PETER HUT

# MONITORING BEHAVIOR OF DAIRY COWS



# Sense of Sensors

Monitoring behavior of dairy cows

Peter Hut

#### **Sense of Sensors**

Monitoring behavior of dairy cows

PhD thesis, Utrecht University – with a summary in Dutch ©Peter Hut, 2022

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# Sense of Sensors

Monitoring behavior of dairy cows

#### Meten is Weten

Gedragsmonitoring van melkkoeien (met een samenvatting in het Nederlands)

## Proefschrift

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# **Chapter 1**

**General Introduction** 

# Chapter 1

## BACKGROUND

### The dairy industry and a growing human population

Worldwide, milk production increased by 64% in the last three decades, and dairy cows are responsible for 81% of global milk production. The Food and Agriculture Organization of the United Nations (FAO) has detailed information on the dairy industry in 73 countries including 115 million dairy farms. Their average herd size is between 2 to 3 cows per farm globally and in 15 countries the average herd size is over 50 cows (FAO, 2019).

In the Netherlands, the number of dairy farms has been decreasing since 1950, from 216,000 to 15,000. Milk production increased from 4.8 billion kg per year to 14 billion kg per year, even though the number of dairy cows since 1980 has decreased from 2.4 million to 1.6 million (fig.1). While the average herd was 13 cows per farm in 1950, the average herd in 2021 was 108 cows (CBS, 2021). This indicates an enormous improvement in efficient milk production.



**Figure 1.** The decrease in numbers of dairy cows and dairy farms with an increased milk production from 1950 until 2020 in the Netherlands. Data source: CBS.

To ensure current and future food production in a sustainable way requires major changes in production and consumption. This means more plant-based protein in the human diet. However, the fact remains that dairy cows can provide animal-based protein from low quality plant-based sources (Bradford, 1999). With rations based on human-inedible plant material, dairy cows are net producers of human-edible protein (Ertl et al., 2015; De Cuyper et al., 2022). Even in the most favorable plant-oriented scenario from the EAT-Lancet Commission Summary Report, an increase in production of dairy products is reported necessary to ensure a planetary health diet in 2050 (EAT Lancet Commission). This means that the demand for milk will not decrease in the near future. In fact, the "Agricultural Outlook 2021-2030", the Organization for Economic Co-operation Development (OECD) and the FAO estimate an increase of 20% in global milk production by 2030.

In the Netherlands, milk production is already highly efficient, with an increased use of technology to support the farmer with milking and daily cow management playing a role. This technological support is often summarized as Precision Livestock Farming (PLF). PLF gives an opportunity for farmers to monitor animal health, welfare, production and reproductive performance. The implementation and development of PLF has great potential to assist in the improvement of efficient, sustainable and healthy dairy food sources, produced by healthy dairy cows on dairy farms with the highest standards for animal welfare (Rutten et al, 2013; Barkema et al., 2015).

#### Sensor technology and cow behavior

Currently, the key elements of PLF include 129 commercial sensor technologies available to monitor dairy cows. Worldwide, these technologies are manufactured by 67 retailers from 21 different countries. Monitoring dairy cows with such sensor technology provides an opportunity to track their behavior, ruminal pH, temperature, and many more relevant characteristics 24/7 (Stygar et al., 2021).

To monitor dairy cow behavior, external sensors (fig. 2) can be attached to the leg, to the neck collar, to the halter, and in the ear, and internal sensors can be in the rumen. Most of these systems are focusing on detecting estrus and thus determining the ideal moment for insemination. For most farms, investment in such systems is economically sound (Rutten et al., 2014). The detection of health disorders by monitoring deviations based on a rolling average of a specific behavioral variable, such as the number of steps, rumination time or eating time, is also possible (Rutten et al., 2013; Stangaferro et al., 2016a,b,c). Others have shown that disease detection 1-2 days before recognition by the farmer was possible because cows with health issues deviate from their circadian patterns, as detected by real time cow positioning data (Veissier et al., 2017).

In addition, sensors provide an opportunity to monitor differences in individual daily behavioral patterns between cows within a dairy herd. With increasing milk production per cow and a growing average herd size, this becomes more and more difficult to observe otherwise. For instance, the world record in milk production during one year was set by dairy cow 'Senz Pralle Aftershock 3918' in her 5<sup>th</sup> lactation, with a total amount of milk produced of almost 35500kg in 2017, which is over 90kg per day. Her farmer mentioned that she spends her day eating, resting and ruminating. She ruminates an hour and a half more daily compared to her herd mates, as measured by sensor technology in which the farmer invested because "keeping a herd of 425 cows healthy requires attentive care and advanced technology" (Hoards Dairyman, 2018). Dairy cows, such as Holstein Friesian, have the genetic potential to produce such amounts if provided a high level of cow comfort and a balanced ration. By studying

complete behavioral profiles, sensor data could aid in clarifying differences between cows in productive and reproductive parameters, and also in disease incidence.

While genetic selection based on milk production has been the main driver of increased milk production in the last decennia, it resulted in an increased incidence of mastitis, lameness, metabolic and reproductive challenges (Bello et al., 2012; Koeck et al., 2014; Barkema et al., 2015). These can be detected early using behavioral profiles with external and internal factors, and studying sensor based time budgets could thus support the effects of management on cow health and welfare.



Figure 2. Several behavioral variables generated by a neck and a leg sensor. Source: Nedap Livestock Management.

#### Time budget - external factors

This technology relies on a combination of specific behavioral variables, summarized in the term "time budget". A time budget is the net response of a cow to the environment (Grant, 2003). The time budget is time divided among activities when milking time is excluded, because a cow cannot control her milking time completely (Gomez and Cook, 2010). The term is commonly used to refer to certain behavioral patterns, where only one or two behavioral parameters are studied (Neave et al., 2017; Kok et al., 2017). It has also been used (e.g. by Grant and Albright 2000) to describe a set of behavioral variables, and this, combined with the results from Matzke (2003), suggests (Grant 2003) that high producing dairy cows require 14h of lying time per day. Whether this value should be aimed for, for every dairy cow at every single moment,

remains to be discussed. Lying is a behavior of preference for dairy cows (Munksgaard et al., 2005). According to a scientific opinion on welfare of dairy cows published by the European Food Safety Authority (EFSA), failing to optimize housing, nutrition, and management risks creating behavioral problems in cows with a high genetic potential for milk production (EFSA, 2009).

Dairy cow behavior is influenced by both external and internal factors. One of the external factors, extensively reported to influence dairy cow health, production, and welfare, is heat stress. For instance, high environmental temperatures lead to lower milk production and have a negative effect on reproductive outcomes (Ray et al., 1992). Heat stress in the dry period also leads to less milk production during the entire subsequent lactation (Fabris et al., 2017), where cooling dry cows during the dry period lead to 4-7.5kg more milk per day (Fabris et al., 2019). However, behavioral adaptation in temperate climates has been the focus of few studies, and those studies do not report the complete time budget in commercial settings. It is possible that when cows produce less milk during warmer periods this could indicate an adaptation of the animal to produce less metabolic heat. This adaptation could also indicate a disruptive pathophysiological process and thus negatively affects their welfare.

#### Time budget - internal factors

A crucial phase for health and welfare in the life of dairy cows is the transition period. This is a 6 week period starting 3 weeks before parturition and ending 3 weeks after parturition (Drackley, 1999). There is a large overlap between this and the first four weeks after calving, when about 75% of dairy cattle diseases occur (LeBlanc et al., 2006) and up to 50% of dairy cows develop one or more diseases in the early postpartum period (LeBlanc, 2010). Historically, postpartum diseases and conditions (hypocalcemia, retained fetal membranes, metritis, ketosis, abomasal displacement, mastitis) were thought to be unrelated problems and addressed as such. A paradigm shift has occurred over time and strong associations between these have been recognized.

The pathophysiological system of disease in the transition period starts with an excessive mobilization of adipose tissue, leading to circulating non-esterified fatty acids (NEFAs) and followed by accumulation of fat in the liver. These fatty acids should be used for ketogenesis, allowing ketone bodies to be used as energy sources. The circulating ketone bodies induce hypophagia: pathophysiological induced reduced feed intake. Lower feed intake is associated with (sub)clinical hypocalcemia and a negative energy balance (NEB) (Drackley, 1999; Coffey, 2002; LeBlanc et al., 2006). These postpartum conditions have a negative impact on production, fertility and welfare (Esposito et al., 2014). There are other connections as well. (Sub)clinical hypocalcemia and (sub) clinical ketosis play a central role in the occurrence of transition diseases such as retained fetal membranes, metritis, abomasal displacement, and mastitis and also seems to be associated with lameness (Raboisson et al., 2014; Rodríguez et al., 2017). Subclinical

hypocalcemia has also been associated with impaired reproductive performance (Caixeta et al., 2017). Subclinical ketosis was associated with impaired fertility as well and with lower milk production (McArt et al., 2012). Subclinical hypocalcemia (Reinhardt et al., 2011; Neves et al., 2017) and subclinical ketosis (Suthar et al., 2013) are experienced by many cows, despite efforts to optimize dry cow and transition rations. Also, some of these transition diseases have been associated with prepartum behavior, i.e. lower feed intake or hypophagia (Huzzey et al., 2007) or reduced lying time (Neave et al., 2018).

An underlying role for inflammatory response in the pathophysiological process of transition diseases has also recently been reported. It appears that an inflammatory response is an underlying cause of hypophagia: circulating inflammatory mediators depress appetite centers of the brain, leading to less feed intake, and as a result, transition diseases occur (Brown and Bradford, 2021; Horst et al., 2021). This inflammation could be triggered by an actual infection such as mastitis, or udder infections that start in the dry period (Dingwell et al., 2003) but are often unnoticed. Inflammatory response could also be caused by stress (Proudfoot et al., 2018), heat stress (Bagath et al., 2019; Marins et al., 2021), pain (Gleerup et al., 2015; Barragan et al., 2018), and sleep or rest deprivation (Proudfoot et al., 2021).

Sensor data and time budget analysis could detect deviations of the normal time budgets. Based on rumination behavior and physical activity, as others have shown, there is a high sensitivity (98%) for abomasal displacement 3 days before actual clinical diagnosis (Stangaferro et al., 2016a). With varying sensitivity (53-91%) this can detect other diseases, like metritis, ketosis and mastitis, between 0.5 and 1.5 days before clinical diagnosis (Stangaferro et al., 2016b,c). Further, because diagnostics based on milk characteristics cannot be executed during the prepartum period, sensor data could even be of greater importance to monitor time budgets of dry cows.

