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VALIDATION OF AN AUTOMATED BEHAVIOR MONITORING COLLAR,
AND EVALUATION OF HEAT STRESS ON LACTATING DAIRY COW BEHAVIOR
WITH ACCESS TO A FREE CHOICE SOAKER

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Lori Nicole Grinter

Lexington, Kentucky

Director: Dr. Joao Costa, Assistant Professor of Animal Sciences

Lexington, Kentucky

2019

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ABSTRACT OF THESIS

VALIDATION OF AN AUTOMATED BEHAVIOR MONITORING COLLAR, AND EVALUATION OF HEAT STRESS ON LACTATING DAIRY COW BEHAVIOR WITH ACCESS TO A FREE CHOICE SOAKER

Precision dairy technologies (PDT) are becoming more accessible and are therefore becoming more common on commercial dairy farms and in dairy research. Prior to any use of PDT, one should understand the precision, accuracy and bias of the device by a validation studies before interpreting the behavior measurements. Thus, the objective of the first section of my thesis is to validate ruminating, feeding and resting measurements of a behavior monitoring collar used in the second section. Precision dairy technology is used in heat stress studies to compare behavior of cows exposed to different heat stress treatments or abatement strategies. Heat stress is an important issue to research because it negatively affects cow behavior, physiology, and therefore production in lactating dairy cows. The objective of the second section is to assess the ability of a free choice soaker to reduce heat stress measured utilizing PDT and compare use of a free choice to a soaker in addition to one of the two treatments 1) no mandatory soakings, or 2) two mandatory soakings.

KEYWORDS: precision dairy technology, heat abatement strategy, soaker

Lori Nicole Grinter
(Name of Student)

04/15/2019
Date

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To Grams

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	iii
LIST OF FIGURES	vi
LIST OF TABLES	vii
CHAPTER ONE: REVIEW OF LITERATURE.....	1
INTRODUCTION	2
HOW DOES HEAT STRESS AFFECT DAIRY COWS?.....	5
Physiological Changes of Heat Stressed Dairy Cows.....	5
Behavioral Changes	9
Cooling Strategies.....	13
VALIDATING PRECISION DAIRY TECHNOLOGY	20
Intra- and Inter-observer Variation	21
Precision, Accuracy, and Bias	22
Importance of Validating Precision Dairy Technology.....	24
CONCLUSIONS.....	30
CHAPTER TWO: TECHNICAL NOTE: VALIDATION OF A BEHAVIOR MONITORING COLLAR'S PRECISION AND ACCURACY TO MEASURE RUMINATION, FEEDING, AND RESTING TIME OF LACTATING DAIRY COWS	45
INTRODUCTION	46
MATERIALS AND METHODS.....	47
RESULTS	50
DISCUSSION	51
ACKNOWLEDGEMENTS.....	54
CHAPTER THREE: VOLUNTARY HEAT STRESS ABATEMENT SYSTEM FOR DAIRY COWS: DOES IT MITIGATE THE EFFECTS OF HEAT STRESS ON PHYSIOLOGY AND BEHAVIOR?	63
INTRODUCTION	64
MATERIALS AND METHODS.....	66
Data Collection	68
Statistical Analysis.....	70
RESULTS	72

Daily Soaker Use	73
Respiration Rate and Panting Score.....	74
Reticulo-Rumen Temperature.....	74
Rumination, Feeding, and Resting Behavior	74
Lying Time, Lying Bouts, and Steps	75
Milk Production	75
Temperature Humidity Index.....	75
DISCUSSION	77
CONCLUSION.....	80
ACKNOWLEDGEMENTS	80
CHAPTER FOUR: Summary of results:	99
CONCLUSIONS.....	100
FUTURE RESEARCH	100
LIST OF REFERENCES	102
VITAE.....	121

LIST OF FIGURES

Figure 1.1. Precision vs. accuracy.	32
Figure 2.1. Regression of rumination, feeding, and resting, comparing the behavior monitoring collar to visual observations during the 240 min observation.....	56
Figure 2.2. Bland-Altman plot illustrating agreement between the behavior monitoring collar and visual observations for ruminating, feeding, and resting	58
Figure 3.1. Experimental pen for dairy cattle	82
Figure 3.2. Individual variation in total daily frequency of voluntary soaker use.....	83
Figure 3.3. Percentage of voluntary soaker cycles soaked the corresponding areas of the cow	84
Figure 3.4. Behaviors after the end of each soaker cycle	85
Figure 3.5. Mean hourly soaker use of cows, with mean daily THI and mean daily THI + 5 h delay.....	86
Figure 3.6. Daily voluntary soaker use depicted against the respective day's mean daily temperature humidity index (THI).....	87
Figure 3.7. Mean displacement actions of cows (cow displaced another cow, or cow was displaced by another cow) for the voluntary soaker, by cow, with mean daily soaker use	88

LIST OF TABLES

Table 1.1. Panting score ethogram.....	33
Table 1.2 Validation studies of precision dairy technologies (PDT) that record ruminating, feeding or resting within the last 20 years.....	34
Table 2.1 Ethogram of behavior classification for visual observations.....	60
Table 2.2. Mean, minimum and maximum time of cows spent ruminating, feeding and resting, as recorded by visual observations and the behavior monitoring collar.....	61
Table 2.3. The results of the precision and accuracy test of rumination, feeding and resting behaviors between visual observations and the behavior monitoring collar	62
Table 3.1. Microclimate conditions of experimental pen	89
Table 3.2. Ethogram followed to define panting	90
Table 3.3. Ethogram used by observers for recording area of cow wet by soaker when recording voluntary soaker use at the pen.....	92
Table 3.4. Result of physiological, behavioral, and milk variables for cows from the mixed linear model	93
Table 3.5. Result of the regression of physiological, behavioral and milk variables for all cows with mean daily temperature humidity index and soaker use/d	95
Table 3.6. Physiological, behavioral, and milk variables used to observe the heat abatement qualities of the two treatments between cows.....	97

CHAPTER ONE:
REVIEW OF LITERATURE

INTRODUCTION

Heat stress is a condition affecting dairy cows, increasing with warmer environmental conditions (Kadzere et al., 2002; Anderson et al., 2013; Bernabucci et al., 2014). Dairy cows are homeothermic animals and therefore maintain their body temperature within a strict thermoregulatory range (West, 2003; Aggarwal and Upadhyay, 2013). When environmental conditions are within the thermo-neutral zone (indicated by McDowell (1972) as 5°C to 25°C), there are minimal associated metabolic costs to regulating the animals body temperature (West, 2003). Temperatures outside of the thermo-neutral zone cause physiological stress to the animal (Allen et al., 2013) because metabolic requirements increase: the animal pants and sweats, and behavioral changes occur to regulate body temperature (Collier et al., 1982). Behavioral changes as a result of heat stress include increased standing time, reduced lying time (Allen et al., 2015), reduced feeding time, reduced rumination time (Bernabucci et al., 2010; Soriani et al., 2013), and shade seeking (Tucker et al., 2008; Schütz et al., 2011).

Increasing relative humidity reduces the effectiveness of evaporative cooling, and high ambient temperatures negate the effectiveness of non-evaporative cooling methods (convection, radiation, conduction) (West, 2003). Dairy cow heat stress studies typically categorize environmental conditions by a temperature humidity index (THI) to incorporate both temperature and humidity using the following formula:

$$\text{THI} = (1.8 \times T^{\circ}\text{C} + 32) - (0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T^{\circ}\text{C} - 26),$$

T = ambient temperature (°C); RH = percentage of relative humidity (NOAA, 1976).

While “heat stress” is a widely used term (West, 2003), THI is used to describe and measure the magnitude of heat stress. Comfortable conditions for dairy cows are considered when THI is ≤ 70 (Armstrong, 1994). Temperature humidity index values of 72 to 75 are typically categorized as mild heat stress; with THI of 75 to 78, cows experience high levels of heat stress; and conditions $\text{THI} \geq 78$ are considered severe (Armstrong, 1994; Kadzere et al., 2002; Chase,

2006). While THI is the most commonly used heat stress indicator, it could be improved by including other environmental effects such as wind speed or solar radiation (Bohmanova et al., 2007). Temperature humidity index does however include air temperature and relative humidity, which are arguably the most critical and easiest environmental measurements to obtain and are highly correlated to production loss associated with heat stress (Ravagnolo et al., 2000; Bohmanova et al., 2007).

Heat stress studies record physiological and behavioral changes of cows to understand heat stress, for example to test or evaluate heat abatement between treatments. Physiological measures such as respiration rate (Schütz et al., 2008) and body temperature (Araki et al., 1984; Anderson et al., 2013) have been found to have a strong positive correlation to THI. Additionally, THI has a strong linear relationship to behavioral changes including; lying (negative), standing (positive) (Cook et al., 2007a; Tucker et al., 2008; Allen et al., 2015), DMI (negative) (Spiers et al., 2004; Rhoads et al., 2009; Soriani et al., 2013), and rumination (negative) (Soriani et al., 2013).

Milk production is reduced in dairy cows exposed to heat stress (e.g. Spiers et al., 2004; Soriani et al., 2013; Bernabucci et al., 2014). Milk production is negatively affected because of physiological changes (Schütz et al., 2011; Anderson et al., 2013), and behavioral changes (Kadzere et al., 2002; Tucker et al., 2008; Allen et al., 2015), such as reduced feed intake (West, 2003; Spiers et al., 2004). A reduction of milk yield from heat stress is an economic problem for the dairy industry, however this could be reduced via heat alleviation options (St-Pierre et al., 2003).

Considering the increasing temperatures extremes worldwide, strategies of heat abatement are important to research for the economy of the dairy industry. Options for heat abatement include use of shade, soaking cows (Tucker et al., 2008; Legrand et al., 2011; Schütz et al., 2011), or fans (Allen et al., 2015). Cooling strategies reduce heat stress; easing respiration

rate (Schütz et al., 2010; Min et al., 2015), reducing body temperature (Schütz et al., 2011), increasing lying time (Allen et al., 2015), increasing rumination time and increasing feeding time (Soriani et al., 2013).

Behavior monitoring precision dairy technology (PDT) such as collars, leg tags, or ear-tags can theoretically measure behavioral changes from heat stress such as rumination, feeding and resting time. Behavior monitoring PDT is also used to remotely supervise dairy cows, to maximize individual animal potential, enhance animal health and wellbeing (Bewley, 2010), and to improve heat detection (Dolecheck et al., 2015). Leg tags can weigh as little as 18 g, and ear-tags can weigh as little as 32 g (Borchers et al., 2016), therefore unlikely to cause discomfort or alter natural cow behavior when worn. Behavior monitoring PDT use built-in accelerometers (Borchers et al., 2016; Caja et al., 2016), microphones (Ambriz-Vilchis et al., 2015), or pressure sensors (Zehner et al., 2017) to measure and record behavior. Automated, continuous recording via PDT can be very useful, as it does not change cow behavior from human interaction or suffer from observer bias, as frequently found when using manual (visual) behavioral observation (Müller and Schrader, 2003). To ensure PDT devices record what is intended, validation of the device is paramount prior to any data interpretation to ensure that the obtained data can be deemed reliable. Validation studies for PDT compare behavior recorded by the technology to a known record of behavior the cow is performing (gold standard, for example by continuous observation) (Norton and Berckmans, 2017), and analyzing agreement between the two data sets. The purpose of validation studies is to analyze the precision, accuracy and bias, to ensure the imprecision, inaccuracy and bias are sufficiently small (Grubbs, 1973).

This review describes heat stress, beginning by defining effects of animal physiology (respiration rate and body temperature), and behavioral changes (standing and lying, and ruminating and feeding), and the effects the physiological and behavioral changes have on milk

production. Following, the review will cover heat abatement strategies of providing shade and soaking cows. Finally, because of the substantial use of PDT throughout, validation of PDT will be discussed; firstly methodology, and finishing with precision, accuracy and bias.

HOW DOES HEAT STRESS AFFECT DAIRY COWS?

When environmental conditions exceed the thermo-neutral zone ($\geq 25^{\circ}\text{C}$ or $\text{THI} \geq 70$) (McDowell, 1972; Armstrong, 1994), dairy cows begin heat stress; a state where metabolic requirements increase to thermo-regulate the animal (West, 2003; Allen et al., 2013). To support thermoregulation, cows increase respiration and panting with increasing temperatures (Rhoads et al., 2009; Schütz et al., 2010; Min et al., 2015). While cows are homeotherms – therefore they maintain body temperature within a narrow window to support cellular metabolic functions (Allen et al., 2013) – body temperature has been found to rise as a result of heat stress (Tucker et al., 2008; Schütz et al., 2010). Cows also change their behavior because of heat stress; cows have been observed to stand longer and lie less (Tucker et al., 2008; Anderson et al., 2013; Allen et al., 2015). Additionally, heat stress negatively affects feeding and rumination behaviors (Kadzere et al., 2002; Bernabucci et al., 2010; Soriani et al., 2013). Because of increased metabolic requirements and reduced feed intake, milk production and welfare are negatively affected by heat stress (West, 2003; Allen et al., 2013; Polsky and von Keyserlingk, 2017).

Physiological Changes of Heat Stressed Dairy Cows

Respiration and Panting

Respiration rate is increased during periods of heat stress (and to a further extent, panting) as a method to dissipate excess heat (Hahn, 1999). Panting is a method of respiratory heat loss that works by air moving over the moist surfaces of the respiratory tract, evaporating and cooling the cow, similar to how sweating cools (Robertshaw, 2006). An increase in panting

increases the speed of moving air and therefore increases evaporation and thus, cooling (Robertshaw, 2006). Respiration rate has been reported as the “best physiological indicator of heat stress in a production setting” by Brown-Brandl et al. (2005); claiming respiration rate has minimal or no delay, it is consistently affected in all conditions, and it is easy and cheap to record. Respiration rate is typically recorded by counting number of flank movements (Rhoads et al., 2009; Legrand et al., 2011; Min et al., 2015), however some studies have utilized automated respiration rate monitors (Brown-Brandl et al., 2005; Strutzke et al., 2018).

Panting observations should be taken alongside respiration rate when assessing heat stress in dairy cows (Gaughan et al., 2000). To record panting, previous studies have recorded panting score (see Table 1.1.) (Mader et al., 2006; Legrand et al., 2011), or simply recorded whether or not open mouth breathing was occurring during the respiration rate observation (Legrand et al., 2011). Panting is used by cows as a means of respiratory heat loss; it is estimated that when temperatures are $> 30^{\circ}\text{C}$, 15% of heat loss of lactating dairy cows is attributed to panting and 85% by evaporative heat loss via the skin (Maia and Loureiro, 2005).

Respiration rate has been reported to have a strong positive correlation to THI (Schütz et al., 2008) and ambient air temperature (Hahn, 1999; Brown-Brandl et al., 2005; Schütz et al., 2008). Additionally, Brown-Brandl et al. (2005) observed a strong positive correlation of respiration rate with an approximately 1 h lag of solar radiation (in a study comparing shade vs. no shade treatments). Gaughan et al. (2000) however found approximately a 2 h lag in respiration rate with air temperature when soakers and fans were used as cooling techniques, explaining that while respiration rate is correlated to air temperature, cooling technique must be considered. Regardless of cooling technique, several authors have used respiration rate as an assessment to compare heat abatement between treatments. Cows have been shown to have significantly higher respiration rates in treatments with no options of heat abatement than cows with options for heat abatement (e.g. Rhoads et al., 2009; Schütz et al., 2010; Min et al., 2015).

Schütz et al. (2011) did however propose respiration rate to be affected by exercise (walking distances in a pastoral setting). Cows walking further distances (2.0 vs. 0.3 km) exhibited higher respiration rates (as also illustrated by Mader et al. (2005) in addition to increased body temperature). Schütz et al. (2011) observed a reduction in respiration rate after cows ceased exercise and heat abatement treatment was applied, which was later discussed as a limitation of comparing cooling strategies between cows in different management conditions or cows performing exercise.

Respiration rate may be an effective measure of assessing heat stress in research (Rhoads et al., 2009; Schütz et al., 2010; Min et al., 2015) and future research should consider including it when assessing heat stress. Future research using respiration rate should additionally consider several measurements of respiration rate throughout the day to observe how the heat stress response of cows is affected.

Body Temperature

Body temperature is another variable used to assess heat stress in dairy cows. Body temperature can be measured in various ways; one of which is by rectal temperature (Bewley et al., 2008; Dikmen et al., 2013; Soriani et al., 2013). Internal temperature has also been recorded by automated temperature data loggers, inserted into abdominal cavities of steers (Brown-Brandl et al., 2005), vaginal cavities (Tucker et al., 2008; Legrand et al., 2011; Schütz et al., 2011), and the reticulorumen (bolus) (Bewley et al., 2008; Liang et al., 2013; Stone et al., 2017; Cantor et al., 2018). Surface body temperature has been measured by infra-red thermometer (Schütz et al., 2011) or automated temperature data logger adhered to the skin surface (Tresoldi et al., 2018).

Internal body temperature for Holstein dairy cows is maintained between approximately 38.6 to 39.0 °C (Piccione and Refinetti, 2003), and follows a pattern of diurnal fluctuations (Piccione and Refinetti, 2003; Bewley et al., 2008; Burfeind et al., 2012).

Additionally, breed and milk yield may influence the body temperature of dairy cows (Stone et al., 2017). While elevated temperatures may be a result of illness and fever (39.4 °C to 39.7 °C (Smith and Risco, 2005; Benzaquen et al., 2007; Wagner et al., 2008)), high body temperature has also been observed in heat stressed cows (Burfeind et al., 2012). Heat stressed cows experience an increase in body temperature in environmental conditions above the thermo-neutral zone as progressively increasing air temperature decreases the efficacy in cows' ability to dissipate heat (Finch, 1986; West, 2003). Thus, with increasing air temperature, cows begin to utilize more evaporative rather than non-evaporative cooling. In high humidity climates, evaporative cooling techniques are significantly reduced, further reducing the cow's ability to dissipate heat when in heat stress (West, 2003). As the cow body temperature deviates further from normal, production is further decreased (Kadzere et al., 2002).

Body temperature has been recorded to have a positive correlation with environmental temperature (within homoeothermic limits) by Araki et al. (1984) and Anderson et al. (2013) which reiterates the lack of effectiveness to dissipate heat outside the thermo-neutral zone (Finch, 1986; West, 2003). Body temperature is also found to be different between ambient and shade conditions, for example Schütz et al. (2011) reported lower body temperature in cows comparing before (no heat alleviation) and after access to sprinklers or shade. However, some studies have not found differences in body temperature between two heat alleviation treatments (e.g. Tucker et al., 2008; Schütz et al., 2010; Legrand et al., 2011). Tucker et al. (2008) found no difference between shade treatments (no shade, 25%, 50% and 99% solar protection) in mean body temperature, or maximum body temperature (at around 1600 h). Minimum body temperature (at around 0830 h) however was lowest in the 99% shade treatment (Tucker et al., 2008). Similarly, Schütz et al. (2010) found no difference between non-shaded and shade (no shade, 2.4 m² or 9.6 m² shade/cow) treatments in mean body temperature or maximum body temperature. Schütz et al. (2010) did not report minimum body temperatures.

Additionally, Legrand et al. (2011) found no difference between treatments (soaker vs. no soaker) of minimum, mean, or maximum body temperature. Soaked cows were however cooler in the evening (1800 to 2059 h) by at least 0.2 °C (Legrand et al., 2011). While mean daily body temperature showed no difference between treatments in Tucker et al. (2008) and Legrand et al. (2011) suggesting no heat alleviation, body temperature was different in the morning (minimum) and evening (maximum). Because there was evidence of heat alleviation throughout the day, it indicates that cows did benefit from shade at times during the day, therefore shade is a mean to alleviate dairy cows' heat stress in some condition.

Future studies should investigate differences of body temperature between treatments throughout the day, and the minimum and maximum body temperatures, in conjunction with respiration rate to diagnose heat stress. Taking body temperature throughout the day could be further investigated in research to consider strategically using cooling techniques at critical time points. Furthermore, future research should consider the method of automated data loggers to record temperature to collect constant temperature of cows throughout the day.

Behavioral Changes

Changes of behavior from heat stress includes lying less in exchange for standing longer (Tucker et al., 2008; Allen et al., 2015) – even after lying deprivation (Schütz et al., 2008). Heat stressed cows also experience reduced feed intake (Spiers et al., 2004; Bernabucci et al., 2010; Soriani et al., 2013) and feeding bouts (Bernabucci et al., 2010); therefore, heat stressed cows ruminate less (Kadzere et al., 2002; Bernabucci et al., 2010; Soriani et al., 2013).

Standing and Lying

Standing and lying time has been recorded in heat stress studies by visual observation such as scan sampling (e.g. Cook et al., 2007b; and Tucker et al. , 2008) or by automated data loggers such as leg tags (e.g. Anderson et al., 2013; Allen et al., 2015; and Johnson et al., 2017).

Once the environmental conditions increase over a THI of 68, or core body temperature increases above 39.2 °C, cows are more likely to stand (Allen et al., 2013). Tucker et al. (2008) observed cows increased standing time and reduced lying and grazing time with increasing solar radiation, regardless of shade treatment (no shade, 25%, 50% and 99% solar protection).

Standing and lying behavior in heat stressed cows was investigated by Allen et al. (2015) by taking body temperature (vaginal) during the transition between lying to standing, the transition of standing to lying, the continuation of lying, and the continuation of standing. The transition from lying to standing was positively correlated with body temperature, and continuing to stand was negatively correlated with cow temperature slightly less than continuing to lie (Allen et al., 2015). The reason is theorized that standing cows increase the surface area of contact with the moving air (Maia and Loureiro, 2005; Allen et al., 2015). This may explain the motivation for cows to stand longer in increasing heat stress conditions, as observed by Tucker et al. (2008). Furthermore, barns with minimal heat abatement strategies has resulted in no difference of standing and lying time in heat stressed cows (Allen et al., 2015), likely attributed the lack of air exchange, and therefore cooling potential of standing than lying. Similarly, a study compared a freestall barn with open sides with no heat abatement to a tunnel ventilated barn and reported the tunnel barn had cooler periods of THI and cows that lay longer, with lower respiration rate and lower udder temperature. In contrast, other studies that provide sprinklers or soakers as a method of heat abatement require cows to stand to use it. Sprinkler use for heat abatement increases with increasing THI, observed by time standing at the feedbunk without feeding (Parola et al., 2012; Chen et al., 2013; Chen et al., 2016) or time at the voluntary sprinklers (Parola et al., 2012) or soakers (Legrand et al., 2011). Furthermore, cows with no heat abatement strategies spend more time around the water trough (Schütz et al., 2010; Legrand et al., 2011). Therefore, while cows may stand for longer and lie for less time in heat stress, regression models with standing or lying time with temperature may

be different for each condition because of the different heat abatement strategies enforced, as identified by Allen et al. (2015).

