THE EFFECT OF HEAT STRESS ON THE BEHAVIOUR OF DAIRY COWS AT THE DRINKER

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Abstract

Heat-stressed dairy cows on pasture will compete for resources that aid cooling, but it is not known how heat stress affects the competition for water by indoor housed cows, or if competition for water can be recorded automatically with an electronic drinking system. The objectives of my thesis were to 1) validate an electronic drinking system to detect social competition between dairy cows at the drinker by identifying the interval between one cow leaving the drinker and another cow taking her place, and 2) evaluate how heat stress affects the behaviour of indoor-housed cows at the drinker, both at group and cow level. For the first objective, 20 cows were monitored for 4-d by video recording and an electronic drinking system. Replacements (defined as when physical contact initiated by one cow causes the other to remove her head from the drinker with the initiator subsequently placing her head in the same drinker), identified by video, were paired with the interval between drinking events of the 2 cows at the electronic drinker, to identify the interval that best predicted replacements. The optimal interval to identify replacements at the drinker was ≤ 29 s. For the second objective, 69 cows were observed over 59 d. The electronic drinking system recorded time spent at the drinker, frequency of visits, water intake, and competitive replacements. The number of replacements a cow was involved in was used to determine her level of competitive success at the drinker (low, medium, high). The temperature-humidity index (THI) was recorded by the local weather station. With increasing THI, cows drank more water, spent more time at the drinker, made more visits, and engaged in more replacements at the drinker. We also found that cows with low competitive success at the drinker shifted their drinking behaviour to avoid the drinker at the hottest and most competitive time of day. These results indicate that competition between dairy cows at the drinker can be accurately

measured with an electronic drinking system, and that drinking behaviour can be used to indicate when cows feel hot.

Lay Summary

Dairy cows change their behaviour during hot weather as a means to cool down. My thesis determined that dairy cows increase their drinking behaviour and engage in more aggressive interactions for access to water when the ambient temperature is high. These changes can be accurately monitored with an electronic drinking system. Understanding behavioural changes during elevated ambient temperatures can detect when dairy cows are too hot.

Preface

The data used in my thesis were collected as part of previous research conducted by Neave et al. (2017, 2018) and Lomb et al. (2018a, 2018b). All procedures were approved by the University of British Columbia Animal Care Committee (protocol A14-0040).

A version of Chapter 2 has been published: McDonald, P.V., M.A.G. von Keyserlingk, and D.M. Weary. 2019. Technical note: Using an electronic drinker to monitor competition in dairy cows. J. Dairy Sci. 102:3495-3500. doi:10.3168/jds.2018-15585. P.V. McDonald and D.M. Weary developed the main ideas for this project. P.V. McDonald wrote the manuscript. M.A.G. von Keyserlingk and D.M. Weary acted in the typical role of supervisors, helping with statistical analysis, and providing input and editing drafts.

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List of Abbreviations

AIC = Akaike information criterion

AUC = area under the curve

C = Celsius

CBT = core body temperature

d = day

DIM = days in milk

FN = false negative

FP = false positive

h = hour

kg = kilogram

m = metre

min = minute

no. = number

RFID = radio-frequency identification

RH = relative humidity

ROC = receiver operating characteristic

s = second

SD = standard deviation

Se = sensitivity

SE = standard error of the mean

Sp = specificity

THI = temperature-humidity index

TN = true negative

TP = true positive

TRP = transient receptor potential

wk = week

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Chapter 1: Introduction

In Canada between 1960 to 2018, the average milk production per Holstein cow, which is the most prominent dairy breed, more than doubled, increasing from 14.1 kg/d to 29.5 kg/d (CDIC, 2019a). One of the consequences of high productivity is increased metabolic heat production and greater susceptibility of dairy cows to heat stress (Kadzere et al., 2002).

Heat stress has detrimental effects; in the most extreme cases, heat stress can result in death (Stull et al., 2008; Vitali et al., 2009). In the United States, approximately 4,000 cattle died in Iowa in the summer of 2011 when the heat index (a measure of how hot it feels when relative humidity [RH] is considered along with ambient temperature) exceeded 43.3 °C (WallacesFarmer, 2011). More recently, an estimated 4,000 to 6,000 dairy cows died in Fresno County, California during a heat wave in June of 2017 (CBS News, 2017).

Heat stress is both a production problem estimated to cost the United States dairy industry $900 million annually (St-Pierre et al., 2003) and also an animal welfare concern (Polsky and von Keyserlingk, 2017). In Western Canada, dairy cows experience heat stress on 40% of summer days (Ominski et al., 2002). Between 2010 - 2015, dairy cows were exposed to heat stress conditions for 135.8 d per year in Southwest Quebec and 95.3 d in Eastern Quebec (Ouellet et al., 2019). Particularly troublesome is that Quebec is the principal dairy producing province, housing 36.8% of all dairy cows in Canada in 2018 (CDIC, 2019b). It seems safe to conclude that the majority of dairy cows in the country are exposed to periods of heat stress for a large proportion of their lifetime. Climate change models predict an increase in global mean surface temperatures of approximately 0.3 - 4.8 $_{o}$C by 2100 (IPCC, 2014); it is likely that heat stress events will occur more often in the future.

The aim of this review is to critically examine the literature on the effect of heat stress on dairy cows, focusing on how changes in behaviour can be used to identify dairy cows are experiencing heat stress. I first describe thermoregulation in animals, how changes in environmental temperatures are detected by the body, how heat exchange occurs, and the resulting effect of heat stress when thermoregulatory mechanisms fail. I will then detail how dairy cows respond to heat stress, both physiologically and behaviourally, ending with particular attention to changes in social behaviour. Lastly, I will discuss how advances in technology can be used to automatically detect dairy cows experiencing heat stress. Throughout this review I will discuss discrepancies between studies in regards to the magnitude of heat stress responses in dairy cows, and show how heat stress negatively affects both dairy farmers and the welfare of their cows. Gaps in the literature will be identified where further research is needed to better understand how dairy cows cope with heat stress and how it can be identified.

1.1 Thermoregulation and Heat Stress

The core body temperature (CBT) for cattle is approximately 38 °C (Collier and Gebremedhin, 2015) and will remain within 1 °C of this in normal ambient conditions (Berman et al., 1985). The thermoneutral zone (surrounded by a upper and lower critical threshold) is defined as the range of ambient temperatures in which an animal can maintain a constant body temperature with minimal energy expenditure (Bernabucci et al., 2010). The thermoneutral zone for dairy cows, based on productivity measures, is an ambient temperature between 5 - 25 °C (Kadzere et al., 2002), although the optimal range for highest efficiency in energy utilization is between 13 - 18 °C (NRC, 1981) .

Heat stress occurs when the ambient temperature is above an animal's thermoneutral zone (Buffington et al., 1981). When the ambient temperature exceeds the upper critical threshold, thermoregulatory mechanisms can no longer compensate for heat gain, causing body temperature to rise (Bernabucci et al., 2010). This zone can vary in dairy cows depending on age, breed, production, diet, previous temperature acclimation, coat characteristics, and amount of tissue insulation (Kadzere et al., 2002).

1.1.1 Ambient temperature detection

As heat flows in the direction of warmer to cooler, mammals need to maintain a relatively constant body temperature higher than the ambient temperature for effective heat flow from the body to the environment, for CBT to remain constant (Collier et al., 2006). Thermoregulation involves 3 pathways that link the external environment to thermoregulatory responses: the afferent pathway, the central nervous system, and the efferent pathways (Collier and Gebremedhin, 2015).

The afferent pathway involves transient receptor potential (TRP) ion channels in the skin that detect thermal energy from the environment (Collier and Gebremedhin, 2015). TRP ion channels have different thermal activation thresholds and can be divided into heat, warm, and cold activated channels (Numata et al., 2011). While specific thresholds have not been described for dairy cows, rats have heat activated TRP ion channels with an activation threshold of 41.5 - 43 °C and humans have heat activated TRP ion channels with an activation threshold of 42 °C. Activation thresholds of different warm TRP ion channels range between, 31 - 39 °C, 25 - 33.6 °C, and 15 - 35 °C.

Information from TRP ion channels is transferred up sensory neurons in the spinothalamic tract to the central nervous system (Collier and Gebremedhin, 2015). The thalamus first receives this sensory information and transfer it to the hypothalamus and the cerebral cortex. The cerebral cortex enables thermal perception and the hypothalamus activates the sympathetic nervous system and endocrine system for thermoregulatory responses. Thermoregulatory mechanisms involve cardiovascular changes, behavioural responses, metabolic responses, and endocrine responses. An increase in body temperature will cause skin blood vessels to vasodilate, sweat glands to produce sweat, endocrine tissue to decrease metabolic rate, and behaviour to reduce activity and appetite.

1.1.2 Heat exchange

Heat exchange occurs through 4 routes: radiation, conduction, convection, and evaporation (Tansey and Johnson, 2015). Radiation occurs when heat is transferred between bodies not in contact, such as ultraviolet light from the sun and infrared radiation from the body. Conduction is heat transferred between objects in contact and convection is when air molecules contacting the body are warmed and this heat is transferred away from the body in a moving gas or liquid (such as movement of air over the body). Evaporative heat transfer occurs when heat is lost as water vapour from the respiratory system and skin.

1.1.3 Assessing heat stress

While directly measuring body temperature is the most effective method of assessing heat stress (fever occurs in cattle when rectal temperature rises above 39 °C; De Rensis et al., 2015), it is not always the most feasible. Measuring environmental conditions is a more practical method that can serve as a proxy for body temperature (Dikmen and Hansen, 2009). Thus, the risk of heat stress in dairy cows is commonly assessed using the temperature-humidity index (THI) (West, 2003), first established by Thom (1959) for humans.

The THI is one value that represents air temperature and RH (Bernabucci et al., 2014). Solar radiation and wind speed can also affect heat stress (Buffington et al., 1981). The THI has many different formulae in the literature {e.g. THI = (1.8 × air temperature in °C + 32) - [(0.55 - 0.0055 × RH in %) × (1.8 × air temperature in °C - 26.8)], NRC, 1971; THI = (0.8 × air temperature in °C) + [(RH in % ÷ 100) × (air temperature in °C - 14.4)] + 46.4, Mader et al., 2006}, and is often classified into different zones to depict when heat stress begins. According to the formula, heat stress is thought to begin at 68 (Zimbelman et al., 2009) or 72 (Armstrong, 1994). A THI of 72 equates to approximately an ambient temperature of 25 °C at an RH of 50%.

How THI measures are used (e.g. mean, minimum, maximum, hours above a certain value) can also affect the results of studies on heat stress in dairy cows (Collier et al., 2011). Many studies have identified different thresholds for when heat stress begins (e.g. mean THI = 72, Igono et al., 1992; number of hours per day with a THI > 74, Linvill and Pardue, 1992; mean THI > 60, Brügemann et al., 2012; maximum THI between 65 - 76, Bernabucci et al., 2014; mean THI > 68, Zimbelman et al., 2009) or different ways of calculating THI that are most associated with heat stress effects on dairy cows (with minimum temperature, Holter et al., 1996; with maximum temperature and minimum RH, Ravagnolo and Misztal, 2000).

Previous work has suggested that heat stress may best be characterized by including THI measures of previous days, opposed to just the day of interest (2 d, West et al., 2003; 3 d, Bouraoui et al., 2002, Hill and Wall, 2017; 2 - 4 d, Spiers et al., 2004; 4 d, Linvill and Pardue, 1992). A lag in response to elevated ambient temperatures is logical, give that it takes time for behavioural and physiological responses to occur (West et al., 2003). In feedlot cattle, increased rectal temperature lags 4 - 5 h after ambient temperature changes, and increased respiratory rate lags 1.5 - 2 h (Brown-Brandl et al., 2003). Vaginal temperature in dairy cows has been shown to lag 2 h behind changes in THI (Kendall et al., 2006). For periods of prolonged heat load, Hahn (1999) suggested that approximately 3 to 4 days are required for cattle's thermoregulatory mechanisms to reduce metabolic heat production, helping the animal to cope with a thermal stressor.

1.2 Physiological Responses to Heat Stress

Thermoneutrality is essential for normal physiologically functioning (Kadzere et al., 2002). When heat gain is greater than heat dissipation, dairy cows have increased respiratory rate to improve evaporative heat loss (Beatty et al., 2006). Heart rate is higher in summer months (70 beats/min) than spring months (64 beats/min) (Bouraoui et al., 2002).

Milk yield and reproduction are sensitive to changes in body temperature because milk synthesis and secretion are sources of metabolic heat production, and heat stress disrupts the hormones important for normal ovarian function (e.g. heat stress depresses luteinizing hormone that triggers ovulation) (Wolfenson and Roth, 2019).

1.2.1 Milk yield

As THI increases, milk yield decreases and milk composition changes with less fat and protein produced (Ravagnolo et al., 2000; Hammami et al., 2013; Bernabucci et al., 2014). Milk yield can decrease by 0.2 kg/d per unit increase in THI ≥ 72 (Ravagnolo et al., 2000), 0.41 kg/d per unit increase in THI ≥ 69 (Bouraoui et al., 2002), 0.88 kg/d per unit increase in the THI measured 2 d previously (West et al., 2003), to as much as 0.96 kg/d per unit increase in THI between 70 to 74 (Hammami et al., 2013).

Discrepancies between estimates of decreased milk yield in response to increased THI, or indeed discrepancies between estimates for any responses to heat stress, may partly be explained by the different conditions that studies were conducted in. For example, some studies have been conducted under Mediterranean (Bouraoui et al., 2002), subtropical (Ravagnolo et al., 2000), hot humid (West et al., 2003), and temperate (Hammami et al., 2013) conditions. Heat stress THI thresholds for dairy cows have been shown to be higher in semiarid climates (ambient temperature ≥ 30 °C at 25 % RH) than hot humid climates (ambient temperature ≥ 23 °C at 75 % RH) (Bohmanova et al., 2007). Cows in temperate climates have lower heat stress thresholds of an ambient temperature of 17.8 °C at 75% RH (Hammami et al., 2013).

Cattle are able to adapt to changes in environmental conditions when these are gradual and prolonged (Collier et al., 2019). The prolonged heat loads in subtropical climates allow better acclimation to environmental conditions (e.g. reduced metabolic rate, changes to the cardiovascular system, more efficient heat loss, and changes to morphology) (Renaudeau et al., 2012). Cattle in temperate climates are less able to acclimate to heat because they are only exposed to acute heat stress events during the summer (Renaudeau et al., 2012). Thus, the lower THI thresholds of dairy cows in temperate versus subtropical climates possibility relate to the different heat tolerances of the animals. This may partly explain why, of the aforementioned studies, Ravagnolo et al. (2000) found the smallest decrease in milk yield (0.2 kg/d) in a subtropical climate, and Hammami et al. (2013) found the largest decrease in milk yield (0.96 kg/d) in a temperate climate. When comparing heat stress studies on dairy cows, it is important to consider the climate that studies were conducted in.