#### The "Sense of Sensors" study

The "Sense of Sensors" research project was started to better understand sensor data of transition cows, with the ultimate aim to support a healthy transition from the dry period to the lactation period for dairy cows and supporting dairy cow welfare. The project first needed to elucidate and clarify the basics of dairy cow behavior on high producing dairy farms. The overall goal of the "Sense of Sensors" study was to determine time budgets of dairy cows and how these are affected by environmental factors and internal factors regarding cow health. The focus for this thesis was on behavioral adaptation as the outcome from internal and external influences. Others studied behavioral variables in relation to production outcomes.

The "Sense of Sensors" study started as a collaboration between Nedap Livestock Management (Groenlo, the Netherlands), Vetvice (Bergen op Zoom, the Netherlands), Wageningen University and Research (Wageningen, the Netherlands) and Utrecht University (Utrecht, the Netherlands), and consisted of two phases. In the first phase, 17 dairy farms were selected. The criterion used was that at least one type of Nedap sensors was already used on that farm. This implied that the antenna and server (the most costly elements) were already present. Next, Nedap provided the other type of sensor: when a leg sensor was already present, neck sensors were provided or vice versa. During this phase, the focus was on the transition period: the additional sensors were attached to cows from around 6 weeks prepartum until 4 weeks postpartum, after which the sensors were removed and reset for the next prepartum cow. Both sensors were validated by previous studies and have high correlations between observed and reported behavioral parameters (0.88-0.97) (Nielsen et al., 2018; Dela Rue et al., 2020; Borchers et al., 2021). During the entire study, sensor data were collected between November 1, 2016 and November 11, 2020.

In phase two of the project, eight farms from the group of 17 farms were identified to be followed more intensively. These farms were located in different areas of the Netherlands. Three of these farms used automatic milking systems and housed their cows indoors; five had a conventional milking system and provided pasture access.

These eight farms were visited weekly to collect cow specific data for a period of one and a half years. All cows were permanently equipped with both sensors to collect additional data. Each cow was scored for body condition and locomotion in the early dry period, in the late dry period, in week four after calving and in week eight after calving. The farmers noted the date and time of calving. Additionally, cows were sampled once for blood calcium within 48h postpartum and twice for blood ketone body concentrations in week one and two postpartum (fig. 3). All these observations in phase two resulted in a data set of over 150Gb of data, consisting of over one billion observations.



Figure 3. Time line of the cow side measurements of the "Sense of Sensors" study. BCS: Body condition score. Locomotion: Locomotion score. BHBA: beta hydroxy butyric acid (ketone body). US: Ultrasound of uterus and ovaries.

#### Scope and outline of this thesis

The scope of this thesis was to elucidate dairy cow behavior, health and welfare, based on sensor data as collected within the "Sense of Sensor" project on commercial dairy farms in the Netherlands.

The daily and lactational time budgets of dairy cows on commercial dairy farms are examined in Chapter 2. The aim was to elucidate behavioral differences between the pre and postpartum periods, as well as the behavioral effects of parity, pasture access and other farm differences. Behavioral differences between day and night, based on a complete time budget as measured by a neck and a leg sensor, were also studied.

How these time budgets of dry and lactating cows were affected by daily environmental temperature and humidity in a temperate and maritime climate is presented in chapter 3. According to most meteorological studies, temperature will rise in the future. If milk production per cow increases as well, the metabolic and behavioral adaptations of dairy cows will be of even higher importance.

Chapters 4 and 5 focus on an extended transition period. In chapter 4, the effects of eating time variables in the transition period on the interval between calving and first service were studied. The aim was to understand how weekly mean eating time and weekly standard deviation of the eating time in an extended transition period were associated with longer or shorter intervals between calving and first service. In chapter 5 how lameness, as defined by locomotion scores, was related to the body condition score and how lameness affected daily time budgets of dry and lactating cows was studied. Another aim was to gain insights in the percentage of lame cows in a commercial setting from the early dry period until peak lactation.

In the summarizing discussion in chapter 6, the aspects of dairy cow behavior and the association with health and welfare were integrated with recent insights regarding the transition period and the pathophysiology of transition disease from other studies. The effects of lameness and heat stress during transition will be proposed and discussed.

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# Sensor based time budgets in commercial Dutch dairy herds vary over lactation cycles and within 24 hours

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#### ABSTRACT

Cows from 8 commercial Dutch dairy farms were equipped with 2 sensors to study their complete time budgets of eating, rumination, lying, standing and walking times as derived from a neck and a leg sensor. Daily sensor data of 1074 cows with 3201 lactations was used from 1 month prepartum until 10 months postpartum. Farms provided data over a 5 year period. The final models (lactational time budget and 24h time budget) showed significant effects of parity, farm and calving season.

When primiparous cows were introduced in the lactational herd, they showed a decrease in lying time of 215 min (95% CI: 187-242) and an increase in standing time of 159 min (95% CI: 138-179), walking time of 23 min (95% CI: 20-26) and rumination time of 69 min (95% CI: 57-82). Eating time in primiparous cows increased from 1 month prepartum until 9 months in lactation with 88 min (95% CI: 76-101) and then remained stable until the end of lactation.

Parity 2 and parity 3+ cows decreased in eating time by 30 min (95% CI: 20-40) and 26 min (95% CI: 18-33), respectively, from 1 month before to 1 month after calving. Until month 6, eating time increased 11 min (95% CI: 1-22) for parity 2, and 24 min (95% CI: 16-32) for parity 3+. From 1 month before calving to 1 month after calving, they showed an increase in ruminating of 17 min (95% CI: 6-28) and 28 min (95% CI: 21-35), an increase in standing time of 117 min (95% CI: 100-135) and 133 min (95% CI: 121-146), while lying time decreased with 113 min (95% CI: 91-136) and 130 min (95% CI: 114-146), for parity 2 and 3+, respectively. After month 1 in milk to the end of lactation, lying time increased 67 min (95% CI: 49-85) for parity 2, and 77 min (95% CI: 53-100) for parity 3+.

Lactational time budget patterns are comparable between all 8 farms, but cows on conventional milking system (CMS) farms with pasture access appear to show higher standing and walking time, and spent less time lying compared to cows on automatic milking system (AMS) farms without pasture access.

Every behavioral parameter presented a 24h pattern. Cows eat, stand and walk during the day and lie down and ruminate during the night. Daily patterns in time budgets on all farms are comparable except for walking time. During the day, cows on CMS farms with pasture access spent more time walking than cows on AMS farms without pasture access. The average 24h pattern between parities is comparable, but primiparous cows spent more time walking during daytime compared to older cows.

These results indicate a specific behavioral pattern per parameter from the last month prepartum until 10 months postpartum with different patterns between parities but comparable patterns across farms. Furthermore, cows appear to have a circadian rhythm with varying time budgets in the transition period and during lactation.

#### **INTRODUCTION**

Continuous monitoring of dairy cattle with sensor technology provides opportunities to detect deviations based on rolling averages (i.e. heat detection) and a better understanding of the behavior of these animals [1-3]. As sensor technology develops, it is also becoming possible to use sensor data for disease detection [4-7], for disease and fertility prediction [8,9], assessment of welfare [10] and decision support in management [11].

Although much research is focused on early detection of disease, some also elucidate zootechnical influences on specific behavior [12-14]. For example, dairy cow behavior differs between farms with automatic milking systems (AMS) and conventional milking systems (CMS) [15]. It also varies with group size and stocking density [16,17] and with pasture access compared to indoor housing [12]. In addition to zootechnical aspects, specific cow attributes present behavioral differences. Behavioral patterns of dairy cows differ in the dry period compared to the lactational period. Further, primiparous cows behave differently compared to multiparous cows [9,18].

Combining several behavioral parameters can lead to a better understanding of the dairy cows' time budget [19,20]. For instance, when cows are lying down rumination time is higher compared to rumination time when cows are not. When cows spent more time ruminating, these periods were associated with less dry matter intake (DMI), indicating that cows do not eat and ruminate at the same time [21]. Time budgets can be approached as a combined set of several behavioral parameters per day and over a certain period in time, such as 24h patterns. For example, dairy cows show a diurnal pattern in feed intake depending on milking time and fresh feed delivery [22]. They also seem to have a circadian pattern, based on individual cow positions [23]. Moreover, they exhibit changes in circadian rhythms that can be used to detect estrus and disease [24].

While others have studied dairy cattle using extensive sensor data, these studies reported only one or two behavioral parameters as time budgets [9,18,25]. Thus, complete time budgets combining data for feeding behavior (eating time and rumination time), lying behavior and walking behavior (standing time and walking time) seem lacking. This is also true of behavioral profiles based on 24h patterns and studies based on sensor data originating from commercial dairy farms.

The goal of this retrospective observational study is to combine sensor data from 2 types of sensors (3-dimensional neck and leg accelerometers) to create a complete time budget of dairy cows throughout the lactation cycle, to gain a better understanding of dairy cow behavior and sensor data in a commercial setting while correcting for parity, milking type and calving season taken into account. In addition, the combined daily sensor data creates a time budget of the daily behavioral pattern allowing the creation of 24h patterns. This reveals the effects of parity, months in lactation and differences between farms, extending previous reports which are mostly studied on a single farm,

allowing comparisons between time budgets among farms. The results of this study could provide a benchmark for different dairy farming systems.

### **MATERIALS AND METHODS**

#### Farms, animals and sensors

All dairy cows on the 8 commercial dairy farms in The Netherlands included in this study were equipped with 2 types of sensors. Details of these farms, with an average herd size of 140 cows, are described in table 1 and in our previous publication on lameness [26]. To monitor feeding behavior (eating time and rumination time), commercially available "Nedap Smarttag Neck" sensors (Nedap, Groenlo, The Netherlands) were attached to the neck collar of each cow and the commercially available "Nedap Smarttag Leg" sensors were attached to one of the front legs of each cow, to monitor walking (walking time and standing time) and lying behavior (lying time). Both sensors were validated by previous studies and have high correlations between observed and reported behavioral parameters (0.88-0.97) [27-29]. In total, 1074 cows with 3201 lactations were available in this study. The use of such sensors in a commercial dairy herd is not considered an animal experiment under Dutch law, hence no formal ethical approval was needed (see also [26]). The number of cows per sensor based behavioral parameter is presented in Figure 1. For visualization purposes, farms were grouped by type of milking system (AMS, N=3 / CMS, N=5) where cows on CMS farms also had pasture access during parts of spring, summer and autumn for at least 120 days/year for at least 6h/day as a part of a subsidized Dutch system to stimulate pasture access for dairy cows. This resulted in 2 groups: the AMS-C (automatic milking system - confined) group and the CMS-P (conventional milking system – pasture access) group.

