heat stress. Researches have demonstrated a significant correlation between milk temperature and ambient temperature, internal body temperature, and animal health (Chaudhari and Singh, 2015; Pohl *et al.*, 2014).

This paper hypothesises that assessment of heat stress can be performed using milk temperature, a variable that is readily available in all AMSs. In addition, this paper is aimed at generating new heat stress thresholds values. These thresholds values are to be determined based on animals' responses of milk production and milk temperature. Influence of different production factors will be considered and compared including milk production level, age, body weight and days in milk. Finally, a machine learning model will be developed for adjusting the threshold values according to animals' production factors.

4.3. Methodology

Data Collection

As described in the first paper of this series (Ji *et al.*, Submitted-a), the data collection was performed in a dairy farm located in Gatton, Queensland, Australia. The AMS farm had about 160 lactating cows with milking robots (LELY Astronaut, Lely Industries NV, Maassluis, the Netherlands). Animals were allowed to freely move between feeding and resting area, while semi-free access to the milking station was managed by the herd management system (LELY T4C, Lely Industries NV, Maassluis, the Netherlands). The system controlled the daily maximum visits of individual animals. The herd management system was linked to AMS to collect information from the individual animal during each milking visit. This information mainly included age, body weight (BW), days in milk (DIM), milk production (daily milk yield, DMY), and milk temperature (MT), as listed in Table 4-1. We collected a dataset of nearly 5 years' (June 2013- November 2017) with information of 126 Holstein lactation cows, which contained 78455 rows of test day's data.

It is demonstrated that the utilization of data from local weather stations outside farm could provide a similar assessment of heat stress as using the data from on-farm measurement (Ji *et al.*, Submitted-a). Therefore, in this study, the climate data from June 2013 to November 2017 was downloaded from local weather station with nearly 8 km distance from the research farm. As discussed in the previous paper of these series (Ji *et al.*, Submitted-a), a model developed by using the relationship between dry bulb temperature (Tdb) and animal responses could provide with enough certainty. For long term analysis in this paper, we used observed daily minimum temperature to represent night time cooling condition, and mean value of Tdb to represent all day thermal condition, as listed in Table 4-1.

Table 4-1 List of variables in data collection

Category	Name	Unit	Definition
Animal status and production	Age	Years	Age of individual animal
	BW	Kg	Body weight of individual animal
	DIM	Days	Days in milk, or lactation days
	DMY	Kg/cow/day	Daily milk production of individual animal
	MT	ºC	Milk temeprature when milking the animal
Environment	Tmin	ºC	Daily minimum temperature, represent the night time cooling
	Tmean	ºC	Daily average temperature, represent the all day thermal condition

Data processing and statistical analysis

The raw data was filtered, cleaned and outliers have been removed using standard statistical methods (Mead, 2017). The analysis was conducted: (1) to identify the specific thresholds for different group of animals with different production factors, (2) to identify the general thresholds for the overall group by including production factors as dependent variables in the model, and (4) to establish a decision tree based on the results from (1), (2) and (3), which was expected to calibrate the threshold dynamically.

- ## Group the animal

Animals were classified into different groups to identify specific thresholds according to the production factors, age, BW, and DIM of individual animals. The multi-phases segmented single linear regression was applied to identify the significant break points for grouping (Chamsaz *et al.*, 2011) and the model was formulated as follows:

$$y = \begin{cases} a_1 + b_1 x + \varepsilon, & (x \le s_1) \\ a_2 + b_2 x + \varepsilon, & (s_1 < x \le s_2) \\ \quad\quad\quad \cdots & \\ a_n + b_n x + \varepsilon, & (s_{n-1} < x \le s_n) \end{cases}$$

Where x is the independent variables of age, BW or DIM and y is dependent variable DMY or MT. The parameters $\boldsymbol{a_i}$ and $\boldsymbol{b_i}$ (i = 1 to n) are the intercept and the slope for different phases; $\boldsymbol{\varepsilon}$ denotes the residual error terms. The threshold or break points ($\boldsymbol{s_i}$, i = 1 to n) and the number of phases (n) determined by comparison between the significant difference of $\boldsymbol{b_i}$ of each phases. The value of $\boldsymbol{s_i}$ is used to group the dataset into sub-groups according to different production factors. For example, three groups of younger than 5 years old, between 5 and 10 years old, and older than 10 years old were generated based on the significant break points of age.

- **Identify the specific thresholds**

Following the first paper of this series (Ji *et al.*, Submitted-a), for each group (categorized with age, BW and DIM), the broken-line single linear regression was applied to determine the threshold values of Tdb. The model was formulated following the equation below:

$$y = \begin{cases} c + \varepsilon, & x \leq THR \\ a + bx + \varepsilon, & x > THR \end{cases}$$

Where c is a constant representing the mean value of dependent variable y i.e. DMY or MY without significant impact from heat stress. The parameters a and b denotes to the intercept and slope of single linear regression when the independent variable is above the threshold (THR), respectively.

- **Identify the general thresholds**

The multi-phases segmented multiple linear regression was applied to estimate the thresholds of Tdb for the whole herd by considering production factors (age, BW and DIM). The model was formulated as follows:

$$y = \begin{cases} a_1 + b_1 x_1, & (x_1 \leq s_1) \\ a_2 + b_2 x_1, & (s_1 < x_1 \leq s_2) \\ & \ldots \\ a_n + b_n x_1, & (s_{n-1} < x_1 \leq s_n) \end{cases} + \sum_{j=1,k=2}^{j=3,k=4} c_j x_k + \varepsilon$$

Where x_1 is independent variable Tdb, x_k (k = 2 to 4) is the independent variable of production factor i.e. age, BW and DIM, y is the dependent variable of animal responses DMY and MT, and ε is the residual error terms. The parameters $a_{1\ to\ n}$ $b_{1\ to\ n}$, and $s_{1\ to\ n}$ are using the same definition as described in the multi-phases segmented single linear regression. The parameter c_j (j = 1 to 3) are the weight (slope) of production factors in the regression. To implement this model, an orthogonal transformation was used to a canonical model (Siegmund and Zhang, 1994). The multiple linear regression is then reduced to a single linear regression by this elimination (Lehmann and Romano, 2006).

- **Determine the stages of heat stress**

According to the specific and general thresholds as identified above, as well as the varied level of cows' responses with temperature exceeding these thresholds. The stages of heat stress impact were determined as follows:

Stage I - thermal comfort zone: no impact from ambient temperature;
Threshold I: ambient temperature with significant increase of MT;
Stage II - innocuous heat stress: the animal's internal body temperature (MT) started to raise as a normal physiological reaction toward heat stress, no extra energy required to cope with heat stress, and no production loss (decline of DMY);
Threshold II: ambient temperature with a significant decline of DMY, this could also be identified as a threshold of MT.
Stage III - effective heat stress: the production performance (DMY) started to decline as the animal requiring extra energy to cope with heat stress.
Threshold III: ambient temperature with a more significant increase of MT or decline of DMY compared with Stage II and III.
Stage IV – critical heat stress: the increase of internal body temperature (MT) and decline of production performance (DMY) became more significant, and mitigation (e.g. ventilation cooling) was necessary to prevent health injury.

As the broken-line regression for each grouped dataset (age, BW and DIM) only generated two phases and one thresholds for each correlation, it was unable to find out the Stage III (effective heat stress), where the increase of MT or decline of DMY was significant but not as serious as Stage IV. Therefore, for the regression applied in each group classified by Age, BW or DIM, only Stage I, II and IV were identified. The multi-phased segmented multiple linear regression for the whole herd determined the status of Stage III (thresholds and slopes).

- **Decision tree for threshold selection**

For Stage IV, the thresholds determined for the whole herd could underestimate the heat stress for specific groups. Thus, mitigation of heat stress using the herd's thresholds might be insufficient. The specified thresholds to ensure the thermal comfort of a specific group of animals could be a better solution. However, same cows might be assigned with a different threshold as they can meet the features of more than one group (e.g. age<4 years, BW>1000 kg and DIM<20 days), which lead them to suit to multiple specific thresholds. Therefore, the thresholds of specific groups were summarized as classification factors. Then, a decision tree was applied to provide an artificial selection of the classes (thresholds) according to the production factors (Age, BW or DIM) of individual animal or sub group of the herd.

$$THR(x) = c_{full} + \sum contrib(x_i, k)$$

Where the THR(x) is the threshold value determined by the decision tree, parameter of c_{full} and $contrib(x, k)$ is the value at the root of the node and the contribution from the k-the feature of variable x_i, respectively. The prediction of decision tree is made by calculating the mean (root) given by the topmost region that covers the whole training set plus the sum of the features' contributions. Dataset were split into 75% for train and 25% for test. Sensitivity, the specificity, balanced accuracy and overall accuracy were applied to validate the model (Berry *et al.*, 2004; Pinzón-Sánchez *et al.*, 2011; Brodersen *et al.*, 2010).
All data processing and statistical analysis were done using R 3.4.3. (R Development Core Team, 2017). The separation of the dataset was performed using the basic function of "split" in R, with 75% for training and 25% for testing. The general linear regression analysis was done by using the basic function of "lm"(Chambers, 1992), while the generalized linear regression analysis was dependent on the function of "glm" (Dobson and Barnett, 2008). The broken-line regression was performed based on the "lm.br" package (Adams, 2017). The multi-phases linear regression was taken by using the "segmented" package (Muggeo, 2008). The decision tree was established by using the "part" package (Therneau *et al.*, 2015).

4.4. Results and Discussion

Group the animal

The whole dataset of the individual animal was grouped to form cohesive clusters that can be meaningfully analysed by multi-phased segmented regression (Figure 4-1 and Table 4-3). In Figure 4-1 [a], the regression between DMY and Age is plotted. The production level of younger animals (age < 4-year) statistically and numerically increased with age. In contrast, the production increase was statistically significant but numerically small for older animals (aged between 4 and 10 years). A significant decline of DMY was found when animals were older than 10 years. The fluctuation of the original dataset in Figure 4-[a] was caused by the impact of lactation curve as plotted in Figure 4-1 [c]. In Figure 4-1 [b], positive correlations can be seen between DMY and BW for first two weight classes (BW <400 kg, 400 to1000 kg, and). However, the correlation under the last group (BW >1000 kg) was negative. Three groups were categorized for each factor as listed in Table 4-2. The correlations between BW, Age and

DIM are shown in Figure 4-2. Both the Age and DIM had a positive correlation with BW, as well as a steady state in the mid of the regression curve. Therefore, the interaction between the three factors was considered in the further analysis of this study.

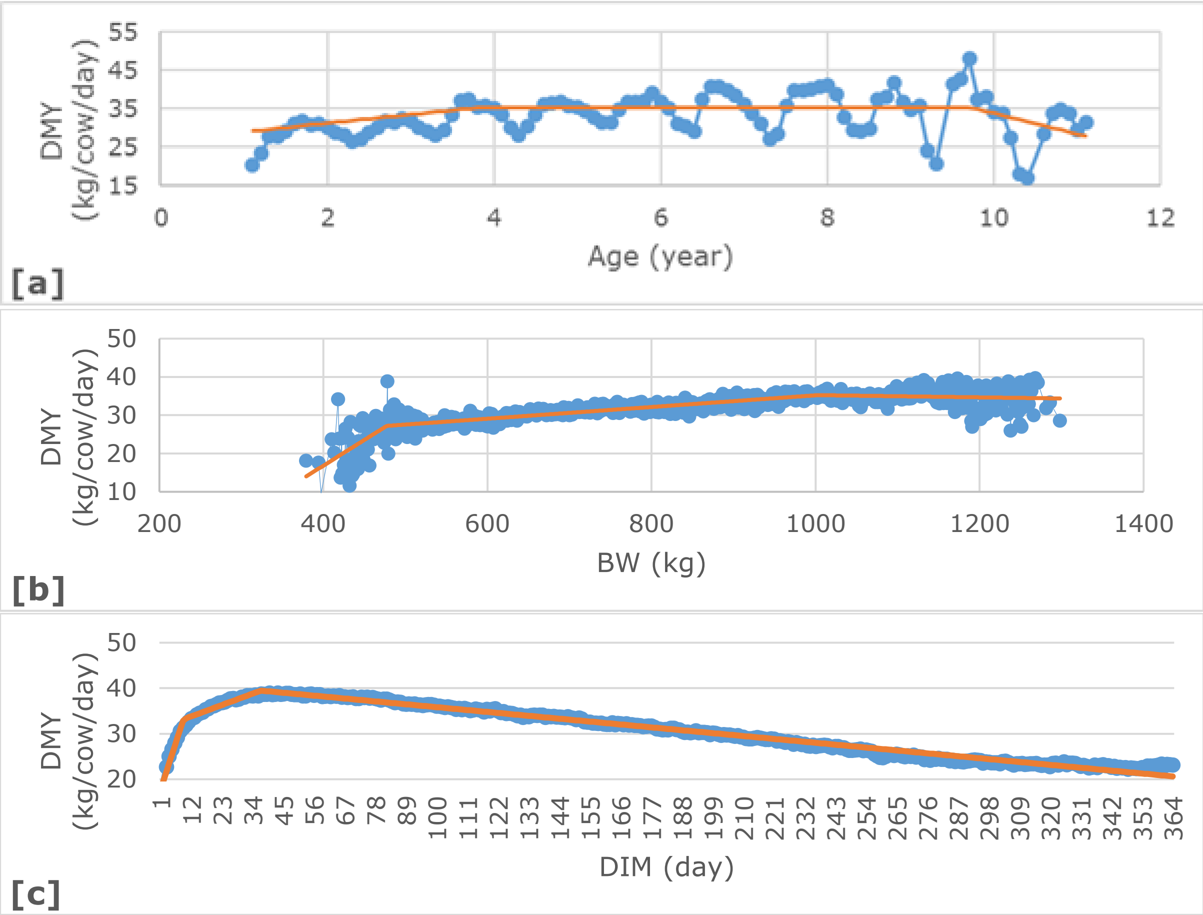

Figure 4-1 Multi-phases linear regression with different production factors. [a] is for Age, [b] is for BW, and [c] is for DIM. The fitted lines are the values calculated from regression models.

Table 4-2 Thresholds and slopes of multi-phases linear regression

Group	**Age**		**BW**		**DIM**	
	THR	Slope (kg/cow/day/year)	THR	Slope (kg/cow/day/kg)	THR	Slope (kg/cow/day/day)
1	<4	2.91	<500	0.13	<10	1.87
2	4 to 10	0.28	500 to 1000	0.02	10 to 40	0.23
3	>10	-5.80	>1000	-0.003	>40	-0.06
R2	0.26		0.77		0.98	

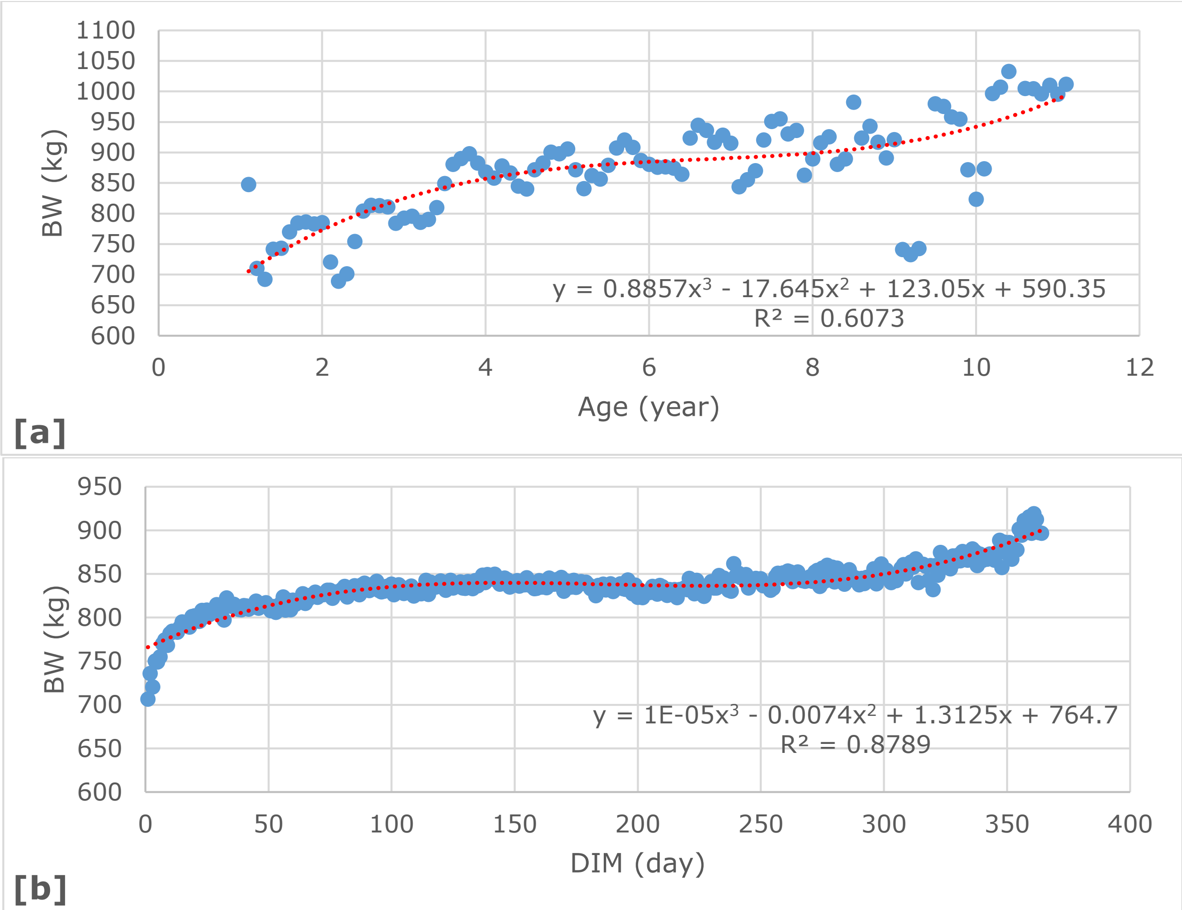

Figure 4-2 Correlation between BW, Age and DIM. [a] – the relationship between BW and Age, [b] – the relationship between BW and DIM.

Identify the thresholds of heat stress

The fitted lines of broken-line regression for different animal groups are shown in Figure 4-3 to 5 and Table 4-3. For the whole herd, the results of multi-phases segmented regression are shown in Figure 4-6 and Table 4-4.

As presented in Table 4-3, the thresholds for different groups of Age indicated the weakened heat tolerance of older cows. The thresholds of Tmin entering Stage IV (critical heat stress) decreased from 12 to 8 °C, as more night time cooling was required for the older cows. A decrease was also found with the thresholds of Tmean and MT with older cows, which was decreased from 15 to 13 °C, and from 39 to 38 °C, respectively. The thresholds of Tmean entering Stage II (innocuous heat stress) showed a different pattern. The cows with Age between 4 and 10 had the lowest thresholds (11 °C). It might be caused by the higher production level (DMY) within this Age class when compared to other age classes (Figure 4-1 [a]). Evidently, cows with higher production level were more prone to experience innocuous heat stress. However, the same threshold of Tmin entering Stage II indicated that the same level of night time cooling ($\leq$ 7 °C) were sufficient for all three groups. The animal with higher production level (Age: 4 to 10) could have the same recovery from heat stress as other groups, if they have access to sufficient cooling. Moreover, in Stage II, older cows (Age > 10) had higher slope value for the increase of MT (+0.13 °C/Tmin, and +0.16 °C/Tmean), as well as the higher slope value for the decline of DMY in Stage IV (-0.90 kg/Tmin, and -1.01 kg/Tmean). The influence of high MT became more important than the influence of high ambient temperature (Tmin and Tmean) when cows were older than 4 years old. The decline of DMY raised from -0.24 kg/MT to -6.61 kg/MT.

The threshold entering Stage II of the group with BW lower than 500 kg was lower than other groups. However, the slope value for the increase of MT was lower, which indicated they were able to dissipate the heat more readily than other groups. This was most likely caused by their smaller body mass, which made their internal temperature change more readily in relation to changes in external temperatures. The threshold of Tmin entering Stage IV had a lower value (10 ^{o}C) for cows with BW less than 500 kg, while the threshold of Tmean had a higher value (16 ^{o}C). It partially implied that this BW cluster would be less likely to enter Stage IV heat stress if they had access to sufficient night time cooling. There is no visible difference between the thresholds entering Stage II or IV for the groups with BW from 500 to 1000 kg and higher than 1000 kg, except 1 ^{o}C (11 vs. 12 ^{o}C) difference between the thresholds of Tmean (MT – Tmean) entering Stage II. Nevertheless, the animal with BW higher than 1000 kg had notable more decline of DMY (-0.94 kg/Tmin, -0.97 kg/Tmean, and -2.81 kg/MT), compared with another group (-0.57 kg/Tmin, -0.53 kg/Tmean, and -0.86 kg/MT). It clearly indicated the reduced heat tolerance of heavy cows. As the DMY had no significant increase when the animal was heavier than 1000 kg (Figure 4-1 [b]), control their BW below 1000 kg could enhance the heat tolerance of the herd, and reduce the risk of heat stress.

During the first 10 days of lactation (DIM<10 days), cows were unlikely to enter the Stage II or III of heat stress as their production (DMY: 25.4 kg/cow/day) had not reached to the high-production level. The correlation between DMY and MT indicated no decline of DMY even with the MT beyond the threshold of 41 ^{o}C, which was already 2^{o}C above the threshold of MT for the group with DIM>40 days. Between 10 and 40 days of lactation (10 days <DIM <40 days), the production (36.7 kg/cow/day) of cows achieved high-production level. These cows had the lowest heat tolerance compared with other periods. The decline of DMY could be as high as 2.63 kg/MT. For the first 40 days of lactation (DMY<40 days), the thresholds identified for Tmin (DMY-Tmin) entering Stage IV were 0 ^{o}C. It might be unrealistic to state that cows during this period requiring the ambient temperature of night time cooling to be lower than 0 ^{o}C. However, it did imply the importance of night time cooling, as cows always had insufficient cooling in this period, thus extra mitigation techniques, such as nutritional manipulations should be applied to prevent heat stress. Moreover, during the first 40 days of lactation, the thresholds to identify Stage II were higher than the thresholds of Stage IV, which indicated the reduced effectiveness of using MT in Stage II to detect heat stress, as the DMY started to decline before the MT increasing. After 40 days lactation, the DMY started decreasing as illustrated in Figure 4-1 [c], while average DMY in this period was still 32 kg/cow/day. As this period lasted for about 300 days, the thresholds identified within this period had similar results as the thresholds from other groups classified by Age or BW.

The regression analysis for the whole herd considered all three factors (Age, BW and DIM) and applied multi-phases segmented regression. Three phases were determined for the regression between MT, DMY and ambient temperature (Tmin and Tmean). Two phases were determined for the regression between DMY and MT. The general threshold for the declining of DMY caused by the increasing of MT was identified as 39 ^{o}C. This value was equal to most of the results identified based on broken line regression for different groups, except the groups with DIM<40 days or Age>10 years. The thresholds of Tmin and Tmean entering Stage III (effective heat stress) were found as 6 and 11 ^{o}C. They were 1 and 2 ^{o}C higher than the thresholds of Stage II (5 and 9 ^{o}C for Tmin and Tmean, respectively). The thresholds entering Stage IV determined for the whole herd (14 and 16 ^{o}C for Tmin and Tmean, respectively) were equal or higher than the maximum thresholds determined by the groups (12 and 16 ^{o}C for Tmin and Tmean, respectively). Therefore, even by considering the production factors in the regression, the thresholds identified for the whole herd in Stage IV could underestimate the heat stress for a specific group of animals. In contrast, the thresholds of Stage II or III could be applied as a more reliable alert of innocuous and effective heat stress, since their values were lower than most of the thresholds identified for specific groups.

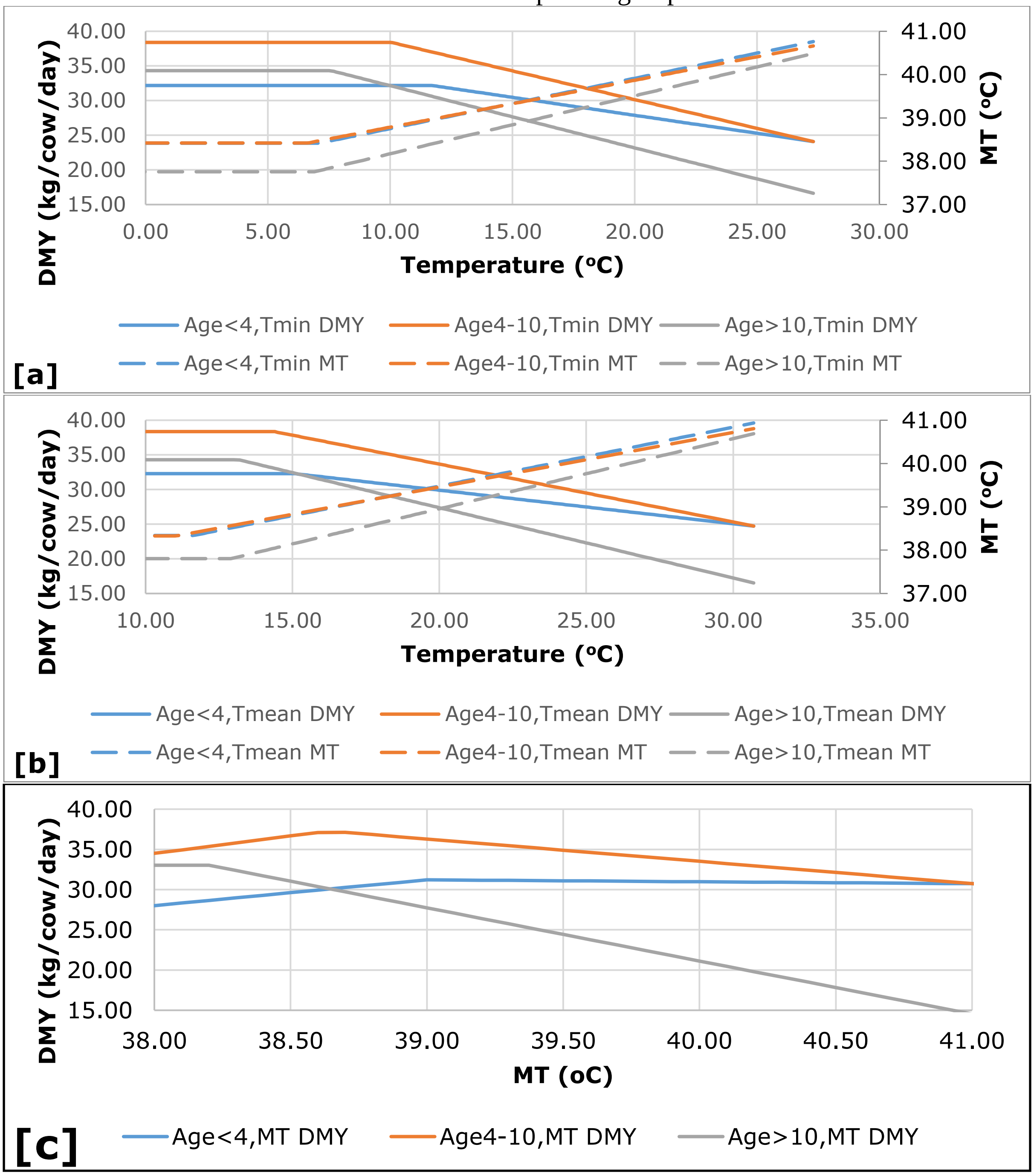

Figure 4-3 Thresholds identification for groups with different Age. Legend format: Group, Independent and dependent variable. [a] fitted lines of regression between DMY and Tmin; [b] fitted lines of regression between DMY and Tmean; and [c] fitted lines of regression between DMY and MT. The value of thresholds and slopes are presented in Table 4-3.

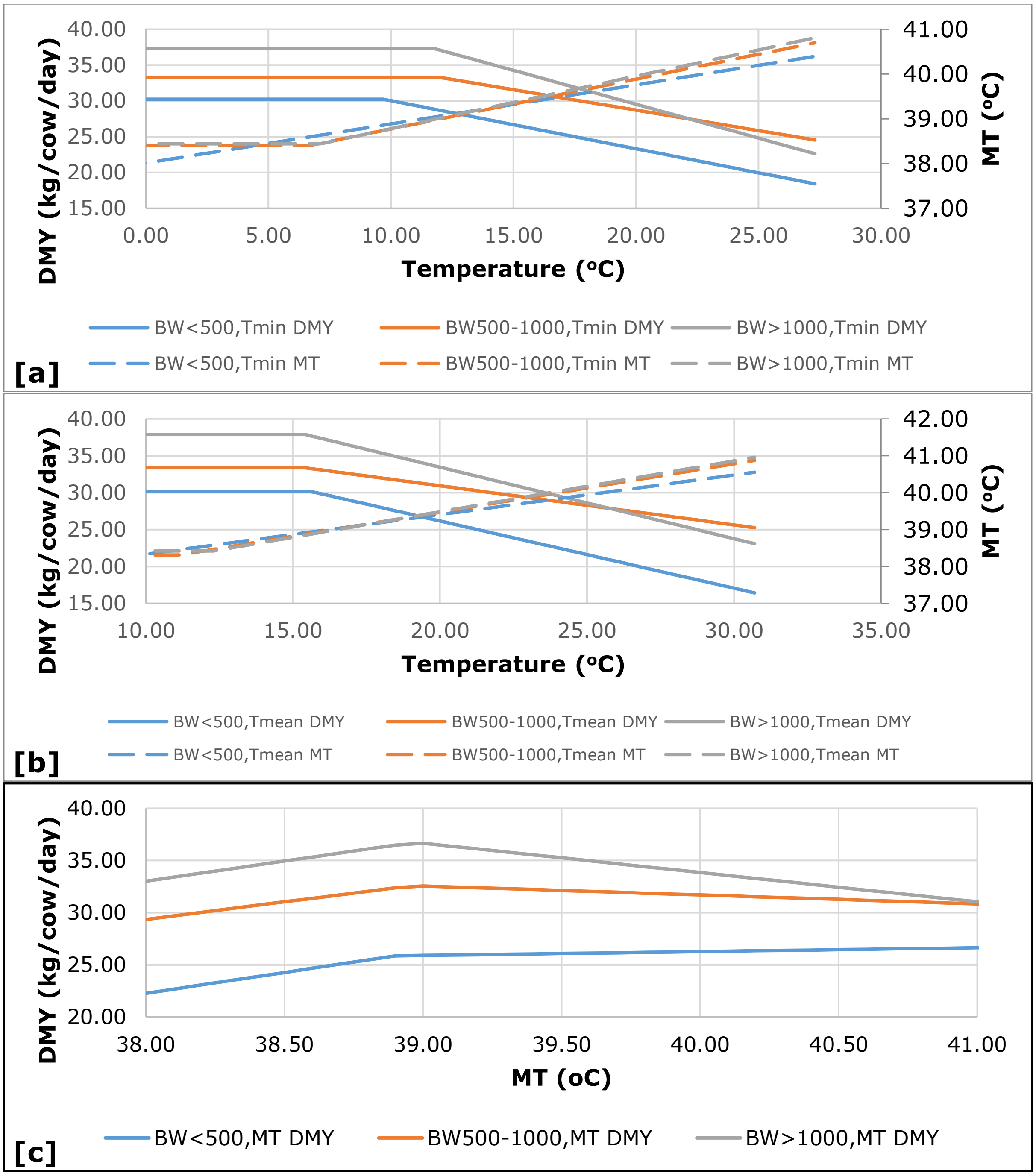

Figure 4-4 Thresholds identification for groups with different BW. Legend format: Group, Independent and dependent variable. [a] fitted lines of regression between DMY and Tmin; [b] fitted lines of regression between DMY and Tmean; and [c] fitted lines of regression between DMY and MT. The value of thresholds and slopes are presented in Table 4-3.