Maintenance energy requirements increase during heat stress, thus reducing energy available for milk synthesis (West, 2003). In addition to the negative impact of decreased milk yield, changes in milk composition are detrimental for Canadian dairy farmers because compensation is based on milk composition (e.g. butterfat, protein, and other solids) (Ouellet et al., 2019).

1.2.2 Reproduction

Heat stress compromises reproduction, including reduced pregnancy success rates and embryonic survival (Hansen, 2007). Around breeding, plasma hormone concentrations undergo a dynamic change that can be negatively affected by heat stress (Ingraham et al., 1974). Ovulation failure is 9 % higher and pregnancy rate is 16 % lower for cows inseminated in the warm season compared to the cool season (López-Gatius et al., 2005). Conception rates can decline as much as 45 % when the average THI on the 2nd day prior to breeding increases from 70 to 84 (Ingraham et al., 1974). The differences between these studies may be due to differing levels of ambient conditions the cows were exposed to; López-Gatius et al. (2005) only measured the effects of season (the warm period was when an average of 20 - 31 days in a month had a maximum temperature above 25 °C), while Ingraham et al. (1974) directly accounted for THI (a THI of 84 equated to above 30 °C).

Further complicating interpretation of reduced pregnancy rates is that heat stress can make estrus behaviour harder to detect due to shortened duration and intensity of visible signs (De Rensis et al., 2015). Estrus is the external sign of ovulation, and identifying estrus is imperative for correct timing of insemination for pregnancy success (Roelofs et al., 2010). Dairy cows in the summer season have a 0.7 h shorter duration of estrus and take 144 fewer steps/h on the day of estrus, compared to cows in the winter season (Sakatani et al., 2012).

The likelihood of pregnancy loss increases by a factor of 2 for every unit increase in the cumulative number of hours with a THI > 85 during gestation days 11 - 20 (Santolaria et al., 2010). During days 21 - 30 of gestation, the likelihood of pregnancy loss increases by a factor of 1 for every unit increase in mean maximum THI (García-Ispierto et al., 2006).

In conclusion, heat stress makes it difficult for dairy cows to maintain their milk production, get pregnant, and stay pregnant. Low milk production and reproduction are two of the main reasons for culling decisions on dairy farms (Hadley et al., 2006; Bell et al., 2010).

1.3 Behavioural Responses to Heat Stress

In addition to physiological responses, changes in behaviour, such as increased standing time, decreased feed intake, seeking shade, and increased water intake, are other means of thermoregulation (Collier and Gebremedhin, 2015; Polsky and von Keyserlingk, 2017). Observing behavioural changes in response to heat stress is valuable because behaviour will change before drops in productivity occur (Schütz et al., 2010).

Standing time increases during heat stress as this behaviour exposes more surface for heat loss, such as sensible water loss, radiating surface area, and air movement via convection (Cook et al., 2007; Allen et al., 2015). Reduced feed intake decreases the amount of heat generated from digestion and absorption of nutrients (Stull et al., 2008), and lowers metabolism and maintenance energy requirements (Blackshaw and Blackshaw, 1994). Dairy cows housed on pasture will readily use shade when it is provided (Kendall et al., 2006), especially when it blocks a greater percentage of solar radiation (Tucker et al., 2008; Schütz et al., 2009), as shade reduces the heat load gained from solar radiation (West, 2003). Increased ingestion of water increases heat loss and combats dehydration (Vizzotto et al., 2015), as evaporative heat loss is the primary method of heat dissipation for dairy cows (Collier et al., 1982). Water has a high heat of vaporization and heat capacity which allows an animal to dissipate and withstand a great amount of heat relative to volume loss and body temperature increase (Murphy, 1992). Ingested water absorbs heat resulting in less heat production, especially when the water is cold (Lanham et al., 1986). Water cools the reticulum (Collier et al., 1982; Bewley et al., 2008; Ammer et al., 2016) and drinking cooler water can decrease rectal temperature (Milam et al., 1986; Wilks et al., 1990).

1.3.1 Standing and lying behaviour

Cook et al. (2007) identified a THI threshold of 68 as when standing and lying behaviour begins to change. As THI increases, time spent standing increases from 2.6 h to 4.5 h/d (Cook et al., 2007) and time spent lying decreases from 10.9 h/d to 7.9 h/d (Cook et al., 2007) or from 9.5 h/d to 6.2 h/d (Nordlund et al., 2019). Lying bout duration also decreases from 50 min/bout to 33 min/bout as THI increases (Nordlund et al., 2019), and can decrease as much as 30 min/day (Allen et al., 2015) As CBT increases from 37.8 °C to above 40.5 °C, standing bout duration increases to more than 60 min (Allen et al., 2015).

Allen et al. (2015) and Nordlund et al. (2019) measured the thermodynamics of these postural changes. Average CBT decreases at a rate of 0.25 °C/h when standing and increases at a rate of 0.50 °C/h when lying (Nordlund et al., 2019). Average CBT is 0.2 °C lower at the end of a standing bout and 0.17 °C higher at the end of a lying bout (Allen et al., 2015).

Nordlund et al. (2019) tried to determine the CBT threshold when dairy cows would end a lying bout; however, the CBT range in which cows ended a lying bout (39.0 - 39.6 °C) overlapped the CBT range in which they chose to lie down (38.6 - 39.2 °C). Cows quite consistency ended their lying bouts after a net gain in CBT of 0.40 - 0.48 °C; thus, the authors concluded that the net increase in CBT during lying may be a more accurate determinant of when a cow choose to end a lying bout, as opposed to a specific CBT threshold.

Lying is a high priority behaviour for dairy cows (Munksgaard et al., 2005) that is compromised by heat stress; in normal conditions dairy cows choose to lie for between 11.2 h/d (Ito et al., 2010) to nearly 13 h/d (Munksgaard et al., 2005; Fregonesi et al., 2007; Ledgerwood et al., 2010). Increased standing time increases the risk of lameness in dairy cattle (Cook and Nordlund, 2009), a malady frequently associated with an abnormal, likely painful, gait (Whay et al., 1997). Thus, heat-stressed dairy cows that spend more time standing may be more susceptible to lameness. Indeed, Cook et al. (2007) reported an increase in the prevalence of lame cows at the end of their summer trial. To compound matters, severely lame cows spend more time lying than non-lame cows (Ito et al., 2010); thus, lame cows may be less able to cope with heat stress (Cook et al., 2007).

1.3.2 Feeding behaviour

Feed intake decreases by 0.11 kg/d for every unit increase in THI between 53 - 76 (Ammer et al., 2018), and by 0.51 kg/d for every unit increase in THI between 72 - 84 (West et al., 2003). Climate controlled trials where dairy cows were subjected to a short period of heat stress after thermoneutral conditions have found different results for the magnitude of feed intake decline. Exposure to experimental heat stress conditions for 4 d resulted in consumption of 14.6 kg less feed (Spiers et al., 2004), and for 7 d resulted in consumption of 6.1 kg less feed (Wheelock et al., 2010), on the last experimental day. Ominski et al. (2002) noted a substantially smaller decline in feed intake of only an average of 1.4 kg/d less over the 5 d of an experimental heat stress period.

A difference between these climate controlled trials was how the experimental heat stress conditions were imposed. During the heat stress periods, Spiers et al. (2004) maintained the THI at a constant 78, while the other studies used a cyclic THI (ambient temperature range of 20 - 32 °C, Ominski et al., 2002; THI range of 72 - 82, Wheelock et al., 2010). Cyclic THI allows for thermal recovery (i.e. reestablishment of normal body temperature) after periods of high temperature through improved heat dissipation when the temperature is lower (Igono et al., 1992). If the night-time ambient temperature drops below 21°C for 3 - 6 h, the decline in milk production due to elevated THI during the day may be mitigated (Igono et al., 1992). The decline in dry matter intake in the Ominski et al. (2002) study may have been a consequence of the 13 h duration of > 21°C ambient night time temperature. In contrast, the decline in feed intake in the Spiers et al. (2004) study may have been a result of the heat stress conditions being severe given the continuous high THI.

Other observed changes in feeding behaviour as a consequence of heat stress include feed sorting (Miller-Cushon et al., 2019) and decreased rumination time (Soriani et al., 2013; Moretti et al., 2017). Normally dairy cows sort in favour of smaller particles (e.g. grain) but in conditions where the THI is high dairy cows sort in favour of longer forage particles, possibly due decreases in rumen pH and possible rumen acidosis (Miller-Cushon et al., 2019). Rumen pH is also affected by the amount of time spent ruminating as increased saliva flow reduces acidosis (Owens et al., 1998); Soriani et al. (2013) found rumination time decreased by 2.2 min for every unit increase in daily maximum THI above 76, possibly compounding the issue of decreased rumen pH during heat stress.

Decreased feed intake can put cows into negative energy balance and make them more susceptible to disease (Contreras et al., 2016). Apart from the negative health consequences of reduced feed intake for a dairy cow, reduced feed intake is a problem for dairy farmers, as decreased feed intake leads to decreased milk yield (Brown et al., 1977).

1.3.3 Seeking shade

On average, dairy cows use shade for 1.8 / 6 day-light h, and this number increases on days with higher solar radiation (Schütz et al., 2009). Dairy cows will use a shade structure that blocks 25% solar radiation for 1.3 / 15.5 day-light h and a shade structure that blocks 99% solar radiation for 3.3 / 15.5 day-light h (Tucker et al., 2008). When offered a choice between shade that blocks 25% or 99% of solar radiation, cows will spend a greater amount of time (72.3%) under the 99% shade (Schütz et al., 2009).

The amount of shade provided also affects usage, when offered 9.6 m^2 of shade/cow, cows spend 2.9 / 5.8 day-light h in shade, but this value decreased to 1.4 h when there was only 2.4 m^2 of shade/cow (Schütz et al., 2010). Even when dairy cows are deprived of lying for 12 h, they will spend more time standing in shade than lying in a non-shaded area with increasing ambient air temperature (Schütz et al., 2008).

Testing in group measures of shade use may be difficult due to competition for simultaneous access (Kendall et al., 2006). When offered 2 shade structures not all cows within groups accessed the shade for the same amount of time; possibly due to increased competition. Schütz et al. (2010) reported that shade use increased with increased space available per cow (9.6 m^2 of shade/cow vs 2.4m^2 of shade/cow). It would be interesting to measure shade use when competition and space are not limiting factors.

While dairy cows will use shade, studies have found mixed results on the effectiveness of shade in reducing body temperature and temperature underneath the shade structure. Kendall et al. (2006), Schütz et al. (2010), and Palacio et al. (2015) all reported no difference in body temperature between shaded and non-shaded cows. In contrast, Kendall et al. (2007) reported a 0.3 °C decline in body temperature after cows were provided shade during a 90-min treatment period where the microclimate under the shade that blocked 93% solar radiation was 1 °C cooler. Unfortunately the authors Kendall et al. (2007) failed to clarify whether 1 °C refers to ambient temperature or black globe temperature. Given that shade has little to no effect on ambient temperature (West, 2003) and that they measured heat load index, it is likely that the authors are referring to a 1 °C cooler black globe temperature. Similarly, when shade blocked 25%, 55%, and 99% of solar radiation the corresponding black globe temperature in the shade was 1, 2, and 3 °C cooler, respectively (Schütz et al., 2009). Kendall et al. (2006) speculates that the lack of any change in body temperature was a consequence of the mild ambient conditions during their study period (average mean ambient temperature = 18.5 °C). Although the average ambient temperature conditions in the studies mentioned above were similar (19.5 °C, Kendall et al., 2007; 16 °C, Schütz et al., 2009; 18 °C, Schütz et al., 2010; 20.7 °C , Palacio et al., 2015). Palacio et al. (2015) speculated that the failure to note any differences in body temperature between shaded and non-shaded cows may be due to the cows using alternative strategies used for cooling, such as increased time spent around the water trough. The area around a water trough may have a cooler microclimate (Palacio et al., 2015), and as shown by Schütz et al. (2010) a preferred area to stand for non-shaded cows as a possible way to decrease body temperature.

There are differences in the how shade was provided between studies that report shade decreasing body temperature and temperature underneath the shade structure (Kendall et al., 2007; Schütz et al., 2009) and those that did not (Kendall et al., 2006; Schütz et al., 2010; Palacio et al., 2015). For example, the shade structure provided by Kendall et al.

(2007) blocked solar radiation both from above and on 3 sides; whereas, the other studies only blocked solar radiation from above. Offering a more expansive shade structure may allow the cows to more effectively reduce their body temperature by minimizing heat from solar radiation. Indeed, Hahn et al. (1963) found that placing shade on top of an animal reduced radiant heat gain from the sun by 45%, but that this increased to 54% when an additional 3 walls were shaded.

Schütz et al. (2009) provided the largest space allowance per cow (16 m^2/cow for 3 cows) compared to the other studies (1.8 m^2/cow for 10 cows, Kendall et al., 2006; 1.25 m^2/cow for 4 cows, Kendall et al., 2007; 2.4 and 9.6 m^2/cow for 10 cows, Schütz et al., 2010; 4.65 m^2/cow for 4 cows, Palacio et al., 2015). The amount of radiative heat transfer between 2 bodies decreases with distance between them (Polder and Van Hove, 1971); the increased space allowance per cow provided underneath the shade structure by Schütz et al. (2009) may have resulted in less radiating heat, thus lowering ambient temperature in the shaded area. Although, there are numerous anecdotal accounts that dairy cows bunch together when heat stressed (e.g. see Drovers, 2015; FarmingIndependent, 2018), a behaviour that increases the radiative heat transfer between animals. To my knowledge there is little known about the motivation for this behaviour. Grouping may be a protective response to stress (Mooring and Hart, 1992) or perhaps a desire of all to be in a preferred environment.

An alternative form of shade provision shown to reduce rectal temperature offered cows access to shade provided by 2 elm trees and one artificial shade structure that collectively blocked 80 % solar radiation (Valtorta et al., 1997). These authors found that shaded cows had a lower rectal temperature (39.3 °C) than non-shaded cows (40.1 °C). Trees may be a better form of shade than artificial shelters because evaporation from leaves offers additional cooling (Van laer et al., 2015). Veissier et al. (2018) also investigated shade provided by trees with shade cloth strung between them and found shaded cows tended to have a lower rectal temperature than non-shaded cows.

Lastly, Kendall et al. (2006) and Schütz et al. (2010) both commented on the challenges associated with providing shade to a group of cows where competition may affect an individual cow within the group from accessing the shade resource. Thus, studies that found no effect of shade on body temperature of cows may not be an accurate representation of the effectiveness of shade.

