Environmental and Transition Stress in Multiparous Holstein Cows

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Abstract

Cows are exposed to a range of environmental and physiological stressors that can affect their welfare, behaviour, milk production, and reproduction. Calving is a particularly critical phase in the dairy production cycle, characterised by significant physiological demands. During this period, reduced feed intake coincides with increased energy requirements for foetal growth and the onset of lactation, predisposing cows to metabolic disorders and diseases. This can negatively impact welfare, productivity, and longevity. Stress is commonly assessed using behavioural observations or biomarkers such as cortisol, though these approaches may be subjective or invasive. Heart rate variability (HRV) provides a non-invasive alternative for evaluating autonomic nervous system activity and physiological stress.

This thesis aimed to assess physiological stress in cows -10 to +7 days around calving using HRV and to investigate associations with transition diseases, cow-level factors, and calf characteristics. Clear temporal changes in HRV were observed. Heart rate increased as calving approached, peaking shortly before parturition, while vagally mediated HRV parameters showed a U-shaped pattern with a nadir around calving. These findings indicate elevated stress during the periparturient period followed by recovery. Carrying twin calves or dairy-sired calves was associated with HRV patterns suggesting greater stress. In contrast, post-partum disease, body condition score, rumen fill, and lactation number showed no significant association with HRV within the study period.

A second objective was to evaluate environmental conditions within a commercial dairy housing system using both commercial and custom-built monitoring devices. Results from the commercial sensors revealed considerable spatial and temporal variation in microclimatic conditions within the sheds. Sheds were typically warmer and less humid than outside, yet the Temperature Humidity Index (THI) frequently exceeded the threshold of 62, particularly in high-density areas such as the collecting yard. Custom-built sensors performed comparably to commercial devices for temperature and humidity but were less reliable overall, particularly for light intensity.

In conclusion, HRV is a practical and sensitive non-invasive tool for assessing physiological stress in dairy cows around calving. Stress levels peak during the immediate peri-calving period, with calf characteristics influencing maternal stress. Environmental monitoring demonstrated variability within housing, with higher-risk areas identified. These findings highlight the potential for targeted interventions, such as cooling systems in high-density areas, and support the integration of HRV and environmental monitoring into precision livestock farming to improve cow welfare, management, and productivity.

Table of Contents

Contents

List of Tables

List of Figures

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Definitions/abbreviations

AIC — Akaike's Information Criterion

ACTH — Adrenocorticotropic hormone

ANOVA — Analysis of variance

ANS — Autonomic nervous system

B — Breed of calf's sire

BCS — Body condition score

BHB — Beta-hydroxybutyrate

BIC — Bayesian Information Criterion

BRS — Baroreflex sensitivity

CO_2 — Carbon dioxide

D — Number of days in relation to calving, mean-centred

D2 — Quadratic component of the number of days in relation to calving, mean-centred

%DET — Percentage of determinism

DHARMa — Residual diagnostics for hierarchical (multi-level/mixed) regression models

DIM — Days in milk

FF — Female/ female twins

FM — Female/ male twins

HF — High frequency power (component of heart rate variability)

HPA — Hypothalamic–pituitary–adrenal axis

HR — Heart rate

HRV — Heart rate variability

IBI — Interbeat interval

ID — Identification

L — Lactation number

L_{max} — Maximum line length in recurrence plot analysis

LF — Low frequency (component of heart rate variability)

LF/HF ratio — Ratio of low-frequency to high-frequency HRV power

MM — Male/ male twins

NASA POWER — National aeronautics and space administration prediction of worldwide energy resources

NEFA — Non-esterified fatty acids

NS — Calf number and sex

PM1 — Particulate matter with aerodynamic diameter ≤1 µm

PM2.5 — Particulate matter with aerodynamic diameter ≤2.5 µm

PM10 — Particulate matter with aerodynamic diameter ≤10 µm

PPD — Post-partum disease

%REC — Percent of recurrence rate

RF — Rumen fill

RMSSD — Root mean square of successive differences between normal heartbeats

RR interval — Time interval between two consecutive R-waves of the electrocardiogram

S (Poincaré plot) — Area of the ellipse in a Poincaré plot of HRV

SD1 — Standard deviation 1 (short-term HRV component in Poincaré plot)

SD2 — Standard deviation 2 (long-term HRV component in Poincaré plot)

SD1/SD2 — Ratio between SD1 and SD2

SDNN — Standard deviation of normal-to-normal interbeat intervals

SDRR — Standard deviation of RR intervals

SEM — Standard error of the mean

THI — Temperature humidity index

UV — Ultraviolet

VLF — Very low frequency power (component of HRV)

Y— Dependent variable

Author's Declaration

"I declare that this thesis is the result of my own work, except where explicit reference is made to other people's contributions. It has not been submitted for any other degree at the University of Glasgow or any other institution."

Jaka Jakob Hodnik

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Chapter 1: Introduction

1.1 Abstract

During their production cycle cows are exposed to many stressors mainly divided into internal and external stressors. External stressors can be roughly divided into management and environmental stressors. Internal stressors are mostly linked to pathological (e.g. disease, pain) or physiological conditions (e.g. metabolic demand, calving). Measuring stress in cows can be performed in different ways from behaviour observations, measuring stress hormones and assessing the activity of the autonomous nervous system using heart rate variability; each method has its own advantages and disadvantages.

The amount of stress cows experience depends on how well they can cope with these stressors. The transition from late gestation into early lactation—collectively defined as the transition period—represents the most critical and challenging phase in the dairy production cycle. While the physiology of this period involves extensive physiological remodelling to support calving and the onset of lactation, it is fundamentally a period of increased stress. When describing these physiological processes, it is important to note that the drop in dry matter intake and the subsequent mobilization of non-esterified fatty acids from body tissue are not merely metabolic shifts; they are intrinsic biological stressors. When tissue mobilization occurs in excess, the liver's capacity for complete oxidation is overwhelmed, generating reactive oxygen species and triggering a pro-inflammatory response. This systemic inflammation acts as a metabolic stressor that further suppresses appetite and drains vital energy. Crucially, this metabolic burden does not happen in a vacuum—it directly overlaps with the acute physical and psychological stress of parturition itself. Therefore, transition diseases such as ketosis, hypocalcaemia, and metritis should not be viewed purely as isolated pathology, but rather as products of a cumulative stress load where environmental, managerial and internal stressors intersect.

The primary aim of this literature review is to evaluate the various environmental, managerial, and animal-level stressors that impact dairy cows during this crucial window. By contextualizing the physiology of calving, lipid mobilization, and periparturient disease risk within the overarching theme of stress, this review aims to clarify how external and internal pressures converge to threaten cow welfare and productivity.

1.2 Stress

1.2.1 Definition of stress

In the early 20th century, stress was defined as a threat to homeostasis and was associated with an acute response of the adrenal gland associated with the "fight or flight response" (Goldstein and Kopin, 2007). In 1936, the definition was broadened to "the nonspecific response of the body to any demand upon it" (Selye, 1976). There are three recognised stages of coping with stress also named the "General Adaptation Syndrome": the alarm reaction (fight or flight response), adaptation, and exhaustion (Selye, 1976). The response to stress was demonstrated to be associated with the hypothalamic-pituitary-adrenocortical axis and release of cortisol from the adrenal cortex in the 1950s (Chrousos et al., 1988). In addition to its physiological role in resistance to stressors, cortisol can also be associated with some pathological changes, such as immune suppression, metabolic dysfunction, reproductive failure, and cellular damage (Chrousos et al., 1988). Each stressor was believed to have a specific response until a specific threshold was reached; then, the response became a nonspecific stress syndrome (Chrousos et al., 1988).

In the 1990s stress was defined as a sensed threat, be it conscious or unconscious to homeostasis, or rather allostasis to account for natural alterations in parameters within acceptable ranges (Sterling and Eyer, 1988, Goldstein and Kopin, 2007). A subset of stress called distress is defined as a cognitive recognition of aversive conditions with a high motivation of the organism to avoid them (Breier et al., 1992). It is dependent on character and intensity of the stressor as recognised by the organism and the organism's perceived ability to deal with it (Goldstein and Kopin, 2007). If stress or distress are continuous or occur repeatedly, they can cause pathologies such as altered behaviour, immunosuppression increasing disease susceptibility or altering the animal's metabolism which can be linked to decreased production and growth, or a combination of all of the above (Chen et al., 2015).

1.2.2 The stress response

Today the stress response is divided in two major pathways. The first is the hypothalamic-pituitary-adrenal axis. In the face of a stressor the paraventricular nucleus of the hypothalamus releases corticotropin-releasing hormone and vasopressin which stimulate the anterior lobe of the pituitary gland to secrete adrenocorticotropic hormone which in turn stimulates the release of glucocorticoids (a class of steroid hormones naturally produced by the adrenal glands, most notably cortisol) from the adrenal cortex.
Glucocorticoids exert negative feedback on the hypothalamus and pituitary to control the response to stress (Smith and Vale, 2006). The second branch of the response is more rapid

and works through the release of catecholamines (a class of hormones and neurotransmitters produced primarily by the nervous system and adrenal glands) from the adrenal medulla. It is called the sympathetic-adrenal medullary axis.

Glucocorticoids have a major role in energy metabolism. In the face of heat stress carbohydrate metabolism is downregulated causing reduced metabolism in peripheral blood leukocytes (Kolli et al., 2014). It impairs glycogen synthesis and insulin-dependent glucose uptake (Mehla et al., 2014). Glucocorticoids also contribute to the balance of anabolism and catabolism of both amino (Revollo and Cidlowski, 2009) and fatty acids (De Guia et al., 2014), they can have both pro and anti-inflammatory action depending on the type of stress (Cruz-Topete and Cidlowski, 2015). Usually, they act in an anti-inflammatory manner by repressing pro-inflammatory genes, causing leukocyte apoptosis and reducing cytokine and interleukin production, but they can also have pro-inflammatory effects in acute stress (Cruz-Topete and Cidlowski, 2015).

Catecholamines (norepinephrine and epinephrine) work on alpha- and beta-adrenergic receptors and affect many organ systems (cardiovascular, respiratory and gastrointestinal tract) usually during acute stress. They can upregulate glucose metabolism by increasing mitochondrial biogenesis and insulin dependent glucose uptake in the cells (Barth et al., 2007). They can also alter immune function through altered neutrophil tracking, regulating cytokine secretion and altering microbial growth (Figure 1-1) (Chen et al., 2015).

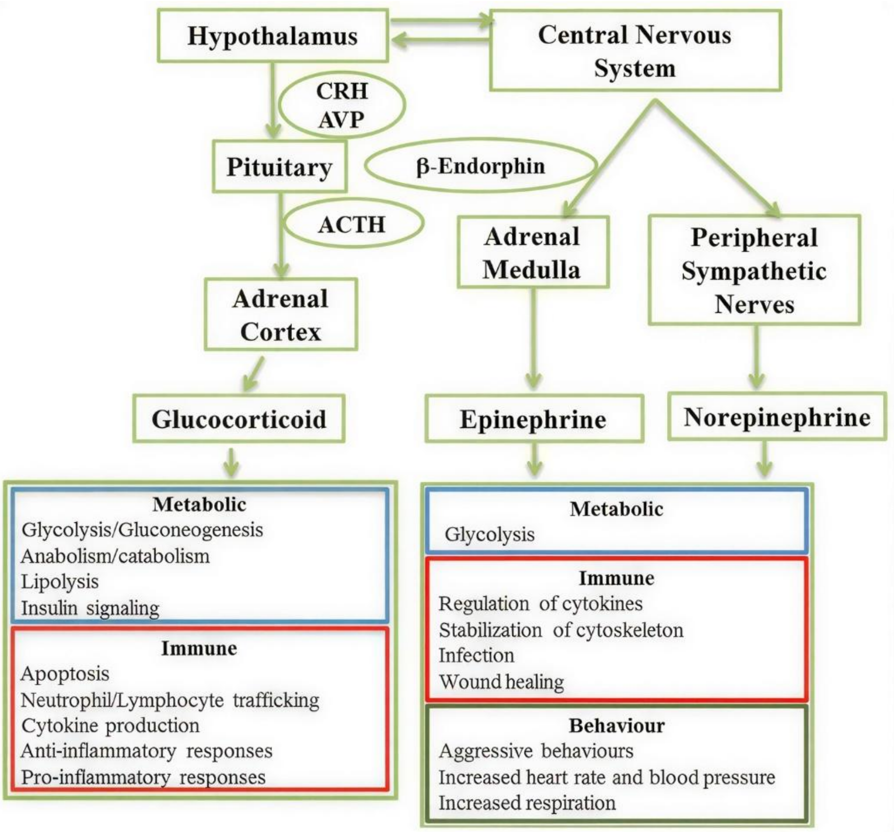


Figure 1-1 Stress axis regulation and influence on cattle metabolism, immunity and behaviour. Reproduced from Chen et al. (2015), published under a CC BY 4.0 licence. The figure uses US terminology ('epinephrine'/ 'norepinephrine') rather than the UK terms ('adrenaline'/ 'noradrenaline')

1.2.3 Measuring stress in cattle

Quantifying the impact of stress on the animal's welfare, immunity, metabolism and production can be difficult as stress does not necessarily manifest on a clinical level; therefore, different methods of assessing stress are needed to make informed decisions to manage or prevent stress (Chen et al., 2015).

1.2.3.1 Behaviour

A non-invasive way of assessing stress is through observing behaviour. Various methods like chute entry order, exit velocity, vocalisation and lying, walking and ruminating behaviour have been studied (Bristow & Holmes, 2007; Grandin, 1997; Haley et al., 2005). Distress associated with pain can be assessed through animal behaviour observations in three different ways: pain-specific behaviour, change in behaviours that the animals are highly motivated to perform (e.g. lying, feeding, play) and assessing preference of choice (Weary et al., 2006). On-farm pain assessment focuses on the observations of a cow's attention, head and ear position, facial expression, response to approach and back position (Gleerup et al., 2015). Other behavioural signs of pain can include changes in gait, postural changes, looking at the painful region, rolling, kicking, grinding teeth and vocalisation (Constable et al., 2017). The simplicity of this method of stress assessment is hindered by its subjectivity (Weary et al., 2009), non-specificity and time and resource intensiveness (Mattachini et al., 2013). To limit subjectivity and resource expenditure changes in behaviour such as standing, lying, walking, feeding and ruminating activity can be measured continuously using accelerometers (Porto et al., 2024). Preference of choice is usually limited to research studies and is impractical to use in a commercial setting (Gleerup et al., 2015). Animals will avoid areas of bad experiences (Grandin and Shivley, 2015). Many stress-related behaviours occur in both short term and long-term stress states so behavioural observation alone may not reliably differentiate acute from chronic stress without additional physiological evidence.

1.2.3.2 Cortisol

Cortisol is a crucial mediator of the stress response. Measurements in various tissues and secretions (blood, saliva, urine, milk, faeces and hair) are used to assess stress levels in cattle (Idris et al., 2021). Blood sampling is the most invasive way of assessing cortisol levels. It is a very acute measure as blood cortisol levels increase rapidly; therefore sampling must take place in minutes after the stressful event. The acuteness of changes can also mean that the act of restraining and sampling and circadian rhythm can influence cortisol levels (Mormède et al., 2007). Measurements from secretions urine, saliva, faeces, and milk are considered less invasive, however, the concentrations of cortisol in saliva, urine and cortisol metabolites measured in faeces have a lag compared to blood cortisol during the time needed to produce and release the secretion (Morrow et al., 2000, Morrow et al., 2002, Hernandez et al., 2014).

Hair cortisol measurement is a non-invasive method of measuring chronic stress (Ghassemi Nejad et al., 2017). It has the advantage of not being influenced by circadian rhythm or disturbance experienced during sampling (Comin et al., 2013) and that long hair can be used to assess particular time periods, dependent on hair growth rate (Heimbürge et al., 2019). However, hair cortisol can be affected by many other factors such as age, hair colour, hair sampling site, hair growth rate and UV exposure (Baier et al. 2019; Heimbürge et al., 2019). Heifer calves have higher concentrations of cortisol compared to adult cows (González-de-la-Vara et al., 2011). Black hair has approximately twice the cortisol concentrations of white hair from the same animal (Otten et al., 2023). Tail hair also has higher concentrations and grows faster compared to shoulder hair (Burnett et al., 2014). Otten et al. (2021) found that UV exposure causes photodegradation of cortisol in white cattle hair and that black hair had higher cortisol concentrations which could be the result of UV protection of eumelanin in black hair. Therefore, access to outdoor areas and conditions during sample transport are important factors to consider when assessing hair cortisol concentrations (Otten et al., 2021). In addition to other physiological factors, the stress of calving increases hair cortisol (Braun et al., 2017). Clinical disease increases hair cortisol concentration, which does not appear to be true in subclinical disease as was found in a study investigating subclinical endometritis (Burnett et al., 2015). Hair cortisol is increased by pain associated with castration, but the increase can be alleviated with the use of pain mediating drugs (meloxicam) (Creutzinger et al., 2017). A study by Tekin et al., (2023) found a positive correlation between average temperature humidity index (THI) levels in the period 4 weeks before to 4 weeks after calving and hair cortisol levels 4 weeks after calving, the THI was calculated using wet and dry bulb temperatures, and the hair was collected from the tail switch. However, the study does not mention how often and where in the shed they measured the environmental parameters or which equipment they used. The study also found a positive correlation between hair cortisol and body condition score but did not find a correlation between cortisol and disease (Tekin et al., 2023).

1.2.3.3 Heart rate variability

An alternative method for assessing stress is heart rate variability (HRV), which can be used to monitor the autonomic nervous system as an indicator of acute and chronic stress in cattle. According to Singh (2018), the heart receives sympathetic innervation from the caudal cervical and upper thoracic ganglia of the sympathetic trunk. Before reaching the heart, postganglionic fibres form cardiac plexuses in the cranial mediastinum. Parasympathetic innervation comes from the vagal nerve directly or indirectly from the recurrent laryngeal nerve. They connect to nerve cells in the heart wall especially within the sinoatrial and atrioventricular nodes (Singh, 2018). The base rhythm of the heart without external stimuli is controlled by the sinoatrial node which is a highly innervated node of modified cardiac fibres. The sinoatrial node has the highest rate of spontaneous activity without external stimuli. It is located beneath the entrance of the cranial vena cava in the right atrial wall. Excitation from the sinoatrial node spreads through atrial muscle fibres to the atrioventricular node located in the septum of the atria. A short delay in signal transfer allows the completion of atrial contraction. From there the excitation spreads rapidly across the whole ventricular myocardium through the atrioventricular bundle which penetrates the fibrous skeleton of the heart and connects the atria and ventricles. The atrioventricular bundle is made of Purkinje fibres which are modified cardiac muscle fibres able to conduct impulses much more rapidly than regular cardiac muscle fibres. The bundle splits into two branches that flow down the intraventricular septum and branch out to reach all parts of the ventricles (Singh, 2018).

The effects of the autonomic nervous system on HR in farm animals were reviewed by von Borell et al. (2007). In short, the ANS is controlled by the hypothalamus and is split into the sympathetic and parasympathetic branches. The sympathetic component increases and the parasympathetic (vagal) component decreases heart rate. The heart rate of an animal at any given time is the reflection of the balance between the two components (Singh, 2018). Sympathetic activity also increases the conduction of the atrioventricular node and contractile force of atrial and ventricular muscle fibres while parasympathetic decreases only the latter (Singh, 2018).

HRV assessments measure the fluctuations of interbeat intervals (IBI). These can then be analysed using different measures like time-domain, frequency-domain and non-linear measures that assess the HRV detected during monitoring periods (Shaffer & Ginsberg, 2017). Specific HRV measures are listed and explained in Table 1. In general, HRV decreases with stress and increases at times when stress is reduced. HRV is superior for assessing the autonomic nervous system and its association with stress compared to measuring plain heart rate as it detects changes at a finer resolution (Tomes et al., 2025). It was shown that heart rate was positively correlated with rectal temperature in heat stressed cattle (Kovács et al. 2018) and HRV was decreased if cattle were exposed to direct sunlight (Bun et al. 2018; Kovács et al. 2018). Therefore, HRV can potentially be used to assess the negative influence of heat stress on the autonomic nervous system. Like cortisol, HRV can also be influenced by many parameters such as production, disease, fear, pain (Kovács et al. 2014), calving (Nagel et al., 2016), body weight, breed, management (Hagen et al., 2005), demeanour (Kovács et al., 2015), standing time, herd size, physical activity (ruminating, standing, lying, feeding) (Kézér et al., 2017), body condition score (BCS), days in milk (DIM), % butterfat (Frei et al., 2022) and rumen fill (von Borell et al., 2007). Therefore, as with cortisol many cow-level parameters have to be recorded when HRV is measured. Both cortisol and HRV are limited by valence issues because they both measure arousal which may reflect stress or excitement.