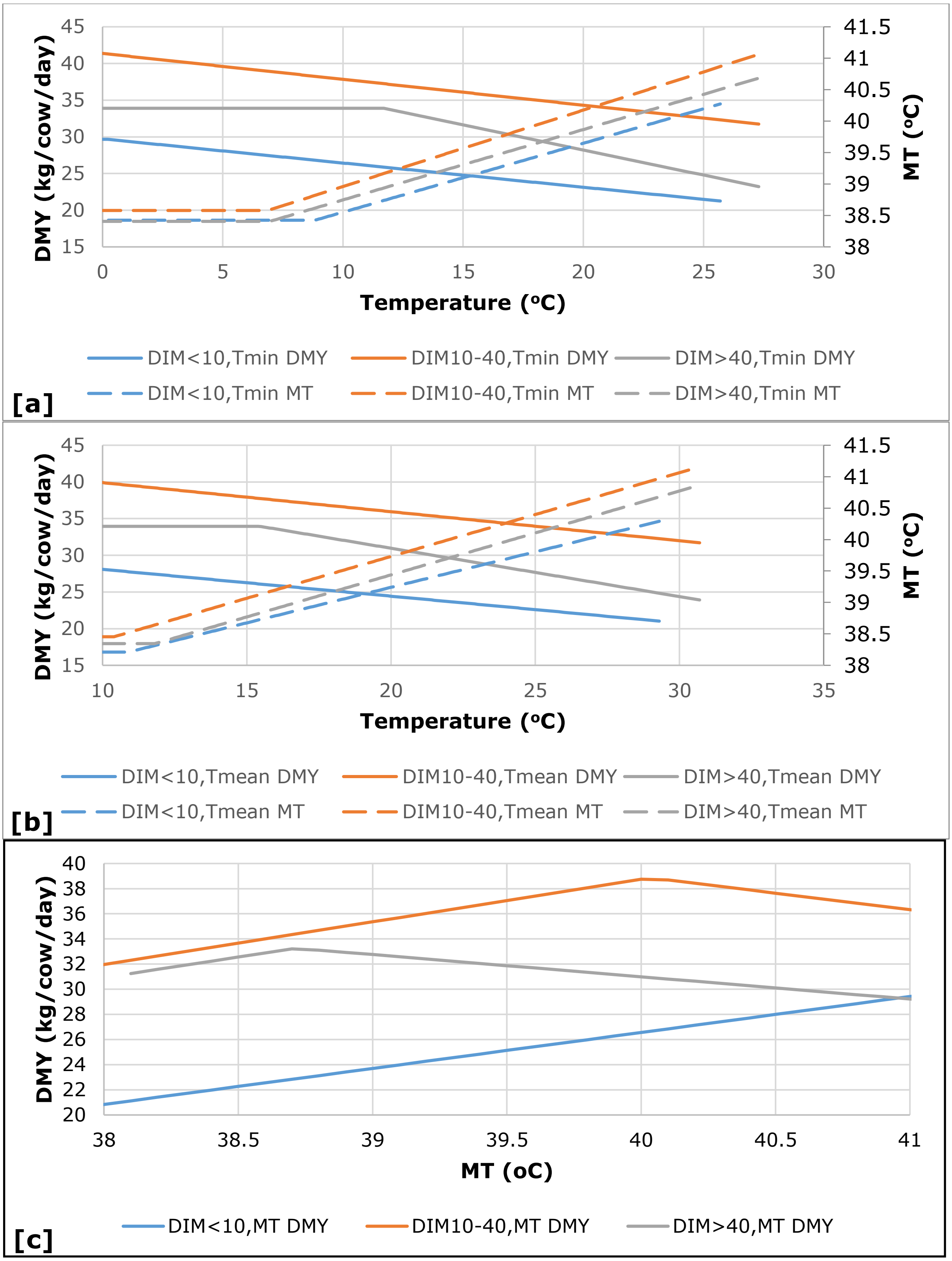

Figure 4-5 Thresholds identification for groups with different DIM. Legend format: Group, Independent and dependent variable. [a] fitted lines of regression between DMY and Tmin; [b] fitted lines of regression between DMY and Tmean; and [c] fitted lines of regression between DMY and MT. The value of thresholds and slopes are presented in Table 4-3.

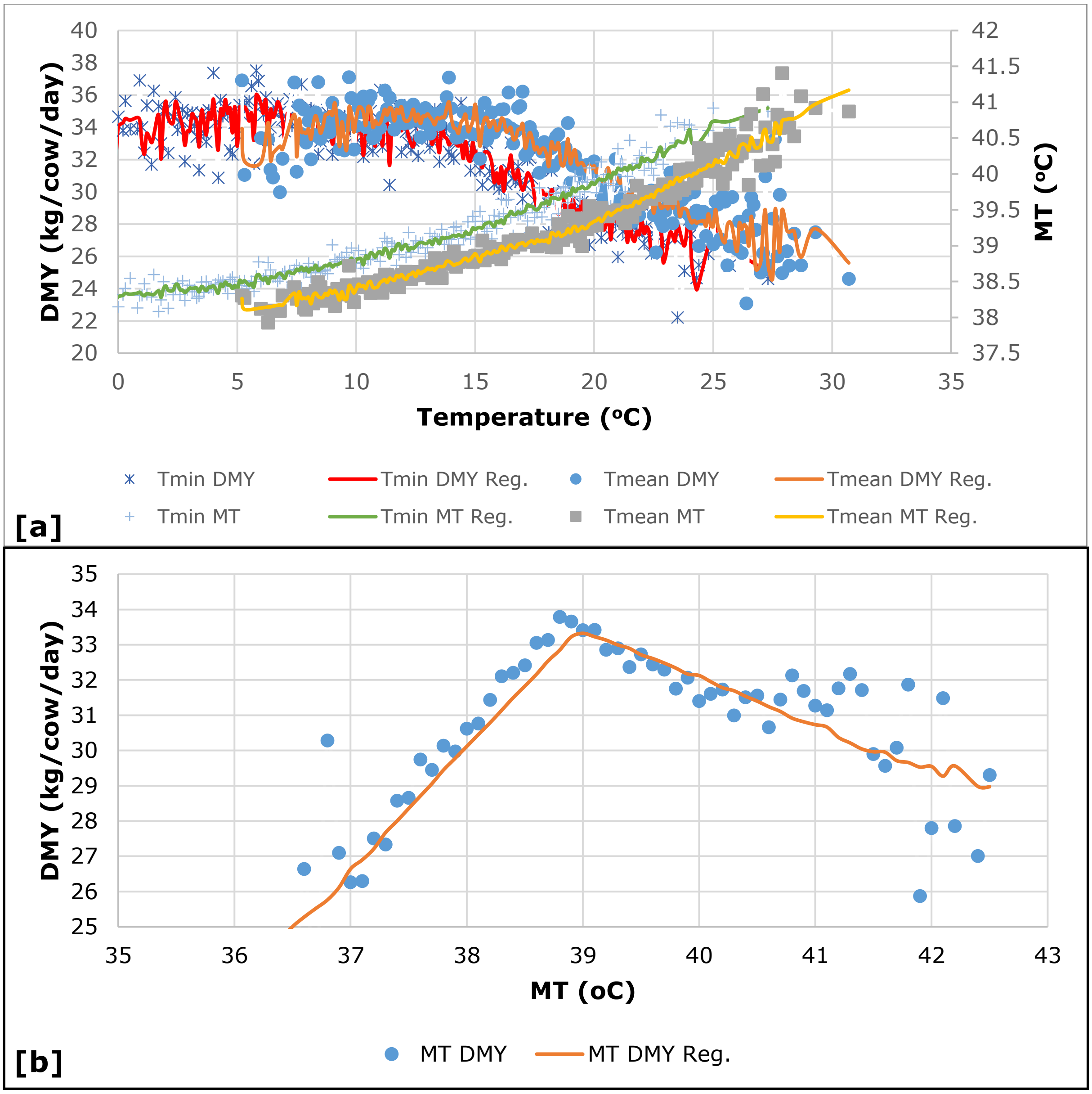

Figure 4-6 Thresholds identification for the whole herd with multi-phases segmented multiple linear regression. Legend format: Independent and dependent variable, original dataset or regression fitted values (Reg.). [a] raw data and fitted lines of regression with Tmin and Tmean; and [b] raw data and fitted line of regression between DMY and MT. The value of thresholds and slopes are presented in Table 4-4.

Table 4-3 Summary of broken line regression for thresholds identification (unit of THR: ºC, unit for Slope: kg/cow/day/ ºC, for all models P<0.05)

	The threshold of Stage II						**The threshold of Stage IV**						**DMY - MT**		
	MT-Tmin			MT - Tmean			DMY-Tmin			DMY - Tmean					
Group of Age	THRs	Slope	R^2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2
<4	7	0.12	0.94	12	0.14	0.94	12	-0.51	0.65	15	-0.48	0.67	39	-0.24	0.72
4 to 10	7	0.11	0.93	11	0.13	0.94	10	-0.83	0.83	14	-0.84	0.8	39	-2.77	0.69
>10	7	0.13	0.79	13	0.16	0.82	8	-0.9	0.57	13	-1.01	0.56	38	-6.61	0.88
Group of BW	THRs	Slope	R^2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2
<500	0	0.09	0.54	8	0.11	0.5	10	-0.67	0.28	16	-0.91	0.33	39	0.37	0.53
500 to 1000	7	0.11	0.94	11	0.13	0.94	12	-0.57	0.8	15	-0.53	0.77	39	-0.86	0.76
>1000	7	0.11	0.88	12	0.14	0.92	12	-0.94	0.68	15	-0.97	0.71	39	-2.81	0.47
Group of DIM	THRs	Slope	R^2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2
<10	9	0.11	0.63	11	0.11	0.67	0	-0.33	0.2	7	-0.37	0.18	41	0	0.69
10 to 40	7	0.12	0.9	10	0.13	0.93	0	-0.35	0.5	5	-0.4	0.48	40	-2.63	0.41
>40	7	0.11	0.93	12	0.13	0.94	12	-0.68	0.8	16	-0.66	0.76	39	-1.8	0.7

Table 4-4 Summary of multi-phases segmented multiple linear regression to identify thresholds of the herd (unit of THR: oC)

	MT-Tmin			MT - Tmean			DMY-Tmin			DMY - Tmean			DMY - MT		
	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2	THRs	Slope	R2
Stage I	<5	0.03	0.96	<9	0.03	0.97	<6	0.23	0.89	<11	0.26	0.85	<39	3.43	0.77
Stage II	5 to 15	0.08		9 to 19	0.09										
Stage III							6 to 14	-0.15		11 to 16	-0.09		>39	-1.21	
Stage IV	>15	0.14		>19	0.16		>14	-0.6		>16	-0.6				

Selection of specific thresholds

As the thresholds for Stage II and III achieved only modest reliability, the selection of specific thresholds was only processed for Stage IV. The specific thresholds for Stage IV in a different group with different age, BW and DIM were reorganized and summarized in Table 4-5.

Table 4-5 Thresholds for critical heat stress for specific production factors (unit of THR: ^{o}C)

DMY-Tmin			
THR	Age	BW	DIM
0	-	-	<40
8	>10	-	-
10	4 to10	<500	-
12	<4	>500	>40
DMY-Tmean			
THR	Age	BW	DIM
5	-	-	10 to 40
7	-	-	<10
13	>10	-	-
14	4 to 10	-	-
15	<4	>500	-
16	-	<500	>40

The model of decision tree is illustrated in Figure 4-7 and 8. The performance of the models were listed in Table 4-6, 94 and 74% overall accuracy were achieved for predicting the threshold values of Tmin and Tmean for the test dataset.

In Figure 4-7, the basic classification (root branch) is presented based on DIM. If animal's DIM was less than 21 days and BW was larger than 517 kg, the threshold would be 0 ^{o}C, which indicated night time cooling may not be sufficient to dissipate heat stress, and extra mitigations might be required. However, if animal's BW was less than 517 kg, night time cooling could be sufficient if the Tmin was lower than 10 ^{o}C. On the other hand, if animals were older than 3.9 years old or less than 577 kg, the threshold of Tmin would be 10 ^{o}C. Otherwise, the animals would not have critical heat stress unless the Tmin was higher than12 ^{o}C.

In Figure 4-8, the basic and second level of classification was performed. As all of the cows were within their first 13 and 33 days of lactation (DIM< 13 and 33 days), these branches together represented 45% of the whole dataset. It suggested more mitigation of heat stress should be provided for the early lactation period, regardless of the BW or Age of the animal. The third level of classification was based on the Age of cows. If the cow was older than 4 years old with BW larger than 947 kg or DIM less than 158 days, the heat stress could happen with Tmean higher than 14 ^{o}C. Before the mid of lactation period (DIM<153 days), cows took heat stress with Tmean larger than 15 ^{o}C. However, for the Age between 1.8 and 2.5 years old, the cows had higher heat tolerance, and the threshold value increased to 16 ^{o}C. This threshold was similar for the cows within the later period of lactation (DIM>153 or 158 days).

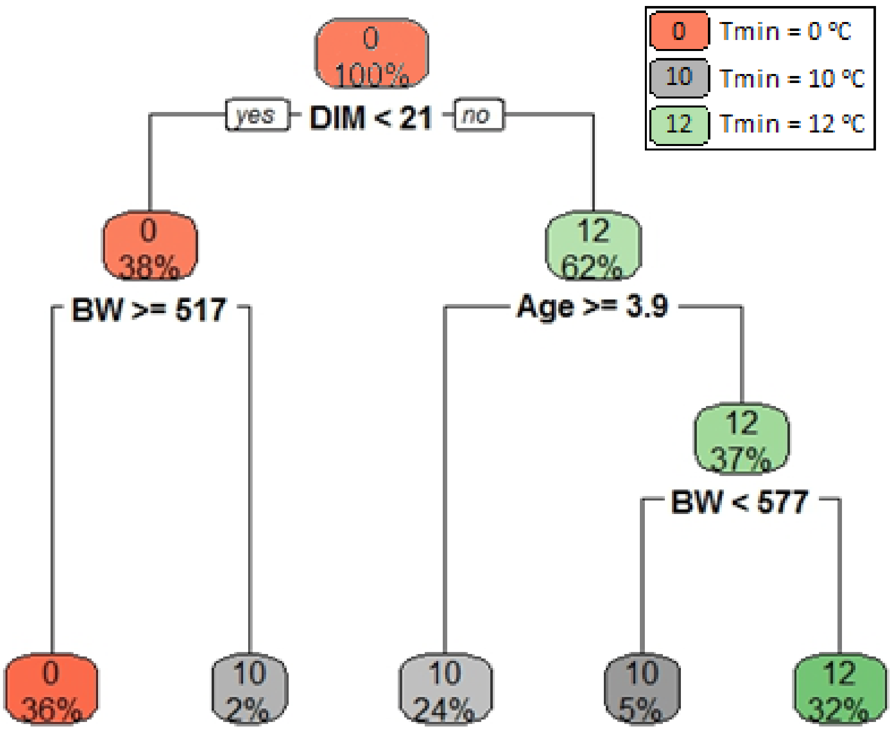

Figure 4-7 Decision tree for threshold selection of Tmin (Stage IV of heat stress). Each node shows a predicted threshold and the percentage of cows assigned in this class.

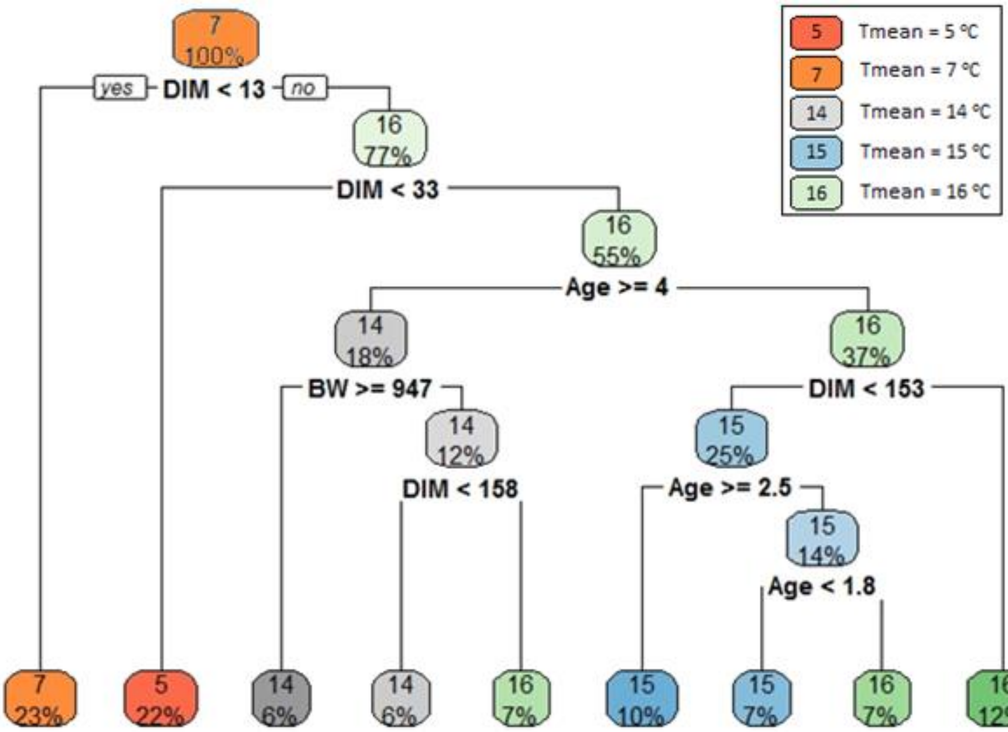

Figure 4-8 Decision tree for threshold selection of Tmean (Stage IV of heat stress). Each node shows a predicted threshold and the percentage of cows assigned in this class.

Table 4-6 Performance of decision tree for different classes

DMY-Tmin				
THR (oC)	Sensitivity	Specificity	Balanced accuracy	Overall accuracy
0	0.97	1	0.99	0.94
8	-	-	-	
10	1	0.92	0.96	
12	0.88	1	0.94	
DMY-Tmean				
THR	Sensitivity	Specificity	Balanced accuracy	Overall accuracy
5	1	1	1	0.79
7	1	1	1	
13	-	-	-	
14	0.67	0.92	0.79	
15	0.48	0.96	0.72	
16	0.79	0.85	0.82	

Discussion of key issues

Previous studies have already developed different indices to evaluate the thermal environment which was dependent on the dry bulb temperature, relative humidity, wind speed and solar radiation (Gaughan *et al.*, 2002; Brown-Brandl *et al.*, 2005; Mader *et al.*, 2010). For the subtropical area in this study, Dikmen and Hansen (2009) and Ji *et al.* (Submitted-a) had already demonstrated the direct application of dry bulb temperature can provide enough accuracy when assessing the thermal condition. Therefore, instead of selecting thermal parameters or comparing the indices, this study aimed to identify the thresholds of heat stress based on the key production factors (Age, BW and DIM) associated with dairy cows. The three production factors were applied to separate the cows in different groups for analysis. The analysis of heat stress for different ages of animals indicated the decrease of heat tolerance with the older cows, even though their production level was lower than the mid age group (4-10 years old). However, as the old cows only took small proportion of the herd population, and the average age of the herd was 3.8 years old, more attentions should be paid to the differences between the first two groups (before 4 vs. 4 to 10 years old). The link between heat tolerance and age of cows provided a statistical relationship, while the internal reasons were the changes of cow's body with age and consequent decline in health status.

The correlation between Age and BW was reported in this study, the younger cows had lower BW than the older cows. The similar relationship was also found by Renquist *et al.* (2006), which measured the BW and body condition score (BCS) of cows with different ages under the different status of production (pre-calving, pre-breeding, at weaning, and midway through the second trimester of pregnancy). The numerically lowest BW was reported at 3 years old, while the peak value was at 8 years old. As reviewed by Kadzere *et al.* (2002), the BW of cows was highly correlated to the heat production and dissipation. The higher BW could lead to more heat production and more difficulties in heat dissipation. A similar mechanism was also demonstrated by the results of this study. For cows with lower BW it was easier to dissipate heat load with night time cooling compared with the ones with higher BW. In addition, the heat tolerance of older and heavier cows might also been reduced by their sub-optimal behaviour pattern and welfare. Studies found increased rate of lameness with older Age and heavier BW (Andersson and Lundström, 1981; Wells *et al.*, 1993), as well as the interaction between lameness and heat stress (Cook *et al.*, 2007a). Therefore, management of the Age and BW of the herd to reduce the proportion of older (>10 years old) and heavier (>1000kg) cows would be able to enhance the heat tolerance of the whole herd. However, the standards to define these thresholds of Age and BW needed to be refined according to specific herd profile in different farms or regions.

Renquist *et al.* (2006) also reported the calving interval of younger cows was longer than older cows. This might explain the higher heat tolerance of younger cows, as they would enter the high-production level with lower frequency compared with older cows. The high-production level was highly related to the lactation days (DIM) as reported by the current study. The DMY increased from 25.4 kg/cow/day to 36.7 kg/cow/day during the first 40 days of lactation and kept an average of 32 kg/cow/day from 41 days to the end of lactation. Cows during the early stage of lactation experienced heat stress more readily compared with later stage (>40 days) cows. Studies had demonstrated that early lactation cows could easily experience a negative energy balance (NEB) of as their nutrients intake is insufficient to maintain the high production level (Rensis and Scaramuzzi, 2003). A decline of DMY in the early stage of lactation with MT higher than 39 ^{o}C was observed (-2.63 kg/MT), while the later stage reduction was -1.8 kg/MT (Table 4-3). However, the decline of DMY along with ambient temperature (Tmin a Tmin) during later lactation was higher than the early stage. This was possibly caused by the production of early lactation cows relied on body stores for a portion of the nutrients, whereas the later lactation cows had to rely more on the nutrients intake (West, 1999). The lag-effect of heat stress during the early stage of lactation could than exacerbate the heat stress in a later stage, even the later stage had lower production level. Therefore, mitigation of heat stress during the early stage of lactation provided more benefit than the later stage. With different mitigation approaches, cooling the cows during first 60 lactation days was found to increase 7 – 8 kg/cow/day of the DMY, whereas the cooling only increase 0.4 – 5 kg/cow/day DMY after 120 days of lactation (Avendano-Reyes *et al.*, 2006; Urdaz *et al.*, 2006; Do Amaral *et al.*, 2008; Do Amaral *et al.*, 2009; Adin *et al.*, 2009; Tao *et al.*, 2011; Ortiz *et al.*, 2015b; Frazzi *et al.*, 2000; Kendall *et al.*, 2006).

The MT was measured as body temperature in this study. It had been demonstrated previously that a reliable correlation between MT and vaginal temperature (VT) exists when predicting fever in cows (Pohl *et al.*, 2014). The threshold of 39 ^{o}C of MT and 39.5 ^{o}C of VT were used to identify the fever of cows performing 0.65 Sensitivity and 0.65 specificities. Moreover, the production loss (DMY decline) with MT higher than 39^{o}C was similar to the rectal temperature (RT) higher than 38.9^{o}C as reported by Johnson and Ragsdale (1963). Therefore, MT could be used to detect body temperature instead of VT and RT monitoring, especially within AMS systems, as VT and RT monitoring is labour intensive, costly and could put extra stress on the animals during the measurement in traditional dairy farming systems (Hale *et al.*, 2003).

Previous studies attempting to define the levels of heat stress only relied on one parameter, such as body temperature or production performance (Eigenberg *et al.*, 2005b; Silva *et al.*, 2007; Hammami *et al.*, 2013). However, one parameter might not be sensitive enough to indicate different levels of heat stress. For example, in this study, the thresholds of MT under critical heat stress (Tmin: 15 ^{o}C and Tmean: 19 ^{o}C) were higher than the thresholds of DMY (Tmin: 14 ^{o}C and Tmean: 16 ^{o}C). Therefore, implementing mitigations techniques based on thresholds of MT might come too late for the cows that are already under considerable heat stress. To solve this problem, levels were re-defined in this study focusing on the changes in internal body temperature and the production performance, as shown in Table 4-7.

Table 4-7 Thresholds of Tmin and Tmean entering different levels of heat stress, for the whole herd

Levels	Tmin (^{o}C)	Tmean (^{o}C)
No stress	<5	<9
Innocuous	5 to 6	9 to 11
Effective	6 to 14	11 to 16
Critical	>14	>16

A selection of thresholds for specific groups within the herd was formulated by using a decision tree. The overall accuracy was 94% for Tmin and 79% for Tmean. The model presented in this manuscript was built based on a very large dataset acquired from a single farm. Therefore, while these results are significant and potentially useful for the dairy industry generally; applying the model for other farms or regions still require more evaluations and modifications. However, it would be relatively easy to generate new thresholds to optimize mitigation procedures according to the profile of specific herds. The mitigations could be optimized via providing more effective, timely cooling and more accurate nutrient management.

4.5. Conclusions

This study performed a modification to the thresholds of heat stress in relation to production factors (Age, BW and DIM) of the cows. A negative correlation was found between heat tolerance, Age and BW. Influence of heat stress during early lactation (DIM<40 days) should be managed on farms carefully, as the effects could last for the rest of lactation period. For the whole herd, the thresholds of heat stress based on internal body temperature and production performance was established for Tmin and Tmean. Decision tree model was built to make a selection of the specific thresholds based on the production factors. The model had overall 94% accuracy with the thresholds of Tmin, and 79% accuracy with the thresholds of Tmean.

Chapter 5. **Modelling of heat stress in a robotic dairy farm. Part 3: animal behaviour and milking performance**

5.1. Abstract

The application of robotic milking system (RMS) has been demonstrated to reduce labour requirements for farmers. The performance of RMS is highly depend on the milking frequency and milking speed, which can vary with different production factors on farms such as the traffic system, thermal comfort and health of cows. Apart from saving labour cost, the RMS also collects a series of data in relation to animal health, welfare and production performance, which could indicate the status of individual animal. However the usage of these datasets is still insufficient. In this study, the dataset provided by RMS was applied to analyse the influence of heat stress on an important behaviour, such as rumination. The farm selected by this study applied semi-free traffic with automated drafting gates to control the milking behaviour of cows. The indicators of animal responses coping with heat stress were detected as rumination time (RT), milk temperature (MT) and daily milk yield (DMY). In addition, the performance of RMS (milking behaviour) were also monitored, including the time of milking (TM), milking frequency (MF), milking duration (MD), milking speed (MS), and milk yield per milking (MY). A new index (rumination efficiency index, REI) was defined as ratio between DMY and RT, which was created to evaluate the efficiency of rumination under heat stress. By using the multiple broken-line regression, it was found that 1^{o}C raising of daily mean temperature could reduce 5.12 minutes of RT, decrease 0.07 kg/cow/hour of REI, and increase 1% of low efficiency milking (milking with MS below 1kg/min or MY below 10kg/milking). Moreover, the study also found cows prefer to milk between 7:00AM to 9:00AM, and 86% of their milking event happened during this time of period. No significant correlation was found between heat stress and the pattern of milking behaviour (such as visiting time pattern). However, delaying the first milking event of the day and controlling milking interval (less than 4 hours) was found to be beneficial for REI and robotic milking performance.

5.2. Introduction

Robotic milking systems (RMS) have been widely applied in modern dairy farms, especially in Europe. One important motivation for installing RMS is labour saving for farmers. Field surveys reported, that the labour saving of RMS farms ranged from 18% to 30% when compared to conventional milking systems (CMS) (Rodenburg, 2012). However, Rodenburg (2012) also pointed out the challenges associated with RMS, including the complexity associated with sorting or restraining cows for individual handling. For example, animals during traditional milking can be inspected and treated individually, while these opportunities for animal handling is not present in RMS, due to the nature of the system. Although, automatic sorting gates (often used in conjunction with RMS systems) can be potentially programmed to separate individual animal from the herd, frequent interaction with farm workers is normally reduced in RMS systems and thus opportunities for regular inspections. Studies also reported that the milking frequency, milking speed and production level of RMS could vary widely depending on cow traffic arrangements (e.g. forced vs. free) , temperature changes, and other operational factors (e.g. stage of lactation and feeding) (Ketelaar-de Lauwere *et al.*, 2000; Hermans *et al.*, 2003; Speroni *et al.*, 2003; Svennersten-Sjaunja *et al.*, 2000; Rodenburg and Wheeler, 2002).