Farm	Herd size	DP cubicle bedding far off	DP cubicle/ yard bedding close up	Average DP length (25%-75% IQR)	Cubicle bedding lactation	Milking system	Pasture access	Production level (kg milk/cow/year)
-	170	Deep litter	Straw yard	41 (31-46)	Deep litter	AMS	No	10786
2	130	Deep litter	Straw yard	39 (30-41)	Deep litter	AMS	No	11177
ŝ	110	Mattress	Mattress	45 (40-51)	Mattress	AMS	No	9341
4	110	Mattress	Straw yard	39 (33-43)	Mattress	CMS	Yes	9314
Ś	140	Deep litter	Deep litter	35 (30-40)	Deep litter	CMS	Yes	9256
9	170	Mattress	Mattress	37 (32-42)	Mattress	CMS	Yes	9243
7	175	Deep litter	Straw yard	42 (32-48)	Deep litter	CMS	Yes	9109
8	120	Mattress	Mattress	45 (37-49)	Mattress	CMS	Yes	9197
AMS = : straw ya	automatic mil rd means a fre	king system. CMS = e-range area. Pasture	conventional milking e access is for lactating	system. DP = dry perio animals only.	d. IQR= interquartile	: range. Deep li	itter is related to c	ubicle systems where a



Figure 1. Distribution of all cows used in this study equipped with both leg and neck sensors per type of farm (AMS=automatic milking system, CMS=conventional milking system) per statistical model (months in milk model and 24h pattern model).

#### Study design

For this retrospective observational study, sensor data of 1074 cows with 3201 lactations was collected over a 5-year period from January 1<sup>st</sup> 2016 until December 31<sup>st</sup> 2020. Sensor data was provided by Nedap Livestock Management (Nedap, Groenlo, The Netherlands). Behavioral parameter sensor data was collected in two different formats in minutes per 2 hours (min/2h) and minutes per 24h (min/24h) data files (CSV). Sensor data per animal was aligned around the day of calving.

The data files with daily summations (min/24h) were averaged per 30 days, creating "months" before and after calving. The day before calving, calving day and the day after calving were used separately as "month 0" because of specific alterations in the behavioral patterns on these days around calving [9]. Month -1 consisted of d -31 until d -2. Month 1 in lactation consisted of d 2 until d 31. Every month in lactation until month 10 consisted of 30-day cycles and do not represent calendar months. The data files with data per 2 hours were used to study the 24h pattern. The numbers of animals differ slightly between models due to sensor data transfer.

#### **Statistical Analysis**

To be able to analyze these data sets (over 150Gb), analysis was carried out using R via the Google Colab system, including packages: "car" [30], "carData" [31], "dplyr" [32], "emmeans" [33], "ggplot2" [34], "gridExtra" [35], "lme4" [36], "lmerTest" [37], "lsmeans" [38], "multcomp" [39], "multcompView" [40], "mvtnorm" [41], "plyr" [42], "readr" [43], "TH.data" [44], "tidyr" [45], and the R Project [46].

All statistical analyses including code scripts can be downloaded at https:// github.com/Bovi-analytics/hut-et-al-2021. Independent variables used were the unique herd and animal identifier, parity of the animal(1, 2, 3+), months in milk (-1 to 10 for monthly analysis, 1-10 for 24h pattern in lactation to exclude dry period effects), calving season (spring: April/May/June, summer: July/August/September, autumn: October/ November/December, winter: January/February/March), and 2 hour blocks (12 blocks from 0 to 22 for the 24h pattern) For visualization purposes, farms were divided in 2 groups: AMS-C (automatic milking system – confined) and CMS-P (conventional milking system – pasture access). A continuous 'months in milk' variable was also added as repeated measures to account for covariance over time. Separate models were built for each of the sensor values: eating time, rumination time, lying time, standing time and walking time. All final model residuals were checked for normal distribution with QQ plots.

First, all explanatory variables were tested in univariable linear mixed effect models taking into account a random effect of each animal nested within the fixed effect of the herd. Each of the univariable models showed a lower Akaike's Information Criterion (AIC) compared to the null model only taking into account the random effect. Multivariable model building was based on AIC. First, a multivariable model was

created with every factor in a complete model. Second, possible pairwise interactions were created between all offered variables. Two final multilevel models were created based on the lowest AIC: one final model per behavioral parameter for the complete daily time budget and a second final model for the 24h pattern. These models had the lowest AIC in every behavioral parameter analysis. The final model (model 1) for time budgets over lactation cycles as independent variable resulted in the following model: months in milk, parity, farm and calving season were used as fixed effects taking into account a repeated effect of months in milk nested within each cow. Biologically relevant interactions were included, namely months in milk with parity, months in milk with farm and months in milk with calving season. The final model (model 2) for the 24h pattern based on 2 hourly sensor data was as follows: model 1 with 2h block as extra fixed effect and interactions between 2h block and parity, 2h block and farm, and 2h block and calving season considering a repeated effect of months in milk nested within each cow. Final model effects were reported and plotted as least square means (LSM) with 95% confidence intervals (95% CI). Multiple comparisons contrasts were adjusted using the Tukey method. Per graph, significant differences (P<0.05) were present when the 95% CI error bars did not overlap.

#### RESULTS

#### Monthly time budget models

The complete time budget of all cows in this study from 1 month before calving until 10 months in milk is presented in figure 2A. These overall estimates show that behavioral parameters have a pattern during the lactational cycle. Lying time decreased from 1 month before calving until 1 month after calving. After, lying time gradually increased towards the end of lactation. Standing time showed an inverse pattern of lying time. While eating time decreased after calving, rumination time increased up to 4 months in milk. Eating time increased after 1 month in milk towards 6 months in milk and seemed to decrease afterwards. Walking time increased the first month after calving and decreased afterwards. In total, daily time budgets changed over the course of lactation with most notable changes from 1 month before until 1 month after calving when cows transitioned from the dry period into the lactational period.

The final model showed significant effects (P<0.001) of parity, farm and calving season. Therefore, the LSM and 95% CI predictions per behavioral parameter for parity groups (1, 2 and 3+), farms and calving season are presented in figures 3-5, all exact estimates are available on the previously reported open access repository.

Eating time of primiparous cows increased with 88 min (95% CI: 76-101) (fig. 3A) from 1 month before first calving until month 9 in milk. Cows in parity group 2 and 3+ spent more time eating (30 min (95% CI: 20-40) and 26 min (95% CI: 18-33))

the first month pre partum compared to 1 month post partum. After the first month, eating time for parity 2 and 3+ increased until 6 months in milk (11 min (95% CI: 1-21) and 24 min (95% CI: 16-32), respectively). Eating time differed between parity groups over the presented period, except for month 3 and for in milk for parity 1 and 2.

For rumination time, primiparous cows had an increase of 69 min (95%CI: 57-82) (fig. 3B) from 1 month before calving to 1 month after calving. A further incline of 33 min (95% CI: 22-44) up to 4 months in milk was present and remained more or less stable during the rest of lactation. Multiparous cows showed an increase of between 17 min (95% CI: 6-28) and 28 min (95% CI: 21-35) from 1 month pre partum to 1 month post partum for parity 2 and 3+, respectively. Towards peak lactation a further increase of 8 min (95% CI: 3-18) for parity 2 and 23 min (95% CI: 15-31) for parity 3+ was present, followed by a decline of around 20 min until the end of lactation for both parity groups.

Standing time of primiparous cows had a large increase (fig. 3C) of 159 min (95% CI: 138-179) between 1 month before calving and 1 month after calving. In month 2 post partum, their standing time decreased by 51 min (95% CI: 33-69), with a further decrease of 48 min (95% CI: 30-66) over the remainder of lactation. Multiparous cows showed a slightly different pattern. Their standing time increased with 117 min (95% CI: 100-135) for parity 2 and with 133 min (95% CI: 121-146) for parity 3+. Towards the end of lactation, standing time decreased with 72 min (95% CI: 53-90) for parity 2 and with 77 min (95% CI: 63-91) for parity 3+.

A large decrease in lying time of 215 min (95% CI: 187-242) was shown by primiparous cows (fig. 3D) from 1 month before calving to 1 month after calving. Their lying time increased by 113 min (95% CI: 89-137) at around 7 months in milk. Older cows show a similar pattern but with less decline after calving (113 min (95% CI: 91-136) for parity 2 and 130 min (95% CI: 114-146) for parity 3+). From month 1 in milk to the end of lactation, multiparous cows increased in lying time with 67 min (95% CI: 49-85) and 77 min (95% CI: 53-100) for parity 2 and 3+, respectively.

The patterns of walking time (fig. 3E) were similar compared to standing time where primiparous cows experienced the largest increase in walking time between 1 month before and 1 month after calving (23 min, 95% CI: 20-26). Until month 10 in milk, walking time decreased with 16 min (95% CI: 13-19). The course for parity 2 and 3+ was similar in pattern. Walking time differed between parity groups over the presented period, except for month -1 for parity 1 and 2.

Differences between farms are illustrated in figure 4 and grouped by color for farm types: AMS-C and CMS-P. Sensor data from the neck sensor (eating and rumination time) showed overlapping patterns between farms, without distinction between the two farm types. The sensor data from the leg sensor (standing, lying and walking time) presented distinction between the two farm types, with cows from AMS-P farms showing higher lying time and lower standing and walking time compared to CMS-P cows.

The effects of calving season on daily time budgets is shown in figure 5. Cows calving in winter show a steeper incline in eating time after calving compared with cows calving in other seasons (fig. 5A). Other behavioral parameters showed similar patterns per season except for walking time. The effects of calving season (fig. 5E) show the effects of pasture access in spring, summer and the first part of autumn for CMS-P cows. These effects were analyzed separately with factor farm as random factor, results are available on the previously mentioned online open access repository.