Table 1-1 Important heart rate variability (HRV) variables summarised after Shaffer & Ginsberg, (2017)

Parameter abbreviation	Explanation	Unit	Influence
Time-domain measurements			
SDNN	Standard deviation of the IBI of normal sinus beats	ms	Increased by respiratory sinus arrhythmia
SDRR	Standard deviation of the IBIs for all sinus beats	ms	Increased by abnormal beats
RMSSD	Root mean square of successive differences between normal heartbeats	ms	Vagally-mediated changes reflected in HRV
Frequency-domain measurements			
VLF band	Very low frequency band	ms^2	Decreased by inflammation, physical activity, thermoregulation, renin–angiotensin pathway, and endothelial influences
LF band	Low frequency band	ms^2	Increased by baroreceptor activity, respiration-related efferent vagally mediated influences
HF band	High frequency band	ms^2	Decreased by respiration rate, stress and increased with vagal tone
LF/HF ratio	Ratio of LF to HF power	%	
Non-linear measurements			
S	Area of the ellipse of a Poincaré plot	ms	Correlates with baroreflex sensitivity (BRS), LF and HF

			power, and RMSSD
SD1	The distance of each point from the y = x axis (SD1), specifies the ellipse's width	ms	Correlates with baroreflex sensitivity (BRS), which is the change in IBI duration per unit change in BP, and HF power. Positively associated with vagal activity. RMSSD is identical to the non-linear metric SD1, which reflects short-term HRV. Represents vagal nerve activity
SD2	Deviation of each point from the y = x + average R–R interval (SD2) specifies the ellipse's length	ms	Correlates with LF power and BRS. Positively associated with sympathetic activity.
SD1/SD2	Ratio between SD1 and SD2	%	Measures the unpredictability of the RR time series. Is correlated with the LF/HF ratio. Positively associated with vagal activity.

1.2.4 Types of stressors assessed in dairy cows

1.2.4.1 Environmental stressors

Cattle are affected by many environmental stressors such as cold, heat, humidity, wind, direct sunlight and photoperiod. Out of these heat stress has been investigated the most. These stressors can affect cattle welfare, behaviour, milk production and reproduction (Bova et al., 2014). Housed dairy cattle are exposed to artificial environmental conditions which limit or alter the expression of natural behaviours such as grazing, roaming, standing, lying, walking and oestrus behaviours (Smid et al., 2020), all of which could impact welfare. Dairy cattle welfare and production can also be affected by the microclimatic conditions in barns (Marumo et al., 2022), which can vary between locations inside the barn depending on the distance from fresh air inlets and areas of animal crowding such as the collecting yard and milking parlour (Schüller and Heuwieser, 2016). Specifically, the combination of environmental and biological factors such as humidity, heat (Brügemann et al., 2012), stocking density (Collins et al., 2018), metabolic activity (Kadzere et al., 2002), lying times (Allen et al., 2015), and selection for high milk yields (Kadzere et al., 2002) can lead to heat stress in housed dairy cattle, even in a temperate climate. Most production dairy cows in Scotland are housed for at least some part of the annual production cycle (Shortall and Lorenzo-Arribas, 2022) and the conditions in the stall may be less favourable (higher Temperature humidity index (THI) in summer) compared to the outdoor environment (Marumo et al., 2022).

Heat stress

Acute heat stress increases cortisol production and turnover (Christison and Johnson, 1972). However, both decrease when exposed to chronic heat stress (7-10 weeks). Values return to normal 9 days after the end of chronic exposure (Christison and Johnson, 1972). In a study on sheep and goat heart rate variability, vagal parameters (RMSSD and HF) were negatively correlated with THI while sympathetic parameters were positively correlated (L_{max} and %DET) (Kitajima et al., 2021). In calves, vagal activity (RMSSD) was negatively associated with THI and was higher in calves which were not provided shade and were subsequently exposed to higher THI (Kovács et al., 2018). However, calves were seen to compensate during the night with higher vagal activity in the non-shaded group. Which could indicate that heat stressed calves need nighttime recovery to cope with high heat stress (Kovács et al., 2018). Heat stress will also affect cattle behaviour as heat stressed cows will have increased respiration rate (Pinto et al., 2020), drinking time (McDonald et al., 2020), standing time (Nordlund et al., 2019) and decreased feed intake (Chang-Fung-Martel et al., 2021), activity and lying times (Nordlund et al., 2019, Allen et al., 2015). A decrease in lying time due to thermoregulation (Allen et al., 2015, Nordlund et al., 2019) can lead to frustration indicated by leg stomping, shifting weight and butting (Cooper et al., 2007). The threshold for increased breathing rate was found to be at THI >68 when animals are lying (Pinto et al., 2020).

Heat stress in dairy herds is associated with decreased feed intake, milk yield, and butterfat and protein content of milk (Gernand et al., 2019). Milk yield, butterfat and protein yield have been shown to decrease at THI thresholds as low as 61, 48 and 58, respectively (Rockett et al., 2023). Heat stress can also affect cattle welfare and can be associated with increased incidence of lameness (Allen et al., 2015), mastitis (Gernand et al., 2019), ruminal acidosis (Mishra et al., 1970), retained placenta, puerperal disorders, digital phlegmona (Gernand et al., 2019) and on-farm mortality (2018; Vitali et al., 2015). The association with these diseases is mostly because of the effect of heat stress on increased standing time for locomotion disease, altered feed ingestion (slug feeding) for acidosis and increased oxidative stress and reduced feed intake for periparturient disease. Heat stress causes reduced oestrus behaviour expression and foetal growth, as well as increased early embryonic death, pregnancy loss and early calving (Gernand et al., 2019), which all lower the fertility of cows. Conception rate of dairy cows was negatively affected at THIs as low as 60 on the day of insemination and the interval from calving to first insemination was negatively affected at THIs as low as 50 (Baccouri et al., 2025, Ojo et al., 2025).

Heat stress in cattle is also known to have delayed effects. For example, it arrests the development of early antral follicles, and it may take 2 months to restore normal fertility after summer heat stress (Herbut et al., 2018). Stress hormones released after heat stress also suppress luteinizing hormone, which inhibits ovulation and decreases oestradiol secretion from dominant follicles, which reduces sexual behaviour and therefore lowers fertility (Sammad et al., 2020). Moreover, Hill and Wall (2017) found that milk yield and weather elements summarised over a week explained variation in milk yield more effectively than test day or three-day summaries.

Scotland has a temperate climate, with summer temperatures usually not exceeding 32°C, average daily winter temperatures above 0°C and an average daily relative humidity of 70 to 90%. However, the temperatures are rising consistent with global warming. The average temperature in Scotland has risen by 0.7 degrees since 1980 (Jenkins et al., 2009). Since Scotland has a temperate climate, it is surprising to find heat stress related effects such as decreased feed intake (Hill and Wall, 2017), milk yield, and butterfat and protein content of milk (Hill and Wall, 2015). The estimated economic loss due to heat stress in Scotland was predicted to be £217 per year for an average Scottish farm in the 2010s and could go up to £1,936-4,760 by the year 2100 depending on the increase in average herd size (Fodor et al., 2018).

Photoperiod

Photoperiod has a variable effect on physiological state in animals. In rats, the adrenal gland shows variable sensitivity to ACTH depending on darkness exposure independent of melatonin (Otsuka et al., 2012). A short-day length was associated with diurnal rhythm of corticosterone (the primary glucocorticoids in rodents) levels, whereas this rhythm was abolished under long-day conditions. Short days were also associated with higher corticosterone levels overall in rats (*Rattus norvegicus*) (Otsuka et al., 2012) and hamsters (*Mesocricetus auratus*) (Workman et al., 2011). Parasympathetic and sympathetic activity was elevated using HRV in hamsters exposed to short days (8h) (Weil et al., 2009). No studies on cortisol and HRV related to light levels and day length have been performed on cows. However, long photoperiods affect the patterns of secretion of hormones that increase milk yield such as Insulin-Like Growth-Factor-I (Dahl et al., 1997; Spicer et al., 2007). Additionally, cows exposed to long light periods (16-18 h) returned to oestrus sooner, consumed more dry matter (Hansen, 1985, Peters et al., 1981) and had lower cortisol levels (Lim et al., 2021) compared to cows exposed to natural photoperiod (9-14h). Day length was also found to be positively correlated with conception rate of cows in temperate climates (Pal et al., 2021). In contrast, a short photoperiod (8 h) in the dry period appears to increase milk yield in the subsequent lactation (Miller et al. 2000). In addition to natural photoperiod, housed cows are exposed to artificial light, which (if strong enough) affects their perception of day and night in the same way as natural light (Buchanan et al., 1992; Muthuramalingam et al. 2006). Cows that are housed under continuous light do not recognise day length anymore and default to a short photoperiod (Dahl et al., 2000). The interaction between photoperiod and heat stress in dairy cattle has not been studied. This interaction would be of interest as increased photoperiod is usually associated with higher environmental temperatures and because they have an opposite effect on milk yield. Therefore, an increase of photoperiod under more favourable ambient temperatures could yield greater increases in milk production. Due to its latitude, Scotland's dairy cattle are subject to large seasonal changes in natural photoperiod. Daylight hours reach 17h in summer and fall to 7h in the winter (Metrological Office National Climate Information Centre). However, the effect of photoperiod variation is mitigated by artificial lighting. Studies from Europe and North America which increased photoperiod length above 12 h/day observed increased milk yield between 0.5 and 3.3 kg/cow/day (Dahl et al., 2000). Production was also increased by ~8 kg/day when light duration was increased from 14.2h (natural photoperiod) to 16h with light intensity of 100 Lux. Additionally, milk fat percentage and total solids were higher (Lim et al., 2021). As light period manipulation is relatively easy and cheap, the benefit of an extended photoperiod could readily be achieved to increase milk yield and improve reproduction in Scottish farms.

Air quality

In rats, exposure to air particulate matter and ozone was associated with an increased ACTH and corticosterone levels (Thomson et al., 2013). In humans, exposure to air pollution (PM 2.5, nitrogen oxide and ozone) decreased vagal parameters of HRV (HF and RMSSD) and increased sympathetic parameters (LF/HF ratio) (Weichenthal et al., 2011, Wu et al., 2010). In housed cattle, air velocity is considered due to the known risks of pneumonia in calves exposed to draughts (Leenen et al., 2020). However, air quality (including concentrations of ammonia and carbon dioxide waste products), which can vary with stocking density (Charpiot et al., 2012) and ventilation rate, are likely to affect production characteristics, health and welfare in adult cattle. Ammonia in the air causes irritation of the mucous membranes of the eyes and the respiratory system leading to increased incidence of infectious disease and poor growth of young animals (Osorio et al., 2009). Ammonia concentrations in a barn depend on temperature, relative humidity and ventilation, livestock density and frequency of manure removal (Herbut & Angrecka, 2014). Therefore, a careful balance is needed when designing ventilation in calf sheds as we want the ventilation to be effective enough to remove toxic gases, but we do not want draughts at calf level. Ammonia concentrations measured in cattle sheds ranged between 0-54 ppm in the past (Clark & McQuitty, 1987); however, average ammonia concentrations did not exceed 10 ppm in more recent studies (Herbut & Angrecka, 2014; Zhao et al., 2005; Zhao et al., 2007). The main route of reducing ammonia concentrations in barns is bedding and manure management (Gilhespy et al., 2009; Pereira et al., 2011). European recommendations suggest that ammonia concentrations should not exceed 20 ppm (CIGR, 1994).

Most studies on the harmful effects of ammonia on production animals have been done in poultry and pigs (Ni et al., 2020). Pigs exposed to ammonia concentrations between 10-150 ppm had increased irritation of nasal mucosa (Urbain et al., 1996), coughing (Stombaugh et al., 1969), isolation of pathogens from the nose and pleurisy (Michiels et al., 2015). Pigs and poultry will avoid areas of high ammonia concentration (Wathes et al., 2002), which was also suggested in a study on cattle (Smith, 2021). In calves, ammonia levels were positively associated with the prevalence of lung consolidation and epithelial cell number found on bronchoalveolar lavage (Leenen et al., 2020). The same study found no correlation of CO_2 (range 0-8000 ppm) in the environment with any of the health outcomes measured (Leenen et al., 2020). However, CO_2 is still a valuable parameter often used to indirectly assess ventilation rate in livestock housing systems (Pedersen et al., 1998). Particulate matter is another common pollutant of the cattle industry having detrimental effects for both animals and humans. It is estimated that feedlot cattle produce 31-60 g/animal/day of PM_{10} particles (Mcginn et al., 2010). The effects of air quality on adult cattle represents a knowledge gap in the literature as we were unable to find any peer reviewed studies on this topic. No study has investigated the effect of ammonia or CO_2 on adult dairy cattle health and production or its interaction with heat stress. Both gases could be used as an objective measure of ventilation efficiency in barns or a measure of air quality provided to the animals as a welfare standard. Currently it is mostly speculated if and how ammonia concentrations affect adult cows, therefore it would be important to define this more clearly. Heat stress can increase both gases in a shed if ventilation if inadequate, however it is not known if they might have a cumulative effect of cattle and therefor, the cutoffs might need to be temperature specific. A masters project that investigated the levels of ammonia in a farm in Scotland found concentrations between 0.4 and 14 ppm (mean 3.89 ppm) which were positively correlated with air temperature (Smith, 2021).

The microclimate as a whole (the combined effect of temperature, relative humidity, light and air quality) is therefore an important but poorly studied factor that could affect dairy cow stress and consequently impact productivity.

1.2.4.2 Management stressors

Milking

Stress behaviour related to milking is seen as hesitation to enter the parlour, tail swishing, increased stepping rate and kicking (Kézér et al., 2015, Davis et al., 2023, Marçal-Pedroza et al., 2023). Based on cortisol levels, milking in a conventional milking parlour does not seem to be more stressful than milking in an automated milking system (Hopster et al., 2002, Gygax et al., 2006). However, more reactive cows have higher cortisol levels in milk (Marçal-Pedroza et al., 2023). Heart rate increased during udder preparation and milking compared to baseline (Davis et al., 2023). Based on RMSSD and LF/HF ratio primiparous cows are more stressed than multiparous cows during milking in an automated milking system (Davis et al., 2023). HF power decreased in cows restrained in the milking stall after milking, and at the same time their stepping rate also increased (Kézér et al., 2015). It seems that driving the cows to the collecting yard is more stressful to cows (based on decreased HF and increased LF/HF ratio) compared to the act of milking (Kovács et al., 2013b, Kovács et al., 2019).

Maternal separation

At the time of separation from their calves, cows usually display stress as increased vocalisation, time spent next to the fence or with the head over the fence, decreased lying time and sniffing/searching (Stěhulová et al., 2008, Neave et al., 2024). Compared to later removal cortisol does not seem to increase after separation if calves are removed 3 days after birth (Hopster et al., 1995). However, in beef cows, cortisol increased on the 3rd day after separation at 25 days after calving (Pérez-Torres et al., 2016). There are no studies on heart rate variability in cows at cow-calf separation. However, heart rate did increase in cows after separation but then returned to baseline in the span of 5-60 min (Hopster et al., 1995, Stěhulová et al., 2008). There was no association with the age of calf at separation (1, 4 or 7 days) or visual contact after separation with heart rate (Stěhulová et al., 2008). Currently, calf separation seems to only have a short-lived effect on dams, which is reduced with earlier separation. However, more studies are needed to address this pressing issue. To the author's knowledge the effects of cow-calf separation on HRV has not been studied yet.

Overstocking

Overstocked cows walk more, lie less, have more lying bouts, ruminate less and have more displacements from feeding and lying spaces (Huzzey et al., 2006, Krawczel et al., 2012, Fustini et al., 2017, Grelet et al., 2022). Overstocking (200%) in the far-off dry period does not seem to affect cortisol levels (Huzzey et al., 2012). However, in the close-up period, overstocking (300%) increased cortisol level but this effect was only evident 15 days before calving (1 week after exposure) (Fustini et al., 2017). In one study overstocking was associated with higher hair cortisol but not saliva cortisol (Grelet et al., 2022). Surprisingly, heart rate and SD2/SD1 ratio were lower and RMSSD and SD1 were higher after 4 weeks in overstocked cows (Grelet et al., 2022). Further studies are needed to determine the effect of overstocking on HRV.

Restraint/isolation

Behavioural response to restraint is highly dependent on the temperament of the animal and her previous experience. Separated animals can respond with agitation and vocalisation (Grandin and Shivley, 2015). Animals have an acute increase in cortisol when exposed to restraint/transport for the first time but the cortisol response will decline at repeated exposure, therefore training can be used to decrease stress (Andrade et al., 2001, Stockman et al., 2011, Szenci et al., 2011). Cattle that have experienced an unpleasant event have an increased heart rate when they return to the area of the event (Pascoe, 1986). When cows were isolated they had an increase in heart rate that decreased after 10-15 min, however, RMSSD did not change (Mandel et al., 2019). In another study both vagal (SDNN, RMSSD, HF) and sympathetic tone (HR, LF, %REC and %DET) were increased during handling (Frondelius et al., 2015). Therefore, level of restraint has to be documented when assessing stress parameters in cattle.

Dry off

If cows are dried off with high milk yield (25-35kg/day) they show signs of distress, vocalisation and delayed involution (Silanikove et al., 2013). Pain associated with milk accumulation can also reduce lying time and lying bouts (Rajala-Schultz et al., 2018). Sudden dry off in high-yielding cows (>15 kg/day at dry off) is also associated with elevated faecal cortisol concentrations (Bertulat et al., 2013). Dry off was associated with lowering of heart rate one week after drying off (Trenk et al., 2015). Incomplete milking for a week in an attempt to reduce milk yield before dry off did not influence the heart rate or HRV (Joest et al., 2025). Therefore, dry off although a physiological process, can be stressful in high yielding cows.

1.2.4.3 Cow level stressors

Disease and Pain

Cows isolate themselves, eat less and lie down more when experiencing illness after calving (Proudfoot et al., 2014). Disease can affect lying behaviour differently: cows with metritis (Neave et al., 2018) and mastitis (Medrano-Galarza et al., 2012) lie down less than healthy cows. Both induced and spontaneous milk fever was associated with elevated cortisol levels in blood (Horst and Jorgensen, 1982). Diseases like clinical and subclinical mastitis, metritis and laminitis or multiple diseases had elevated hair cortisol levels compared to healthy mid lactation cows (Comin et al., 2013). Calves with diarrhoea have decreased vagal and sympathetic activity (Mohr et al., 2002). The results of studies of HRV and bovine spongiform encephalopathy have been contradictory with some studies finding increased RMSSD and HF power (Little et al., 1996) activity, while others reported no changes (Konold et al., 2011). Contrary to expectations, chronic lameness increased RMSSD and HF and decreased mean heart rate, LF/HF and SD2/SD1 (Kovács et al., 2015a). Lameness is associated with increased lying duration which would increase vagal and decrease sympathetic activity (Frondelius et al., 2015).

Calving

Cows calving indoors prefer to isolate themselves (Proudfoot et al., 2014). This behaviour was only observed in primiparous cows on pasture (Jensen et al., 2022). Based on measures of the output of the hypothalamo-pituitary-adrenal (HPA) axis i.e., cortisol concentrations in blood, saliva, milk and urine (Mormède et al., 2007) and visual observation of cow behaviour (Jurkovich et al., 2024), it has been reported that transition period cows are under physiological stress, with a peak on the day of calving (Nikolić et al., 2003, Arfuso et al., 2023). Studies of HRV during calving have concluded that cows show a decrease in vagal activity 12-24h before the first clinical signs of calving (restlessness), suggestive of increased physiological stress (Kovács et al., 2015c). This is followed by an increase in vagal tone during the second stage of calving (Kovács et al., 2015c, Nagel et al., 2016), which could be associated with the release of oxytocin needed for uterine contraction and expulsion of the calf. Sympathetic tone was increased in the late stage of calving and 4-8h after calving (Kovács et al., 2015c), which the authors proposed was associated with pain due to the uterine contractions needed to expel the placenta. In these studies, heart rate also increased with restlessness and reached a peak at calving likely due to the physical demands of calving (Kovács et al., 2015c, Nagel et al., 2016), after which it was seen to gradually decrease. Maternal physiological stress was reported to increase with calf birth weight, but the effects of other calf characteristics such as breed, sex or number were not assessed in those studies (Kovács et al., 2015c). These factors are expected to affect HRV as cows carrying twins and oversized calves (compared to the dam's pelvic size) have higher cortisol concentrations at calving compared to cows carrying single normal sized calves, (Patel et al., 1996, Sano et al., 2023). The HRV studies of Kovács et al. (2015c) and Nagel et al. (2016), however, only focused on calving, and did not assess stress in the wider transition period.

1.3 Transition

1.3.1 Definition of the transition period

The transition period in dairy cattle is defined as the 3 weeks before and after parturition (Drackley, 1999). It represents a period of major physiological change, including reduced feed intake and rumination time (Cook et al., 2021) combined with increased energetic demand to support foetal growth, colostrum and milk production (Wankhade et al., 2017). The transition period is when many metabolic and infectious diseases present, and transition period health has a significant influence on subsequent milk production and reproductive performance (Cook et al., 2024).