The impact of milking system on cow health and comfort is another consideration when evaluating RMS. It has been found that cows that are not disturbed frequently in RMS avoid the test person less frequently than cows in CMS (Rousing 2005), which implied the cows might have better and longer resting times (Rodenburg, 2012). The health parameters including fertility, metabolism, body condition, lameness frequency and udder health reportedly did not change significantly due to installation of RMS in a dairy farm (Hillerton *et al.*, 2004). The stress levels during milking are also reported to be similar on RMS or CMS farms, while a higher stress level was detected during milking on a RMS farm, which was detected by higher milk cortisol concentrations (Hagen *et al.*, 2005).

Heat stress is well known to cause reduction in animal welfare and production performance generally (West, 2003; Zimbelman, 2007). When dealing with heat stress, a study found that RMS could exacerbate heat stress of primiparous cows (Speroni *et al.*, 2006). However, the same study also found that RMS can increase milk production during heat stress in multiparous cows. Studies also found that providing cooling facilities in the milking station of RMS could increase the visits of cows in the hot season, thus raise the utilization of milking robots (John *et al.*, 2016).

In comparison with most CMS systems, the RMS system has an advantage of providing more specific data related to animal health, welfare and production performance. In essence, RMS systems can generate significant amounts of useful information in relation to animal responses, in addition to saving labour during milking for the farmers. High frequency and long-term data are always readily available by RMS. Thus, routine detection of health problems (e.g. mastitis) might be offered to farmers, if data could be systematically collected and analysed in RMS (LeBlanc, 2016). However, the selection of key indicators within the data stream and modelling of these indicators are still problematic and had limited success such as lameness detection (Pastell and Madsen, 2008).

The data stream provided by the RMS can be also used for the analysis of the heat stress impact. For example, rumination time (RT) is one of the key indicators of the heat stress which can be recorded by the RMS system for an individual cow. Moallem *et al.* (2010) indicated that the heat stress (high temperature-humidity index, THI) could depress RT. The depressed RT reduced feed intake and in turn decreased milk production. When the maximum THI was higher than 76, nearly 2.2 min reduction of RT per daily maximum THI unit was reported by Soriani *et al.* (2013).

Therefore, this study was implemented to analyse an RMS database obtained of a dairy farm in Queensland, Australia over five-year period. The primary indicators of heat stress were daily rumination time, milking frequency, and milking speed of an individual cow. Moreover, the time (at hourly frequency) of each milking visit of each cow were recorded and involved in the analysis. The overall aim of this study was to identify the influence of heat stress on both animal response and milking performance in an RMS and to provide recommendations for RMS operations to mitigate heat stress. Finally, an estimation of financial benefit will be given for the adjusted RMS operations on this dairy farm. It was hoped that the results of this study will improve milking efficiency in RMS and reduce the negative impact of heath stress in these systems.

5.3. Methodology

Data collection

- ### Farm, animal and climate

The field measurement was undertaken in an RMS dairy farm located in Gatton, Queensland, Australia. As described in previous papers of this series (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b),the farm had three milking robots (LELY Astronaut, Lely Industries NV, Maassluis, the Netherlands) that were connected to a herd management software system (LELY

T4C, Lely Industries NV, Maassluis, the Netherlands). From June 2013 to November 2017, the herd management system recorded the data of production variables (e.g. daily milk yield, DMY) for the individual cow, which was made available to the researchers working on this study. Approximately 160 Holstein dairy cows were held on the farm during this period and the different variables predetermined by the system were recorded routinely. Variables related to the climate condition of the farm were collected from a local weather station, approximately 8km away from the farm.

- **Measurement of rumination time**

Rumination time was selected as an indicator for heat stress in this study to detect the health and welfare status of dairy cows. This variable was measured by using tag monitor (Lely Qwes-HR, Lely Industries BV, Maassluis, the Netherlands) as shown in Figure 5-1. The monitoring device was attached to the upper part of the neck as part of the routine management procedure of the farm. The system was part of the commercially available Lely product range. A straps were arranged in a way to maximise animal welfare, prevent false movements and mechanical damage. The monitor contained an acceleration sensor, tuned rumination microphone, a microprocessor and memory. Rumination time was calculated based on the recording of the general activity index, as well as the vocal signals. The rumination monitor used in this study was validated by previous study (Lindgren, 2009). The monitor recorded rumination time of the cow in blocks of 2 hours. Wireless transmission using infrared communication was invoked when a cow was moving close to the receiver installed in the milking station. After each visit, the daily rumination time was updated in the herd management system and was made available for download.

Figure 5-1 Positioning of the collar used for measuring rumination time (photo by Ji, 2017)

- **Measurement of milking behaviour**

The RMS study farm had free traffic between feeding and resting area, as well as semi-free access to the milking station, as shown in Figure 5-2. Water troughs were positioned only in the milking station and passageway leading to the feeding area, which encouraged the animals to move to the milking area. The semi-free access provided to the milking stations (controlled by the drafting gate) was to enhance the milking efficiency of animals and milking robots via normalising the frequency of the visits by the same cow. For example, if a cow visited the robot with very high frequency (e.g. ten times per day), the efficiency of the milking robot the resting time available for that animal would decline, resulting in further decrease of production efficiency. Therefore, the herd management system controlled access to the milking station to prevent high frequency visits by the same animal. This strategy is routinely used in commercially available robotic milking systems. On the study farm RMS, the cows were only allowed to visit the robots every 5 hours if their milk yield reached the threshold level (e.g.

15kg/milking). The variables describing milking behaviour of the individual cow were recorded by the herd management system and included the time of visit, milking duration, milking speed, milk yield per visit, milking frequency and the number of cows milked per day.

Figure 5-2 Layout of farm traffic. The letter “1” indicates the passageway toward milking station; the letter “G” indicates the drafting gate to control animal access to milking station; the letter “2” indicates the passageway of rejected cows, and the letter “3” indicates the general passageway of existing milking station. No limitation of access or forced movement between feeding and resting area. Adapted form google map (https://www.google.com.au/maps/@-27.5045056,152.4202448,143m/data=!3m1!1e3)

Data processing and statistical analysis

The dataset obtained in this study included age, body weight (BW), days in milk (DIM), daily milking frequency (MF), time, milking duration (MD), milking speed (MS), milk yield (MY) and milk temperature (MT) of individual milking visit of 126 lactating cows for a 5-year period. The whole dataset about animals contained 249489 rows of test day’s results. By excluding the noise data, which was higher or lower than the mean value plus or minus three times the standard deviation, 228087 rows of data remained for the animal. In addition, the climate data included daily maximum, minimum, and mean of dry bulb temperature (Tdb) from the local weather station. The single correlation test was performed between different variables to obtain a basic analysis of their interactions before further modelling. Table 5-1 lists the abbreviation and definition of all collected variables.

Table 5-1 List of variables in data collection

Category	**Name**	**Unit**	**Definition**
Animal status and production	Age	Years	Age of individual animal
	BW	Kg	Body weight of individual animal
	DIM	Days	Days in milk, or lactation days
	DMY	Kg/cow/day	Daily milk production of individual animal
	MT	oC	Milk temeprature when milking the animal
	MF	Times	Frequency of milking visit per day
	MD	Minutes	Duration of robotic milking per visit

Robotic milking performance	MS	Kg/min	Milking speed of each robotic milking
	MY	Kg/cow/visit	Milk production of each robotic milking
	TM	Hour in day	The time of robotic milking
	LEM	%	Proportion of robotic milking with low efficiency in daily robotic milking (MS < 1kg/min or MY < 10kg/milking)
Environment	Tmin	oC	Daily minimum temperature, represent the night time cooling
	Tmax	oC	Daily minimum temperature, represent the maximum heat stress
	Tmean	oC	Daily average temperature, represent the all day thermal condition

- ## Analysis of temporal patterns

As the first step, the dataset was transformed into different time frames to analyse the diurnal, monthly or seasonal changes or distributions of the previously mentioned variables. The statistically significant differences were tested using a t-test. Duncan's multiple range test was applied to detect the statistically the different variables with different time patterns.

- ## Rumination efficiency index (REI)

Previous studies have confirmed a decline in rumination time due to heat stress (e.g. Soriani *et al.* (2013). The insufficient rumination of cows can cause sub-optimal metabolic conditions and ultimately reduce the milk production. In this paper, a new index is developed to provide an integrated indicator of heat stress based on rumination time. The index formulated as follows:

$$\boldsymbol{REI = \frac{DMY}{RT}}$$

The "Rumination efficiency index" (REI) is designed to evaluate the efficiency of rumination as the amount of milk produced in relation to animal rumination per hour (kg/cow/hour). RMS Low values of this index is assumed to indicate animal health/welfare problems due to heat stress, because animals might be unable to produce sufficient amounts of milk even with enough rumination time. The evaluation of heat stress was performed using the dataset of the individual animal. Single and multiple linear regression with multi-phases segments were applied for the analysis following the model below:

$$\boldsymbol{y = \begin{cases} a_1 + b_1 x_1, & (x_1 \leq s_1) \\ a_2 + b_2 x_1, & (s_1 < x_1) \\ & \dots \\ a_n + b_n x_1, & (s_{n-1} < x_1 \leq s_n) \end{cases} + \sum_{j=1,k=2}^{j=3,k=4} c_j x_k + \varepsilon}$$

Where y is the dependent variable representing the animal responses and the performance of robotic milking, x is the independent variables including thermal condition and animal production factors, x_1 is Tdb. More specific description of this method was presented in the previous papers of this series (Ji *et al.*, Submitted-b). The threshold value of Tdb with a significant decline of rumination time and REI was identified within this model.

- ## Impact of heat stress on robotic milking performance

The indicators of the performance of robotic milking stations were selected as milking frequency, milking speed, time of milking, and a number of animals milked. The relationship between these performance indicators and heat stress was analysed using general linear regression.

All data processing and statistical analysis were done using R 3.4.3. (R Development Core Team, 2017). The analysis of multi-phase linear regression and general linear regression was done by using the same functions and packages introduced by Ji *et al.* (Submitted-b). The Duncan's multiple range test was taken by using the package "Agricola" (Mendiburu Delgado, 2009).

Estimation of financial benefit for the adjusted RMS

The potential income increase associated with individual milk production and saving of energy cost were estimated. The daily income of milk production was calculated by multiplying the DMY, number of cows and the local milk price (Gresham, 2018). The energy cost was estimated as the product of MF, LEM, number of cows, energy cost of each robotic milking (Calcante *et al.*, 2016), and the local energy price (O'Neill, 2018).

5.4. Results

Description of the dataset

The basic description of the variables used in this paper is listed in Table1. For the period between June 2013 and November 2017, the mean of ambient temperature ranged from 13.1 to 27.8 ^oC. The mean values of BW, Age and DIM were 828.9 kg, 3.7 years old and 136.7 days in milk, respectively. With the RMS, cows preferred to be milked around 08:20 AM with about 2.8 times of milking per day. The robotic milking averagely took 287.2 seconds for each milking including 115.6 seconds for positioning and cleaning the teats. The average milking speed was 2.9 kg/min, with DMY between 18.4 and 39.4 kg/cow/day. The cows required 255 to 535 minutes for daily rumination, and their milk temperature was around 39.1 ^oC.

Table 5-2 Summary of descriptive statistics

Category	Var. [a]	Unit	min	max	mean	std.dev
Thermal condition	Tmin	^oC	-1.6	27.3	13.1	5.6
	Tmax	^oC	13.5	45.7	27.8	5.2
	Tmean	^oC	5.2	30.7	17.5	5.0
Production factors	BW	Kg	379.0	1395.0	828.9	170.0
	DIM	Days	1.0	364.0	136.7	89.7
	Age	Years	1.1	11.1	3.7	1.8
Robotic milking	TM	Hour in day	0.0	23.0	8.2	3.4
	MF	Times	1.0	6.0	2.8	0.9
	MD	Seconds	85.0	746.3	287.2	96.5
	TD	Seconds	62.0	575.7	115.6	33.1
	MS	Kg/min	0.0	9.8	2.9	1.1
Behaviour, Health & Production performance	RT	Minutes	255.0	535.0	401.5	47.3
	MT	^oC	28.3	42.5	39.1	0.8
	DMY	Kg/cow/day	18.4	39.4	29.5	3.8

[a] Definition of the variables – Tmin/max.mean: daily minimum/maximum/mean temperature; BW: body weight; DIM: days in milk; Age: age of cow; TM: time of milking; MF: milking frequency; MD: milking duration; TD: treatment duration; MS: milking speed; RT: rumination time; MT: milk temperature; DMY: daily milk yield.

The monthly patterns of thermal environment, animal behaviour and production performance indexes are plotted in Figure 5-3. The patterns of MT and Tmean had a similar trend. However, MT reached the highest value (in March) with one month lag compared to the Tmean (in February). After the hot season (from December to February), the DMY increased from March to August with decreasing Tmean and MT. The highest DMY was achieved between August and October with increasing Tmean and MT from June to August. In Figure 5-3 [b] and [c], the DIM of the herd was lower between April and June compared to the other months, which suggested the start of lactation (early lactation) during this period. The cows required more rumination (RT) and more milking (MF) in their early lactation period. The efficiency of rumination (REI) was also increased during this time, with the highest REI observed in September after cold season. The speed of milking (MS) decreased in June and July and returned to the highest value on September, which might be related to the changing of both lactation status and the thermal condition. The patterns of RT, MF and MS all decreased to the lowest value during the hot season, which indicated the influence of heat stress. However, the REI decreased to its lowest value with 2 months after the hot season (on April), which was partially correlated to the early lactation (lower DIM on April). However, it might also indicate the lag effect of heat stress, which requires more analysis to distinguish between these two influences. In Figure 5-3 [d] the total number of daily milking behaviours declined to the lowest number in February and started increasing from March to June. A slight decrease has happened during July, August and September, which might be caused by the discomfort of cold stress. However, the ratio of low-efficiency milking (LEM: robotic milking with the speed lower than 1kg/min or milk yield lower than 10kg/milking) was declined after the hot season and kept at a constant level during the cold season.

The diurnal patterns of robotic milking performance were shown in Figure 5-4 and Table 5-3. In Figure 5-4 [a], the MS was higher than 3.1 kg/min after 11:00 AM and the milk yield per milking (MY) reached to the peak (18.5 kg/milking) at night time (19:00 PM). However, the Figure 5-4 [b] implied the 80% of the daily milking was finished between 04:00 AM and 10:00 AM. Although the LEM decreased from 70% to 10% during the early morning, most of the milking was finished before the cows achieved the highest MS and MY as shown in Figure 5-4 [a]. As the average MF was about 2.8 times per day, the performance of the first, second and third robotic milking was summarised in Table 5-3. The MS and MY were higher with later milking, therefore, even the number of third milking only accounted for 12% of the total milking behaviour of the day, the production accounted for 24% of the daily milk production.

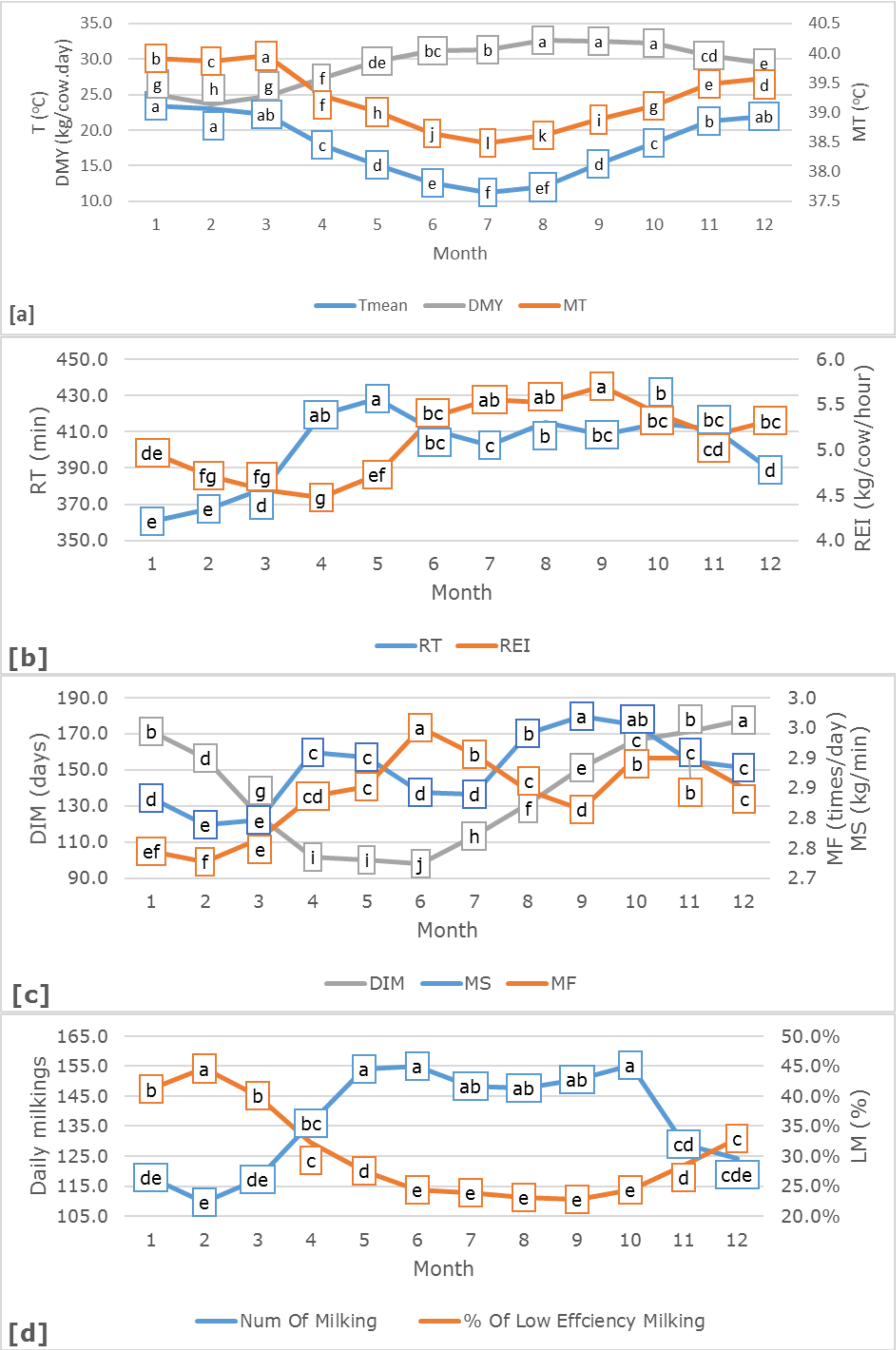
T (ºC)
DMY (kg/cow.day)
MT (ºC)
Month
Tmean
DMY
MT
[a]
RT (min)
REI (kg/cow/hour)
Month
RT
REI
[b]
DIM (days)
MF (times/day)
MS (kg/min)
Month
DIM
MS
MF
[c]
Daily milkings
LM (%)
Month
Num Of Milking
% Of Low Effciency Milking
[d]

Figure 5-3 Monthly patterns of variables. [a] – the pattern of DMY, Tmean and MT; [b] – the pattern of RT and REI; [c] – the pattern of DIM, MF and MS; [d] – the pattern of daily milking and low-efficiency milking. LEM - Percentage of low-efficiency milking among the daily total milking, the low-efficiency milking is defined with milking speed lower than 1 kg/min or milk yield per visit lower than 10kg/visit.

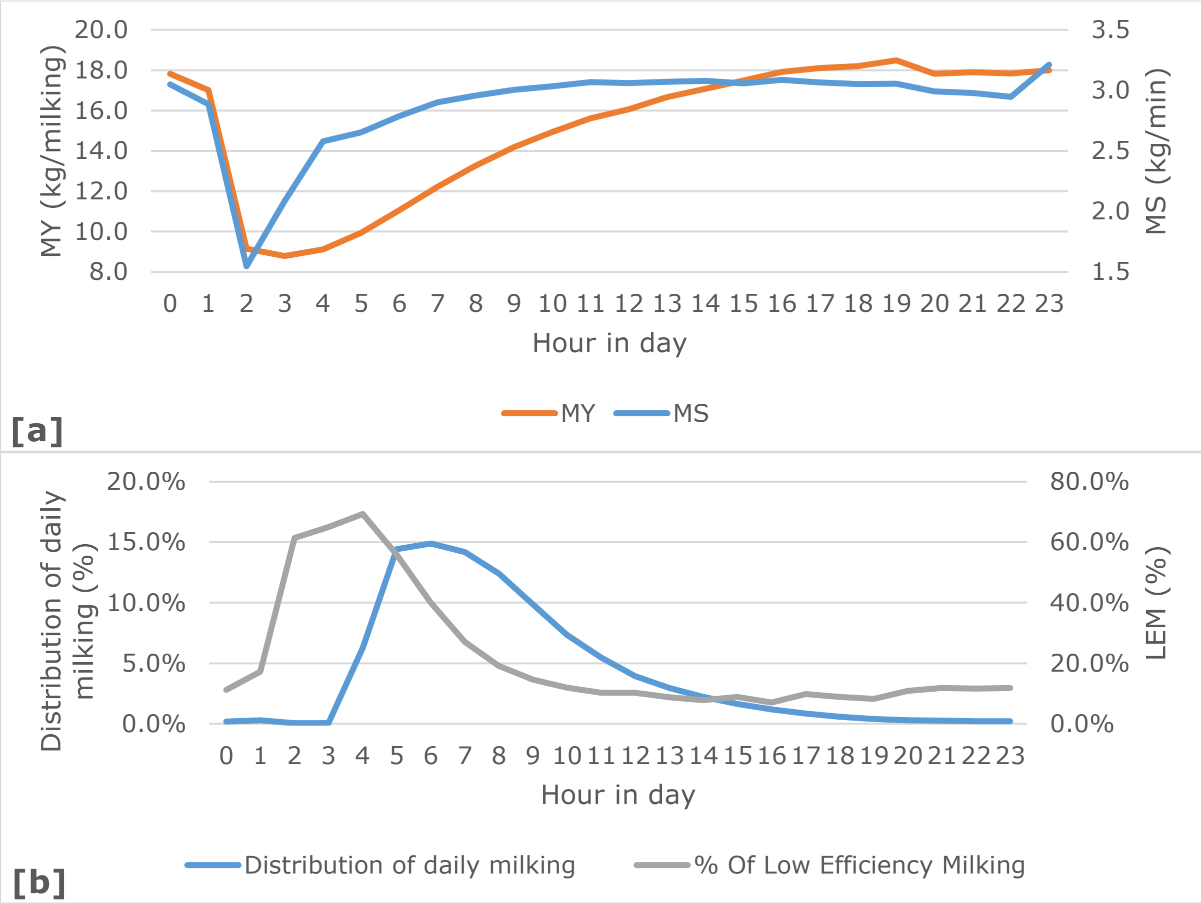

Figure 5-4 Diurnal patterns of robotic milking performance. [a] – pattern of MY and MS; [b] – a pattern of milking and low-efficiency milking. The low-efficiency milking is defined with milking speed lower than 1 kg/min or milk yield per visit lower than 10kg/visit. LEM - Percentage of low-efficiency milking among the daily total milking, the low-efficiency milking is defined with milking speed lower than 1 kg/min or milk yield per visit lower than 10kg/visit.

Table 5-3 Average performance of first, second and third robotic milking

Var.	MS	MY	TM	The proportion in daily milking event	The proportion in daily milk production
Unit	Kg/min	Kg	Time	%	%
1st	2.8	11.3	06:42 AM	38	29
2nd	2.9	13.4	08:48 AM	36	32
3rd	3.0	14.4	09:12 AM	12	24

Regression analysis

Table 5-4 shows the results of two phases segmented single linear regression between thermal condition, animal behaviours and robotic milking performance. These regressions also identified the breakpoints (thresholds) after which the influence of thermal condition (slope) would be significant. The heat stress significantly reduced the rumination time of cows by 6.5-9.5 min/^{o}C when Tmin was higher than 14^{o}C or Tmean was higher than 19^{o}C. Moreover, the efficiency of rumination (REI) started to decline with lower Tmin and Tmean (6 and 8 ^{o}C,

respectively). The performance of robotic milking also decreased under heat stress. The MS of robotic milking was reduced by 0.01-0.07 kg/min/Tmean with Tmin higher than 17°C or Tmean higher than 28°C. The percentage of low-efficiency milking (LEM) was increased with 2.3-2.4%/Tmean when Tmin exceeded 12°C or Tmean exceeded 16°C.
However, the thermal condition had no significant impacts on MF and TM. This indicated the cows would not adjust their milking pattern for coping with heat stress. However, the interval between every two milking (MI) decreased with higher Tmin and Tmean. It possibly indicated the cows concentrated their milking in the early morning under heat stress. Therefore, they could have less movement and more rest during the hot day-time. Moreover, as the water troughs were only provided on the way to the milking station, and cows required more drink of water under heat stress, which increased the ratio of their visiting to the drafting gate. As the performance of each robotic milking was reduced, the herd management system (drafting gate) allowed more access of cows to the milking robot when they were moving for water. This also decreased the interval between every two milking.

Table 5-4 Summary of two phases segmented single linear regression between thermal condition, animal behaviours and robotic milking performance

	Unit	Tmin				Tmean			
		Threshold	Slope	Sign.[b]	R^2	Thr	Slope	Sign.	R^2
RT	min	14	-6.49	***	0.35	19	-9.45	***	0.52
REI	Kg/cow/hour	6	-0.06	**	0.19	8	-0.04	**	0.14
MS	Kg/min	17	-0.01	*	0.08	28	-0.07	*	0.04
LEM	%	12	2.32	***	0.77	16	2.41	***	0.74
MF	Times	NS				NS			
TM	Hour in day	NS				NS			
MI[a]	Hours	0	-0.01	*	0.06	5	-0.01	*	0.09

a – Milking interval: the time interval between every two milking of the cow

b – Levels of significant: "***" – P<0.001, "**" – P<0.01, "*" – P<0.05, NS – Nonsignificant

The simple correlation coefficients between the variables representing production factor, animal behaviour and robotic milking performance are listed in Table 5-5. It is still difficult to generate highly reliable conclusions of these correlations only depending on simple correlation, as they had interactions. However, some preliminary conclusions in relation to the change of RT, REI, robotic milking (TM, MF, LEM, MS, and MI) and DMY can be found as follows.

RT: The cows with heavier BW had more RT (0.03min/BW), while the increase of DIM and Age reduced the RT (-0.19min/DIM and -9.95min/Age). Moreover, the later milking or two milking with a long interval (higher TM and MI) could also reduce the RT of cows (-2.54min/TM and -2.95min/MI).

REI: By analysis the correlation between these variables and REI, heavier cows (larger BW) had lower REI (-0.0034kg/cow/hour/BW), while the later milking and long interval (higher TM and MI) could increase the REI of cows (0.06kg/cow/hour/TM and 0.30kg/cow/hour/MI). In addition, the REI was also declined with a higher frequency of milking (-0.27kg/cow/hour/MF).

Robotic milking: The higher MF increased the LEM (6.08%/MF) and decreased the MS (-0.09kg/min/MF), which lead to low robotic milking efficiency. In contrast, enough RT or higher REI could reduce the ratio of LEM (-0.05%/RT and -3.27%/REI). It was also found the later milking or longer interval (higher TM and MI) were able to reduce the LEM (2.96%/TM and -1.50%/MI). The positive correlation between MF and RT (0.0007 times/RT) indicating sufficient rumination could guarantee the cows to be milked with more times per day. The negative correlation between TM and MF (-2.46hour/MF) implied the cows which took more milking per day started their milking behaviour earlier than the others.

DMY: The DMY had positive correlations with RT and MF (0.02kg/cow/day/RT and 4.79kg/cow/day/MF), and negative correlations with TM and MI (-1.20kg/cow/day/TM and -1.11kg/cow/day/MI). Therefore, to provide better milk production from the cows, the robotic milking should be concentrated and finished during the early morning (TM) with lower interval (MI) and higher frequency (MF), which could allow the cows to have enough rumination time (RT) for the rest of the day.

Table 5-5 Correlation matrix between production factors, animal behaviours and robotic milking performance
(Levels of significant: "***" – P<0.001, "**" – P<0.01, "*" – P<0.05. X – Independent variable. Y – Dependent variable)

Var.			**Y**										
			DIM	BW	Age	RT	REI	LEM	MF	MS	TM	MI	DMY
			Days	Kg	Years	min	Kg/cow/hour	%	Times	Kg/min	Hour in day	Hours	Kg/cow/day
X	DIM	Days	1	0.1859 ***	0.0023 ***	-0.1889 ***	-0.0104 ***	1.6232 ***	-0.0209 ***	0.0001 ***	0.0062 ***	0.0035 ***	-0.0436 ***
	BW	Kg	-	1	-	0.0360 ***	-0.0034 ***	-0.0341 ***	0.0001 ***	0.0001 ***	0.0001 *	-0.0003 ***	0.0127 ***
	Age	Years	-	29.8552 ***	1	-9.9476 ***	0.3194 ***	-11.527 ***	-0.0216 ***	-0.0176 ***	0.0459 ***	0.0326 ***	1.2427 ***
	RT	min	-	-	-	1	-	-0.0544 ***	0.0007 ***	0.0007 ***	-	-	0.0208 ***
	REI	Kg/cow/hour	-	-	-	-	1	-3.2681 ***	-	0.0174 ***	-	-	-
	LEM	%	-	-	-	-	-	1	-	-	-	-	-
	MF	Times	-	-	-	58.8600 ***	-0.2673 **	6.0812 ***	1	-0.0941 ***	-2.4600 ***	-1.5854 ***	4.7861 ***
	MS	Kg/min	-	-	-	-	-	-	-	1	-	-	-
	TM	Hour in day	-	-	-	-2.5407 ***	0.0662 ***	-2.9631 ***	-	0.0213 ***	1	0.6026 ***	-1.1992 ***
	MI	Hours	-	-	-	-2.9450 ***	0.3009 ***	-1.4955 ***	-	0.0047 ***	-	1	-1.1067 ***
	DMY	Kg/cow/day	-	-	-	-	-	-	-	0.0152 ***	-	-	1

The interactions (positive or negative correlations) between part of these variables such as the correlation between RT and MI cannot be ignored. For practical reasons, it was still necessary to know which variables should be prioritised for management control under commercial farm conditions. In this case, multi-phases segmented multiple linear regression (**Model 1 to 9**) was applied to select the important variables with higher significant impacts as shown in Table 5-6. These models mainly have three categories, for the regression of RT (Model 1, 2 and 3), REI (Model 4, 5 and 6) and LEM (Model 7, 8 and 9). In each category, the difference are using Tmean, MT or MI as the segmented variable to formulate the regression.