**Figure 2.** A,B: Overall time budget (eating, rumination, lying, walking and standing) based on least square means (LSM) with 95% confidence intervals (95% CI) of all cows on 8 commercial dairy farms in The Netherlands from 1 month before calving until 10 months in milk with "month 0" consisting of d-1, d0 and d+1 (A) and their overall 24h pattern time budget (B).



**Figure 3.** A,B,C,D,E: Time budget parameters in minutes per day (min/day) based on least square means (LSM) with 95% confidence intervals (95% CI) grouped by parity (1, 2 and 3+) on 8 commercial dairy farms in The Netherlands from 1 month before calving until 10 months in milk with "month 0" consisting of d-1, d0 and d+1.



**Figure 4.** A,B,C,D,E: Time budget parameters in minutes per day (min/day) based on least square means (LSM) with 95% confidence intervals (95% CI) per farm grouped by color: red= AMS-C, blue = CMS-P (automatic milking system – confined, and conventional milking system – pasture access) on 8 commercial dairy farms in The Netherlands from 1 month before calving until 10 months in milk with "month 0" consisting of d-1, d0 and d+1.


**Figure 5.** A,B,C,D,E: Time budget parameters in minutes per day (min/day) based on least square means (LSM) with 95% confidence intervals (95% CI) grouped by calving season (Spring, Summer, Autumn, Winter) on 8 commercial dairy farms in The Netherlands from 1 month before calving until 10 months in milk with "month 0" consisting of d-1, d0 and d+1.

#### 24h time budget models

The daily time budget based on 2 hourly sensor data blocks is presented in figure 2B. During daytime, cows spent most time eating, standing and walking, while lying and rumination occurred mostly during the night.

The final model showed significant effects (P<0.001) of parity, farm and calving season. The LSM and 95% CI predictions per 24h pattern of each behavioral parameter for parity groups (1, 2 and 3+), farms and calving season are presented in figures 6-8, all exact estimates are available on the previously reported open access repository.

Cows in parity group 3+ spent less time eating (32 min/2h (95% CI: 32-33)) during the entire 24h course compared to younger cows (38 min/2h (95% CI: 37-38) (fig. 6A), while rumination patterns (fig. 6B) are more or less comparable across parities. During the night, cows in parity group 3+ spent less time lying (fig. 6D) compared with the other groups (71 min/2h (95% CI: 70-72) versus 77 min/2h (95% CI: 75-79)), but lying time during the morning was higher (57 min/2h (95% CI: 56-58)) in the group of older cows compared to younger cows (53 min/2h (95% CI: 52-54)). Parity groups showed lower walking time (fig. 6E) with increasing parity during the 24h pattern with at noon, for example, 5.3 min/2h (95% CI: 5.2-5.4) for parity 1, 4.5 min/2h (95% CI: 4.4-4.6) for parity 2, and 3.9 min/2h (95% CI: 3.8-4.0).

The 24h patterns of the different farms were very similar with a daytime pattern of mainly eating (20-50 min/2h), standing (50-100 min/2h) and walking (3-8 min/2h) during the day while during the night rumination (45-65 min/2h) and lying (50-90 min/2h) were most dominant (fig.7). Cows from AMS-C farms showed less walking time (3-4 min/2h) during the morning compared to cows from CMS-P farms (4-8 min/2h).

Daily patterns separated by calving season only showed differences for walking time: cows that calved in winter or spring showed higher activity during the daytime of 5.5 min/2h (95% CI: 5.4-5.6) versus 4.5 min/2h (95% CI: 4.4-4.6) for cows that calved in summer or autumn (fig. 8).



**Figure 6.** A,B,C,D,E: 24h pattern in minutes per 2 hours (min/2hours) based on least square means (LSM) with 95% confidence intervals (95% CI) grouped by parity: 1, 2 and 3+ on 8 commercial dairy farms in The Netherlands from 00:00AM until 22:00PM.



**Figure 7.** A,B,C,D,E: 24h pattern in minutes per 2 hours (min/2hours) based on least square means (LSM) with 95% confidence intervals (95% CI) per farm grouped by color: red= AMS-C, blue = CMS-P (automatic milking system – confined, and conventional milking system – pasture access) on 8 commercial dairy farms in The Netherlands from 00:00AM until 22:00PM.



**Figure 8.** A,B,C,D,E: 24h pattern in minutes per 2 hours (min/2hours) based on least square means (LSM) with 95% confidence intervals (95% CI) grouped by calving season (Spring, Summer, Autumn, Winter) on 8 commercial dairy farms in The Netherlands from 00:00AM until 22:00PM.

#### DISCUSSION

The goal of this study was twofold. First, we wanted to study how time budgets of dairy cows vary between the dry period and the lactational period, and vary over the lactation period. Second, we wanted to study the daily activity pattern. In this study we used sensor data of 1074 cows with 3201 cow lactations collected over a period of 5 years from 8 commercial Dutch dairy farms. The resultant sensor data of multiple parities per individual cow was modeled as cow nested within the fixed effect of the farm as a random factor and corrected for repeated observations over time. Our results show that primiparous cows present vastly different time budgets compared to multiparous cows, from 1 month before calving throughout lactation. Primiparous and multiparous cows present a distinct 24h pattern of lying and ruminating during the night and walking, standing and eating during the day.

Others have already studied sensor data in the transition period (from 3 weeks before until 3 weeks after calving) per day or week while we studied 1 month before and 1 month after calving [18,25]. While our data shows comparable patterns as other studies of baseline behavioral sensor data output, we considered the last 31 days before calving, summed and averaged in min/day (and d-1, d0 and d+1 were studied separately as "month 0"). Because of the effect of calving on behavioral patterns, the day before calving, the day of calving and the day after calving were modeled as a separate "month" to prevent interference with baselines before and after calving [47-49]. Behavioral patterns in the transition period are subject to change [50]. Our data corroborated this, presenting differences between month -1 and month +1, even if our data includes healthy as well as less healthy cows. Others reported such transition patterns as useful parameters to relate to transition diseases [50-52].

Parity differences in behavioral parameters were described earlier, although mostly related to the transition period [9,18,53]. Where we only studied eating time, others studied meals per day, visits per meal, meal size, meal time, DMI and feeding rate. Younger cows spent more time eating with more meals, more visits, lower DMI, and lower feeding rate compared to older cows. Although that study was performed on 1 farm, our results on 8 farms for eating time are consistent with more eating time for primiparous cows compared to older cows [54]. Additionally, primiparous cows showed smaller bite size compared to multiparous cows, which could explain higher eating time with less DMI [55]. Primiparous cows also showed improved health and production when housed in a separate group the first month after calving [56]. All cows have energy requirements for milk production, but primiparous cows differ metabolically because they need energy for growth as well [57]. This suggests that the 24h patterns of primiparous cows were revealing more eating time and longer walking time patterns as the quantified effect of hierarchical differences between primiparous and multiparous cows. Primiparous cows also have less weight than older animals which might result in

evasive behavior when conflicts for feed, milking order or resting places arise especially after introduction to the milking herd for the first time [58-60]. Combining these effects on behavior, health, production and growth, it could be advisable to house primiparous cows separate from multiparous cows, which is relatively simple to implement in larger herds [61].

While others have studied the effect of different housing and milking systems on lying behavior, daily behavioral patterns are complex and dynamic combinations of zootechnical circumstances, stocking density, ration and management [14,62,63]. For lying time the same trajectory was seen by others who report a drop in the early post partum period and a gradual rise towards the end of lactation [64]. The only behavioral parameter that follows the lactation curve is rumination indicating that peak production correlates with peak rumination and does not seem to coincide with peak lying time.

Our results suggest that differences among farms were associated with the management type. We suggest that these differences were most likely influenced by pasture access on CMS farms. However, when separating for calving season, the pasture access effect on walking time was especially strong for winter and spring calving cows. Previous studies have shown conflicting results regarding the effects of pasture access on behavior. Some report that cows on pasture have higher lying times [65] where others show lower lying, standing, and rumination times and higher eating time [66]. Our results show lower lying time and higher standing and walking time on farms with pasture access. Less lying and more standing time in our study could indicate higher waiting times before milking on CMS-P farms compared to AMS-C farms [20]. Also, other farm management differences as cubicle size and cubicle bedding could confound these results. On eating time, it probably takes more time to ingest the same amount of dry matter while grazing compared to complete ration feeding indoors. On AMS farms, cows have a more continuous flow of eating compared to farms with grazing [15]. However, eating and rumination times between AMS-C and CMS-P farms overlapped greatly. All farms fed a PMR (partial mixed ration) which typically contained 75% grass silage, 25% maize silage supplemented with different protein sources and balanced concentrates. In the CMS-P group, cows also had pasture access as part of the feeding strategy, which was clearly illustrated in walking time variation. Unfortunately, these detailed feed and ration data were not available and these effects could not be studied further.

The 24h patterns in this study present a clear diurnal rhythm per behavioral parameter. This pattern cannot be observed when utilizing sensor data on a daily scale. The main behavioral variations occurred in eating, standing and walking during the day and rumination and lying during the night. A nightly lying and rumination pattern described earlier seems consistent with our data [21]. We expect that cows in our study are able to present simultaneous lying and feeding behavior because these farms had neither overstocking in cubicles nor feeding places. Farms differed in the times of milking and fresh feed delivery, as well as in rational differences. These were not recorded. Differences between farms, as presented by AMS-C and CMS-P groups, were most clear in leg sensor data. Leg sensor data from cows in the CMS-P group showed more daily variations compared to cows in the AMS-C group. The daily patterns, however, are comparable between both groups. For instance, standing time has a peak in the morning and at the end of the afternoon in both groups. In the AMS-C group, cows have a voluntary milking system, while this is an obligatory moment in the CMS-P group.

Diurnal patterns in fully grazing systems are to our knowledge unknown. Circadian rhythms based on an indoor positioning system showed that deviations from this rhythm were useful to detect disease and estrus expression [23, 24]. This could imply that sensor data which monitors 24h patterns could give rise to specific algoritms for early disease detection in individual animals. Furthermore, our data provides a benchmark for sensor data to use in decision support in daily management such as feeding or monitoring welfare in lying and standing.