1.3.2 Physiology of calving

Three weeks before calving, dry matter intake starts to decrease with the greatest drop in the days close to calving (Grummer et al., 2004), which leads to mobilisation of body reserves. Non-esterified fatty acids (NEFA) are mobilised from adipose tissue and can be used for energy in tissues (Herdt, 2000). Excess NEFA can be cleared by the liver in three ways: complete oxidation- via entry into the tricarboxylic acid cycle, partial oxidation leading to the production of ketone bodies, and re-esterification into triglycerides, which are either stored in the liver or exported as very-low density lipoproteins (Grummer, 1993). The liver has limited capacity for complete oxidation and to export NEFA therefore most NEFA are converted to ketone bodies such as acetate, beta-hydroxybutyrate (BHB) and acetone (Drackley, 1999). When NEFA supply exceeds the complete oxidation and export capacity of the liver, cows are likely to develop ketosis and fatty liver disease (Herdt, 2000). Increased NEFA oxidation in the liver may also contribute to negative energy balance as it reduces appetite (Allen et al., 2009). Lipolysis in adipose tissue stimulates a proinflammatory response (Contreras et al., 2015). Oxidation of NEFA in the liver results in the production of reactive oxygen species that lead to proinflammatory cytokine production (Sordillo et al., 2009). After calving the uterine tissue is compromised and bacteria reach the endometrium through the open cervix which also leads to an inflammatory response (Chapwanya et al., 2012). All these processes result in a systemic inflammation response in the cows, causing an additional drain on energy and calcium (Bradford et al., 2015).

1.3.3 Transition disease risk factors

Transition disease and/or their diagnosis is shown in Table 1-2. Risk factors for transition disease can be split into cow and herd level. Cow level risk factors are BCS, parity, gestation length, physiological parameters and dry matter intake (Daros et al., 2022). Higher BCS (>3.25/5) is associated with subclinical ketosis and abomasal displacement whereas low BCS (<3/5) or loss of BCS during the dry period are associated with metritis and retained placenta (Shaver, 1997, Daros et al., 2022). Primiparous cows have a higher incidence of uterine disease while multiparous cows have more metabolic diseases (Daros et al., 2022). Uterine disease in first calving heifers is mostly associated with the higher incidence of dystocia (Mee, 2008). Multiparous animals have an impaired neutrophil function which could leave them more susceptible to infectious diseases (Gilbert et al., 1993). They also have a reduced numbers of parathyroid hormone and vitamin D receptors which leads to a higher incidence of hypocalcemia (Goff, 2014). Parity is also a risk factor for subclinical ketosis which could be related to higher metabolic demand at the end of lactation or higher lipid mobilization in multiparous compared to primiparous cows (Daros et al., 2022). Shorter gestation length has been associated with higher retained placenta incidence which could be due to the immaturity of placentomes (Laven and Peters, 1996) or hormonal imbalances (Beagley et al., 2010). High NEFA values before calving have been associated with subclinical ketosis incidence (Ospina et al., 2013). High BHB after calving is associated with clinical ketosis, displaced abomasum and uterine disease (Overton et al., 2017). Behaviour such as lying was found to be associated with dystocia, subclinical hypocalcaemia, metritis and ketosis but not subclinical ketosis (Daros et al., 2022), although the direction of relationship may be disease specific.

Herd level risk factors can be split into management and ambient factors. Regrouping and overcrowding interrupt the group hierarchy and increase antagonistic interactions, which in turn disrupt feeding and lying behaviour (Proudfoot et al., 2009, Schirmann et al., 2011). Antagonistic behaviour around the feed bunk is associated with increased risk of metritis and subclinical ketosis (Huzzey et al., 2007, Goldhawk et al., 2009). Lameness also reduces feed intake which will increase beta-hydroxybutyrate (Vergara et al., 2014). Heat stress is associated with an increased incidence of retained placenta, metritis and mastitis (Menta et al., 2022).

In addition to the risk factors stated above, certain transition diseases are risk factors for other transition diseases. For example, animals with delayed or persistent subclinical hypocalcaemia are more prone to developing subclinical ketosis, metritis and displaced abomasum than normocalcaemic cows (Mcart and Neves, 2020), and cows with ketosis, retained placenta or metritis have a higher risk of abomasal displacement (Shaver, 1997). Negative energy balance before or after calving is associated with transition disease. Increased NEFA before calving (NEFA >0.4 mmol/L) are associated with increased risk of displaced abomasum, retained placenta, culling and reduced milk production in the first 4 months of the lactation cycle. Elevated serum BHB in the first 2 weeks after calving (BHB >1.2-1.4 mmol/L) is associated with increased risk of displaced abomasum, metritis, clinical ketosis, endometritis, prolonged postpartum anovulation, increased severity of mastitis, and lower milk production in early lactation (Leblanc, 2010).

Table 1-3 Definition or diagnosis of transition disease

Transition disease	Definition/Diagnosis
Retained placenta	Failure to pass foetal membranes within 12 or 24 h after calving (Daros et al., 2022)
Metritis	Characterized by foul watery vaginal discharge with or without fever and with or without signs of systemic illness between 0 to 21 DIM (Daros et al., 2022)
Subclinical ketosis	Blood beta-hydroxybutyrate >0.96 to 1.4 mmol/L or milk >0.15 mmol/L
Displaced abomasum	Detected using percussion with simultaneous auscultation
Clinical hypocalcaemia	Determined based on clinical signs of recumbency, fine tremors over the flanks, subnormal body temperature and cold extremities and a reduced blood calcium level. Delayed or persistent subclinical hypocalcaemia is defined as total calcium levels ≤2.20 mmol/L at 4 DIM (Mcart and Neves, 2020)

1.3.4 Impact of the transition period on future production

Leblanc (2010) reports that 30-50% of dairy cows are affected by some sort of infectious or metabolic disease around calving and all dairy cows experience insulin resistance, reduced feed intake, lipid mobilization, weight and body condition loss, hypocalcaemia, immunosuppression and bacterial contamination of the uterus. Poor transition results in increased transition disease incidence which can result in poorer milk yield and reproductive performance and earlier culling (Dubuc and Denis-Robichaud, 2017). Cows with clinical hypocalcaemia had a 2.19kg/d decreased milk yield in the first 3 milk recordings. Moreover, many cows with clinical hypocalcaemia leave the herd early which might introduce survival bias to these results (Probo et al., 2018; Venjakob et al., 2018). Cows with delayed subclinical hypocalcaemia had a higher likelihood of being culled in the first 60 days in milk (Mcart and Neves, 2020). Transient subclinical hypocalcaemia was associated with higher milk production compared to normocalcaemic cows and cows with delayed and persistent subclinical hypocalcaemia (Mcart and Neves, 2020). Poorer conception rates at first service were found with hyperketonemia, endometritis, prolonged anovulation and displaced abomasum (Dubuc and Denis-Robichaud, 2017). First service conception rate and the hazard of pregnancy by 150 DIM were also impaired by subclinical hypocalcaemia at 4 DIM (Seely and Mcart, 2023). Retained placentas and purulent vaginal discharge were associated with pregnancy loss. Postpartum culling is associated with hyperketonemia, retained placenta and displaced abomasum (Dubuc and Denis-Robichaud, 2017). Therefore, the transition period is the most critical period of the production cycle and must be manged well in order to set up cows for optimal production and reproduction in the subsequent lactation.

1.4 Conclusion

In summary, the literature review demonstrates that cows experience many external and internal stressors and that the transition period is an inevitable flashpoint for physiological stress in dairy cattle. The key findings of this review highlight that while traditional biomarkers like blood cortisol effectively capture acute spikes in stress, they are often limited by invasive sampling methods. Heart rate variability (HRV) emerges as a highly sensitive, non-invasive alternative for tracking autonomically mediated stress, yet its application across the broader periparturient window—and its direct relationship with specific transition diseases and calf characteristics—remains sparse and poorly defined in existing research. Furthermore, while microclimatic environmental variables (such as THI, photoperiod, and air quality) are suspected to induce stress, there is a distinct knowledge gap regarding how these indoor housing conditions effect HRV and how they vary within sheds and how they interact with transition cow welfare. These findings directly inform the research objectives of this thesis. To bridge these gaps, the following chapters investigate the temporal dynamics of physiological stress in multiparous cows using non-invasive HRV monitoring around calving, while simultaneously validating localized environmental monitoring systems to better identify and mitigate multi-factorial stress in commercial dairy housing.

Chapter 2: Assessing Stress in the Transition Period Using Heart Rate Variability in Multiparous Holstein Cows

2.1 Abstract

The transition period is a critical part of the production cycle of a dairy cow, characterised by major physiological changes and events that can act as stressors. Heart rate variability (HRV) can be used to assess the response of the autonomous nervous system to stressors. The aim of this study was to examine the dynamics of HRV parameters in the transition period and to explore associations with transition disease, and cow- and calf characteristics. HRV was opportunistically measured in 134 Holstein multiparous cows across a period 10 days before to 7 days after calving, using a wearable heart rate monitor. Cow and calf characteristics for these animals were retrospectively extracted from the farm management system. Within a week after calving, cows were clinically examined and the incidence of transition diseases recorded. Data were analysed by fitting Linear mixed-effects models to examine the relationship between HRV parameters and cow- and calf-level characteristics.

Temporal changes in HRV parameters were observed that were associated with days relative to calving. Mean heart rate peaked just before calving (day -1.5). Both the root mean square of successive differences between normal heartbeats and standard deviation 1 showed a quadratic concave up (u-shaped) relationship during the transition period with a turning point near parturition (day +1.2). The ratio of standard deviation 2 to standard deviation 1 declined in a linear fashion throughout the study period. These changes indicate that cows experienced increasing stress as calving approached, and that HRV returned to early-transition period levels after calving. Carrying twins and dairy-sired calves was also associated with HRV patterns suggestive of greater stress. Transition disease and cow-level parameters did not show significant associations with HRV parameters studied, in the period from 10 days before to 7 days after calving.

This study highlights the utility of HRV as a non-invasive measure of autonomic nervous system activity and stress in dairy cows. It shows that during the transition period, the greatest physiological stress occurs in the final run-up to and during calving, while physiological stress is also associated with calf characteristics.

2.2 Introduction

The transition period in dairy cattle is defined as the three weeks before and after parturition (Drackley, 1999). It represents a period of major physiological change, including reduced feed intake and rumination time (Cook et al., 2021) combined with increased energetic demand to support foetal growth, colostrum and milk production (Wankhade et al., 2017). During the transition period multiparous cows are at high risk of many metabolic and infectious diseases, and transition period health has a significant influence on subsequent milk production and reproductive performance (Cook et al., 2024).

Based on measures of the output of the hypothalamo-pituitary adrenal (HPA) axis i.e., cortisol concentrations in blood as well as blood products (saliva, milk, urine) (Mormède et al., 2007), and the visual observation of cow behaviour (Jurkovich et al., 2024), it has been reported that transition period cows are under physiological stress, with a peak on the day of calving (Nikolić et al., 2003, Arfuso et al., 2023). It is of note, however, that animal handling and the collection of invasive samples can affect acute stress assessments (Hopster et al., 1999). As exposure to stressors can activate the autonomic nervous system, heart rate variability (HRV) provides an alternative non-invasive method to assess stress. HRV, which involves analysing fluctuations in the interbeat intervals (IBI) using time-domain, frequency-domain and/or non-linear measures provides a measure of the balance between sympathetic and parasympathetic nervous system activity and has been used as an indicator of acute and chronic stress in human and animals (Von Borell et al., 2007, Shaffer and Ginsberg, 2017). In cattle, HRV has been reported to be associated with a variety of parameters including production, disease, fear and pain (Kovács et al., 2014), calving (Nagel et al., 2016), body weight, breed and management (Hagen et al., 2005), demeanour (Kovács et al., 2015b), herd size and physical activity (ruminating, standing, lying, feeding) (Kézér et al., 2017), body condition score (BCS), days in milk and % butterfat (Frei et al., 2022) and rumen fill (Von Borell et al., 2007).

Studies of HRV during calving have concluded that cows show a decrease in vagal activity 12-24h before stage one of calving (restlessness), suggestive of increased physiological stress (Kovács et al., 2015c). This is followed by an increase in vagal tone during the second stage of calving (Kovács et al., 2015c, Nagel et al., 2016), which could be associated with the release of oxytocin needed for uterine contraction and expulsion of the calf. Sympathetic tone increased in the stage three of calving and 4-8h after calving (Kovács et al., 2015c), which the authors proposed was associated with pain due to the uterine contractions needed to expel the placenta. In these studies, heart rate also increased with restlessness and peaked at calving likely due to the physical demands of parturition (Kovács et al., 2015c, Nagel et al., 2016), after which it gradually decreased. Maternal physiological stress was reported to increase with calf birth weight, but the effects of other calf characteristics such as breed, sex or number were not assessed in those studies (Kovács et al., 2015c). These factors are expected to affect HRV as cows carrying twins and oversized calves (relative to the dam's pelvic size) have higher cortisol concentrations at calving than cows carrying single normal-sized calves (Patel et al., 1996, Sano et al., 2023). The HRV studies of Kovács et al. (2015c) and Nagel et al. (2016), however, only focused on calving and did not assess stress across the wider transition period, or the effects of transition diseases. It has been shown that after calving, cows have increased concentrations of the inflammatory marker haptoglobin, and that haptoglobin concentrations remain higher for longer in cows that experience transition disease like retained placenta, metritis, mastitis, ketosis, or lameness (Qu et al., 2014). To the best of the authors' knowledge no studies have assessed the influence of transition disease on HRV.

The aim of the present study was to assess stress across the transition period using HRV and to assess if stress in the transition period was associated with transition disease (ketosis, hypocalcaemia, retained placenta, displaced abomasum and metritis) and cow and calf characteristics. We hypothesised that the HRV parameters which reflect parasympathetic nervous system activity will reach their lowest point on the day of calving and return to baseline in the days after calving. The opposite was expected for HRV parameters linked with sympathetic nervous system activity. We hypothesised that cows with transition disease would have lower HRV (more stress) compared to healthy cows because transition diseases are associated with inflammation and pain. As beef sired, male and twin calves take up more space in the abdomen in the last days of gestation and are associated with reduced maternal dry matter intake (Bell and Roberts, 2007), we hypothesised that cows carrying beef-sired, male and twin calves would experience greater physiological stress and have lower measures of HRV than dams carrying dairy-sired, female and single calves. We also hypothesised that higher lactation number and BCS and lower rumen fill would be associated with higher HRV.

2.3 Materials and methods

2.3.1 Ethics licence

This research was approved by the University of Glasgow, School of Biodiversity, One Health and Veterinary Medicine Ethics and Welfare Committee (EA31(a)/16 and EA38/21).

2.3.2 Animals

The study was conducted between August 2023 and August 2024 at a commercial Holstein dairy farm in the United Kingdom. At this farm, cows were housed all year round and milked three times a day with a mean standard lactation (305 days) milk yield of more than 10,000 L/cow. Animals were dried off, on average, 46 days before calving and were moved to the close-up/calving pen 17 days before calving. They were moved to the fresh pen immediately after calving and stayed there for 3-7 days. Calves were removed within an hour of birth, which minimises the likelihood that calf separation contributed to the stress responses observed. Both the close-up and fresh pens are 240 m^2 deep-straw-bedded yards, each with 20 m of feeding and 3.8 m of water space.

Data pertaining to individual cows and their calves were extracted from the farm's management software, DairyComp 305 (VAS, USA) and monthly milk recordings (Cattle Information Service, UK). Low genetic merit cows were bred using British Blue and Wagyu beef semen, whereas in genetically superior dairy animals sexed Holstein semen was used.

2.3.3 Diet

Cows were fed once a day between 8.00-9.00 AM with a total mixed ration (Table 2-1) that covered the cows' nutritional needs and matched the US National Research Council recommendations (National Research, 2001). Feed was pushed up every 2 h. Water access was *ad libitum*.

Table 2-1 Composition of the daily diets fed to cows in the close-up dry period (around 3 weeks before calving until calving) and 3-7 days after calving (fresh cow).

Ingredient	Close-up diet (kg/day)	Fresh cow diet (kg/day)
Wheat straw	5.5	1.2
Whey permeate		2.7
Rapeseed meal		1
Pot Ale syrup		5.8
Milking cow minerals		0.22
Salt		0.07
Limestone	0.25	0.26
Maize ground		5.4
Supergrains	4	21.4
Barley coarse rolled	3	6
Glycerol		0.3
Soya high protein		0.9
Soybean hulls pellet		1
Grass silage	13.5	21.05
Mycotex		0.01
Biochlor	0.85	
Acid buffer	0.15	
Dry cow minerals	0.15	
CholiGem	0.03	
Magnesium Chloride	0.1	
Soybean meal	0.75	
Water	7	
Total	35.280	67.310

2.3.4 Data collection

Data were opportunistically collected during routine weekly farm visits by author JJH. Each week, five animals were selected based on their expected calving date and signs of approaching parturition (swollen vulva and full udder or loose tail head ligaments). Primiparous cows were not included, as they were housed separately without handling facilities. Multiparous cows were also targeted as more transition diseases were expected. Data were collected from each animal on two occasions, once during the period 0-10 days before, and once during the period 1-7 days after, calving. These cutoffs were chosen as cows were, on average, moved to the close-up pen 17 days before the expected calving date, and measurements >10 days before calving might have been influenced by the stress of relocation and the need to establish social hierarchy within the group. Seven days after calving the cows were moved into the lactation pens which do not have head yokes. Body condition and rumen fill (each measured on a 5-point scale) were scored using the UK Agriculture and Horticulture Development Board (AHDB) scoring system. As part of routine management and to facilitate bedding of the straw yards each day while feeding, cows were caught in head yokes. During this time H7 heart rate sensors were attached to the animals using Polar Equine Electrode belts and interbeat interval (IBI) data were collected for 30 min using a paired Polar V800 watch (Polar Electro Öy, Finland). Ultrasound gel was used to improve contact between the electrodes and the skin. The animals were not restrained for more than 2 h at a time.

All animals were observed daily by farm staff and underwent a clinical examination by author JJH 1-7 days after calving and any health conditions were recorded. Retained placenta was defined as failure to expel the foetal membranes within 12 h after calving. Metritis was characterised by a foul smelling, liquid discharge with pyrexia (>39.0°C). Ketosis was assessed on urine samples obtained via urethral catheterisation and using test strips (KetoStix, Bayer Diagnostics Europe Ltd., Dublin, Ireland). A displaced abomasum was detected using percussion with simultaneous auscultation. Hypocalcaemia was determined based on clinical signs of recumbency, fine tremors over the flanks, subnormal body temperature and cold extremities.

2.3.5 Interbeat interval (IBI) data processing

From the 30-min recording the 5-min interval between the 20th and 25th minute of data collection was selected for analysis. This period was chosen as it allowed the animals to become accustomed to the belt and supplied stationary conditions for HRV measurement (Von Borell et al., 2007). The HRV parameters (Table 2-2) were analysed with the Kubios HRV Standard 3.5.0. software (Kubios Oy, Kuopio, Finland). Abnormal intervals between two successive IBIs were filtered out using the artefact correction feature. If, within a sample, more than 5% of the beats were corrected, the closest alternative 5-min period was selected. Frequency domain parameters were adjusted to bovine specific breathing rates according to von Borell et al. (2007) and Kovács et al. (2015c).

Table 2-2 Measured heart rate and heart rate variability (HRV) parameters with abbreviations, units, physiological meaning and domain definition.

Parameter	Abbreviation	Unit	Physiological meaning (Gross et al., 2011; Shaffer and Ginsberg, 2017)	Domain
Mean heart rate	Mean HR	Beats/min	Combination of vagal and sympathetic activity	Time-domain
Root mean square of successive differences between normal heartbeats	RMSSD	ms	Vagal-dependent changes in the sympathovagal balance, short-term HRV	Time-domain
Standard deviation 1 of the distance from the y = x axis in a Poincaré plot	SD1	ms	Reflects short-term HRV. Represents vagal nerve activity	Non-linear measure
Standard deviation 2 of each point from the y = x + mean R–R interval in a Poincaré plot	SD2	ms	Reflects moderate- and long-term HRV. Correlates with low frequency power and baroreflex sensitivity	Non-linear measure
Standard deviation 2/Standard deviation 1 ratio	SD2/SD1 ratio		Reflects the sympathovagal balance	Non-linear measure
High-frequency power (normalised)	HF		Vagal activity, respiratory sinus arrhythmia	Frequency-domain
Low-frequency/High-frequency ratio	LF/HF ratio		Reflects the sympathovagal balance	Frequency-domain

Time-domain: Quantifies the number of HRV observations during a monitoring period, Frequency-domain: Quantifies the number of HRV observations across different frequency components, Non-linear measures: Measures the unpredictability of HRV patterns within a sample period (Shaffer and Ginsberg, 2017)

2.3.6 Power analysis

Based on a human meta-analysis (Quintana, 2017) and the variance in HRV parameters in dairy cattle (Frei et al., 2022), it was determined that 21, 61 or 233 samples would be required to detect large, medium or small effect sizes respectively, with 80% power and a P value of 0.05 (Quintana, 2017).

2.3.7 Statistical analysis

Statistical analysis was performed using R statistical software version 4.2.0 (2025). Parameters in Table 2-2 were log transformed using the natural logarithm. To examine the relationship between HRV parameters in Table 2-2 and cow and calf characteristics, Linear mixed-effects models were fitted using Restricted Maximum Likelihood using the "lme4" package.