1.3.4 Drinking behaviour

Multiple studies have found that drinking behaviour increases during elevated ambient temperatures and THI (e.g. Murphy et al., 1983; Meyer et al., 2004; Beatty et al., 2006; Cook et al., 2007; Cardot et al., 2008; Ammer et al., 2018). As THI increases from 56 to 78, drinking time can double, from 0.26 h/d to 0.5 h/d (Cook et al., 2007). In an average THI range between 53 - 76, water intake increases by 0.96 - 1.08 L/d and drinking frequency increases by 0.12 - 0.23 bouts/d per rising THI unit (Ammer et al., 2018). Water intake increases by 0.84 L/d per °C increase in average ambient temperature and by 1.89 L/d per °C increase in maximum ambient temperature (Meyer et al., 2004). In regards to minimum ambient temperature, water take increases by 1.2 L/d per °C increase (Murphy et al., 1983; Meyer et al., 2004).

Drinking behaviour can also increase when alternative forms of cooling are limited. Compared to cows with access to shade, unshaded cows spend more time around the water trough as heat load increases (0.7 / 5.8 h for unshaded cows versus 0.1 - 0.3 / 5.8 h for shaded cows, Schütz et al., 2010; 0.8 / 3 h for unshaded cows versus 0.1 / 3 h for shaded cows, Palacio et al., 2015). Unfortunately, other measures of drinking behaviour, such as water intake, were not included in these studies.

For non-productivity related indicators of heat stress in dairy cows, changes in drinking behaviour are less frequently used than changes in feeding and resting behaviour (Galán et al., 2018). However, the substantial increases in water intake, time spent drinking, and frequency of visits to the drinker in response to heat stress indicate that drinking behaviour is a valuable indicator of heat stress in dairy cows, and is worthy of further research.

1.4 Social Behaviour and Aggression

Dominance is established among cows based on wins and losses in competitive interactions (i.e. dominant cows will be more successful in winning aggressive interactions and subordinate cows will experience more losses) (Friend and Polan, 1974; Kondo and Hurnik, 1990). In competitive situations, dominant cows have priority access to valuable resources (Galindo and Broom, 2000), such as feed when there is competition at the feedbunk (Friend and Polan, 1974). The peak time for competition at the feeder and motivation to feed is greatest right after food is delivered (Huzzey et al., 2007); dominant cows will spend more time at the feeder during the 120 min following provision (Val-Laillet et al., 2008a). Subordinate cows will choose to consume lesser quality feed alone than feed next to a dominant cow (Rioja-Lang et al., 2009). Apart from the negative effect of competition limiting access to important resources such as feed (Olofsson, 1999; DeVries et al., 2004), competition can also be a source of physiological stress (Friend et al., 1979; Hasegawa et al., 1997; Huzzey et al., 2012; Hetti Arachchige et al., 2014).

Aggression and competition in dairy cows will increase as group size increases and space allowance decreases (Kondo et al., 1989), after regrouping (Kondo and Hurnik, 1990; von Keyserlingk et al., 2008), when there is less space per cow at the feedbunk (Olofsson, 1999; DeVries et al., 2004; Collings et al., 2011) or feed is temporally restricted (Collings et al., 2011), when freestalls are overstocked (Huzzey et al., 2006; Fregonesi et al., 2007; Lobeck-Luchterhand et al., 2015; Winckler et al., 2015) and in confinement housing versus on pasture (O'Connell et al., 1989). Different cows will compete for different resources (e.g. feed, lying stalls, mechanical brush) based on their motivation for that resource (Val-Laillet et al., 2008b).

Much of the previous literature on competition between dairy cows has focused on feed and lying stalls; competition for water has received little attention, despite water being one of the most important nutrients for dairy cows (NRC, 2001).

1.4.1 Competition for cooling resources

Shade and water are valuable cooling resources for a heat-stressed dairy cow (Dash et al., 2016), and cattle become more aggressive when warm (Coimbra et al., 2012). When there is less shade available per cow, dairy cows engage in aggressive interactions to gain access to shade (Schütz et al., 2010; Stivanin et al., 2019). Dairy cows engage in an average of 0.2 competitive interactions/h when there is 10 m^2 of shade per cow, but this increases to 0.5 competitive interactions/h when there is only 2 m^2 of shade per cow (Stivanin et al., 2019). In a group of 10 cows during a 5.8 h observation period, Schütz et al. (2010) found the total number of aggressive interactions per m^2 of shade increased from 3.2 when there was 9.6 m^2 of shade per cow to 10.7 when there was 2.4 m^2 of shade per cow.

Competition near the water trough also increases when shade is not provided (Vizzotto et al., 2015) or provided in small amounts (Stivanin et al., 2019). While shaded cows will engage in an average of 0.12 competitive events per day to gain proximity to the water trough, this number increases to 0.35 for unshaded cows (Vizzotto et al., 2015). When the amount of shade per cow decreases from 10 m^2 to 2 m^2, the average number of competitive events per cow near the water trough increases from 0.2/h to 0.8/h (Stivanin et al., 2019).

The majority of studies that have looked at competition between dairy cows at the drinker have focused on cows on pasture. As of 2014 (the most recent statistics available), only 7.5% of dairy farms in the United States used pasture as the primary type of housing for lactating dairy cows (USDA, 2016). A larger proportion of dairy cows (20%) were housed in freestalls (an indoor, loose housing system) with no outdoor access. In Canada in 2018, 26.2% of farms used freestall housing and 73.8% of farms used tie stall housing (a system where dairy cows are tethered in place at one stall; CDIC, 2019c). Thus, research is required on competition at the drinker for free stall housed dairy cows.

1.5 Automation in the Dairy Industry

Technologies allow for continuous, automatic, real-time monitoring of animal health and behaviour (Rutten et al., 2013). Some common devices in use in the dairy industry include milk flow sensors (Rutten et al., 2013), radio-frequency identification (RFID) tags, neck collars, and accelerometer leg bands (Grinter et al., 2019). These systems can be used to monitor cow activity, milk yield, udder inflammation, and milk components (Borchers and Bewley, 2015).

The increased demand for these technologies is due to increasing herd sizes making it less feasible for dairy farmers to individually monitor cows (Berckmans, 2014), and increasing labor costs (Rutten et al., 2013). Integration of automatic technologies can provide farmers with an early alert of potential health problems that might be difficult to detect otherwise (Rutten et al., 2013).

1.5.1 Validation of automatic technologies to monitor behaviour

Before automatic technologies can be incorporated, it is important to validate that they measure what they intend to (Grinter et al., 2019). The consequences of an ill-performing automatic system are that farmers may decrease their response to alerts, or waste time attending to cows that are not sick (Grinter et al., 2019). Evaluation of the accuracy of sensors to detect milk yield components, udder diseases, and estrus has received considerable interest (e.g. see Gebre-Egziabher et al., 1979; Koelsch et al., 1994; de Mol et al., 1997; Maatje et al., 1997; Norberg et al., 2004; Hovinen et al., 2006), and efforts are increasing to automatically detect cow behaviour, such as feeding (DeVries et al., 2003; Chapinal et al., 2007), standing (Ledgerwood et al., 2010), lying (Ledgerwood et al., 2010), resting (Bikker et al., 2014), and location in the barn (Gygax et al., 2007).

RFID ear tags that identify individual animals can be coupled with electronic feed bins (Chapinal et al., 2007) or a radio frequency monitoring system (DeVries et al., 2003; Chizzotti et al., 2015) to accurately detect feeding behaviour. Accelerometer data loggers can accurately record standing and lying behaviour (Ledgerwood et al., 2010). Ruminating and resting behaviour can be monitored with accelerometer data loggers attached to RFID ear tags (Bikker et al., 2014). Radar technology can also be used to automatically track the location of dairy cows in a free stall barn (Gygax et al., 2007). By combining data from electronic feed bins and accelerometer data loggers, Thompson et al. (2017) was able to estimate the time that dairy cows spent away from their pen to be milked.

Research is also being conducted to develop systems that automatically detect social behaviour. Competitive events at the feeder can be accurately identified using electronic feed bins (Huzzey et al., 2014). Data from electronic feeds can also be combined with data from electronic water bins to detect agonistic interactions (Foris et al., 2019). Social interactions between dairy cows can be inferred with a surveillance system using image segmentation and tracking methods (Guzhva et al., 2016). Image analysis has also been used to detect aggressive behaviours in pigs (Chen et al., 2019).

Automatically detecting social behaviour between dairy cows is valuable because changes in social behaviour are a sign of problems, such as disease (e.g. see Huzzey et al., 2007; Goldhawk et al., 2009; Sepúlveda-Varas et al., 2016) or an inadequate environment (e.g. too few lying stalls or feeding places). Validation of systems that automatically detect social behaviour is of particular interest to this thesis because conventional methods to record social behaviour involve video analysis, and this technique is time consuming and subject to observer error and bias. Automatic systems are a more efficient and objective method to record social behaviour.

1.5.2 Automatic detection of heat stress

Decreased rumination time (Soriani et al., 2013; Moretti et al., 2017), panting (Beatty et al., 2006), and increased respiratory rate (Ominski et al., 2002; Spiers et al., 2004) are established characteristics of a dairy cow experiencing heat stress. Schirmann et al. (2009) validated an electronic rumination monitoring system with data loggers attached to neck collars to accurately measure rumination times. This system was then used by Moretti et al.

(2017) to determine the relationship between THI and rumination time. Abeni and Galli (2017) also explored whether a neck collar equipped with a rumination data logger could monitor changes in rumination time due to heat stress. Panting score, identified with accelerometer based tags on neck collars, can provide an automated measure of heat load, based on a comparison of measures from the collar and intravaginal body temperature monitoring loggers (Bar et al., 2019). Respiratory rate sensors highly correlate with visual observations of respiratory rate (Atkins et al., 2018); this technology has the potential to be used in further studies on heat stress.

To date, no work has been done to determine if changes in social behaviour measured electronically can be used to indicate if dairy cows are experiencing heat stress.

1.6 Objectives and Hypotheses

My thesis will investigate the behaviour of dairy cows at the drinker with the aim to: 1) validate an electronic drinking system to automatically record dairy cow behaviour at the drinker, in particular competition for this resource and, 2) use this validated technology to assess whether drinking behaviour is associated with elevated THI. I hypothesize that 1) a short interval of time between sequential visits of 2 cows at one drinker can be used to automatically identify competitive events at the drinker and 2) that water intake, frequency of visits to the drinker, time spent at the drinker, and competitive events at the drinker will increase along with increasing THI.

Chapter 2: Using an Electronic Drinker to Monitor Competition in Dairy Cows

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2.1 Introduction

Monitoring behaviour using video analysis can be time consuming and subject to observer error and bias. Technology can be used to automate some types of monitoring. For example, in agricultural systems, radio frequency monitoring systems can accurately record feeding frequency, duration, and intake (see Chizzotti et al., 2015). Data from automatic systems has also been used to make inferences about social interactions (see Guzhva et al., 2016). Social competition is often assessed during feeding by recording ‘displacements’, typically defined as when physical contact from 1 cow (actor) results in the recipient cow (reactor) withdrawing from the feed bunk (DeVries et al., 2004). This event can be further defined as a ‘replacement’ if the actor then takes the place of the previous cow at the feeder. Huzzey et al. (2014) found that replacements could be accurately identified with automated feeders by screening the data for ≤ 26 s intervals between sequential visits of 2 cows at the same feed bin within the interval. This method has since been used to quantify social interactions in studies on feedbunk stocking density (Crossley et al., 2017) and introduction of postpartum cows into new groups (Jensen and Proudfoot, 2017).

Drinkers may also be useful for monitoring social competition. During periods of elevated ambient temperature when cows increase water intake and decrease feed intake (Kadzere et al., 2002), aggressive interactions are high (Coimbra et al., 2012) resulting in more competition at, and close to, the water source (Vizzotto et al., 2015). Especially during heat stress, water is an important resource for dairy cows and competition can result in dominant animals preventing subordinate animals from access (Coimbra et al., 2012). Drinkers are also typically provided at high stocking rates, likely leading to more displacement events (DeVries et al., 2004). To our knowledge, no work to date has investigated whether data from drinking systems that electronically track individual animal attendance can be used to assess social competition.

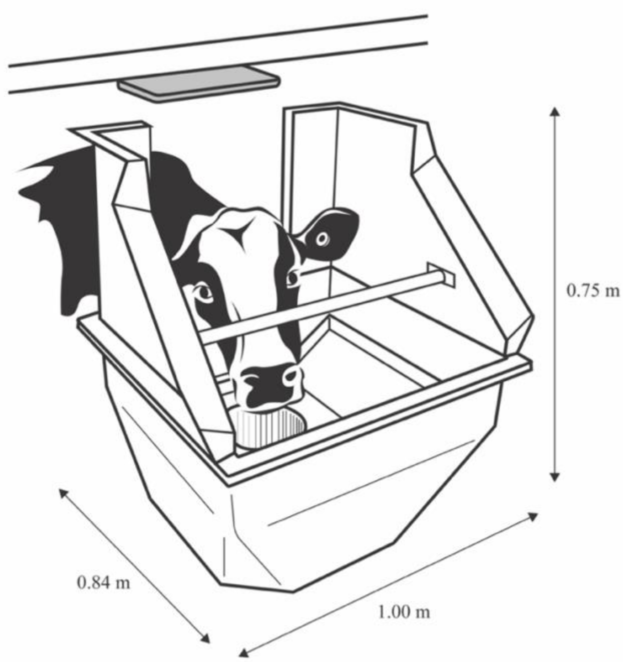
The objective of this study was to determine if the interval between one cow leaving the drinker and another cow taking her place could accurately identify competitive 'replacements' at the drinker. The specific aims were to assess a range of intervals using receiver operating characteristic (ROC) curves and, on this basis, recommend a specific interval that can best identify replacements at the drinker.