#### CONCLUSIONS

This study presented the variability in time budget from the late dry period to the late lactation cycle. Time budgets differ between first, second and older cows, particularly eating time. As first parity cows showed different time budgets compared to older animals, these young animals might need specific management to better adapt to the milking herd. Time budgets of cows from different farm types were comparable. Finally, the dairy cows in this study showed a 24h pattern per behavioral parameter, indicating dairy cow behavior has a diurnal or circadian aspect.

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#### **Author Contribution Statement**

PH collected the data. PH wrote the paper. Analysis was carried out by SK, MH and PH, supervised by MN. The study was designed by PH, SK, MH, ES, and MN. JH acted as external advisor because of his specific expertise in this topic, but did not have

any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. All authors read and approved the final manuscript.

### **Conflict of Interest Statement**

JH is co-owner of Vetvice BV (Bergen op Zoom, The Netherlands). All authors declare that they have no conflict of interest related to the study discussed in this manuscript.

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# **Chapter 3**

# Heat stress in a temperate climate leads to adapted sensor-based behavioral patterns of dairy cows

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# ABSTRACT

Most research on heat stress has focused on (sub)tropical climates. The effects of higher ambient temperatures on the daily behavior of dairy cows in a maritime and temperate climate are less studied. With this retrospective observational study, we address that gap by associating the daily time budgets of dairy cows in the Netherlands with daily temperature and temperature-humidity index (THI) variables.

During a period of 4 years, cows on 8 commercial dairy farms in the Netherlands were equipped with a neck and leg sensors to collect data from 4,345 cow lactations regarding their daily time budget. The time spent eating, ruminating, lying, standing, and walking was recorded. Individual cow data were divided into three datasets: (1) lactating cows from 5 farms with a conventional milking system (CMS) and pasture access, (2) lactating cows from 3 farms with an automatic milking system (AMS) without pasture access, and (3) dry cows from all 8 farms.

Hourly environment temperature and relative humidity data from the nearest weather station of the Dutch National Weather Service was used for THI calculation for each farm. Based on heat stress thresholds from previous studies, daily mean temperatures were grouped into 7 categories:  $0 = (< 0^{\circ}C)$ ,  $1 = (0-12^{\circ}C)$ , reference category),  $2 = (12-16^{\circ}C)$ ,  $3 = (16-20^{\circ}C)$ ,  $4 = (20-24^{\circ}C)$ ,  $5 = (24-28^{\circ}C)$  and  $6 = ( \ge 28^{\circ}C)$ ; Temperature-humidity index values were grouped as follows: 0 = (THI < 30), 1 = (THI 30- 56, reference category), 2 = (THI 56-60), 3 = (THI 60-64), 4 = (THI 64-68), 5 = (THI 68-72) and  $6 = (THI \ge 72)$ . To associate daily mean temperature and THI with sensor-based behavioral parameters of dry cows and of lactating cows from AMS and CMS farms, we used generalized linear mixed models. In addition, associations between sensor data and other climate variables, such as daily maximum and minimum temperature, and THI were analyzed.

On the warmest days, eating time decreased in the CMS group by 92 min/d, in the AMS group by 87 min/d, and in the dry group by 75 min/d compared with the reference category. Lying time decreased in the CMS group by 36 min/d, in the AMS group by 56 min/d, and in the dry group by 33 min/d. Adaptation to daily temperature and THI was already noticeable from a mean temperature of 12°C or a mean THI of 56 or above, when dairy cows started spending less time lying and eating and spent more time standing. Further, rumination time decreased, although only in dry cows and cows on AMS farms. With higher values for daily mean THI and temperature, walking time decreased as well. These patterns were very similar for temperature and THI variables.

These results show that dairy cows in temperate climates begin to adapt their behavior at a relatively low mean environmental temperature or THI In the temperate maritime climate of the Netherlands, our results indicate that daily mean temperature suffices to study the effects of behavioral adaptation to heat stress in dairy cows.

Key words: dairy cow, heat stress, sensor data, time budget

#### **INTRODUCTION**

If current climate change continues without mitigation measures, temperatures are estimated to increase by 4 °C by the year 2100 (Naumann et al., 2021). In addition to the gradual overall increase in temperature, heatwaves in Europe are increasingly frequent (Schär et al., 2004). Finally, in dairy cattle, endogenous heat is generated by high-producing cows due to their high metabolism (Kadzere et al., 2002; Hansen, 2007). A combination of increasing milk production with higher metabolic heat production and increasing external temperature could result in more and longer periods of heat stress in dairy cows.

Heat stress can be measured in various ways. For example, heat stress in cattle can be identified using environmental temperature as a sole parameter, since it correlates with rectal temperature (Dikmen and Hansen, 2009). Meteorological variables that are used to monitor heat stress are often based on a combination of temperature and relative humidity: the Temperature Humidity Index (THI), a unit first reported as a discomfort index for humans (Thom, 1959). Historically, heat stress in dairy cattle is indicated by a cut-off value of 72 for THI and 28°C or above (Armstrong, 1994; Dikmen and Hansen, 2009), which is deemed to indicate stressful climatic conditions (McDowell et al., 1976). When calculating this boundary, humidity normally weighs more heavily in the equation in humid climates, while in dry climates, the temperature suffices (Bohmanova et al., 2007); different ranges for the thermoneutral zone of cows have been given. A review in dairy cattle shows that heat stress can be present from a THI value of 68 (de Rensis et al., 2015). According to a study in temperate and maritime climatic regions, heat stress threshold values were found at a mean THI of 60 or a mean daily temperature of 16°C (Brügemann et al., 2012).

Higher ambient temperatures during the dry period results in decreased milk production in the following lactation because of compromised mammary development in the late dry period compared with cows that are cooled (Tao et al., 2011). Higher ambient temperature also increases disease incidence postpartum (Tao and Dahl, 2013), and results in decreased reproductive performance in the following lactation (Avendaño-Reyes et al., 2010; Thompson and Dahl, 2012). Moreover, heat stress in the dry period has a negative effect on fetal growth and immune function in the calf (Tao et al., 2012), resulting in decreased milk production during the productive life of the offspring, thus having a negative effect over generations (Dado-Senn et al., 2020).

Cows try to adapt to increasing ambient temperature by altering their behavior. By decreasing lying time and increasing standing time, cows expose a greater surface area to the air to cool as much as possible (Schütz et al., 2011; Allen et al., 2015). Increased standing time is associated with a higher risk for lameness (Cook et al., 2007; Cook and Nordlund, 2009). As the THI increases, their dry matter intake (DMI) decreases, resulting in reduced milk production (West, 2003; Bohmanova et al., 2007). During heat stress induced in climate chambers, cows' respiration rate and internal body temperature increase (de Andrade Ferrazza et al., 2017), and their energy requirements also increase (NRC, 2021). Thus, this decreased DMI and increased energy requirements leads to a deeper negative energy balance in early lactation cows, which has a negative correlation with production, reproduction, and health (Baumgard and Rhoads, 2012; Bernabucci et al., 2014).

For early identification, investigation, and management of heat stress, thorough monitoring is essential. Several commercial sensor systems are available to monitor dairy cattle (Stygar et al., 2021). Monitoring data collected during heat stress show that cows decrease rumination when THI increases (Soriani et al., 2013; Moretti et al., 2017). Rumination begins to decrease from a THI of 52 (Müschner-Siemens et al., 2020), yet studies reporting the effects of higher ambient temperatures in temperate climates on the complete time budget (feeding, lying and standing behavior) of dairy cows are lacking. The time budget varies over the transition period and is known to differ between dry and lactating cows, between parity groups (Huzzey et al., 2005; Neave et al., 2017; Hut et al., 2019), between cows on farms with automatic milking systems (AMS) and cows on farms with conventional milking systems (CMS) (Wagner-Storch and Palmer, 2003), and between cows on farms with or without pasture access (Roca-Fernández et al., 2013); however, these differences could also be influenced by climatic conditions.

To address several gaps in understanding outlined above, the objective of this retrospective observational field study was to associate climate variables with complete time budgets of dairy cows on commercial dairy farms with different husbandry systems in a temperate maritime climate.

#### **MATERIALS AND METHODS**

#### Farms, animals and sensors

Data were collected from 4,345 cow lactations between January 1, 2017, and November 4, 2020, on 8 dairy farms with free stall barns in the Netherlands. On 3 farms in this study, cows were milked with an automatic milking system (AMS) and had no pasture access. The other 5 milked with a conventional milking system (CMS) and the lactating herd had pasture access for at least 120 d annually for at least 6 h per day, whereas the dry cows had no pasture access. The farms contributing to this study can be considered representative of the modern Dutch dairy industry. For further details of the farms, see Table 1 and Hut et al. (2021). Farms differed in the exact times of milking and fresh feed delivery, as well as in the exact ration composition. All farmers fed a partial mixed ration (PMR) that typically contained 75% grass silage and 25% maize silage, supplemented with different protein sources and balanced concentrates. Dry cows were fed low-energy diets based on roughage from the milking herd, diluted with straw or hay. None of these farms had cooling systems; instead, ll farms had a combination of natural ventilation (open sides with open roof ridge) and 1 or more fans. Cows on CMS farms were milked twice per day. Depending on the available sensor data, the number of cow lactations varied between 2,821 and 2,847 for CMS farms, and between 1,338 and 1,498 for AMS farms. The number of dry periods varied between 3,616 and 3,676 cow lactations for both farms.

Cows on all 8 farms were equipped with 2 commercially available sensors from Nedap Livestock Management: a neck sensor (Nedap Smarttag Neck) that collected data regarding eating and rumination time (Borchers et al., 2021), and a leg sensor (Nedap Smarttag Leg), that collected data concerning lying, standing, and walking time (Nielsen et al., 2018). On these farms, not every pregnant heifer was equipped with both sensors before first calving. The use of such sensors in a commercial dairy herd is not considered an animal experiment under Dutch law; therefore, formal ethical approval was not necessary.