Rumination and Feeding

Heat stressed cows feed less and therefore ruminate less (Kadzere et al., 2002; Bernabucci et al., 2010; Soriani et al., 2013). Feed data of heat stressed cows has been measured by weighing feed refusals (Spiers et al., 2004; Rhoads et al., 2009; Chen et al., 2016), visual observation (Legrand et al., 2011), or scan sampling techniques (e.g. every 10 min for grazing cows) (Schütz et al., 2010). Additionally, rumination time has been recorded in heat stress studies via automated data logging collars (Soriani et al., 2013). Feeding is important to support the nutritional requirements of the cow, as well as the caloric requirements of milk production. Heat stress changes feeding behavior by reducing DMI and feeding bouts/d (Bernabucci et al., 2010). A reduction in feed bouts may risk ruminal acidosis from a large influx of feed, resulting in a rapid decrease of rumen pH, and not allowing the rumen time to be buffered by saliva (Bernabucci et al., 2010; Palmonari et al., 2010). Rumination is similarly important because it stimulates saliva production (Bernabucci et al., 2014) which has buffering agents, that maintains a healthy rumen pH (Bernabucci et al., 2010; Palmonari et al., 2010). Heat stressed cows risk acidosis because of increased respiration rate (losing saliva to drool), and reduced saliva production from reduced feeding bouts and rumination. Increased respiration rate also increases the risk of acidosis by more CO₂ being exhaled, reducing pH of the rumen (Bernabucci et al., 2010). Additionally, increased body temperature redirects blood flow from the gastrointestinal tract to the peripheral parts of the body, potentially reducing the absorption of volatile fatty acids, therefore lowering rumen pH and further increasing risk of acidosis (Bernabucci et al., 2010).

Feeding and rumination time is decreased in cows in heat stress environments compared to thermo-neutral environments. Spiers et al. (2004) found cows in heat stress (confirmed by

higher respiration rate and rectal temperature) reduced DMI after the onset of heat stress, whereas thermo-neutral control cows maintained DMI. Likewise, Rhoads et al. (2009) found heat stressed cows reduced DMI compared to thermo-neutral treatment cows across 9 days of the study. Similarly, Soriani et al. (2013) observed a negative linear relationship with feeding and rumination time; DMI and rumination to daily maximum THI.

Future research should explore the relationship of THI, DMI, feeding time and rumination time. Additionally, Kendall et al. (2007), Schütz et al. (2011), and Chen et al. (2016) discuss cows dislike getting their head wet (shown by head wetting avoidance behaviors). Chen et al. (2016) reports avoidance behaviors increased feed bout length to avoid crossing the water barrier with the head which resulted in reduced feed bouts which may result in acidosis (as discussed by Bernabucci et al. (2010) and Palmonari et al. (2010)). This raises further research questions of interference to feeding behavior of cows during heat stress studies when sprinklers are over the feedbunk as they may serve as a deterrent.

Milk Production

Energy balance is dictated by DMI and the energy required for cellular function, metabolism maintenance and milk production. Negative energy balance occurs when the energy required is not met by energy intake (Drackley, 1999). One effect of reduced available energy, and the consequent negative energy balance, for cows is reduced milk production (Drackley, 1999). Physiological and behavioral changes caused by heat stress affecting metabolic processes and DMI therefore negatively affect milk production (Spiers et al., 2004; Rhoads et al., 2009; Soriani et al., 2013). Findings by Rhoads et al. (2009) and Bernabucci et al. (2010) show that a decrease in DMI due to heat stress only accounts for approximately a third of the consequential decrease in milk yield. Other factors attributed to metabolic and physiological changes from heat stress such as changes in hormones, absorption of energy from feed, and lipid and protein metabolism (Bernabucci et al., 2010). Spiers et al. (2004) observed

cows to have a reduced milk yield 2 d after the onset of heat stress was applied (d 0) and observed a continued reduction of milk until d 4 (end of study). Soriani et al. (2013) reported similar negatively correlated results of milk yield reduction during heat stress, and additionally explained that milk yield tended to follow an inverse trend of THI.

The reduction of milk production by heat stress is a tremendously expensive issue; it was estimated to cost the United States alone approximately \$1,507 million/year without implemented heat abatement (St-Pierre et al., 2003). St-Pierre et al. (2003) estimated milk loss production to be over 1000 kg/cow per year for six of the United States (AL, AR, FL, GA, MS, and OK) and over 2000 kg/cow per year for two states (LA and TX). Thus, research investigating heat abatement strategies and the relationship of heat stress and milk yield is very important for the profitability of dairies for producers.

The presented studies in this review are valuable to the dairy industry and scientific community as they increase knowledge and understanding regarding the relationship of heat stress and milk yield. Further research should investigate change in milk yield with heat abatement strategies that are more effective and target the individual animal. Also, research should investigate the most effective methods to reduce the negative impact of heat stress has on milk yield.

Cooling Strategies

Two heat abatement strategies to be discussed in this section are the provision of shade and the soaking of cows (therefore increasing heat dissipation (West, 2003)). It is important to note that some of the methods covered in this section may be predominantly related for confinement housed cows.

Shade

Shade provides cows with a partial or complete barrier of solar radiation, providing a cooler microclimate (Tucker et al., 2008) that reduces heat stress, and is preferred over no shade by lactating dairy cows (Tucker et al., 2008; Schütz et al., 2011). The motivation to seek shade is so strong, that after 12 h of lying deprivation, cows choose to stand in shade (facilities didn't support lying) than to lie in no shade (Schütz et al., 2008). Shade has been measured to reduce heat stress by reducing body temperature and respiration rate. Schütz et al. (2011) found that after just 10 min in a shaded environment cows reduced surface body temperature, whereas surface body temperature in a no shade treatment increased. Schütz et al. (2010) found lower respiration rates in cows under full shade or with varying amounts of shade per cow compared to cows provided with no shade, despite the shaded areas having a higher THI than the no shade areas. Conversely, Tucker et al. (2008) found no difference in mean vaginal temperature between no shade and different densities of shade, but reported a lower observed minimum temperature (minimum found at 0828 h) for cows given 99% solar radiation protection. While average temperature was not different, lower minimum vaginal temperature indicates there were differences in heat alleviation between treatments, demonstrating the advantage to provide shade.

Temperature humidity index may not however be the best measurement of environmental conditions in studies comparing shade to no shade. The THI does not consider solar radiation or wind speed and has been suggested to only be used as a summary of weather conditions by Brown-Brandl et al. (2005). Schütz et al. (2011) found cows preferred shade than no shade, increasing with ambient temperature, solar radiation, and wind speed. However, Schütz et al. (2011) found no relationship with humidity, therefore no relationship was found between shade preference and THI. Similarly, Tucker et al. (2008) noticed cows spent an increasing amount of time in the areas with increased solar radiation protection. While there were no differences of mean lying or standing time between treatments, this study occurred

during mild THI (Tucker et al., 2008), which may indicate that it is important to always provide shade to cows.

Building on the concept of shade, Schütz et al. (2010) explored the effects of different shaded area sizes for 10 cows and reported cows with a larger shaded area (total area: 9.6 m²/cow) spent over twice the amount of time utilizing the shade compared to cows with a smaller shaded area (2.4 m²/cow). Additionally, Schütz et al. (2010) found that cows provided with 2.4 m² of shade reverted to congregating around the water trough, a behavior observed in cows in the no shade group. More shade per cow decreased respiration rate but had no effect on body temperature (Schütz et al., 2010).

These studies support the hypothesis that shade helps alleviate heat stress, and therefore somewhat reduces negative effects of heat stress on dairy cows. Knowing shade reduces heat stress, and there are minimum requirements of shade per cow (Schütz et al., 2010), future research should investigate requirements of shade per cow, so enough shade can be made suitably available to alleviate heat stress for all cows in a group or herd.

Sprinklers and Soakers

Water has been used to cool cows via sprinklers (e.g. Schütz et al. (2011) and Tresoldi et al. (2018)), soakers (Legrand et al., 2011), or sprinklers above the feed alley (e.g. Chen et al. (2016) and Johnson et al. (2017)). Soakers and sprinklers are very similar because they use evaporative cooling by wetting the cow (Moran, 2005; Chaiyabutr et al., 2008) however, sprinklers are typically on a timed, cyclic system (Schütz et al., 2011; Chen et al., 2013; Chen et al., 2016). Soakers have incorporated into heat stress studies by voluntary use soakers that turn on only when in use by cows (Legrand et al., 2011), however manually activated sprinklers can be used for restrained cows for a specific length of time as reported by Tresoldi et al. (2018).

While spraying water may increase humidity in the microclimate (Lin et al., 1998; Schütz et al., 2011), most sprinkler and soaker studies have reported heat abatement from the water use, regardless of THI. Legrand et al. (2011) found cows with access to a shaded, voluntary soaker had lower respiration rates between 1700 and 1900 h, lower vaginal temperature between 1700 to 2059 h compared to cows with no access to a soaker. Additionally, panting was only observed in cows with no access to the soaker (Legrand et al., 2011). Similarly, Schütz et al. (2011) compared soaking cows for 10 min in the holding pen before milking to no sprinklers and found respiration rates and surface temperature to be reduced after 10 minutes of the sprinkler treatment compared to the no sprinkler treatment. Chen et al. (2016) also found lower vaginal temperature for cows with sprinklers (in a shaded barn) between 1300 to 1500 h and between 1700 to 2000 h than cows with no sprinklers. Marcillac-Emberson et al. (2009) however found heifers with access to sprinklers (no shade) to have the same rectal temperature as heifers with shade, but in contrast to the other studies mentioned, heifers to had higher respiration rate in the sprinkler treatment. Marcillac-Emberson et al. (2009) and Schütz et al. (2011) both compared sprinklers with no shade, however Schütz et al. (2011) had grass flooring and used lactating dairy cows, whereas Marcillac-Emberson et al. (2009) used heifers and dirt flooring. The results from Legrand et al. (2011) and Chen et al. (2016) indicate sprinklers provide better heat abatement when cows are additionally given shade.

Effect of heat stress abatement by sprinkler duration was investigated by Tresoldi et al. (2018), who reported respiration rate and shoulder skin temperature was reduced regardless of duration of sprinkler exposure (0.5, 1.5, 3, and 13 min) with shade compared to shade only. However, cows exposed to longer times (3 and 13 min) of sprinkler use retained lower respiration rates until the end of the observation period (30 min), and cows had lower respiration rates than the shade only treatment for 21 min (after 1.5 min sprinkler), and 12 min

(after 0.5 min). Tresoldi et al. (2018) concluded that longer sprinkler exposure cooled cows better; however, the results seem to indicate that any water use helps reduce heat stress.

Interestingly, very little is known for the ideal amount of water, droplet size, or duration of water to efficiently cool cows (Schütz et al., 2011; Tresoldi et al., 2018), however regardless of water used in most sprinkler and soaker studies, all reported heat abatement. Legrand et al. (2011) used two shower heads supplying 7.25 L/min each, Schütz et al. (2011) used five sprinklers supplying 0.43 L/min each. and Tresoldi et al. (2018) supplied cows with 4.9 L/min via an unspecified number of sprinklers. Chen et al. (2016) however used three treatments to investigate usefulness of different water flows and droplet size; control (no water), 1.3 L/min (450 µm average droplet size), and 4.9 L/min (660 µm average droplet size). Chen et al. (2016) showed no difference in body temperature between the two sprinkler treatments, however significant differences in body temperature between sprinkler and no sprinkler treatments was observed. This indicates that sprinklers are better than no sprinklers, however future research should investigate methods to use soakers on cows that do not elicit the previously observed heat wetting avoidance behavior observed by Kendall et al. (2007), Schütz et al. (2011), and Chen et al. (2016). Future research including soakers should additionally include respiration rate with body temperature to explore differences of efficiency in cooling cows with differing water delivery.

Cow Preference Between Soakers and Sprinklers

While Schütz et al. (2011) found shaded sprinklers to be more effective for heat abatement compared to shade without sprinklers or no shade or sprinklers, cows preferred shade than sprinklers, and were indifferent between sprinklers and ambient conditions. This is surprising given the dramatic reduction of heat stress symptoms soakers and sprinklers provide, as demonstrated by Schütz et al. (2011), Legrand et al. (2011), and Tresoldi et al. (2018). Chen et al. (2016) explained that it was possible that cows disliked the sprinklers because cows had

to pass through water, and water exposure to the head was explained to be dissatisfactory. This resulted in fewer, but longer feed bouts/d (not affecting DMI or daily time at the feed bunk compared to cows with no sprinklers). Legrand et al. (2011) used high-flowrate soakers, activated by cows standing on the platform below the soaker, while Schütz et al. (2011) and Chen et al. (2016) used sprinklers on timed cycles at low-flowrates, and had to wet their head walking through the activated sprinkler. Chen et al. (2016) addressed this, explaining the evasive behavior occurring because walking through the sprinkler (and wetting their head) may have been perceived as an obstacle or “expense” in return of the “payoff” of being fed. Perhaps design of the cow activated soaker by Legrand et al. (2011) had a positive preference because it was not perceived as an “obstacle” because it soaked the cow on the body after the cows’ head passed through without wetting the head, allowing the cow to control head wetting after soaker activation. Legrand et al. (2011) found cows to use the voluntary soaker for more than half of the instances on the head, however also included that there was extreme variation in soaker use occurred between cows, which could be from cows avoiding head wetting. Legrand et al. (2011) suggested soakers should be considered regardless of the variation found because of the considerable reduction to heat load to cows that used it.

Conversely, sprinklers or soakers could be integrated on dairies for cooling of the microclimate, rather than the cows themselves. Marcillac-Embertson et al. (2009) observed heifers moved away from the sprinklers when they were activated (agreeing with Schütz et al. (2011) and Chen et al. (2016) about evasive behavior with sprinklers), heifers spent more of their time in the sprinkler area during the hottest times of the day. This may indicate the heifers found this area to have a more comfortable environment than the areas of the pen with no sprinklers. Future research should therefore investigate cow preference to soakers by studying motivation for soaking or variance of soaker use between cows, possibly by comparing ‘forced’ or ‘group level’ soakings (e.g. on the feed bunk or at the milking parlor) with voluntary soaker

use. Future research could additionally investigate whether cows experiencing elevated effects of heat stress, such as increased respiration rate and body temperature, seek to use heat abatement more than cows experiencing less heat stress. Lastly, environmental microclimates could further be investigated as a method of cooling the environment, specifically for cows that avoid using voluntary soakers.

Free Choice Heat Abatement

Voluntary heat abatement options such as a free choice soaker in Legrand et al. (2011) supports the modern concept of free choice and cow self-management, and the consumer pressure from animal friendly industries (Webster, 2001; Pow et al., 2014). Supplying animals such as cows with the free choice to use equipment for their own wellbeing removes the subjectivity from the animal – such as in automatic milking systems (Holloway, 2007), and returns some of the freedom and choice to cows by removing some of the daily automation (Webster, 2001; Holloway et al., 2014). Legrand et al. (2011) investigated a free choice soaker where cows could use the soaker at their own leisure, providing a more individualized heat abatement strategy compared to herd level compulsory soakers such as in Schütz et al. (2011), or applying water while restrained as in Chen et al. (2015). Individualized heat abatement strategies should be considered because cows have different, individualized tolerances of heat stress because of genetics (Aguilar et al., 2009; Liang et al., 2013; Alfonzo et al., 2016), parity (Aguilar et al., 2009; Stone et al., 2017), milk production (Liang et al., 2013; Stone et al., 2017), body size, and hair structure (Alfonzo et al., 2016). Thus, future research should investigate use of individualized, voluntary heat abatement strategies to observe what designs are preferred and used by cows, and if it provides heat abatement at varying THI. Additionally, future research should investigate whether cows with low heat tolerance qualities (genetics, parity, milk production, body size or hair structure) do in fact utilize voluntary use heat abatement tools more than cows with higher heat tolerance qualities.

VALIDATING PRECISION DAIRY TECHNOLOGY

Precision dairy technology can be used with farm equipment – such as sort gates, production measurements, or automated scales – and can be worn by the animal. Wearable, behavior monitoring PDT are designed to measure cow behaviors autonomously, while reducing human error. Commercially-available, wearable PDT devices to monitor behavior include collars, ear-tags, and leg bands (Borchers et al., 2016, Caja et al., 2016). Dairy cow behavior such as rumination, feeding and resting are amongst variables that PDT can record. Monitoring cow behavior using PDT gives producers or researchers an early alert to provide attention to an animal that has deviated from normal patterns of behavior (Norton and Berckmans, 2017). Changes in behavior often occur for reasons needing human intervention, such as estrus (Dolecheck et al., 2015, Shahriar et al., 2016) or illness (Schirmann et al., 2016, Stangaferro et al., 2016a).

The purpose of validating PDT is to evaluate how correct it measures behavior. Validation studies compare behavior data recorded by the technology to a known measure of behavior such as visual observation or to another validated technology, known as the gold standard (Norton and Berckmans, 2017). Precision dairy technologies are predominantly validated against human recording of behavior. Two commonly utilized methods of recording observations are by live observation (i.e. Schirmann et al., 2009; Elischer et al., 2013; Bikker et al., 2014) or video recording (i.e. Ledgerwood et al., 2010; Ambriz-Vilchis et al., 2015; Zobel et al., 2015).

Behaviors being observed must be categorized and defined; typically using an ethogram (i.e. Bikker et al., 2014; Ambriz-Vilchis et al., 2015; Zehner et al., 2017). Ethograms are not only important to provide a definition of all start, stop, and duration requirements for all behaviors that will be observed to improve replicability of a study, but also to reduce inter-observer variability or bias (Fraser and Rushen, 1987). Ethograms must include explanations

of categorical behaviors (Fraser and Rushen, 1987); for example what the categorical behavior of “resting” includes. Additionally, the differentiation between bouts of behavior would also be required (Fraser and Rushen, 1987). Agreement of defining behaviors between observers has typically been tested before observations of the study begin, allowing adjustments to be made to the ethogram to reduce inter-observer variation (Schirmann et al., 2009; Bikker et al., 2014; Borchers et al., 2016).

Intra- and Inter-observer Variation

Behavioral observation is a time consuming and labor demanding activity, therefore it is common to use multiple observers in research studies, and during studies of lengthy observations multiple observers in research trials. However, differences in recording behaviors can occur between one or more observer (inter-observer) regardless if behavior is observed live or from video. High levels of inter-observer variation can reduce replicability (Stoler and Schiffman, 2001), and make the results of a study of very little use (Viera and Garrett, 2005). Using a single observer can completely remove inter-observer variability, however behaviors can be recorded differently by one observer (intra-observer variability) by recording behavior differently between one time point and another, particularly during long studies.

One method of assessing observer variation is by a kappa statistic. Kappa statistics are a quantitative measure of the magnitude of agreement between observers and is calculated by accounting for the measure of agreement that occurred (“observed”) and agreement by chance (“expected”) (Fleiss and Cohen, 1973; Viera and Garrett, 2005). The value of kappa statistics varies from -1 to +1; negative meaning poorer than chance, zero indicating exactly chance agreement, and positive meaning better than chance agreement (Fleiss and Cohen, 1973). Kappa agreement has been defined as poor: < 0.00; slight: 0.00 to 0.20; fair: 0.21 to 0.40; agreement: 0.41 to 0.60; substantial agreement: 0.61 to 0.80; and almost perfect: 0.81 to 1.00 (Landis and Koch, 1977). Kappa statistics has been used to assess inter- (O’Driscoll et al.,

2008; Bikker et al., 2014; Borchers et al., 2016) and intra observer variation (Zobel et al., 2015) in validation studies utilizing dairy cows.

Another method of assessing variation of observers is using Pearson correlation coefficient. Pearson correlation coefficient is an assessment of a linear relationship between two observers (Lawrence and Lin, 1989). Hinkle (1988) defined an interpretation of the results as namely; negligible: 0.00 to 0.30; low: 0.30 to 0.50; moderate: 0.50 to 0.70; high: 0.70 to 0.90; and very high: 0.90 to 1.00. Pearson correlation has successfully been used in validation studies to assess inter-observer agreement (Schirmann et al., 2009; Elischer et al., 2013; Borchers et al., 2016).

Inter-observer variation is measured by comparing observations between individual observers while watching the same animal. Intra observer is assessing variation of one observer to themselves by re-watching the same recorded observation and comparing behaviour recorded at the same time, therefore is not possible to do this for live observations. Elischer et al. (2013), Borchers et al. (2016) and Schirmann et al. (2009) made live observations for their validation studies, which has advantages of being able to move with the animal to retain view (especially important in subtle behaviors like rumination). Ambriz-Vilchis et al. (2015) and Zobel et al. (2015) watched recorded observations, which would allow observers to re-watch the same recording and therefore assess intra observer variation, in addition to (where appropriate) compare inter-observer variation between observers.

Precision, Accuracy, and Bias

Precision and accuracy – although often used interchangeably and incorrectly – are different (see Figure 1.1) and need to be assessed along with bias to truly validate a PDT. Precision is a measure of variance between measurements of a device, accuracy measures the magnitude of correctness the device has to the true value, and bias assesses whether the device consistently over- or under-estimates the true value (Walther and Moore, 2005). The purpose

of a validation study is to understand the possible error of measurement (Grubbs, 1973); this being imprecision, inaccuracy, or bias of a PDT.