$Y_{ijklmno} = D_i + D_i^2 + NS_j + B_k + L_l + RF_m + BCS_n + PPD_o + (1|COW)$

Y- dependent variable

D- number of days in relation to calving, mean-centred (i= -10 -7 d)

D^2- quadratic component of the number of days in relation to calving, mean-centred (i= -10 - 7d)

NS- calf number and sex (j=twins, single male, single female)

B- breed of calf's sire (k=dairy, beef)

L-lactation number (l=2-11)

RF- rumen fill (m=1-5)

BCS- body condition score at the time of each sampling (n=2-4 in 0.25 intervals)

PPD- post-partum disease (o=present/absent)

(1|COW)- random intercept of cow identity

Due to low individual incidences, transition diseases were grouped together into a single category for the analysis. Twins were grouped together regardless of sex and breed for the same reason (Table 2-3). Wagyu- and British Blue-sired calves were grouped together into a single beef calf category as the model did not detect a difference between the two. Collinearity was assessed on the full model using the "vif" function in the "car" package (Fox and Weisberg, 2019). Models were compared using Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC). Stepwise variable selection was performed manually to achieve the lowest AIC and BIC values on Maximum Likelihood models. Model comparisons were conducted using likelihood ratio tests between nested mixed-effects models to assess the significance of the removal of fixed effects. The model with the lowest AIC, BIC, and with a non-significant impact on overall fit, was selected. Restricted Maximum Likelihood was refitted on the final model. Only fixed effects relevant to the final model are presented in Results. Statistical significance was set at $p<0.05$. Diagnostics and goodness-of-fit were assessed using the "simulateResiduals" function in the "DHARMa" package (Hartig, 2016). The "glht" function in the "multcomp" package (Hothorn et al., 2008) was used to perform pairwise comparisons between twin, singleton male and singleton female for each model where calf number and sex were retained. P-values were adjusted according to the Holm-Bonferroni method. The turning point of the models was calculated by dividing the coefficient of the linear component of days to calving by the coefficient of the quadratic component of days to calving multiplied by two. Centred estimates were converted to the original scale by adding the centred day value (from the model) to the mean day to calving.

Table 2-3 Descriptive statistics of calf sex and sire breed from 134 cows calving between August 2023 and August 2024.

	Female	Male	Twin	Total calving events
Dairy	15	2	1 (FF)	18
Beef	50	55	11 (4 FF, 5 FM, 2 MM)	116
Total	65	57	12	134

FF- female/ female twins, FM- female/ male twins, MM- male/ male twins

2.4 Results

2.4.1 Descriptive statistics

Data were collected from a total of 134 multiparous Holstein cows 4 ± 1.7 days (mean ± SD) before and 3.2 ± 2.7 days after calving. The median number of lactations in the study population was 3 (range 2-11) and the mean milk yield in the previous lactation was 14,193 kg (range 8,170-22,430 kg). The median BCS of the study population was 3.25 before and 3 after calving. 19 animals had one or more transition disease, hypocalcaemia (n=1), ketosis (n=9), retained placenta (n=9), metritis (n=5) and displaced abomasum (n=2). Calf sex, breed and twinning descriptive statistics are presented in Table 2-3. Figure 2-1 shows the studied HRV parameters across the transition period and Tables 2-4 – 2-10 present the outputs of the Linear Mixed effects Models to test the effects of cow and calf characteristics on HR and HRV through time.

2.4.2 Transition Disease

None of the HRV parameters studied either prior to or after calving were associated with transition disease incidence.

2.4.3 Mean heart rate

Mean HR showed a significant quadratic relationship with days relative to calving, decreasing over the transition period until a turning point at day -1.5 (Figure 2-1A). Cows carrying twins (regardless of sex) had a higher HR than cows carrying single female calves (twins vs. single female 92±3 vs. 85±1 beats/min [raw arithmetic mean ± SEM], multiple comparison test p=0.027). There was no significant difference in HR between cows carrying male calves and cows carrying twins (single male vs twins 87±1 vs. 92±3 beats/min [raw arithmetic mean ± SEM], multiple comparison test p=0.147) or single female calves (single male vs. single female 87± 1 vs 85± 1 beats/min [raw arithmetic mean ± SEM], multiple comparison test p=0.446) (Table 2-4). The breed of the calf's sire, lactation number of the dam, rumen fill and BCS were not associated with mean HR.

Table 2-4 Linear Mixed effects Model to assess variation in mean heart rate (natural-log transformed) in relation to days relative to calving, calf breed, birth type, lactation number, rumen fill, body condition score and disease status. Variables tested but not significant ($p > 0.05$) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown. Data were collected from 134 cows spanning the period from 10 d before to 7 d after calving. Cow ID was fitted as a random effect.

Model Terms	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	4.538	0.031	138.900	146.819	<0.001
Days in relation to calving					
Linear term	-0.003	0.002	148.500	-1.753	0.082
Quadratic term	-0.001	<0.001	270.900	-2.583	0.011
Birth type					
Twins	Reference				
Female	-0.084	0.033	132.300	-2.558	0.012
Male	-0.062	0.033	133.000	-1.849	0.067
Lactation	-0.016	0.008	148.900	-1.496	0.137
Rumen fill	0.016	0.010	230.600	1.553	0.122
BCS	-0.002	0.020	174.200	-0.088	0.930
Transition disease Yes/No	0.042	0.027	207.900	1.527	0.128

2.4.4 HRV- Root mean square of successive differences between heartbeats (RMSSD)

Dam and calf characteristics that were associated with variation in RMSSD are shown in Table 2-5. RMSSD showed a quadratic relationship with days relative to calving, decreasing over the transition period until a turning point at +1.2 days (Figure 2-1B). Dams carrying dairy calves had lower RMSSD than cows carrying beef crosses (Table 2-5). Cows carrying single calves - whether female (single female vs. twins 5.7±0.2 vs. 4±0.4 ms [raw arithmetic mean ± SEM], multiple comparison test $p<0.001$) or male (single male vs. twins 5.2± 0.2 vs 4± 0.4 ms [raw arithmetic mean ± SEM], multiple comparison test $p=0.04$) had higher RMSSD compared to cows carrying twins. Cows carrying male calves showed a numerically lower RMSSD than cows carrying female calves (single male vs. single female 5.2±0.2 vs. 5.7± 0.2 ms [raw arithmetic mean ± SEM], multiple comparison test $p=0.1$). Lactation number, rumen fill, and BCS were not significantly related to RMSSD.

Table 2-5 Linear Mixed effects Model to assess variation in root mean square of successive differences between normal heartbeats (RMSSD) (natural log transformed) in relation to days relative to calving, calf breed, birth type measured in 134 Holstein cows in a period 10 d before to 7 d after calving with cow ID fitted as a random effect. Variables tested but not significant ($p > 0.05$) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown.

Model Term	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	1.265	0.096	131.482	13.126	<0.001
Days in relation to calving					
Linear term	-0.010	0.005	144.932	-1.878	0.062
Quadratic term	0.003	0.001	210.107	2.726	0.007
Breed					
Beef	Reference				
Dairy	-0.225	0.084	116.305	-2.676	0.009
Birth type					
Twins	Reference				
Female	0.378	0.103	124.264	3.674	<0.001
Male	0.253	0.104	125.247	2.444	0.016

2.4.5 HRV - High frequency (HF)

Table 2-6 shows the dam and calf characteristics that were tested for associations with variation in the HF domain. Cows carrying single male calves had a numerically lower HF than cows carrying twins (regardless of sex) (single male vs. twins 7.6± 0.5 vs. 10.2± 1.3 [raw arithmetic mean ± SEM], multiple comparison test p=0.061).

Table 2-6 Linear Mixed effects Model to assess variation in high frequency domain (natural-log transformed) in relation to birth type measured in 134 cows in a period 10 d before to 7 d after calving with cow ID fitted as a random effect. Variables tested but not significant (p > 0.05) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown.

Model Terms	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	2.201	0.168	134.038	13.127	<0.001
Birth type					
Twins	Reference				
Female	-0.309	0.182	133.141	-1.697	0.092
Male	-0.413	0.185	133.980	-2.238	0.027

2.4.6 HRV- LF/HF ratio

A summary of the results of the statistical analysis testing whether dam and calf characteristics were associated with variation in LF/HF ratio is shown in Table 2-7. Cows carrying single male calves had a numerically higher LF/HF ratio compared to cows carrying twins (regardless of sex) (single male vs. twins 21.5±1.8 vs. 12.3±1.4 [raw arithmetic mean ± SEM], multiple comparison test p=0.08).

Table 2-7 Linear Mixed effects Model to assess variation in low frequency/high frequency ratio (natural-log transformed) in relation to birth type measured in 134 cows in a period 10 d before to 7 d after calving with cow ID fitted as a random effect. Variables tested but not significant (p > 0.05) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown.

Model Terms	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	2.347	0.183	133.469	12.828	<0.001
Birth type					
Twins	Reference				
Female	0.291	0.1987	132.562	1.464	0.145
Male	0.428	0.201	133.411	2.127	0.035

2.4.7 HRV - SD1

Dam and calf parameters that were associated with variation in SD1 are reported in Table 2-8. SD1 showed a quadratic relationship with days relative to calving, decreasing as calving approached until a turning point at +1.2 days (Figure 2-1E). SD1 was lower in dams carrying dairy calves than in those carrying beef crosses ($p=0.009$). SD1 was higher in cows carrying single calves than cows carrying twins (regardless of sex) (multiple comparison test: twins 2.8±0.3 ms vs. female single 4±0.2 ms, $p<0.001$ and male single 3.7±0.1 ms, $p=0.04$, [raw arithmetic mean ± SEM]). SD1 tended to be lower in cows carrying male calves than in cows carrying female calves (single male vs. single female 3.7±0.1 vs. 4±0.2 ms [raw arithmetic mean ± SEM], multiple comparison test $p=0.1$).

Table 2-8 Linear Mixed effects Model to assess variation in SD1 (natural-log transformed) in relation to days in relation to calving, calf breed and birth type measured in 134 cows in a period 10 d before to 7 d after calving with cow ID fitted as a random effect. Variables tested but not significant ($p > 0.05$) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown.

Model Terms	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	0.920	0.096	131.483	9.540	<0.001
Days in relation to calving					
Linear term	-0.010	0.001	144.936	-1.877	0.063
Quadratic term	0.003	0.001	210.113	2.727	0.007
Breed					
Beef	Reference				
Dairy	-0.225	0.084	116.3	-2.675	0.009
Birth type					
Twins	Reference				
Female	0.378	0.103	124.264	3.674	<0.001
Male	0.253	0.104	125.247	2.444	0.016

2.4.8 HRV - SD2

Dam and calf parameters evaluated in relation to variation in SD2 are presented in Table 2-9. SD2 showed a negative linear association with days relative to calving, with a tendency toward a quadratic relationship and a turning point at +4.6 days. Dams carrying beef cross calves had higher SD2 than dams carrying dairy calves (p=0.006). Cows carrying twins (regardless of sex) had a lower SD2 compared to cows carrying single female (twins vs. single female 13.3±1.3 vs. 18.6±0.7 ms [raw arithmetic mean ± SEM], multiple comparison test p=0.001) or male (twins vs. single male 13.3±1.3 vs. 17.4±0.7 ms [raw arithmetic mean ± SEM], multiple comparison test p=0.038) calves. There was no difference in SD2 between cows carrying single male and female calves (single male vs. single female 17.4±0.7 vs. 18.6±0.7 ms [raw arithmetic mean ± SEM], multiple comparison test p=0.191).

Table 2-9 Linear Mixed effects Model to assess variation in SD2 (natural-log transformed) in relation to days in relation to calving, calf breed and birth type measured in 134 Holstein cows in a period 10 d before to 7 d after calving with cow ID fitted as a random effect. Variables tested but not significant (p > 0.05) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown.

Model Terms	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	2.468	0.106	135.741	23.306	<0.001
Days in relation to calving					
Linear term	-0.021	0.005	146.016	-3.960	<0.001
Quadratic term	0.002	0.001	205.898	1.7	0.09
Breed					
Beef	Reference				
Dairy	-0.259	0.093	121.150	-2.801	0.006
Birth type					
Twins	Reference				
Female	0.393	0.113	128.719	3.464	0.001
Male	0.277	0.114	129.617	2.434	0.016

2.4.9 HRV - SD2/SD1 ratio

A summary of the results of the statistical analysis testing whether dam and calf parameters were associated with variation in SD2/SD1 is shown in Table 2-10. The SD2/SD1 ratio showed a negative linear association with days relative to calving, with a tendency toward a quadratic relationship and a turning point at -5.1 days (Figure 2-1G).

Table 2-10 Linear Mixed effects Model to assess variation in SD2/SD1 (natural-log transformed) ratio in accordance with days in relation to calving measured in 134 cows in a period -10 d before to 7 d after calving with cow ID fitted as a random effect. Variables tested but not significant ($p > 0.05$) were removed from the final model (except where retained as lower-order terms in polynomial structures) and are not shown.

Model Terms	Estimate (β)	Std. Error	df	t-value	p-value
Intercept	1.56	0.020	193.200	80.002	<0.001
Days in relation to calving					
Linear term	-0.012	0.003	164.800	-3.762	<0.001
Quadratic term	-0.001	0.000	234.7	-1.750	0.081

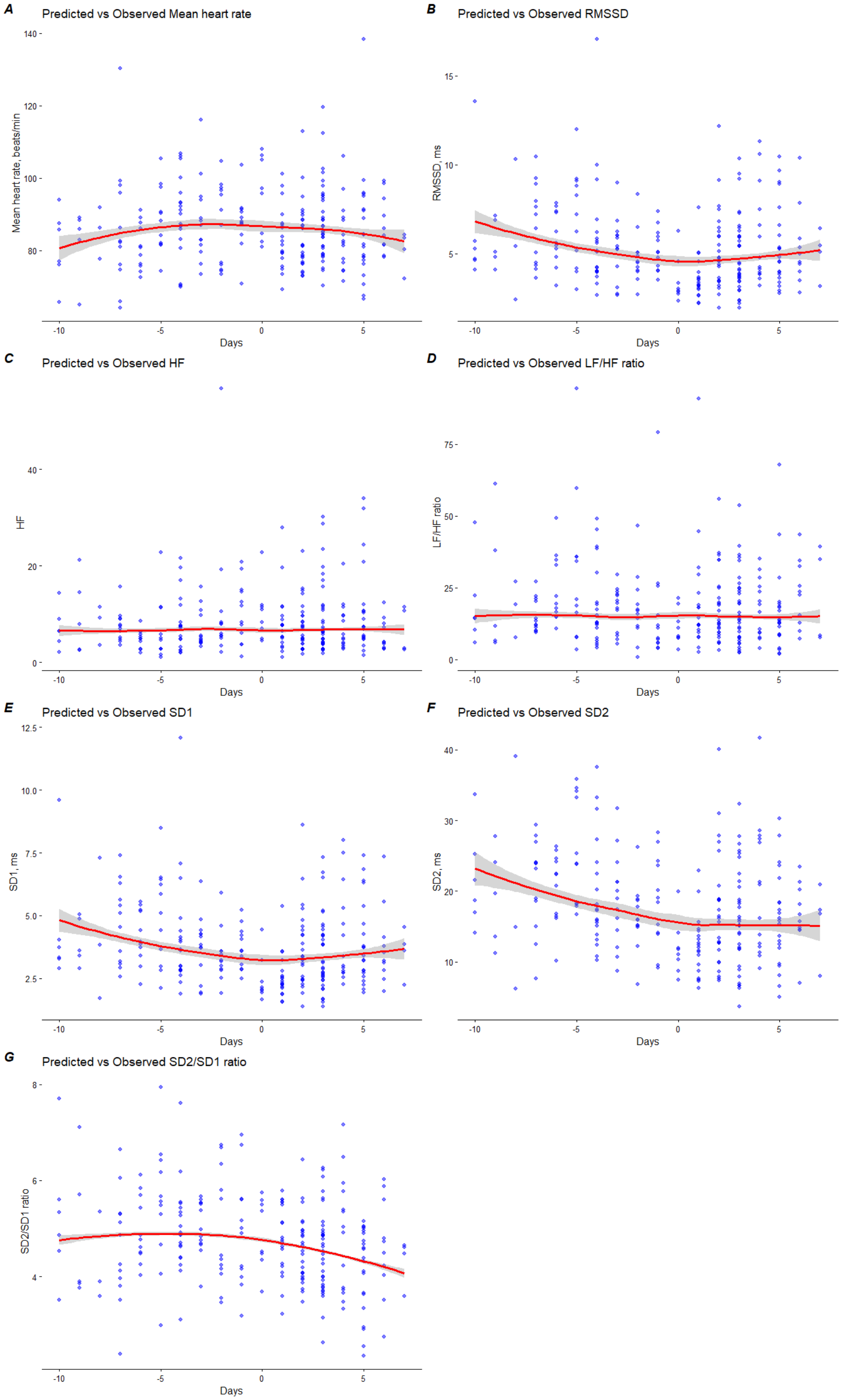


Figure 2-1 Observed heart rate and heart rate variability (HRV) parameters (blue dots) compared with predicted values (red line) in relation to calving, measured in 134 Holstein

cows from 10 days before to 7 days after calving. Grey bands represent 95% confidence interval. HRV parameters comprise: RMSSD (Root mean square of successive differences between normal heartbeats), SD1 (Standard deviation 1), SD2 (Standard deviation 2), SD2/SD1 ratio (Standard deviation 2/Standard deviation 1 ratio), LF/HF ratio (Low frequency/High frequency ratio) and HF (High-frequency power normalised).

2.5 Discussion

This study demonstrated that during the transition period, specifically around the time of calving, multiparous cows had reduced HRV indicative of higher physiological stress and that variation in HRV was more strongly associated with calf as opposed to cow characteristics. It was seen that carrying twins and dairy-sired calves was associated with more physiological stress than single and beef sired calves.

The ability of cows to cope with the physical demands of late gestation, parturition and early lactation is commonly assessed through indicators of metabolic health and has been linked with the incidence of infectious diseases (Drackley, 1999). Further, transition period health has been linked to subsequent milk production and reproductive performance (Drackley, 1999). Therefore, understanding the physiological and pathological changes occurring during this period is of critical importance for both animal health and welfare, as well as economic productivity. To address this, and to gain a measure of the physiological stress that the animals were experiencing, heart rate and heart rate variability (HRV) were opportunistically studied in dairy cattle across the narrow transition period (10 days before to 7 days after calving).

The observed changes in mean HR - an increase as calving approached and a decrease after calving – generally agree with previous studies (Trenk et al., 2015, Kojima et al., 2024). However, the turning point occurred 1.5 days before calving in our study whereas it has previously been reported to occur on the day of calving (Kojima et al., 2024). While tissue remodelling, hormonal changes and the pain and discomfort associated with calving would be expected to be comparable across both studies, a number of factors may have differed, such as genetic background, nutrition and/or environmental conditions, and these may have altered the temporal positioning of the period of maximum physiological stress. Furthermore, heart rate can be influenced by physical activity, and this was not accounted for in either study. In the study performed by Kojima et al., (2024) the animals were unrestrained in a maternity pen compared to the study performed by Trenk et al., (2015) and our cows which were restrained in head yokes. Therefore, the additional stress of restraint during the late stage of gestation could have influenced the turning point in our study.

In the current study, analysis of the HRV parameters RMSSD and SD1, which are accepted indicators of parasympathetic activity, revealed a quadratic concave up (u-shaped) relationship during the transition period. This suggests that physiological stress first increased but then declined across the transition period, but in this case the turning point was on day +1.2. This result supports the conclusions of Kojima et al. (2024), namely that peak physiological stress occurred when animals are experiencing pain, extensive tissue remodelling, and face the increasing metabolic demands associated with the onset of lactation and further supports the interpretation that cows experience the greatest stress response at the time of calving (Hudson et al., 1976). These results are broadly in agreement with those of acute studies of HR and HRV at calving, which reported that HR and vagal activity increased at the time of calf expulsion and that sympathetic activity peaked 4-8 h after calving (Kovács et al., 2015c, Nagel et al., 2016) and returned to pre-calving levels (96-72 h before calving) within 48 h.

The changes in the ratio of SD2/SD1, which is often used as a measure of sympathovagal balance (Shaffer and Ginsberg, 2017), showed a different profile compared to RMSSD and SD1 with a linear decline observed throughout the study period. In our study, we also observed a linear decline in SD2 during the 10 days before to 7 days after calving. These longer-term changes (slower return to normal values or even linear decline) in HRV potentially indicate that the timeline (96-72h before and 48h after calving) used by Kovács et al. (2015c) could have underestimated the baseline values of these parameters. In contrast, frequency domain parameters did not follow the same pattern as the other parameters nor those reported by Kovács et al. (2015c); instead, they remained stable throughout the study. The patterning of the HRV results obtained in this study were similar to those we have reported previously (Frei et al., 2022) as such it might be that some of the differences between studies may be the result of the HRV measuring techniques used. For example, it may be a feature of the posture of the cows as in the study by Kovács et al. (2015c) the cows were lying down, whereas in the current study and that by Frei et al. (2022) the cows were standing and it has been reported that cows have a higher HF and lower LF/HF ratio when lying down (Frondelius et al., 2015).