2.2 Materials and Methods

All procedures were approved by the University of British Columbia (UBC) Animal Ethics Committee (protocol A14-0040). In September of 2013, lactating, Holstein dairy cows (n = 20, with a mean ± SD parity of 3.1 ± 2.2 and a range of 1 - 8 lactations) at the UBC Dairy Education and Research Centre (Agassiz, BC, Canada) were observed for 4 consecutive 24-h periods (mean ± SD ambient temperature = 19.7 ± 4.3 $_{o}$C; range = 12.9 - 29.3 $_{o}$C). Average days in milk (DIM) at the start of the observation period (mean ± SD) was 11.1 ± 6.9 (range = 1 - 23) and average milk production over the first 21 d postpartum was 24.8 ± 9.5 kg/d (range = 7.9 - 41.5 kg/d). Two of the observation periods were assigned as the Baseline Set (mean ± SD ambient temperature = 18.9 ± 3.8 $_{o}$C; range = 12.9 - 25.4 $_{o}$C), and used to identify the optimal interval; the 2 other periods were assigned as the Validation Set (mean ± SD ambient temperature = 20.5 ± 4.7 $_{o}$C; range = 13.9 - 29.3 $_{o}$C), and thus used to validate the interval. As elevated ambient temperatures affect competitive behaviour at the drinker (Vizzotto et al., 2015), the 24-h observation periods were assigned to either the Baseline Set or the Validation Set based on mean daily temperature. The Baseline Set was comprised of the more moderate 2 days (mean ± SD ambient temperature of Baseline Set period 1 = 18.3 ± 3.9 $_{o}$C and Baseline Set period 2 = 19.5 ± 3.7 $_{o}$C) and the Validation Set was comprised of the warmest and coolest days (mean ± SD ambient temperature of Validation Set period 1 = 24.0 ± 3.6 $_{o}$C and Validation Set period 2 = 16.9 ± 2.2 $_{o}$C). In this way, the interval could be assessed in a range of summer conditions. All animals were housed together in a 24-freestall pen equipped with 12 Insentec feed bins and 2 Insentec water bins (see Neave et al., 2017, for a full description of the facility). Animals had *ad libitum* access to water and were milked and fed twice per day at approximately 0700 and 1700 h and 0800 and 1600h, respectively. Feed was formulated according to NRC (2001) guidelines. As animals were part of larger studies by Neave et al. (2017, 2018) and Lomb et al. (2018a, 2018b), the sample size was kept to the stocking density (20 cows/pen) required for those studies. Group size and composition were constant during the observations and all animals present in the pen were included in data collection and analysis. Temperature was recorded continuously at 1 h intervals by the Environment Canada local weather station situated approximately 500 m from the barn.

Each cow was fitted with an ear tag transponder (High Performance ISO Half Duplex Electronic ID Tag; Allflex Canada, Saint-Hyacinthe, Quebec, Canada) that allowed the electronic drinking system (Roughage Intake Control system; Insentec B.V., Marknesse, The Netherlands; Figure 2.1) to record the time each cow entered and left a water bin (see Chapinal et al., 2007, for validation of the system). Social behaviour was monitored using video cameras (CCTV camera, model WV-BP330; Panasonic, Osaka, Japan) positioned 6 m above the feed alley of the pen. Cows were identified with unique alphanumeric symbols (1 marking on the back and 1 on the right and left sides), such that it was always possible to identify each cow at the water bin; red lights (100W) facilitated recording at night. A replacement at a water bin was recorded by the video observer when physical contact initiated by one cow caused the receiving cow to entirely remove her head from the water bin, with the actor subsequently placing her head in the same water bin within 60 s. The same observer rescored one of the periods to assess intra-observer reliability and found this to be high (Cohen's kappa = 0.95). Earlier work has shown that this definition of a replacement also produces high inter-observer reliability (Schirmann et al., 2011).

Figure 2.1. The electronic water bins and drinking system (Roughage Intake Control system; Insentec B.V., Marknesse, the Netherlands) used to calculate the interval between sequential drinking events of 2 cows at the same water bin.
For this system, each cow is fit with a unique passive ear tag transponder (pictured in figure) that is detected by the radio-frequency identification reader above the water bin to allow access to the water. For each visit to the water bin, the system records the cow number, the water bin number, the time the cow entered and left the water bin, the duration of the visit, and the water intake.



2.3 Statistical Analyses

All statistical analyses were performed in SAS (version 9.4; SAS Institute Inc., Cary, NC). The interval between drinking events was used as the independent variable and the occurrence of a replacement or not (as identified from video) was used as the binary outcome variable. To determine the interval between cows drinking at a water bin that best identifies replacements, PROC LOGISTIC was used to construct ROC curves. ROC curves allow for a graphical assessment of the true-positive rate (sensitivity) and false positive rate (1-specificity) for different thresholds of a test (Fawcett, 2006). Sensitivity (Se) is defined as the proportion of positive events correctly identified and specificity (Sp) is the proportion of negative events correctly identified (Metz, 1978). The results of a binary classification test (true positive [TP], true negative [TN], false positive [FP], false negative [FN]) are arranged into a 2 x 2 table called a confusion matrix, from which Se and Sp can be calculated.

Ranging between 0 and 1, the area under the curve (AUC) represents the diagnostic accuracy of the ROC curve as a whole with all possible threshold values (Greiner et al., 2000). An AUC ≤ 0.5 represents a test with outcomes worse than chance and an AUC ≥ 0.9 is highly accurate.

For a continuous measurement, a threshold must be determined to differentiate between a positive and negative test result (Gardner and Greiner, 2006). However, Se and Sp are inversely related and the choice of cut-off affects the weight given to each parameter. Methods of determining an optimal cut-off point from a ROC curve are the Youden index (that maximizes the sum of Se and Sp; Youden, 1950), Distance to (0,1) (the value closest to the "perfect" 100% Se and 100% Sp point; Perkins and Schisterman, 2006), and Se = Sp (the point where Se and Sp are roughly equal; Greiner et al., 1995).

In the Baseline Set, ROC curves were constructed for 5 cows with the most replacement events as the reactor to examine between cow variability, and then again for the 20 cows pooled as a group. Individual ROC curves were only constructed for these 5 cows as the others engaged in too few replacements as a reactor (range = 1 - 7) to create reasonable ROC curves. Individual and group (n = 20) ROC curves were analyzed separately to explore how the optimal interval differs at individual and group levels. The ROCPLOT macro was used to generate the 3 metrics (Youden Index, Distance to [0,1], Se = Sp) considered in determining the optimal cut-off point. The group ROC was used for further analyses. Se, Sp, TP, TN, FP, and FN were calculated for each potential cut-off point. The value with the lowest number of FP was chosen as the optimal interval in an *a priori* decision based on the nature of the data, as the number of non-replacement events (negative events) greatly outweighed the number of replacement events (positive events). To test the performance of the optimal interval, it was then applied to the Validation Set using ROC analysis for examination of Se and Sp.

2.4 Results

In the Baseline Set, 5 cows had a total of 174 drinking events with 65 replacements and 109 non-replacements (see Table 2.1). All AUC estimates were > 0.8 indicating moderate to high test accuracy. Estimates for individual cut-offs ranged from 20 to 36 s; however, regardless of cut-off, most values were associated with high Se and Sp. Using data from all 20 cows, there was a total of 745 drinking events with 124 replacements and 621 non-replacements. The group ROC curve had an AUC of 0.91 (95% CI: 0.89 - 0.93), and the optimal cut-off point ranged from 29 to 46 (s) depending on the criterion used (see Figure

2.2). A cut-off of 29 s was associated with the lowest number of FP (see Table 2.2), so this value was then applied to the Validation Set.

Table 2.1. The results from the receiver operating characteristic curve analysis of individual cows (*n* = 5) in the Baseline Set and the associated measures of area under the curve (AUC) and different interval cut-off points (s) between 2 cows drinking at the same water bin to identify replacements at the drinker with sensitivity (Se) and specificity (Sp).
For each cow, the cut-off (s) identified by the Youden Index and the point closest to 100% Se and 100% Sp (Distance to [0,1] metric) was the same and listed in the first row, the cut-off (s) identified by the point where Se and Sp are roughly equal (Se = Sp metric) is listed underneath.

Cow	No. of intervals	No. of replacements[1]	AUC (95% CI)[2]	Cut-off (s)	Se (95% CI)[3]	Sp (95% CI)[3]
1	26	13	0.97 (0.93-1.00)	28	1.00 (0.75-1.00)	0.92 (0.75-0.99)
				26	0.92 (0.64-1.00)	0.92 (0.75-0.99)
2	48	10	0.91 (0.83-1.00)	28	0.90 (0.56-1.00)	0.83 (0.70-0.93)
				27	0.80 (0.44-0.97)	0.83 (0.70-0.93)
3	28	19	0.93 (0.86-1.00)	31	1.00 (0.82-1.00)	0.86 (0.67-0.96)
				27	0.84 (0.60-0.97)	0.86 (0.67-0.96)
4	39	14	0.83 (0.72-0.94)	36	1.00 (0.77-1.00)	0.67 (0.50-0.81)
				26	0.71 (0.42-0.92)	0.74 (0.58-0.87)
5	33	9	0.94 (0.87-1.00)	24	1.00 (0.66-1.00)	0.85 (0.68-0.95)
				20	0.89 (0.52-1.00)	0.88 (0.72-0.97)

1 As the reactor
2 Wald 95% CI
3 Clopper-Pearson 95% CI

Figure 2.2. The group (*n* = 20) receiver operating characteristic curve of different interval cut-off points (s) between 2 cows drinking at the same water bin sequentially to identify replacements at the drinker in the Baseline Set.

Points labeled by different interval cut-offs (s) with symbols to mark those identified as the optimal cut-off (s) by the 3 metrics: the Youden Index (Y), the point closest to 100% sensitivity and 100% specificity (D), and the point where sensitivity and specificity are roughly equal (=).

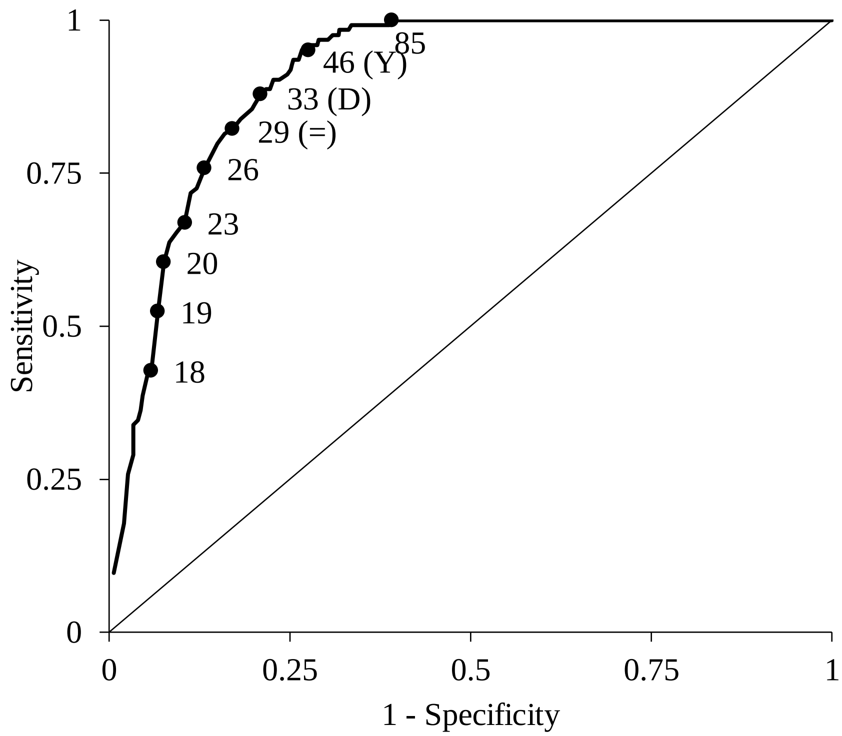


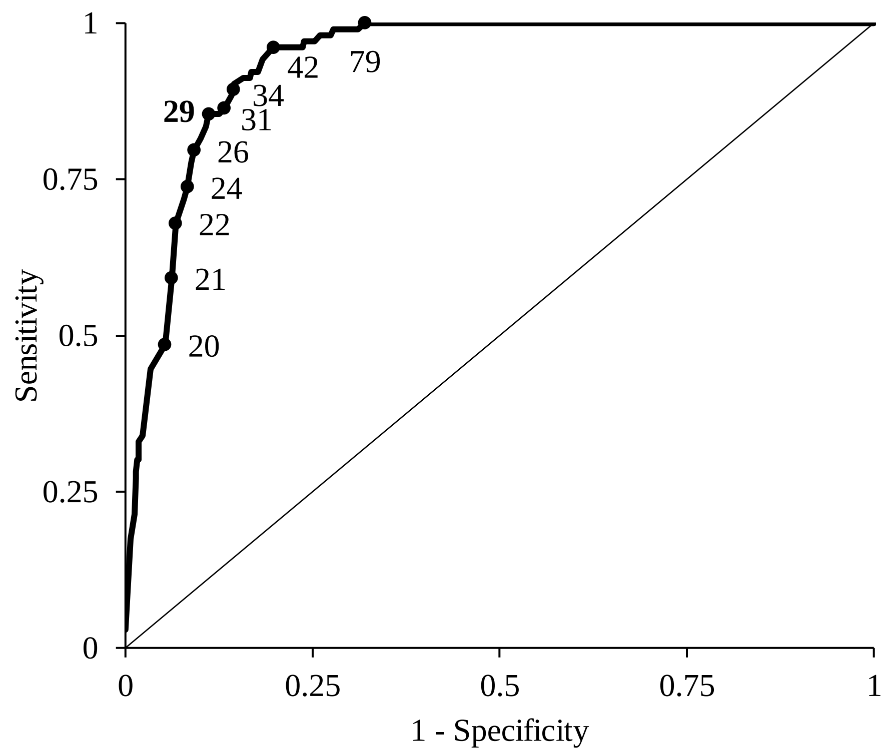
Table 2.2. The optimal cut-off interval (s) between 2 cows drinking at the same water bin to identify replacements at the drinker as identified by the Youden Index, the point closest to 100% sensitivity (Se) and 100% specificity (Sp) (Distance to [0,1] metric), and the point where Se and Sp are roughly equal (Se = Sp metric).
Data are from the Baseline Set of observations on 20 cows over two 24-h periods. For each cut-off (s) we show the Se, Sp, and confusion matrix values (true positive [TP], true negative [TN], false positive [FP], false negative, [FN]). The interval chosen as the optimal cut-off (s) is in bold.

Metric	Cut-off (s)	Se (95% CI)[1]	Sp (95% CI)[1]	TP[2]	TN[3]	FP[3]	FN[2]
Youden Index	46	0.95 (0.90-0.98)	0.73 (0.70-0.77)	118	455	166	6
Distance to (0,1)	33	0.88 (0.81-0.93)	0.79 (0.76-0.82)	109	491	130	15
Se = Sp	**29**	**0.82 (0.74-0.89)**	**0.83 (0.80-0.86)**	**102**	**515**	**106**	**22**

[1]Clopper-Pearson 95% CI
[2]Number of events classified as TP or FN out of a total of 124 positive events
[3]Number of events classified as TN or FP out of a total of 621 negative events

In the Validation Set, the 20 cows had a total of 669 drinking events with 103 replacements and 566 non-replacements. The group ROC curve had an AUC of 0.94 (95% CI: 0.91 - 0.96) and the 29 s cut-off point performed well with a Se of 0.85 (95% CI: 0.77 - 0.92) and a Sp of 0.89 (95% CI: 0.86 - 0.91) (see Figure 2.3).