The fitted values from Model 1 to 9 are displayed in Figure 5-5 and 5-6 along with the original dataset for the regression. In Figure 5-5 [a], with the increasing of Tmean or MT, RT increased to the maximum value of 446 min and then decreased to the minimum value of 252 min. In Figure 5-5 [b], the REI decreased from the initial maximum value of 7.2kg/cow/hour to the minimum value of 4.1kg/cow/hour. The increased REI after exceeding the threshold of Tmean (25°C) or MT (41°C) could not return to the initial level. LEM increased from nearly 15 to 45%, which represented a serious reduction of robotic milking performance caused by heat stress (Figure 5-5[c]). In Figure 5-4, within 4 hours of MI, the decrease of RT and LEM, as well as the increase of REI can be seen clearly. The data points are concentrated around the regression curve, however, with more than 4 hours of MI, the trend becomes inverse and the data points are divergent.

Models of RT: In Model 1, Tmean had a negative influence on RT when it was lower than 11°C or higher than 23°C, which partially indicated the thermal discomfort caused by cold and/or heat stress. The effects of cold stress (-0.27 min/Tmean) was less serious than the heat stress (-5.12min/Tmean). Older Age, higher MT, later TM_1, and longer MI were all leading to a decreased of RT. However, the influence of Age and MT had more significant influence than the influence of TM_1 and MI. In Model 2, the significant decline of RT happened (-61.05min/MT) when MT was higher than 40 °C. Age and TM_2 also had negative impacts on RT. However, the strongest significant influence was found between RT and TM_3. This implied a delay of third milking could possibly increase the RT of cows. In Model 3, the milking with an interval longer than 4 hours increased the RT, which partially agreed with the benefit of delaying the third milking in Model 2. However, the influence of MI in Model 3 was less significant than the influence of Age and DMY.

Models of REI: In Model 4, the efficiency of rumination (REI) reduced when Tmean was between 13 and 25°C showing the influence of heat stress (-0.07 kg/cow/hour/Tmean). The increase of REI (0.06kg/cow/hour/Tmean) when Tmean was higher than 25°C could be explained by the decrease of RT becoming more dramatic than the one of DMY. It indicated the heat stress became more serious than the previous two stages, as rumen health might be compromised by the heat stress. Moreover, the significant reduction of REI was also found with the increase of DIM, BW and MT (-0.09kg/cow/hour/DIM, -0.04kg/cow/hour/BW, and -0.47kg/cow/hour/MT). In Model 5, the increase of REI (2.01 kg/cow/hour/MT) was also found when MT was higher than 41 °C, which again indicated a higher drop rate of RT than DMY in this stage. The delay of second milking benefited REI (0.65kg/cow/hour/TM_2). However, in Model 6, this influence became negative (-0.84kg/cow/hour/TM_2), while the delay of first milking became positive (1.5kg/cow/hour/TM_1). As reported in Model 3, 4 hours was identified as a threshold point. The REI significantly decreased with MI longer than 4 hours. Moreover, for the influence of MI longer than 4 hours, the significant level in Model 4 was higher than the one in Model 1.

Models of LEM: Only two stages identified by the regression of LEM in Model 7, 8 and 9. According to Model 7 and 8, the percentage of low-efficiency milking was significantly increased with Tmean higher than 17 °C (1%/Tmean) or MT higher than 39 °C (10%/MT). In Model 9, the milking interval of 4 hours was also determined as the threshold. The LEM started to increase with 8%/MI. In Model 7 to 9, the delay of first and second milking was demonstrated with reduction of LEM, however, the delay of third milking increased the LEM. The regression analysis from Model 1 to 9 demonstrated the controlling of milking interval might enhance the performance of robotic milking by increasing the REI of cows and decreasing the LEM of robotic milking. To maximize the benefit, the interval between every two milking needs to be controlled for around 4 hours. Moreover, the regression also demonstrated that most of the influence on RT and LEM from the lactation stage (DIM) was not as significant as internal body temperature (MT) and other production or management factors (i.e. MI).

In addition, according to the regression of Model 1, 6, 7 and 9, adjusting the time of first milking to the later morning could reduce the RT (-10.06min/TM_1), but increase the REI (1.5kg/cow/hour/TM_1), and decrease the LEM (-3%/TM_1). Delaying the time of second milking could reduce both RT (-30.25min/TM_2, Model 2) and REI (-0.85kg/cow/hour/TM_2, Model 6). Therefore, to improve the herd management, delaying the first milking and reducing the interval between first and second milking (less than 4 hours) might enhance the cows' production and the robotic milking performance. As shown in Figure 5-7, the adjusted milking interval can potentially increase about $255 daily income of milk production and save about $135 daily cost of robotic milking with low efficiency.

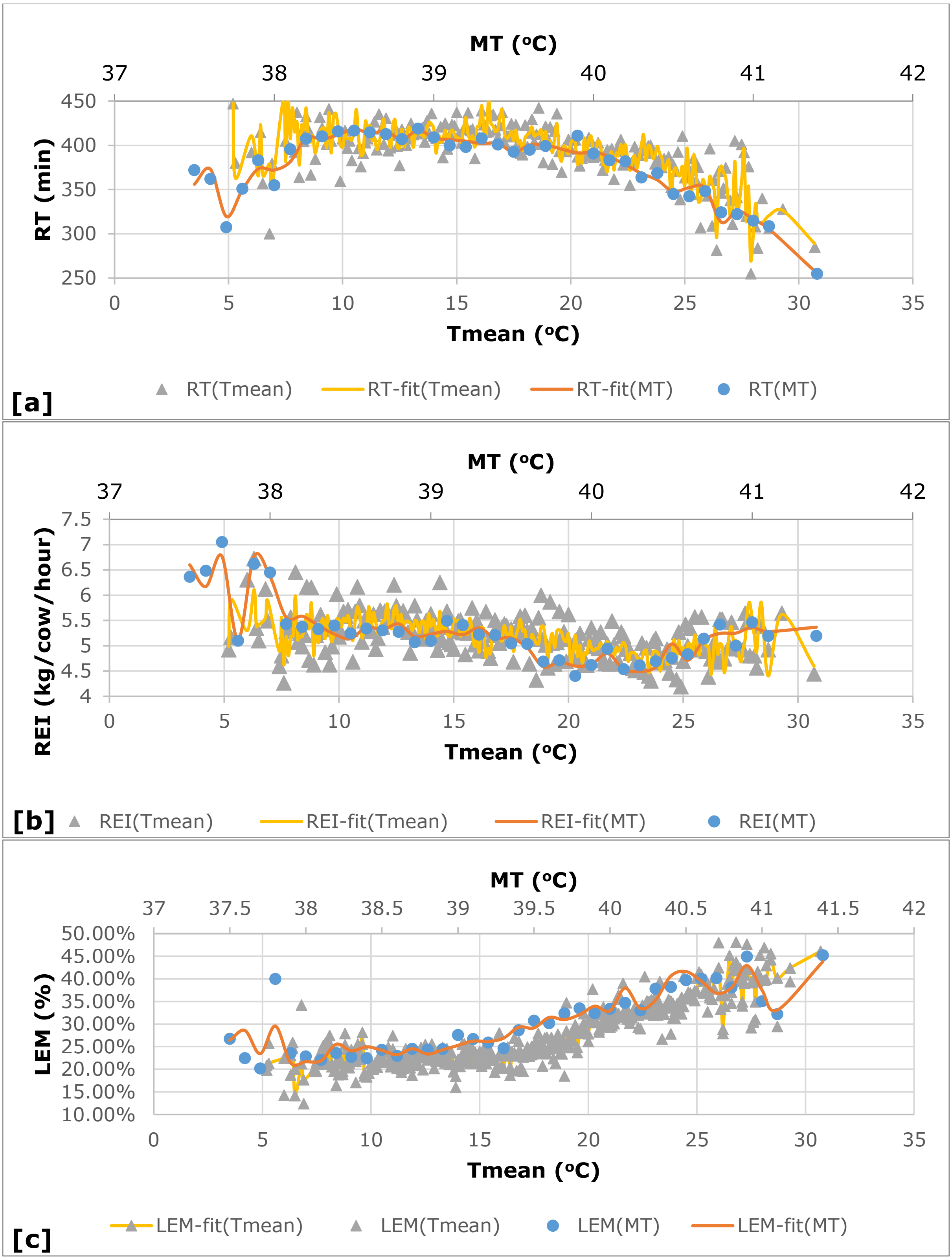

Figure 5-5 The multi-phases segmented regression of Tmean and MT. [a] – the regression for RT; [b] – the regression for REI; [c] – the regression for LEM.

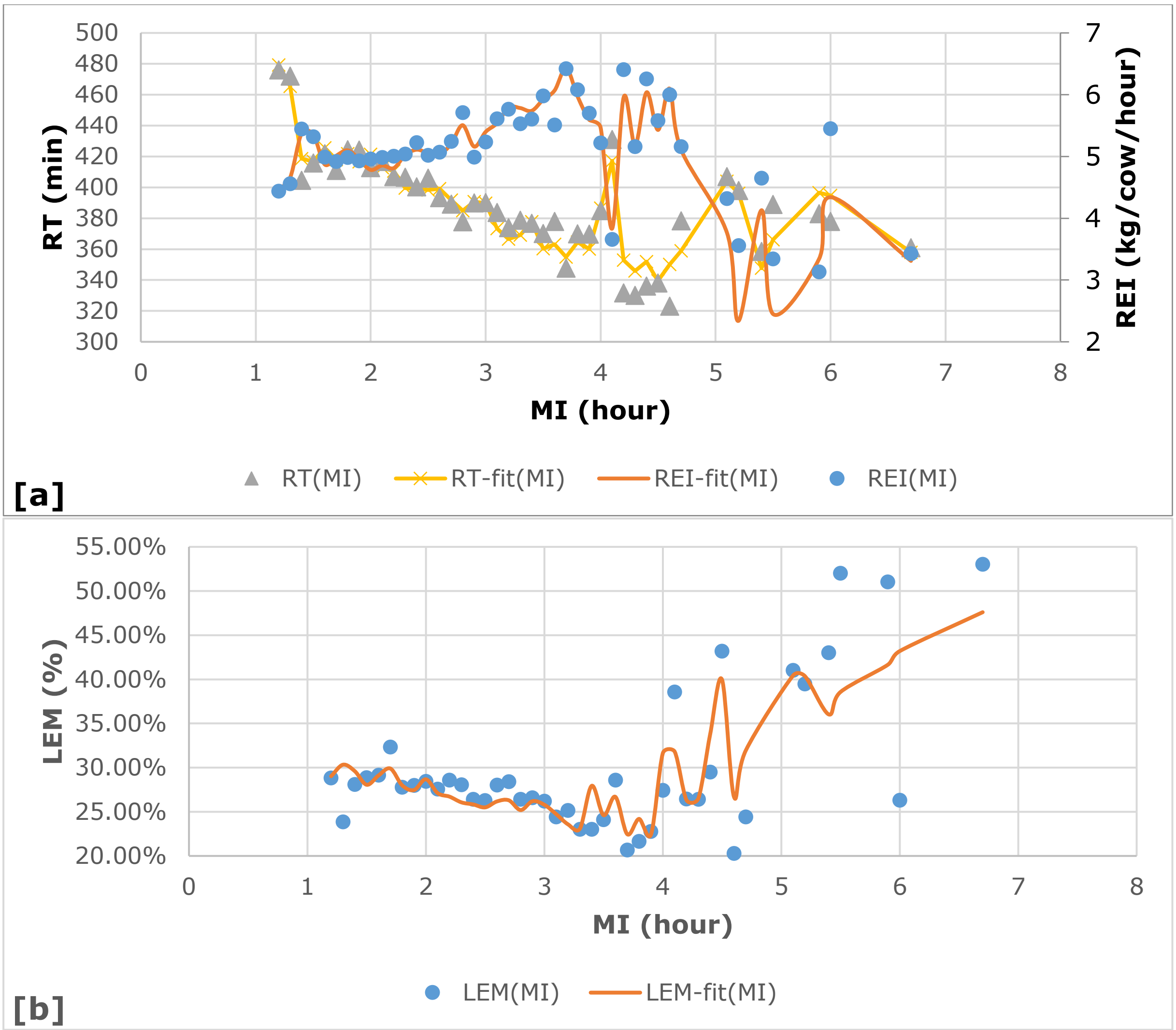

Figure 5-6 The multi-phases segmented regression of MI. [a] – the regression for RT and REI; [b] – the regression for LEM.

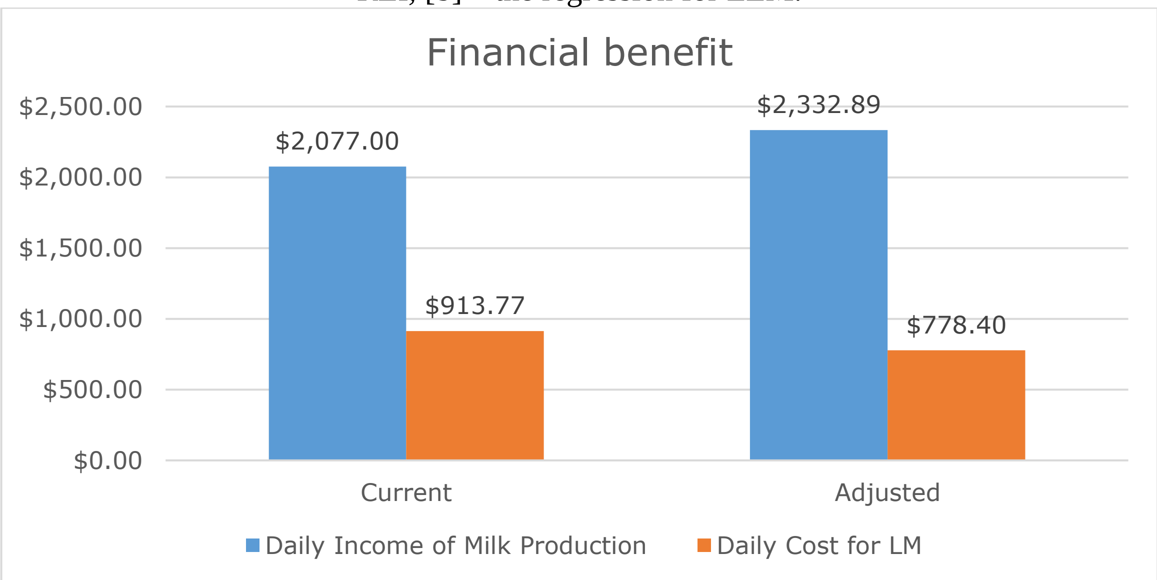

Figure 5-7 Estimation of potential financial benefit with adjusted milking interval. The estimation was fomulated assuming 126 cows on farm, 0.56 $/kg as the milk price, 34.26 kWh/milking as the energy cost and 0.28 $/kWh as the energy price.

Table 5-6 Result of multi-phases segmented regression for the analysis of RT, REI and LEM

RT													
Model 1[b]	THRs	Tmean	DIM	BW	Age	MF	DMY	MT	TM_1[b]	TM_2	TM_3	MI	R2
Slope	<11	-0.27	0.52	0.13	-66.39	NS	9.02	-27.73	-10.06	NS	NS	-12.26	0.77
	11 to 23	3.05											
	>23	-5.12											
Sig.[a]	***		***	***	***		***	***	**			**	
Model 2	THRs	MT	DIM	BW	Age	MF	DMY	MT	TM_1	TM_2	TM_3	MI	R2
Slope	<38	65.8	NS	NS	-69.74	NS	7.8	NS	NS	-30.25	43.55	NS	0.96
	38 to 40	14.49											
	>40	-61.05											
Sig.	**				*		**			**	***		
Model 3	THRs	MI	DIM	BW	Age	MF	DMY	Tmean	TM_1	TM_2	TM_3	MT	R2
Slope	<1	-501.69	NS	NS	-132.65	NS	9.59	NS	NS	NS	NS	NS	0.89
	1 to 4	-14.49											
	>4	9.92											
Sig.	*				**		**						
REI													
Model 4	THRs	Tmean	DIM	BW	Age	MF	MT	TM_1	TM_2	TM_3	MI		R2
Slope	<13	0	-0.09	-0.04	1.31	NS	-0.47	NS	NS	NS	0.29		0.48
	13 to 25	-0.07											
	>25	0.06											
Sig.[a]	***		***	***	***		**				***		
Model 5	THRs	MT	DIM	BW	Age	MF	MT	TM_1	TM_2	TM_3	MI		R2
Slope	<41	-0.24	-0.01	-0.01	2.47	NS	NS	NS	0.65	NS	NS		0.95
	>41	2.01											
Sig.	***		*	***	***				***				
Model 6	THRs	MI	DIM	BW	Age	MF	MT	TM_1	TM_2	TM_3	MT		R2
Slope	<4	1.63	-0.01	-0.01	3.61	NS	NS	1.5	-0.84	NS	NS		0.9
	>4	-2.14											
Sig.	***		***	***	**			***	***				
LEM													
Model 7	THRs	Tmean	DIM	BW	Age	MF	MT	TM_1	TM_2	TM_3	MI		R2
Slope	<17	0	0.0006	-0.02	NS	NS	NS	-3.21	-2.54	2.88	NS		0.81
	>17	1.02											

Sig.[a]	***		**	***				**	**	**		
Model 8	THRs	MT	DIM	BW	Age	MF	Tmean	TM_1	TM_2	TM_3	MI	R2
Slope	<39	0	NS	0.001	NS	NS	NS	NS	-6.75	NS	NS	0.87
	>39	10.23										
Sig.	***			*					***			
Model 9	THRs	MI	DIM	BW	Age	MF	Tmean	TM_1	TM_2	TM_3	MT	R2
Slope	<4	-1.01	NS	NS	NS	NS	0.01	-5.58	NS	2.01	NS	0.75
	>4	8.09										
Sig.	**						*	*		*		

[a] – Levels of significant: "***" – P<0.001, "**" – P<0.01, "*" – P<0.05, NS – Non-significant

[b] – TM_1 – the time of first milking, TM_2 – the time of second milking, TM_3 – the time of third milking,

Discussions on the key issues

As the routine monitoring of RT is readily available for farmers via commercially available instruments, previous researchers have used RT to predict the health and production performance of dairy cows. For example, Soriani *et al.* (2012) found that RT decreased to the minimum level at calving day for both primiparous (PR) and pluriparous (PL) dairy cows (from 463 to 263min in PR, and from 522 to 278min in PL). In their research, RT raised to 504min in PR and 562min in PL in early lactation days, which was found to be positively correlated with DMY. This study and Bar (2010) also reported the reduced RT before calving could result in a reduced RT after calving for the cows taking a greater ratio of calving disease (e.g. endometritis or ketosis). In this paper, DIM had a negative correlation with RT for the whole herd, as the average DIM for the herd was about 90days. However, a clear increase in RT from March to May observed (Figure 5-1) when the DIM was decreasing. It suggested that the early lactation caused a relatively higher RT as was reported by Soriani *et al.* (2012).

In the assessment of the heat stress, Soriani *et al.* (2013) demonstrated the negative correlation between RT and temperature humidity index (THI), which was -2.2min RT/THI. Moreover, it was found that nearly 60% of daily rumination happened during night-time when the heat stress was not as serious as day-time (-1.128 and -5.191min/ THI, for night-time and day-time, respectively)(Soriani *et al.*, 2012; Soriani *et al.*, 2013). Previous research has reported that the ambient temperature (Tmin or Tmean) can provide the same performance as THI for predicting heat stress in the sub-tropical region (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b). In the current study, the regression between RT and Tmin had a higher slope (-6.49min/Tmin) than the one for the regression between RT and Tmean (-9.45min/Tmean). As the proportion of rumination activities during day-time and night-time was unknown from the current study, it was still difficult to conclude that more cooling during day-time could increase the daily rumination, in comparison with more cooling during night-time. However, the regression analysis of this study still indicated that cows had less heat stress during night-time, therefore additional heat stress mitigation should be performed during the day-time, which is the routine mitigation strategy on farms anyhow.

Identifying the thresholds of different stages in heat stress were important for providing accurate mitigations. The regression of Model 1 in the current study identified 11°C of Tmean with RT starting to decrease significantly. This value was equal to the threshold of effective heat stress (with significant decreasing of DMY, -0.09kg/cow/day/Tmean) as reported by Ji *et al.* (Submitted-b). However, the significant increase of RT was found with Tmean between 11 and 23 °C (3.05min/Tmean). Therefore, the threshold identified by RT for predicting heat stress had inconsistency with the threshold identified by DMY. In this case, using the threshold of Tmean identified by REI in Model 4 could gain more consistency. The threshold was 13°C with a slope of -0.07kg/cow/hour/Tmean when exceeding. This was between the thresholds of effective and critical heat stress (11 to 16°C) as found by Ji *et al.* (Submitted-b), which implied another stage of heat stress when the cows' milk production started to decrease even with sufficient rumination time. This could be explained by their reduced ruminal digestion efficiency (Christopherson and Kennedy, 1983) or unwillingness to move and milk under heat stress (Klaas *et al.*, 2003). The RT started to decrease with -5.12min/Tmean when Tmean was higher than 23 °C. However, the decline of RT became more significant than the decline of DMY, as the REI started to increase with 0.06kg/cow/hour/Tmean when Tmean was higher than 25 °C. This might indicate compromised health and/or welfare of the animals, as the reduction of RT became irrelevant with the decline of the production performance (DMY). However, due to the time and financial limitations of this study, it was impossible to conduct physiological experiments on cows. Therefore, further studies are needed to understand the biochemical or metabolic mechanism driving these identified trends.

The positive correlation between RT and MF is shown in Table 5-5, which was 58.86 min more rumination time with 1-time increase of milking frequency. Österman and Redbo (2001) found the cows with 3 milking per day had more lying down rumination than the cows with 2 milking per day. This could be explained by the relief of udder pressure with higher MF, which enhanced the comfort of lying down, and increased the RT. However, without other behaviour monitors, we were unable to classify the specific behaviour (lying down, getting up, standing or moving) during rumination in the current study. As the first and second milking behaviour accounted for 74% of daily milking event and the herd could be considered as having 2 milking per day, thus more milking was recommended according to the positive correlation between RT and MF. In addition, the impact of MF on RT was insignificant when compared with the impact of thermal condition (e.g. Tmean) and other factors (e.g. DIM and BW) in Model 1-3. The MF and TM were also found with insignificant correlation with thermal condition (Table 5-4). This suggested that the cows could not adjust their milking behaviours (MF or TM) to cope with heat stress. Moreover, only adjusting the MF could not lead to an increase in RT under heat stress.

In RMS, cow traffic is a key factor to ensure a competitive efficiency of milking compared with RMS (John *et al.*, 2016). For semi-free with guided traffic system, cows had to pass through a selection unit (drafting gate) before entering the milking station or feeding area. The control of the milking interval (MI) was one of the important management strategies for optimizing the traffic system and milk production. However, the correlation between MI and production performance (e.g. DMY) had different values from different studies (Penry *et al.*, 2018; Hogeveen *et al.*, 2001; Mollenhorst *et al.*, 2011). The farm evaluated in this study applied a control algorithm that disallowed cows to revisit the milking stations within 5h interval if they have reached a certain milk yield pre-set by the system (e.g. 15 kg/milking for 2 milking per day). The milk yield was set based on their lactation status. Thus for cows in early lactation, higher milk yield would be set, resulting in more frequent milking permitted by the system. However, the result shown in Figure 5-2 and Table 5-3 implied the control of MI did not meet the expectation of five hours between two milking. It can be related to the low-efficiency milking (LEM) in early mornings, as most of the cows did not fulfil their estimated milk yield (nearly 60% LEM between 05:00 AM to 07:00 AM). The animals were allowed to revisit the milking robot several times within 5 hours in the morning. However, the analysis of Model 3, 6, and 9 found the MI between 1 and 4 hours could reduce the RT (-14.49min/MI), but increase the REI (1.63kg/cow/hour/MI) and decrease the LEM (-1.01%/MI). As the average REI was around 4-6kg/cow/hour, while the RT was between 350 to 450 minutes, the increase of REI (nearly 17%) was larger than the reduction of RT (nearly 4%). Therefore, four hours window can be applied as the control threshold of milking interval instead of the five hours with more benefits for REI. After applying the suggested adjustments, an estimation of the potential financial benefits is shown in Figure 5-5. Daily earnings on this farm can potentially increase with approximately $400 due to the combined effects of increased income and reduced cost. As this profit increase was only estimated in relation to a particular farm, the results might vary on other farms. Therefore, the implementation of these recommendations might need to be modified according to the specific circumstances of other farms (i.e. climate and breeding). However, even if only 50% of the potential benefits can be realised, a significant income increase can still be expected when applying such adjustments to RMS, especially on farms with larger number of cows (holding 1000-10000 cows).

Another reason for the early morning milking with low efficiency was caused by the position of the watering trough, as the cows had to move to pass the drafting gate for drinking. The drafting gate then allowed the cows to enter milking station as their daily recording of milk yield was refreshed in the early morning although they were not under the best status of milking as shown in Figure 5-2. According to the analysis of Model 1, 6, 7 and 9, delay the first milking by 1 hour would reduce nearly 2.5% of RT, but increase nearly 30% of REI and decrease 3% of LEM. Therefore, delaying the time of first milking and adjusting the milking interval to 4 hours would improve the herd production and robotic milking performance for the farm of this study. However, general recommendations still require more data and analysis collected from different farms applying RMS.

5.5. Conclusions

This study focused on the influence of heat stress on animal behaviours and the performance of robotic milking machines. According to the regression analysis, when exceeding the thresholds, 1°C raising of daily mean temperature decrease 5.12 minutes of rumination time, reduce 0.07kg/cow/hour of rumination efficiency, and increase 1% of low-efficiency milking. Moreover, for the herd traffic of RMS observed by this study, the time of daily first milking was recommended to be delayed, whereas the milking interval was suggested to be 4 hours to maximize the benefits on milk production and robotic milking performance.

Chapter 6. **Modelling of heat stress in a robotic dairy farm. Part 4: Lag and cumulative effect of heat stress**

6.1. Abstract

The assessment of heat stress is usually conducted by using thermal comfort indices (TCIs) that calculates an integrated value of temperature, humidity, wind speed and solar radiation. However, the influence of heat stress is not only related to the intensity but also related to the duration of heat stress. This study was implemented to develop thermal indices which could quantify both the intensity and duration of heat stress. The analysis was conducted using the production data collected on a robotic farm, and climate data obtained from data-loggers on the farm and also from local weather stations. The thresholds associated with daily maximum, minimum and mean temperatures with heat stress were identified by using single broken-line regressions between daily milk yields (DMY) and daily mean temperatures. An intensity duration index (IDI) was proposed to evaluate daily short-term heat stress by multiplying the mean temperature of the heat stress period with the duration of the period. The threshold of IDI was than detected by using multi-phases segmented regression between DMY, IDI and other essential production factors (age, body weight and days in milk). Multiple levels of heat stress were identified by the thresholds of IDI with a different decline rate of DMY (-0.01 to -0.13 kg/cow/oC). For long-term heat stress, the lag and cumulative effect of heat stress were demonstrated by the negative correlation between heat stress during the dry-off period and the production performance of the next lactation period. By analysing the correlation between test day's DMY and temperature of previous days, the lag effect was found to be 3-4 days. However, the cumulative effect could last around 2 months. Next, the heat stress mean temperature ($T_{HS\ mean}$) was established by calculating the mean of maximum/minimum temperature of the period with heat stress during the 2 months before the test day. The regression between test day's DMY and the newly developed $T_{HS\ mean}$ was demonstrated to be higher R2 (0.73-0.77) than the regression with the same day's temperature (0.65-0.68).

6.2. Introduction

Assessment of the heat stress in dairy cows is necessary for developing mitigation strategies to maintain health and productivity of the system. As reviewed in the previous papers of this series (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b; Ji *et al.*, Submitted-c), a number of thermal comfort indices (TCIs) have been established via modelling thermal parameters and cow responses (LCI, 1970; Buffington *et al.*, 1981; Mader *et al.*, 2006; Brown-Brandl *et al.*, 2005; Gaughan *et al.*, 2008b). By using the published TCIs, the effect of heat stress on production efficiency can be predicted and evaluated, but only for conditions that they have been developed. However, when they applied for the prediction of cattle responses under different thermal environments, the accuracy of TCIs are always constrained (Bohmanova *et al.*, 2007; Silva *et al.*, 2007; Kendall *et al.*, 2006; Hammami *et al.*, 2013; Li *et al.*, 2009). In sub-tropical regions, past studies have even suggested the direct application of ambient temperature (dry bulb temperature, Tdb) (Dikmen and Hansen, 2009) instead of the application of TCIs. This is so, because ambient temperature measurements can provide similar performance to TCIs with fewer measurements, thus making ambient temperature measurements a more economical proposition. .