We anticipated that HRV parameters would show associations with transition disease and the period after calving as they are likely associated with pain and stress. Qu et al. (2014) reported increased inflammation in cows with transition disease shown by increased concentrations of the inflammatory marker haptoglobin for 7-14 days after calving and increased beta-hydroxybutyrate in the first week after calving. However, the more prolonged elevation of these markers, relative to the markers of stress used in the current study or cortisol used by Hudson et al. (1976) may reflect the fact that these markers can be stimulated by the genomic actions of cortisol which are longer lasting (Yeager et al., 2004, Corvo et al., 2020). The inflammation and pain associated with calving, combined with negative energy balance due to the increased energy demands of milk production, may result in animals experiencing more or different forms of chronic stress which might have masked the effect of transition disease.

In contrast to our hypothesis, RMSSD and SD1 were lower in cows carrying dairy calves, which suggests higher physiological stress in dams with dairy than beef calves. One possible explanation is the fact that dams carrying beef-cross calves have a longer gestation length than dams with dairy calves (Basiel et al., 2024). Twin pregnancies also have a reduced gestation length (Echternkamp and Gregory, 1999) and were associated with reduced RMSSD and SD1 in this study. In a study by Cook et al. (2024) the principal component of gestation length and days in close up showed an asymmetric, tick-shaped quadratic association with disease incidence and a positive association with milk production. Taken together, these findings suggest that the shorter gestation length observed in cows carrying dairy calves could result in a shorter dry and close-up period, which may contribute to the higher stress levels recorded in these animals.

We also considered whether maternal stress might vary according to pregnancy type, specifically twin versus single pregnancy. We had hypothesised that twin pregnancies would be associated with greater physiological stress as the combined birth weight of twins exceeds that of single male or female calves (Kertz et al., 1997). Twin pregnancies are therefore expected to impose a greater physiological load. Indeed, the 50 to 70% greater energy demand reported for twin pregnancies (Koong et al., 1982, Nishida et al., 1997) and a more pronounced decrease in dry matter intake before calving (Bell and Roberts, 2007) were consistently associated with lower HRV (RMSSD, SD1, SD2) and higher HR, patterns that may indicate higher physiological stress (Kovács et al., 2015c). The results of the current study supported our hypothesis and are also in agreement with findings pertaining to HPA axis activity (Patel et al., 1996) all of which suggest that carrying twin calves is associated with higher physiological stress.

Maternal factors of rumen fill, lactation number and BCS were not significantly associated with variations in HR or any of the studied HRV parameters. While the lack of an association between HRV and BCS differs from the findings of Frei et al. (2022), who reported significantly higher HF in cows with higher BCS, the cows in the two studies were in different physiological states. In Frei et al. (2022) the cows were lactating and therefore in a state where metabolic demands and nutritional intake could be mismatched, leading to metabolic stress (Broster and Broster, 1998) whereas in the current study the animals' metabolic demands may have been more closely matched to their nutritional intake. The lack of association between lactation group and HRV parameters in our study also contrasts with Kovács et al. (2015a), who reported lower RMSSD in second lactation cows compared with third and fourth lactation animals. This discrepancy may reflect the fact that their measurements were taken in mid-lactation cows under lesser metabolic load. We had hypothesised that lower HRV would be seen in cows with transition disease compared to healthy cows. This relationship was not seen with any HRV indicators related to increased sympathetic or decreased parasympathetic nervous system activity. The lack of a relationship, however, may reflect the low incidence rate of transition diseases in the animals in the current study during the narrow transition period. While changes in HRV have been linked to disease in cattle, for example, cows with fever after calving have an increased mean HR, total power, LF and LF/HF ratio (Aoki et al., 2020), a link between HRV parameters and disease is also questioned by the results of a study in calves with diarrhoea, which showed decreased vagal and sympathetic activity (Mohr et al., 2002). Furthermore, studies of HRV and bovine spongiform encephalopathy have produced contradictory results with some reporting increased RMSSD (Pomfrett et al., 2004) and HF power (Little et al., 1996), while others found no changes (Konold et al., 2011). Studies have also reported that vagal tone was increased during handling and lameness (Frondelius et al., 2015, Kovács et al., 2015a). Contrary to expectations, chronic lameness was associated with increased RMSSD and HF, and decreased mean heart rate, LF/HF and SD2/SD1 (Kovács et al., 2015a). Lameness is associated with increased lying which may increase vagal and decrease sympathetic activity (Frondelius et al., 2015).

When interpreting the results of this study, it is important to acknowledge several limitations. The work was conducted on a relatively small number of multiparous cows, all from a single farm, and each animal was sampled only twice. Primiparous cows were not included, as they were housed separately without handling facilities, which means that some variability associated with lactation number may not have been captured. In most field studies, farm itself is a significant variable (Frei et al., 2022), as management, environment and nutrition differ across sites. Therefore, the generalisability of our findings is limited and further studies are needed to assess whether similar patterns are observed elsewhere. Additional data, such as calf birth weight or the combined weight of twin calves, would allow more precise conclusions on how calf weight relates to HRV parameters. Likewise, continuous measurement of HRV would provide a clearer picture of changes across the transition period. Finally, sampling a larger cohort, including cows with transition disease, would improve the ability to detect smaller associations between HRV and transition disease outcomes.

The study found that dairy sired calves and twins were associated with more stress which could be considered when making breeding decisions to minimise stress in the transition period and possibly subsequently improve later production. Therefore, keeping dairy semen use to a minimum by only breeding enough cows to secure enough replacements heifers for the herd and by using sexed semen might not only be a sound economic but also a welfare decision. The termination of twin pregnancies is more controversial; however, extra care could be provided (e.g. extra lying and feeding space, longer dry and close-up period) to cows known to be carrying twins to minimise stress around the time around calving. Cows carrying twins could also be nutritionally managed as a separate group as is common practice in sheep farming, due to extra metabolic demand and reduce feed intake (Planet-Dubé et al., 2025).

2.6 Conclusions

This study highlights the utility of heart rate variability (HRV) as a non-invasive measure of autonomic nervous system activity and stress in dairy cows. Based on changes in HRV parameters observed in cows across the narrow transition period, there was a switch from parasympathetic to sympathetic dominance during the pre-parturient period, suggesting heightened stress around calving. This pattern aligns with previous studies measuring HRV and cortisol concentrations.

Calf characteristics, particularly twin pregnancies, were associated with significantly reduced HRV and increased HR, consistent with elevated maternal stress. In contrast, maternal factors (BCS, rumen fill and lactation number) showed no association with HRV indices or mean HR. HRV was not associated with disease incidence, which may reflect study design or sampling constraints. Overall, these findings emphasise the importance of supportive management strategies for animals carrying twins to improve both welfare and subsequent productive and reproductive performance.

Chapter 3: Assessing environmental variables in a commercial dairy herd that could impact performance and welfare: validation of a custom-built sensor against a commercial device

3.1 Abstract

Cattle are affected by many environmental stressors, which can affect cattle welfare, behaviour, milk production and reproduction. This study investigated the microclimatic variation across different pens of a commercial dairy farm. Environmental parameters (temperature, relative humidity, and light intensity) were monitored inside five adult cow pens and the milking collecting yard using commercial loggers over a 13-month period and compared against regional satellite weather data. To assess broader air quality metrics, four custom-built sensors recording additional variables (ammonia and particulate matter sizes PM_1, $PM_{2.5}$, PM_{10}) were trailed for two months. Indoor barn temperatures and Temperature Humidity Index (THI) values were consistently higher than outdoor measurements (up to 4.0°C and 7.1 units higher, respectively). Significant microclimatic drops occurred when pens were emptied for milking, while severe, acute environmental spikes were recorded in the high-density collecting yard, where THI values frequently exceeded stress thresholds (THI> 62) even during winter. Due to differences in building orientation and design, cows at identical geographical coordinates experienced discrepancies in day length of up to 6 hours at the 100-lux production threshold. The air quality metrics remained safely below regulatory limits. The custom-built sensors demonstrated lower operational reliability and data-recording durability than commercial devices. However, the custom sensors exhibited strong statistical agreement with commercial loggers for temperature (R^2 = 0.80–0.96), indicating high potential for affordable temperature mapping. Overall, these findings highlight that regional weather data heavily underestimates indoor cattle stress, emphasizing the need for pen level environmental measurements to safeguard cow welfare and maximize herd productivity.

3.2 Introduction

Cattle are affected by many environmental stressors such as cold, heat, humidity, wind, direct sunlight and photoperiod. These stressors can affect cattle welfare, behaviour, milk production and reproduction (Bova et al., 2014). Most production dairy cows in Scotland are housed for at least some part of the annual production cycle (Shortall and Lorenzo-Arribas, 2022). Housed dairy cattle are exposed to artificial microclimatic conditions in barns (Marumo et al., 2022). Housed cows are exposed to artificial light, which (if strong enough) affects their perception of day and night in the same way as natural light (Buchanan et al., 1992; Muthuramalingam et al. 2006). Cows that are housed under continuous light do not recognise day length anymore and default to a short photoperiod (Dahl et al., 2000).

Most environmental studies on the effect of the shed environment on adult cows are related to heat stress and production (Hill and Wall, 2015, Gernand et al., 2019). Research that studied the effect of the environment on dairy cow production usually use information from nearby weather stations. Data from the nearest weather stations may be limited by temporal gaps and low spatial resolution. National Aeronautics and Space Administration Prediction of Worldwide Energy Resources (NASA POWER) a satellite based environmental data collection tool is a good alternative as it provides reliable information at a higher spatial resolution (Rockett et al., 2023). Compared to the data of the outside environment, sheds are usually hotter and less humid (Schüller et al., 2013). Therefore, the microclimate in the barn would represent a more accurate representation of the environmental stressors cows are exposed to.

Environmental parameters inside sheds are dependent on shed design and the assessment of environmental parameters will be influenced by sensor placement as proximity to ventilation inlets will influence results (Schüller and Heuwieser, 2016). The environment animals are exposed to depends on the distance from fresh air inlets and areas of animal crowding such as the collecting yard and milking parlour (Schüller and Heuwieser, 2016). There are few studies that have assessed the microclimate in calf sheds (Leenen et al., 2020, Mahendran et al., 2023) but little work has been done on adult cow sheds. A study on microclimate variation in adult cows sheds throughout the year found that insulated barns had a significantly higher indoor temperature during winter compared to uninsulated barns (Teye et al., 2008). However, the conditions in barns may be less favourable in summer as the temperature humidity index (THI) may be higher (Marumo et al., 2022). Stationary cow environment loggers can underestimate the heat stress experienced by cows. Studies usually place a sensor in one location in the animals' pen (Gernand et al., 2019), which might miss important variation in environmental parameters in areas where animals comingle at a higher stocking density such as the collecting yard. In one study, cow mounted loggers were above a THI threshold of 72 for 13% longer compared to stationary logger (Schüller and Heuwieser, 2016). The same study also found a decrease in temperature, RH and THI in the pens when the animals were being milked. Therefore, when studying the microclimate inside a barn, the location of animals inside the barn has to be accounted for when using stationary loggers. Commercial loggers usually offer higher reliability that is guaranteed by the manufacturer, which comes at a cost. However, the barn environment is specific and might the sensor might come into contact with animals which might prove to be too harsh even for commercial sensors. Therefore, less expensive custom-build sensors might be a good alternative as they can be more easily replaced and they may offer a broader range of measured variables for a fraction of the price. However, the reliability and accuracy of these sensors need to be validated as they don't come with a guarantee from the manufacturer.

The aims of this study were to assess the variation of temperature, humidity and light intensity inside sheds of a commercial dairy farm in Scotland. We also aimed to assess the influence of the presence of animals in the pen on temperature and humidity inside the pen and to compare the microclimate inside the shed between different pens and to the outside environment. Our last aim was to assess the durability and accuracy of custom-build sensors compared to commercial sensors.

3.3 Methods

Farm

Work was conducted on a commercial dairy farm, milking 800 cows. The dairy cows were housed all year round and they were milked three times a day with an average standard lactation (305 days) milk yield of over 10,000 L/cow. The farm managed their dairy cows in four milking groups (fresh [until 7-10 days in milk (DIM)], primiparous [7-10 DIM – until pregnant or milk drops below 30 L/day], high-yielding ("Highs") [7-10 DIM – until pregnant or milk drops below 30 L/day], and mid-yielding ("Mids") [pregnant or milk below 30 L/day]), and two dry cow groups (far-off [7 - 3 weeks before calving] and close-up [3 weeks before calving]). Milking was conducted between the hours of 3:30 and 7:04, 11:30 and 15:11, and 19:31 and 23:00. The fresh cows entered the parlour first followed by the Primiparous, Highs and Mids. Milking of each group took approximately 60 minutes except for the fresh group which was milked in 10 min (Table 3-1). Cows were confined to the collecting yard in high stocking density conditions at milking.

Table 3-1 Average milking start and stop times per pen and milking. The farm milked three times a day.

	Start 1	Stop 1	Start 2	Stop 2	Start 3	Stop 3
Fresh	03:30:26	03:40:09	11:30:20	11:37:20	19:31:00	19:34:30
Primiparous	03:42:34	04:32:13	11:41:00	12:37:40	19:37:50	20:30:30
Highs	04:34:30	05:41:09	12:39:20	13:47:50	20:32:50	21:38:30
Mids	05:44:09	07:04:04	13:50:40	15:11:30	21:42:10	23:00:20
Total	04:22:55	05:14:24	12:25:20	13:18:35	20:20:57	21:10:57

Housing

Cows were housed in a free stall cubicle system except for the fresh cows which were housed in a straw yard. The barns were positioned in E-W orientation. They were steel framed covered by spaced (10 cm) corrugated composite sheets and had several skylights seen in Figure 3-1. The main shed (Close up/Fresh, Primiparous and Highs) was open on the south side for the length of the Highs pen (70m) (Figure 3-). The rest of the shed was covered by corrugated vented metal panels. The panels were separated by one empty panel length to aid ventilation. The roof had an open ridge down most of its length excluding the parlour area. The Highs pen was the only pen with 4 fans installed every 15m, angled towards the animals over the cubicles on the south side. The Mids shed was open on both long sides and had an open ridge roof. The Top shed was open on the north side, and the rest of the sides were covered by corrugated vented metal panels. The floors were solid grooved concrete. Slurry was scraped 3 times a day while the cows were being milked. The stocking capacity of the pens can be seen in Table 3-2. All the lactation pens were usually overstocked from 133 to 173% based on lying space (1.1 cubicle or 11-14 m^2 per cow), 120 to 201% based on feeding space (0.56-0.66 m per cow) and 123 to 290% based on water space (0. 65-0.09 m per cow) (Tyson, 2017, Jones and Kammel, 2017).

Table 3-2 Stocking capacity of the pens based on lying (1.1 cubicle or 12 m2 per cow), feed (0.6 m per cow) and water (0.09 m per cow) space (Tyson, 2017, Jones and Kammel, 2017).

Pen	**Lying spaces (animals)**	**Feed space (animals)**	**Water space (animals)**
Primiparous	168 (153)	75m (125)	16.5m (183)
Highs	200 (182)	140m (233)	13m (144)
Mids	238 (216)	150m (250)	11.5m (128)
Close up/Fresh	20/20	21m (35)/ 21m (35)	3.8m (38)/3.8m (38)

Environmental data collection

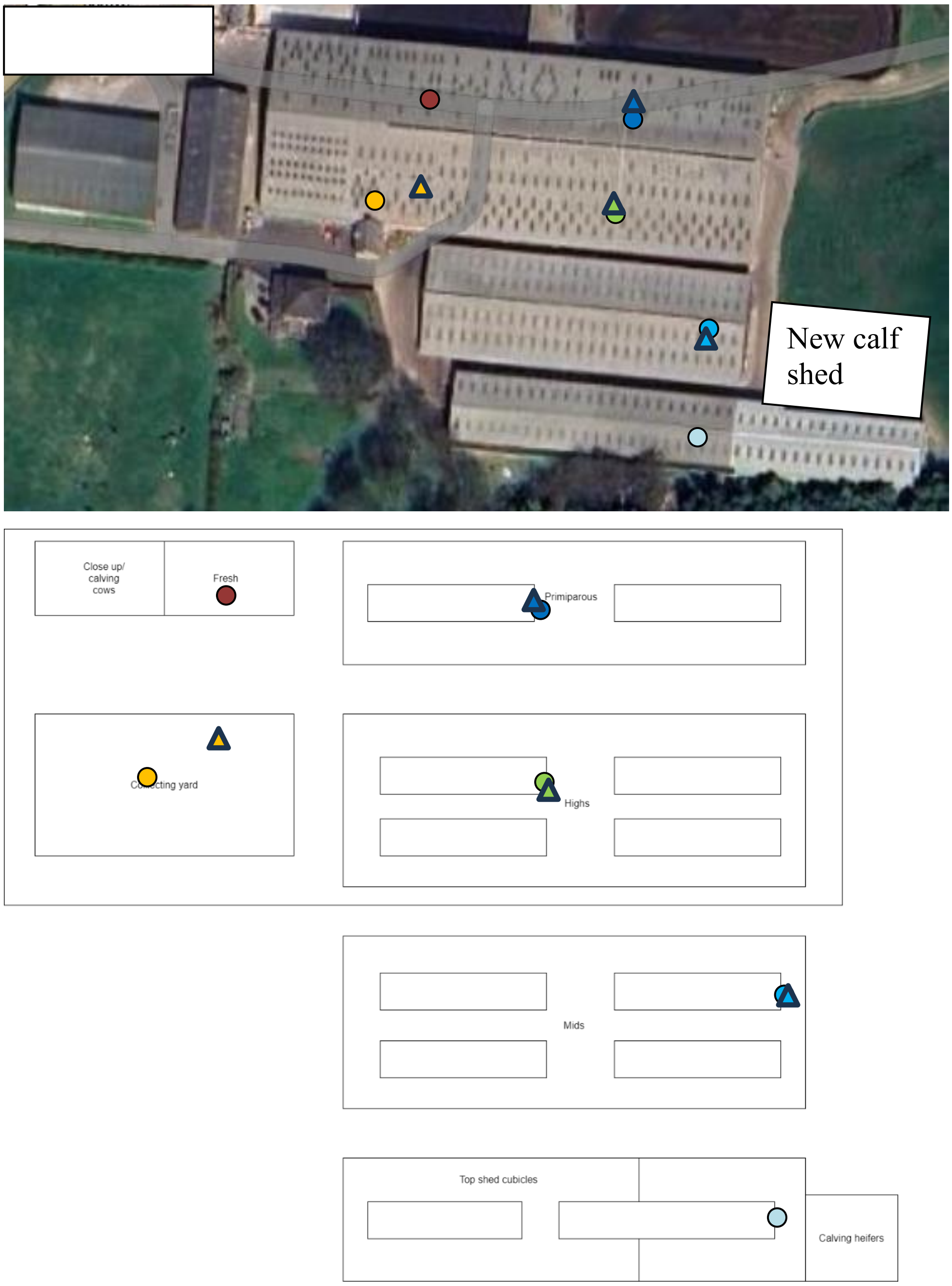
Commercial data loggers HOBO MX1104 (Onset Computer Corporation, USA) were used to collect data on the temperature (range -20-70°C, accuracy ±0.20°C from 0° to 50°C), relative humidity (range 0%-100% at -20° to 70°C, accuracy ±2.5% from 10% to 90%) and light (range 0-167,731 lux, accuracy ±10% typical for direct sunlight) across the areas in which cows were housed (Closeup/Fresh, Primiparous, Highs, Mids, Tops shed) and milked (Collecting yard) (Figure 3-1). The loggers were placed slightly above cow level, and away from direct sunlight and draughts. The data logger measured the environmental parameters in the area near water troughs. Data were collected every 30 min from 27/7/2023 to 29/8/2024. However, some intermittent data points were lost where there were battery or sensor failures.

Four custom-built Enviro + Air Quality data loggers (Pimoroni, UK) were used to record temperature (range -40-85°C, accuracy ±0.5°C), relative humidity (range 0-100%, accuracy ±3%), light levels (0.01–64,000 lux, accuracy not given), air pressure (range 300-1100 hPa, ±1hPa), ammonia $(NH)_3$ (range 1-300 ppm, accuracy- Not given) and air particles PM 1, 2.5 and 10 (range 0-500 μg/m^3, accuracy ± 10 μg/m^3). The custom-built loggers were also placed slightly above cow level, and away from direct sunlight and draughts in the Primiparous, Highs and Mids pens and the collecting yard. The data logger measured the environmental parameters in the area near water troughs. Custom-built data loggers collected environmental data every 30 min between 1/10/2023 and 27/11/2023.

The Temperature humidity index (THI) for both types of sensors was calculated using the following formula (NRC, 1971):

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

where T is the temperature, and RH is the relative humidity. Day was defined by light intensity higher than 100 lux for day length calculations.



Custom-build (triangles) / commercial sensors (circles):

- Close up/Fresh
- Primiparous
- Highs
- Mids
- Top shed-Close up heifers and small cows
- Collecting yard

Figure 3-1 Location of commercial and custom-built sensors on farm and in pens. Commercial sensors recorded temperature, relative humidity and light and custom-build sensors recorded temperature, relative humidity, light levels, air pressure, ammonia and air particles.