Figure 2.3. The group (*n* = 20) receiver operating characteristic curve of different interval cut-off points (s) between 2 cows drinking at the same water bin sequentially to identify replacements at the drinker in the Validation Set.
Points labeled by different interval cut-offs (s). The optimal cut-off (s) identified in the Baseline Set is in bold.



2.5 Discussion

This is the first study to investigate whether social competition at the drinker can be measured with an electronic drinking system. Using a ≤ 29 s interval between 2 cows sequentially drinking from the same water bin, replacements could be identified with 82 - 85% Se and 83 - 89% Sp. Similar results were found by Huzzey et al. (2014) with respect to electronic feed bins, identifying a 26 s interval as optimal for identifying replacements. The

ROC curve analysis differed slightly between the 2 studies with Huzzey et al. (2014) using the Distance to (0,1) metric (point closest to 100% Se and 100% Sp). The current study evaluated 3 different metrics (Youden Index, Distance to [0,1], Se = Sp), and the point at which Se and Sp are roughly equal was chosen. Comparing 3 commonly used metrics for identifying an optimal cut-off point allowed for more thorough selection of the interval with the best Se, Sp, and confusion matrix values based on the test results and prevalence of replacement events.

In a binary classification test, determining the optimal cut-off and weight given to Se and Sp depends on event prevalence and priority given to avoiding FP or FN (Greiner et al., 2000). If negative events greatly outnumber positive events, and the consequences of FP are determined to be more detrimental than the consequences of FN, more weight should be placed on Sp. In the current study, non-replacements occurred at 6 times the rate of replacements (and non-replacements should normally far exceed replacements under most management conditions), so we considered the interval that had the lowest number of FP (identified by the Se = Sp metric) as optimal. The Youden Index applies an equal weight to both Se and Sp (Greiner et al., 2000) and, in the case of the current study data set for the group (*n* = 20) ROC curve, yielded the greatest number of FP. For the ROC curves of individual cows (*n* = 5), where the balance between positive and negative events was less pronounced, the interval identified by the Youden Index had a Sp equal to that of the Se = Sp point in 3 of the cows and a greater Se in all 5 cows. Theoretically, the value closest to the "perfect" (0,1) point should result in the least number of misclassified events as either FP or FN (Perkins and Schisterman, 2006) but the current study found the point where Se and Sp are roughly equal had the lowest number of FP. We refrained from assessing accuracy, which is the fraction of all events classified correctly, given that it weighs FP and FN equally (Weiss and Provost, 2003) and has an inherent bias when the number of negative events greatly outweighs positive events (He and Garcia, 2009). A classifier can be highly accurate in identifying the many negative events correctly, while failing to detect the smaller percentage of positive events. A highly specific interval to identify replacements at the drinker is important to avoid FP and the resulting exaggerated estimation of competition, while there are many fewer chances to generate FN.

Feed and water are very different resources over which competitive behaviour may differ. For example, feed is often delivered once or twice per day, but water is typically available throughout the day. Dairy cows spend more time feeding than drinking per day (5 versus 0.3 h/d) (Dado and Allen, 1994), but daily water intake is greater than feed intake (83.6 kg/d vs. 20.6 kg/d for lactating cows) (Cardot et al., 2008). Dairy barns also typically provide more linear space per cow to eat than to drink. Despite these differences in how the resources are provided and used, the optimal cut-off to identify replacements was similar. One reason for this similarity may be that the physical design of the drinkers used in the current study was identical to that of the feeders used by Huzzey et al. (2014). Future studies should examine the effect of different feeder and drinker designs, as this is likely to affect competition. As data for this study was collected during the summer, it would also be interesting to see if the optimal cut-off to identify replacements at the drinker changes in cooler weather conditions.

There are some limitations with the automatic detection of behaviour as described in this study. Twenty-nine replacements identified by video were not paired with electronic data, as the electronic drinking system did not register the identity of 1 of the 2 cows involved in the event. Chapinal et al. (2007) noted similar occurrences when validating the water recording system of the same manufacturer. As can be seen in the ROC curve analysis of individual cows (n = 5) in the Baseline Set (see Table 2.1), the automatic detection of replacements at the drinker with a ≤ 29 s interval between sequential visits of 2 cows at the same drinker may work better for some cows compared to others. Binary classification tests will also inevitably result in some misclassifications (Metz, 1978).

2.6 Conclusion

In conclusion, this study provides the first evidence that social competition at the drinker can be measured using an electronic drinking system. An interval of ≤ 29 s between 2 cows drinking from the same water bin sequentially identified competitive replacements with high Se and Sp. Automated measures of drinking behaviour may be particularly useful for studies on competition during heat stress. Automatically detecting competition is a novel use of an electronic drinking system.

Chapter 3: Hot Weather Increases Competition Between Dairy Cows at the Drinker

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3.1 Introduction

Heat stress occurs when heat load, accumulated both metabolically and from the environment, is higher than the animal's ability to dissipate this heat (Bernabucci et al., 2010). The risk of heat stress in dairy cows is commonly assessed with the THI that represents climatic conditions based on air temperature and RH (Bernabucci et al., 2014). Heat stress was thought to occur when the THI exceeded 72 (Armstrong, 1994), but more recent work has shown that dairy cows begin to experience heat stress at a THI of 68 (Zimbelman et al., 2009). Heat stress can lead to decreased milk yield (Bouraoui et al., 2002), low fertility (Biffani et al., 2016), increased standing time (Allen et al., 2015), decreased feed intake and increased water intake (Beatty et al., 2006).

Agonistic social behaviour, such as competition, can occur over resources that aid in cooling (Vizzotto et al., 2015), such as seeking shade and increasing water intake (Dash et al., 2016). Schütz et al. (2010) found that dairy cows engaged in aggressive interactions to gain access to shade and, when not provided with shade, spent more time standing within 4.5 m of the water trough. Dairy cows will also compete for proximity to a water source (Vizzotto et al., 2015). Coimbra et al. (2012) found that more agonistic interactions occurred during warmer hours; however, while data were collected on agonistic interactions at various resources, the results were not reported separately for the number of events that took place over access to water. These studies were all conducted with dairy cows housed on pasture.

Competition for water has yet to be studied in indoor housing, despite aggression being more common indoors (O'Connell et al., 1989).

Competition over resources can be detrimental to cows, for example, by reducing the number of times that cows are able to visit the feeder (DeVries et al., 2004) and the amount of time spent feeding (Olofsson, 1999). Not all cows experience these negative effects equally; cows with lower social rank are disproportionally affected by competition (Olofsson, 1999; DeVries et al., 2004; Proudfoot et al., 2009). During competition at the feedbunk, cows of lower social rank lose more feeding time than dominant cows (Olofsson, 1999), eat faster (Proudfoot et al., 2009), and avoid the feedbunk at peak times of day (Olofsson, 1999; DeVries et al., 2004). While not studying the direct effects of competition due to elevated ambient temperature, Coimbra et al. (2012) found that dominant cows were able to monopolize a water trough when it was located in a narrow (and presumably more easily defensible) corridor versus when in an open paddock. It is unknown how social rank affects drinking behaviour and competition at the drinker during heat stress.

This observational study addresses objectives at both the group and cow level. Our objective at the group level was to describe the association between increasing THI and behaviour of indoor, loose-housed dairy cows at the drinker, with particular attention to competition for this resource. Our objective at the cow level was to describe the relationship between THI and drinking behaviour of individual cows, based on their level of competitive success at the drinker. We predicted that: 1) periods with high THI would lead to increased water intake, frequency of visits to the drinker, time spent at the drinker, and competitive events at the drinker and, 2) that cows with lower levels of competitive success at the drinker would be most affected. We also explored how the distribution of drinking behaviour throughout the day differed for cows with different levels of competitive success at the drinker.

3.2 Materials and Methods

3.2.1 Animals and housing

All procedures were approved by the UBC Animal Care Committee (protocol A14-0040). Data were collected during June to August of 2014 at the UBC Dairy Education and Research Center (Agassiz, BC, Canada), as part of a larger study by Neave et al. (2017, 2018) and Lomb et al. (2018a, 2018b). The barn was a passively ventilated 2,230 m^2 wood frame building with 4 rows of free stalls consisting of 120 stalls divided into 10 pens of 12 stalls each (5 pens on each side of the building separated by a center feed-alley). The side walls (facing northwest and southeast) had a 1.2 m concrete wall with 1 m of open space above. The end walls (facing northeast and southwest) were open. This barn design was typical of farms in this region.

A total of 69 lactating, Holstein dairy cows (with a mean ± SD parity of 2.6 ± 1.7 and a range of 1 to 9 lactations) were followed. Average milk production was 38.1 ± 12.0 kg/d (mean ± SD, range = 4.0 - 66.0 kg/d). Animals were housed together in a mixed-parity group for 3 wk after calving, except for 8 cows (with a mean ± SD DIM of 231 ± 124.0 d) used as "filler" cows to maintain group size. Group size was kept constant at 20 animals but group composition was dynamic, with cows entering the pen within 24 h after calving and leaving approximately 21 d later. The pen was equipped with 12 Insentec (Insentec, Marknesse, the Netherlands) feed bins, 2 Insentec water bins, and 24 freestalls with pasture mats (Pasture Mat, Promat Inc., Woodstock, Ontario, Canada) covered with 5 cm of sand bedding. The pen did not have any form of heat abatement. Animals had *ad libitum* access to water and were milked and fed twice per day at approximately 0700 and 1700 h and 0800 and 1600h, respectively. Feed was formulated according to NRC (2001) guidelines.

3.2.2 Drinking behaviour and social competition measures

Insentec water bins (Roughage Intake Control system; Insentec B.V., Marknesse, the Netherlands) were used to continuously monitor drinking behaviour and social competition. Each cow was fitted with an electronic ear tag (High Performance ISO Half Duplex Electronic ID Tag by Allflex Inc. St. Hyacinthe, Quebec, Canada), which the Insentec System used to allow and record individual access to each of the drinkers (see Chapinal et al., 2007, for a full description and validation of the system). Measures included water intake, frequency of visits to the drinker, and time spent at the drinker. To determine if drinking behaviour was affected by feeding behaviour, the Insentec feed bins were also used to continuously monitor feed intake.

Social competition at the drinker was recorded using the concept of competitive replacements. A replacement is defined as when physical impact from one cow (actor) results in the recipient cow (reactor) withdrawing her head from the drinker, with the actor then taking the place of the previous cow at the drinker. Physical impact is normally assessed via video analysis, but in the current study replacements were identified with an algorithm that used data from the electronic drinking system to identify cases where the interval between 2 cows sequentially drinking from the same drinker was ≤ 29 s. This method of quantifying social competition was validated by McDonald et al. (2019) for identifying replacements at the drinker with high sensitivity and specificity; cows in the current study were kept under the same housing conditions as cows in this validation study.

The level of competitive success of each cow was determined from an index of success in competitive interactions at the drinker, as described by Galindo and Broom (2000). This index ranges from 0 to 1 and is calculated by dividing the total number of replacements in which the cow was an actor by the total number of replacements in which she was an actor or reactor. Cows with an index < 0.4 were classified as having low success, those with an index ≥ 0.4 and ≤ 0.6 were classified as having medium success, and those with an index > 0.6 as having high success.

3.2.3 Temperature and humidity measures

Ambient temperature and RH were recorded continuously at 1 h intervals by the Environment Canada weather station situated approximately 500 m from the barn. Hourly THI was calculated using the following formula: THI = (1.8 × temperature + 32) - [(0.55 - 0.0055 × RH) x (1.8 × temperature - 26)], with temperature measured in ^{o}C and RH measured in % (NRC, 1971).

3.3 Statistical Analyses

All statistical analyses were conducted in SAS (Version 9.4; SAS Institute Inc., Cary, NC). Values reported are means ± SE unless otherwise noted. Adjusted R^2 values are presented to account for the number of effects in the models.

3.3.1 Group-level drinking behaviour

For the group-level analysis, day was used as the observational unit (*n* = 63). Four days were excluded due to pen disturbances leaving 59 d in the analysis. For the analysis of drinking behaviour (time spent at the drinker, frequency of visits to the drinker, water intake), measures from all cows in the pen (*n* = 20) were averaged to create one observation per day and, for the analysis of social competition at the drinker, the number of replacements was summed. As dairy cows show a time lag in their response to heat stress (West et al., 2003), and following Hill and Wall (2017) who found that weather averaged over 3 d explained feed intake better than current day or 7 d means, we calculated moving averages for daily mean and maximum THI over a 3 d period (weather spanning the day behaviour was measured plus the previous 2 days).

A linear regression was performed to test the predictive value of THI on behaviour at the drinker. Plots of residuals were examined to ensure approximate normal distribution and determine whether transformations of the data were required. The 3 d moving averages of mean and maximum THI were compared to determine which THI parameter had the best fit. The best fitting model was chosen as the one with the lowest Akaike information criterion (AIC), where a change in AIC of ≥ 4 from the model with the minimum information criteria was considered a meaningful difference (Burnham and Anderson, 2001). Quadratic models were fitted for all dependent variables (time spent at the drinker, time spent at the drinker per visit, frequency of visits to the drinker, number of competitive replacements) by adding a squared term of the independent variable (THI), except for water intake, where a linear model was retained. To improve interpretation of the beta coefficients, the independent THI variable was centered prior to creating the squared term (Dalal and Zickar, 2012).

For time spent at the drinker, frequency of visits to the drinker, and number of competitive replacements at the drinker, the maximum THI averaged over 3 d generated lower AIC values than the average mean THI over 3 d. For water intake, the AIC values for mean and maximum THI were similar, but the model with maximum THI had a higher R^2, so all analysis were based on maximum THI.

The vertex of the parabola in the quadratic models (i.e. the maximum THI threshold at which dairy cow drinking behaviour began to change) was determined by taking the first derivative of the quadratic function, resulting in the formula: $x = - \beta_x / 2 \times \beta_{x2}$

3.3.2 Cow-level drinking behaviour

For the cow-level analysis (*n* = 69 cows), 30 cows were excluded due to health disorders (metritis, retained placenta, mastitis, lameness, ketosis, milk fever; see Lomb et al., 2018a, for a full description of disease diagnosis) or missing health data. Two cows were involved in too few competitive interactions at the drinker (*n* < 8; Hohenbrink and Meinecke-Tillmann., 2012) to accurately calculate level of competitive success and were also excluded, leaving 37 cows in the analysis. Seven cows were classified as having high competitive success at the drinker, 25 were classified as having medium success, and 5 were classified as having low success.