Farm no.	No. of dairy cows	Milking system <sup>1</sup>	Pasture access	Start data collection	End data collection
1	140	CMS	Yes	01-01-2017	09-04-2019
2	180	AMS	No	01-01-2017	04-11-2020
3	170	CMS	Yes	01-01-2017	04-11-2020
4	115	CMS	Yes	01-01-2017	04-11-2020
5	125	AMS	No	19-05-2017	04-11-2020
6	120	CMS	Yes	02-06-2017	04-11-2020
7	110	AMS	No	13-05-2017	04-11-2020
8	176	CMS	Yes	01-01-2017	03-11-2020

Table 1. Details of the 8 farms in this study

1 AMS = automatic milking system; CMS = conventional milking system.

#### Study design

Sensor data were provided by Nedap Livestock Management (Groenlo, the Netherlands) per behavioral parameter in minutes per 15-min time block. These data were summed to create daily totals for each of the 5 behavioral parameters, expressed in minutes per day. For each cow and lactation, all sensor data that were available between 21 d before calving and 305 d after calving were included. Days in milk, based on the day of calving, were categorized in 6 groups as follows: <0 d (DIM = 0): the prepartum transition period; 0 to 21 d (DIM = 1): the postpartum transition period; 21 to 60 d (DIM = 2): fresh cows; 61 to 120 d (DIM = 3): peak lactation; 121 to 200 days (DIM = 4): mid lactation; and >200 d (DIM = 5): late lactation. Parity had 8 levels: 1, 2, 3, 4, 5, 6, 7, and  $\geq$ 8.

The individual cow data were divided into 3 datasets: (1) dry cows from all 8 farms, (2) lactating cows from the 5 CMS farms, and (3) lactating cows from the 3 AMS farms.

Ambient temperature (expressed in °C) and ambient relative humidity (expressed as a percentage) were recorded hourly by the Dutch National Weather Service (KNMI) at various locations. For each farm, the recordings of the nearest weather station were used. The THI was calculated following the NRC (National Research Council), 1971:

THI = (1.8 \* Temperature + 32) - (0.55 - 0.0055 \* Relative air humidity) \* (1.8 \* Temperature - 26).

To be able to study effects of heat stress on time budgets of cows, temperature and THI were classified into groups based on the different cut-off values found in other studies for the thermoneutral zone (Kadzere et al., 2002; Brügemann et al., 2012). To allow the study of a change in daily time budget before reaching those cut-off values, we classified the mean and maximum THI values per day into 7 groups as follows: 0 (THI <30), 1 (THI 30-56, reference category), 2 (THI 56-60), 3 (THI 60-64), 4 (THI 64-68), 5 (THI 68-72) and 6 (THI  $\geq$ 72). The mean and maximum temperatures per day were also classified into 7 groups. The classification for temperature was as follows: 0 (<0°C), 1 (0-12°C, reference category), 2 (12-16°C), 3 (16-20°C), 4 (20-24°C), 5 (24-28°C) and 6 ( $\geq$ 28°C).

Grouping of temperature and THI values per increments of 3 and 5, and minimum and maximum temperature and THI values were analyzed as well (all models and results available at https://github.com/Bovi-analytics/Hut-et-al-2022).

#### **Statistical Analysis**

The effect of climate variables on average lying and standing time, the median of log-transformed walking time (for normal distribution) and the average eating and rumination time (in minutes per cow per day) were analyzed using generalized linear mixed models.

The temperature (mean/maximum) or THI (mean/maximum) variable was included as the main effect, with reference category 0 to 12°C for temperature and 30 to 56 for THI. All behaviors were corrected for cow-related factors: parity (1-8), DIM category (0-5), farm and design-related factors such as month and year, all as fixed effects.

"Cow" was included as a random effect to correct for multiple observations per cow, and "Day" was included as a random effect to correct for day-specific conditions that may influence time budgets. No model reduction strategy was applied. For all models, residuals were plotted to check for normality. A 95% profile (log-)likelihood confidence interval was calculated for each estimate. Data was analyzed in Python with R scripts (version 4.1.2; R Core Team, 2019) via the Google Colab platform, including packages glmmTMB (Brooks et al., 2017), dplyr (Wickham et al., 2021), plyr (Wickham, 2011), ggplot2 (Wickham, 2016), emmean (Lenth, 2021), and Ismeans (Lenth, 2016).

#### RESULTS

#### **Descriptive statistics**

We collected sensor data from 4,345 cow lactations monitored on 8 dairy farms in the Netherlands from 2017 to 2020. In Figure 1, the data are plotted per month and present sensor data for lying, standing, walking, eating, and rumination time. With increasing temperature and THI in spring and summer, a pattern is seen of less time lying and more time standing and walking compared with patterns in autumn and winter. No clear annual pattern was observed in eating and rumination time. Furthermore, the monthly climate variables indicate that temperature and THI follow similar patterns, whereas humidity is relatively stable in the Netherlands.

In Figure 2, we present an overview of sensor data of dry versus lactating cows. On average, lactating cows spent less time lying and more time standing, walking, eating, and ruminating than dry cows. Dry and lactating cows showed similar annual patterns in lying, walking and standing, but at different levels. They were less similar in terms of annual patterns of eating time and rumination time. In months where the THI has the highest values, lactating cows spent less time eating and more time ruminating, whereas dry cows spent less time ruminating and more time eating.

To obtain insight into the variability in eating and rumination time in lactating cows, this group was further divided into lactating cows on CMS farms (Figure 3A) and lactating cows on AMS farms (Figure 3B), as these two farm types differed in pasture access during the warm period of the year.



**Figure 1.** Overall sensor and climatic data from 2017 to 2020 in means per month on 8 dairy farms in the Netherlands. Sensor data of 4,345 cow lactations consists of daily lying, standing, walking, eating, and rumination time in minutes per day. Climatic data consists of mean and maximum daily temperature-humidity index (THI), mean and maximum daily ambient temperature (Temp; °C), and mean and maximum daily air humidity (relative humidity; RH, %), mean always being the lowest value in the graphs.



**Figure 2.** Daily sensor data from 2017 to 2020 of daily lying, standing, walking, eating, and rumination time in average minutes per day per month on 8 dairy farms in the Netherlands. Overview of monthly data of (A) lactating cows (n = 4,345 cow lactations); and (B) dry cows (n = 3,676 dry periods) is presented.



**Figure 3.** Daily sensor data from 2017 to 2020 of daily lying, standing, walking, eating, and rumination time in average minutes per day per month on 8 dairy farms in the Netherlands. Overview of monthly data of (A) cows with pasture access (n = 2,847), milked with a conventional milking system (CMS); and (B) cows without pasture access (n = 1,498), milked with an automatic milking system (AMS).

#### Statistical analysis

The mixed model analysis showed increasing effects of temperature and THI on the time budget of lactating and dry cows. Higher average daily temperature and higher THI corresponded to more pronounced effects on sensor data for all measured variables, with cows lying and eating less. These results of the mixed model analyses per cow group are presented in Figure 4, Figure 5 as well as Tables 2, 3, 4, 5, and 6.

On average, lactating cows on CMS farms spent 612 min/d lying. Their lying time decreased 8 min/d when the THI reached 56 and decreased gradually to 566 min/d when the THI  $\geq$ 72 (Figure 4A). Lactating cows on AMS farms spent on average 688 min/d lying. Lying time decreased with 6 min/d beginning when the THI reached 56 and decreased gradually to 627 min/d when the THI  $\geq$ 72 (Figure 4B). Dry cows spent on average 664 min/d lying, and this decreased by 8 min/d beginning with a THI of 56-60 and reaching 630 min/d when the THI  $\geq$ 72 (Figure 4C).

Lactating cows on CMS farms spent on average 773 min/d standing, cows on AMS farms 727 min/d standing and dry cows 680 min/d (Figure 4D, E, F). The standing time increased when the daily mean THI increased and the effect was inverse to the decrease in lying time.

The walking time of lactating cows on CMS farms decreased as THI increased, starting with a THI >64, in contrast to AMS or dry cows (Figure 4G, H, I). The AMS and dry cows only showed decreased walking time at THI  $\geq$ 72, the highest THI class (Figure 4H, I).

On average, lactating cows on CMS farms spent 323 min/d eating and those on AMS farms 348 min/d (Figure 5A, B). Dry cows spent 374 min/d eating (Figure 5C). Eating time decreased as mean daily THI increased. Eating time decreased 5 min/d for lactating cows on CMS farms when the mean daily THI reached 60 and continued decreasing until it totaled 75 min/d less time eating when THI was  $\geq$ 72 (Figure 5A). Lactating cows housed on AMS farms spent 4 min/d less time eating when the average daily THI was  $\geq$ 56, and 70 min/d less when the THI reached  $\geq$ 72 (Figure 5B). The average daily eating time of dry cows decreased as well, from 6 min/d beginning at a THI value of 64, to 41 min/d at a THI value  $\geq$  72(Figure 5C).

Lactating cows on CMS farms spent around 573 min/day ruminating. Beginning at a THI of 68, their rumination time increased 12 min/day reaching 14 min/day by THI  $\ge$  72 (fig. 5D). This is in contrast with lactating cows on AMS farms (542 min/day), where rumination decreased 9 minutes beginning at a THI  $\ge$  72 (fig. 5E). In contrast, in dry cows (559 min/day) a decrease of 5 min/day was present from a THI of 56 and 9 min/day less at THI of  $\ge$  72 (fig. 5F).



**Figure 4.** Predicted least squares mean with 95% confidence intervals of daily lying time (A–C), standing time (D–F), and median walking time (G–I) in minutes per day plotted against daily mean temperature-humidity index (THI). Left-hand panels present lactating cows on farms with conventional milking systems (CMS, n = 2,821 cow lactations), middle panels present lactating cows milked with an automatic milking system (AMS, n = 1,338 cow lactations), and right-hand panels present dry cows from all 8 farms (dry, n = 3,616 cow dry periods). THI group 0 represents THI <30; group 1: 30–56; group 2: 56–60; group 3: 60–64; group 4: 64–68; group 5: 68–72; group 6:  $\geq$ 72. Colors darken as THI values increase.



**Figure 5.** Predicted least squares mean with 95% confidence interval of daily eating time (A–C) and rumination time (D–F) in minutes per day plotted against daily mean temperature-humidity index (THI). Left-hand panels present lactating cows on farms with conventional milking systems (CMS, n = 2,847 cow lactations), middle panels lactating cows milked with an automatic milking system (AMS, n = 1,498 cow lactations), and right-hand panels present dry cows from all 8 farms (n = 3,676 cow dry periods). THI group 0 represents THI <30; group 1: 30–56; group 2: 56–60; group 3: 60–64; group 4: 64–68; group 5: 68–72; group 6:  $\geq$ 72. Colors darken as THI values increase.