Outside environmental information

The daily average relative humidity (in %), temperature (in °C), from 27/7/2023 to 29/8/2024 were downloaded from National Aeronautics and Space Administration Prediction of Worldwide Energy Resources (NASA POWER) (https://power.larc.nasa.gov/). THI was calculated as above. NASA POWER provides solar and meteorological data sets for any location based on satellite observations and models. NASA POWER data are averaged over 0.5-degree latitude by 0.625-degree longitude geographical grids. The data was obtained from the POWER Project's Hourly version 2.5.25 on 19/12/2025. NASA POWER was selected as the preferred weather data source as weather stations have a low spatial resolution and can have site-to-site variability due to human or machine error (Rockett et al., 2023).

Statistical analysis

Data were analysed using R statistical software 4.2.0 (R-Core-Team, 2025). Graphs were made using the “ggplot2” package (Wickham, 2016). Descriptive statistics were produced to describe the variation of all the measured variables between pens and the outdoor environment throughout the year for the commercial sensors. One-way ANOVA (using the “aov” function) and post hoc pairwise comparisons using Tukey adjustment were used to compare pens. The averages for subtraction of variables between pen and outdoor (NASA POWER) when animals were in the pens and when they were out for milking based on Table 3-1 for the Primiparous, Highs and Mids pens and the collecting yard were compared between pens using a linear mixed-effects model and post hoc pairwise comparisons using Tukey adjustment using the “emmeans” package. The Close-up/Fresh pen was not included as the milking was too short to be captured by the environmental sensors. Graphs were also made for the comparing the daily variation of all the measured parameters between pens throughout the year. Comparison between the commercial and custom-build sensors was performed using linear regression models. The linear regression models and the linear mixed-effects model were run using the “lme4” package.

3.4 Results and Discussion

Dynamics of environmental parameters throughout the year using commercial sensors for indoor conditions and NASA POWER data for outdoor conditions on a dairy farm in Scotland (27/7/2023-29/8/2024)

Temperature

Both indoor and outdoor temperature showed a clear decline during winter and reached their lowest point in January 2024. Peak temperature was reached in September 2023 (Figure 3-2). The Top shed with close-up heifers and smaller statured cows had the lowest temperature, most likely due to the lower stocking density and openness of the shed ($p>0.001$, Table 3-3). The Primiparous shed was the hottest on average ($p>0.001$). This pen had no fans and was relatively closed off compared to other pens. The highest recorded temperature was in the collecting yard (Table 3-3). The temperature in the sheds was numerically higher than the outdoor temperature for most of the year (Figure 3-2). Surprisingly, the lowest measurement of temperature recorded was in the Top shed (Table 3-3). This was most likely due to a recording error as this was lower than the lowest recorded temperature outdoor. The mean temperature of the shed was 1.9˚C and 4˚C higher in the coldest and hottest pen compared to the outdoor temperature. A Finnish study found that uninsulated sheds had a 3 ˚C higher temperature compared to the outdoor temperature (Teye et al., 2008). In Estonia, uninsulated sheds had a similar temperature compared to the outdoor during summer but a 3-4 ˚C higher temperature in winter (Pajumägi et al., 2007). A study from small dairy holdings in Vietnam, found an association of summer ambient temperature inside sheds with eave hight and altitude (Bang et al., 2021).

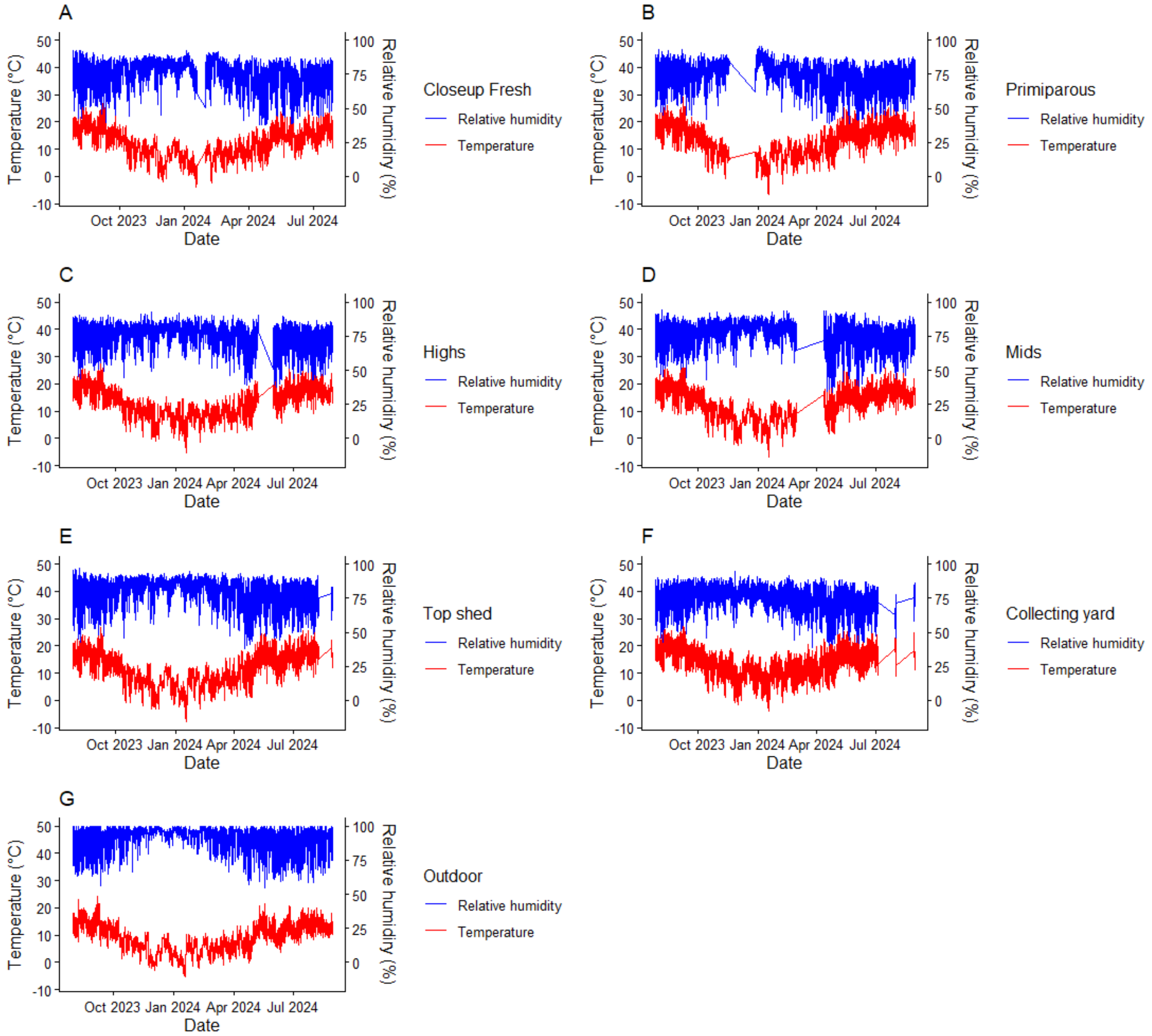


Figure 3-2 Variation in temperature and relative humidity between pens and throughout the year using commercial sensors for indoor conditions and NASA POWER data for outdoor conditions on a dairy farm in Scotland.

Table 3-3 Variation in temperature between pens throughout the year using commercial sensors for indoor conditions and NASA POWER data for outdoor conditions on a dairy farm in Scotland. The lowest value is coloured blue and the highest red.

	Min (˚C)	Median (˚C)	Mean (˚C)	Max (˚C)
Close-up/Fresh	-4.1	11.7	11.6	28.4
Primiparous	-6.5	13.3	12.9	29.1
Highs	-5.1	12.4	12.5	28.8
Mids	-6.7	11.9	11.7	29.4
Top shed	-7.6	10.8	10.8	26.9
Collecting yard	-4.1	12.7	12.6	29.9
Outdoors	-5.45	9.0	8.9	24.3

Temperature in the pens showed daily variation with a peak between 15:00 and 16:00 and a nadir around 6:00. Temperature appeared to depend on the presence of animals in the pens as the temperature dropped when the animals were being milked Table 3-4. Further, the temperature in the collecting yard had three distinct daily peaks, each of which coincided with milking times (Figure 3-3). During milking the density of the animals was greater in the collecting yard compared to the pens which could explain the greater range. The shape of the temperature curve in the Mids pen differed from the others in that it seemed to have plateaued when the others peaked (Figure 3-3). This might have been the result of the position of the sensor as it was located on the east side of the pen compared to the middle of the pen position in other pens (Figure 3-). The outside temperature peaked around 13:00 and reached its nadir at around 03:00, which is 2-3 hours earlier than inside the pens. This could be the result of the buildings additionally being heated by the sun.

Table 3-4 Mean differences in pen and outdoor temperature, relative humidity, and Temperature Humidity Index (THI) throughout the year between periods when animals were inside versus outside the pen on a dairy farm in Scotland. Differences were assessed using a linear mixed-effects model, followed by post hoc pairwise comparisons of estimated marginal means with Tukey adjustment (n=52,699).

3	**Temperature difference (˚C)**			**Relative humidity difference (%)**			**THI difference**		
	In	Out	P- value	In	Out	P- value	In	Out	P- value
Primparous	3.5	2.7	<0.001	-15.5	-15.3	0.537	6.2	5	<0.001
Highs	3.9	3.2	<0.001	-15.7	-14.1	<0.001	7	5.7	<0.001
Mids	3.2	2.4	<0.001	-13.9	-12.3	<0.001	5.7	4.2	<0.001
Collecting yard	6.1	2.8	<0.001	-16.2	-14.5	<0.001	10.5	5.3	<0.001

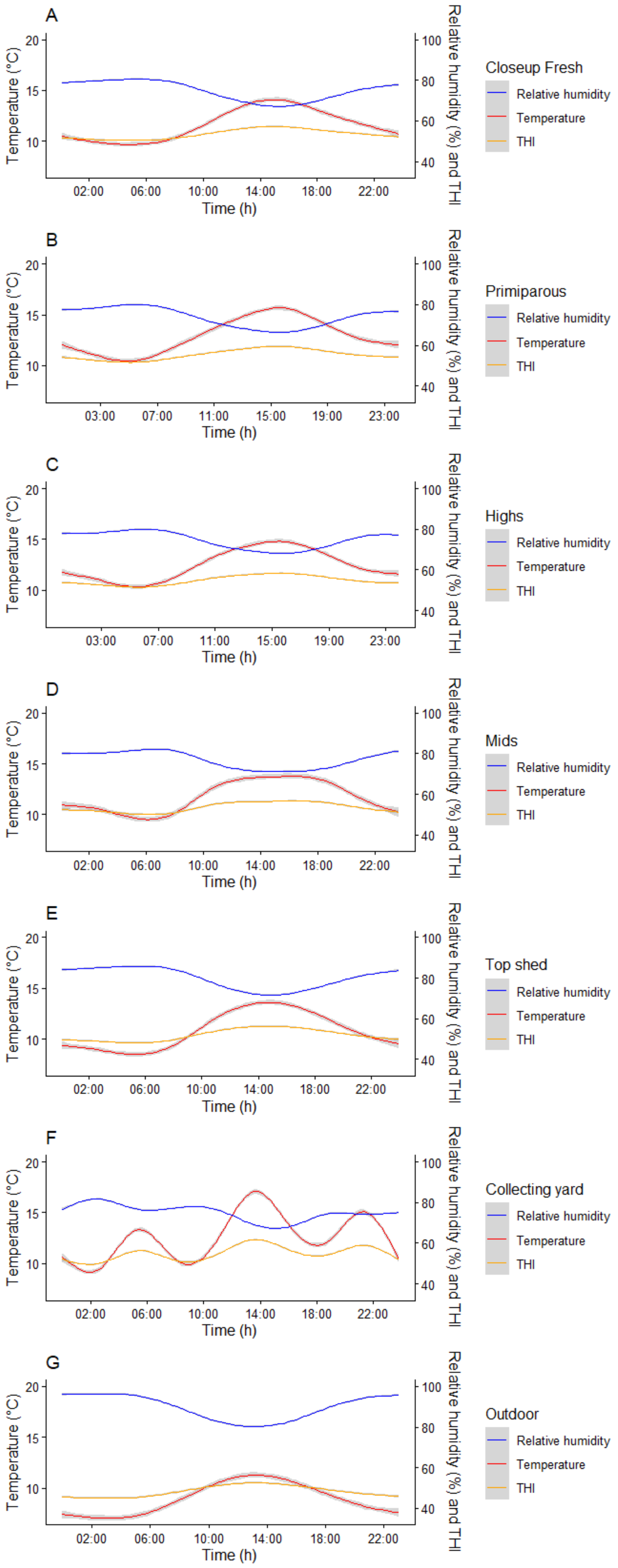


Figure 3-3 Average daily variation in temperature, relative humidity and THI between pens

throughout the year using commercial sensors for indoor conditions and NASA POWER data for outdoor conditions on a dairy farm in Scotland.

Relative humidity

Compared to temperature, relative humidity (inside and outside) was more stable throughout the year but showed large variations during each day inside the sheds (Figure 3-2). The outside environment was more humid than any of the pens and the relative humidity dropped when animals were in the pens (Table 3-4), suggesting that relative humidity is influenced more by temperature than by the presence of animals. On average the outside air was 10 and 16% more humid than the most and least humid pen, respectively. The Top shed was the most and the Primiparous pen the least humid ($p<0.001$) which is expected as relative humidity is negatively related to temperature (Table 3-5). The Top shed pen was the most open to the outside environment.

Table 3-5 Variation of relative humidity between pens throughout the year using commercial sensors for indoor conditions and NASA POWER data for outdoor conditions on a dairy farm in Scotland. Lowest value is coloured blue and highest red.

	Min (%)	Median (%)	Mean (%)	Max (%)
Close-up/Fresh	37.7	77.5	74.8	100
Primiparous	39	75.9	73.9	95.8
Highs	37.6	77	74.7	92.4
Mids	34	79.1	77	94.1
Top shed	37.8	82.9	79.9	96.7
Collecting yard	38.5	76.7	75.1	94.5
Outdoors	54.4	93.7	89.9	100

Relative humidity in the pens was inversely related with temperature, reaching its peak around 06:00 and its nadir around 15:00 (Figure 3-4). The same was also true for the collecting yard, which was surprising as relative humidity was expected to rise due to the presence of animals. The ventilation in the collecting yard might have been sufficient to lower the extra humidity from the animals but not effect overall temperature and relative humidity. The relative humidity in the pens increased due to the absence of animals during milking and the decrease of the temperature in the pen (Table 3-4). Outdoor, the peak of relative humidity was reached around 3:00 and nadir around 13:00, which is 2-3 h earlier than inside the pens. This is most likely following the increase of the temperature in the pens. A study in open uninsulated sheds found 3-8% higher relative humidity levels in sheds compared to outdoor in winter (Pajumägi et al., 2007). This does not agree with the findings of our study.

Temperature humidity index

THI followed a similar trend to temperature (Figure 3-3). As for temperature the pen with the lowest average THI was the Top shed pen and the highest average THI was the Primiparous pen (Table 3-6). However, the highest THI value was recorded in the collecting yard. Most likely due to being empty for some time when there was no milking and the higher stocking density for short periods during the day when milking. The average THI throughout the year was above 50 in all pens Table 3-6. THI has been related to fertility in dairy cattle, and the THI cut-off for fertility impairment can be as low as 50 for the interval from calving to first insemination and 60 for conception rate (Ojo et al., 2024). Milk yield and milk solid yields are also impaired above THI 62 (Hammami et al., 2013). The cutoff of 62 was regularly exceeded in most pens from June until mid-September. The threshold was often exceeded in the collecting yard even in winter. However, the exposure might not be long enough to cause detrimental effects. The average peak THI in the collecting yard throughout the year was 63 at mid-day milking (Figure 3-3). The outdoor THI was lower than any of the pens. On average the outside THI was 3.7 and 7.1 units lower than the THI in the lowest and highest pens, respectively. Haskell et al., (2013) found that cows prefer to use outdoor loafing areas when THI increases as the THI in the loafing area was lower compared to inside.

Table 3-6 Variation in Temperature Humidity Index (THI) between pens throughout the year using commercial sensors for indoor conditions and NASA POWER data for outdoor conditions on a dairy farm in Scotland. The lowest value is coloured blue and highest red.

	Min	Median	Mean	Max
Close-up/Fresh	28.3	53.7	53.4	77.8
Primiparous	24	56.2	55.4	79.7
Highs	27	54.8	54.8	78.6
Mids	23.6	53.9	53.5	79.6
Top shed	21.6	52.1	52	75.1
Collecting yard	29.3	55.2	55	81.1
Outdoors	22.2	48.7	48.3	72.3

Given the formula used THI is mostly associated with temperature changes. It was positively influenced by the presence of the animals in all pens (Table 3-4).

Light and day length

Day length with light intensity above 100 lux varied throughout the year and reached its peak at the end of June and its nadir at the end of December (Figure 3-6). The longest day was around 14h and thus never exceeded the 16h per day threshold that would increase milk production based on the literature (Dahl et al., 2000; Lim et al., 2021). The day length in the Top shed pen was substantially longer compared to the others especially the Mids pen and the collecting yard. In the Highs pen day length did not decline after June 2024, which coincides with the installation of new lights on the 4/6/2024. There were days in winter when the 100-lux threshold [threshold for increasing milk yield (Lim et al., 2021)] was not reached (Figure 3-6). Light intensity showed seasonal variation with a peak in summer and a nadir in winter (Figure 3-5). As there were random spikes of high light intensity throughout the year and the pens (Figure 3-5) which we speculate were errors in recording or interference, the median was compared between pens. This showed that the Mids was the darkest and the close-up/Fresh pen the lightest pen Table 3-7. Variation in light intensity was also seen in another study which reported a decrease of light intensity toward the North side of the shed and as a result of shadows from other buildings (Pajumägi et al., 2007). The average light intensity for the two sheds studied was 1350 and 176 lux in winter and 116 and 1270 in the summer, respectively (Pajumägi et al., 2007). Interestingly, the average light intensity was opposite for the sheds depending on season. The authors did not explain this result even though the light intensity of the outdoor measurement was higher at the first shed in both seasons. There was also no data on artificial lighting in the studied sheds. Our sheds appeared to be darker compared to the sheds in the former study as our median light intensity ranged from 59 to 227.

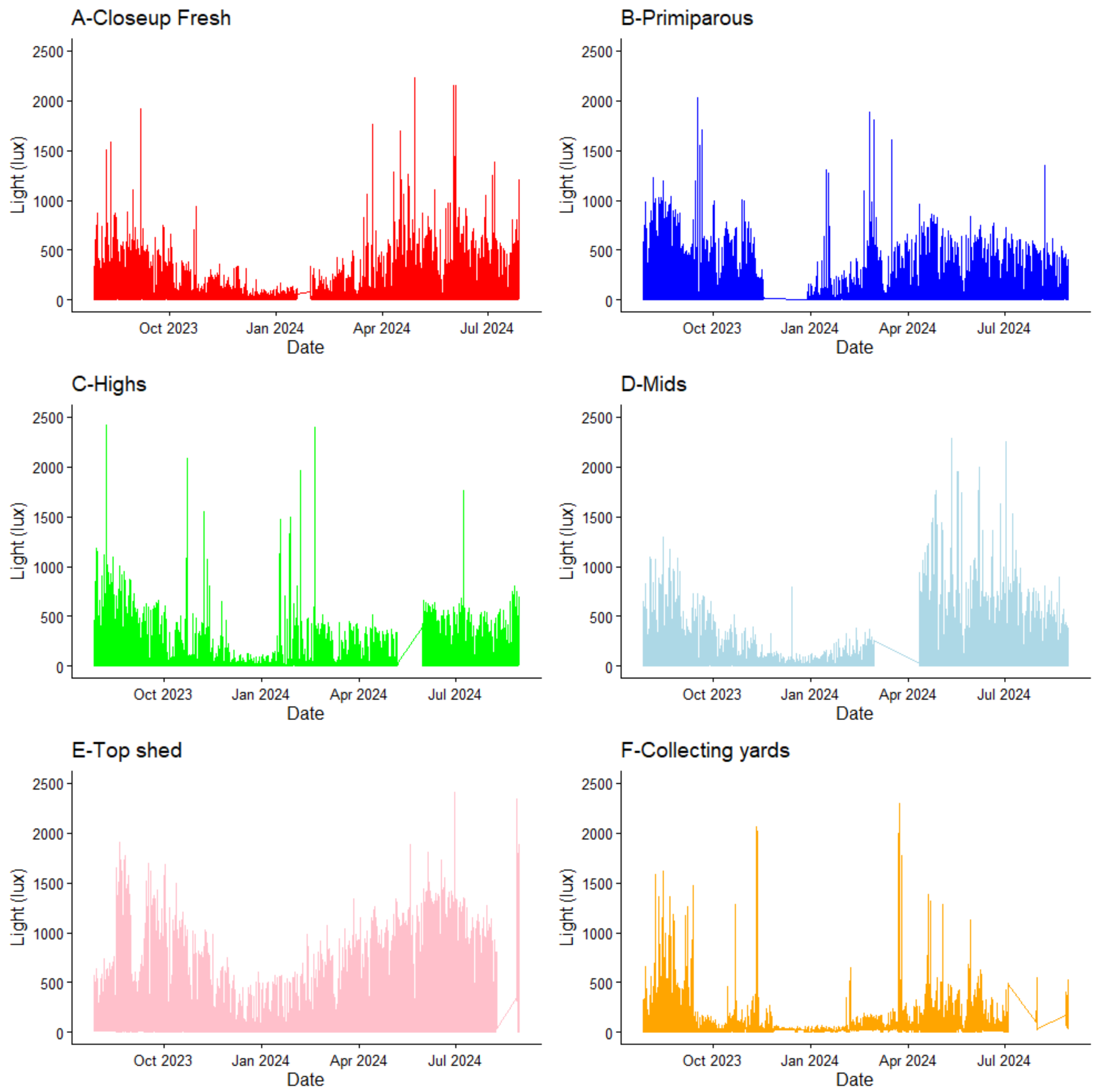


Figure 3-5 Variation in light intensity between pens throughout the year using commercial sensors on a dairy farm in Scotland.