Average daily measures of drinking behaviours for each cow (time spent at the drinker, frequency of visits to the drinker, water intake, number of competitive replacements as an actor or reactor) were calculated. The distributions of each drinking behaviour per day were screened for the presence of outliers. Extreme outliers (i.e. more than 3 times outside the interquartile range) were deemed water bin malfunctions and removed from the data set. Differences in drinking behaviour in relation to increasing THI between cows with different levels of competitive success at the drinker were analyzed using a mixed model, with day relative to calving specified as a repeated measure and a first-order autoregressive covariance structure. Fixed effects were level of competitive success at the drinker, daily milk yield, and 3 d maximum THI for the models with time spent at the drinker, time spent at the drinker per visit, and frequency of visits to the drinker as outcome measures.

Along with ambient temperature, feed intake has also been shown to be predictive of water intake (see Cardot et al., 2008); feed intake was explored as a potential explanatory variable for water intake, time spent at the drinker, and frequency of visits to the drinker. Feed intake was not highly correlated with time spent at the drinker (r = 0.07), or frequency of visits to the drinker (r = 0.08), but was correlated with water intake (r = 0.65); we thus considered feed intake in our analysis of water intake in the cow level models.

A multivariable linear mixed model was used to assess the effect of THI on water intake, controlling for level of competitive success at the drinker, feed intake and milk yield. Analysis of variance was performed to assess model fit, where the final model was compared to a model that was the same except that the variables THI and level of competitive success at the drinker were removed one by one, and both together. The final model that included both THI and social rank decreased the residual sum of squares when compared to the models without one or both these variables. The total variation in water intake accounted for by each explanatory variable was 19.2% for THI, 10.4% for milk yield, 39.9% for feed intake, and 12.2% for level of competitive success at the drinker (measured with the partial omega squared, which is the proportion of variance in the dependent variable accounted for by the independent variable, analogous to adjusted R_2, and adjusted for bias). Feed intake was thus included as a fixed effect for the model with water intake as the outcome measure.

Biologically plausible interactions with maximum THI were examined (level of competitive success at the drinker, daily milk yield, feed intake), and retained in the model when $P < 0.05$. Plots of residuals were examined to ensure approximate normal distribution and determine whether transformations of the data were required. Studentized residuals greater than 3 or less than -3 were investigated as potential outliers (Dohoo et al., 2003).

Outliers were deleted if they were the first or last day a cow was in the pen, as it was likely the cow was not in the pen the whole day. Collinearity was not a problem in the models.

To normalize residuals, a natural log transformation was applied to time spent at the drinker and time spent at the drinker per visit, and a square root transformation was applied to frequency of visit to the drinker; water intake did not require transformation.

3.3.3 Exploratory analysis on the distribution of drinking behaviour

The daily time distribution of each drinking behaviour per cow was also examined based on level of competitive success at the drinker. Each 24-h time period was divided into six 4-h segments (0000 - 0400, 0400 - 0800, 0800 - 1200, 1200 - 1600, 1600 - 2000, 2000 - 2400 h). The 4-h bin size was chosen to improve interpretation of the time trends in drinking behaviour, as the differences between the drinking behaviours of cows based on level of competitive success at the drinker were less clear with smaller bins. Analyses were separated into 2 categories based on whether the 3 d maximum THI was above or below 72, to account for how the daily distribution of each drinking behaviour was affected by high THI.

To identify times of peak competition at the drinker, the average number of competitive events that occurred during each 4-h segment was calculated per day. Water intake, time spent at the drinker, and frequency of visits to the drinker, and the proportion of each behaviour performed in each 4 h time segment out of the total daily expression of each behaviour, were calculated per cow.

3.4 Results

Daily mean and maximum ambient temperature, RH, and THI over the entire trial period are summarized in Table 3.1. The THI was ≥ 68 on 45 days and ≥ 72 on 28 days of the 59 days included in our analysis.

Table 3.1. Mean, SD, and range of average and maximum daily values for ambient temperature, relative humidity (RH), and the temperature-humidity index (THI).

	24-h mean			24-h maximum		
Variable	Mean	SD	Range	Mean	SD	Range
Temperature, ^{o}C	18.8	3.1	12.5 - 24.8	24.2	4.8	13.4 - 32.6
RH, %	74.4	8.1	56.8 - 95.3	95.3	3.6	77.0 - 99.0
THI	64.0	4.4	54.7 - 72.4	70.9	5.9	56.3 - 81.3

3.4.1 Group-level drinking behaviour

The average time spent at the drinker was 25.6 ± 1.53 min/d (range: 11.7 - 54.0 min/d), the average time spent at the drinker per visit was 81.0 ± 2.35 s/visit (range: 54.6 - 115.3 s/visit), the average frequency of visits to the drinker was 19.0 ± 0.6 no./d (range: 10 - 31 no./d), the average water intake was 96.9 ± 1.06 kg/d (range: 77.3 - 112.7 kg/d), and the average number of competitive replacements was 54.0 ± 4.5 no./d (range: 12 - 172 no./d).

As maximum THI increased, cows spent more time at the drinker (R^2_{Adj} = 0.77, df = 56, intercept = 21.52 ± 1.01, β_{linear} = 1.92 ± 0.14, P_{linear} < 0.0001, $\beta_{quadratic}$ = 0.16 ± 0.03, $P_{quadratic}$ < 0.0001; Figure 3.1A), visited the drinker more often (R^2_{Adj} = 0.76, df = 56, intercept = 17.42 ± 0.38, β_{linear} = 0.73 ± 0.05, P_{linear} < 0.0001, $\beta_{quadratic}$ = 0.05 ± 0.01 $P_{quadratic}$ < 0.0001; Figure 3.1B), drank more water (R^2 = 0.20, df = 57, intercept = 96.02 ± 1.32, β_{linear} = 0.72 ± 0.19, P_{linear} = 0.0004; Figure 3.1C), and engaged in a greater number of competitive replacements at the drinker (R^2_{Adj} = 0.75, df = 56, intercept = 41.41 ± 3.06, β_{linear} = 5.52 ± 0.44, P_{linear} < 0.0001, $\beta_{quadratic}$ = 0.48 ± 0.08, $P_{quadratic}$ < 0.0001; Figure 3.2). Time spent at the drinker per visit also increased along with maximum THI (R^2_{Adj} = 0.53, df = 56, intercept = 76.50 ± 2.25, β_{linear} = 2.49 ± 0.32, P_{linear} < 0.0001, $\beta_{quadratic}$ = 0.17 ± 0.06, $P_{quadratic}$ = 0.005). For the quadratic models, time spent at the drinker and the number of competitive replacements at the drinker began to increase at a THI of 65; frequency of visits to the drinker began to increase at a THI of 64.

Figure 3.1. The average (A) time spent at the drinker (min/d), (B) frequency of visits to the drinker (no./d), and (C) water intake (kg/d) per cow in relation to the maximum temperature-humidity index (THI) averaged over the day of observation and the two previous days.

Each point shows the average drinking behaviour over all 20 cows in the group for each day of observation.

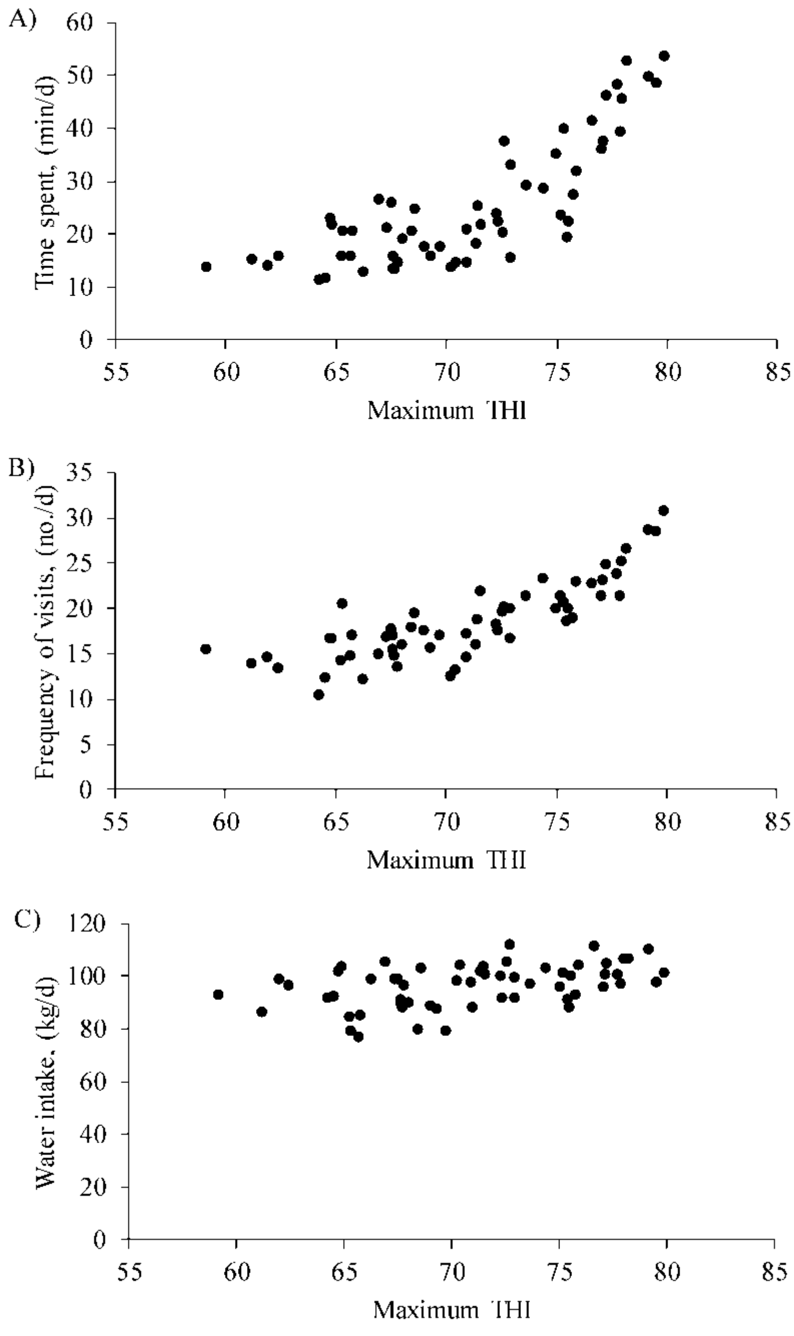
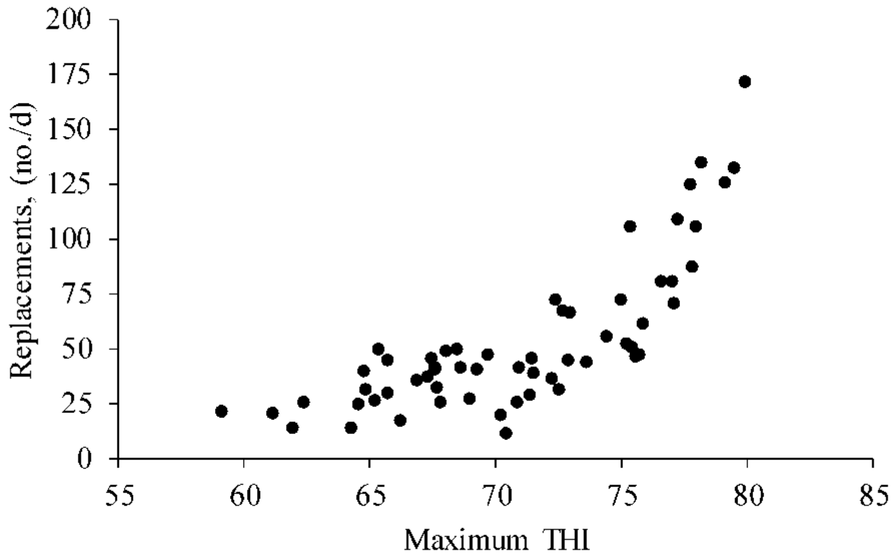


Figure 3.2. The total number of competitive replacements at the drinker (no./d) in relation to the maximum temperature-humidity index (THI) averaged over the day of observation and the two previous days.
Each point shows the sum of replacements over all 20 cows in the group for each day of observation.



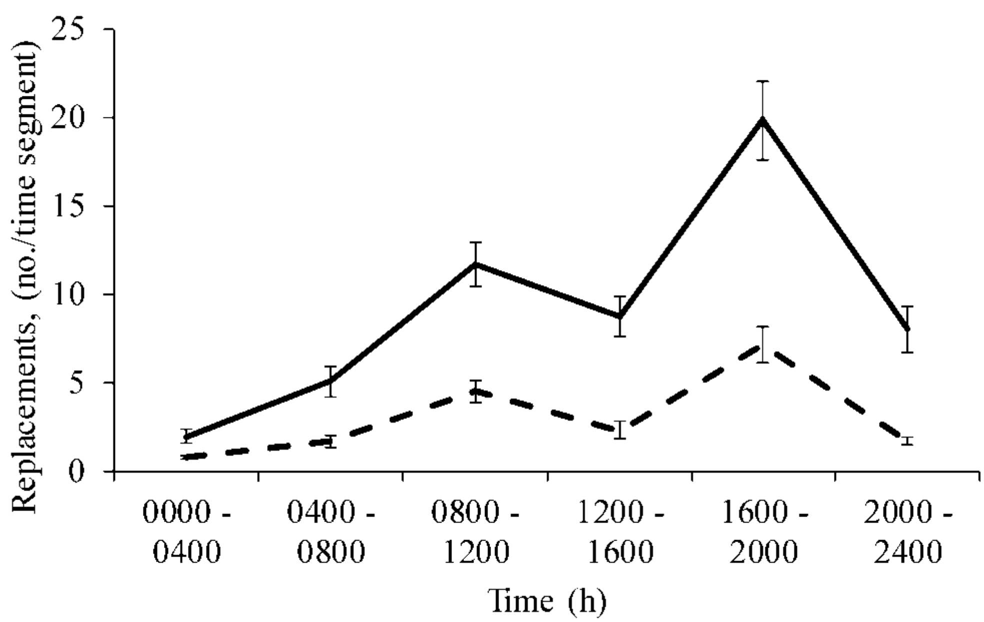
3.4.2 Cow-level drinking behaviour

Cows with higher levels of competitive success at the drinker drank more water ($F_{2,34}$ = 13.60, $P < 0.0001$), cows with greater feed intake drank more water ($F_{1,539}$ = 425.18, $P <$ 0.0001), and cows with greater milk yield drank more water ($F_{1,539}$ = 16.83, P = 0.0001). There was a positive interaction between milk yield and THI for frequency of visits to the drinker, where cows with greater milk yield made more visits to the drinker as THI increased ($F_{1,539}$ = 28.51, $P < 0.0001$), but level of competitive success did not affect frequency of visits to the drinker ($F_{2,34}$ = 2.75, P = 0.08). There was a positive interaction between milk yield and THI for time spent at the drinker, where cows with greater milk yield spent more time at the drinker as THI increased ($F_{1,539}$ = 15.90, $P < 0.0001$), but level of competitive success at the drinker did not affect time spent at the drinker ($F_{2,34}$ = 1.29, P = 0.29). Cows with greater milk yield had longer visits to the drinker ($F_{1,540}$ = 23.68, $P < 0.0001$), but level of competitive success did not affect time spent at the drinker per visit ($F_{2,34} = 0.86$, $P = 0.43$). The interaction between level of competitive success at the drinker and THI was not significant for water intake, frequency of visits to the drinker, time spent at the drinker, or time spent at the drinker per visit.