Effects of the average daily mean temperatures of lactating and dry cows showed similar patterns as average daily mean THI. See supplemental materials (https://github. com/Bovi-analytics/Hut-et-al-2022) and Tables 2 through 6 for the effects of average daily mean temperature on daily sensor data. Effects of daily maximum and minimum temperature and THI, as well as the mean temperature and THI of the previous 2 d, on the different sensor data, were also evaluated in linear mixed model analyses. The responses from the 2 days prior to the day of measurement were less clear than the reported adaptation in daily time budget on the particular day. The time budgets of cows were most strongly influenced by a higher mean daily temperature and THI on the particular day. Additionally, different categorial classifications for temperature and THI showed similar effects as the presented results (results not shown).

Model <sup>1</sup>		Estimates (95% CI) <sup>2</sup>	
THI	CMS	AMS	Dry
Intercept	612 (592; 632)	688 (662; 714)	664 (646; 682)
< 30	-1 (-4; 1)	-19 (-25; -12)	-14 (-24; -4)
30-56	Ref.	Ref.	Ref.
56-60	-8 (-9; -7)	-5 (-7; -4)	-8 (-12; -4)
60-64	-9 (-10; -8)	-8 (-10; -6)	-8 (-12; -3)
64-68	-22 (-24; -21)	-19 (-21; -17)	-9 (-14; -4)
68-72	-30 (-31; -28)	-37 (-41; -34)	-14 (-21; -7)
≥ 72	-46 (-48; -43)	-61 (-66; -56)	-34 (-45; -23)
Temperature °C			
Intercept	611 (592; 631)	688 (662; 715)	665 (647; 683)
< 0	-3 (-4;-1)	-15 (-18; -12)	-11 (-17; -5)
0-12	Ref.	Ref.	Ref.
12-16	-7 (-8; -6)	-3 (-5; -1)	-9 (-13; -5)
16-20	-13 (-14; -11)	-9 (-11; -7)	-9 (-13; -4)
20-24	-27 (-29; -26)	-28 (-31; -25)	-13 (-19; -7)
24-28	-43 (-45; -40)	-57 (-62; -52)	-32 (-42; -23)
≥ 28	-36 (-41; -31)	-56 (-67; -46)	-33 (-51; -15)

**Table 2.** Lying time (estimates in min/d; 95% CI in parentheses): associations from 6 multivariable models between daily lying time of cows in the CMS, AMS, and dry groups with mean daily temperature-humidity index (THI) groups and mean daily ambient temperature (°C) groups; estimates reflect change in lying time compared with the intercept

1 Cow-related and design-related factors were included in all models.

2 CMS = lactating cows on farms with conventional milking system with pasture access; AMS = lactating cows on farms with automatic milking system without pasture access; Dry = dry cows on both farms without pasture access.

Model <sup>1</sup>		Estimates (95% CI) <sup>2</sup>	
THI	CMS	AMS	Dry
Intercept	773 (754; 792)	727 (702; 753)	680 (663; 698)
< 30	5 (2; 7)	22 (16; 28)	20 (11; 29)
30-56	Ref.	Ref.	Ref.
56-60	3 (2; 4)	2 (0; 4)	5 (1; 9)
60-64	6 (5; 7)	7 (4; 9)	6 (2; 10)
64-68	20 (19; 22)	11 (8; 13)	6 (1; 11)
68-72	33 (31; 34)	38 (34; 41)	12 (6; 19)
≥ 72	58 (55; 60)	62 (57; 67)	44 (34; 54)
Temperature °C			
Intercept	773 (754; 793)	727 (701; 753)	679 (662; 696)
< 0	4 (3; 6)	18 (15; 20)	16 (10; 22)
0-12	Ref.	Ref.	Ref.
12-16	1 (0; 2)	1 (-1; 3)	3 (-1; 7)
16-20	9 (9; 10)	7 (5; 10)	6 (2; 11)
20-24	27 (25; 28)	25 (23; 28)	9 (4; 15)
24-28	49 (46; 51)	59 (54; 64)	22 (13; 30)
≥ 28	51 (47; 56)	59 (57; 67)	63 (47; 80)

**Table 3.** Standing time (estimates in min/d; 95% CI in parentheses): associations from 6 multivariable models between daily standing time of cows in the CMS, AMS, and dry groups with mean daily temperature humidity index (THI) groups and mean daily ambient temperature (°C) groups; estimates reflect change in standing time compared with the intercept

1 Cow-related and design-related factors were included in all models.

2 CMS = lactating cows on farms with conventional milking system with pasture access; AMS = lactating cows on farms with automatic milking system without pasture access; Dry = dry cows on both farms without pasture access.

1	1.			
Model <sup>1</sup>	Estimates (95% CI) <sup>2</sup>			
THI	CMS	AMS	Dry	
Intercept	39 (37; 42)	36 (33; 39)	37 (35; 40)	
< 30	1.02 (1.02; 1.03)	1.03 (1.02; 1.05)	1.06 (1.03; 1.09)	
30-56	Ref.	Ref.	Ref.	
56-60	1.02 (1.01; 1.02)	1.02 (1.01; 1.02)	1.07 (1.05; 1.08)	
60-64	1.02 (1.01; 1.02)	1.02 (1.02; 1.03)	1.08 (1.06; 1.10)	
64-68	0.97 (0.97; 0.97)	1.00 (1.00; 1.01)	1.05 (1.04; 1.07)	
68-72	0.91 (0.91; 0.92)	0.99 (0.98; 1.00)	1.05 (1.03; 1.07)	
≥ 72	0.84 (0.84; 0.85)	0.98 (0.96; 0.99)	0.96 (0.93; 1.00)	
Temperature °C				
Intercept	40 (37; 42)	35 (32; 38)	37 (35; 40)	
< 0	1.02 (1.01; 1.02)	1.04 (1.03; 1.04)	1.02 (1.00; 1.04)	
0-12	Ref.	Ref.	Ref.	
12-16	1.01 (1.00; 1.01)	1.01 (1.00; 1.01)	1.09 (1.08; 1.11)	
16-20	1.01 (1.01; 1.01)	1.02 (1.02; 1.03)	1.11 (1.10; 1.13)	
20-24	0.92 (0.92; 0.93)	1.00 (1.00; 1.01)	1.09 (1.07; 1.11)	
24-28	0.81 (0.81; 0.82)	1.00 (0.98; 1.01)	1.01 (0.98; 1.04)	
≥ 28	0.80 (0.78; 0.80)	0.95 (0.93; 0.98)	0.90 (0.85; 0.95)	

**Table 4.** Walking time (ratio; 95% CI in parentheses): associations from 6 multivariable models between daily walking time of cows in the CMS, AMS, and dry groups with mean daily temperature-humidity index (THI) groups and mean daily ambient temperature (°C) groups; estimates reflect change in walking time compared with the intercept

1 Cow-related and design-related factors were included in all models.

2 CMS = lactating cows on farms with conventional milking system with pasture access; AMS = lactating cows on farms with automatic milking system without pasture access; Dry = dry cows on both farms without pasture access.

Model <sup>1</sup>		Estimates (95% CI) <sup>2</sup>	
THI	CMS	AMS	Dry
Intercept	323 (308; 338)	348 (328; 369)	374 (363; 385)
< 30	1 (0; 3)	0 (-3; 3)	3 (-1; 8)
30-56	Ref.	Ref.	Ref.
56-60	-1 (-2; -1)	-4 (-5; -3)	1 (-1; 2)
60-64	-5 (-6; -4)	-10 (-11; -9)	-2 (-4; 0)
64-68	-22 (-23; -21)	-17 (-18; -16)	-6 (-9; -4)
68-72	-51 (-52; -50)	-41 (-42; -39)	-22 (-25; -18)
≥ 72	-75 (-76; -73)	-70 (-72; -68)	-41 (-46; -36)
Temperature °C			
Intercept	322 (307; 338)	346 (325; 367)	374 (363; 385)
< 0	4 (3; 5)	2 (0; 3)	2 (-1; 5)
0-12	Ref.	Ref.	Ref.
12-16	-3 (-3; -2)	-5 (-5; -4)	0 (-2; 1)
16-20	-8 (-9; -8)	-12 (-12; -11)	-2 (-4; 0)
20-24	-40 (-41; -39)	-30 (-31; -28)	-14 (-16; -11)
24-28	-67 (-68; -66)	-56 (-58; -54)	-34 (-38; -29)
≥ 28	-92 (-95; -89)	-87 (-92; -83)	-75 (-84; -67)

**Table 5.** Eating time (estimates in min/d; 95% CI in parentheses): associations from 6 multivariable models between daily eating time of cows in the CMS, AMS, and dry groups with mean daily temperature-humidity index (THI) groups and mean daily ambient temperature (°C) groups; estimates reflect change in eating time compared with the intercept

1 Cow-related and design-related factors were included in all models.

2 CMS = lactating cows on farms with conventional milking system with pasture access; AMS = lactating cows on farms with automatic milking system without pasture access; Dry = dry cows on both farms without pasture access.

Model <sup>1</sup>		Estimates (95% C	<b>I</b> ) <sup>2</sup>	
THI	CMS	AMS	Dry	
Intercept	573 (562; 583)	542 (528; 556)	559 (549; 568)	
< 30	-5 (-6; -3)	5 (2; 8)	10 (4; 15)	
30-56	Ref.	Ref.	Ref.	
56-60	-1 (-2; -1)	-3 (-4; -3)	-5 (-7; -3)	
60-64	0 (-1; 1)	-3 (-4; -2)	-8 (-10; -5)	
64-68	1 (0; 2)	0 (-1; 1)	-9 (-12; -6)	
68-72	12 (11; 13)	-4 (-5; -2)	-5 (-9; -2)	
≥ 72	14 (13; 16)	-9 (-11; -7)	-9 (-14; -3)	
Temperature °C				
Intercept	575 (564; 586)	548 (533; 563)	556 (546; 566)	
< 0	2 (1; 3)	0 (-1; 1)	17 (14; 21)	
0-12	Ref.	Ref.	Ref.	
12-16	-1 (-2; -1)	-3 (-3; -2)	-7 (-9; -5)	
16-20	-2 (-2; -1)	-3 (-4; -2)	-11 (-14; -9)	
20-24	8 (8; 9)	-2 (-3; -1)	-12 (-15; -8)	
24-28	13 (12; 15)	-6 (-8; -4)	-6 (-11; -1)	
≥ 28	12 (10; 15)	-26 (-31; -22)	-18 (-27; -8)	

**Table 6.** Rumination time (estimates in min/d; 95% CI in parentheses): associations from 6 multivariable models between daily rumination time of cows in the CMS, AMS, and dry groups with mean daily temperature-humidity index (THI) groups and mean daily ambient temperature (°C) groups; estimates reflect change in rumination time compared with the intercept

1 Cow-related and design-related factors were included in all models.

2 CMS = lactating cows on farms with conventional milking system with pasture access; AMS = lactating cows on farms with automatic milking system without pasture access; Dry = dry cows on both farms without pasture access.