Pearson correlation coefficient [r] (Schirmann et al., 2009; Borchers et al., 2016; Zehner et al., 2017), and coefficient of determination [R^2] (Schirmann et al., 2009; Ambriz-Vilchis et al., 2015) are commonly used in validation studies. These methods test for linear relationships (strength of relationship between PDT and observation) (Giavarina, 2015). Therefore these are measurements of precision – how repeatable the results are (Grubbs, 1973; Peduzzi et al., 1995) – thus, further tests must be used to assess accuracy and bias.

Accuracy tests assess how correct PDT records behavior in comparison to the true value (typically the observation in a validation study) (Grubbs, 1973; Peduzzi et al., 1995). Methods of accuracy assessment for PDT used in validation studies are; regression (slope) (Ambriz-Vilchis et al., 2015), bias correction factor [C_b] (Borchers et al., 2016), sensitivity, specificity (Wolfger et al., 2015; Zehner et al., 2017), positive- (Wolfger et al., 2015; Zehner et al., 2017) and negative-predictor values (Wolfger et al., 2015), and accuracy (Zehner et al., 2017). Accuracy assessment compares ability of the PDT to report the same value as the observation, taking only magnitude of accuracy into account (Walther and Moore, 2005). Thus, precision and bias need to be calculated in addition to accuracy.

Bias is the trend of the PDT to consistently over or under-estimate animal behavior (Peduzzi et al., 1995). Bland-Altman analysis a common method to assess bias in validation studies using PDT (Ambriz-Vilchis et al., 2015; Wolfger et al., 2015; Zehner et al., 2017). The Bland-Altman plot quantifies the agreement of two measurements (PDT and observation) by studying the mean difference, and illustrating limits of agreement (Bland and Altman, 1995a; b). Bland-Altman plots however only indicate bias and range of agreement for 95% of recorded measurements (Giavarina, 2015), therefore does not calculate precision or accuracy.

Importance of Validating Precision Dairy Technology

Several validation studies have highlighted the importance of validating PDT by indicating the differences between PDT simultaneously being worn on the same cow. Tsai (2017) reported mean rumination to differ by approximately 3 h/d between three technologies; mean steps/d differed more than 2000 steps between three technologies; and mean lying time differed by approximately 3,5 h/d between four technologies. Similarly, Borchers et al. (2016) observed differences in recorded behavior by PDT by analyzing agreement of PDT worn by cows simultaneously during their validation study. Agreement was as low as $r = 0.83$ between two PDT recording lying time. Rumination had very high agreement with observers for one PDT, however another PDT worn simultaneously recorded differently, yielding much less agreement with observers. Similarly, feeding differed between two PDT with agreement of observers (Borchers et al., 2016). These results are an example as to why validation studies are important to verify how correct the PDT is recording before using on farm or reporting results in research. Ruminating, feeding and resting are very important behaviors that need to be measured correctly and consistently for research and for producers.

Technology Recording Rumination Time

Some of the PDT that record rumination time that have been assessed for precision and accuracy include collars (e.g. Schirmann et al. (2009); Elischer et al. (2013); and Ambriz-Vilchis et al. (2015)), pressure sensors (e.g. Beauchemin et al. (1989); Kononoff et al. (2002); and Zehner et al. (2017)) or ear-tags (e.g. Bikker et al. (2014); Wolfger et al. (2015); and Borchers et al. (2016)), outlined in Table 1.2. Recording rumination via PDT has been found to range between different technologies from poor correlation (e.g. Kononoff et al. (2002) and Wolfger et al. (2015)) to very high correlation (e.g. Schirmann et al. (2009); Borchers et al. (2016); and Zehner et al. (2017)).

An accelerometer collar (collar 1) for rumination measurement validated by Schirmann et al. (2009) via live observation in a confinement setting utilizing dairy cows resulted in very high precision with very little bias (Table 1.2). Collar 1 however was validated in another study by Goldhawk et al. (2013) to investigate its application in beef cattle (tie stall and loose housed feedlot). Goldhawk et al. (2013) found collar 1 to have negligible to low results for the tie stall cattle, and negligible results for the loose housed feedlot cattle (Table 1.2). This indicates that collar 1 could be used for research or by producers for dairy cows, however collar 1 should be used with caution (or modified) if it were to be used on beef cattle. Similarly Elischer et al. (2013) and Ambriz-Vilchis et al. (2015) assessed a different collar (collar 2) using a microphone to detect rumination time via live observation with lactating dairy cows and had differing results (Table 1.2). Elischer et al. (2013) found moderate precision for lactating dairy cows in freestalls with mattresses and 24 h access to pasture, with concentrate feed fed at the automatic milking system. Ambriz-Vilchis et al. (2015) conducted three trials with lactating dairy cows; 1 and 2) freestall barn, partial mixed ration fed, and 3) pasture. Ambriz-Vilchis et al. (2015) found different results collar 2 than Elischer et al. (2013); the first two trials resulted in high precision, and the third trial resulted in low precision.

Similarly, pressure sensor devices built into halters have been validated with a variety of results between studies. Beauchemin et al. (1989) and Zehner et al. (2017) found high precision for rumination in lactating dairy cows in confinement housing, however Kononoff et al. (2002) reported much lower precision (Table 1.2). Both Zehner et al. (2017) and Rombach et al. (2018) validated the same noseband pressure sensor (pressure sensor 2) in different management styles (confinement and pasture, respectively), and similarly to the rumination validation with collars, they found differing results. Zehner et al. (2017) found very high agreement between pressure sensor 2 and live observations, whereas Rombach et al. (2018)

found moderate agreement between pressure sensor 2 and live observations in the pasture setting.

Validation studies assessing accelerometer ear-tags have also ranged in correlation with live observations from poor (Wolfger et al., 2015) to very good (Bikker et al., 2014; Borchers et al., 2016) (Table 1.2). Ear-tag 1 has been validated for rumination by Bikker et al. (2014), and Borchers et al. (2016) in confinement settings, by Pereira et al. (2018) in a pastoral setting, and Wolfger et al. (2015) in feedlot steers. Bikker et al. (2014) indicated very high precision, Borchers et al. (2016) indicated moderate precision, Pereira et al. (2018) indicated moderate precision, and Wolfger et al. (2015) indicated low precision for ear-tag 1. A different accelerometer ear-tag (ear-tag 2) has been validated in lactating dairy cows housed in freestalls by Borchers et al. (2016) and Reiter et al. (2018) via live and video observation, respectively, and both found very high agreement between ear-tag measured and observed rumination.

Results from previous validation studies with PDT measuring rumination indicate production setting may be important to consider for validity of PDT. Additionally, PDT that have been validated with high precision for rumination time with lactating dairy cows does not necessarily translate to working with high precision for beef cattle. Therefore, future studies using PDT to record rumination should consider validating any technology if it is going to be used in a different management system or with a different production animal than previously validated with. This is necessary to ensure the PDT is reliable in the different management style, housing system or production animal.

Technology Recording Feeding Time

Feeding time has been also been recorded by collars (e.g. Krawczel et al. (2012); and Benaissa et al. (2017)), pressure sensors (e.g. Zehner et al. (2017); and Rombach et al. (2018)), and ear-tags (e.g. Bikker et al. (2014); Wolfger et al. (2015); and Borchers et al. (2016)). Agreement of feeding with observation with PDT also range from little agreement (Kononoff

et al., 2002) to very high agreement (e.g. Wolfger et al. (2015); and Zehner et al. (2017)), outlined in Table 1.2.

The use of collars to record feed time in dairy cows have been extensively investigated with very positive results. The same collar (collar 3) has been validated by Krawczel et al. (2012) and Benaissa et al. (2017) for feeding time in freestall dairies (Table 1.2). Krawczel et al. (2012) used lactating dairy cows and live, 3 min scan sampling observations to assess the validity of collar 3, whereas Benaissa et al. (2017) used video and a mix of lactating and non lactating dairy cows. Both studies indicated acceptable precision and accuracy; Krawczel et al. (2012) used a Bland-Altman plot that indicated no bias), and Benaissa et al. (2017) indicated very high sensitivity and specificity values.

Pressure nose sensors have also been validated and resulted in very high agreement for assessing feeding time in lactating dairy cows in confinement settings. The validation of a pressure sensor with 1 min scan sampling (Beauchemin et al., 1989), and live observation (Zehner et al., 2017) have found very high agreement. Rombach et al. (2018) validated the same pressure nose band sensor (pressure sensor 2) as Zehner et al. (2017), however Rombach et al. (2018) validated the product with pastoral cows, which resulted in a lower, moderate agreement with the live observations (Table 1.2). A different nose pressure sensor (pressure sensor 3) previously was found to have very high precision in measuring eating time in grazing sheep was validated by Kononoff et al. (2002) in confinement housed lactating dairy cows via 5 min scan sampling. Kononoff et al. (2002) however found *P*-values indicating difference (non-agreement) between the pressure nose band to the 5 min scan sampling (Table 1.2). Scan sampling for observing feeding behavior has very high correlation with continuous sampling methods for 1 and 5 min (Mitlöhner et al., 2001) as used by Beauchemin et al. (1989) and Kononoff et al. (2002), therefore should not have effected validity testing of these PDT.

Bikker et al. (2014), Wolfger et al. (2015), Borchers et al. (2016), and Pereira et al. (2018) have all validated the same accelerometer ear-tag (ear-tag 1) for recording feeding. Bikker et al. (2014) used lactating dairy cows in confinement housing, Wolfger et al. (2015) used feedlot beef steers, Borchers et al. (2016) used lactating dairy cows in confinement housing, and Pereira et al. (2018) used lactating dairy cows in a pastoral setting. Despite the different management practises between these studies, they all indicated high levels of precision between the ear-tag with live observation (Table 1.2).

Precision dairy technologies that have been validated indicate there are reliable (high precision, high accuracy, or minimal bias) technologies commercially available for use. However any PDT should also be considered for validation (or re-validation) before use to verify the validity of them within the destined management style on the animals being monitored before use, particularly when being used in research.

Technology Recording Resting Time

Resting time is an addition of stationary standing and lying behaviors, therefore few technologies record this exact variable. Bikker et al. (2014) validated resting for ear-tag 1 using an accelerometer with lactating dairy cows in confinement housing via live observation and resulted with very high precision for this behaviour (Table 1.2). Zambelis et al. (2019) validated the same ear-tag (ear-tag 1) as Bikker et al. (2014) in lactating dairy cows in a tiestall barn. Zambelis et al. (2019) reported ear-tag 1 (in comparison to visual observation) to have negligible precision with low accuracy for rumination time; moderate precision and low accuracy for feeding time; and very high precision with moderate accuracy for resting time (Table 1.2). Ear-tag 1 was also validated by Bikker et al. (2014), Borchers et al. (2016), and Pereira et al. (2018) in lactating dairy cows, and by Wolfger et al. (2015) in beef cattle, however the resting behavior was not included in the validation.

Future validation of resting time needs to be investigated so that it may be compared to other studies (such as by breaking resting into standing and lying behaviors). Because resting may be the sole output variable from some PDT (as opposed to it being broken into standing and lying behaviors), researchers should consider that may lead to limitations in discussing results by disabling the comparison to other studies that used PDT that separate standing and lying variables from resting. Comparing resting time to resting time however, such as between treatments on the same study (in research) or comparing a cow to its own data (in research or on farm), has no disadvantage providing it has been validated in the same management style and on the same animal production type as intended use.

CONCLUSIONS

Heat stress can negatively affect cow physiology, behavior, and milk production, therefore heat stress is an issue for cow welfare and producer economics. Therefore, dairies should invest in the implementation of heat abatement tools to control these deleterious effects when environmental conditions exceed the thermo neutral zone. Heat stress abatement strategies influence the behaviour of dairy cows, especially feeding, ruminating, and resting, and these directly impact milk yield and animal welfare. Research investigating heat abatement methods, such as shade, soaking and sprinklers, found that it has the potential to diminish heat stress effects.

Soaking dairy cows has been found to drastically cool cows, however this method is often delivered via timed sprinklers which may not be perceived favorably by dairy cows. Voluntary soaker use has not shown head avoidance behaviour, however, use of voluntary soakers is varied between cows. Additionally, cows have different heat tolerance levels from individual genetics, therefore cows predisposed to have a higher or lower tolerance to heat stress could benefit from individual level cooling strategies.

As mentioned, PDT should be used to measure cooling techniques and treatments on heat stressed cows to consistently and constantly record cow behavior, however PDT requires validation to assess precision, accuracy and bias. Validation is important for researchers to confidently compare results of treatments of heat abatement strategies, adding to knowledge of better or more preferred cooling techniques. Therefore, the objectives for this thesis is to firstly validate measurements of ruminating, feeding and resting determined by a behavior monitoring collar so it can be used to record behavior in a study investigating the heat abatement qualities of a voluntary soaker in the second section. The objectives of the second section are to assess cow preference for a voluntary soaker adjacent to the home pen, and quantify behavior changes that have been previously correlated to heat stress between two treatments of 1) a free

choice soaker with cows given two mandatory soakers, vs 2) free choice soaker use with no mandatory soakers.

Figure 1.1. Precision vs. accuracy, adapted from Viera and Garrett (2005). A represents low accuracy and low precision. B illustrates high precision and low accuracy. C shows low precision and high accuracy. D demonstrates high precision and high accuracy.

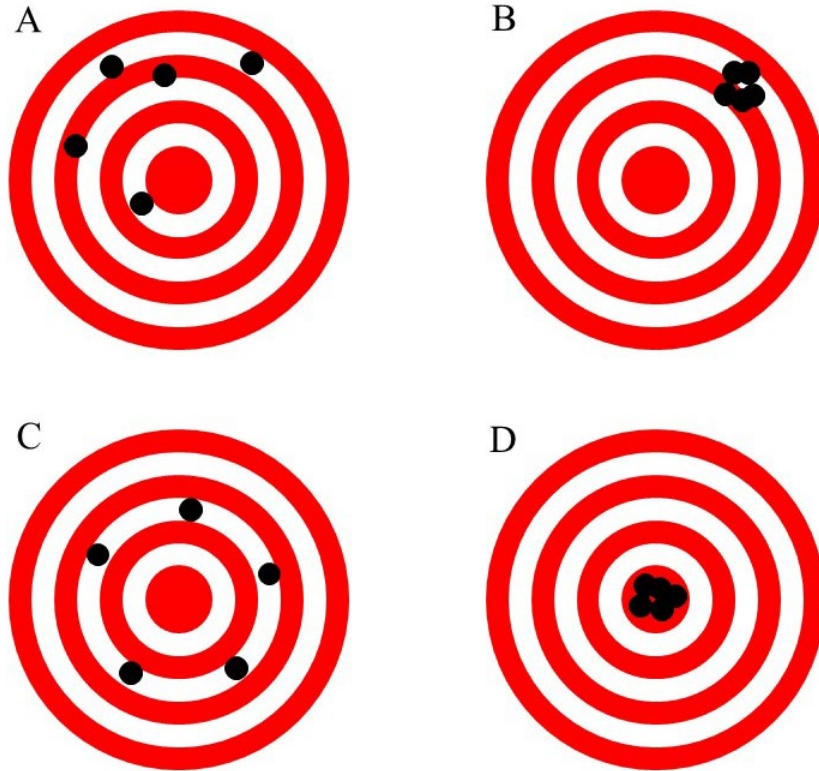


Table 1.1. Panting score ethogram by Mader et al. (2006). Note: half scores between two number scores were also used by Mader et al. (2006) when an animal was considered to fit between the description of two scores.

Score	Description
0	Normal respiration
1	Elevated respiration
2	Moderate panting and/or presence of drool or small amount of saliva
3	Heavy open-mouthed panting; saliva usually present
4	Severe open-mouthed panting accompanied by protruding tongue and excessive salivation; usually with neck extended forward

Table 1.2 Validation studies of precision dairy technologies (PDT) that record ruminating, feeding or resting within the last 20 years. Included is the behavior validated, the precision, accuracy, and bias measurement.

Author/s, PDT validated, and supporting information	Precision measurement	Accuracy measurement	Bias measurement
<p>Ambriz-Vilchis et al. (2015). Collar 2 (Lely, Maassluis, the Netherlands). Validated for rumination in dairy cows in a freestall barn (trial 1 and 2) and in a pastoral system (trial 3). Trial 1 used video and direct observations, and trial 2 and 3 only had direct observations.</p>	<p>Only given for trial 1 (direct observations): $R^2 = 0.66$; $P < 0.001$</p>	<p>Trial 1 (video observations): Bland-Altman 95% LOA = 27 to -24 min, including approx. 95% data points. Slope = 1.08 (not different from 1 ($P = 0.71$)). Trial 1 (direct observations): Bland-Altman 95% LOA = 20 to -33 min, including approx. 95% data points. Slope = 1.02 (not different from 1 ($P = 0.72$)). Trial 2: Bland-Altman 95% LOA = 20 to -32 min, including approx. 95% of data. slope = 0.93 (not different from 1 ($P = 0.63$)). Trial 3: Bland Altman 95% LOA = 53 to -51 min, including approx. 95% of data points. Slope = 0.57 (not different from 1 ($P = 0.06$)).</p>	<p>Bland-Altman showed Collar 1 recorded 1 min longer than video observations and 6 min shorter than visual observation for trial 1; 3 min shorter than visual observation for trial 2; and 1 min longer than visual observation for trial 3 (2 h observation period).</p>

Table 1.2 (continued)

<p>Benaissa et al. (2017). Collar 3 (Onset Computer Corporation, Pocasset, MA) validated for feeding with dairy cows housed in individual cubicles.</p>	<p>Algorithm K-nearest neighbors: precision = 88%. Algorithm Naïve Bayes: precision = 84%. Algorithm Support vector machine: precision = 92%.</p>	<p>Algorithm K-nearest neighbors: sensitivity = 96%; accuracy = 86%. Algorithm Naïve Bayes: sensitivity = 95%; accuracy = 84%. Algorithm Support vector machine: sensitivity = 98%; accuracy = 91%.</p>	
<p>Bikker et al. (2014). Ear tag 1 (Agis Automatisering BV, Harmelen, The Netherlands) validated for rumination, feeding and resting in dairy cows in a confinement setting.</p>	<p>Rumination: $r = 0.93$; $CCC = 0.93$; $\kappa = 0.85 \pm 0.01$; $\kappa_{max} = 0.99$. Feeding: $r = 0.88$; $CCC = 0.75$; $\kappa = 0.77 \pm 0.03$; $\kappa_{max} = 0.88$. Resting: $r = 0.98$; $CCC = 0.97$; $\kappa = 0.86 \pm 0.02$; $\kappa_{max} = 0.96$.</p>	<p>Rumination: $CCC = 0.93$; $\kappa = 0.85 \pm 0.01$; $\kappa_{max} = 0.99$. Feeding: $CCC = 0.75$; $\kappa = 0.77 \pm 0.03$; $\kappa_{max} = 0.88$. Resting: $CCC = 0.97$; $\kappa = 0.86 \pm 0.02$; $\kappa_{max} = 0.96$.</p>	<p>Rumination: $C_b = 1.0$. Feeding: $C_b = 0.86$. Resting: $C_b = 0.99$.</p>

Table 1.2 (continued)

<p>Borchers et al. (2016). Ear tag 1 (Agis Automatisering BV, Harmelen, The Netherlands) validated for rumination and feeding in dairy cows housed in freestall barns.</p>	<p>Rumination: $r = 0.69$ ($P < 0.01$); $CCC = 0.59$. Feeding: $r = 0.88$ ($P < 0.01$); $CCC = 0.82$.</p>	<p>Rumination: $CCC = 0.59$. Feeding: $CCC = 0.82$.</p>	<p>Rumination: $C_b = 0.69$. Feeding: $C_b = 0.88$</p>
<p>Borchers et al. (2016). Ear tag 2 (gmbh, Jutogasse, Austria) validated for rumination in dairy cows housed in freestall barns.</p>	<p>$r = 0.97$ ($P < 0.01$); $CCC = 0.96$.</p>	<p>$CCC = 0.96$</p>	<p>$C_b = 0.97$</p>
<p>Büchel and Sundrum (2014). Pressure sensor 5 (bitsz engineering gmbh, Zwickau, Germany) validated for rumination and feeding in dairy cows in tethered housing.</p>	<p>Rumination: $r = 0.86$ ($P < 0.001$); $R^2 = 0.74$ ($P < 0.001$). Feeding: $r = 0.87$ ($P < 0.001$); $R^2 = 0.75$ ($P < 0.001$).</p>	<p>Accuracy (rumination and feeding) = 87%</p>	<p>Bland Altman mean difference = 3.56, 95% LOA: -12.6 to 19.8 (rumination); mean difference = 0.46, 95% LOA: -2.67 to 3.59 (feeding)</p>

Table 1.2 (continued)

Burfeind et al. (2011). Pressure sensor 3 (SCR Engineers Ltd., Netanya, Israel) validated for rumination in dairy calves and heifers housed individually (2.0 m × 1.2 m up to 63 d old; 12.8 m x 4.7 m 85 d and older)	25 ± 2 d old: $r = 0.65$ ($P < 0.01$); $R^2 = 0.42$. 42 ± 2 d old: $r = 0.70$ ($P < 0.01$); $R^2 = 0.49$. 62 ± 1 d old: $r = 0.89$ ($P = 0.01$); $R^2 = 0.79$. 95 ± 10 d old: $r = 0.47$ ($P < 0.01$); $R^2 = 0.22$. 185 ± 1 d old: $r = 0.72$ ($P < 0.01$); $R^2 = 0.53$. 282 ± 7 d old: $r = 0.88$ ($P < 0.001$); $R^2 = 0.77$.	25 ± 2 d old: t -test = 2.99 ($P = 0.01$). 42 ± 2 d old: t -test = 0.30 ($P = 0.77$). 62 ± 1 d old: t -test = 0.53 ($P = 0.60$). 95 ± 10 d old: t -test = 3.96 ($P < 0.001$). 185 ± 1 d old: t -test = 2.36 ($P = 0.03$). 282 ± 7 d old: t -test = 2.18 ($P = 0.05$).	
Chapinal et al. (2007). Electronic feed bin 1 (Insentec, Marknesse, the Netherlands) validated for feeding in prepartum and lactating dairy cows in loose housing.	$R^2 = 1.0$; $P < 0.001$	Slope = 1.07 (not different from 1 ($P < 0.02$)).	
Chizzotti et al. (2015). Electronic feed bin 2 (Intergado Ltd., Contagem, Minas Gerais, Brazil) validating feeding in dairy cows in freestall housing.	Per visit: $R^2 = 0.99$; per 4 h period: $R^2 = 0.99$.	Per visit: slope = 1.002; per 4 h period: slope = 1.007.	