The limitation of TCIs performance is also related to the method of data collection and processing. Previous indices only utilised the data of test day, however, it was shown that thermal conditions in the days preceding the measurements of cow responses were more important than the condition on the day of measurement (West *et al.*, 2003; Garcia-Ispierto *et al.*, 2007a; Morton *et al.*, 2007b) because of lag in animal response. In addition, the processing of TCIs' value as daily maximum or mean value ignored the cooling-off period (night-time cooling) (Vitali *et al.*, 2009) that allows the cow to recover from heat stress (Scott *et al.*, 1983). In a study of short-term (acute) heat stress in a climate chamber, Garner *et al.* (2017) found the cows exposed to heat-wave conditions with temperature humidity index (THI) of 74-84, for four days resulted in reduced milk production by 53%. Moreover, the cows required at least seven days within thermal neutral conditions (THI 55-61) to return to the pre-experimental production performance. In practical dairy farming, acute heat stress can easily occur without enough cooling in the summertime. Moreover, unlike in laboratory studies, cows may not have enough recovery period under thermal neutral conditions. Thus the combined effects of heat stress over an extended period of time can potentially lead to long-term (chronic) effects. Previous study has demonstrated that the reproduction performance of cows could be reduced by 20-30% in summer and the observed negative after-effects could persist during the autumn period as well (Rensis and Scaramuzzi, 2003). In relation to the long-terms effect on milk production; the current understanding is still insufficient. However, studies have found that heat stress experienced by cows during the dry-off period could reduce milk production of the next lactation period (Do Amaral *et al.*, 2009; Tao *et al.*, 2011). To evaluate health condition under heat stress, blood sample tests were taken for nearly 80 days around calving (from -35 to +42 days, 0 is the day of caving) and the results demonstrated the long lasting (chronic) effects of heat stress on lactating cows (Do Amaral *et al.*, 2009).

Therefore, both of the severity and duration of heat stress need to assessed in order to improve the overall evaluation of heat stress effects of cows. While evaluating the severity and duration of heat stress, both the lag and cumulative effects can be studied. Modelling efforts of cumulative effects are still rare in the literature on dairy cows (e.g. two weeks (Berry *et al.*, 1964), 24-48 hours (Spiers *et al.*, 2004), and 2 days (West *et al.*, 2003). For heat stress on human, Rocklov *et al.* (2012) developed a model of temperature-related mortality. The model included heat-intensity, as well as the heat duration effects in consecutive three days. They demonstrated a better correlation between heat stress and mortality by adding the duration effect. Kong *et al.* (2010) also developed a statistical method for the analysis of nonlinear long-term cumulative effects. The performance of this model was tested by describing the effect of air pollutants on respiratory disease, and immunity of human against influenza in France. In medical science, the weighted cumulative exposure model (WCE) was developed to estimate the effects of time-varying exposures (Sylvestre and Abrahamowicz, 2009). The formulation of these time-related indices was referenced and modified in this study to develop an index for cows.

This paper is therefore aimed at evaluating the effect of heat stress on dairy cows with different time lags and durations while the impact of heat stress during the dry-off period is also included. Based on a large dataset (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b; Ji *et al.*, Submitted-c) of thermal conditions and cow responses from a commercial robotic dairy farm, a new index was developed based on heat stress intensity and duration. The ultimate aim of the study was to quantify the cumulative effect of heat stress and provide a better assessment of the likely production loss associated with cumulative heath stress on dairy farms.

6.3. Materials and Methods

Data collection

The data collection was undertaken in a robotic dairy farm using robotic milking system (RMS). The details of the farm, cow and measurement were explained in detail in associated publications (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b; Ji *et al.*, Submitted-c), so only a brief description will be given here. In this study, the short-term impact of heat stress was analysed by using the thermal data collected on the farm using HOBO data-loggers (Onset Computer, Bourne, MA) between April 2016 and November 2017 with data logging interval of 0.5 hours. The long-term effects were identified by using the data downloaded from the local weather station, which provided the daily result of temperature measurements. The manager of the study farm has aimed to provide a consistent and standardised diet to the cows throughout the year. Cows on this farm were predominantly fed in the buildings using a total ration diet. Therefore the impacts of seasonal pasture and forage availability were not considered in this study. The name and definition of variables and indices utilized in this paper are listed in Table 6-1.

Table 6-1 List of variables and indices

Category	Name	Unit	Definition
Animal status and production	Age	Years	Age of individual animal
	BW	Kg	Body weight of individual animal
	DIM	Days	Days in milk, or lactation days
	DMY	Kg/cow/day	Daily milk production of individual animal
	LD	Days	Lactation duration
	Total MP	Kg/cow	The sum of daily milk production over the lactation period
	Total MF	oC	The sum of daily milking frequency over the lactation period
	Total Fat	Kg/cow	The sum of daily fat content over the lactation period
	Total Protein	Kg/cow	The sum of daily protein content over the lactation period
Environment	Tmin	°C	Daily minimum temperature, represent the night time cooling, which was measured by local weather station
	Tmax	°C	Daily minimum temperature, represent the maximum heat stress, which was measured by local weather station
	Tmean	°C	Daily average temperature, represent the all day thermal condition, which was measured by local weather station
	T_daytime	°C	Average temperature between 06:00 AM and 18:00PM, which was measured by on-farm data-loggers
	T_nighttime	°C	Average temperature between 18:00PM and 06:00 AM+1, which was measured by on-farm data-loggers
	T_allday	°C	Average temperature between 06:00 AM and 18:00PM, which was measured by on-farm data-loggers
	IDI	°C * hour	Intensity duration index
	Tmax or min WCEmean	°C	Daily maximum or minimum temperature adjusted by weighted cumulative effect
	Tmax or min HSmean	°C	Mean of maximum or minimum temperature during the days under heat stress
	NumHSTmin	Days	Duration of long-term heat stress with daily minimum temperature exceeding the threshold
	NumHSTmax	Days	Duration of long-term heat stress with daily maximum temperature exceeding the threshold

Data processing and statistical analysis

The collected data was filtered by excluding outliers following the procedures described in previous papers of this series (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b; Ji *et al.*, Submitted-c). Moreover, dataset to link heat stress during the dry-off period and the production performance of cows in the follow-up lactation period was created from another 5 years' dataset, based on an individual recording of 126 Holstein lactating cows held on the farm.

- ## Intensity duration index (IDI) for short-term heat stress

The duration under general thermal conditions with a temperature of 15, 20, 25 and 30 °C was used for the basic analysis of the relationship between the intensity of thermal conditions and the duration. Then, the duration of heat stress was calculated as the hours or days with heat stress. The heat stress was identified as a temperature higher than the threshold values identified for the herd in a previous study (Ji *et al.*, Submitted-b). For short-term effect, the mean value of Tdb during day-time (6:00AM-18: 00 PM), night-time (18:00 PM to 6:0AM+1), and all-day was used to compare with the threshold and calculate the hours under heat stress. The mean temperature of the hours identified with heat stress was then calculated and multiplied by the duration, which was defined as the Intensity Duration Index (IDI) of heat stress. Multi-phases segmented linear regression between daily milk yield (DMY) and the independent factors of intensity duration index (IDI), age, body weight (BW) and days in milk (DIM) was applied to identify the threshold of IDI, which followed same procedures as described by Ji *et al.* (Submitted-b). The thresholds were finally described in figures with intensity and duration as the x and y-axis.

- **Statistical modelling for evaluation of the lag effect**

In this study, the 'lag effect' used in the analysis was defined as the effect of heat stress during a given period before the day when DMY was recorded. This lag effect can be as short as a day or can stretch over a number of months/weeks. Two steps were undertaken to evaluate the lag effect. First, the relationship between cow responses (e.g. DMY) on the test day and the thermal conditions (Tdb) on the days before were tested using a single correlation coefficient test. The range of proceeding days was chosen between -1 to -90 days.

Next, the relationship between heat stress during the dry-off period and the production performance of cow in the coming lactation period was tested using multiple linear regression with backwards stepwise selection as formulated below:

$$y = a + bAge + cBW + dDD + \sum e_i HD_i + \varepsilon$$

Where y is the dependent variable of cow production performance including lactation duration, total milk production, total fat/protein ratio of the milk, and the number of milking. The parameter 'a' to 'e' are the intercept and slopes for age, BW, dry-off duration (DD) and heat stress duration (HD), respectively. The variable of HD_i included six time durations with different level of heat stress. Three of the durations were defined with daily maximum Tdb ranges of 20-25, 25-30, >30 °C. The other three were defined with daily minimum Tdb of 15-20, 20-25, >25 °C.

- **Define and evaluation of the long-term cumulative effect index**

The long-term cumulative effect index was established by modifying the WCE model developed for medical science (Sylvestre and Abrahamowicz, 2009) and the model developed for assessing heat stress mortality (Rocklov *et al.*, 2012). The first model was adjusted from the WCE model by defining a new weight curve as follows:

$$T_{WCE\ mean} = \sum_{t \le u} \frac{w(u-t)T_t}{u}$$

$$w(u-t) = \frac{Cor(T_t, DMY_0)}{Cor(T_0, DMY_0)}$$

Where, $T_{WCE\ mean}$ is the mean value of dry bulb temperature adjusted by weighted cumulative effect, 'u' is the time duration of the cumulative effect, t is time, u-t is the lag days of the effect (u-t equal to 0 means the current day's effect), T_t is the average temperature at time t (t≤u), w(u-t) is the weight of the heat stress intensity, T_0 and DMY_0 are the temperatures and milk yield of current day . In this study, the w(u-t) is defined as the ratio between the effect of previous days' heat stress and the effect of the current day's heat stress. The effect was calculated as the coefficient of temperature in the regression with DMY.

The second model was adjusted from the model of heat stress mortality

$$T_{HS\,mean} = \sum_{t \le u} \frac{h(u-t)T(t)}{d}$$

$$h(u-t) = \begin{cases} 1, & T(t) \ge Thr \\ 0, & T(t) < Thr \end{cases}$$

Where, $T_{HS\ mean}$ is the mean value of dry bulb temperature of the days under heat stress, u is the number of days to be considered in the calculation, d is the number of days with heat stress, u-t is the lag days of the effect (if u-t equals 0 means the current day), T(t) is the dry bulb temperature at time t (t≤u), h(u-t) is a value to identify heat stress. The Thr is the thresholds of heat stress identified using the method from the previous analysis (Ji *et al.*, Submitted-a; Ji *et al.*, Submitted-b; Ji *et al.*, Submitted-c).

The $T_{wce\ mean}$ and $T_{HS\ mean}$ were evaluated comparing their performance of predicting production loss in the multiple linear regression. The formulation of this model was illustrated as below:

$$y = a + bAge + cBW + dDIM + \ eT + \ \varepsilon$$

Where y is the dependent variable of cow responses (e.g. DMY). The parameter a, b, c, and d are intercept and slopes of the basic model with cow production factors of Age, BW, days in milk (DIM) and rumination time (RT). The parameter e is the slope of thermal factor (T), which are daily temperature or the cumulative effect index ($T_{WCE\ mean}$ or $T_{HS\ mean}$).

All data processing and statistical analysis performed using R 3.4.3. (R Development Core Team, 2017). The application of functions and packages were described in detail by Ji *et al.* (Submitted-a); Ji *et al.* (Submitted-b); Ji *et al.* (Submitted-c).

6.4. Results and discussion

Description of the observed dataset

The raw dataset of DMY, daily minimum temperature (Tmin) and daily maximum temperature (Tmax) are presented in Figure 6-1. It can be seen that the values of DMY are changing in relation to Tmin and Tmax, but a lag or delay can been seen between the different curves. This could indicate that thermal conditions are no necessarily the primary factors affecting herd production, but the single linear regression between DMY and temperature (Tmin and Tmax) was still statistically significant (R^2 =0.31) Interestingly, this R^2 was higher than the R^2 of regression between DMY and DIM (R^2 = 0.29). However, it is recognised that the interactions between thermal conditions and other factors (e.g. DIM) can also influence herd production. For example, cows with early-lactation stage (DIM<40) entering heat stress (high Tmin and Tmax) might yield more milk than in their later-lactation stage (DIM>100) without heat stress. These kind of interactions were considered by multiple linear regression in the follow up analysis. As DIM (days in milk) is a time related factor (which is important in the modelling of DMY), time related factors of heat stress (duration of heat stress) may also gain meaningful impacts in the analysis. Such factors are also expected to qualify and quantify the lag or delay that might exist in relation to the impact of heat stress.

To calculate the time related factors of heat stress, the thresholds identified in previous studies were modified for this study (Ji *et al.*, Submitted-b) as shown in Figure 6-2. For the long-term analysis, thresholds of Tmin and Tmax were found as 11.1 and 21.3 ^oC. For the short-term analysis, thresholds of day-time, night-time and all-day mean temperature were determined as 22.4, 17.3 and 20.4 ^oC. The number of days (duration) with Tmin and Tmax higher than 11.1 and 21.3 ^oC during the previous two months of each test day are displayed in Figure 6-3. The long-term heat stress categorized into three levels from this figure:

- Level I: the duration of night-time and day-time heat stress start increasing;

- Level II: the duration of day-time heat stress reached to the peak value, while the one of night-time heat stress keep increasing;
- Level III: the duration of day-time and night-time heat stress all reached to the peak value.

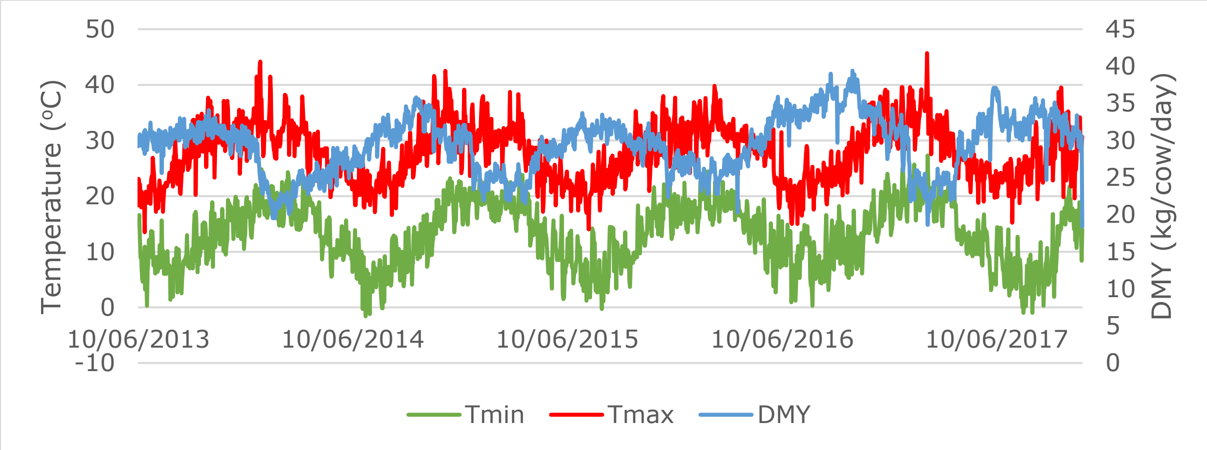

Figure 6-1 The observed DMY, Tmin, and Tmax. Data from 2016-August to 2017-November was applied for short-term heat stress analysis.

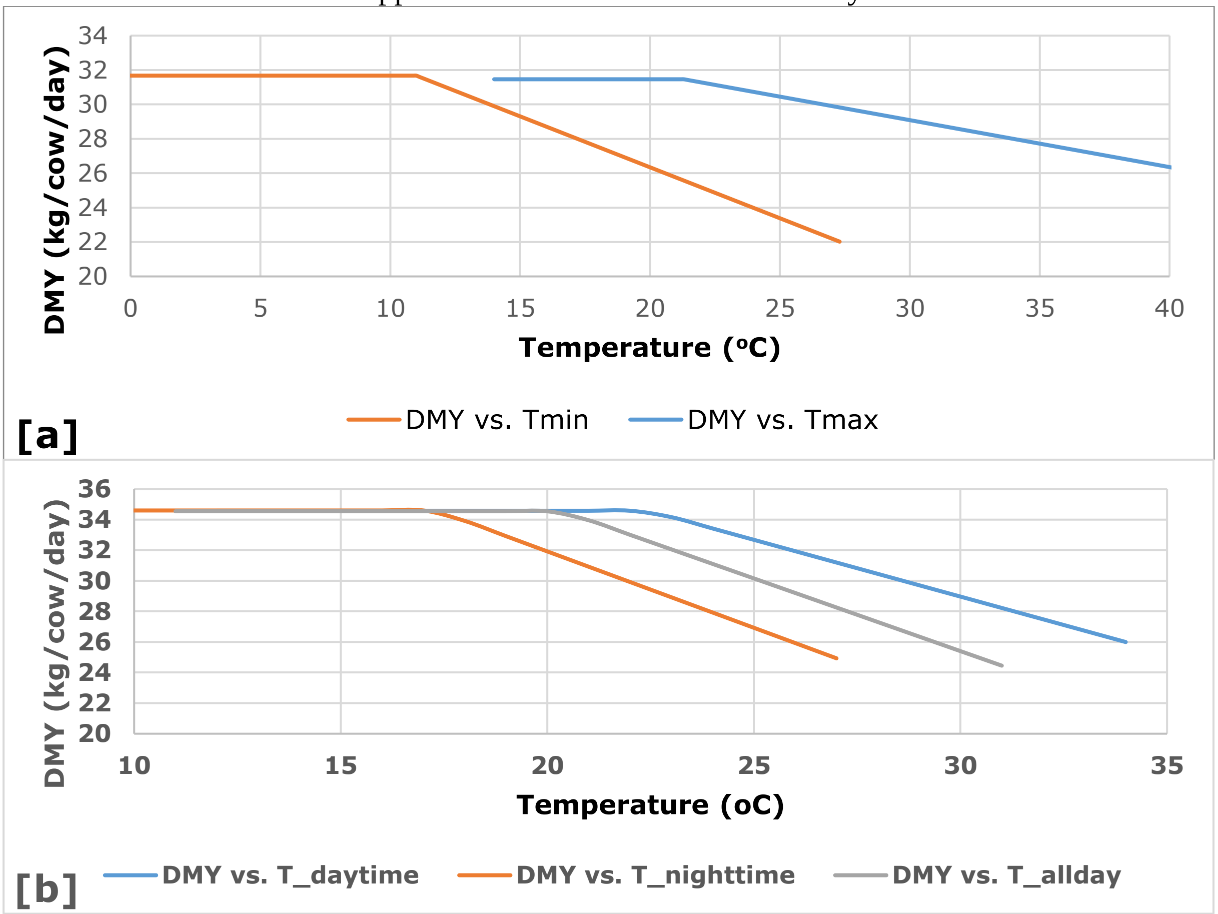

Figure 6-2 Threshold identification for long/short term period, using broken-line single linear regression. [a] – For the long-term period, the threshold of Tmax and Tmin are 11.1 and 21.3 ^{o}C. [b] – For short-term period the thresholds for day-time (T_day-time), night-time (T_night-time) and daily temperature (T_all-day) are 22.4, 17.3, and 20.4 ^{o}C.

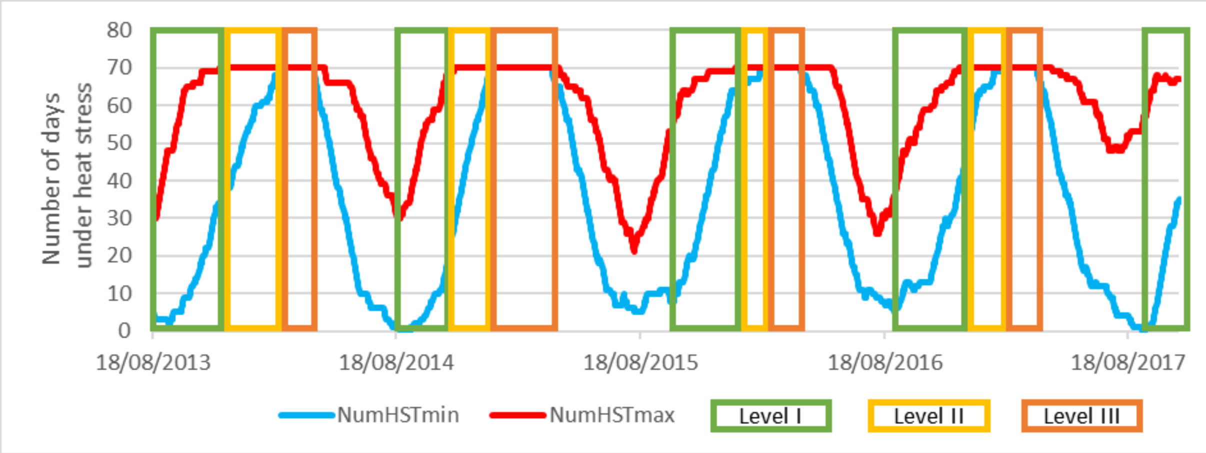

Figure 6-3 The number of the day in heat stress for the previous two months period before test day. NumHSTmin: the number of days with night-time heat stress when daily Tmin higher than the threshold value (11.1 °C). NumHSTmax: the number of days with day-time heat stress when daily Tmax higher than the threshold value (21.3°C).

Influence of short-term heat stress

The regression between DMY and durations of heat with a temperature higher than 15, 20, 25 and 30 °C were analysed and shown in Figure 6-4. For the day-time thermal condition (Figure 6-4 [a]), there was no significant correlation between DMY and the duration with a temperature higher than 15 °C. The decline of DMY also did not occur when cows remained less than 2 hours in a day-time temperature higher than 20 °C or 4 hours with a day-time temperature higher than 25 °C. When exceeding these time ranges, significant declines of DMY were observed when day-time temperature above 20°C (-0.26 kg/cow/day/hour), and 25 °C (-0.41 kg/cow/day/hour). With temperatures higher than 30°C, a significant decline of DMY (-0.37 kg/cow/day/hour) was observed from the beginning, which indicated no tolerance existed in comparison with encountering 20 °C or 25 °C. The decline of DMY were -3.84, -3.19 and -2.26 kg/cow/day/hour when the duration of heat stress was longer than 8, 10 and 11 hours with a day-time temperature higher than 20, 25 and 30 °C.

For the night-time thermal conditions (Figure 6-4[b]), the DMY also significantly declined (-0.04 kg/cow/day/hour) if the duration with a temperature higher than 15 °C was longer than 10 hours. The decline of DMY with a temperature higher than 20 °C had two phases (-0.17 and -0.67 kg/cow/day/hour), with a threshold of 5 hours. For other levels of night-time thermal condition (25 and 30 °C), the decline of DMY were -0.76 and -2.05 kg/cow/day/hour when the duration of heat stress was higher than 0 hours.

For the all-day thermal condition (Figure 6-4 [c]), the duration less than 22, 8 and 4 hours with a temperature higher than 15, 20 and 25 °C had a non-significant impact on DMY. With the all-day mean temperature higher than 20°C, the decline of DMY had two phases (-0.19 and -0.89 kg/cow/day/hour) with a threshold of 20 hours. The same level of DMY's decline was found with a temperature higher than 25 and 30 °C, which were -0.60 kg/cow/day/hour.

The correlation between DMY and the duration of cooling with a temperature below 15, 20, 25 and 30 °C were analysed and displayed in Figure 6-5. For the day-time cooling (Figure 6-5 [a]), the duration longer than 1, 2, 3 and 5 hours with a temperature below 15, 20, 25 30 °C provide a significant increase of DMY, which were 2.72, 2.94, 1.21 and 1.80 kg/cow/day/hour. For the night-time cooling (Figure 6-5 [b]), the cooling with the temperature only below 30 °C had a non-significant increase of DMY. The thresholds of duration with other night-time cooling (temperature below 15, 20 and 25 °C) were 1, 4, and 6 hours. The significant increase in DMY with a duration below these thresholds were 2.91, 1.32 and 1.24 kg/cow/day/hour. For the all-day cooling (Figure 6-5 [c]), the increase of DMY were 2.78, 0.7, 0.5, and 0.4

kg/cow/day/hour with 1, 6, 20 and 24 hours of cooling when temperature below 15, 20, 25 and 30 ^{o}C, respectively.

According to the analysis in Figure 6-4 and 5, the correlation between the temperature and the thresholds of duration was obvious. The general mechanism between the intensity (temperature) and duration could be expressed as an inverse function. Therefore, the threshold of their product (IDI) was identified using multi-phases segmented multiple linear regression. The dataset and regression results were plotted in Figure 6-6. The R^2 were 0.63, 0.74 and 0.76 for the modelling of day-time, night-time and all-day. With the regression for day-time and night-time, the thresholds of IDI were identified as 184 and 251. These two values were illustrated with the inverse function of temperature (15 to 40 ^{o}C) and duration (0 to 12 hours) in Figure 6-7. The short-term heat stress was then categorized into 3 levels based on the thresholds of day-time and night-time IDI, which were non-heat stress, heat stress during day-time but release during night time, and heat stress during both day-time and night-time. Moreover, it was also found that the decline of DMY when day-time IDI exceeding the threshold (184) was only -0.03 kg/cow/day/^{o}C*hour, while for the night-time (IDI higher than 251) the decline was -0.10 kg/cow/day/^{o}C*hour. However, when IDI higher than 251, the cows were entering both day-time and night-time heat stress, it was difficult to prove that the impact of night-time heat stress was being more important than day-time heat stress. Using the IDI thresholds of all-day thermal condition (IDI 170, 529 and 728), 4 levels of heat stress were categorized considering both the intensity (15 to 40 ^{o}C) and duration (0 to 24 hours) of heat stress. The decline of DMY changed from non-significant (positive value) to -0.13 kg/cow/day/^{o}C*hour.

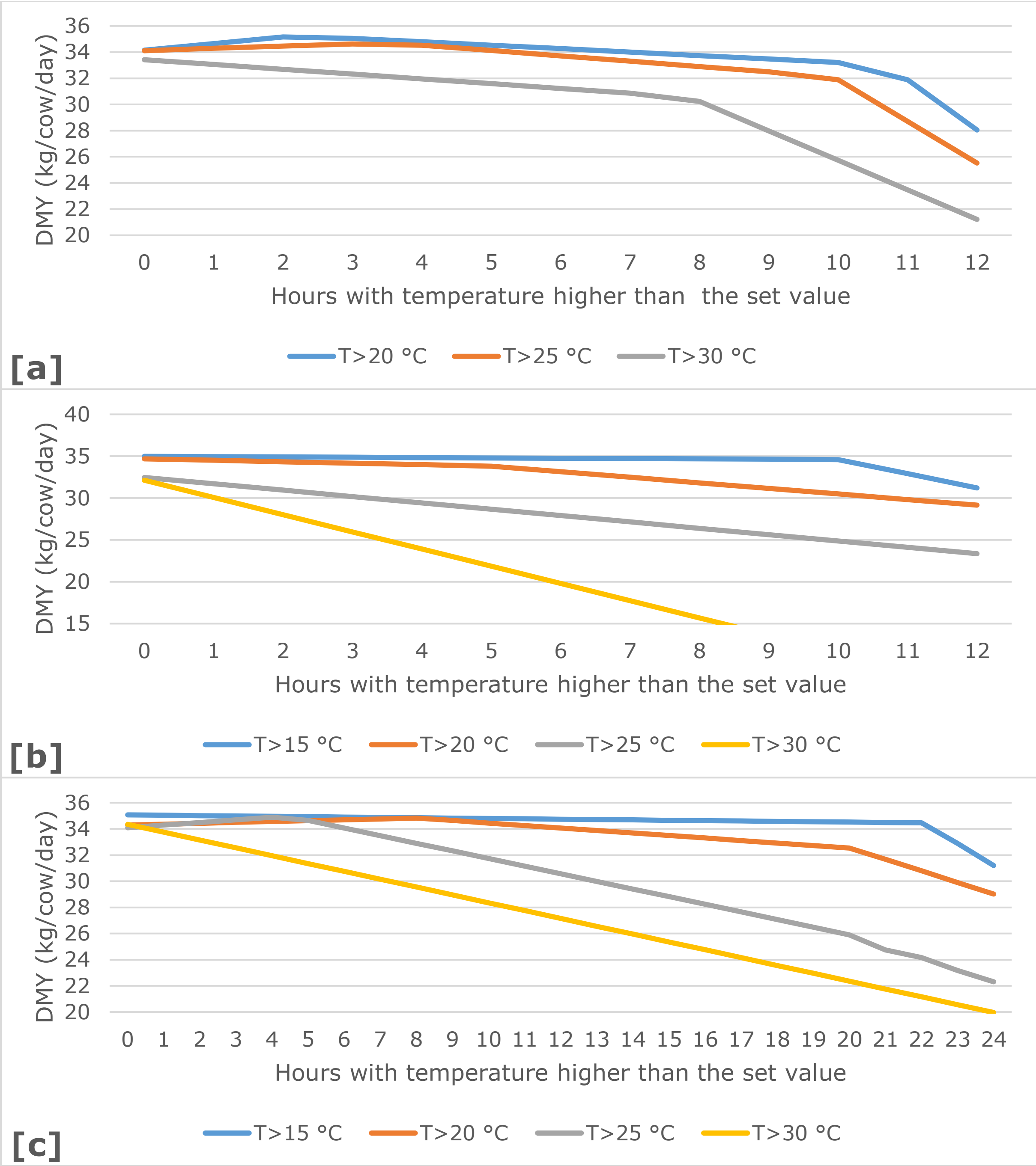

Figure 6-4 Multi-phases segmented single linear regression between DMY and the duration of heat with a temperature higher than different level (15, 20, 25 and 30 ºC). [a] – for the day-time durations, heat with a temperature higher than 15 ºC has no significant impact; [b] – for the night-time durations, and [c] for the overall daily durations.