3.4.3 Exploratory analysis of the distribution of drinking behaviour

Peak times of competition at the drinker were between 0800 - 1200 h and 1600 - 2000 h (Figure 3.3). On days where the THI was < 72, the average number of replacements that occurred at each peak was roughly similar (0800 - 1200 h = 4.5 no./4 h, 1600 - 2000 h = 7.2 no./4 h), but on days where the THI was ≥ 72, the average number of replacements appeared to be higher in the afternoon (0800 - 1200 h = 11.7 ± 1.3 no./4 h, 1600 - 2000 h = 19.8 ± 2.2 no./4 h; no inferential tests were performed for the exploratory analysis).

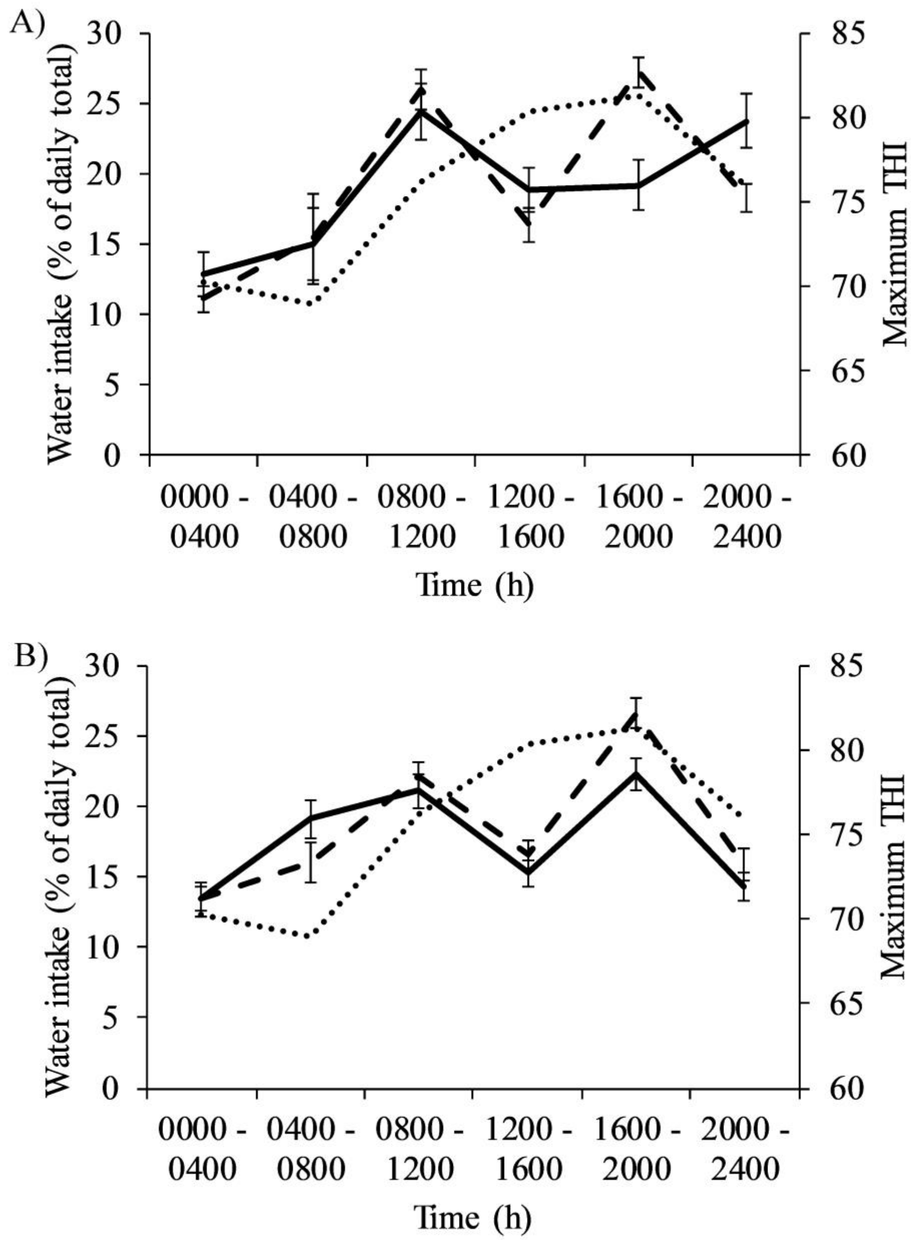
Figure 3.3. The average number of replacements at the drinker that occurred per day during each 4-h segment when the temperature-humidity index was ≥ 72 (solid line) versus < 72 (dashed line).



The distribution of water intake for cows of low and high competitive success at the drinker are depicted in Figure 3.4. On days where the maximum THI was < 72, regardless of level of competitive success at the drinker, all cows had peaks in the proportion of water intake between 0800 - 1200 h (high success: 22.1 ± 1.1 %, medium success: 26.6 ± 1.0 %, low success: 26.0 ± 1.4 % of total daily water intake) and 1600 - 2000 h (high success: 26.6 ± 1.0 %, medium success: 27.5 ± 0.6, low success: 27.2 ± 1.1 % of total daily water intake). When the maximum THI was ≥ 72, both high and medium success cows maintained peaks of water intake between 0800 - 1200 h (high success: 21.1 ± 1.2 %, medium success: 26.0 ± 0.6 % of total daily water intake) and 1600 - 2000 h (high success: 22.3 ± 1.1 %, medium: 22.8 ± 0.6 % of total daily water intake). Low success cows maintained one peak of water intake between 0800 - 1200 h (24.4 ± 2.0 % of total daily water intake) but the second peak shifted to 2000 - 2400 h (23.7 ± 1.9 % of total daily water intake).

On days where the maximum THI was < 72, the distribution of time spent at the drinker and frequency of visits to the drinker was not affected by level of competitive success at the drinker; all cows had peaks in the proportion of time spent at the drinker between 0800 - 1200 h (high success: 20.6 ± 0.9 %, medium success: 22.9 ± 0.7 %, low success: 24.1 ± 1.2 % of total time spent at the drinker) and 1600 - 2000 h (high success: 24.3 ± 1.0 %, medium success: 23.4 ± 0.7%, low success: 23.5 ± 1.3 % of total time spent at the drinker), and peaks in the proportion of frequency of visits to the drinker between 0800 - 1200 h (high success: 19.8 ± 1.3%, medium success: 22.5 ± 0.7%, low success: 26.7 ± 1.4 % of total daily frequency of visits to the drinker) and 1600 - 2000 h (high success: 28.6 ± 1.4 %, medium success: 25.9 ± 0.7%, low success: 26.3 ± 1.6 % of total daily frequency of visits to the drinker).

Figure 3.4. The distribution of water intake (measured as the proportion of total daily intake that occurred during each 4-h segment) when the temperature humidity index (THI) was ≥ 72 (solid line) or < 72 (dashed line) for cows with (A) low competitive success at the drinker and (B) high competitive success at the drinker.
Values represent the average of all cows at each level of competitive success. Maximum THI during each time segment is represented with a dotted line.



When the maximum THI was ≥ 72, high and medium success cows maintained peak time spent at the drinker between 1600 - 2000 h (high success: 24.3 ± 1.4 %, medium: 25.3 ± 0.7 % of total daily time spent at the drinker), while low success cows had an earlier peak between 1200 - 1600 h (26.5 ± 2.4 % of total daily time spent at the drinker). High and medium success cows also maintained peak frequency of visits to the drinker between 0800 - 1200 h (high success: 19.0 ± 1.6 %, medium success: 22.5 ± 0.7 % of total daily frequency of visits to the drinker) and 1600 - 2000 h (high success: 27.2 ± 1.8, medium success: 25.5 ± 0.8 % of total daily frequency of visits to the drinker). Low success cows maintained only one peak in frequency of visits to the drinker at 0800 - 1200 h (22.6 ± 1.7 % of total daily frequency of visits to the drinker).

3.5 Discussion

This is the first study to examine how THI relates to behaviour at the drinker for indoor, loose-housed dairy cattle. As THI increased, dairy cows spent more time at the drinker, visited the drinker more often, increased water intake, and engaged in more competitive behaviour at the drinker. In addition to contributing to the literature that shows that dairy cows increase their drinking behaviour (Ammer et al., 2018) and become more aggressive (Pilatti et al., 2019) when hot, the results of this study suggest that socially subordinate cows may be more vulnerable to increasing THI, due to shifting their drinking behaviour to different times of the day.

Temperature is well known to be associated with water intake in dairy cows (Murphy et al., 1983; Meyer et al., 2004; Cardot et al., 2008). Other studies have found that drinking time (Cook et al., 2007), frequency (Ammer et al., 2018), and intake (McDowell et al., 1969; Beatty et al., 2006; Ammer et al., 2018) increased with increasing THI and ambient temperature. Body water is lost as a result of panting and sweating (Kadzere et al., 2002), and ingestion of water increases heat loss and combats dehydration (Vizzotto et al., 2015). Compared to time spent at the drinker and frequency of visits, the current study found less effect of increasing THI on water intake. Apart from actually consuming water, dairy cows may also be attracted to the cooling effect of water on their skin and in the area around the drinker (Palacio et al., 2015), as passing air over water leads to evaporative cooling (West, 2003). Increased time spent at a resource without greater intake may also suggest that cows are attempting to defend a resource that they are highly motivated to obtain (Val-Laillet et al., 2008b).

The quadratic relationship between THI and time spent at the drinker, frequency of visits to the drinker, and competition at the drinker indicates that changes in behaviour become more pronounced when the maximum THI over a 3 d period exceeds approximately 65. Similarly, Brown-Brandl et al. (2005) noticed a quadratic relationship between ambient temperature and feed intake in cattle, identifying a threshold of 18.5 $_{o}$C (roughly corresponding to a THI of 64 in the current data set). However, these authors noted that there may be insufficient data points to accurately identify this breakpoint, and this may also be an issue in the current study. Our values and that of Brown-Brandl et al. (2005) are lower than the THI of 68 (Zimbelman et al., 2009) or 72 (Armstrong, 1994) that are more commonly associated with heat stress, suggesting that dairy cows may be more vulnerable to heat stress than previously thought. Interestingly, unlike the other drinking behaviours studied, the relationship between THI and water intake remained linear, suggesting that time spent at the drinker, frequency of visits to the drinker, and competition at the drinker may be more useful to signify heat stress.

Despite our hypothesis that level of competitive success at the drinker would affect the rate of change in drinking behaviours during high THI, the interaction of level of competitive success at the drinker with THI was not significantly related to water intake, frequency of visits to the drinker, and time spent at the drinker. This result suggests that the increase in these drinking behaviours due to increasing THI does not depend on the level of competitive success of the cow. However, our exploratory analysis revealed that increasing THI did shift how cows with low competitive success at the drinker distributed their drinking behaviour throughout the day. On days where THI was < 72, cows of all levels of competitive success at the drinker had similar patterns of drinking behaviour. However, when the THI was ≥ 72, the distribution of drinking behaviour for high and medium success cows remained relatively unchanged, but low success cows had a lower proportion of drinking behaviour from 1600 - 2000 h. The 1600 - 2000 h time segment was both when the daily THI reached its maximum and when competition at the drinker was greatest. Thus, cows with low competitive success at the drinker appeared to shift their drinking behaviour to avoid peak times of day. We caution readers that our results are limited by a low sample size of cows at each level of competitive success at the drinker; future work should expand on these findings with more cows.

Increased competition at the drinker may negatively affect the ability of subordinate cows to deal with heat stress. Similar to the results of the current study, Olofsson (1999) observed that during competition at the feedbunk cows of lower social rank adjusted their behaviour to a greater extent than did dominant cows, for example, by altering their feeding times to avoid feeding at busy times of the day. The negative implication of this shift in feeding behaviour is the risk of consuming feed of less nutritional value due to sorting (DeVries et al., 2004). If subordinate cows need to redistribute their drinking behaviour to avoid peak times of day, this could reduce their ability to cope during high temperatures;

water ingestion is an effective way for cows to reduce their body temperature (Coimbra et al., 2012).

The implications of competition at the drinker, and a shift in drinking behaviour away from the hottest part of the day, have yet to be determined. It can be postulated that the inability to drink at a preferred time may lead to feelings of frustration; as Polsky and von Keyserlingk (2017) suggested, frustration could be caused by the inability to perform thermoregulatory behaviour during heat stress. Competition is also a source of physiological stress and can lead to increased activity and heart rate (Hetti Arachchige et al., 2014), perhaps contributing to heat load for cows that compete for access to the drinker. Additionally, the timing of water intake is related to the timing of feed intake. Ominski et al. (2002) found that heat-stressed cows fed in the morning consumed a greater proportion of their daily water intake during the day, and evening fed cows consumed a greater proportion during the night. A shift in drinking behaviour could be related to the negative consequence of a shift in feeding behaviour discussed above.

Inclusion of feed intake in our analysis of drinking behaviour at the cow level allowed us to rule out the possibility that increased drinking behaviour was solely in response to changes in feeding behaviour. Time spent at the drinker and frequency of visits to the drinker were not related to feed intake, but increased in response to THI. While water intake was associated with feed intake, we found that water intake increased along with increasing THI, even after controlling for feed intake.

High THI is used to infer when dairy cows may be experiencing heat stress; however, using environmental factors alone assumes that all cows are affected equally. There is likely individual variation in the THI threshold when dairy cows become heat stressed (Sánchez et al., 2009). Individual characteristics, such as level of milk production (Ravagnolo and Misztal, 2000), breed (Pereira et al., 2014), coat length (Dikmen et al., 2008), and size (Busby and Loy, 1996) can affect a cow's heat tolerance. In the current study, individual milk yield affected drinking behaviour, where greater milk yield was associated with increased water intake, and time spent at the drinker and frequency of visits to the drinker increased especially in response to increasing THI, suggesting that high producing dairy cows are more susceptible to elevated ambient temperatures (see Kadzere et al., 2002). Using individual measures, such as behaviour at the drinker, could allow for individualized management.