Table 3-7 Variation in light intensity between pens throughout the year using commercial sensors on a dairy farm in Scotland. The lowest value is coloured blue and the highest red.

	Min	Median	Mean	Max
Close-up/Fresh	0	30	89	2236
Primiparous	0	18	107	2033
Highs	0	15	82	2423
Mids	0	4	88	2282
Top shed	0	21	227	2408
Collecting yard	0	27	59	2293

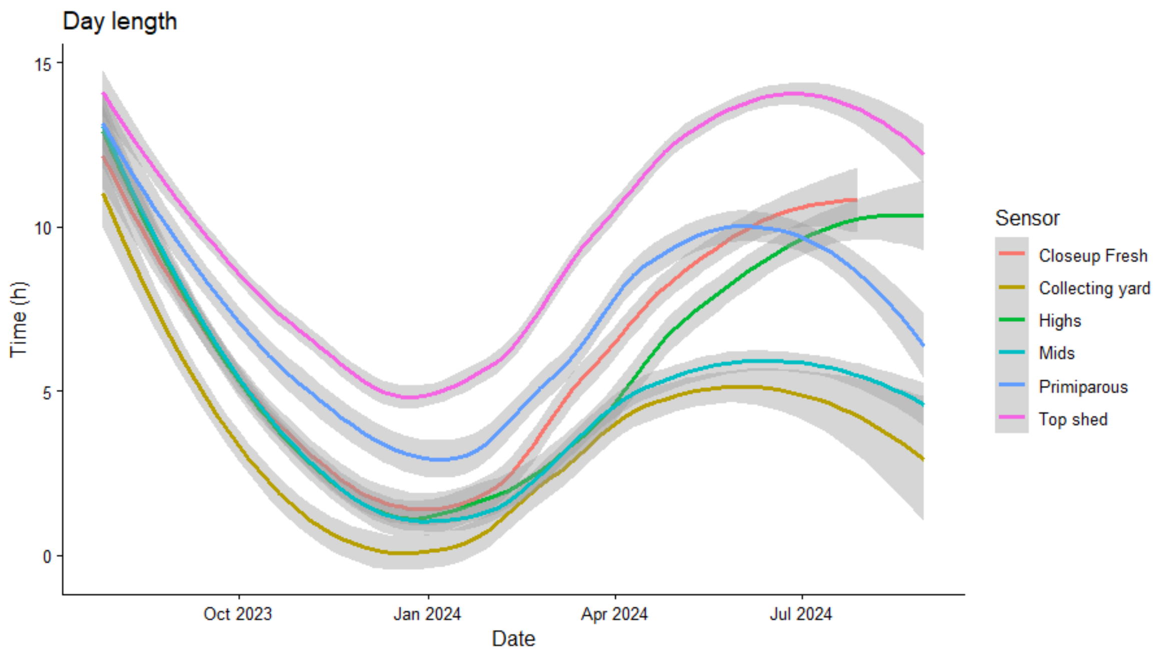


Figure 3-6 Variation in day length with light intensity above 100 lux between pens throughout the year using commercial sensors on a dairy farm in Scotland. Data for the Closeup/Fresh pen are missing from August 2024 because the sensor malfunctioned.

The peak in light intensity varied between pens, which could be the result of the positioning of the sensor and the shed design (Figure 3-7). Therefore, cows on the same geographical location experienced a day length difference of 6 h at the threshold of 100 lux. Given the known positive effect of daylength on milk production this could represent a significant negative production influence (Dahl et al., 2000; Lim et al., 2021).

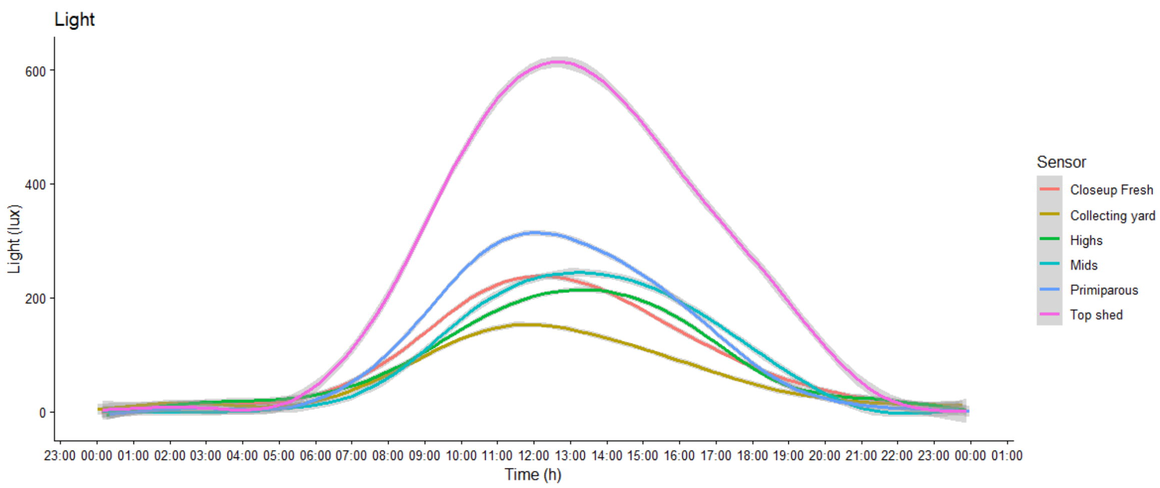


Figure 3-7 Variation in light intensity between pens throughout the year using commercial sensors

Dynamics of environmental parameters in autumn measured using custom-built sensors

The custom-built sensors were trailed in October and November 2023. In addition to the parameters measured by the commercial sensors the custom-built sensors could measure air pressure, ammonia and air particles. They were less reliable with regard to data recording than the commercial sensors, indicated by missing data seen as straight lines in Figures 3-7 to 3-11. Therefore, their use was ended after 2 months. Air pressure showed little variation between pens (Figure 3-8) as expected as air pressure is not influenced by shed design or animal density.

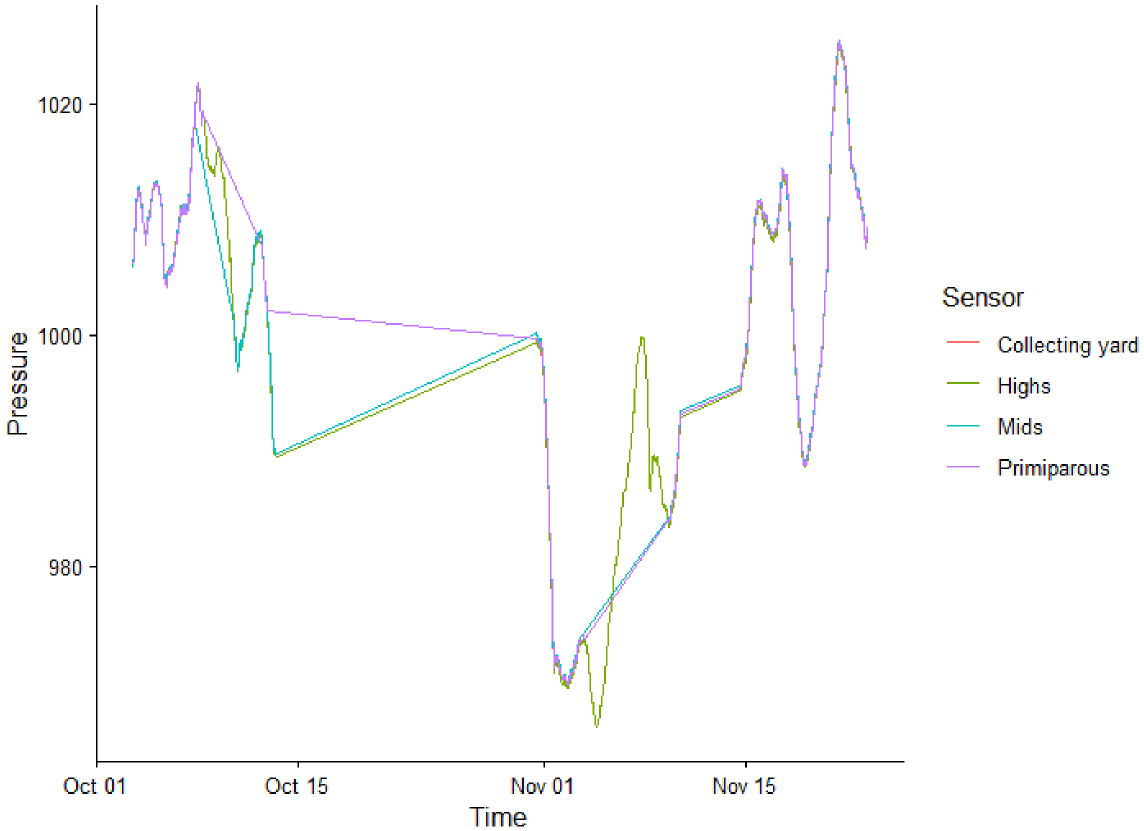


Figure 3-8 Variation in air pressure (mBar) between pens using custom-built sensors on a dairy farm in Scotland.

Ammonia showed some variation throughout the testing period; however, it stayed relatively low with an average of 6.4 ppm and the highest peak reaching 18 ppm. European recommendations suggest that ammonia concentrations should not exceed 20 ppm (CIGR, 1994). The highest values were recorded in the Primiparous and Highs pens (Figure 3-9). High levels did not seem to be related to any managerial changes in the pens. Therefore, it was most likely due to error or random cow interference. In another study ammonia values averaged at 5 ppm, and values were the highest 5-7 m above cow level (Teye et al., 2008).

An earlier study on the same farm as the present study found concentrations between 0.4 and 14 ppm (mean 3.89 ppm). Ammonia was positively related with air temperature (Smith, 2021). Our results show a higher mean ammonia concentration compared to the study done by Smith (2021), which could be due to different equipment used in both studies.

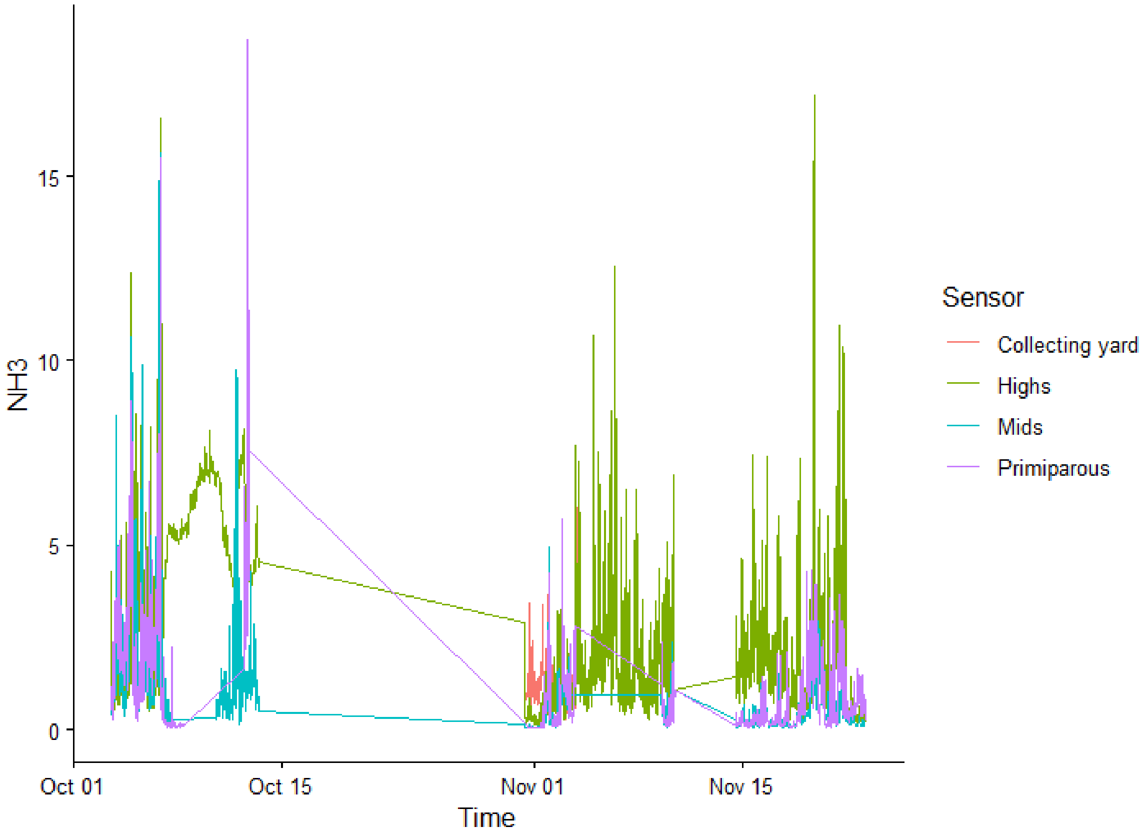


Figure 3-9 Variation in ammonia- NH_3 (ppm) between pens measured using custom-built sensors on a dairy farm in Scotland.

The measurements for all three particulate matter sizes were relatively low. The sensors did not exceed 15 µg/m^3 for any PM size at any time during the operation of the sensors (Figure 3-10, Figure 3-11,Figure 3-12). There were two peaks in PM elevation which lasted for a few days: one in the Mids between the 10^{th} and 13^{th} of October and the second one at the beginning of November (2^{nd} to the 3^{rd}) in the collecting yard. The peaks could not be attributed to any managerial procedure in the pens. Therefore, it was most likely due to error or random cow interference. The peak did not exceed either of the EU or WHO recommendations of 50 and 45 µg/m^3 (EU, 2008; WHO, 2021). $PM_{2.5}$ are most often associated with negative health outcomes in humans as they are small enough to penetrate deep into the lungs and even penetrate into the blood stream (Sethi et al., 2026).

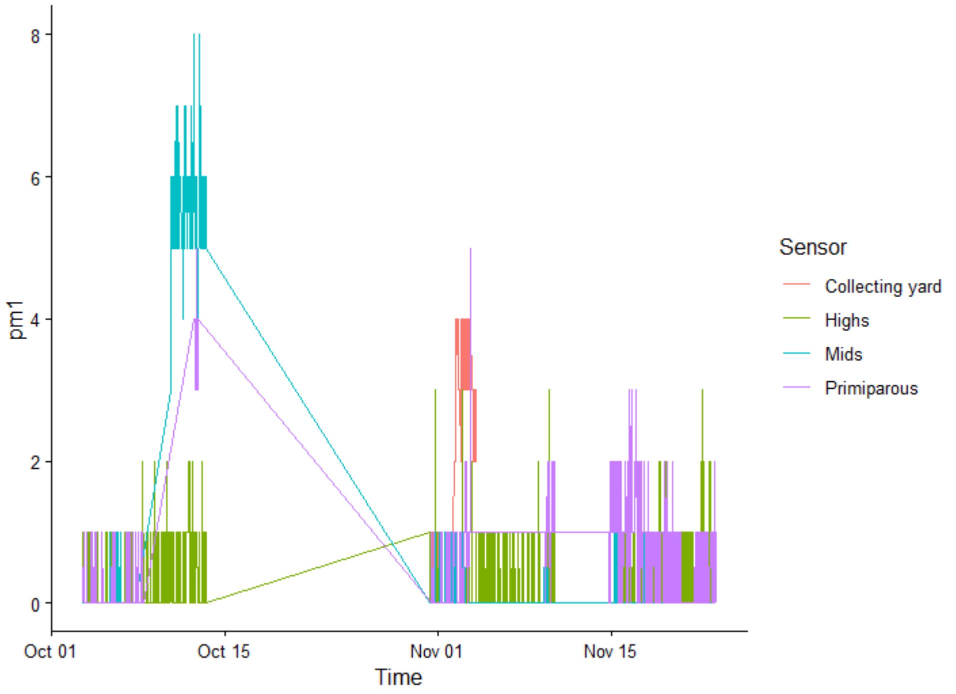


Figure 3-10 Variation in PM 1 particles (µg/m^3) between pens measured using custom-built sensors on a dairy farm in Scotland.

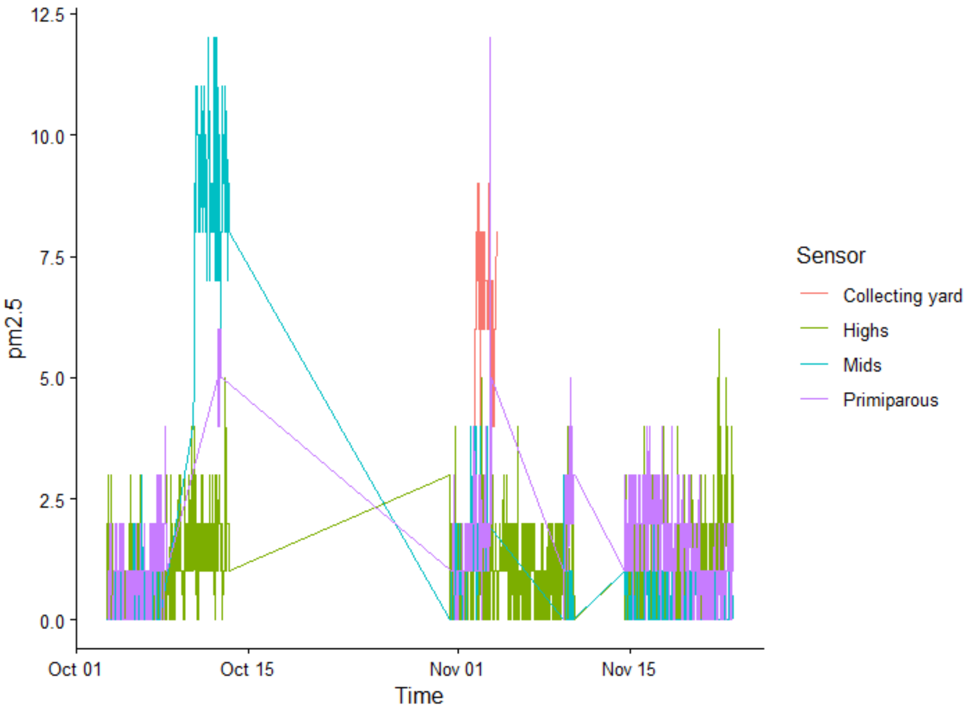


Figure 3-11 Variation in PM 2.5 particles (µg/m^3) between pens measured using custom-built sensors on a dairy farm in Scotland.

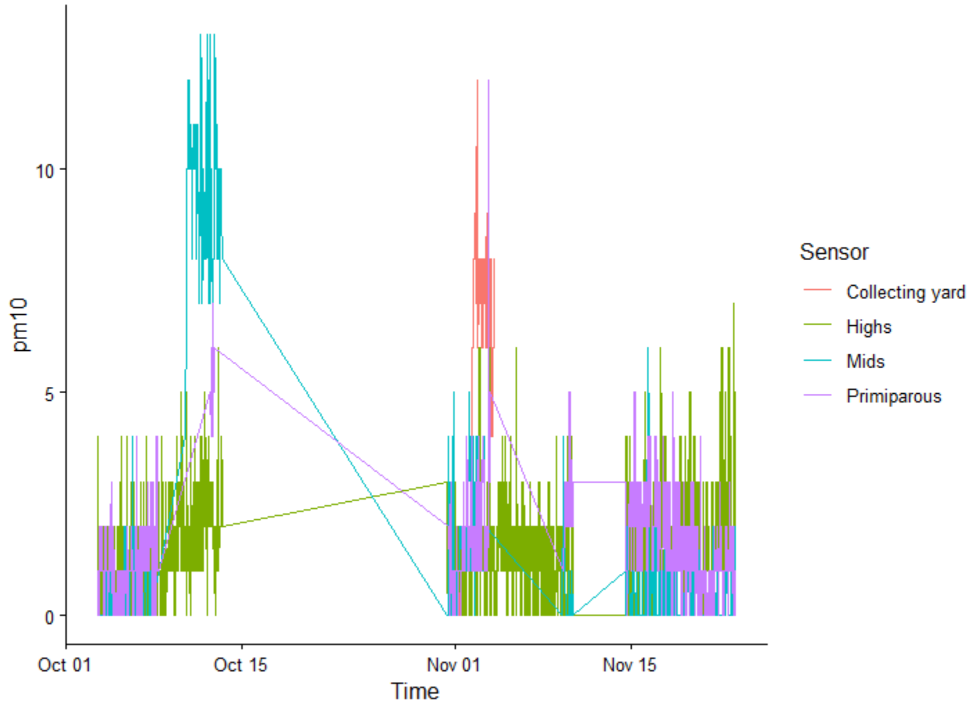


Figure 3-12 Variation in PM 10 particles (µg/m^3) between pens measured using custom-built sensors on a dairy farm in Scotland.