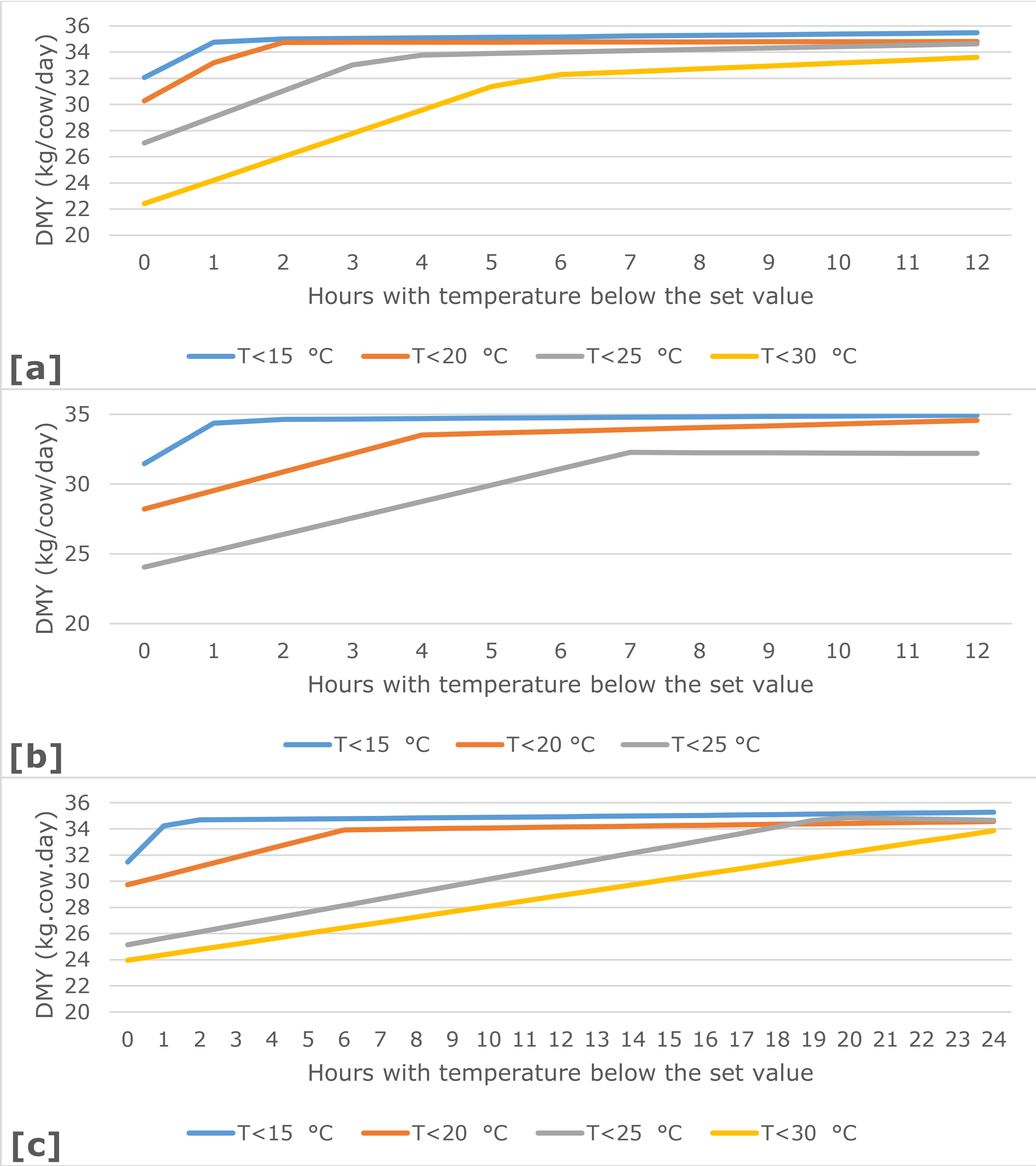

Figure 6-5 Multi-phases segmented single linear regression between DMY and the duration of cooling with a temperature lower than different level (15, 20, 25 and 30 ^{o}C). [a] – for the day-time durations; [b] – for the night-time durations, cooling to keep the temperature lower than 30^{o}C has no significant impact, and [c] for the overall daily durations.

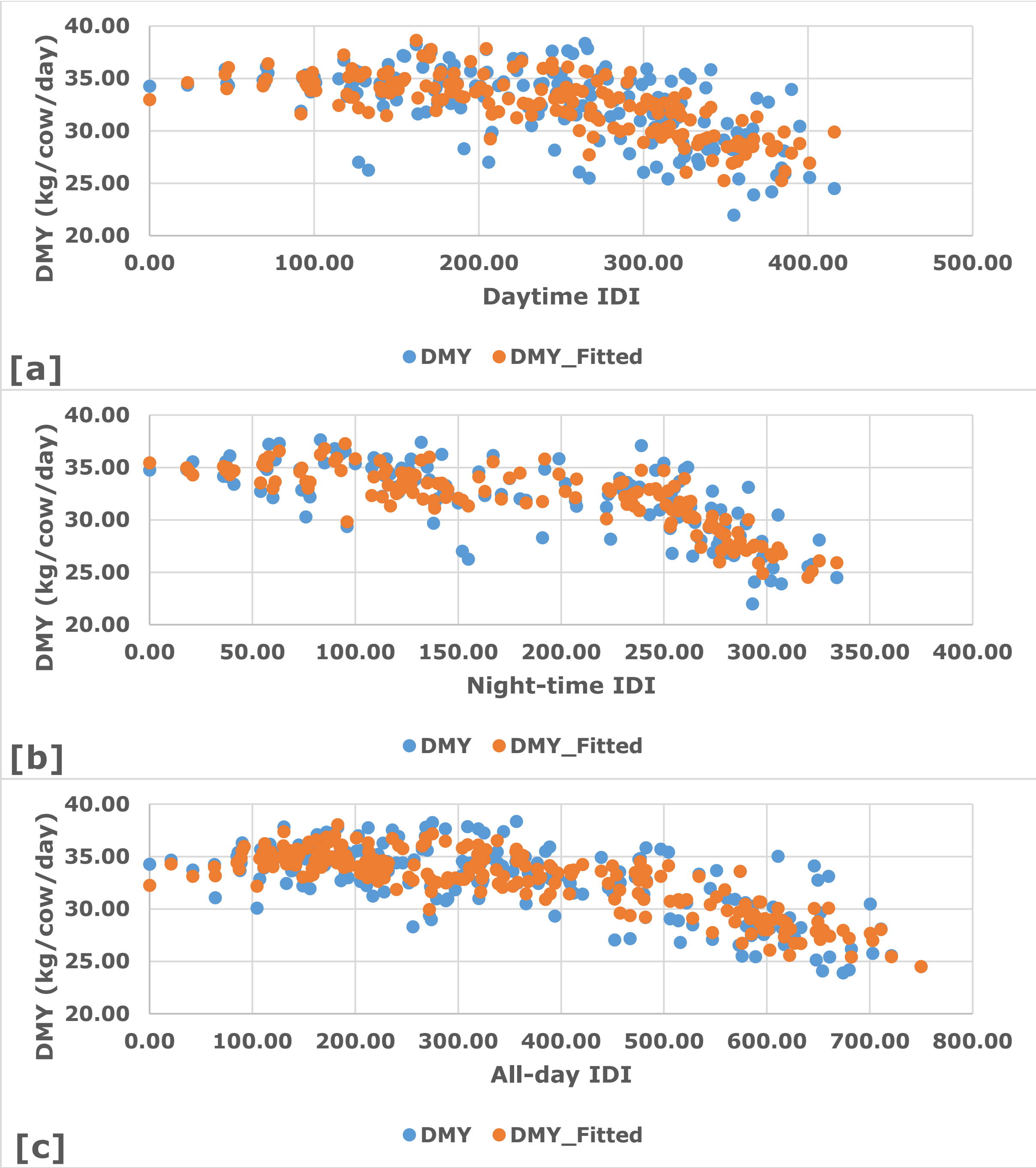

Figure 6-6 Multi-phases segmented regression between DMY and IDI. [a] – for the day-time, two phases identified with a threshold value 184 ^{o}C*hour, slope value before and after threshold are 0.02 and -0.03 kg/cow/day/ ^{o}C*hour, R2 equals 0.63. [b] – for the night time, two phases identified with a threshold value 251 ^{o}C*hour, slope value before and after threshold are 0.01 and -0.10 kg/cow/day/ ^{o}C*hour, R2 equal to 0.74. [c] – for the all-day time, four phases identified with thresholds value 170, 529 and 728 ^{o}C*hour, slope value for the four phases are 0.02, -0.01, -0.03 and -0.13 kg/cow/day/ ^{o}C*hour, R2 equals to 0.75.

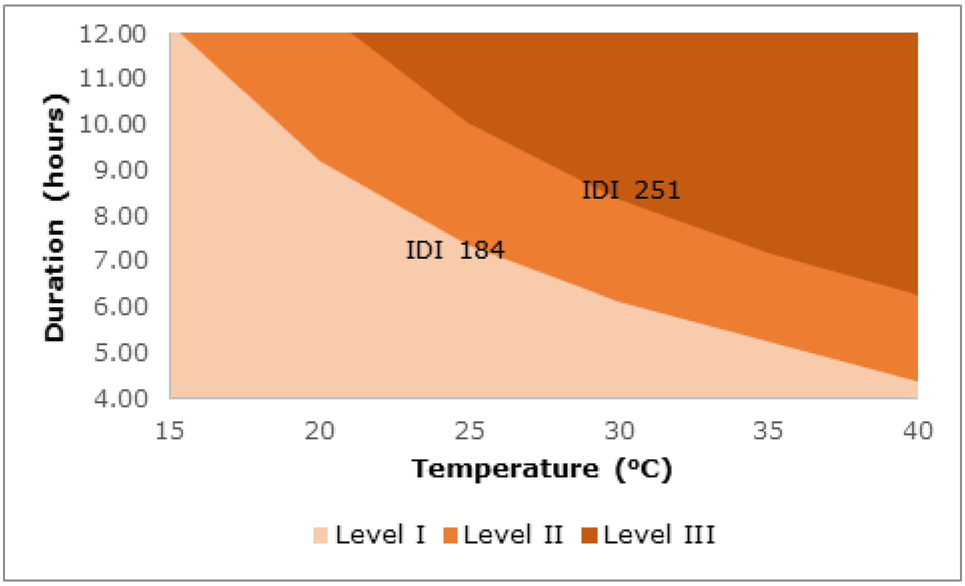

Figure 6-7 Differently coloured areas showing the IDI phases related to temperature and duration of day-time and night-time heat stress. Level I: thermal comfort during day-time and night-time with short duration of heat stress; Level II: heat stress during the day-time, but release during night-time; and Level III: heat stress happened during both day-time and night-time.

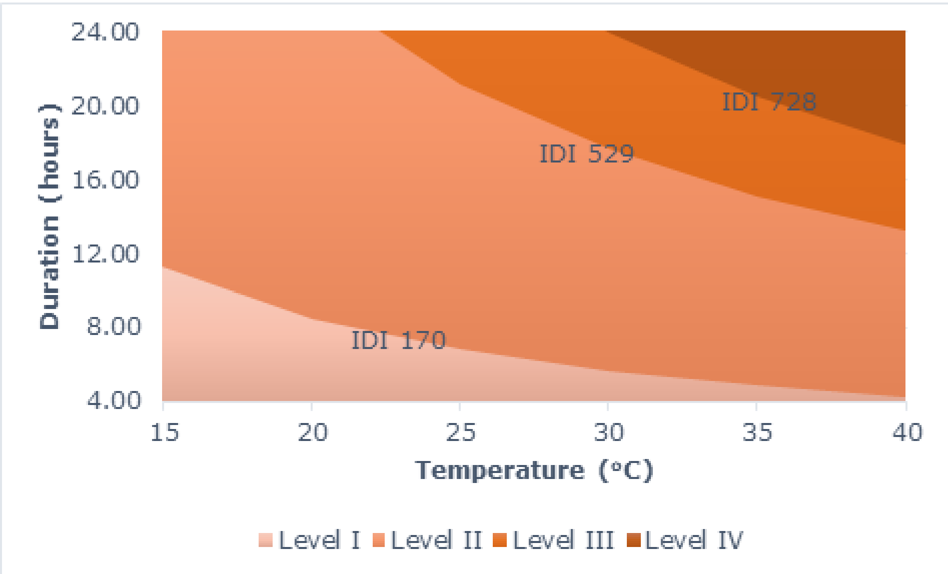

Figure 6-8 Differently coloured areas showing the IDI phases related to temperature and duration of all-day heat stress. Level I: thermal comfort with DMY 0.02 kg/cow/day/ °C*hour; Level II: effective heat stress with DMY -0.01 kg/cow/day/ °C*hour; Level III: critical heat stress with DMY -0.03 kg/cow/day/ °C*hour; and Level IV severe heat stress with DMY -0.13 kg/cow/day/ °C*hour.

Influence of long-term heat stress

The R^2 and slope of single linear regressions between the DMY of test day and the Tmin and Tmax of the previous day (-90 to 0 days) were displayed as curves in Figure 6-9 [a] to show the changes of the lag effect. The short-term lag effect could be demonstrated according to the increasing of R^2 and decreasing of slope from 0 days to -3 or -4 days. The R^2 of regression with Tmin increased from 0.31 to 0.34, while the one of regression with Tmax increased from 0.14 to 0.20. The slope of regression with Tmin decreased from -0.37 to -0.40 kg/cow/day/°C, whereas the one of regression with Tmax decreased from -0.27 to -0.33 kg/cow/day/°C. The long-term lag effect was also determined as the R^2 kept increasing, while the direction of slope was varied. . The R^2 of regression with Tmin achieved the maximum value of 0.39 by the time of -28 days, while the one of regression with Tmax reached to the maximum value of 0.34 by the time of -45 days. In the meantime, the slope decreased to -0.42 and -0.44 kg/cow/day/^oC for Tmin and Tmax, respectively. The regression including Age, BW, DIM (using the data of test day), Tmin and Tmax (using the data of 0 to -90 days) was then applied to recheck the lag effect when considering multiple factors, which is shown in Figure 6-9 [b]. A 4 days short-term lag effect was also demonstrated by the increasing of R^2 (0.68 to 0.69) and decreasing of slope (-0.18 to -0.19 and 0 to – 0.05, kg/cow/day/^oC for Tmin and Tmax, respectively). For the long-term lag effect, the R^2 reached to its maximum value of 0.74 at -60 days, and the slope of Tmax decreased to the minimum value of -0.15 kg/cow/day/^oC at -66 days. However, the slope of Tmin increased after 4 days, which indicated the long-term lag effect of Tmin was less than the one with Tmax.

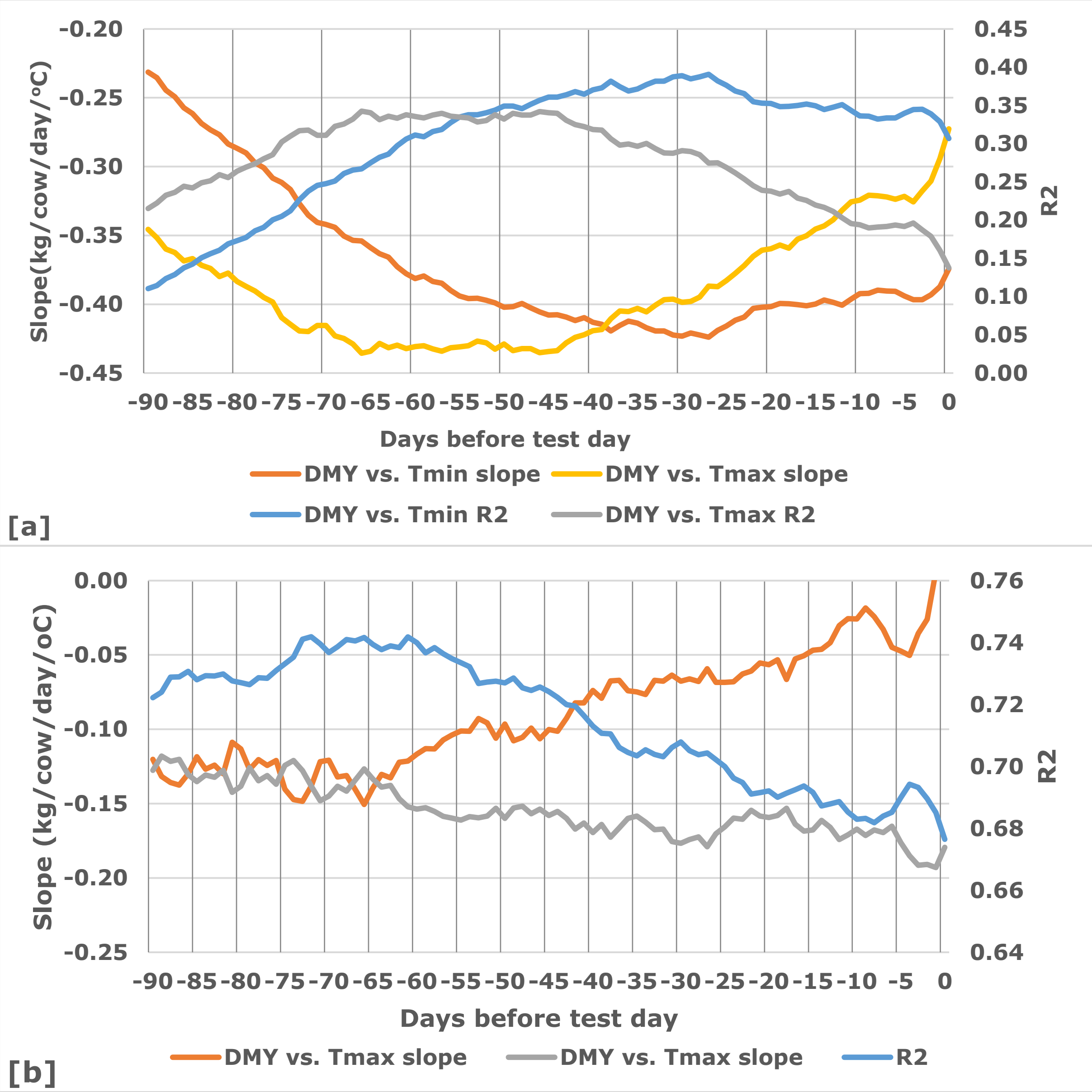

Figure 6-9 Slope and R2 of the linear regression between DMY and the temperature (Tmin and Tmax) of 0 to -90 days before test day. [a] – using the single linear regression; [b] – using the multiple linear regression including Age, BW, DIM, Tmin and Tmax in one modelling.

Table 6-2 Multiple linear regression between production performance after dry off period and heat stress in dry off period

Production after dry off days	R2	Intercept	BW	Age	Dry off Duration	Dry off Tmin	Number of days with Tmin 15-20	Number of days with Tmin 20-25	Number of days with Tmin>25	Dry off Tmax	Number of days with Tmax 20-25	Number of days with Tmax 25-30	Number of days with Tmax>30
LD	0.36	180.25 ***	NS	NS	11.54 ***	NS	2.16 .	-3.14 *	-28.23 *	NS	-12.78 ***	-11.40 ***	-9.18 **
Total MF	0.27	719.42 ***	-0.29 .	NS	28.09 **	NS	7.21 .	-8.39 .	-66.06 .	-59.93 *	-31.18 *	-27.47 **	-24.13 *
Total MP	0.31	3119.58 *	NS	NS	282.99 *	NS	132.86 **	-1075.26 **	NS	-692.86 .	-272.52 *	-260.84 **	-264.17 *
Total Fat	0.30	558.33 *	NS	43.20 *	47.19 **	NS	10.54 .	-14.10 *	-122.07 *	NS	-49.76 **	-43.62 **	-36.44 *
Total Protein	0.33	713.19 ***	NS	NS	38.12 ***	NS	NS	-10.93 *	NS	NS	-42.82 ***	-36.61 ***	-31.09 **

Significant label: "***" - $P<0.001$, "**" - $P<0.01$, "*" - $P<0.05$, "." – $P<0.1$

Table 6-3 Comparing the models of DMY using different thermal parameters

Model Num	Intercept	Slope of Independent					R2
		DIM	BW	Age	Thermal parameter		
1	3.59 ***	-0.08 ***	-0.001 .	8.71 ***	-	-	0.65
2	3.52 ***	-0.06 ***	-0.001 *	8.81 ***	Tmin	-0.18 ***	0.68
3	3.95 ***	-0.07 ***	-0.001 *	8.87 ***	Tmax	-0.05 **	0.65
4	12.54 ***	-0.05 ***	0.002 **	5.98 ***	Tmin WCE mean	-0.30 ***	0.77
5	14.92 ***	-0.04 ***	0.002 **	7.09 ***	Tmax WCE mean	-0.24 ***	0.73
6	22.40 ***	-0.05 ***	0.003 ***	6.03 ***	Tmin HS mean	-0.93 ***	0.77
7	17.29 ***	-0.04 ***	0.002 *	7.36 ***	Tmax HS mean	-0.50 ***	0.73
8	10.15 ***	-0.04 ***	0.002 ***	5,64 ***	NumHSTmin	-0.07 ***	0.77
9	7.98 ***	-0.06 ***	NS	7.72 ***	NumHTTmax	-0.08 ***	0.70

Significant label: “***” - P<0.001, “**” - P<0.01, “*” - P<0.05, “.” – P<0.1

The regression between production performance after a dry-off period and the heat stress during the dry-off period was summarized in Table 6-2. The BW and Age only had a significant impact on total milking frequency (MF) and fat percentage. The duration of dry-off had a significant positive impact on all production performance. No significant correlations were found with the mean of Tmin during the dry-off period. However, the duration with different levels of Tmin had different significant impacts. The duration with Tmin between 15 and 20 ^{o}C had a significant positive impact, while the duration with Tmin higher than 20 ^{o}C had significant negative impacts. The mean Tmax during the dry-off period and the duration with different level of Tmax all had negative effects on total MF and milk production (MY). The results from this Table 6-explain the lag effect of heat stress, as the heat stress during the dry-off period significantly affected the production performance of the next lactation period.

The results of multiple linear regression with different thermal parameters were shown in Table3. The regression with DIM, BW and Age (Model 1) had the same R^2 as the regression include Tmax as the thermal parameter (Model 3). However, the newly created parameters HSmean and WCEmean (Model 4-7) improved the modelling and gained higher R^2 (0.70-0.77). The maximum slope of thermal parameters was found with HSmean calculated according to the Tmin higher than the threshold value, which was -0.93 kg/HSmean. It was also interesting to find the model using the historical duration of days in heat stress (Model 8 and 9) can also provide higher R^2 than Model 1-3, which indicated the duration of heat stress should be taken more seriously than the intensity of heat stress.

Discussions of key issues

The importance of taking into account the duration of heat stress was demonstrated in this study. The analysis found the time-related heat stress could be categorized as short-term (hours in heat stress) and long-term effect (days in heat stress).

For the short-term effect, the duration (e.g. less than 4 hours) with a day-time temperature higher than 25^{o}C (Figure 6-4 [a]) did not lead to the significant decline of DMY, which indicated that cows could adapt to the heat stress within a limited duration **(**West, 2003). However, with a duration of longer than 8 hours, the DMY could have significant decline with a temperature higher than 20 ^{o}C. These variations indicated the potential inadequacy of heat stress mitigation techniques (e.g. ventilation) when only considering thermal parameters. Time-related heat stress with a same value of IDI had the same level of influence on cows' DMY e.g. 6 hours with a day-time temperature higher than 30 ^{o}C and 9 hours with a day-time temperature higher than 20^{o}C. This proposed index (IDI) enabled a more comprehensive evaluation of heat stress. The day-time IDI (184) was lower than the night-time IDI (251), which suggests the ability of animals to endure higher temperature and longer duration of heat stress at night-time. This could be caused by the reduced solar radiation at night-time (Spain *et al.*, 2001), as well as the reduced production activities (Allen *et al.*, 2015). However, as the day-time temperature was always higher than the night-time temperature, most of the days with night-time IDI of greater than 251 could result in a greater IDI in the following day. In our study, it was theoretically impossible to exclude the influence of day-time heat stress when analysing the data of night-time heat stress, as we assumed that the potential negative effects of day-time heat stress already impacted on the experimental animals. Therefore, the decline of DMY with night-time IDI higher than 251 (-0.10 kg/cow/day/^{o}C*hour) was 3 times greater than those with day-time IDI higher than 184 (-0.03 kg/cow/day/^{o}C*hour). This might be so, because the night-time heat stress could potentially account for both day-time and night-time heat stress. The all-day IDI identification generated four levels of time-related heat stress (Fig 8). The level IV with all-day IDI greater than 728 had the same level of decline (-0.13 kg/cow/day/^{o}C*hour) in DMY with the night-time IDI higher than 251 (-0the .10 kg/cow/day/^{o}C*hour), as it represented both day-time and night-time heat stress.

As the study farm did not have any cooling facilities, the analysis of cooling duration was based on the natural changes in weather parameters, as shown in Figure 6-5. The DMY started to significantly increase by applying a cooling strategy that relied on shorter cooling duration but using lower temperatures. However, the DMY under different cooling condition and duration only improved to the same level as the DMY before heat stress, which was a very interesting finding of this study. This suggested that cooling of cows might only alleviate the decline of their milk production, rather than enhancing their production performance. This is again, an important finding of this study. The general cooling strategy employed on modern dairy farms mainly include all-day cooling, cooling with segmented periods or cooling before feeding and milking (Silva and Maia, 2011; Avendano-Reyes *et al.*, 2006; Avendaño-Reyes *et al.*, 2012). However, most of the current cooling strategies tend to apply constant duration of cooling. It might be necessary to develop a dynamic cooling pattern according to the ambient temperature, as overcooling would not benefit the milk production. Moreover, the cows kept under conditions of long-term over cooling might have a compromised heat tolerance, as overcooling might limits their ability to adapt to heat stress (Mader *et al.*, 2007). Therefore, further studies are needed under practical conditions (i.e. farms with appropriate cooling facilities) to verify the results of this study (Figure 6-5). This could in turn, provide a potential optimization of cooling strategies (i.e. dynamic duration of ventilation) by considering the combination of animals' self-adaptation to heat stress and their need for cooling.

In relation to the long-term effect, this paper demonstrated two specified categories including the lag effect and cumulative effect. The lag effect was found as 3-4 days between the increase of temperature (Tmin or Tmax) and the decline of DMY. The lag days identified in the current study was longer than the one (1-3 days) reported by West *et al.* (2003), which might be caused by the different heat tolerance between the study animals. It is assumed that in Australia cows develop certain level of tolerance to heat stress over a period of time. Previous works (e.g. West *et al.* (2003) have not reported any correlation between DMY and the lag effect with longer than 3 days, thus it was unclear whether the correlation decreased after 3 days or increases. In the current study, the correlation between DMY and lag effect of temperature decreased after 3-4 days. However, the value increased 6-7 days and reached to the maximum level of 1-2 months lag. It was impossible to consider this increase as a lag effect, as it was difficult to forecast that the test day's milk production could be influenced by temperatures 30-60 days before the test day. However, the increased correlation indicated a cumulative effect. As described in Figure 6-3, the test day with relatively low ambient temperature (e.g. after the hot season) might have a high historical duration of heat stress (the days under heat stress before the test day). Garner *et al.* (2017) reported that after 4 days of heat stress, 7 days recovery period without heat stress was necessary for cows to return to their production performance before the heat stress. However, when entering the hot season, the daily temperature kept increasing which would not provide recovery period for the cows, and their production performance was influenced by the heat stress cumulatively. The analysis of heat stress impact during the dry-off period in this study also demonstrated the possibility of a long-term cumulative effect. The analysis found that the duration of heat stress during the dry-off period had a more significant impact on productivity than the mean of temperature. Therefore, appropriate mitigation against heat stress during dry-off period should be as important as the mitigation in lactation period, enabling a well-prepared body condition for the subsequent lactation, such as improving innate and acquiring immune status (Do Amaral *et al.*, 2011).

To quantify the cumulative effect, WCEmean and HSmean temperature indices were developed in this study. The correlation between DMY and such new indices were greater than the one between DMY and test day's temperature. As the indices considering both intensity and duration of heat stress were rare in publications, it was difficult to compare the new indices with the indices from other studies. However, these new indices might be able to improve previously published indices in the future, such as THI (Davis and Mader, 2003). As the weighted cumulative effect model was used in medical science (Sylvestre and Abrahamowicz, 2009); the weight curve could not be applied to heat stress. The WCEmean temperature was calculated by using the correlation coefficient (slope value) of temperature in past days as the weight. More studies will be required to find accurate weight of temperatures in past days. The HSmean temperature was recommended by this study, as it just calculated the mean temperature of the days in heat stress, and achieved the same performance as the WCEmean temperature in the regression with DMY.

6.5. Conclusions

In this study, the time-related heat stress was investigated as a short-term and long-term effect. For short-term effect, the intensity-duration index (IDI) was developed for day-time, night-time, and all-day heat stress. The thresholds of IDI were illustrated as areas of heat stress quantifying different durations and temperatures. For long-term heat effect, the lag effect with 3-4 days between temperature and milk production was determined. The cumulative effect was found to last for 1-2 months. Heat stress mean temperature ($T_{HS\ mean}$) was developed to quantify the cumulative effect, which provides better correlation with DMY (R^2 = 0.73 – 0.77) compared with the mean temperature of test day (R^2 = 0.65 – 0.68). Further studies are required to link the short-term and long-term effect. Another recommendation of the study would be to refine the short-term IDI by considering the long-term cumulative effects which were not considered in the current study.

Chapter 7. General conclusion

7.1. Conclusions

This research conducted a number of analysis and modelling in relation to heat stress on a dairy farm with RMS. The outcome from analysis and modelling demonstrated several applicable ways to apply routinely collected dataset from RMS for a more accurate heat stress assessment. By applying and comparing the published TCIs using the collected dataset, this study found:

- A general threshold for high-producing lactation cows (DMY = 31 kg/cow/day) was identified by this study as THI greater than 64, which indicated a basic alert of heat stress for farm managers.
- Instead of using TCIs, which need to measure several thermal parameters and animal responses indicators, the prediction of heat stress using Tdb can provide similar good performance for subtropical climate region.
- The government's online dataset of climate parameters can be applied to reduce the cost of implementing on-farm measurement, which was proven to achieve similar accuracy in forecasting heat stress.