Table 1.2 (continued)

<p>Devries et al. (2003). Feed alley monitoring system (Growsafe, growsafe Systems Ltd., Airdrie, AB, Canada) validated for feeding in dairy cows in freestall housing.</p>	<p>$R^2 = 0.98$; $P < 0.001$</p>	<p>Slope = 0.63; slope did not differ from one ($P > 0.3$)</p>	
<p>Elischer et al. (2013). Collar 2 (Lely, Maassluis, the Netherlands) validated for rumination in dairy cows given freestalls with mattresses and 24 h access to pasture.</p>	<p>$r = 0.65$; $P < 0.001$</p>	<p>Bland Altman 95% LOA = 36.59 and -28.56 min, approx.. 90% of data points within LOA. Slope = 0.88.</p>	<p>Bland Altman: Collar 1 recorded 4.01 min longer rumination (2 h observation).</p>
<p>Goldhawk et al. (2013). Collar 1 (SCR Engineers Ltd., Netanya, Israel) validated for rumination with yearling beef heifers in tie stalls and yearling beef steers in a loose housed feedlot.</p>	<p>$r = 0.41$; $P < 0.001$; $CCC = 0.30 \pm 0.05$; $P < 0.001$.</p>	<p>$CCC = 0.30 \pm 0.05$; $P < 0.001$.</p>	<p>Collar 1 underestimated rumination by 9.8 ± 18.7 min (2 h observation).</p>
<p>Kononoff et al. (2002). Pressure sensor 3 (Triangle Digital Services, London, UK) validated for rumination and feeding with dairy cows in individual stalls.</p>		<p>Rumination: t-test = 8.8 min ($P = 0.09$). Feeding: t-test = 42.9 min ($P = <0.01$).</p>	

Table 1.2 (continued)

Krawczel et al. (2012). Collar 3 (Onset Computer Corporation, Pocasset, MA) validated for feeding in dairy cows in a freestall barn.		Bland Altman plot reported $R^2 = 0.01$; $P = 0.13$	Bland Altman plot values not reported
Pereira et al. (2018). Ear tag 1 (Agis Automatisering BV, Harmelen, The Netherlands) validated for rumination and feeding in dairy cows in a pastoral setting.	Rumination: $r = 0.72$ ($P < 0.001$); $CCC = 0.71$. Feeding: $r = 0.88$ ($P < 0.001$); $CCC = 0.88$.	Rumination: $CCC = 0.71$. Feeding: $CCC = 0.88$.	Rumination: $C_b = 0.99$. Feeding: $C_b = 0.99$.
Reiter et al. (2018). Ear tag 2 (gmbh, Jutogasse, Austria) validated for rumination with dairy cows in a freestall barn.	$r > 0.99$; $P < 0.01$	t -test = -2.10 ; $P = 0.04$	Bland Altman: ear tag 2 recorded 16 s shorter rumination than observed (1 h observation).

Table 1.2 (continued)

<p>Rombach et al. (2018). Pressure sensor 2 (Nydegger and Bollhalder, 2010, Agroscope, Itin+Hoch gmbh, Liestal, Switzerland) validated for rumination and feeding with dairy cows on pasture (approximately ¾ time) with free stall access.</p>		<p>Supplemented cows; V0.7.3.2: rumination: MPE = 43; LOA = -98 to 101; slope = 1.0. Feeding: MPE = 71.0; LOA = -150 to 120; slope = 1.02. Supplemented cows; V0.7.3.11: rumination: MPE = 48.5; LOA = -98 to 101; slope = 0.99. Feeding: MPE = 87.0; LOA = -192 to 148; slope = 1.01. Grazing cows; V0.7.3.2: rumination: MPE = 79.2; LOA = -161 to 140; slope = 1.00. Feeding: MPE = 90.3; LOA = -191 to 161; slope = 0.99. Grazing cows; V0.7.3.11: rumination: MPE = 17.1; LOA = -34 to 33; slope = 1.01. Feeding: MPE = 43.6; LOA = -112 to 62; slope = 1.02.</p>	<p>Supplemented cows; V0.7.3.2: rumination: underestimated by 1.6 min. Feeding: underestimated by 15.3 min. Supplemented cows; V0.7.3.11: rumination: overestimated by 1.9 min. Feeding: underestimated by 21.6 min. Grazing cows; V0.7.3.2: rumination: underestimated by 10.8 min. Feeding:</p>
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Table 1.2 (continued)

			underestimated by 15.1 min. Grazing cows; V0.7.3.11: rumination: underestimated by 0.4 min. Feeding: underestimated by 24.9 min.
Ruuska et al. (2016). Pressure sensor 2 (Nydegger and Bollhalder, 2010, Agroscope, Itin+Hoch gmbh, Liestal, Switzerland) validated for rumination and feeding with dry, dairy cows in tiestalls with peat bedding.	Rumination: $R^2 = 0.93$. Feeding: $R^2 = 0.94$.	Rumination: slope = 0.88. Feeding: slope = 0.98.	
Schirmann et al. (2009). Collar 1 (SCR Engineers Ltd., Netanya, Israel) validated for rumination in dairy cows housed individually in open pens (trial 1 and 2) and stall housing (trial 3).	Trial 1: $r = 0.96$ ($P < 0.001$); $R^2 = 0.93$ ($P < 0.001$). Trial 2: $r = 0.92$; $P < 0.001$; $R^2 = 0.86$; $P < 0.001$. Trial 3: $r = 0.96$ ($P < 0.001$).	Trial 1 and 2: Bland Altman 95% LOA = 13.8 to -14.7 min, approx. 95% of data points included in LOA. Not given for trial 3.	Trial 1 and 2: Collar 1 recorded 0.45 min longer rumination (2 h observation). Not given for trial 3.

Table 1.2 (continued)

<p>Wolfger et al. (2015); Ear tag 1 (Agis Automatisering BV, Harmelen, The Netherlands) validated for rumination and feeding with beef steers on a feedlot.</p>	<p>One min filter: rumination: CCC = 0.41 (0.20-0.58). Feeding: CCC = 0.75 (0.61-0.84). No filter: Ruminating: $r = 0.69$ ($P < 0.0001$). Feeding: $r = 0.27$ ($P = 0.18$). Resting: $r = 0.89$ ($P < 0.0001$).</p>	<p>1 min filter: rumination: CCC = 0.41 (0.20-0.58); sensitivity = 48 (35-63); specificity = 94 (91-95). Feeding: Specificity = 96 (94-98); CCC = 0.75 (0.61-0.84); sensitivity = 93 (91-94); specificity = 94 (91-95). No filter: rumination: CCC = 0.44 (0.23-0.60); sensitivity = 49 (34-64); specificity = 96 (94-98). Feeding: CCC = 0.79 (0.61-0.85); sensitivity = 95 (93-96); specificity = 76 (69-85).</p>	
<p>Zambelis et al. (2019). Ear tag 1 (Agis Automatisering BV, Harmelen, the Netherlands) validated for ruminating, feeding and resting with dairy cows in tiestalls.</p>		<p>Ruminating: <i>t</i>-test: sensor = 39.1 ± 0.05; visual observation = 30.4 ± 0.06; $P < 0.0001$. Feeding: <i>t</i>-test: sensor = 6.1 ± 0.03, visual observation = 16.7 ± 0.04; $P < 0.0001$. Resting: <i>t</i>-test: sensor = 31.6 ± 0.06, visual observation = 30.7 ± 0.06; $P = 0.08$.</p>	

Table 1.2 (continued)

<p>Zehner et al. (2017). Pressure sensor 2 (Nydegger and Bollhalder, 2010, Agroscope, Itin+Hoch gmbh, Liestal, Switzerland) validated for rumination and feeding in dairy cows in loose housing with cubicles.</p>	<p>Converter version V0.7.2.0: rumination: $r_s = 0.91$ ($P < 0.001$). Feeding: $r_s = 0.86$ ($P < 0.001$). Converter version V0.7.3.2: rumination: $r_s = 0.96$ ($P < 0.001$). Feeding: $r_s = 0.96$ ($P < 0.001$).</p>	<p>Converter version V0.7.2.0: rumination: accuracy = 0.95. Feeding: accuracy = 0.92. Converter version V0.7.3.2: rumination: accuracy = 0.90. Feeding: accuracy = 0.88.</p>	<p>Bland Altman showed pressure sensor 2 recorded 2.34 min shorter for rumination and 4.56 min longer for feeding time for converter version V0.7.2.0., and 0.79 min longer for rumination and 2.20 min longer for feeding time for converter version V0.7.3.2 (2 h observation).</p>
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LOA (limit of agreement)

r (Correlation coefficients)

R^2 (coefficients of determination)

RMSE (root mean squared error)

CCC (concordance correlation coefficient)

C_b (bias correction factor)

κ (Kappa)

MPE (mean prediction error)

r_s (Spearman nonparametric correlation coefficient)

CHAPTER TWO:

TECHNICAL NOTE: VALIDATION OF A BEHAVIOR MONITORING COLLAR'S
PRECISION AND ACCURACY TO MEASURE RUMINATION, FEEDING, AND
RESTING TIME OF LACTATING DAIRY COWS

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INTRODUCTION

Wearable, behavior-monitoring, precision dairy technologies (PDT) autonomously monitor cow behavior, while minimizing human interference or human error. Common commercially available wearable PDT for behavior monitoring include collars, ear tags, and leg bands; however, others are also available (Borchers et al., 2016; Caja et al., 2016). Behaviors such as rumination, feeding, and resting time are among the variables that PDT can record in cows. Monitoring dairy cattle behavior using PDT gives producers or researchers an early alert to provide attention to an animal that has deviated from normal patterns of behavior (Norton and Berckmans, 2017). Predominant causes for deviations in behavior are estrus (Dolecheck et al., 2015; Shahriar et al., 2016) or illness (Schirmann et al., 2016; Stangaferro et al., 2016a).

It is important to validate all PDT to understand their precision and accuracy before taking measurements or applying them to cattle management or research. Validation compares the PDT with a known (or gold standard) measurement of behavior (such as visual observation) to understand its precision and accuracy (Norton and Berckmans, 2017). To validate precision of PDT against visual observation, previous studies have successfully used Bland-Altman plots (Schirmann et al., 2009; Elischer et al., 2013; Zehner et al., 2017), correlation coefficients, or regressions (Bikker et al., 2014; Wolfger et al., 2015; Borchers et al., 2016). Accuracy has been assessed in previous validation studies of PDT by analyzing the slope of the regression line (Ambriz-Vilchis et al., 2015; Chizzotti et al., 2015). However, accuracy is often not reported or tested in studies validating PDT. Therefore, the purpose of this study was to validate the precision and accuracy of a behavior monitoring collar (BMC; MooMonitor+, Dairymaster, Co. Kerry, Ireland) measuring ruminating, resting, and feeding time in lactating dairy cows.

MATERIALS AND METHODS

The study was conducted at the University of Kentucky Coldstream Research Dairy Farm (Lexington), and approved by the Institutional Animal Care and Use Committee (protocol number 2017–2724). Cows were housed in a compost bedded-pack barn that was tilled twice per day. The herd was milked twice/d (0430 and 1530 h). Cows were fed a TMR, formulated to follow the NRC guidelines (NRC, 2001) to meet or exceed the requirements of lactating dairy cows producing at least 39 kg of milk/d. All cows were fed the same TMR twice per day (approximately at 0730 and 1430 h) for the duration of the study, via a feed alley with headlocks, and had ad libitum access to fresh water from water troughs.

The number of cows on the study was determined following methods of Friedman (1982) and adapted by Borchers et al. (2016). Twenty-four cows were determined necessary to enroll to the study from a power test, calculated to attain power ($1 - \beta$) of 0.90, and a type I error probability (α) of 0.05 (2-sided) utilizing variances of data reported in Borchers et al. (2016).

The 24 Holstein cows were selected by using a criteria requiring cows from the first home pen (first milking group at 0530 and 1630 h) to be over 30 DIM and have a locomotion score of 1 or 2. Cow locomotion score was obtained weekly using a 5-point scoring method (1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, and 5 = severely lame, developed by Sprecher et al. (1997). Cows were randomly selected weekly using the set criteria for observation times occurring in the next 7 d to ensure cows remained eligible for the study. The 24 cows (mean \pm SD; DIM: 196 ± 101 ; parity: 2.0 ± 1.1 ; and milk yield: 40.0 ± 9.8 kg/d) were fitted with BMC 196.2 ± 101.2 d before observation. One cow chosen changed eligibility (developed a locomotion score of ≥ 3) and therefore was replaced to retain the required sample size of 24 cows.

The mechanical measurements made by the BMC used in this study were recorded by the working part of the device (121 g; 7.25 × 8 × 3.25 cm), positioned Ionmicro electro-mechanical system accelerometer is built into the device; the accelerometer continuously records the animal's movements, which are translated by an algorithm into a nonstop record of behavior. Data were wirelessly transmitted to a base station (located in the barn) every 15 min (as opposed to being stored on the device). The base station in turn transmits data to an Internet-based cloud service (online storage service). In the event of short-term (depending on herd size) connection failure to the Internet-based cloud service, the barn base station can store data and upload when the connection is restored. Life expectancy of batteries in these BMC is up to 10 yr. Behavior-monitoring collars were synchronized (for time and communication to the base station) up to 7 d before being observed. Any BMC failing to synchronize was replaced, and the corresponding cow was not reconsidered for observation until 21 d post-BMC replacement to allow the device to calculate baseline values.

Every cow was observed for two 2-h periods (0700 to 0900 h, and 1900 to 2100 h) within a 24-h timeframe to attempt to record a range of behaviors and account for diurnal variation (DeVries et al., 2003). Observations occurred between September 2017 and April 2018. One cow was observed at a time. All observations were completed in the cow's home pen (approximately 621 m² pen with a 202 m² feed alley, stocked with approximately 58 ± 4 cows). A single observer completed all observations for the study to avoid any inter-observer variance. The observer was positioned within a clear field of view of the focal cow to ensure constant view of the cow's head and muzzle, and without interfering with the cow's natural behavior. A multifunction, radio frequency synchronized atomic watch (Casio, Casio America Inc., Dover, NJ) was used to manually record durations of behavior (hh: mm: ss). The rumination, resting, and feeding behaviors were observed following a previously constructed ethogram, which is provided in Table 1.

Each cow's 240-min observation was summed by behavior (rumination, feeding, and resting) to assess agreement of visually recorded behavior to BMC data. All analyses were performed with SAS (version 9.4, SAS Institute Inc., Cary, NC), using the cow as the experimental unit.

Recorded data from the BMC were supplied from the company in 15-min blocks (summed by behavior). No data were missing from the BMC for any period.

Descriptive analyses were performed, and data were verified for normality using the PROC UNIVARIATE procedure and probability distribution plots. No outliers were detected (data points beyond 3 SD from the mean) transformations were deemed necessary.

Precision was analyzed by a Pearson correlation coefficient (r) and linear regression coefficient of determination (R^2). Results of the Pearson correlation coefficient and coefficient of determination were categorized by Hinkle (1988; 0.00 to 0.30 = namely negligible; 0.30 to 0.50 = low; 0.50 to 0.70 = moderate; 0.70 to 0.90 = high; and 0.90 to 1.00 = very high). Linear regressions were used to calculate the coefficient of determination, and linear regressions with a restricted zero intercept were used to calculate the slope of the relationship between the BMC and visual observation data. Data from the BMC were considered precise if the r and R^2 were high (>0.70). Additionally, the ρ_c was calculated for all behaviors following Lin (1989). Results of the Lin's ρ_c were categorized by McBride (2005; <0.90 = poor; 0.90 to 0.95 = moderate; 0.95 to 0.99 = substantial; >0.99 = almost perfect). Data from the BMC were considered accurate if the r and R^2 were high (>0.70), Lin's ρ_c was classified at least as moderate (>0.90), and the slope of the regression analysis (not different from 1) and Bland-Altman plots were deemed accurate.

Bland-Altman plots (Bland and Altman, 1995a,b) were created for each behavior in Excel [Excel 2016 (v.16.0), Microsoft Corp., Redmond, WA]. The difference of collar and observed behaviors (collar – observed) for each cow's 240-min observation was used to

calculate average bias of the 24 observed cows' difference. Standard deviation of the difference of collar and observed behaviors was calculated from all cow's 240-min observation. Standard deviation was then used to calculate the lower and upper limits of agreement [$\text{bias} \pm (1.96 \times \text{SD})$]. The x-axis showed the mean of the observed and collar recorded behavior $[(\text{observed} + \text{collar})/2]$ for each of the 24 cows plotted. The y-axis showed the difference of the observed compared with the collar-recorded behavior ($\text{collar} - \text{observed}$) for each of the 24 cows plotted. The BMC was considered accurate if the slope from the linear regressions did not differ significantly from 1, and if the 95% interval of agreement included 0 for mean bias from the Bland-Altman plots.

RESULTS

Descriptive data measured by visual observation and BMC are presented in Table 2.2.

The r were 0.99, 0.93, and 0.94 ($P < 0.001$) for rumination time, feeding time, and resting time, respectively. The R^2 were 0.97, 0.85, and 0.88 ($P < 0.001$) for rumination time (Figure 2.1a), feeding time (Figure 2.1b), and resting time (Figure 2.1c), respectively. The slope of regression was found to be 0.90 (CI: 0.87–0.93) for rumination time; 0.77 (0.72–0.83) for feeding time; and 1.13 (1.07–1.19) for resting time. Concordance correlation coefficients (ρ_c) were 0.95, 0.80, and 0.82 for rumination time, feeding time, and resting time, respectively. A Bland-Altman plot was used to assess the differences between the collar and visual observations for rumination (Figure 2.2a), feeding (Figure 2.2b), and resting (Figure 2.2c). The 95% confidence interval of the Bland-Altman plot encompassed all but one cow's observations for both rumination and feeding time, and all cows' resting time observations. Mean differences (BMC – observation) of the plots indicated whether the BMC was overestimating (positive bias) or underestimating (negative bias) behavior compared with visual observations. The results of the mean differences were rumination time: -7.57 ± 6.31 min; feeding time: 15.81 ± 11.84 min; and resting time: -13.03 ± 9.37 min. The mean differences did, however, include

zero within the 95% interval of agreement, indicating no difference between the BMC and visual observation. Precision and accuracy criteria results are shown in Table 2.3.

DISCUSSION

Precision dairy technology is useful to monitor behavior, or get alerts for abnormal cow behavior (Soriani et al., 2012). Cows have been reported to ruminate less if they are (or later become) diagnosed with metabolic disease(s) (Schirmann et al., 2016; Stangaferro et al., 2016a), or mastitis (Stangaferro et al., 2016b), or during the time around calving (Soriani et al., 2012; Calamari et al., 2014). Cows developing health disorders typically change activity to an extent that algorithms of the PDT can detect and report deviations of cow behavior, in comparison to normal (Stangaferro et al., 2016a). The MooMonitor+ collar used in this study yielded a high correlation of automated observations for feeding, ruminating, and resting behaviors in comparison to a trained observer. This finding is important for potential future large-scale implementation of the collar on-farm, because the automatic collection of data is precise. The BMC should detect the deviation in behavior, thus giving the producer an early warning to respond appropriately for the individual cow as needed.

Feeding and rumination time were in agreement with the visual data in this study. Precisely quantifying feeding and rumination is important because these behaviors decrease in cows that have been (or later become) diagnosed with metabolic disease (Goldhawk et al., 2009; Schirmann et al., 2016; Stangaferro et al., 2016a). The correlation of behavioral changes with metabolic disease gives producers the opportunity to detect onset of metabolic disease earlier because of the behavioral changes, which is important for cow welfare, maintaining milk production, and maintaining reproduction rates on farm. Additionally, feeding and rumination behavior deviating from normal during the period around calving may negatively affect cow reproduction (Wiltbank et al., 1962; Roche et al., 2000; Wiltbank et al., 2015).

Although BMC feeding data were highly correlated with visual observations, further research should investigate the accuracy of PDT in different management and housing conditions.

The BMC found resting behavior to be in agreement with visual observation. Animals with metabolic diseases such as ketosis have been reported to lie more (Sepúlveda-Varas et al., 2014; Itle et al., 2015) or to be lethargic or depressed (Hart, 1988), making this behavior important to precisely assess over longer periods of time to detect animals at risk.

We found the BMC to be very precise in measuring behavior of dairy cows, and it performed similarly to or better than other commercially available behavior monitoring technologies. An accelerometer ear tag (ear tag 1) was validated by Bikker et al. (2014; freestall-housed dairy cows, TMR and partial mixed ration fed), Wolfger et al. (2015; steers housed on an outdoor dirt floor, bunk fed), Borchers et al. (2016; freestall-housed dairy cows, TMR fed), and Pereira et al. (2018; grazing dairy cows). All authors found good correlation for feeding when validating ear tag 1; however, the BMC validated in this study had better precision. Bikker et al. (2014) had very similar, high correlations for resting. Additionally, Bikker et al. (2014) reported ear tag 1 to have similar very high precision of rumination as the BMC; however, Wolfger et al. (2015), Borchers et al. (2016), and Pereira et al. (2018) reported a much lower correlation for rumination when validating ear tag 1. Future research could compare the performance of PDT in different management styles and environments for precision and accuracy. Borchers et al. (2016) additionally validated 2 other accelerometer behavior-monitoring ear tags (ear tag 2 and ear tag 3), both reporting very high correlations for feeding and rumination behaviors, respectively. A collar using a combination of a microphone and an accelerometer was validated by Schirmann et al. (2009) was also very highly precise in addition to having a similar percentage of cows included in the 95% interval agreement of the Bland-Altman plot. A validation of a commercially available noseband pressure sensor by Zehner et al. (2017) reported similar values of accuracy and precision as the BMC in this study

for ruminating and feeding. The BMC validated in this study had the same or higher accuracy as the noseband pressure sensor, depending on version of converter used by Zehner et al. (2017). The noseband pressure sensor studied by Zehner et al. (2017) was less precise at measuring feeding time when compared with the BMC.