Several management strategies are available to mitigate heat stress (Collier et al., 2006), but cows often have little individual control over access to cooling options. Heat abatement strategies that allow individual choice may be beneficial, and the results of the current study suggest that simply providing cows better access to water provides one such opportunity for cow agency.

There are limitations to the current study. THI data were obtained from the local weather station and not from inside the barn. This may have underestimated THI which is typically higher inside a barn (Shock et al., 2016). Different microclimates can also exist within a barn (Collier et al., 2006). Schüller and Heuwieser (2016) found that the average THI at cow level loggers was 2.3 points higher than at other loggers placed elsewhere in the barn. Additionally, the Insentec water bins are different than typical water troughs on commercial dairy farms, and trough design affects drinking behaviour (Pinheiro Machado Filho et al., 2004). Lastly, data were collected as part of a larger study (Neave et al. 2017, 2018; Lomb et al. 2018a, 2018b); the dynamic group composition required for that study may have increased competition (see von Keyserlingk et al., 2008). In the current study group composition was changed slowly (approximately 2 to 4 cows were moved every few days); it is unknown how much of an effect this has on competitive behaviour. Regrouping frequently happens in commercial herds, thus making the results of the current study applicable to on-farm situations. The current study was observational; future work should consider experimentally varying heat load to allow stronger causal inferences about the effects of heat stress on drinking behaviour. In addition, measures of individual body temperature would allow for a more sensitive analysis, taking into account that different cows may be more affected by heat stress.

3.6 Conclusion

With increasing THI cows spent more time at the drinker, had a greater frequency of drinker visits, drank more water, and engaged in more competition at the drinker. Cows with low competitive success at the drinker shifted their drinking behaviour on hot days to avoid the drinker during times of high competition. These behaviours may be useful biomarkers of heat stress, especially for farms where attendance at the drinker can be monitored electronically.

Chapter 4: General Discussion

In this chapter, I will discuss my research findings and how they contribute to a deeper understanding of how dairy cows change their drinking behaviour in response to elevated ambient temperatures, and how the novel use of an electronic drinking system can be used to indicate that dairy cows are too hot. I will also describe the strengths and limitations of my research, and provide suggestions for further research in this area.

4.1 Summary

Chapter 1 provided an overview of the literature on the physiological and behavioural effects of heat stress on dairy cows, and the potential for integration of advanced technologies for the automatic detection of heat stress. Changes in dairy cow behaviour are important means of thermoregulation (Collier and Gebremedhin, 2015) and observing these changes as an indicator of heat stress is valuable given that behaviour will change before drops in productivity occur (Schütz et al., 2010; Polsky and von Keyserlingk, 2017). Little work to date has examined how social behaviour is affected by heat stress. Automated technologies exist that can monitor changes in rumination time (Abeni and Galli, 2017) and panting score (Bar et al., 2019) in response to increasing THI, but this had yet to be extended to automatic detection of competition for cooling resources, such as water.

The results of my thesis show that increasing THI is associated with increased water intake, more visits to the drinker, more time spent at the drinker, and a greater number of competitive events at the drinker. At the highest THI observed in this study (79.9), the total number of competitive events at the drinker reached a high of 172 no./d, as measured by the electronic drinking system. On hot days, subordinate cows shifted their drinking behaviour to avoid the drinker at times of peak heat load and competition. These results suggest that competition at the drinker is a) an indicator that dairy cows are too hot, and b) that subordinate cows may be less able to cope with heat stress, due to limited access to water.

These results agree with previous work showing that dairy cows increase their drinking behaviour when heat stressed (Cook et al., 2007; Ammer et al., 2018), compete for cooling resources (Schütz et al., 2010; Vizzotto et al., 2015; Stivanin et al., 2019), and that subordinate animals are at a disadvantage when competition is high (Olofsson, 1999; DeVries et al., 2004). This study provides the first evidence that indoor-housed dairy cows compete for access to water when it is hot, and that this competitive behaviour can be monitored automatically with an electronic drinking system.

4.2 Strengths and Limitations

A strength of my work is the unique use of ROC curve analysis in Chapter 2 to validate the methodology used in Chapter 3 for the automatic detection of social competition between dairy cows at the drinker. This allowed me to collect data on dairy cow behaviour over an entire summer (59 d) for 24 h/d over a wide range of environmental conditions. Otherwise, identifying these events through visual analysis would have taken an inordinate amount of time. Previous work using video analysis to record the social behaviour of dairy cows in response to elevated ambient temperatures has been limited to much shorter time frames (e.g. 5.8 h observations on 20 d, Schütz et al., 2010; 24 h observations on 8 d, Coimbra et al., 2012; 15.5 h observations on 5 d, Vizzotto et al., 2015; 7 h observations on 5 d, Stivanin et al., 2019). Observing dairy cow behaviour over longer periods is important given the time lag in their responses to changes in ambient temperature (West et al., 2003).

ROC curves were first used by the British Air Force in World War II for radar signal detection to accurately distinguish signals on radar scans between enemy aircrafts versus noisy interference (Carter et al., 2016). ROC curves were subsequentially adopted by the medical community for decision making in diagnostic tests in humans (e.g. see Lusted, 1971; Gavin et al., 1989; Bortheiry et al., 1994; Heffler et al., 2006). ROC curves have also been applied to decision making in disease diagnosis for dairy cows. For example, optimal thresholds have been identified for the concentration of progesterone in milk to discriminate between pregnant and non-pregnant cows (Faustini et al., 2007) and for serum concentrations of non-esterified fatty acids and β-hydroxybutyrate to detect diseases associated with calving (Ospina et al., 2010).

Much of the research using ROC curve analysis has focused on disease detection; less literature is available on applying ROC curve analysis to studying behaviour. A 10% decline in rumination time was identified as the optimal threshold to predict day of calving (Clark et al., 2015). The optimal threshold to identify competitive events at the feeder was identified as when there was a ≤ 26s interval between 2 cows sequentially visiting 1 feed bin (Huzzey et al., 2014). The optimal thresholds for feeding time and number of competitive events instigated at the feedbunk to predict metritis was defined as 302 min/d and 16.5 no./d, respectively, where metritic cows would be below these values (Patbandha et al., 2012). Statistical techniques, such as ROC curve analysis, have the potential to identify optimal thresholds of changes in dairy cow behaviour to identify or predict heat stress.

Another strength of my thesis is that it is the first to highlight that subordinate cows may be more negatively affected by increasing THI than dominate or moderate cows, due to increased competition at the drinker. Previous work on competition between dairy cows has mainly focused on feed (Olofsson, 1999; DeVries et al., 2004; Huzzey et al., 2006; Proudfoot et al., 2009; Collings et al., 2011) and lying stalls (Fregonesi et al., 2007; Winckler et al., 2015). During competition at the feedbunk, subordinate cows have less feeding activity at peak times of day (DeVries et al., 2004), shorter feeding times (Olofsson, 1999), and eat at a faster rate (Proudfoot et al., 2009). During competition for lying stalls, subordinate cows spent more time lying down during the day, which is less preferable than lying at night, and spent more time standing in the crossovers (Winckler et al., 2015). It is important to consider how subordinate cows may be disproportionally affected during competition at the drinker, due to the potential implication of limited access to water decreasing the ability of subordinate cows to cope during elevated ambient temperatures.

There are two main limitations of the research in my thesis. Firstly, the study in Chapter 3 was observational, which prevents any causal relationships from being determined. Observational studies have impaired internal validity (e.g. the ability to measure what is intended) because they are prone to bias (over or undervaluing a variable; Hoppe et al., 2009) and the association identified between exposure and outcome could be affect by an unaccounted covariate (Grimes and Schulz, 2002). For high quality evidence, an experimental design should include control, randomization, and replication, all of which observational studies do not provide (Hoppe et al., 2009). However, observational studies can have good external validity because the observational protocol matches what would occur in real-life scenarios, and provide valuable information for further study (Hoppe et al., 2009).

Secondly, without measures of individual body temperature, we cannot definitively say dairy cows were in fact experiencing heat stress, as the definition of heat stress is commonly based on physiological measures. Heat stress occurs when rectal temperature rises above normal body temperature (De Rensis et al., 2015), but environmental conditions are only imperfectly related to body temperature (Dikmen and Hansen, 2009). Heat tolerance is affected by individual cow characteristics, such as parity (Maust et al., 1972; Bernabucci et al., 2014), level of milk production (Ravagnolo and Misztal, 2000; Ravagnolo et al., 2000; Sánchez et al., 2009), and likely other cow-level factors. Thus, it is likely that not all cows reacted equally to the THI conditions observed. However, it is safe to say that the dairy cows in this study were experiencing hot weather conditions.

4.3 Future Directions

My thesis demonstrates that increased drinking behaviour in dairy cows, particularly competition at the drinker, is associated with elevated THI. Future work should include a randomized controlled trial to provide stronger causal evidence of the relationship between heat stress and these behaviours. Additionally, in the following section, I will expand upon suggestions for future research to a) validate other technologies to automatically identify dairy cows are experiencing elevated heat load, b) determine whether changes in drinking behaviour can be used to identify other ailments in dairy cows, and c) move away from an all-or-nothing view of heat stress and place more emphasis on ensuring the thermal comfort of dairy cows, before any threshold of heat stress is reached.

Electronic drinkers were validated in Chapter 2 to automatically record social competition at the drinker. Social competition at the drinker was associated with elevated THI in Chapter 3. Thus, the number of competitive events a dairy cow engages in at an electronic drinker could be used to automatically predict if she is experiencing heat stress. Similarly, rumination time (Schirmann et al., 2009; Moretti et al., 2017) and panting score (Bar et al., 2019) recorded automatically have the potential to predict heat stress. Future work should explore validating other behavioural indicators that can be measured electronically to predict heat stress. Additionally, incorporating summed indicator variables would create a more accurate model to predict heat stress (Bar et al., 2019). Automatically recording individual cow behaviour will allow for movement away from environmental measures of elevated ambient temperatures to individual level measures of changes in behaviour for predictive models of heat stress.

The work described in my thesis shows that drinking behaviour changes in relation to heat stress. Future work should explore if drinking behaviour is indicative of other ailments in dairy cows. Changes in feeding (Urton et al., 2005; Huzzey et al., 2007; González et al., 2008; Sepúlveda-Varas et al., 2016) and lying behaviour (Hassall et al., 1993; Juarez et al., 2003; Walker et al., 2008; Ito et al., 2010) have been studied and found to be indicative of disease in dairy cows. The majority of work focused on changes in drinking behaviour has focused on the time around calving (Huzzey et al., 2007; Goldhawk et al., 2009; Jawor et al., 2012). Monitoring changes in drinking behaviour may be useful to identify sick cows, especially when data can be measured electronically (Lukas et al., 2008; Kramer et al., 2009).

Lastly, throughout my thesis work, it became apparent to me that the literature examining the effects of ambient temperatures on dairy cows typically considers heat stress as an "all or nothing" occurrence. Previous work categorized THI into different levels (e.g. THI < 70 no heat stress, $70 \leq$ THI < 75 light effect, $75 \leq$ THI < 78 moderate effect, THI ≥ 78 extreme effect; Bellagi et al., 2017), which implies that below a particular heat stress threshold, but within the thermoneutral zone (the range of ambient temperatures where normal body temperature can be maintained without changes in metabolic heat production or evaporative heat loss), dairy cows are not experiencing any negative effects. However, within the thermoneutral zone lies the zone of thermal comfort (Silanikove, 2000). The thermoneutral zone and thermal comfort zone are terms that are often used interchangeably (e.g. see Ravagnolo et al., 2000; Palacio et al., 2015; Polsky and von Keyserlingk, 2017), but I suggest these terms do not actually refer to the same environmental conditions for dairy cows.

In the human literature, thermal comfort is defined as “a condition of mind which expresses satisfaction with the thermal environment” (ASHRAE, 1997) and this involves a minimum rate of nervous signals firing from thermal receptors in the skin to the hypothalamus (Höppe, 2002). For a lightly clothed person at an office work metabolic rate, a suggested thermal comfort zone is an ambient temperature between 19.5 - 21.9 $_{o}$C, while the thermoneutral zone is a wider range between 14.8 - 24.5 $_{o}$C (Kingma et al., 2014). This is much below the ambient temperature threshold (a heat index ≥ 32 $_{o}$C) above which heat stress will occur (Blazejczyk et al., 2012). It is deemed important to ensure thermal comfort for humans, and surveys are often done to assess how participants rate their satisfaction with their thermal environment (e.g. see Wyon et al., 1968; Han et al., 2007; Hwang and Lin, 2007; Zhang et al., 2007). It is clear that, in addition to preventing heat stress, a lot of emphasis is placed on ensuring thermal comfort for humans long before heat stress would occur.

The difficultly with applying the concept of thermal comfort from humans to dairy cows is that thermal comfort is typically assessed using verbal reports, and zones of comfort may vary among individuals (Wyon et al., 1968). Observations of animal behaviour can shed light on their environmental preferences (Kirkden and Pajor, 2006). In Chapter 3, I identified that dairy cow drinking behaviour began to change at a THI of approximately 65. This may not be the threshold where detrimental physiological effects occur, but perhaps this is the point where dairy cows begin to feel heat related discomfort and begin seeking out cooling resources.

In an effort to avoid the difficulty of assessing the subjective environmental preference of dairy cows, Silanikove (2000) decided that the zone of thermal comfort would best be restated as the zone of optimal thermal well-being (defined solely by optimal physiological functioning). However, how an animal feels is an important component of animal welfare (Fraser et al., 1997) that has received little attention in the heat stress literature, except for a review by Polsky and von Keyserlingk (2017) where the authors described that heat stressed negatively affects animal welfare in part by causing negative feelings (e.g. thirst, frustration, discomfort).

The most suitable way to relate ambient temperature to positive animal welfare is to ensure an animal is within the zone of thermal comfort (Silanikove, 2000), as the zone of thermal comfort involves both positive feelings about the thermal environment, based on the definition of thermal comfort for humans, and normal physiological functioning. However, the ambient temperature range comprising the zone of thermal comfort for dairy cows has yet to be defined. I encourage future work to explore how dairy cows feel in their thermal environment to gain a better understanding of the thermal comfort zone for dairy cows, before any threshold of heat stress is reached.

4.4 Conclusions

The research in my thesis contributes to the limited body of literature examining the effect of elevated THI on the behaviour of dairy cows. Specifically, my work shows that dairy cows increase their drinking behaviour, which increases competition at the drinker, in response to increasing THI. The effect of heat stress on dairy cows is often approached from a physiological perspective; however, research on behavioural indicators that dairy cows are feeling uncomfortably warm has the potential to improve cow welfare, even in the absence of negative physiological effects of heat stress. My thesis also illustrates that behavioural changes at the drinker can be monitored electronically, which can aid in the development of automatic technologies that create early alert systems for farmers.

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