To establish a dynamic threshold algorithm, this study reported several new findings as follows:

- By using the indicators of MT and DMY, four stages of heat stress were identified including Stage I - no stress, Stage II - innocuous heat stress, Stage III - effective heat stress and Stage IV - critical heat stress, which described a gradual coping of cows against heat stress. These levels were categorized with different thresholds of daily minimum and mean Tdb.
- Decision tree algorithm was formulated to estimate specific threshold of Stage IV - critical heat stress. By input different BW, DIM and Age of cows, the algorithm could modify the threshold value and indicate different heat tolerances or cooling demands with 79-94% overall accuracy.

By using the dataset recording individual cow's health and production performance, the analysis of this study found:

- A new index REI was developed for evaluating the efficiency of cows' rumination that was influenced by heat stress. The value of REI significantly decreased with the raising of temperature. However, when temperature exceeded 25 ^{o}C, the value started increasing due to the reduction of RT becoming more significant than the decline of DMY, which possibly indicated a more compromised health/welfare status of cows.
- As the farm observed by this study applied semi-free traffic, there was no forced movement of cows. All of their milking events happened voluntarily. It was found that 86% of the milking event occurred between 07:00AM and 09:00AM, which had non-significant correlation with heat stress.
- Adjustment of milking interval and time of milking were found can be able to benefit cows' REI, as well as the performance of robotic milking (reduce the rate of low efficiency milking). This proposed adjustment was estimated to potentially increase income of the study farm by approximately $400 per day.

To deal with the lag and cumulative effects of heat stress within short and long term periods, this study found:

- For short-term heat stress, another new index IDI was established to quantify the cumulative and lag effect of heat stress by involving time as a parameter in the model. The decline of DMY along with the raising of IDI ranged from -0.01 to 0.13 kg/cow/day/IDI.

- For long-term heat stress, the mean temperature of days under heat stress before test day was found to be able to quantify a nearly 2 months' cumulative effect. The duration of heat stress during dry-off period was also demonstrated to have significant impact on the production performance of the following lactation period.

7.2. Limitations

This study has been conducted to demonstrate the possibility of developing better heat stress assessment and prediction relying on the management database of RMS, which routinely records health and production information of individual cow. However, a number of limitations should be acknowledged in relation to data collection and the results of analysis. These limitation may include:

- The on-farm measurement of thermal parameter was occasionally interrupted due to electricity interrupt, internet failure and farm management issues. Therefore, the comparison between on-farm measured dataset and online climate dataset was only performed during a set period of time. Nonetheless, the researchers involved in this study are confident that the on-line climatic data can be used effectively for on farm heat stress management within practical precision.
- The accuracy of sensors in RMS measuring cows' health and production indicators were not tested. Therefore the reliability of such measurements' results was only maintained by routine calibration of RMS conducted by farmers and technicians of the manufacturer. However, the system error or uncertainty of measurements was minimized by the large number of daily replications.
- The identification and quantification of cows' behaviour (i.e. milking event) were only depended on the data measured by RMS, while no video recording or vision observation were taken for rechecking. Again, it was assumed that the large data set used compensated for any potential measurement inaccuracies.
- The feed and nutrition supply for the cows of this study was confirmed by farm managers to be standardized and was not influenced by seasonal forage availability. However, the farmers did not provide any specific nutritional or feed intake information. Therefore, nutritional variables were not considered in the analysis. It was assumed that the standardised diet would eliminate any nutritional variability within the dataset.
- The programming of traffic control system was not made available for us. Therefore the adjustment of control algorithm (i.e. optimized interval of milking events) was not implemented and evaluated under practical farm condition. Further studies need to be undertaken to verify the calculated results.
- The understanding of cows' responses to heat stress was obtained by data analysis and no physiological and biochemical measurements were performed to gain deeper understanding of the potential internal metabolic reactions of cows. However, the main aim of this study was to generate practical outcomes that can be implemented under on-farm conditions. Therefore further (potentially laboratory based) studied will be needed to look into the underlining physiological responses of cows to heat stress.
- Although the number of cows observed by this study was large (approximately 150 lactating cows) and the duration of measurements lasted for nearly 5 years, the universality of the results from this study is still need to be verified. In addition, modification of models might be necessary if and when outcomes of this study implemented in the future on other farms.

7.3. Further research

This study also identified several related areas with further research interest, which are:

- Future studies are required to not only develop new TCIs, but also establish reliable approach to modify the models considering specific farm and climate conditions.
- Research similar to this study should be replicated in multiple farms with RMS, which could generate more generalized results and suggestions for practical applications. The proposed study farms should be selected from different climate regions.
- The models established by this study can be refined by including more parameters associated with physiological reactions of cows. However, to consider the feasibility in practical applications, this kind of data should be collected from commercial devices which has automated measurement functions such as rumen bolus.
- Research related to ventilation system can apply similar analysis performed in this study, which may be able to generate a dynamic ventilation pattern that can save energy cost and prevent the loss of heat tolerance due to over-cooling.
- Optimization of RMS management was predicted to benefit the milk production and robotic milking performance. However, more studies are required to implement such adjustments in practical RMS operations and evaluate the financial outcomes.
- Further study should consider the combined effect of short-term and long-term heat stress influence, thus a more comprehensive duration and intensity index could be developed to provide a more accurate estimation of cumulative and lag effects.

References

Adams, M. (2017). lm. br: An R Package for Broken Line Regression.

Adin, G., Gelman, A., Solomon, R., Flamenbaum, I., Nikbachat, M., Yosef, E., Zenou, A., Shamay, A., Feuermann, Y. & Mabjeesh, S. (2009). Effects of cooling dry cows under heat load conditions on mammary gland enzymatic activity, intake of food and water, and performance during the dry period and after parturition. *Livestock Science* 124(1): 189-195.

Al-Katanani, Y. M., Webb, D. & Hansen, P. (1999). Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. *Journal of Dairy Science* 82(12): 2611-2616.

Allen, J., Anderson, S., Collier, R. & Smith, J. (2013).Managing heat stress and its impact on cow behavior. In *28 th Annual Southwest Nutrition and Management Conference*, 68.

Allen, J. D., Hall, L. W., Collier, R. J. & Smith, J. F. (2015). Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. *J Dairy Sci* 98(1): 118-127.

Alphonce, E., Kisangiri, M. & Kaijage, S. (2017). Design and Analysis of Smart Sensing System for Animal Emotions Recognition.

Anderson, S., Bradford, B., Harner, J., Tucker, C., Choi, C., Allen, J., Hall, L., Rungruang, S., Collier, R. J. & Smith, J. (2013). Effects of adjustable and stationary fans with misters on core body temperature and lying behavior of lactating dairy cows in a semiarid climate. *Journal of Dairy Science* 96(7): 4738-4750.

Andersson, L. & Lundström, K. (1981). The influence of breed, age, body weight and season on digital diseases and hoof size in dairy cows. *Transboundary and Emerging Diseases* 28(2): 141-151.

Avendano-Reyes, L., Alvarez-Valenzuela, F., Correa-Calderón, A., Saucedo-Quintero, J., Robinson, P. & Fadel, J. (2006). Effect of cooling Holstein cows during the dry period on postpartum performance under heat stress conditions. *Livestock Science* 105(1): 198-206.

Avendaño-Reyes, L., Hernández-Rivera, J., Álvarez-Valenzuela, F., Macías-Cruz, U., Díaz-Molina, R., Correa-Calderón, A., Robinson, P. & Fadel, J. (2012). Physiological and productive responses of multiparous lactating Holstein cows exposed to short-term cooling during severe summer conditions in an arid region of Mexico. *International Journal of Biometeorology* 56(6): 993-999.

Baeta, F., Meador, N., Shanklin, M. & Johnson, H. (1987). Equivalent temperature index at temperatures above the thermoneutral for lactating dairy cows. *American Society of Agricultural Engineers (USA)*.

Banhazi, T. (2013). Seasonal, diurnal and spatial variations of environmental variables in Australian livestock buildings. *Australian Journal of Multi-disciplinary Engineering* 10(1): 60-69.

Banhazi, T., Aarnink, A., Thuy, H., Pedersen, S., Hartung, J., Payne, H., Mullan, B. & Berckmans, D. (2009a). Review of the consequences and control of high air temperatures in intensive livestock buildings. *Australian Journal of Multi-disciplinary Engineering* 7(1): 63-78.

Banhazi, T., Kitchen, J. & Tivey, D. (2009b). Potential of using infrared thermography for determination of skin wetness and thus perceived thermal comfort of pigs. *Australian Journal of Multi-disciplinary Engineering* 7(1): 47-54.

Banhazi, T., Seedorf, J., Rutley, D. L. & Pitchford, W. S. (2008a). Identification of risk factors for sub-optimal housing conditions in Australian piggeries: Part 1. Study justification and design. *Journal of Agricultural Safety and Health* 14(1): 5-20.

Banhazi, T., Seedorf, J., Rutley, D. L. & Pitchford, W. S. (2008b). Identification of risk factors for sub-optimal housing conditions in Australian piggeries: Part 2. Airborne pollutants. *Journal of Agricultural Safety and Health* 14(1): 21-39.

Banhazi, T., Seedorf, J., Rutley, D. L. & Pitchford, W. S. (2008c). Identification of risk factors for sub-optimal housing conditions in Australian piggeries: Part 3. Environmental parameters. *Journal of Agricultural Safety and Health* 14(1): 41-52.

Banhazi, T., Vranken, E., Berckmans, D., Rooijakkers, L. & Berckmans, D. (2015).Word of caution for technology providers: Practical problems associated with large scale deployment of PLF technologies on commercial farms. In *7th European Conference on Precision Livestock Farming*, Vol. 1, 105-111 (Ed I. Halachmi). Leuven, Belgium: Wageningen Academic Publishers

Banhazi, T. M. & Banhazi, A. (2015).Long term monitoring of growth rates and environmental parameters on selected European pig farms. In *8th European Conference on Precision Livestock Farming*(Eds D. Berckmans and M. Guarino). Milan, Italy: University of Milan.

Banhazi, T. M., Lehr, H., Black, J., Crabtree, H., Schofield, P., Tscharke, M. & Berckmans, D. (2012). Precision livestock farming: an international review of scientific and commercial aspects. *International Journal of Agricultural and Biological Engineering* 5(3): 1-9.

Banhazi, T. M., Tscharke, M., Ferdous, W. M., Saunders, C. & Lee, S.-H. (2011). Improved image analysis based system to reliably predict the live weight of pigs on farm: Preliminary results. *Australian Journal of Multi-disciplinary Engineering* 8 (2): 107-119

Bar, D. (2010).Daily rumination time and calving diseases. In *Proc. 26th World Buiatrics Congress, Santiago, Chile. Accessed November*, Vol. 7, 2012.

Benezra, M. (1954).A new index for measuring the adaptability of cattle to tropical conditions. In *Journal of Animal Science*, Vol. 13, 1015-1015: AMER SOC ANIMAL SCIENCE 1111 NORTH DUNLAP AVE, SAVOY, IL 61874.

Benjamin, M. (1981). Fluid and electrolytes. *Outline of Veterinary Clinical Pathology. Iowa State Univ. Press, Ames*: 213-228.

Berman, A. (2005). Estimates of heat stress relief needs for Holstein dairy cows. *Journal of Animal Science* 83(6): 1377-1384.

Berman, A., Folman, Y., Kaim, M., Mamen, M., Herz, Z., Wolfenson, D., Arieli, A. & Graber, Y. (1985). Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate. *Journal of Dairy Science* 68(6): 1488-1495.

Berman, A. & Horovitz, T. (2012). Radiant heat loss, an unexploited path for heat stress reduction in shaded cattle. *J Dairy Sci* 95(6): 3021-3031.

Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N. & Nardone, A. (2014). The effects of heat stress in Italian Holstein dairy cattle. *J Dairy Sci* 97(1): 471-486.

Berry, E. A., Hogeveen, H. & Hillerton, J. E. (2004). Decision tree analysis to evaluate dry cow strategies under UK conditions. *Journal of dairy research* 71(4): 409-418.

Berry, I., Shanklin, M. & Johnson, H. (1964). Dairy shelter design based on milk production decline as affected by temperature and humidity. *Transactions of the ASAE* 7(3): 329-0331.

Bianca, W. (1962). Relative importance of dry-and wet-bulb temperatures in causing heat stress in cattle. *Nature* 195: 251.

Bianca, W. (1963). Rectal temperature and respiratory rate as indicators of heat tolerance in cattle. *The Journal of Agricultural Science* 60(01): 113-120.

Biby, W. W. T. I. F. J. B. T. R. (2010).Interrelationships of Heat Stress and Reproduction in Lactating Dairy Cows. In *High Plains Dairy Conference*.

Black, J., Sara Willis, S. & Banhazi, T. M. (2016).Estimation of Accuracy Needed for Live Weight and Feed Intake Measurements in Precision Livestock Farming Systems Using Auspig Simulation Software In *First Asian Precision Livestock farming conference* in-print (Ed C. Wang). Beijing, China China Agricultural University

Blazquez, N., Long, S., Mayhew, T., Perry, G., Prescott, N. & Wathes, C. (1994). Rate of discharge and morphology of sweat glands in the perineal, lumbodorsal and scrotal skin of cattle. *Research in veterinary science* 57(3): 277-284.

Bohlouli, M., Shodja, J., Alijani, S. & Eghbal, A. (2013). The relationship between temperature-humidity index and test-day milk yield of Iranian Holstein dairy cattle using random regression model. *Livestock Science* 157(2-3): 414-420.

Bohmanova, J., Misztal, I. & Cole, J. B. (2007). Temperature-humidity indices as indicators of milk production losses due to heat stress. *J Dairy Sci* 90(4): 1947-1956.

Bohmanova, J., Misztal, I., Tsuruta, S., Norman, H. & Lawlor, T. (2005). National genetic evaluation of milk yield for heat tolerance of United States Holsteins. *Interbull Bulletin* (33): 160.

Bond, T., Kelly, C., Morrison, S. & Pereira, N. (1967). Solar, atmospheric, and terrestrial radiation received by shaded and unshaded animals. *Transactions of the ASAE* 10(5): 622-0625.

Bouraoui, M. B. S. R. (2009). Heat Stress in Tunisia: Effects on dairy cows and potential means of alleviating it. *South African Journal of Animal Science*.

Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M. n. & Belyea, R. (2002). The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Animal Research* 51(6): 479-491.

Brodersen, K. H., Ong, C. S., Stephan, K. E. & Buhmann, J. M. (2010).The balanced accuracy and its posterior distribution. In *Pattern recognition (ICPR), 2010 20th international conference on*, 3121-3124: IEEE.

Brosh, A. (2007). Heart rate measurements as an index of energy expenditure and energy balance in ruminants: A review1. *Journal of Animal Science* 85(5): 1213-1227.

Brown-Brandl, T., Eigenberg, R., Nienaber, J. & Hahn, G. L. (2005). Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, Part 1: Analyses of indicators. *Biosystems engineering* 90(4): 451-462.

Brown-Brandl, T., Nienaber, J., Eigenberg, R., Hahn, G. & Freetly, H. (2003). Thermoregulatory responses of feeder cattle. *Journal of Thermal Biology* 28(2): 149-157.

Bryant, J., Lopez-Villalobos, N., Pryce, J., Holmes, C., Johnson, D. & Garrick, D. (2007). Environmental sensitivity in New Zealand dairy cattle. *Journal of Dairy Science* 90(3): 1538-1547.
Buffington, D., Collazo-Arocho, A., Canton, G., Pitt, D., Thatcher, W. & Collier, R. (1981). Black globe-humidity index (BGHI) as comfort equation for dairy cows. *Transactions of the ASAE* 24(3): 711-0714.
Calcante, A., Tangorra, F. M. & Oberti, R. (2016). Analysis of electric energy consumption of automatic milking systems in different configurations and operative conditions. *Journal of Dairy Science* 99(5): 4043-4047.
Carabano, M.-J., Logar, B., Bormann, J., Minet, J., Vanrobays, M.-L., Díaz, C., Tychon, B., Gengler, N. & Hammami, H. (2016a). Modeling heat stress under different environmental conditions. *Journal of Dairy Science* 99(5): 3798-3814.
Carabano, M. J., Logar, B., Bormann, J., Minet, J., Vanrobays, M. L., Diaz, C., Tychon, B., Gengler, N. & Hammami, H. (2016b). Modeling heat stress under different environmental conditions. *J Dairy Sci* 99(5): 3798-3814.
Chambers, J. (1992). Linear models, eds. *JM Chambers and TJ Hastie, Wadsworth & Brooks/Cole*.
Chamsaz, M., Perai, A., Asadpour, S. & Hosseini Shahidi, R. (2011). Comparison of the 3-phase segmented linear regression and artificial neural network models to predict broiler hatchability. *Journal of Applied Poultry Research* 20(4): 447-453.
Chanchai, W., Chanpongsang, S. & Chaiyabutr, N. (2010). Effects of misty-fan cooling and supplemental rbST on rumen function and milk production of crossbred Holstein cattle during early, mid and late lactation in a tropical environment. *Animal science journal* 81(2): 230-239.
Chaudhari, B. K. & Singh, M. (2015). Relationship between udder, skin and milk temperature in lactating Murrah buffaloes during the hot-humid season. *Buffalo Bulletin* 34(2): 181-188.
Christopherson, R. & Kennedy, P. (1983). Effect of the thermal environment on digestion in ruminants. *Canadian Journal of Animal Science* 63(3): 477-496.
Church, J. S., Hegadoren, P. R., Paetkau, M. J., Miller, C. C., Regev-Shoshani, G., Schaefer, A. L. & Schwartzkopf-Genswein, K. S. (2014). Influence of environmental factors on infrared eye temperature measurements in cattle. *Res Vet Sci* 96(1): 220-226.
Coates, D., Mannetje, L. & Seifert, G. (1987). Reproductive performance and calf growth to weaning of Hereford and Belmont Red cattle in subtropical, subcoastal Queensland. *Australian Journal of Experimental Agriculture* 27(1): 1-10.
Collier, R., Annen, E., Armstrong, D. & Wolfgram, A. (2003). Evaluation of two evaporative cooling systems for dairy cattle under semi-arid conditions. *J. Anim. Sci., 2003a* 81: 18.
Collier, R., Dahl, G. & VanBaale, M. (2006). Major advances associated with environmental effects on dairy cattle. *Journal of Dairy Science* 89(4): 1244-1253.
Cook, N., Bennett, T. & Nordlund, K. (2005). Monitoring indices of cow comfort in free-stall-housed dairy herds. *Journal of Dairy Science* 88(11): 3876-3885.
Cook, N., Mentink, R., Bennett, T. & Burgi, K. (2007a). The effect of heat stress and lameness on time budgets of lactating dairy cows. *Journal of Dairy Science* 90(4): 1674-1682.
Cook, N. B., Mentink, R. L., Bennett, T. B. & Burgi, K. (2007b). The effect of heat stress and lameness on time budgets of lactating dairy cows. *J Dairy Sci* 90(4): 1674-1682.
Costa, N., Accioly, J. & Cake, M. (2003). Determining critical atmospheric ammonia levels for cattle, sheep and goats–a literature review. *Meat & Livestock Australia Ltd: North Sydney*.
Cummins, K. (1998). Bedding plays role in heat abatement. *Dairy Herd Manage* 35(6): 20.
Das, R., Sailo, L., Verma, N., Bharti, P. & Saikia, J. (2016). Impact of heat stress on health and performance of dairy animals: A review. *Veterinary world* 9(3): 260.
Daskalov, P. I., Arvanitis, K. G., Pasgianos, G. D. & Sigrimis, N. A. (2006). Non-linear Adaptive Temperature and Humidity Control in Animal Buildings. *Biosystems Engineering* 93(1): 1-24.
Davis, S. & Mader, T. L. (2003). Adjustments for wind speed and solar radiation to the temperature-humidity index. *Nebraska Beef Cattle Reports*: 224.
DeShazer, J. A., Hahn, G. L. & Xin, H. (2009).Basic principles of the thermal environment and livestock energetics. In *Livestock Energetics and Thermal Environment Management*, 1-22: American Society of Agricultural and Biological Engineers.
Dhuyvetter, K., Kastens, T., Brouk, M., Smith, J. & Harner III, J. (2000).Economics of cooling cows. In *Proc. Heart of America Dairy Management Conference, St. Joseph, MO*, 56-71.
Dikmen, S., Alava, E., Pontes, E., Fear, J., Dikmen, B., Olson, T. & Hansen, P. (2008). Differences in thermoregulatory ability between slick-haired and wild-type lactating Holstein cows in response to acute heat stress. *Journal of Dairy Science* 91(9): 3395-3402.
Dikmen, S. & Hansen, P. J. (2009). Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? *J Dairy Sci* 92(1): 109-116.
Do Amaral, B., Connor, E., Tao, S., Hayen, J., Bubolz, J. & Dahl, G. (2009). Heat-stress abatement during the dry period: Does cooling improve transition into lactation? *Journal of Dairy Science* 92(12): 5988-5999.

Do Amaral, B., Connor, E., Tao, S., Hayen, M., Bubolz, J. & Dahl, G. (2011). Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. *Journal of Dairy Science* 94(1): 86-96.

Do Amaral, B., Hayen, J., Connor, E., Tao, S. & Dahl, G. (2008). Heat stress abatement for dry cows: Does cooling improve transition into lactation? *Journal of Dairy Science* 91: 379.

Dobson, A. J. & Barnett, A. (2008). *An introduction to generalized linear models.* CRC press.

Eigenberg, R., Brown-Brandl, T. & Nienaber, J. (2007). Development of a livestock weather safety monitor for feedlot cattle. *Applied Engineering in Agriculture* 23(5): 657-660.

Eigenberg, R., Brown-Brandl, T., Nienaber, J. A. & Hahn, G. L. (2005a). Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, Part 2: Predictive relationships. *Biosystems Engineering* 91(1): 111-118.

Eigenberg, R., Hahn, G., Nienaber, J., Brown-Brandl, T. & Spiers, D. (2000). Development of a new respiration rate monitor for cattle. *Transactions of the ASAE* 43(3): 723.

Eigenberg, R. A., Brown-Brandl, T. M. & Nienaber, J. A. (2008). Sensors for dynamic physiological measurements. *Computers and Electronics in Agriculture* 62(1): 41-47.

Eigenberg, R. A., Brown-Brandl, T. M., Nienaber, J. A. & Hahn, G. L. (2005b). Dynamic Response Indicators of Heat Stress in Shaded and Non-shaded Feedlot Cattle, Part 2: Predictive Relationships. *Biosystems Engineering* 91(1): 111-118.

Ferreira, F., Gennari, R., Dahl, G. & De Vries, A. (2016). Economic feasibility of cooling dry cows across the United States. *Journal of Dairy Science* 99(12): 9931-9941.

Frazzi, E., Calamari, L., Calegari, F. & Stefanini, L. (2000). Behavior of dairy cows in response to different barn cooling systems. *Transactions of the ASAE* 43(2): 387.

Gantner, V., Mijić, P., Kuterovac, K., Solić, D. & Gantner, R. (2011). Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo* 61(1): 56.

García-Ispierto, I., López-Gatius, F., Bech-Sabat, G., Santolaria, P., Yániz, J., Nogareda, C., De Rensis, F. & López-Béjar, M. (2007). Climate factors affecting conception rate of high producing dairy cows in northeastern Spain. *Theriogenology* 67(8): 1379-1385.

Garcia-Ispierto, I., Lopez-Gatius, F., Bech-Sabat, G., Santolaria, P., Yaniz, J. L., Nogareda, C., De Rensis, F. & Lopez-Bejar, M. (2007a). Climate factors affecting conception rate of high producing dairy cows in northeastern Spain. *Theriogenology* 67(8): 1379-1385.

Garcia-Ispierto, I., Lopez-Gatius, F., Santolaria, P., Yaniz, J. L., Nogareda, C. & Lopez-Bejar, M. (2007b). Factors affecting the fertility of high producing dairy herds in northeastern Spain. *Theriogenology* 67(3): 632-638.

Garner, J., Douglas, M., Williams, S., Wales, W., Marett, L., DiGiacomo, K., Leury, B. & Hayes, B. (2017). Responses of dairy cows to short-term heat stress in controlled-climate chambers. *Animal Production Science* 57(7): 1233-1241.

Gaughan, J., Goopy, J. & Spark, J. (2002). Excessive heat load index for feedlot cattle. *Sydney: MLA*.

Gaughan, J., Mader, T. & Gebremedhin, K. (2012).Rethinking heat index tools for livestock. In *Environmental physiology of Livestock*, 243-265: Wiley-Blackwell Chichester.

Gaughan, J., Mader, T. L., Holt, S. & Lisle, A. (2008a). A new heat load index for feedlot cattle. *Faculty Papers and Publications in Animal Science*: 613.

Gaughan, J. B., Mader, T. L., Holt, S. M. & Lisle, A. (2008b). A new heat load index for feedlot cattle. *J Anim Sci* 86(1): 226-234.

Gebremedhin, K. G., Wu, B. & Perano, K. (2016). Modeling conductive cooling for thermally stressed dairy cows. *Journal of Thermal Biology* 56: 91-99.

Ghavi Hossein-Zadeh, N., Mohit, A. & Azad, N. (2013). Effect of temperature-humidity index on productive and reproductive performances of Iranian Holstein cows. *Iranian Journal of Veterinary Research* 14(2): 106-112.

Givens, R. (1965). Height of artificial shades for cattle in the Southeast. *Transactions of the ASAE* 8(3): 312-0313.

Gorniak, T., Meyer, U., Sudekum, K. H. & Danicke, S. (2014). Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate. *Arch Anim Nutr* 68(5): 358-369.

Grant, R. (2012). Economic Benefits of Improved Cow Comfort. *Novus Int. St. Charles, MO*.

Green, J. A. (2011). The heart rate method for estimating metabolic rate: review and recommendations. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 158(3): 287-304.

Gresham, D. (2018).Farmgate milk price. Dairy Australia.

Hagen, K., Langbein, J., Schmied, C., Lexer, D. & Waiblinger, S. (2005). Heart rate variability in dairy cows—influences of breed and milking system. *Physiology & behavior* 85(2): 195-204.

Hahn, G. (1976). Shelter engineering for cattle and other domestic animals. *Progress in biometeorology. Division B. Progress in animal biometeorology*.

Hahn, G. (1999). Dynamic responses of cattle to thermal heat loads. *Journal of Animal Science* 77: 10.

Hahn, G., Mader, T., Gaughan, J., Hu, Q. & Nienaber, J. (1999). Heat waves and their impacts on feedlot cattle. *ICB11* 1.

Hahn, L., Kelly, C. F., McKillop, A. & Bond, T. (1962). relation of roof size to temperatures of irradiated metal roof sections exposed to forced convection. *ARS-42*.

Hale, K. D., Weigent, D. A., Gauthier, D. K., Hiramoto, R. N. & Ghanta, V. K. (2003). Cytokine and hormone profiles in mice subjected to handling combined with rectal temperature measurement stress and handling only stress. *Life sciences* 72(13): 1495-1508.

Hamada, T. (1971). Estimation of lower critical temperatures for dry and lactating dairy cows. *Journal of Dairy Science* 54(11): 1704-1705.

Hammami, H., Bormann, J., M'Hamdi, N., Montaldo, H. H. & Gengler, N. (2013). Evaluation of heat stress effects on production traits and somatic cell score of Holsteins in a temperate environment. *J Dairy Sci* 96(3): 1844-1855.

Hansen, P. (2007). Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. *Theriogenology* 68: S242-S249.

Henry, A., Arunachalam, S., Sivakumar, T. & Jagatheesan, P. (2014). Correlation of temperature humidity index with milk yield of crossbred jersey cows. *Indian Journal of Field Veterinarians (The)* 9(4): 74-77.

Herbut, P. & Angrecka, S. (2012). Forming of temperature-humidity index (THI) and milk production of cows in the free-stall barn during the period of summer heat. *Animal Science Papers & Reports* 30(4).

Herbut, P. & Angrecka, S. (2018). Relationship between THI level and dairy cows' behaviour during summer period. *Italian Journal of Animal Science* 17(1): 226-233.

Hermans, G., Ipema, A., Stefanowska, J. & Metz, J. (2003). The effect of two traffic situations on the behavior and performance of cows in an automatic milking system. *Journal of Dairy Science* 86(6): 1997-2004.

Hillerton, J., Dearing, J., Dale, J., Poelarends, J., Neijenhuis, F., Sampimon, O., Miltenburg, J. & Fossing, C. (2004). Impact of automatic milking on animal health. *Automatic Milking, a better understanding. Meijering A, Hogeveen H, De Koning CJAM (Eds.), Wageningen Academic Publishers, The Netherlands*: 125-134.

Hillman, P., Lee, C. & Willard, S. (2005). Body temperature versus microclimate selection in heat stressed dairy cows. *Trans. Am. Soc. Agric. Eng., 2003b* 48(2): 795.

Hoffmann, I. (2010). Climate change and the characterization, breeding and conservation of animal genetic resources. *Animal genetics* 41(s1): 32-46.

Hogeveen, H., Ouweltjes, W., De Koning, C. & Stelwagen, K. (2001). Milking interval, milk production and milk flow-rate in an automatic milking system. *Livestock Science* 72(1): 157-167.

Huang, C., Tsuruta, S., Bertrand, J., Misztal, I., Lawlor, T. & Clay, J. (2008). Environmental effects on conception rates of Holsteins in New York and Georgia. *Journal of Dairy Science* 91(2): 818-825.

Igono, M., Bjotvedt, G. & Sanford-Crane, H. (1992). Environmental profile and critical temperature effects on milk production of Holstein cows in desert climate. *International Journal of Biometeorology* 36(2): 77-87.

Ji, B., Banhazi, T., Ghahramani, A., Les, B., Wang, C. & Li, B. (Submitted-a). Modelling of heat stress in a robotic dairy farm. Part 1: Thermal comfort indices as the indicators of production loss. *Biosystems Engineering*.