Precise PDT can make a dairy more efficient by automating animal monitoring and alerting producers to cow behavior change (El-Osta and Morehart, 2000). Precision dairy technologies with low precision can lead to producers not acting on alerts (Eckelkamp, 2018). Inaccurate PDT will have either low sensitivity (true positives) or low specificity (true negatives), meaning it fails to alert (false negative) for a cow with abnormal behavior, needing attention, or falsely alerts (false positive) for cows with normal behavior, respectively. Eckelkamp (2018) noted that producers would often not act on alerts if the alerts were not believed. Additionally, PDT with low precision has the potential to be very expensive, for example by missed estrus detection, missed health events (Hogeveen et al., 2010; Rutten et al., 2013), or distributing treatment to healthy animals (Burfeind et al., 2011).

Accurate PDT provides real-time cattle monitoring tools for producers and data recording for management and comparisons (Norton and Berckmans, 2017). Highly accurate PDT can especially help producers running larger dairies without compromising integrity of animal care (Norton and Berckmans, 2017). Accuracy opens an opportunity for data to be compared across the industry, for example by comparing records between multiple farms, or for cross-sectional research. None of the three behaviors tested in this study met the slope criteria (slope not different from 1), and only rumination met the ρ_c criteria established for accuracy. Most studies regarding validation of automated behavior monitoring devices have not presented data regarding the accuracy of the device. Future research should investigate the factors that affect accuracy of PDT. Additionally, few validation studies have investigated the

accuracy of PDT; thus, there is a need to validate PDT in different environments and to determine how the accuracy of the technology is affected.

Overall, PDT aims to precisely record changes in behavior, therefore potentially detecting health or wellbeing issues before producers may have otherwise visually noticed the cow requiring intervention (Norton and Berckmans, 2017). Benefits of earlier observation or otherwise unnoticed cattle illness include preventing expensive treatments (Mazeris, 2010), reducing large production losses (Mazeris, 2010; Steensels et al., 2017), improving treatment implementation time (Goff, 2008; Sheldon et al., 2008; Lomander et al., 2012), and improving animal well-being (Steensels et al., 2017). Additional to producer uses, PDT with high accuracy can also aid researchers of dairy cows to collect a comprehensive data set. Practical PDT like the BMC in this study can be worn with little disruption to the cow, and take readings of behavior consistently, 24 h/d. Being able to rely on such a device could help researchers collect constant and consistent data without having to rely on visual observation. This could remove some limitations in dairy cow behavior studies such as inter- and intra-observer differences, enabling collection from all cows simultaneously and enabling data collection overnight in low visibility.

To our knowledge, this is the first validation study of the MooMonitor+ for ruminating, resting, and feeding in a confinement setting for lactating dairy cows. In this study, the BMC performed precisely, with very high correlations for ruminating, feeding, and resting behaviors.

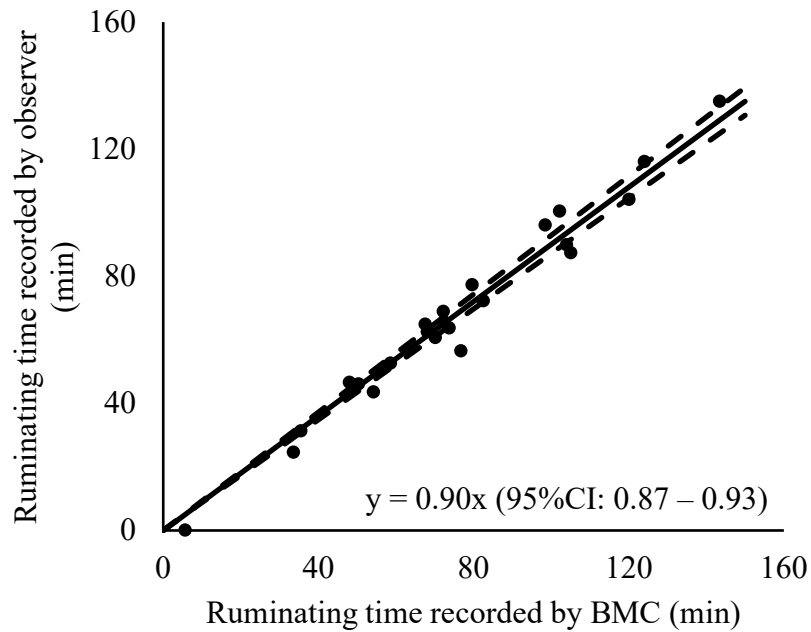
ACKNOWLEDGEMENTS

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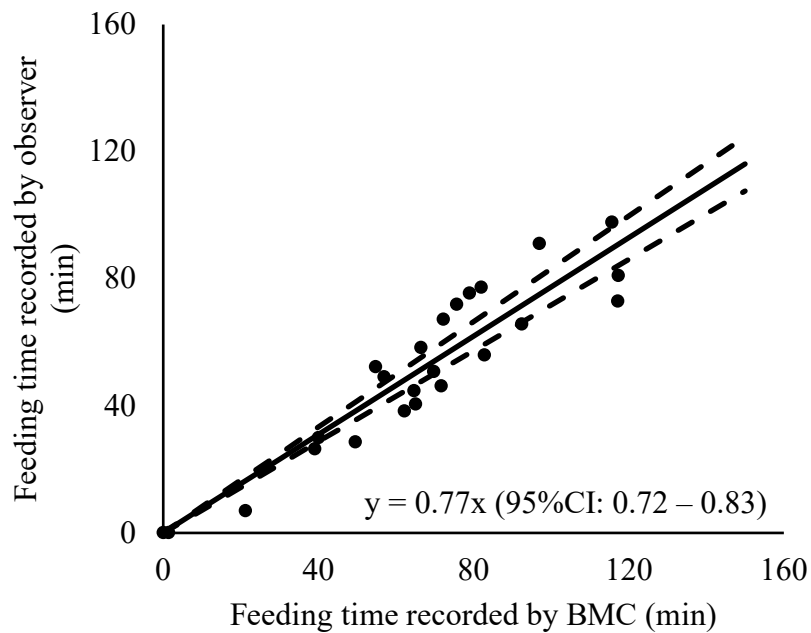
Kalamazoo, MI) for helpful discussions on the topic of this study. We also thank Olga Vsevolozhskaya, Michelle Arnold, and Eric Vanzant from the University of Kentucky for their contribution in this project. This project was funded by DairyMaster (Co. Kerry, Ireland), through a research project partnership with the Dairy Science Program at the University of Kentucky.

Figure 2.1. Regression of rumination (a), feeding (b), and resting (c), comparing the behavior-monitoring collar (BMC; *x*-axis) with visual observations (*y*-axis). Data points indicate total minutes each cow spent performing the corresponding behavior during the 240-min observation.

a.



b.



c.

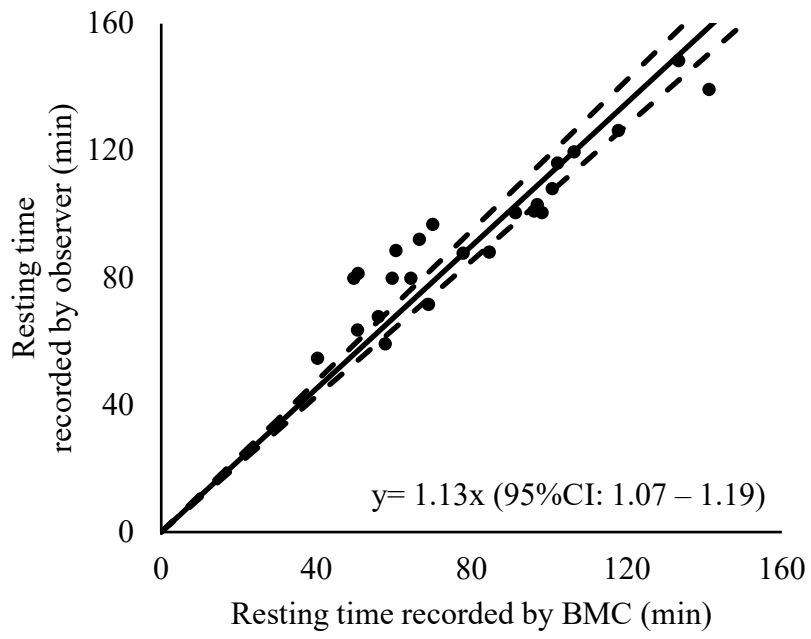
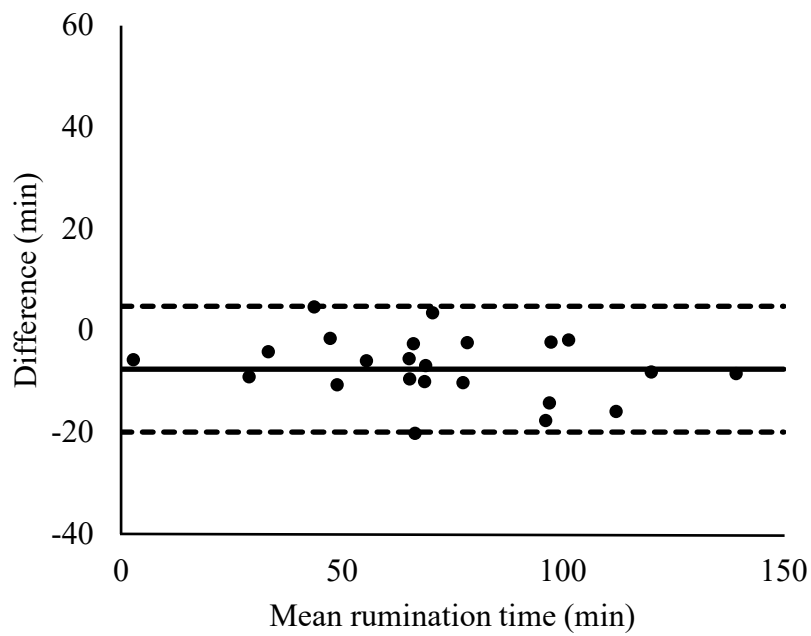
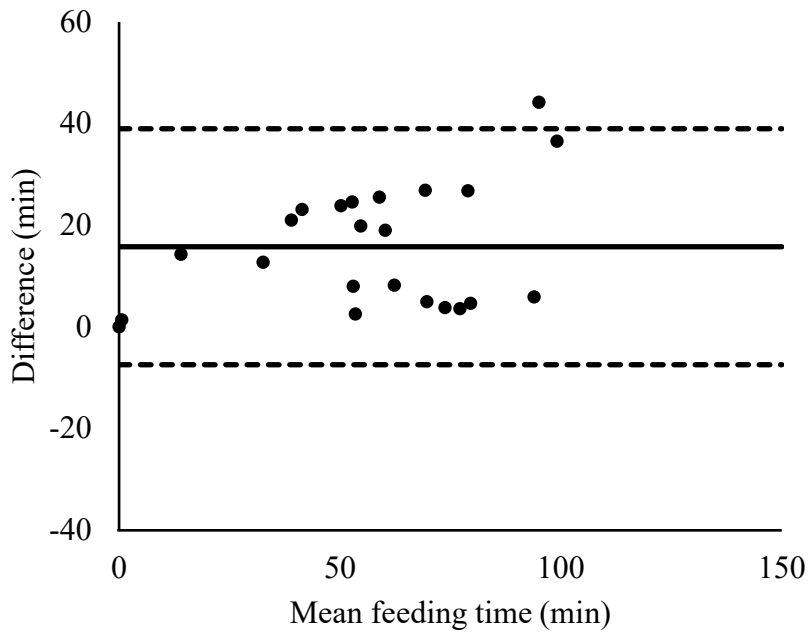


Figure 2.2. Bland-Altman plot illustrating agreement between the behavior-monitoring collar (BMC) and visual observations for ruminating (a), feeding (b), and resting (c). For all graphs, the x-axis is the mean of BMC and visual observation and the y-axis is the difference between BMC-recorded behavior and visual observation (BMC – observed). Every data point on the graph is the result of each cow’s agreement for the corresponding behavior.

a.



b.



c.

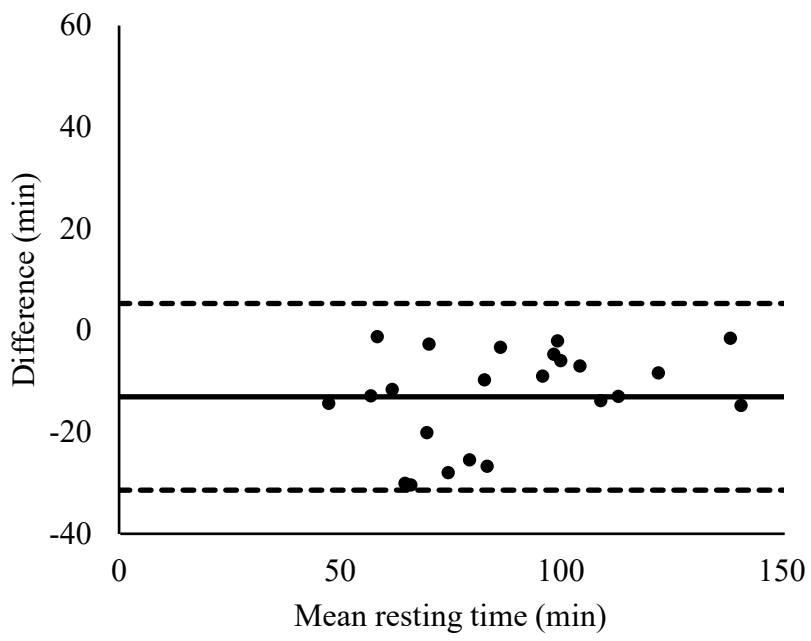


Table 2.1. Ethogram of behavior classification for visual observations

Behavior	Classification
Ruminating	Regurgitation and re-mastication of a bolus with a rhythmic jaw movement. A break between bolus exchanges of ≥ 5 s was recorded as a different activity.
Resting	Includes lying and standing behavior. A lying event was defined as any time the cow was lying with all four limbs on the ground. Lying events begun when the posterior end contacted the ground, and finished when the posterior end was off the ground. A standing activity was categorized by the cow standing static for ≥ 5 s, with all four hooves on the ground.
Feeding	Cow with muzzle in contact with feed, including sorting, smelling, and chewing feed (not stopping for ≥ 5 s).
Other	Any other activity such as drinking, walking, grooming, licking, rubbing, interacting with other cows.

Table 2.2. Mean, minimum, and maximum time (min) of lactating dairy cows spent ruminating, feeding, and resting, as recorded by visual observations and the behavior-monitoring collar (BMC)¹

	Visually recorded observation			BMC recorded observation		
	Mean ± SD (% ± SD)	Minimum	Maximum	Mean ± SD (% ± SD)	Minimum	Maximum
Ruminating	70.1 ± 31.0 (29.2 ± 12.9%)	0.0 (0%)	135.0 (56%)	77.0 ± 32.1 (32.1 ± 13.4%)	5.7 (2.4%)	143.5 (59.8%)
Feeding	48.0 ± 25.6 (20.0 ± 10.7%)	0.0 (0%)	91.0 (37.9%)	65.5 ± 31.3 (27.3 ± 13.0%)	0.0 (0%)	117.4 (48.9%)
Resting	93.4 ± 23.8 (38.9 ± 9.9%)	54.7 (22.8%)	148.3 (61.8%)	79.7 ± 27.3 (33.2 ± 11.4%)	40.3 (16.8%)	141.3 (58.9%)

¹The percentage of time spent displaying the corresponding behavior of the 240-min observation is given in parentheses.

Table 2.3. The results of the precision and accuracy test of rumination, feeding, and resting behaviors between visual observations and the behavior-monitoring collar (BMC)¹

	Ruminating	Feeding	Resting
Pearson correlation coefficient (r)	Yes	Yes	Yes
Coefficient of determination (R ²)	Yes	Yes	Yes
Concordance correlation coefficient (ρ_c)	Yes	No	No
Slope of the linear regression	No	No	No
Bland-Altman Plots	No	No	Yes
All criteria	No	No	No

¹Data from the BMC were considered precise if the r and R² were high (>0.70). The BMC was considered accurate if the slope from the linear regressions did not differ significantly from 1, if visual analysis presented no bias on the Bland-Altman plots, and if all difference data were within the 95% interval of agreement.

CHAPTER THREE:

VOLUNTARY HEAT STRESS ABATEMENT SYSTEM FOR DAIRY COWS: DOES IT
MITIGATE THE EFFECTS OF HEAT STRESS ON PHYSIOLOGY AND BEHAVIOR?

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INTRODUCTION

Cows in environmental conditions that exceed their thermoneutral zone (5 to 25°C) (McDowell, 1972) have increased metabolic requirements, increase respiration rate (RR), sweat, and pant to regulate body temperature (Collier et al., 1982). Temperature humidity index (THI) is a common method of assessing heat stress affecting dairy cows, as it is being shown to be highly associated to production losses and behavioral changes (Ravagnolo et al., 2000; Bohmanova et al., 2007). Production losses have been found to occur at around $THI \geq 72$ (Armstrong, 1994; Chase, 2006); however, behavioral and motivational changes were found to occur at lower THI, such as ≥ 68 (De Rensis et al., 2015).

Heat stress reduces DMI (Spiers et al., 2004; Bernabucci et al., 2010; Soriani et al., 2013) and feeding bouts (Bernabucci et al., 2010), therefore heat stressed cows ruminate less (Kadzere et al., 2002; Bernabucci et al., 2010; Soriani et al., 2013). This can have consequences for rumen pH because more feeding bouts and ruminating produces saliva which acts as a pH buffer, important for healthy rumen pH (Bernabucci et al., 2010). Heat stress additionally affects cow behavior including preference for standing rather than lying (Tucker et al., 2008; Allen et al., 2015), even after lying deprivation (Schütz et al., 2008), which is a welfare issue. The increase of maintenance metabolism (Collier et al., 1982) and reduction in DMI (Spiers et al., 2004; Bernabucci et al., 2010; Soriani et al., 2013) during heat stress, heat stressed cows are often in a state of negative energy balance (Drackley, 1999). Negative energy balance subsequently diminishes milk production (Spiers et al., 2004; Soriani et al., 2013) – the primary source of income to dairy producers – and is therefore a serious economic issue for the dairy industry.

It is important to investigate options for cooling heat stressed dairy cows to improve milk production to support dairy farm economics and to improve cow welfare during periods of heat stress. Options for heat abatement have previously included the use of shade, fans, and

soaking the cows, which typically reduce the negative effects of heat stress on physiology and behavior (Schütz et al., 2011; Chen et al., 2016; Tresoldi et al., 2018). For decades, one of the most efficient methods of cooling dairy cattle was based on repeated soaking to attain maximal water trapping in the coat, followed by its rapid evaporation (Flamenbaum et al., 1986; Chen et al., 2016). Water as a method of cooling is often delivered via automated cycling sprinklers installed above feed alleys; however, cows may perceive them as a deterrent or obstacle because wetting their head is uncomfortable (Chen et al., 2016). Conversely, cows have not been observed to display head wetting avoidance behavior in a voluntary use soaking system (Legrand et al., 2011).

Dairy cattle respond to an increase in heat load differently to one another. Voluntary soaking stations for heat stress abatement provides freedom of choice and cow self-management, which focuses on individual cow needs instead of the group. Other voluntary use equipment (e.g. automatic milking systems) are perceived as advantageous because removes the necessity of daily laborious tasks (e.g. daily milking), and adds freedom of choice whilst avoids herding and interaction with humans (Webster, 2001; Holloway et al., 2014). Because cows have different, individualized tolerances of heat stress, it seems logical to offer heat abatement at an individually self-management level, such as by a voluntary use soaker station. Cow heat tolerance differs between individuals because of genetics (Aguilar et al., 2009; Liang et al., 2013; Alfonzo et al., 2016), parity (Aguilar et al., 2009; Stone et al., 2017), milk production (Liang et al., 2013; Stone et al., 2017), body size, hair structure (Alfonzo et al., 2016), and many other factors. Thus, animals may have different requirements and therefore motivation to use heat stress abatement tools. Thus, the first objective of the study was to assess the heat abatement capability of voluntary soaking of cows by assessing cow physiology (RR, body temperature), behavior (rumination, feeding, resting, and lying time, and steps/d), and milk production. The second objective was to compare voluntary soaker use of cows between

treatments two mandatory soakers at the parlor with voluntary soaker use, in comparison to voluntary soaker use with no mandatory soakers). Lastly, this study aimed to determine the relationship between voluntary soaker use and THI.

MATERIALS AND METHODS

The study was conducted from July 10th to October 3rd, 2018 at the University of Kentucky Coldstream Research Dairy Farm (Lexington, KY, USA; Lat: 38.1103759, Long: -84.5164302), and approved by the Institutional Animal Care and Use Committee of the University of Kentucky (Protocol # 2018-2914).

Cows were housed in a compost bedded pack barn that was tilled twice/d (approximately 0520 to 0550 h, and 1415 and 1510 h). Layout and approximate measurements of barn and fan placement is illustrated in Figure 3.1. Each side of the barn was equipped with two 4.9 m fans (Powerfoil X3.0, Big Ass Fans, KY, USA) and six 91 cm fans over the feed alley. Cows on this study were housed in one pen. Cows were fed a TMR formulated following the National Research Council (NRC) guidelines (NRC, 2001) to meet or exceed the requirements of lactating dairy cows producing at least 39 kg of milk daily. Composition of the TMR *as fed* was 40.7% corn silage, 27.8% lactating cow grain mix, 23.6% alfalfa silage, 5.1% cotton seed, 1.8% alfalfa hay, and 1.0% mineral mix. Cows were fed *ad libitum* twice per day at approximately 0800 and 1400 h. Orts were removed once per day before the afternoon feeding. Animals had *ad libitum* access to fresh water provided from a self-filling water trough located in the feeding alley. Milking occurred twice daily at 0730 and 1800 h. A summary of environmental conditions during the experimental period is given in Table 3.1.