Agreement between commercial and custom-built sensors

The temperatures measured by the commercial and custom-built sensors were strongly related as most of the variation of the custom-built sensors was explained by the commercial sensor R^2 of 0.8-0.96 in the same pens (all p<0.001; Figure 3-13).

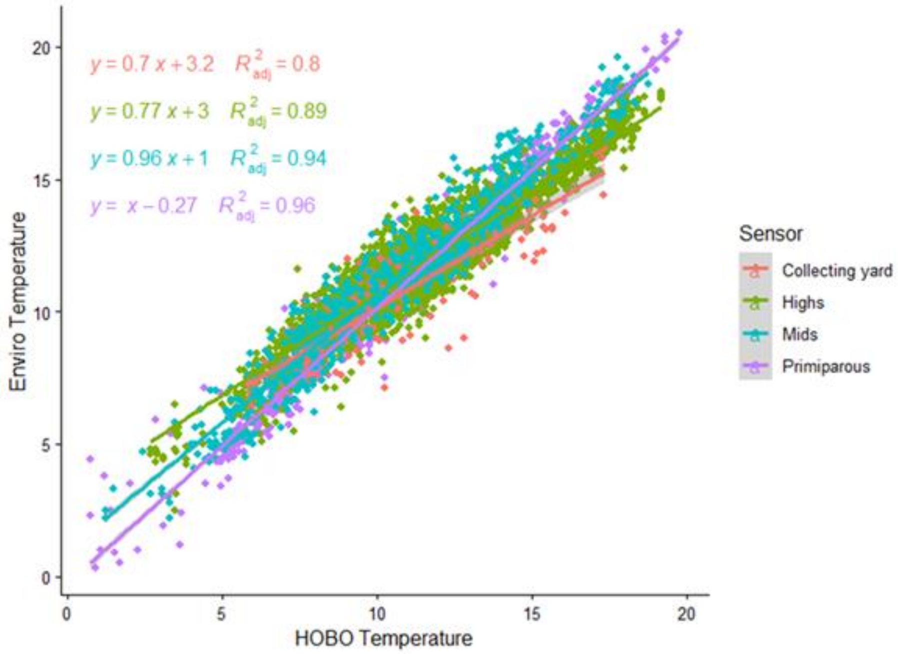


Figure 3-13 Agreement in temperature (˚C) between commercial (HOBO) and custom-built (Enviro) sensors on a dairy farm in Scotland.

Relative humidity had fair to moderate relation between the two types of sensors as moderate amount of the variation of the custom-built sensors was explained by the commercial sensor R^2 was lower at 0.33 to 0.76 (Figure 3-14). However, all the p-values were all below 0.001. Which means that the sensors respond differently to changes in relative humidity or that there might have been different influences on the sensors even though they were placed on the same location in most cases. The collecting yard had the lowest agreement between the two sensors which could be explained by the different positioning of the sensors due to the access to an electrical socket.

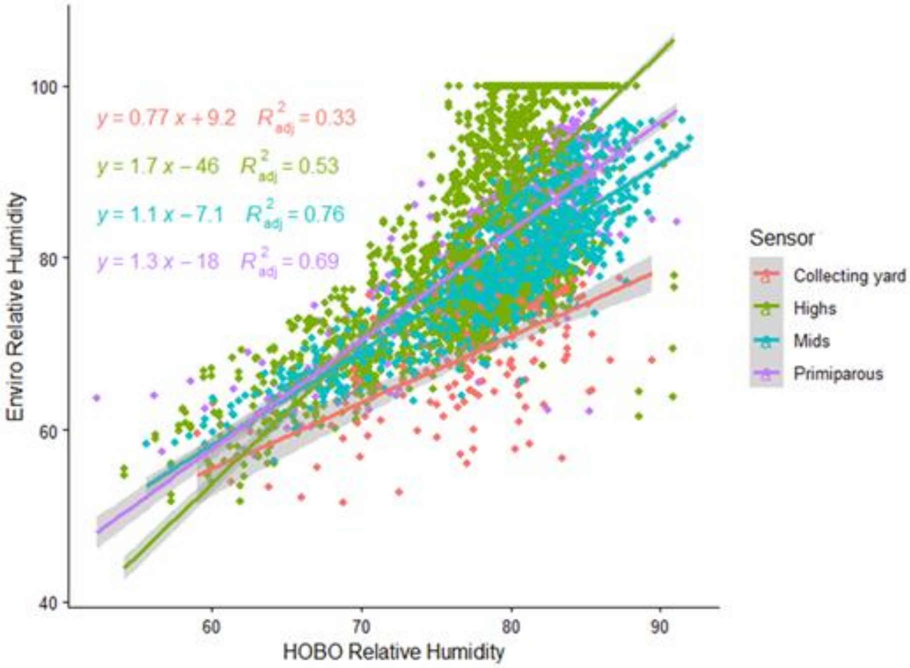


Figure 3-14 Agreement in relative humidity (%) between commercial (HOBO) and custom-built (Enviro) sensors on a dairy farm in Scotland.

Light intensity agreement seemed to vary more between commercial and custom-built sensors compared to temperature and relative humidity, which could be the result of a slightly different orientation of the sensors as the sensors are very sensitive. Agreement was also fair to moderate between sensors as variation of the custom-built sensors was moderately explained by the commercial sensor R^2 between 0.42 and 0.79 (Figure 3-15). The correlation p-values were all below 0.001. Again, the poorest agreement was seen in the collecting yard most likely due to the different positioning of the sensors.

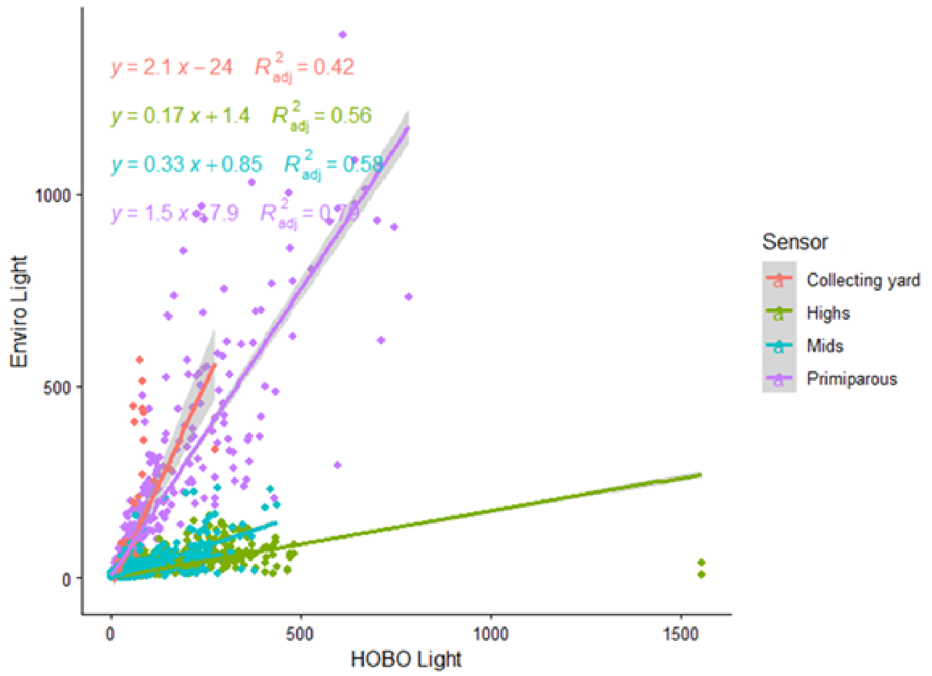


Figure 3-15 Agreement in light intensity (lux) between commercial (HOBO) and custom-built (Enviro) sensors on a dairy farm in Scotland.

3.5 Conclusion

In conclusion, this study demonstrates that indoor loggers reveal significant, highly granular microclimatic variation across different pens of a commercial dairy farm, proving that regional outdoor weather station data heavily underestimates the actual environmental stressors experienced by housed cattle. Crucially, the presence and high stocking density of cows directly influenced indoor microclimates, as evidenced by clear temperature and THI drops when pens were emptied for milking, and acute, corresponding microclimatic spikes within the collecting yard. From an industry perspective, these findings emphasize that dairy management cannot rely on blanket environmental assumptions; instead, mitigating localized barn microclimates through precise housing design, optimized ventilation in high-density areas like collecting yards, and corrective lighting is paramount to eliminating hidden stressors, safeguarding cow welfare, and maximizing herd productivity. While the custom-built sensors provided broader diagnostic capabilities—confirming that ammonia and particulate matter remained safely below regulatory thresholds—they suffered from poor operational durability and lower data-recording reliability compared to commercial devices. Nonetheless, the high statistical agreement for temperature (R^2 = 0.80-0.96) suggests that budget-friendly, custom-built devices hold strong potential for localized environmental mapping, provided their physical housing and connectivity are capable of withstanding the harsh realities of commercial dairy barn environments.

Chapter 4: General Discussion

4.1 Overview

Cattle welfare is becoming more important in today's society and is demanded by the consumer (Alonso et al., 2020). Therefore, researchers are looking for new ways of assessing welfare and stress in cattle (Chen et al., 2015) to allow us to design system to mitigate stress and improve animal welfare. Most welfare assessments are based on visual observations that only record a snapshot of the situation and are relatively subjective, therefore precision livestock farming technologies could be used to provide objective and continuous monitoring of welfare (Molina et al., 2020). Heart rate variability could be such a technology; however, work needs to be conducted to better understand how different HRV parameters change in cows under good and bad welfare conditions (Kovács et al., 2014). HRV parameters, especially RMSSD, HF power, SD1, are good at detecting changes in autonomic arousal, but they cannot tell whether that arousal is positive or negative (Kovács et al., 2014).

Valence (good vs. bad welfare) is not directly measurable via HRV alone. HRV is an index of autonomic neurocardiac regulation, but not whether that reflects good or bad welfare. Glucocorticoids also suffer from the same valence problem.

Furthermore, the technology for a reliable wearable device that would record HRV for cows and allow long-term continuous monitoring and not interfere with daily activities does not exist yet and would need to be developed.

As increasingly many dairy cattle are housed all their lives, in artificial environmental conditions which limit or alter their expression of natural behaviours (Smid et al., 2020), and which could negatively impact welfare, it is our responsibility to provide them with an environment that will maximise welfare, which in turn underpins good production. Therefore, the assessment and understanding of the cow environment and how it impacts on the cows for designing more suitable buildings or for early intervention when conditions become adverse in existing buildings is crucial.

4.2 Using heart rate variability to assess stress

Heart rate variability has been used as a means of assessing stress in dairy cows related to management and veterinary procedures, pain, milking, calving and diseases (Von Borell et al., 2007, Kovács et al., 2014, Kovács et al., 2015c). Compared to other stress-measuring techniques (behaviour assessment and HPA-axis measurements), HRV can detect rapid changes and is a more precise identification of stress; it can also collect continuous data and minimally interferes with the daily operations on farm (Kovács et al., 2014). HRV measurements can vary with rectal temperature (Kovács et al. 2014), exposure to direct sunlight (Bun et al. 2018), production, disease, fear, pain (Kovács et al. 2014), calving (Nagel et al., 2016), body weight, breed, management (Hagen et al., 2005), demeanour (Kovács et al., 2015), standing time, herd size, physical activity (ruminating, standing, lying, feeding) (Kézér et al., 2017), BCS, DIM, % butterfat (Frei et al., 2022) and rumen fill (von Borell et al., 2007). Therefore, behaviour need to be observed continuously and cow level parameters accounted for. Furthermore, the action potential of muscles from free roaming cattle, electromagnetic interference from the environment and arrhythmias can cause noise in HRV measurements (Berntson and Stowell, 1998), therefore the data have to be screened for any artifacts. In our study we did account for BCS, DIM, rumen fill, breed, standing time, herd size and physical activity as animals were sampled from the same farm during the same activity (feeding) and BCS and rumen fill were recorded. Data were also filtered for any abnormal recordings using the in-built filter in the HRV analytical software. Our HRV data did not seem to be influenced by the season of measurement, even though we observed substantial variation in the environmental parameters in the shed throughout the year. Our type of study was not the optimal one to analyse these most likely minor influences as we sampled cows around calving when they are exposed to so many different stressors. Therefore, sampling of mid-lactation cows would be better suited for this purpose.

While the relationship between disease and HRV has been studied, it does not appear to be consistent. It would be expected that diseased animals would experience more stress and have reduced HRV. In this regard, a study in calves with diarrhoea showed decreased vagal and sympathetic activity (Mohr et al., 2002). Studies of HRV and bovine spongiform encephalopathy, however, have produced contradictory results with some reporting increased RMSSD (Pomfrett et al., 2004) and HF power (Little et al., 1996), while others found no changes (Konold et al., 2011). Chronic lameness was also associated with increased RMSSD and HF, and decreased mean heart rate, LF/HF and SD2/SD1 (Kovács et al., 2015a). This apparent contradiction may have arisen as HRV can also be influenced by activity and lameness is associated with increased lying duration which may increase vagal and decrease sympathetic activity (Frondelius et al., 2015). There is a lack of understanding of the relationship between HRV and disease in cattle. In our study we could not find a relation between transition disease and HRV parameters. Therefore, future work is needed to shed more light on this complex yet very important topic.

As with disease the results of HRV assessments associated with management have also been at times contradictory. For example, calves disbudded without local anaesthesia had an increased LF/HF ratio and HR in the 5 min after disbudding compared to pre-disbudding, indicating increase sympathetic activity (Stewart et al., 2008). The same group also found a decrease in RMSSD after disbudding, and that the effect of decreased vagal activity (decrease in HF, increase in LF/HF ratio) was delayed if local anaesthetic was used (Stewart et al., 2009). However, when calves were surgically castrated without local anaesthesia, they had an initial 2 min increase in HR then an increase in RMSSD, HF and decrease in LH/HF ratio (Stewart et al., 2010). The authors associated the increase in parasympathetic activity during castration with deep visceral pain when the spermatic cords are pulled and torn or as a response to increased sympathetic activity to control cardiac output and blood pressure. Response to unmitigated castration was also found to be age specific in one study, which found a decrease in RMSSD and HF in 6 week old calves but not in 3 or 6 month old calves (Bergamasco et al., 2021). The effect of both painful management procedures on HRV parameters was alleviated with the use local anaesthetic. There seems to be a beneficial effect of using local anaesthetics and pain relieving drugs during routine management procedures. However, further studies should investigate the optimal age and pain mitigation for these procedures in regard to HRV.

Handling also causes stress detected by HRV measurements (Frondelius et al., 2015), however it can be mitigated by gentle human interaction (Schmied et al., 2008) or positive handling experiences (Waiblinger et al., 2004). On the farm in our study, the cows were used to being handled and did no show any adverse effects to having the belts attached, therefore we speculated that if there was and effect of handling it was minimal and the same for all cows. Different phases of milking seem to cause variable amounts of stress with one study finding increased vagal activity during milking (Kovács et al., 2014) and reduced vagal activity before release while another by the same group found no difference (Kovács et al., 2013a). There was also no difference in stress experienced by the cows in between automatic and conventional milking parlours (Kovács et al., 2014). Restricted feeding increased stress at the time of feeding in preweaned calves (Prokop et al., 2025). Therefore, further studies are needed before HRV data could be used to design cow-friendlier housing and management procedures and improve overall welfare.

4.3 Monitoring housing environmental parameters and their effect on cows

The outside environment is subject to seasonal variation throughout the year. Housed animals are shielded from some of that variation by the sheds they are kept in (Teye et al., 2008). Environmental parameters inside sheds are dependent on shed design and the assessment of environmental parameters will be influenced by sensor placement as proximity to ventilation inlets will influence results (SchüLler and Heuwieser, 2016). A study on microclimate variation throughout the year found that insulated barns had a significantly higher indoor temperature during winter compared to uninsulated barns (Teye et al., 2008). Few studies have assessed the microclimate in calf sheds (Leenen et al., 2020, Mahendran et al., 2023) but little work has been done on adult cow sheds. Most environmental studies and effect of the shed environment on adult cows are related to heat stress and production (Hill and Wall, 2015, Gernand et al., 2019). Our study found that both temperature and THI in the sheds were higher compared to the outdoor environment and that THI thresholds for decreased production and reproduction were exceeded even in winter months. Given the know detrimental effects of heat stress on cows, further studies on the effect of shed design and animal management on the microclimate in the shed are warranted.

Stationary cow environment loggers can underestimate the heat stress experienced by the cows. In one study, cow mounted loggers were above a THI threshold of 72 for 13% longer compared to stationary logger (Schüller and Heuwieser, 2016). The same study also found a decrease in temperature, RH and THI in the pens when the animals were being milked, which is in agreement with the results from our study. Compared to the data of the nearest weather station, sheds were hotter and less humid (Schüller et al., 2013). This is consistent with our findings when comparing the logger data to the NASA POWER data.

4.4 Study strengths, limitations and future directions

The project has several important strengths. It used heart rate variability (HRV) as a non-invasive and objective physiological indicator of autonomic arousal, avoiding the possible subjectivity of behavioural assessment and the sometime invasive sampling for cortisol measurements. Data were collected from 134 multiparous Holstein cows under commercial farm conditions, demonstrating the practical execution and relevance of the study and findings. The longitudinal design of collecting HRV measurements around calving enabled temporal changes in physiological stress around calving to be assessed, while linear mixed-effects modelling accounted for repeated measurements and cow-level variation. In addition, the project incorporated environmental monitoring within the housing system, combining commercial sensors with custom-built devices to assess multiple microclimatic parameters (temperature, humidity, light, ammonia and particulate matter) and to evaluate the devices' reliability. Continuous environmental data collected across multiple pens and compared with external weather data allowed the study to identify spatial and temporal variation in the barn microclimate and validate low-cost monitoring technologies, supporting their potential use in precision livestock farming and welfare monitoring.

The study also had several limitations. The HRV study was conducted on a single farm in a relatively small number of multiparous cows and each animal was sampled only twice. Primiparous cows were not included, as they were housed separately without handling facilities, which means that some variability associated with lactation number was not captured. In most field studies, farm itself is a significant variable (Frei et al., 2022), as management, environment and nutrition differ across sites. Therefore, the generalisability of our findings is limited and further studies are needed to assess whether similar patterns are observed elsewhere. Additional data, such as calf birth weight or the combined weight of twin calves, would allow more precise conclusions on how calf weight relates to HRV parameters. Likewise, continuous measurement of HRV would provide a clearer picture of changes across the transition period. Finally, sampling a larger cohort, including cows with transition disease, would improve the ability to detect smaller associations between HRV and transition disease outcomes. Even though the Polar sensors have been used in other studies to assess HRV (Frei et al., 2022) they are limited to short term (24h) HRV measurements (Kovács et al., 2014). A Holter ECG, which records continuous electrocardiogram signals, could provide more detailed data over a longer time period.

The environmental data collection was also limited to one farm and one year. Environmental parameters can vary between different farms and even different sites in the same pen (Schüller and Heuwieser, 2016), which means the observations could differ between different farms and different shed designs. Future work should be done on how different shed types are affected by different environmental conditions. The sensors were also positioned above cow level to limit interference by the animals, which could have influenced the results. Outdoor environmental data were extracted from the NASA POWER project averaged data over 0.5-degree latitude by 0.625-degree longitude geographical grid. The commercial environmental sensor used in the pens could have provided more farm specific and comparable results. The custom-built sensors and the internet connection were not very reliable, which is why limited data were collected. Nevertheless, custom-built sensors could be a more affordable open-source environmental data collection system under the right conditions. However, the farm environment proved sufficiently harsh that even the commercial data loggers failed intermittently, as evidenced by the multiple instances of missing data.

4.5 Conclusion

This thesis demonstrates that heart rate variability is a useful, non-invasive tool for assessing physiological stress in multiparous Holstein cows during the transition period. Clear temporal changes in heart rate and HRV parameters were observed, with evidence of increasing stress as calving approached and recovery in the days following parturition. These findings support the concept that the peri-calving period represents the peak of physiological stress within the transition window. Stress responses during the transition period were more strongly associated with calf characteristics, particularly twinning and sire breed, than with cow-level factors or the incidence of clinically-detected transition disease. This suggests that physical and metabolic demands related to pregnancy and parturition may play a greater role in shaping autonomic stress responses than early postpartum disease status within the timeframe studied.

Environmental monitoring highlighted marked variation in microclimatic conditions within dairy housing, reinforcing the importance of considering environmental stressors alongside physiological measurements. Collectively, these results indicate that integrating HRV with environmental and management data has potential to enhance the assessment of cow welfare and resilience during the transition period. Future research should focus on longer monitoring periods, larger populations, and integration with behavioural and production data to fully realise the value of HRV within precision livestock farming systems.

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