Ji, B., Banhazi, T., Ghahramani, A., Les, B., Wang, C. & Li, B. (Submitted-b). Modelling of heat stress in a robotic dairy farm. Part 2: Identify of the specific thresholds with production factors. *Biosystems Engineering*.

Ji, B., Banhazi, T., Ghahramani, A., Les, B., Wang, C. & Li, B. (Submitted-c). Modelling of heat stress in a robotic dairy farm. Part 3: Animal behaviour and milking performance. *Biosystems Engineering*.

Ji, B., Banhazi, T., Perano, K., Ghahramani, A., Bowtell, L., Wang, C. & Li, B. (Submitted-d). A review on measuring, assessing and mitigating heat stress of housed dairy cow with precision livestock farming techniques. *Biosystems Engineering*.

John, A., Clark, C., Freeman, M., Kerrisk, K., Garcia, S. & Halachmi, I. (2016). Milking robot utilization, a successful precision livestock farming evolution. *animal* 10(9): 1484-1492.

Johnson, H. & Ragsdale, A. (1963). *Temperature-humidity effects including influence of acclimation in feed and water consumption of Holstein cattle.*

Johnson, H., Ragsdale, A., Berry, I. & Shanklin, M. D. (1963). Temperature-humidity effects including influence of acclimation in feed and water consumption of Holstein cattle. *Mo. Agric. Exp. Stn. Bull* 846.

Johnson, H. D. (1965). Environmental temperature and lactation (with special reference to cattle). *International Journal of Biometeorology* 9(2): 103-116.

Johnson, H. D. (1987).Bioclimatology and the Adaptation of Livestock.

Kadzere, C., Murphy, M., Silanikove, N. & Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science* 77(1): 59-91.
Kanjanapruthipong, J., Junlapho, W. & Karnjanasirm, K. (2015a). Feeding and lying behavior of heat-stressed early lactation cows fed low fiber diets containing roughage and nonforage fiber sources. *J Dairy Sci* 98(2): 1110-1118.
Kanjanapruthipong, J., Junlapho, W. & Karnjanasirm, K. (2015b). Feeding and lying behavior of heat-stressed early lactation cows fed low fiber diets containing roughage and nonforage fiber sources. *Journal of Dairy Science* 98(2): 1110-1118.
Kendall, P. E., Nielsen, P. P., Webster, J. R., Verkerk, G. A., Littlejohn, R. P. & Matthews, L. R. (2006). The effects of providing shade to lactating dairy cows in a temperate climate. *Livestock Science* 103(1-2): 148-157.
Ketelaar-de Lauwere, C., Hendriks, M., Zondag, J., Ipema, A., Metz, J. & Noordhuizen, J. (2000). Influence of routing treatments on cows' visits to an automatic milking system, their time budget and other behaviour. *Acta Agriculturae Scandinavica, Section A-Animal Science* 50(3): 174-183.
Klaas, I. C., Rousing, T., Fossing, C., Hindhede, J. & Sorensen, J. (2003). Is lameness a welfare problem in dairy farms with automatic milking systems? *ANIMAL WELFARE-POTTERS BAR THEN WHEATHAMPSTEAD-* 12(4): 599-604.
Kong, E., Tong, H. & Xia, Y. (2010). Statistical modelling of nonlinear long-term cumulative effects. *Statistica Sinica*: 1097-1123.
Kuczynski, T., Blanes-Vidal, V., Li, B., Gates, R. S., Alencar Naas, I. d., Moura, D. J., Berckmans, D. & Banhazi, T. M. (2011). Impact of global climate change on the health, welfare and productivity of intensively housed livestock. *International Journal of Agricultural and Biological Engineering* 4(2): 1-22.
Kumar, A. & Hancke, G. P. (2015). A zigbee-based animal health monitoring system. *IEEE sensors Journal* 15(1): 610-617.
Lanham, J., Coppock, C., Milam, K., Labore, J., Nave, D., Stermer, R. & Brasington, C. (1986). Effects of drinking water temperature on physiological responses of lactating Holstein cows in summer. *Journal of Dairy Science* 69(4): 1004-1012.
LCI (1970).Patterns of transit losses. (Ed I. Livestock Conservation). Omaha, NE.
LeBlanc, S. (2016). 0034 Opportunities and challenges for herd health and reproduction with robotic milking. *Journal of Animal Science* 94(suppl_5): 16-17.
Lee, D. H. K. (1953). Manual of field studies on the heat tolerance of domestic animals. *Manual of field studies on the heat tolerance of domestic animals.* (38).
Legates, J., Farthing, B., Casady, R. & Barrada, M. (1991). Body temperature and respiratory rate of lactating dairy cattle under field and chamber conditions. *Journal of Dairy Science* 74(8): 2491-2500.
Legrand, A., Schütz, K. & Tucker, C. (2011). Using water to cool cattle: Behavioral and physiological changes associated with voluntary use of cow showers. *Journal of Dairy Science* 94(7): 3376-3386.
Lehmann, E. L. & Romano, J. P. (2006). *Testing statistical hypotheses.* Springer Science & Business Media.
Lemerle, C. & Goddard, M. (1986). Assessment of heat stress in dairy cattle in Papua New Guinea. *Tropical animal health and production* 18(4): 232-242.
Li, S., Gebremedhin, K. G., Lee, C. N. & Collier, R. J. (2009).Evaluation of thermal stress indices for cattle. In *2009 Reno, Nevada, June 21-June 24, 2009*, 1: American Society of Agricultural and Biological Engineers.
Lindgren, E. (2009). Validation of rumination measurement equipment and the role of rumination in dairy cow time budgets.
Maak, K. & von Storch, H. (1997). Statistical downscaling of monthly mean air temperature to the beginning of flowering of Galanthus nivalis L. in northern Germany. *International Journal of Biometeorology* 41(1): 5-12.
Mader, T. L., Davis, M. & Brown-Brandl, T. (2006). Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science* 84(3): 712-719.
Mader, T. L., Davis, M. S. & Gaughan, J. B. (2007). Effect of sprinkling on feedlot microclimate and cattle behavior. *Int J Biometeorol* 51(6): 541-551.
Mader, T. L., Johnson, L. J. & Gaughan, J. B. (2010). A comprehensive index for assessing environmental stress in animals. *J Anim Sci* 88(6): 2153-2165.
Maltz, E., Medini, N., Bercovitch, A., Parmet, Y., Halachmi, I., Antler, A. & Edan, Y. (2011).Predicting calving time of dairy cows by behaviour sensor. In *European Conference on Precision Livestock Farming (ed. C Lokhorst and D Berckmans)*, 464-475.
Marciniak, A. (2014). The use of temperature-humidity index (thi) to evaluate temperature-humidity conditions in freestall barns. *Journal of Central European Agriculture* 15(2): 73-83.
Martin, J., Harner, J. & Smith, J. (2012). Water system design considerations for modern dairies. *Kansas State University Extension*.

Mattachini, G., Riva, E. & Provolo, G. (2011). The lying and standing activity indices of dairy cows in free-stall housing. *Applied Animal Behaviour Science* 129(1): 18-27.

Matulla, C., Scheifinger, H., Menzel, A. & Koch, E. (2003). Exploring two methods for statistical downscaling of Central European phenological time series. *International Journal of Biometeorology* 48(2): 56-64.

McGovern, R. & Bruce, J. (2000). A model of the thermal balance for cattle in hot conditions. *Journal of Agricultural Engineering Research* 77(1): 81-92.

Mead, R. (2017). *Statistical methods in agriculture and experimental biology.* Chapman and Hall/CRC.

Mendiburu Delgado, F. d. (2009).Una herramienta de análisis estadístico para la investigación agrícola. Universidad Nacional de Ingeniería, Lima (Perú). Facultad de Ingeniería Industrial y de Sistemas. Sección de Postgrado.

Meyer, M., Smith, J., Harner, J., Shirley, J., Titgemeyer, E. & Brouk, M. (2002). Performance of lactating dairy cattle in three different cooling systems. *Applied Engineering in Agriculture* 18(3): 341.

Milani, M. R. M., Hense, A., Rahmani, E. & Ploeger, A. (2015). A Survey of the Relationship between Climatic Heat Stress Indices and Fundamental Milk Components Considering Uncertainty. *Climate* 3(4): 876-900.

Miron, J., Adin, G., Solomon, R., Nikbachat, M., Zenou, A., Shamay, A., Brosh, A. & Mabjeesh, S. (2008). Heat production and retained energy in lactating cows held under hot summer conditions with evaporative cooling and fed two rations differing in roughage content and in vitro digestibility. *animal* 2(6): 843-848.

Miron, J., Adin, G., Solomon, R., Nikbachat, M., Zenou, A., Yosef, E., Brosh, A., Shabtay, A., Asher, A. & Gacitua, H. (2010). Effects of feeding cows in early lactation with soy hulls as partial forage replacement on heat production, retained energy and performance. *Animal feed science and technology* 155(1): 9-17.

Moallem, U., Altmark, G., Lehrer, H. & Arieli, A. (2010). Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. *Journal of Dairy Science* 93(7): 3192-3202.

Mollenhorst, H., Hidayat, M., Van den Broek, J., Neijenhuis, F. & Hogeveen, H. (2011). The relationship between milking interval and somatic cell count in automatic milking systems. *Journal of Dairy Science* 94(9): 4531-4537.

Moore, A. D. & Ghahramani, A. (2014). Climate change and broadacre livestock production across southern Australia. 3. Adaptation options via livestock genetic improvement. *Animal Production Science* 54(2): 111-124.

Moran, D. S., Pandolf, K. B., Shapiro, Y., Heled, Y., Shani, Y., Mathew, W. & Gonzalez, R. (2001). An environmental stress index (ESI) as a substitute for the wet bulb globe temperature (WBGT). *Journal of Thermal Biology* 26(4): 427-431.

Moretti, R., Biffani, S., Chessa, S. & Bozzi, R. (2017). Heat stress effects on Holstein dairy cows' rumination. *animal* 11(12): 2320-2325.

Morton, J., Tranter, W., Mayer, D. & Jonsson, N. (2007a). Effects of environmental heat on conception rates in lactating dairy cows: Critical periods of exposure. *Journal of Dairy Science* 90(5): 2271-2278.

Morton, J. M., Tranter, W. P., Mayer, D. G. & Jonsson, N. N. (2007b). Effects of environmental heat on conception rates in lactating dairy cows: critical periods of exposure. *J Dairy Sci* 90(5): 2271-2278.

Mote, S., Chauhan, D. & Ghosh, N. (2016). Effect of environment factors on milk production and lactation length under different seasons in crossbred cattle. *Indian Journal of Animal Research* 50(2): 175-180.

Muggeo, V. M. (2008). Segmented: an R package to fit regression models with broken-line relationships. *R news* 8(1): 20-25.

Nabenishi, H., Ohta, H., Nishimoto, T., Morita, T., Ashizawa, K. & Tsuzuki, Y. (2011). Effect of the temperature-humidity index on body temperature and conception rate of lactating dairy cows in southwestern Japan. *Journal of Reproduction and Development* 57(4): 450-456.

Nienaber, J. & Hahn, G. (2007). Livestock production system management responses to thermal challenges. *International Journal of Biometeorology* 52(2): 149-157.

Nonnecke, B., Foote, M., Miller, B., Fowler, M., Johnson, T. & Horst, R. (2009). Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of preruminant calves. *Journal of Dairy Science* 92(12): 6134-6143.

O'Neill, B. (2018).Average Electricity Costs per kWh. CANSTAR BLUE.

Oetting, E., Spain, J. & Sampson, J. (2002). The effects of strategic cooling on thermal balance of late gestation dairy cows. *J. Dairy Sci* 85(Suppl 1): 309.

Olson, T., Lucena, C., Chase, C. & Hammond, A. (2003). Evidence of a major gene influencing hair length and heat tolerance in cattle. *Journal of Animal Science* 81(1): 80-90.

Ortiz, X., Smith, J., Rojano, F., Choi, C., Bruer, J., Steele, T., Schuring, N., Allen, J. & Collier, R. J. (2015a). Evaluation of conductive cooling of lactating dairy cows under controlled environmental conditions. *Journal of Dairy Science* 98(3): 1759-1771.

Ortiz, X. A., Smith, J. F., Rojano, F., Choi, C. Y., Bruer, J., Steele, T., Schuring, N., Allen, J. & Collier, R. J. (2015b). Evaluation of conductive cooling of lactating dairy cows under controlled environmental conditions. *J Dairy Sci* 98(3): 1759-1771.

Österman, S. & Redbo, I. (2001). Effects of milking frequency on lying down and getting up behaviour in dairy cows. *Applied Animal Behaviour Science* 70(3): 167-176.

Overton, M., Sischo, W., Temple, G. & Moore, D. (2002). Using time-lapse video photography to assess dairy cattle lying behavior in a free-stall barn. *Journal of Dairy Science* 85(9): 2407-2413.

Pastell, M. & Madsen, H. (2008). Application of CUSUM charts to detect lameness in a milking robot. *Expert Systems with Applications* 35(4): 2032-2040.

Penry, J., Crump, P., Hernandez, L. & Reinemann, D. (2018). Association of milking interval and milk production rate in an automatic milking system. *Journal of Dairy Science* 101(2): 1616-1625.

Perano, Binxin Wu, Kifle G. Gebremedhin & Wright., P. (2017).Economic Returns for Different Cooling Systems for Dairy Cattle. . In *Animal Environment and Welfare, Oct 23-26, 2017*.

Perano, K. M., Usack, J. G., Angenent, L. T. & Gebremedhin, K. G. (2015a). Production and physiological responses of heat-stressed lactating dairy cattle to conductive cooling. *J Dairy Sci* 98(8): 5252-5261.

Perano, K. M., Usack, J. G., Angenent, L. T. & Gebremedhin, K. G. (2015b). Production and physiological responses of heat-stressed lactating dairy cattle to conductive cooling. *Journal of Dairy Science* 98(8): 5252-5261.

Pereyra, A. V. G., May, V. M., Catracchia, C. G., Herrero, M. A., Flores, M. C. & Mazzini, M. (2010). INFLUENCE OF WATER TEMPERATURE AND HEAT STRESS ON DRINKING
WATER INTAKE IN DAIRY COWS. *Chilean Journal of Agricultural Research* 70(2): 328-336.

Phillips, C. (2008). *The welfare of animals: the silent majority.* Springer Science & Business Media.

Phillips, C. J. (2018). *Principles of cattle production.* CABI.

Pinzón-Sánchez, C., Cabrera, V. & Ruegg, P. (2011). Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. *Journal of Dairy Science* 94(4): 1873-1892.

Pohl, A., Heuwieser, W. & Burfeind, O. (2014). Assessment of milk temperature measured by automatic milking systems as an indicator of body temperature and fever in dairy cows. *Journal of Dairy Science* 97(7): 4333-4339.

Pollard, B., Dwyer, M., Fitzgerald, A., Gentry, P., Henderson, D. & Collier, R. (2004).Effects of ambient temperature and solar radiation on skin evaporative water loss in dairy cattle. In *Journal of Dairy Science*, Vol. 87, 198-198: AMER DAIRY SCIENCE ASSOC 1111 N DUNLAP AVE, SAVOY, IL 61874 USA.

Provolo, G. & Riva, E. (2009). One year study of lying and standing behaviour of dairy cows in a frestall barn in Italy. *Journal of Agricultural Engineering* 40(2): 27-34.

Qi, Q. & Deng, S. (2009). Multivariable control of indoor air temperature and humidity in a direct expansion (DX) air conditioning (A/C) system. *Building and Environment* 44(8): 1659-1667.

R Development Core Team (2017).R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Radoń, J., Bieda, W., Lendelová, J. & Pogran, Š. (2014). Computational model of heat exchange between dairy cow and bedding. *Computers and Electronics in Agriculture* 107: 29-37.

Renaudeau, D., Collin, A., Yahav, S., De Basilio, V., Gourdine, J. & Collier, R. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *animal* 6(5): 707-728.

Renquist, B., Oltjen, J., Sainz, R. & Calvert, C. (2006). Effects of age on body condition and production parameters of multiparous beef cows. *Journal of Animal Science* 84(7): 1890-1895.

Rensis, F. D. & Scaramuzzi, R. J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow—a review. *Theriogenology* 60(6): 1139-1151.

Rhoad, A. O. (1944). The Iberia heat tolerance test for cattle. *Tropical Agriculture* 21(9): 162-164.

Robertshaw, D. & Vercoe, J. (1980). Scrotal thermoregulation of the bull (Bos sp.). *Crop and Pasture Science* 31(2): 401-407.

Rocklov, J., Barnett, A. G. & Woodward, A. (2012). On the estimation of heat-intensity and heat-duration effects in time series models of temperature-related mortality in Stockholm, Sweden. *Environmental Health* 11(1): 23.

Rodenburg, J. (2012).The impact of robotic milking on milk quality, cow comfort and labor issues. In *Natl. Mastitis Counc. Annu. Meet. Proc. St. Pete Beach, FL*, 126-137: Natl. Mastitis Counc., Madison, WI.

Rodenburg, J. & Wheeler, B. (2002).Strategies for incorporating robotic milking into North American herd management. In *Proceedings of the first North American Conference on Robotic Milking, Toronto, Canada, pp III-18–III-32*.

Roenfeldt, S. (1998). You can't afford to ignore heat stress. *Dairy manage* 35(5): 6-12.

Roland, L., Drillich, M., Klein-Jobstl, D. & Iwersen, M. (2016). Invited review: Influence of climatic conditions on the development, performance, and health of calves. *J Dairy Sci* 99(4): 2438-2452.

Rudder, T., Bean, K., Lapworth, J. & Seifert, G. (1976).Commercial evaluation of a tropical breed developed experimentally. In *Proceedings of the Australian Society of Animal Production*, Vol. 11, 89-92.

Rungruang, S., Collier, J., Rhoads, R., Baumgard, L., de Veth, M. & Collier, R. J. (2014). A dose-response evaluation of rumen-protected niacin in thermoneutral or heat-stressed lactating Holstein cows. *Journal of Dairy Science* 97(8): 5023-5034.

Scharf, B., Johnson, J., Weaber, R. & Spiers, D. (2012). Utilizing laboratory and field studies to determine physiological responses of cattle to multiple environmental stressors. *Journal of Thermal Biology* 37(4): 330-338.

Schmidt, S., Bowers, S., Dickerson, T., Graves, K. & Willard, S. (2004).Assessments of udder temperature gradients pre-and post-milking relative to milk production in Holstein cows as determined by digital infrared thermography. In *Journal of Dairy Science*, Vol. 87, 460-461: ELSEVIER SCIENCE INC 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA.

Schuller, L. K., Burfeind, O. & Heuwieser, W. (2014). Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature-humidity index thresholds, periods relative to breeding, and heat load indices. *Theriogenology* 81(8): 1050-1057.

Schütz, K. E., Rogers, A. R., Cox, N. R. & Tucker, C. B. (2009). Dairy cows prefer shade that offers greater protection against solar radiation in summer: Shade use, behaviour, and body temperature. *Applied Animal Behaviour Science* 116(1): 28-34.

Scott, I., Johnson, H. & Hahn, G. (1983). Effect of programmed diurnal temperature cycles on plasma thyroxine level, body temperature, and feed intake of Holstein dairy cows. *International Journal of Biometeorology* 27(1): 47-62.

Seedorf, J., Hartung, J., Schröder, M., Linkert, K., Pedersen, S., Takai, H., Johnsen, J. O., Metz, J., Koerkamp, P. G. & Uenk, G. (1998). Temperature and moisture conditions in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research* 70(1): 49-57.

Segnalini, M., Bernabucci, U., Vitali, A., Nardone, A. & Lacetera, N. (2013). Temperature humidity index scenarios in the Mediterranean basin. *Int J Biometeorol* 57(3): 451-458.

Segnalini, M., Nardone, A., Bernabucci, U., Vitali, A., Ronchi, B. & Lacetera, N. (2011). Dynamics of the temperature-humidity index in the Mediterranean basin. *Int J Biometeorol* 55(2): 253-263.

Sevi, A., Annicchiarico, G., Albenzio, M., Taibi, L., Muscio, A. & Dell'Aquila, S. (2001). Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. *Journal of Dairy Science* 84(3): 629-640.

Seyfi, S. U. (2013). Hourly and seasonal variations in the area preferences of dairy cows in freestall housing. *J Dairy Sci* 96(2): 906-917.

Shoshani, E. & Hetzroni, A. (2013). Optimal barn characteristics for high-yielding Holstein cows as derived by a new heat-stress model. *animal* 7(01): 176-182.

Siegmund, D. O. & Zhang, H. (1994). Confidence regions in broken line regression. *Lecture Notes-Monograph Series*: 292-316.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science* 67: 1-18.

Silanikove, N., Maltz, E., Halevi, A. & Shinder, D. (1997). Metabolism of Water, Sodium, Potassium, and Chlorine by High Yielding Dairy Cows at the Onset of Lactation 1, 2. *Journal of Dairy Science* 80(5): 949-956.

Silva, R. G. d. & Maia, A. S. C. (2011). Evaporative cooling and cutaneous surface temperature of Holstein cows in tropical conditions. *Revista Brasileira de Zootecnia* 40(5): 1143-1147.

Silva, R. G. d., Morais, D. A. E. F. & Guilhermino, M. M. (2007). Evaluation of thermal stress indexes for dairy cows in tropical regions. *Revista Brasileira de Zootecnia* 36(4): 1192-1198.

Smith, J., Bradford, B., Harner, J., Potts, J., Allen, J., Overton, M., Ortiz, X. & Collier, R. J. (2016). Short communication: Effect of cross ventilation with or without evaporative pads on core body temperature and resting time of lactating cows. *Journal of Dairy Science* 99(2): 1495-1500.

Smith, J., Harner, J., de Haro Marti, M., Sheffield, R., Zulovich, J., Pohl, S., Fulhage, C., Nicoli, D., Hetchler, B. & Jacobson, L. (2007).Comprehensive evaluation of a low-profile cross-ventilated freestall barn. In *Proceedings of the 8th Western Dairy Management Conference, America*, 127-147.

Soldatos, A. G., Arvanitis, K. G., Daskalov, P. I., Pasgianos, G. D. & Sigrimis, N. A. (2005). Nonlinear robust temperature–humidity control in livestock buildings. *Computers and Electronics in Agriculture* 49(3): 357-376.

Soriani, N., Panella, G. & Calamari, L. (2013). Rumination time during the summer season and its relationships with metabolic conditions and milk production. *Journal of Dairy Science* 96(8): 5082-5094.

Soriani, N., Trevisi, E. & Calamari, L. (2012). Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. *Journal of Animal Science* 90(12): 4544-4554.

Spain, J. & Spiers, D. (1996). Effects of supplemental shade on thermoregulatory response of calves to heat challenge in a hutch environment. *Journal of Dairy Science* 79(4): 639-646.

Spain, J., Spiers, D. & Sampson, J. (2001).A study to compare nighttime cooling strategies on commercial dairy. In *Livestock Environment VI, Proceedings of the 6th International Symposium 2001*, 41: American Society of Agricultural and Biological Engineers.

Sparke, E., Young, B., Gaughan, J., Holt, S. & Goodwin, P. (2001). Heat load in feedlot cattle.

Speroni, M., Abeni, F., Capelletti, M., Migliorati, L. & Pirlo, G. (2003). Two years of experience with an automatic milking system. 2. Milk yield, milking interval and frequency. *Italian Journal of Animal Science* 2(sup1): 260-262.

Speroni, M., Pirlo, G. & Lolli, S. (2006). Effect of automatic milking systems on milk yield in a hot environment. *Journal of Dairy Science* 89(12): 4687-4693.

Spiers, D., Spain, J., Sampson, J. & Rhoads, R. (2004). Use of physiological parameters to predict milk yield and feed intake in heat-stressed dairy cows. *Journal of Thermal Biology* 29(7): 759-764.

Spurlin, K., Spiers, D., Ellersieck, M. & Spain, J. (2002). Effects of simultaneous evaluation of cooling strategies on production responses and intake behavior during heat challenge in dairy cattle. *J. Dairy Sci* 85(Suppl 1): 206.

St-Pierre, N., Cobanov, B. & Schnitkey, G. (2003). Economic losses from heat stress by US livestock industries1. *Journal of Dairy Science* 86: E52-E77.

Svennersten-Sjaunja, K., Berglund, I. & Pettersson, G. (2000).The milking process in an automatic milking system, evaluation of milk yield, teat condition and udder health. In *Robotic milking: Proceedings of the International Symposium held in Lelystad, The Netherlands, 17-19 August, 2000*, 277-288: Wageningen Pers.

Sylvestre, M. P. & Abrahamowicz, M. (2009). Flexible modeling of the cumulative effects of time-dependent exposures on the hazard. *Statistics in medicine* 28(27): 3437-3453.

Tao, S., Bubolz, J., Do Amaral, B., Thompson, I., Hayen, M., Johnson, S. & Dahl, G. (2011). Effect of heat stress during the dry period on mammary gland development. *Journal of Dairy Science* 94(12): 5976-5986.

Tarazón-Herrera, M., Huber, J., Santos, J., Mena, H., Nusso, L. & Nussio, C. (1999). Effects of bovine somatotropin and evaporative cooling plus shade on lactation performance of cows during summer heat stress. *Journal of Dairy Science* 82(11): 2352-2357.

Therneau, T., Atkinson, B. & Ripley, B. (2015). Recursive partitioning for classification, regression and survival trees. An implementation of most of the functionality of the 1984 book by Breiman, Friedman, Olshen and Stone. *Institute for Statistics and Mathematics*.

Thom, E. C. (1959). The discomfort index. *Weatherwise* 12(2): 57-61.

Thompson, I. & Dahl, G. (2012). Dry-period seasonal effects on the subsequent lactation. *The Professional Animal Scientist* 28(6): 628-631.

Thorat, B., Thombre, B. & Narwade, S. (2016). Studies on effect of climatic parameters on monthly lactation milk yields in Deoni cattle of Maharashtra, India. *Indian Journal of Animal Research* 50(1): 31-34.

Tucker, C. B., Rogers, A. R. & Schütz, K. E. (2008). Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. *Applied Animal Behaviour Science* 109(2-4): 141-154.

Turnpenny, J., McArthur, A., Clark, J. & Wathes, C. (2000a). Thermal balance of livestock: 1. A parsimonious model. *Agricultural and forest meteorology* 101(1): 15-27.

Turnpenny, J., Wathes, C., Clark, J. & McArthur, A. (2000b). Thermal balance of livestock: 2. Applications of a parsimonious model. *Agricultural and forest meteorology* 101(1): 29-52.

Urdaz, J., Overton, M., Moore, D. & Santos, J. (2006). Technical note: Effects of adding shade and fans to a feedbunk sprinkler system for preparturient cows on health and performance. *Journal of Dairy Science* 89(6): 2000-2006.

Uzal Seyfi, S. (2013). Seasonal variation of the lying and standing behavior indexes of dairy cattle at different daily time periods in free-stall housing. *Anim Sci J* 84(10): 708-717.

Van Overbeke, P., De Vogeleer, G., Brusselman, E., Pieters, J. & Demeyer, P. (2015). Development of a reference method for airflow rate measurements through rectangular vents towards application in naturally ventilated animal houses: Part 3: Application in a test facility in the open. *Computers and Electronics in Agriculture* 115: 97-107.

Vitali, A., Segnalini, M., Bertocchi, L., Bernabucci, U., Nardone, A. & Lacetera, N. (2009). Seasonal pattern of mortality and relationships between mortality and temperature-humidity index in dairy cows. *J Dairy Sci* 92(8): 3781-3790.

Wang, F. X., Shao, D. F., Li, S. L., Wang, Y. J., Azarfar, A. & Cao, Z. J. (2016). Effects of stocking density on behavior, productivity, and comfort indices of lactating dairy cows. *J Dairy Sci* 99(5): 3709-3717.

Wathes, C., Jones, C. & Webster, A. (1983). Ventilation, air hygiene and animal health. *Veterinary Record* 113(24): 554-559.

Wells, S., Trent, A., Marsh, W., McGovern, P. & Robinson, R. (1993). Individual cow risk factors for clinical lameness in lactating dairy cows. *Preventive veterinary medicine* 17(1-2): 95-109.

West, J. (2003). Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science* 86(6): 2131-2144.

West, J., Mullinix, B. & Bernard, J. (2003). Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. *Journal of Dairy Science* 86(1): 232-242.

West, J. W. (1999). Nutritional strategies for managing the heat-stressed dairy cow. *Journal of Animal Science* 77(suppl_2): 21-35.

Wheelock, J., Rhoads, R., VanBaale, M., Sanders, S. & Baumgard, L. (2010). Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science* 93(2): 644-655.

Wiersma, F. & Armstrong, D. (1989). Evaporative cooling dry cows for improved performance. *Arizona dairy newsletter-University of Arizona, Cooperative Extension Service (USA)*.

Willis, S., Black, J. & Banhazi, T. (2016).Estimation of Production Losses associated with Short Term Growth Rate Reduction and Sub-Optimal Thermal Conditions on Pig Farms Using Auspig Simulation Software. In *Asian Conference on Precision Livestock Farming (PLF-Asia 2016)*, Vol. 1, 45-52 (Eds G. Zhang, L. Zhao, C. Wang, W. Zheng, Q. Tong, D. Berckmans and K. Wang). Beijing, China: China Agricultural University.

Yousef, M. K. (1985). *Stress physiology in livestock. Volume III. Poultry.* CRC Press Inc.

Zimbelman, R., Rhoads, R., Rhoads, M., Duff, G., Baumgard, L. & Collier, R. (2009).A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. In *Proceedings of the Southwest Nutrition and Management Conference, Arizona*, Vol. 2627, 158168.

Zimbelman, R. J. C. R. B. (2007).Heat Stress Effects on Cattle: What We Know and What We Don’t Know. In *22nd Annual Southwest Nutrition & Management Conference*.