Fifteen lactating Holstein cows were randomly chosen from the herd from the criteria of confirmed pregnant (60 d check) and mid lactation. At the time of enrollment (d 1 of training), cows were (mean \pm SD) DIM: 233 \pm 38; parity: 2 \pm 1; weight: 673 \pm 69 kg; milk yield: 38.0 \pm 5.4 kg/d. Cows were split into eight pairs (one cow in pair H), balanced for parity,

milk production, and bodyweight. Cows were moved from other pens within the barn to the study pen 2 d before the training period (all cows were moved from pens other than the study pen).

Cows were assigned to one of the two treatments by random block design for eight, 1 week treatments. Treatments were: 1) mandatory soaker treatment: two mandatory soakings/d with access to the voluntary soaker throughout the day; and 2) no mandatory soaker treatment: voluntary soaker access throughout the day (no mandatory soakers). Mandatory soakers were given by sorting cows via a sorting gate at the exit of the milk parlor via an alley equipped with a double motion sensor cattle soaker (Cool Sense, Edstrom, WI, USA). All cows could access the voluntary soaker adjacent to the pen at any time except during milking and alley scraping (mean \pm SD: 18.3 \pm 1.6 h/d access). The voluntary soaker was adjacent to barn as an extension of the feed alley walkway (Figure 3.1), with a grooved concrete floor and 75% covered shade cloth overhead.

All cows were trained to use the voluntary soaker during a 4 week training period. Training for the voluntary soaker involved the same individual encouraging cows to pass through the soaker 3x/d (1000, 1230, and 1500 h). Training was considered complete after a cow voluntarily used the soaker at two separate instances within 3 d (monitored via video footage), or after two weeks of training. All cows that were included in the training period were deemed trained and no cow was removed from the experiment. Cows were acclimated to the mandatory soaker for the 4 week training period by using the sort gate and mandatory soaker exit alley from the parlor under a motion sensor soaker. After the training period, the 8 week of treatments were applied.

The mandatory and the voluntary soakers were identical model and setting. Both soakers were set to a 5 s cycle; water flow rate was approximately 4.1 L/5 s soaker cycle via two shower heads (accumulatively). The soaker system was activated once both motion sensors

were activated. The soaker at the exit alley was installed as per manufacturer instructions; two motion sensors were installed above the exit alley approximately 1.8 m apart to allow one cow to activate the soaker while walking through the alley (soaking only one cow at a time). However, the voluntary soaker was modified; both motion sensors were next to each other, immediately adjacent to the shower heads. This modification was made so a cow could activate both motion sensors (and therefore another soaker cycle) while standing under the water flow.

Data Collection

Physiological measurements of the cows measured during the study were reticulo-rumen temperature (RT), RR, and panting score. Reticulo-rumen temperature measurements were collected with an automated data logging bolus (Herdstrong TruCore, DVM Systems, CO, USA) which has been previously validated for recording of RT (Bewley et al., 2008). The company supplied RT data after removing temperature changes from drinking using their algorithm. Boluses were assigned to cows 6 ± 1 d before d 1 of the training period. Respiration rate was recorded by counting flank movements for 1 min following methodology of Rhoads et al. (2009) and Min et al. (2015). Respiration rates were recorded 3x/d; before morning milking (approximately 0645 h), midday (approximately 1230 h), and before evening milking (approximately 1720 h). The observer recording RR was stationed approximately 5 m from the focal cow, and ensured flank movements were visible for the duration of the observation. At the same time, panting score was recorded using a pre-defined ethogram (Table 3.2). Two observers recorded RR and panting scores (observer 1: 88%; observer 2: 12% of the observations). Observer 1 trained observer 2 for RR and panting score. High interobserver agreement was achieved for RR and panting score as defined by Hinkle (1988) ($r = 0.98$ and 0.87 , $P < 0.001$; $R^2 = 0.96$ and 0.75 , $P < 0.001$; respectively), and no difference ascertained by a Bland-Altman plot.

Behavioral data were collected from all cows via automated data loggers. Rumination (min/d), feeding (min/d), and resting (min/d) were recorded for each cow by a behavior monitoring collar (MooMonitor+, Dairymaster, Kerry, Ireland). The collar has previously been validated for all the utilized behaviors (Grinter et al., 2019). Collars were assigned to cows as per farm protocol (306 ± 197 d before the beginning of the training period). A behavior monitoring leg tag (AfiTagII, Afimilk, Kibbutz Afikim, Israel) was used to monitor lying time (min/d), lying bouts (bouts/d), and steps (steps/d). The leg tag has previously been validated for lying time, lying bouts, and steps (Higginson et al., 2010). Leg tags were assigned to cows as per farm protocol (> 30 d before d1 of the training period).

Daily milk yield was recorded during each milking and summarized by day, using an automatic meter (AfiMilk, Afimilk, Kibbutz Afikim, Israel). Milk fat and protein were measured at each milking using an in-line milk analyzer (AfiLab, Afimilk, Kibbutz Afikim, Israel) that had previously been validated (Kaniyamattam and De Vries, 2014). A 3 d rolling average was calculated each day for milk yield, milk fat, and milk protein by taking the mean value of a day with the previous 2 d.

Use of the voluntary soaker was monitored 24 h per day for the duration of the study via video footage (Hikvision, model: DS-2CD2342Wd-I, Hangzhou Hikvision Digital Technology Co. Ltd., Hangzhou, China). Video was recorded and later played back to record soaker use by each animal. Four observers recorded voluntary soaker use and recorded cow ID, time of cycle activation (hh:mm:ss), and area of cow wet following an ethogram (Table 3.3). In addition, observers recorded what happened at the end of each soaker cycle: continued use, displacement (by a cow or farm personnel), by the cow's choice (no other cows present), or unknown (not obvious whether displacement or cow's own choice). In any event of displacement, the cow displacing and the cow that was displaced were both recorded. Voluntary soaker use was summed by experimental day (0000 to 2359 h) and averaged by hour

for each and all animals during the experimental period. To determine interobserver reliability, 15, 1 h blocks of video were simultaneously watched by all observers to determine the use of the soaker by each cow during the period, very high ($r > 0.95$) correlation and very high ($R^2 > 0.95$) linear regressions were used to deem observer reliability for discerning soaker use per day.

Ambient temperature and relative humidity was obtained by a portable weather station (HOBO External Temperature/Relative Humidity Data Logger – U23-002, Onset Computer Corporation, Bourne, MA, USA) located in the study pen. Wind speed was recorded by an anemometer (Model 20250-22, Digi-Sense, Cole-Parmer, IL, USA) located in the study pen. The calculation for THI includes air temperature ($T^{\circ}\text{C}$) in Celsius, and relative humidity (RH) were performed using the following formula:

$$\text{THI} = (1.8 \times T^{\circ}\text{C} + 32) - (0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T^{\circ}\text{C} - 26),$$

$T^{\circ}\text{C}$ = ambient temperature ($^{\circ}\text{C}$); RH = percentage of relative humidity (NOAA, 1976).

Hourly THI weather values were averaged to calculate mean daily THI, and the maximum daily value was taken for daily maximum THI. Additionally, mean daily THI was averaged by hour for the duration of the experimental study to calculate mean hourly THI. Wind speed data was calculated on a mean daily basis. Daily precipitation data were collected from the University of Kentucky Agronomy Research Farm (Lat: 38.1341919, Long: -84.4962154; approximately 4.8 km from the University of Kentucky Coldstream Research Dairy Farm) and summed by day.

Statistical Analysis

Data Preparation. All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Before analysis, data were checked using the UNIVARIATE procedure in SAS and probability distribution plots to assess normality.

Any values of lying time = 0 min/d or > 1440 min/d were removed because they were considered recording error by the device; the 1st (< 366 min) and 99th percentile (> 1014 min) of data were also removed (Stone et al., 2017). Seven percent of lying bout data were removed because the device reported 0 lying bouts/d, which was considered recording error.

Originally there were 28,498 recordings for RT. Any hour where mean RT was < 35.6°C or > 42.2°C were removed for biological relevance, as outlined by Bewley et al. (2008). This step removed 8,654 data points. After this, a further 190 were removed because they were outlier points exceeding 3 SD of the mean, following the same data preparation steps as Bewley et al. (2008), resulting in 19,654 points.

Data from cows detected having mastitis (two cows) or in estrus (one cow) by standard farm procedures during the study were removed the day before detection, day of detection, and day after detection.

Statistical Analyses. Data were analyzed with SAS (version 9.4, SAS Institute, Cary, NC, USA). The effect of treatment was determined by an analysis of variance (ANOVA) using mixed linear models (MIXED procedure) in SAS. The fixed effects in the model included treatment (mandatory soakings or no mandatory soakings), soaker use/d, pair (A-H), mean daily THI, DIM, daily milk yield (kg/d), and interaction of treatment with mean daily THI. Study day was specified as a repeated measure. Cow was used as subject. The model used an autoregressive (AR -1) model structure (smallest AIC structure and consistent with the data structure). Effects with a p -value > 0.30 were removed from the model using a stepwise backward elimination process starting with the least contributing effect. Treatment, soaker use/d, pair and mean daily THI remained as a fixed effect regardless of significance. Significance was declared at $P \leq 0.05$, and trends were defined as $P \leq 0.10$.

When the fixed effect of voluntary soaker use/d was significant for any outcome variable (RR, panting score, RT, rumination, feeding, resting, lying, steps, milk yield, milk

protein percent, and milk fat percent), an additional linear regression analysis (PROC REG) was performed for that variable, with voluntary soaker use/d as the explanatory variable. The regression analyzed the relationship between voluntary soaker use and voluntary soaker + 2 mandatory soakings per day when cows were applied the mandatory soakings treatment (total soaker use) with the response variable to fully assess the influence of cooling by soakers. In addition, when the fixed effect of THI was significant for any outcome variable (soaker use/d, RR, panting score, RT, rumination, feeding, resting, lying, steps, milk yield, milk protein percent, and milk fat percent), an additional linear regression analysis (PROC REG) was performed for that outcome variable (response variable) with THI (explanatory variable).

Linear regression analyses (PROC REG) were also used to model (univariate) the relationship of the distribution of mean soaker use by hour, RR, and panting score (response variables) against THI (explanatory variable). Temperature humidity index was modeled with increasing THI in 1-h increment delays to find the closest fitting model (lowest *P*-value with highest R^2 value) to explain the relationship the response variables had with THI.

Lastly, linear regression analyses (PROC REG) was used to model the relationship of total displacement events (either actor or reactor) (response variable) at the voluntary soaker with total soaker use (explanatory variable) for the duration of the experimental period.

RESULTS

The effects of treatment on physiological, behavioral and milk variables are outlined in Table 3.4. Treatment had minimum effects on measures of physiology and behavior. Briefly, the only variable that differed with treatment was daily rumination time.

The effects of treatment of soaker use/d and temperature humidity index with the physiological, behavioral and milk variables are outlined in Table 3.5. Briefly, soaker use per day influence some variables related to heat stress measurements; we found differences in soaker use/d for minimum daily respiration rate, mean and maximum reticulo-rumen

temperature, rumination time, resting time, steps/d, and daily milk yield. Temperature humidity index had a significant relationship with all variables except for milk protein percent, outlined in Table 3.5. Results of the regression analyses for those variables that were affected by daily soaker use or temperature humidity index (Table 3.5) are presented in Table 3.6. Detailed results information is provided below.

Daily Soaker Use

Treatment did not influence soaker use; the frequency of voluntary soaker use during the no mandatory soakings treatment was approximately 15 voluntary soakings/d, similar to the two mandatory soakings treatment of about 12 voluntary soakings/d ($F_{1,14} = 1.74$; $P = 0.21$; Table 3.4).

There was large individual variation in voluntary soaker use, ranging from 0 to 227 soakings/d, (mean \pm SD) 13 ± 30 voluntary soakings/d, Figure 3.2. Four cows had a maximum use of less than 10 soakings/d, while four other cows had a maximum of more than 100 soakings/d, Figure 3.2. Total voluntary soaker use was predominantly on the back area, followed by the side, rump, neck and head, and licking was the least common soaker use area, Figure 3.3. At the end of each 5 s soaker cycle, cows typically continued to use the soaker, Figure 3.4. Cows were least likely to leave the soaker because of displacement (by cow or farm personnel), followed by leaving by choice, Figure 3.4. The main reason cows left the soaker was “unknown” (observer unable to differentiate between leaving by choice versus a displacement; Figure 3.4). Cows with a greater frequency of soaker use were more likely to displace another cow from the soaker ($F_1 = 9.28$; $P < 0.01$) or be displaced from the soaker ($F_1 = 30.61$; $P < 0.001$). Hourly voluntary soaker use during the day is graphed for descriptive purposes in, Figure 3.5; soaker use peaked between 1900 to 2000 h, and nadir (excluding hours manipulated by limited access) was between 1100 to 1200 h.

Respiration Rate and Panting Score

Treatment did not affect RR minimum ($F_{1,14} = 2.65$; $P = 0.13$), mean ($F_{1,14} = 3.29$; $P = 0.09$), or maximum ($F_{1,14} = 0.06$; $P = 0.81$), Table 3.4. Soaker use did affect RR minimum ($F_{1,752} = 5.51$; $P = 0.02$), therefore, a regression analysis was performed and found a positive relationship between soaker use and RR minimum (Table 3.6). There was however no difference in soaker use and mean RR ($F_{1,736} = 0.97$; $P = 0.33$) or maximum RR ($F_{1,751} = 0.11$; $P = 0.74$) therefore, no regression analysis was performed.

Treatment did not affect panting score minimum ($F_{1,14} = 0.45$; $P = 0.51$), mean ($F_{1,14} = 3.36$; $P = 0.09$), or maximum ($F_{1,14} = 0.34$; $P = 0.57$) (Table 3.4). Soaker use did not affect panting score minimum ($F_{1,696} = 0.31$; $P = 0.58$), mean ($F_{1,736} = 0.47$; $P = 0.49$) or maximum ($F_{1,751} = 0.24$; $P = 0.63$). Therefore, no regression analysis was performed for any panting score variables.

Reticulo-Rumen Temperature

Treatment did not affect RT minimum ($F_{1,14} = 0.05$; $P = 0.83$), mean ($F_{1,14} = 2.20$; $P = 0.16$), or maximum ($F_{1,14} = 2.99$; $P = 0.11$); Table 3.4. Soaker use did not affect RT minimum ($F_{1,637} = 2.38$; $P = 0.12$), however mean ($F_{1,637} = 19.37$; $P < 0.001$) and maximum RT ($F_{1,637} = 25.62$; $P < 0.001$) was positively correlated with increasing voluntary soaker use (Table 3.6).

Rumination, Feeding, and Resting Behavior

Cows ruminated for more minutes per day during the two mandatory soakings treatment (558.6 ± 5.2) compared to the no mandatory soakings treatment (543.4 ± 5.2 min/d; $F_{1,14} = 11.14$; $P < 0.01$; Table 3.4). Additionally, there was a relationship with daily rumination and daily soaker use ($F_{1,752} = 11.28$; $P < 0.001$), therefore a regression was performed.

Treatment did not affect daily eating time ($F_{1,14} = 0.12$; $P = 0.74$); nor did daily soaker use affect daily eating time ($F_{1,752} = 0.01$; $P = 0.94$) (Table 3.4), therefore no regression was performed and found a negative association.

The two mandatory soakings treatment had a tendency to have less daily resting time compared to the no mandatory soakings treatment (mandatory soakings: 560.3 ± 4.0 , no mandatory soakings: 570.6 ± 4.0 min/d; $F_{1,14} = 4.21$; $P = 0.06$). Resting time increased with increasing voluntary soaker use ($F_{1,697} = 39.05$; $P < 0.001$) (Table 3.6).

Lying Time, Lying Bouts, and Steps

The two mandatory soakings treatment had a tendency to result in longer daily lying time than the no mandatory soakings treatment (mandatory soakings: 673.0 ± 5.9 ; no mandatory soakings: 661.3 ± 5.9 min/d, $F_{1,13} = 4.3$; $P = 0.06$), however lying bouts were not affected by treatment ($F_{1,13} = 1.25$; $P = 0.28$; Table 3.4). There was no relationship with daily lying time ($F_{1,682} = 5.08$; $P = 0.25$) or daily lying bouts ($F_{1,641} = 0.15$; $P = 0.70$) with daily soaker use, therefore no regression analysis was performed for either variables. Steps/d were not affected by treatment ($F_{1,13} = 1.30$; $P = 0.28$; Table 3.4); however, steps/d increased with increasing voluntary soaker use/d ($F_{1,416} = 17.46$; $P < 0.001$; Table 3.6).

Milk Production

Milk yield ($F_{1,14} = 0.23$; $P = 0.64$), milk protein percentage ($F_{1,14} = 0.95$; $P = 0.35$), and milk fat ($F_{1,14} = 2.36$; $P = 0.15$) were not affected by treatment. No relationship was found between soaker use and milk yield ($F_{1,698} = 5.93$; $P = 0.02$), milk protein percentage ($F_{1,751} = 0.00$; $P = 0.95$), and milk fat ($F_{1,750} = 0.01$; $P = 0.90$). Therefore, regression analyses were not performed for these variables.

Temperature Humidity Index

Temperature humidity index had a positive relationship with voluntary soaker use ($F_{55,697} = 5.23$; $P < 0.001$), illustrated in Figure 3.6, and the formulae for the regression model is presented in Table 3.6. The regression analyses evaluating voluntary soaker use to THI with no time delay were not significant ($R^2 = 0.02$; $P = 0.48$). The model fit increased as 1 h delays

were added to THI until the optimum model representing voluntary soaker use was found at THI + 5 h delay ($R^2 = 0.37$; $P < 0.01$; Figure 3.6). Regression models were also significant with delays of + 2 to + 8 h, however R^2 values were lower and P -values were larger compared to THI + 5 h. Shower use became not significant again at and after a delay of 9 h was added to THI (THI + 9 h: $R^2 = 0.09$; $P = 0.15$).

Temperature humidity index affected physiological and behavioral variables, and almost all milk variables in the mixed model (Table 3.5). The regression to explain the relationship between variables with a significant relationship (response variable, y) with THI (explanatory variable, x) are given in Table 3.6. Temperature humidity index had a positive relationship with RR; minimum ($F_{55, 752} = 14.86$; $P < 0.001$), mean ($F_{55, 736} = 33.20$; $P < 0.001$), and maximum RR ($F_{55, 751} = 38.66$; $P < 0.001$). Temperature humidity index had a positive relationship with panting score; minimum ($F_{55, 696} = 8.08$; $P < 0.001$), mean ($F_{55, 736} = 23.10$; $P < 0.001$), and maximum panting score ($F_{55, 751} = 17.87$; $P < 0.001$). Temperature humidity index had a positive relationship with minimum RT ($F_{55, 637} = 4.57$; $P < 0.001$), mean ($F_{55, 637} = 5.94$; $P < 0.001$), and maximum RT ($F_{55, 637} = 5.94$; $P < 0.001$). Temperature humidity index had a negative relationship with daily rumination time ($F_{55, 752} = 20.45$; $P < 0.001$) and daily feeding time ($F_{55, 752} = 13.31$; $P < 0.001$). Temperature humidity index had a positive relationship with daily resting time ($F_{55, 697} = 15.38$; $P < 0.001$). Temperature humidity index had a negative relationship with daily lying time ($F_{55, 682} = 16.81$; $P < 0.001$), however a positive relationship with daily lying bouts ($F_{55, 641} = 1.69$; $P < 0.01$), yet the regression of THI with lying bouts was not significant ($P = 0.38$). Temperature humidity index had a positive relationship with daily steps ($F_{48, 416} = 8.97$; $P < 0.01$). Temperature humidity index affected milk yield ($F_{55, 698} = 4.60$, $P < 0.001$), however the regression analysis was not significant. Temperature humidity index did not affect milk protein percent ($F_{55, 751} = 0.88$; $P = 0.72$), however it did negatively affect milk fat ($F_{55, 750} = 5.42$, $P < 0.001$).

DISCUSSION

Voluntary soaking opportunities were provided to dairy cows during a time of elevated temperature humidity index. This study is the first to compare the use of a voluntary soaker method with or without a mandatory cooling opportunity for dairy cows, with fans and shade. We found limited to no differences of heat stress alleviation between the two treatments of 1) two mandatory soakings at the exit of the milking parlor compared to voluntary soaker use, and 2) no mandatory soakings with voluntary soaker use. We did not find an additional heat abatement between treatment and voluntary soaker use, nor within any physiological variables measured, including respiration rate (associated with heat stress e.g. Rhoads et al. (2009), Schütz et al. (2010), and Min et al. (2015)).

The only behavioral variable affected by treatment was rumination. Shorter daily rumination has been suggested as an indication of heat stress (e.g. in Kadzere et al. (2002), Bernabucci et al. (2010), and Soriani et al. (2013)), and was observed in the non mandatory soaking treatment in comparison to longer daily rumination time in the compulsory soaking treatment. In addition to the difference in rumination time between treatments, there was a positive relationship between soaker use and rumination time. Because rumination had a negative relationship with THI (reducing daily rumination time with increasing THI), the positive relationship between soaker use (which had a positive relationship with THI) and rumination time may suggest that soaker use somewhat mitigated negative effects of heat stress on rumination. However, because no differences between treatments for respiration rate, panting score, reticulo-rumen temperature, daily feeding time, daily resting time, daily lying time or bouts, steps/d, or milk production and components, the results suggest cows assigned either treatment had a comparable level of heat alleviation. Future research could however investigate the magnitude of heat alleviation of heat soakers for cows on dairies with different

heat abatement strategies (e.g. without fans, with sprinklers over the feedbunk, without shade) or include a treatment of no voluntary soaker.

While there was no difference in voluntary soaker use between treatments, it was highly, positively correlated with temperature humidity index, similar to previous studies that offered a voluntary soaker (Legrand et al., 2011), voluntary sprinklers (Parola et al., 2012), or voluntary use of sprinklers over the feed bunk (Parola et al., 2012; Chen et al., 2013; Chen et al., 2016). This suggests cows may be more motivated to use a voluntary soaker during periods of elevated heat stress potential, however Parola et al. (2012), Chen et al. (2013), and Chen et al. (2016) used constantly running sprinklers (as opposed to the voluntary soaker in the current study and Legrand et al. (2011)). Constantly running water, or water cows cannot escape, may be perceived as an obstacle because their heads would be wet, which has been associated with discomfort (Chen et al., 2016). For instance, cows show head wetting avoidance behaviors such as lowered heads or keeping their heads outside of the sprinkler (Kendall et al., 2007; Schütz et al., 2011; Chen et al., 2016). Cows have also been observed standing with heads through headlocks when sprinklers over the feed bunk were activated, despite not feeding (Chen et al., 2013), and moving out of the sprinkler radius when sprinklers were activated (Marcillac-Emberson et al., 2009). In support of this work, we also found that cows wet their head and neck much less than other body areas when using the voluntary soaker. Conversely, Legrand et al. (2011) found that cows had their heads near the voluntary soaker heads for more than half of the time when in the soaker. This difference in behavior may be a result of flow rate or water droplet size between Legrand et al. (2011) and the current study. Legrand et al. (2011) speculated this may be a result of cows having control over the water source (as opposed to sprinklers in Schütz et al. (2011), Chen et al. (2013), and Chen et al. (2016)), though it is not evident in the current study. Future research should explore the motivation of cows to use a

voluntary use soaker, or experiment with different soaker structures (e.g. water flow, droplet size, design to avoid cow ears and heads).

High variation of voluntary soaker use between cows was observed in the current study; one cow using it for 227 cycles in one day compared to a cow using the voluntary shower for 0 cycles in one day. Similarly, Schütz et al. (2011) and Legrand et al. (2011) found some cows did not seek further heat alleviation from a voluntary choice soaker. Legrand et al. (2011) speculated that individual variation in soaker use may be related to a lack of learning of the cooling properties of water; however in each of these studies, including our own, cows were previously accustomed to water. Additionally, steers naïve to sprinklers have been shown to use sprinklers for their cooling properties (Parola et al., 2012). High variation in soaker use could also be because cows experience heat stress differently because of different genetics (Aguilar et al., 2009; Liang et al., 2013; Alfonzo et al., 2016), milk variables (Liang et al., 2013; Macciotta et al., 2017; Stone et al., 2017), body size, or hair structure (Alfonzo et al., 2016). In the current study we attempted to account for such individual variation in heat stress tolerance (breed, parity, milk yield), but we were unable to consider differences in genetics that may have contributed to voluntary soak use variability. Differences in genetics and therefore heat tolerance could be a reason for the high variation in daily soaker use. Voluntary cooling options provide cows with the choice of when to use the soaker, and importantly, if they want to use the soaker at all. Water use has been shown to sufficiently reduce heat stress in cows (Schütz et al., 2011; Chen et al., 2016; Tresoldi et al., 2018), therefore a voluntary soaker may offer a good opportunity for particularly heat intolerant cows to alleviate heat stress. Further research however should investigate different voluntary cow soaker designs, individual motivation of cows for voluntary soakings, and the combination with other cooling strategies. Future research should also consider comparing cows with genetic testing for heat tolerance

genes to investigate whether some cows that are less heat tolerant use the voluntary soaker, or if soaker use is simply preference.

CONCLUSION

Limited changes were found in this study of physiological or behavioral differences between the treatments of two mandatory soakings with a free choice soaker, and use of a free choice soaker with no mandatory soakings. While there was a difference of longer rumination time in the two mandatory soakings with a free choice soaker treatment indicated the possibility of heat alleviation, there were no other physiological, behavioral or production differences between treatments. Therefore, because of the limited differences physiologically, behaviorally, or in production, we conclude the results indicated in equal heat alleviation between the treatments. Soaker use was however highly and positively correlated with temperature humidity index, and daily soaker use was best fit when modeled with a 5 h delay of temperature humidity index.

We encourage future research to investigate potential advantages of a voluntary soaker by comparing it to cows with no voluntary soaker, and different levels of heat abatement (such as with and without shade and fans) to further explore heat abatement at the individual level. Furthermore, future studies are necessary to understand cow preference to soaker or sprinkler design to investigate why some cows prefer the soaker more than others.

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Ireland), through a research project partnership with the Dairy Science Program at the University of Kentucky.

Figure 3.1. Experimental pen for dairy cattle ($N = 15$): 155 m² compost bedded pack pen and 50 m² feed alley. Cows were enrolled with two heat alleviation treatments: two mandatory soakings/d (mandatory soakings) via the exit alley of the milking parlor with access to a voluntary soaker, and no mandatory soakings/d (no mandatory soakings) with access to a voluntary soaker. The voluntary use soaker was located immediately adjacent to the feed alley of the pen in an area approximately 20 m², with a grooved concrete surface and shade overhead. Circle annotated fans are attached to the roof of the barn and rotated air flow down, towards pack. Arrow annotated fans are mounted above the feed bunk headlocks, and air flow follows the arrow direction.

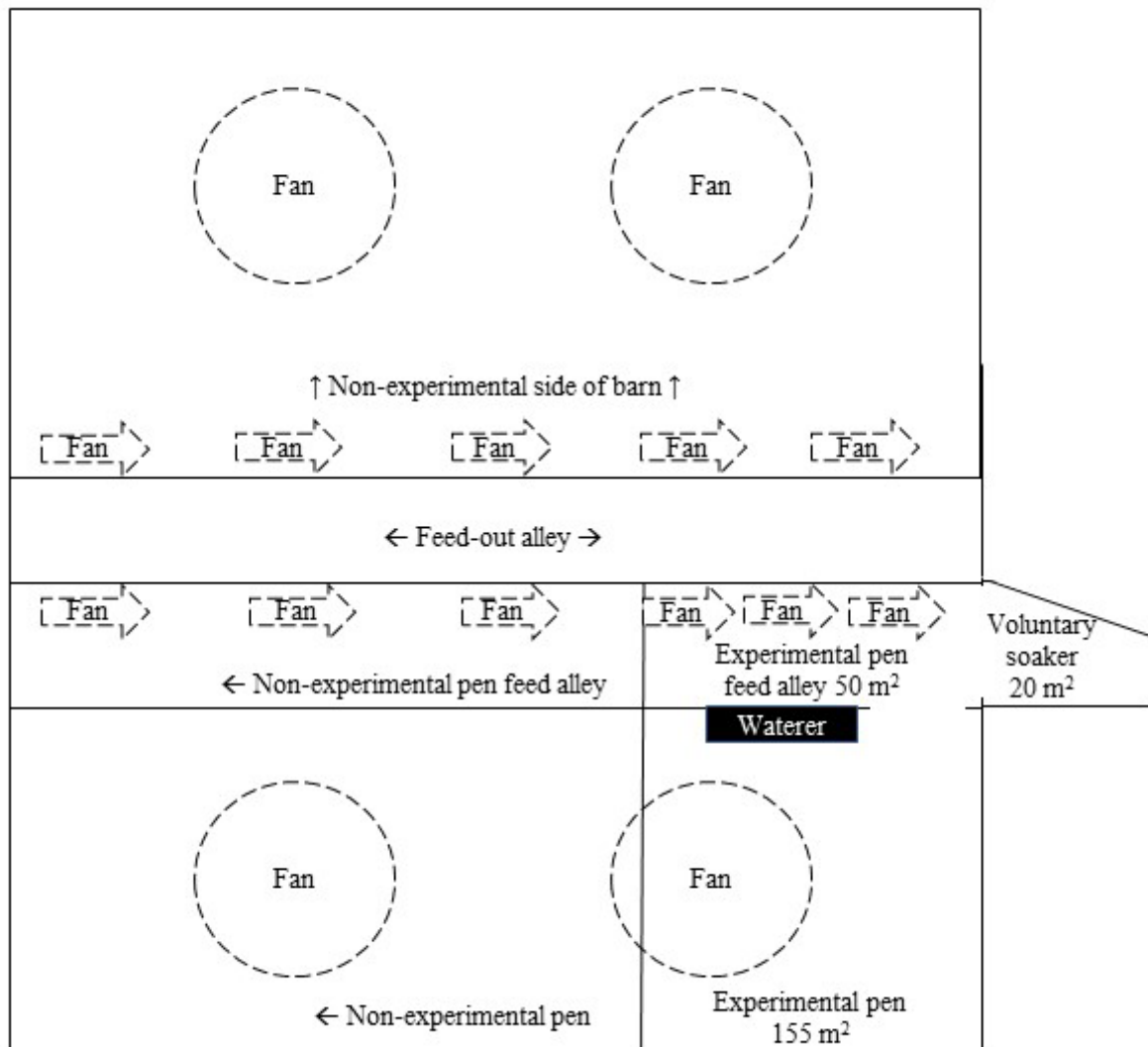


Figure 3.2. Individual total daily frequency of voluntary soaker use (in 5 s cycles) assessed by 24h video recording of dairy cattle ($N = 15$) enrolled with two individualized cooling strategy treatments. Treatments were 1) two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and 2) no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment found, therefore data presented is for the duration of the experimental period (8 weeks, from August to October, in Lexington, KY, USA), regardless of treatment.

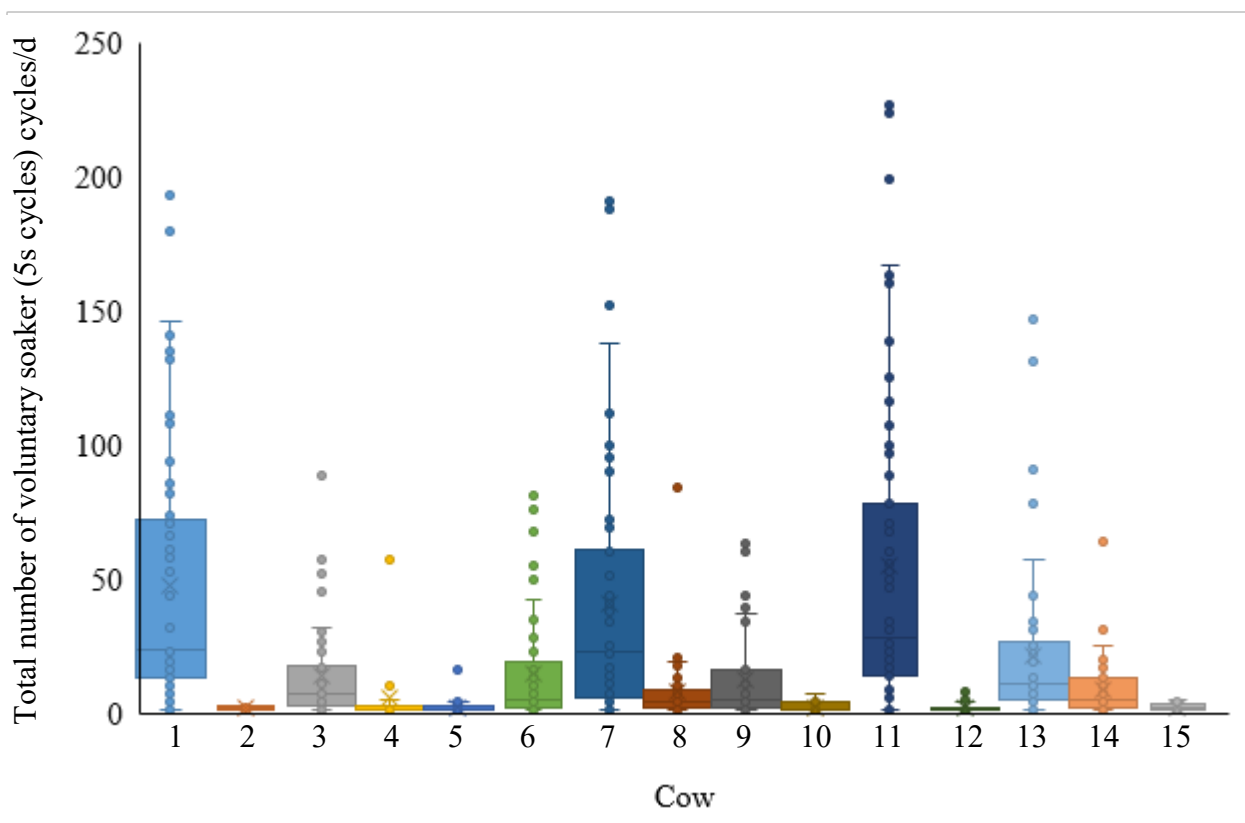


Figure 3.3. Percentage of cycles soaked areas of the cow from the voluntary soaker (x axis) during the experimental period (8 weeks, from August to October, in Lexington, KY, USA) assessed by 24h video recording. Each point represents each cow's ($N = 15$) percent of time soaking the corresponding area. Treatments were: two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment found, all data presented.

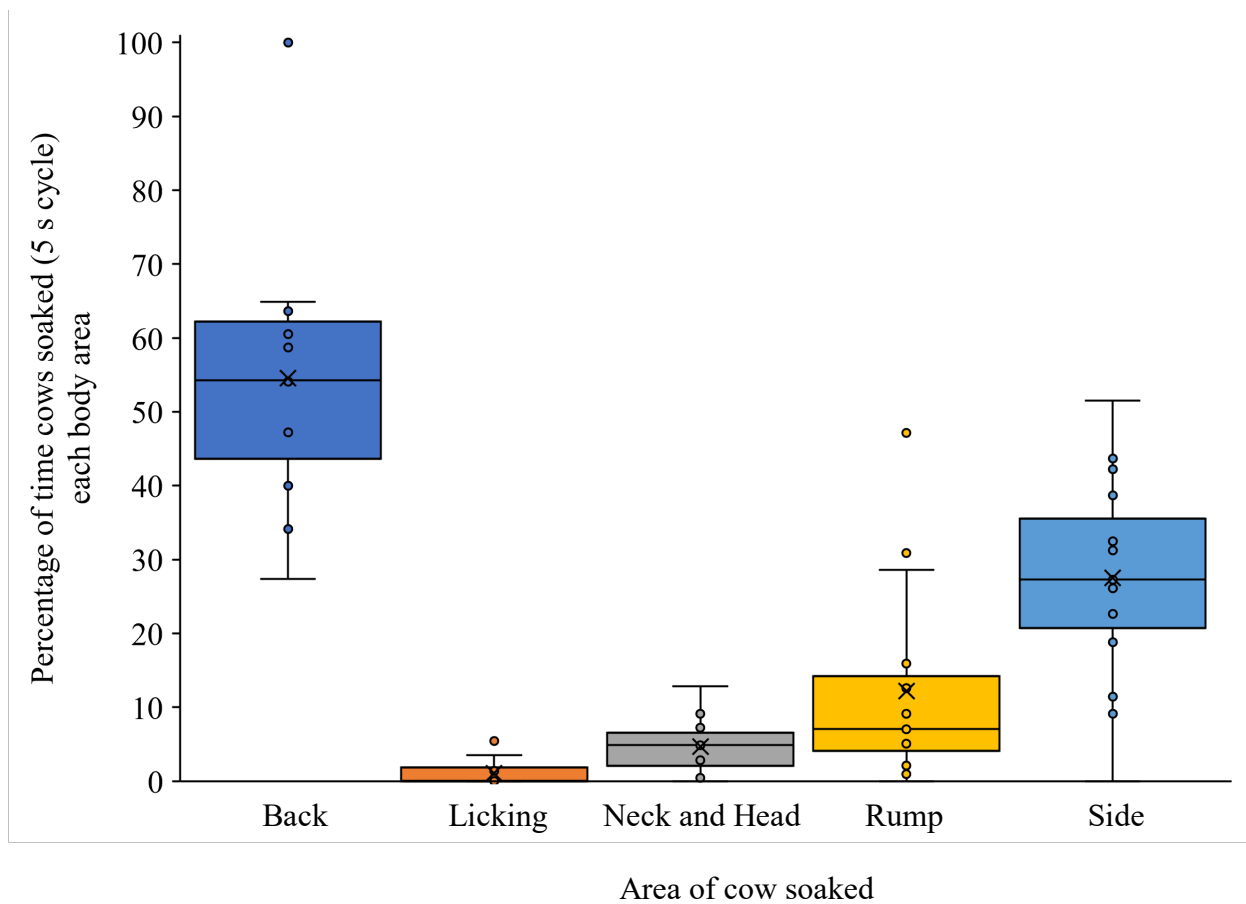
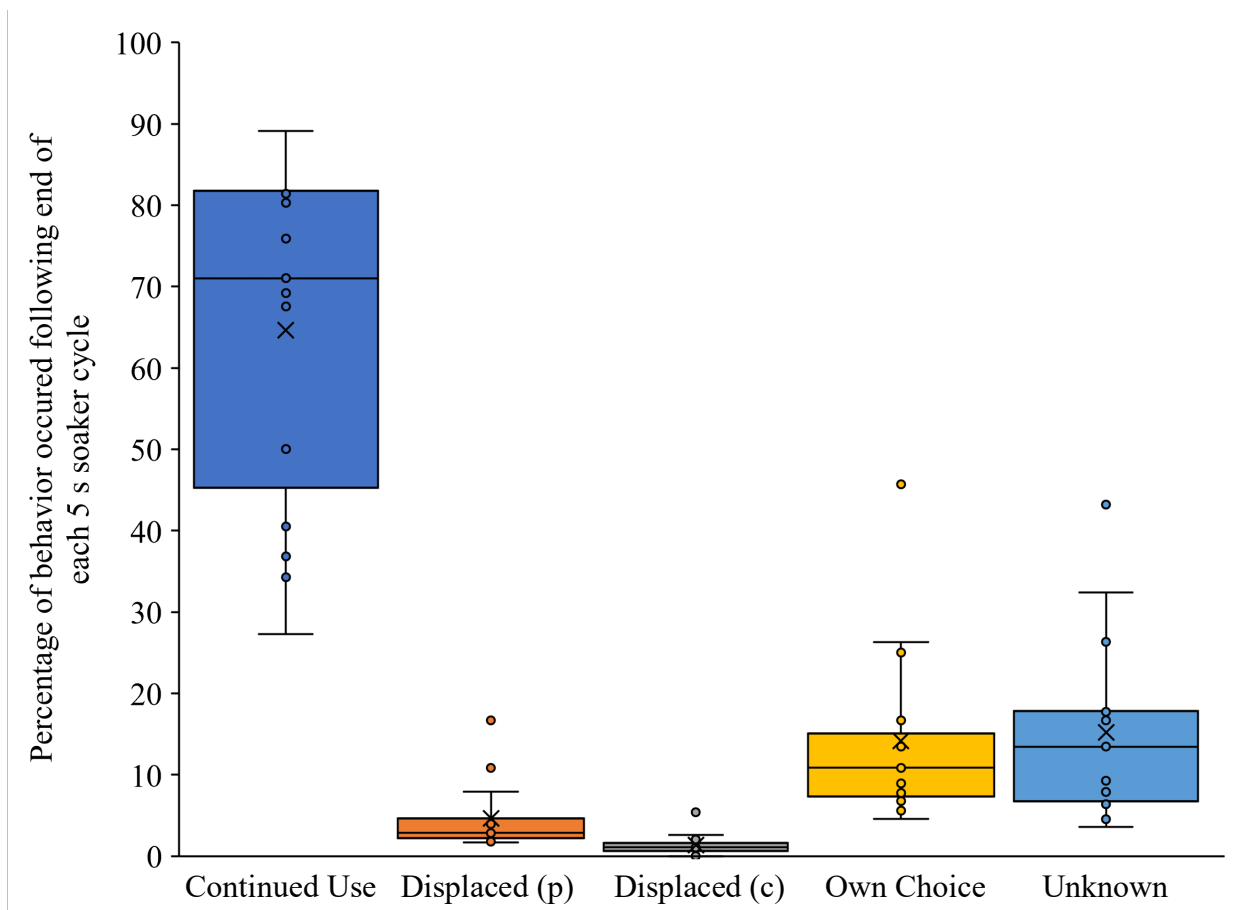
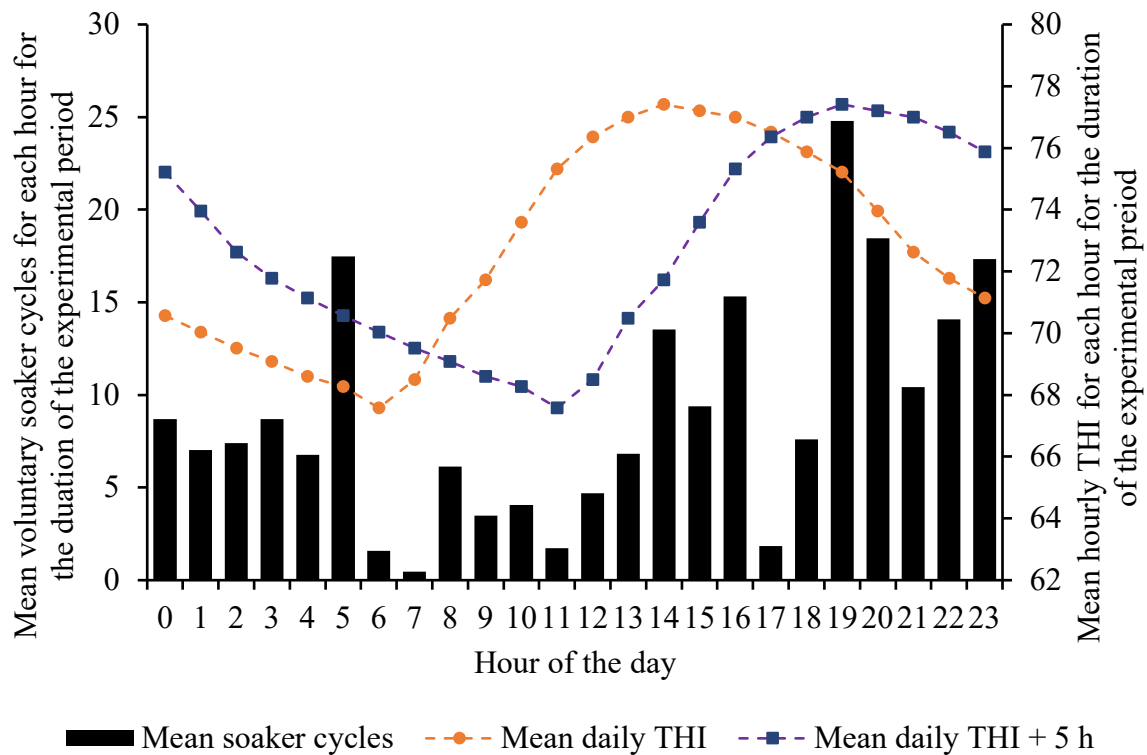


Figure 3.4. Behaviors¹ recorded at the end of each 5 s soaker cycle, assessed by 24h video recording during the experimental period (8 weeks, from August to October, in Lexington, KY, USA). Each point represents the percent of time each cow ($N = 15$) performed the corresponding behavior. Treatments were: two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment were found; therefore all data is presented.



1. If cows “continued use” the soaker was activated again for another cycle. Leaving the soaker area because of being displaced is indicated below, by farm personnel “displaced (p)”, or by another cow “displaced (c)”. A cow that left by “own choice” left with no other cows in the vicinity of the soaker to influence choice for leaving. An “unknown” reason was recorded when the observer was unable to attribute displacement or cow’s own choice.

Figure 3.5. Mean hourly soaker use by cows ($N = 15$) as recorded from 24h video recording (solid bars; left axis)¹ with THI (line graph; right axis). Mean daily THI is depicted by the dotted line with circles, and mean daily THI + 5 h delay is depicted by the dotted line with squares. Mean daily THI + 5 h delay was found to be the best predictor of mean hourly soaker use by the regression analysis ($P < 0.01$). Treatments were: two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment were found; therefore, all data is presented.



1. Cows were unable to access the soaker for approximately 5.7 ± 1.6 h/d. Soaker usage effected by being locked in the pen for other cows to milk (approximately 0600 to 0730 h and 1645 to 1750 h), leaving the pen for milking (approximately 0720 to 0740 h and 1750 to 1820 h), and while the alley was being scraped (approximately 0430 to 0510 h and 1500 to 1550 h). Cows were fed twice/d at approximately 0730 and 1430 h, however access to the soaker was not limited at feeding times.

Figure 3.6. Daily voluntary soaker use (y axis) depicted against the respective day's mean daily temperature humidity index (THI) (x axis). Values for each cow's ($N = 15$) daily soaker use is represented by a different annotation. Daily soaker use was recorded by 24 h surveillance of the soaker. Treatments were: two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment found, all data presented.

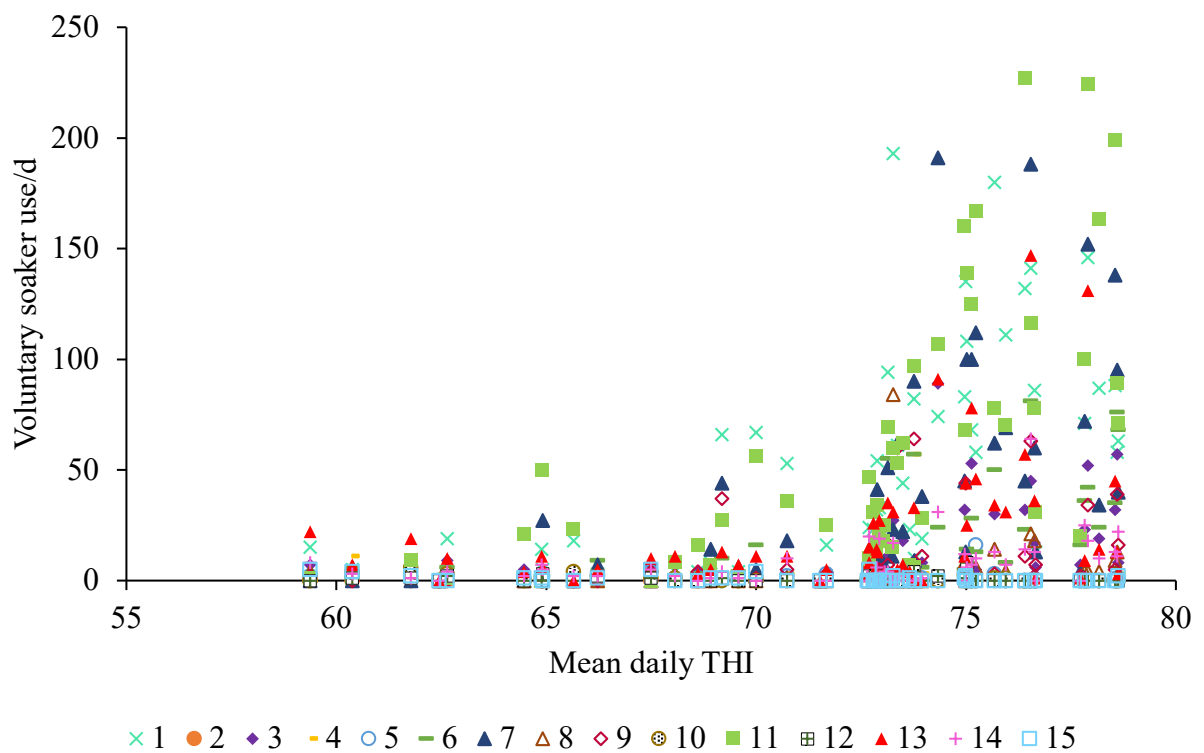


Figure 3.7. Mean displacement actions of cows ($N = 15$) for the voluntary soaker, by cow (left y axis). Depicted is the number of times a cow displaced another cow (diagonal striped bars), or that a cow was displaced by another cow (horizontal striped bars). Additionally, mean soaker use is displayed by the scatter graph (right y axis). Displacements and soaker use was recorded by 24 h surveillance of the soaker. Treatments were: two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment found, therefore all data presented.

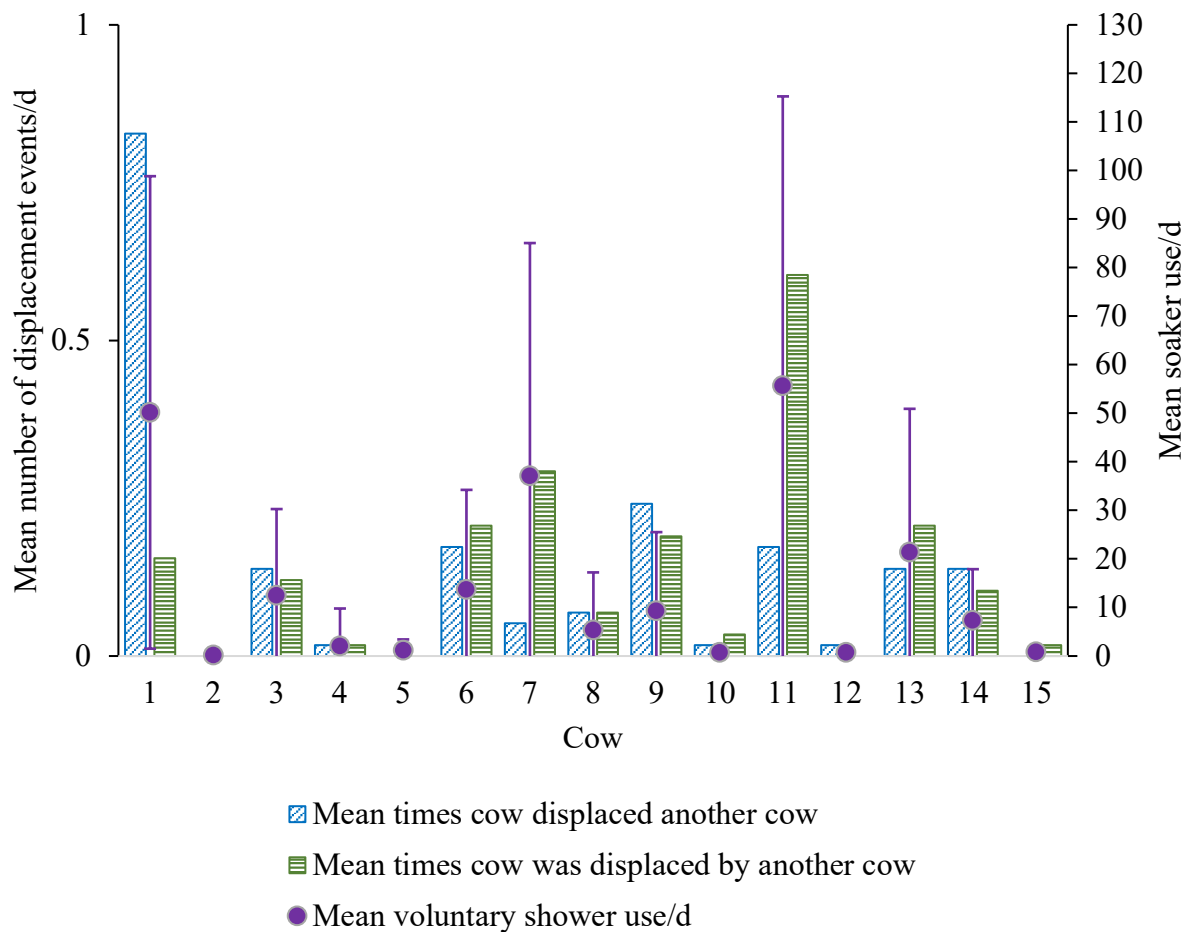


Table 3.1. Microclimate conditions (daily mean, standard deviation (SD), daily minimum and daily maximum) of experimental pen. Daily environmental conditions calculated from 0000 to 2359 h each day during the treatment period.

	Daily (24 h)			
	Mean	SD	Minimum	Maximum
Temperature (°C) ¹	23.5	3.2	15.3	28.0
Relative humidity (%) ¹	71.7	6.2	45.0	81.3
Precipitation (mm) ²	8.1	16.5	0.0	71.1
Wind speed (m/s) ¹	0.3	0.4	0.0	1.9
THI ¹	71.9	4.9	59.4	78.6

¹Measurements taken from inside the experimental cows' home pen.

²Measurements obtained from the University of Kentucky Agronomy Research Farm (Lat: 38.1341919, Long: -84.4962154).

Table 3.2. Ethogram followed to define panting. Panting score was recorded live at the same time respiration rate was taken, 3x/d; before morning milking, midday, and before evening milking during the treatments. Heat alleviation treatments were: two mandatory soakings/d via the exit alley of the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker. For each panting score observation, observers were stationed approximately 5 m from the focal cow and ensured flank movements were visible for the duration of the observation.

Breathing behavior	Approximate respiration rate (breaths/min)	Panting score
No panting – normal. Difficult to see chest movement.	<40	0
Slight panting, mouth closed, no drool or foam. Easy to see chest movement	40-70	1
Fast panting, drool or foam present. No open mouth panting	70-120	2
Fast panting, drool or foam present. Occasional open mouth, tongue not extended	70-120	2.5
Open mouth and some drool present. Neck extended, head may be up	120-160	3
Open mouth and excessive drooling. Tongue out slight, occasionally fully extended for short periods. Neck extended and head usually up	120-160	3.5
Open mouth with tongue fully extended for prolonged periods and excessive drooling. Neck extended and head up	>160	4

Table 3.2. (continued)

Open mouth with tongue fully extended for prolonged periods.		
Head held down, drooling may cease. Cattle “breathe” from flank	Variable (may decrease)	4.5

Table 3.3. Ethogram used by observers for recording area of cow wet by soaker when recording voluntary soaker use at the pen. Recording of soaker use was made by observing daily video recordings (24 h/d) of the soaker area for the duration of the experimental period.

Area wet	Definition
Rump	Cow wet anywhere between (and including) rump and hip bones.
Neck and head	Cow wet anywhere between (and including) shoulders and end of nose.
Back	Cow wet between hip and shoulder bones, or if cow is in motion and wets a combination of rump, neck and head, and back areas.
Side	If cow wets any area, but only on one side (water does not spill over spine to other side).
Licking	If cow only licks water, and water does not wet the head.

Table 3.4. Physiological, behavioral, and milk variables (mean \pm SE) used to observe the heat abatement qualities of the two treatments between cows (N = 15). Treatments were 1) two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and 2) no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen.

	No mandatory soakings	Mandatory soakings	SEM	<i>P</i> -value
Soaker use	14.8	12.4	1.4	0.21
Respiration rate:				
Minimum	42.9	44.0	0.50	0.13
Mean	56.4	57.3	0.41	0.09
Maximum	67.5	67.7	0.52	0.81
Panting score:				
Minimum	0.59	0.61	0.02	0.51
Mean	1.05	1.08	0.02	0.09
Maximum	1.48	1.50	0.02	0.57
Reticulo-rumen temperature:				
Minimum daily	39.03	39.03	0.01	0.83
Mean daily	39.60	39.58	0.01	0.16
Maximum daily	40.12	40.08	0.02	0.11
Rumination time (min/d)	543.4	558.6	5.2	< 0.01
Feeding time (min/d)	173.4	174.2	5.9	0.74
Resting time (min/d)	570.6	560.3	4.0	0.06
Lying time (min/d)	661.3	673.0	5.9	0.06

Table 3.4. (continued)

Lying bouts (bouts/d)	10.8	11.0	0.16	0.28
Steps (steps/d)	2113.6	2172.4	50.0	0.28
Milk:				
Yield (kg/d)	36.2	36.5	0.6	0.64
Protein (%)	2.95	2.94	0.02	0.35
Fat (%)	3.89	3.87	0.02	0.15

Table 3.5. Result of physiological, behavioral, and milk variables for cows ($N = 15$) with the fixed effects of soaker use/d and temperature humidity index from the mixed linear model. If the result was significant from this model, a regression was performed with the fixed effect (soaker use/d or temperature humidity index) as the explanatory variable, and the significant variable (physiological, behavioral, and milk variables) was the response variable. Treatments were 1) two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and 2) no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment found, therefore all data presented.

Variable:	Soaker use/d		Temperature humidity index	
	<i>P</i> -value	Further testing for regression?	<i>P</i> -value	Further testing for regression?
Soaker use/d	-	-	< 0.001	Yes
Respiration rate:				
Minimum	0.02	Yes	< 0.001	Yes
Mean	0.33	No	< 0.001	Yes
Maximum	0.74	No	< 0.001	Yes
Panting score:				
Minimum	0.58	No	< 0.001	Yes
Mean	0.49	No	< 0.001	Yes
Maximum	0.63	No	< 0.001	Yes
Reticulo-rumen temperature:				
Minimum daily	0.12	No	< 0.001	Yes
Mean daily	< 0.001	Yes	< 0.001	Yes

Table 3.5. (continued)

Maximum daily	< 0.001	Yes	< 0.001	Yes
Rumination time (min/d)	< 0.001	Yes	< 0.001	Yes
Feeding time (min/d)	0.94	No	< 0.001	Yes
Resting time (min/d)	< 0.001	Yes	< 0.001	Yes
Lying time (min/d)	0.25	No	< 0.001	Yes
Lying bouts (bouts/d)	0.70	No	< 0.01	Yes
Steps (steps/d)	< 0.001	Yes	< 0.001	Yes
Milk:				
Yield (kg/d)	0.02	Yes	< 0.001	Yes
Protein (%)	0.95	No	0.72	No
Fat (%)	0.90	No	< 0.001	Yes

Table 3.6. Result of the regression of physiological, behavioral and milk variables for all cows ($N = 15$) (dependent variable; y axis) with mean daily temperature humidity index (THI) and soaker use/d (independent variable; x axis). Regression analyses were only performed when THI or soaker use/d were significant in the mixed model. Treatments were: two mandatory soakings/d exiting the milking parlor with access to a voluntary soaker, and no mandatory soakings/d with access to a voluntary soaker located immediately adjacent to the feed alley of the pen. No effects of treatment found, therefore all data presented.

	Soaker use/d	R^2	Mean daily THI	R^2
Soaker use				
Daily	-	-	$y = -96.6 + 1.5x$	0.06
Respiration rate				
Minimum	$y = 41.8 + 0.2x$	0.08	$y = -72.2 + 1.6x$	0.32
Mean daily	-	-	$y = -92.1 + 2.1x$	0.57
Maximum	-	-	$y = -131.4 + 2.8x$	0.50
Panting score				
Minimum	-	-	$y = -3.20 + 0.05x$	0.24
Mean daily	-	-	$y = -4.03 + 0.07x$	0.53
Maximum	-	-	$y = -4.92 + 0.08x$	0.48
Reticulo-rumen temperature				
Minimum	-	-	$y = 38.51 + 0.007x$	0.03
Mean daily	$y = 39.56 + 0.003x$	0.09	$y = 38.12 + 0.021x$	0.57
Maximum	$y = 40.05 + 0.005x$	0.12	$y = 37.74 + 0.033x$	0.46
Rumination (min/d)	$y = 562.7 - 0.4x$	0.008	$y = 1275.1 - 9.9x$	0.16
Feeding (min/d)	-	-	$y = 413.9 - 3.3x$	0.05

Table 3.6. (continued)

Resting (min/d)	$y = 553.2 + 0.6x$	0.02	$y = -140.8 + 9.7x$	0.13
Lying (min/d)	-	-	$y = 1583.8 - 12.6x$	0.26
Lying bouts/d	-	-	$y = 9.75 + 0.02x$	0.001
Steps/d	$y = -3.239 + 0.009x$	0.04	$y = -59.9 + 30.6x$	0.04
Milk				
Yield	$y = 36.48 + 0.035x$	0.02	$y = 29.65 + 0.10x$	0.005
Protein (%)	-	-	-	-
Fat (%)	-	-	$y = 4.90 - 0.01x$	0.04

CHAPTER FOUR:

Summary of results:

Validation of an automated behavior monitoring collar, and evaluation of heat stress on lactating dairy cow behavior with access to a free choice soaker

CONCLUSIONS

In conclusion for the studies completed for this thesis, precision dairy technologies are a tool that can be utilized for research to consistently and continuously record cow behavior, providing they have sufficient precision, accuracy and no systematic bias. Additionally, dairy cattle need heat stress abatement tools when any environmental conditions exceed the thermo neutral zone to minimize the negative effects of heat stress on cow welfare and milk production. The first original research study showed that the behavior monitoring collar could precisely measure rumination, feeding, and resting time. The second original research study indicated that soaker use may reduce heat stress in dairy cows, however the study showed no differences in heat alleviation of cows given free choice to a soaker in addition to one of the two treatments 1) no mandatory soakings, or 2) two mandatory soakings.

FUTURE RESEARCH

Future research using any precision dairy technology should consider performing a validation study when utilizing the PDT in a new management system or with different breeds as it can vary. This is important to ensure the technology performs precisely, accurately, and without bias before relying on the data collected from the technology. Many validation studies have investigated the precision of the PDT, however some lack investigation of accuracy of the device. Thus, in the future the investigation of the factors influencing the accuracy of PDT should be investigated and hopefully resolved.

We encourage future research to explore different levels of heat abatement to investigate potential advantages of a voluntary soaker for heat abatement at the individual level. Future research with voluntary soakers should include various heat abatement strategies, including a negative control of no heat abatement to assess the absolute advantages of the voluntary soaker for heat abatement for dairy cows. Furthermore, because of the high variation observed in voluntary soaker studies, future studies are necessary to understand cow

preferences for soaker, which may include sprinkler design, method of voluntary water activation, water pressure, and water droplet size.

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VITAE

Lori Grinter grew up in Hamilton, New Zealand where she completed her Bachelor of Science majoring in biology at Waikato University. Upon graduating in 2016, Lori worked at as a research assistant at DairyNZ, a co-operatively owned company by New Zealand farmers for research of dairy cows.

In 2017, Lori moved to Lexington, Kentucky to peruse her Master of Animal Science majoring in dairy science, at the University of Kentucky, with funding from Dairymaster, Ireland. During the time Lori was at the University of Kentucky, she was a member of the Animal and Food Science Graduate Association (AFSGA), the American Dairy Science Association Graduate Student Division (ADSA-GSD. Lori was a manager for the dairy group fundraiser at the state fair, a teaching assistant for the fourth year undergraduate dairy production class, and attended the international dairy study abroad trip.

PEER REVIEWED PUBLICATIONS

Grinter, L.N., M.R. Campler, and J.H.C. Costa. 2019. Technical note: Validation of a behavior monitoring collar's precision and accuracy to measure rumination, feeding, and resting time of lactating dairy cattle. *J. Dairy Sci.* 102(4):3487-3494. <https://doi.org/10.3168/jds.2018-15563>.

SCIENTIFIC ABSTRACTS

Grinter, L.N., and J.H.C. Costa. 2018. Automatic measurement of ruminating, feeding and resting behavior in group-housed dairy cattle: validation of a novel collar system. 2018 Congress of the International Society for Applied Ethology (ISAE), Ethology for Health and Welfare. Abstract number: 28418. July 30 to August 3, 2018. Charlottetown, Prince Edward Island, Canada. Poster presentation.

M.D. Adamczyk, L.N. Grinter, A.R. Lee, J.M. Bewley, and J.H.C. Costa. 2018. Automatic feed push-up frequency effects on dairy cattle behavior and milk production. American Dairy Science Association (ADSA) Annual Meeting. Abstract number: 74753 (M15). June 24 to 27, 2018. Knoxville, Tennessee. Graduate student poster presentation competition.

M.D. Adamczyk, L.N. Grinter, A.R. Lee, J.M. Bewley, and J.H.C. Costa. 2018. Case Study: Automatic feed push-up frequency effects on dairy cattle behavior and milk production. Graduate Student Association Poster Symposium, Master's Division. May 31, 2018. Lexington, Kentucky. Poster presentation competition.

Grinter, L.N., and J.H.C. Costa. 2018. Validation of a behavior monitoring collar system for dairy cows; can it measure feeding, ruminating and resting reliably? Tri-State Dairy Nutrition Conference. April 16 to 18, 2018. Fort Wayne, Indiana. Oral and poster presentation competition.

Grinter, L.N. and J.M. Bewley. 2017. Evaluation of a neck-mounted behavior monitor to detect metabolic diseases around the time of calving. University of Kentucky Animal and Food Science Graduate Student Association Poster Symposium, Master's Division. May 25, 2017. Lexington, Kentucky. Poster presentation competition.