#### DISCUSSION

The aim of the current study was to quantify the effect of ambient temperature and THI on the daily time budget of dairy cows in a temperate and maritime climate. Our results showed a direct effect of ambient temperature and THI variables on cow behavior. With increasing daily temperature and THI, cows spend less time lying, eating, and walking. Standing time increased and the effects on rumination time were inconclusive. Dairy cows adapted to increasing climatic parameters beginning with a daily mean temperature between 12°C and 16°C or a daily mean THI between 56 and 60.

Lying is a behavior of preference for dairy cows (Munksgaard et al., 2005). Reduced lying time (7 min/d less) was observed from a temperature between 12 and 16°C and between a THI of 56 and 60, and lying time declined further to as much as 40 min/d less when the mean temperature was  $\geq 28^{\circ}$ C, and to 48 min/d less when the THI was  $\geq 72$ . In a trial of 6 d, an increase in THI from 68.5 to 79 resulted in a decrease in lying time of 3h/d (Nordlund et al., 2019). This is consistent with the 3h/d decrease in lying time at a THI of 68 found by Cook et al. (2007). Our results show that this decrease in daily lying time starts at lower daily mean temperatures than is reported in previous studies.

Standing time showed the inverse effect of higher temperature and THI variables: it increased when THI increased in all cow groups studied (CMS, AMS, dry). This indicates longer weight-bearing periods with increasing ambient temperatures, potentially increasing the risk of claw health issues (Cook et al., 2007; Cook and Nordlund, 2009; Sanders et al., 2009).

Walking time showed a slight decrease with increasing climate variables, mainly in the CMS group. In the temperate climate of the Netherlands, pasture access coincides with the high temperature and THI period and was expected to confound the association between higher ambient temperatures and walking. Indeed, the absolute effect on daily walking time seems greater in the current study in lactating cows on CMS farms with pasture access than in dry cows and cows from AMS farms without pasture access. Other farm management differences could also be associated with these results, such as the distance to the milking parlor. To our knowledge, no other studies have shown an association between THI and walking time. However, decreased lying and walking times during periods of higher ambient temperature indicate a longer time standing idle in such periods.

Our results on reduced eating time could indirectly indicate reduced DMI as climate variables increased in this study and reduced DMI could lead to lower milk production. A correlation between higher ambient temperatures and lower milk production has been reported by others (Bohmanova et al., 2007; Rhoads et al., 2009; Brügemann et al., 2012). In our study, lactating cows from both AMS (confined) and CMS (pasture access) farms showed adaptation in the form of less time spent eating, beginning at a mean daily temperature of 16°C or a THI of 56, whereas dry cows started adapting in this way from 20°C or a THI of 64. The earlier adaptation of lactating cows could be caused by the extra metabolic heat production caused by milk production. Reduced feed intake starting from an ambient temperature of 25°C has been shown previously (Kadzere et al., 2002), and might be explained by the amount of milk produced, differences between climate regions, or adaptational opportunities from rising ambient climate variables.

In another study in a temperate climate, rumination time was found to decline starting at a THI of 52 (Müschner-Siemens et al., 2020), whereas results on rumination time in our study were inconclusive. However, different rumination patterns manifested for cows on AMS (confined) and CMS (pasture access) farms, as well as for dry cows on both types of farms. We studied lactating cows on AMS and CMS separately to show

the seasonal effect on rumination that could be caused by pasture access and to prevent confounding, as much as possible, by various farm management differences in our study. We hypothesized that pasture access might lead to some misclassification of rumination times, potentially caused by a higher respiration rate, panting (Li et al., 2020; Yan et al., 2021), or various head and neck movements associated with grazing activity. The neck sensor used in our study to generate eating and rumination time data was validated for eating time during pasture access (grazing) but not for rumination time during pasture access (Dela Rue et al., 2020). Our study is the first to investigate heat stress with this specific sensor, where pasture access coincides with higher temperature and THI values. The fact that cows without pasture access showed an expected decrease in rumination time of around 20 min/d under higher environmental temperatures indeed suggests some misclassification in rumination time for cows with pasture access, which showed an increase of almost 15 min/d (Müschner-Siemens et al., 2020).

Different levels of heat stress are commonly indicated by cut-off values or particular grouping of THI variables. Mild heat stress is generally thought to start at a THI of 72 (Armstrong, 1994) or at a THI of 68 (de Rensis et al., 2015). We studied different groups of temperature and THI variables because we wanted to test the robustness of our models and to avoid the information bias generated by a single cut-off value. Furthermore, we associated temperature and THI variables (minimum, mean, and maximum) 1 and 2 d before the daily time budgets based on the 5 behavioral parameters because one negative effect of heat stress is a 2-d delayed decrease in milk production (West, 2002).

Windchill on dairy cows is generally studied using THI as a standard parameter. This does not consider air velocity and sunlight, which are also important contributing factors (Mader et al., 2006; Polsky and von Keyserlingk, 2017; Herbut et al., 2018). Furthermore, differences between farms with ventilation and cooling in confined systems or farms offering pasture access can lead to different adaptations to increasing ambient temperatures within the same climate region. In our study, on CMS farms, cows had pasture access for a minimum of 6h/d for at least 120 d/yr. They still showed differences in their time budgets compared with cows from AMS farms: cows that are housed inside year-round showed lower reactions to the increase in THI. However, others showed higher temperatures indoor (+2.6°C) compared with temperatures outdoor (Marumo et al., 2021). We assume that in our study, the indoor-housed cows showed less adaptation to higher THI values because they were not exposed to direct sunlight. None of the farms with pasture access provided shade, suggesting that the stronger adaptation might be related to sun exposure. Dairy farmers in temperate climates could potentially improve animal welfare and production outcomes if they provided shade for cows with pasture access (Van Laer et al., 2015).

Although THI is often used in research, mean or maximum temperature would be easier to monitor in daily farm management. As our results demonstrate,

in a temperate and maritime climate, temperature parameters and THI show similar adaptation effects. We studied only indirect adaptive effects measured by sensors, not the direct physiological effects of heat stress; moreover, daily THI  $\geq$ 72 occurred less frequently during the 4-y study period compared with other studies in other climatic zones. Our data show that dairy cows begin to adapt to rising ambient temperatures at lower temperatures than previously reported. This means that farmers in a temperate maritime climate should begin to support dairy cows through interventions in radiation, convection, evaporation, and conduction (Kadzere et al., 2002) from a mean ambient temperature of 12°C to 16°C or a mean THI of 56 to 60 and higher.

Mean daily temperatures of  $\geq 28^{\circ}$ C occurred even less frequently due to the relatively constant high humidity. Furthermore, the cows showed less clear adaptation patterns on days with a high maximum temperature. Their response could depend on the duration of daily periods with a high temperature, because a desert climate with a cool period of less than 21°C for 3 to 6 h will minimize the effect of heat stress on decreased milk production (Igono et al., 1992). In a temperate maritime climate, days with high minimum temperature or THI seldom occur, making THI less suitable in this climate zone.

#### CONCLUSIONS

In this study, we quantified the effects of ambient temperature and THI on the daily time budget of dairy cows. Cows began to adapt their daily time budgets beginning at a temperature of 12°C and a THI of 56. As climate variable values increased, cows spent less time lying, eating, and walking and more time standing. Results for rumination time were inconclusive. In temperate maritime climates, a mean temperature between 12°C and 16°C or a mean THI between 56 and 60 might warrant supportive measures to reduce potential heat stress. In the temperate maritime climate of the Netherlands, daily mean temperature is sufficient to study the effects of behavioral adaptation to heat stress of dairy cows.

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# **Chapter 4**

# Sensor based eating time variables of dairy cows in the transition period related to the time to first service

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# ABSTRACT

In dairy cattle, reproductive diseases and infertility are some of the most important reasons for culling, where postpartum negative energy balance (NEB) reduces reproductive performance. This single cohort observational study reports the association between eating time and the interval between calving and first service in 2036 dairy cows on 17 commercial farms in The Netherlands. Cows were equipped with a commercially available neck sensor (Nedap, Groenlo, The Netherlands), that measured the time cows spent eating, from 28 days (d) before until 28 d after parturition. Primiparous cows spent a mean of +45 minutes (min) eating time per day ante partum and +15 min eating time post partum more than multiparous cows. A Cox proportional hazard model was used to analyze eating time variables in relation to the interval between calving and first service. From 4 weeks before until 4 weeks after calving eating time variables per week were used. Weeks -4, -3 +3 and +4 were used as weeks with stable eating time patterns and therefore the mean eating time per week and the standard deviation of the mean eating time per week were used. Weeks -2, -1, +1 and +2 were addressed as periods with unstable eating patterns and therefore the slope in eating time per week and the residual variance of the slope per week were modeled. Significant results were the mean eating time in week -4 and +3 where in both weeks higher eating time lead to a higher hazard for first service. Difference between primiparous and multiparous cows were also significant with a higher hazard for first service for primiparous cows. Week 4 post partum presented a significant difference between eating time of primiparous cows and multiparous cows. These results display how eating time variables in the transition period could be related to the interval between calving and first service, and that there is a relation between mean eating time in week -4, +3, +4 and the interval between calving and first insemination.

Key words: dairy cow, eating time, transition period, negative energy balance, reproduction

# **INTRODUCTION**

One of the most important factors involved in culling of dairy cattle is impaired fertility (Chiumia, Chagunda et al., 2013; Pinedo, De Vries et al., 2010). Poor reproductive performance, infertility and diseases of the genital tract in the early post partum period are often linked to the negative energy balance (NEB) (Sheldon et al., 2006; McArt et al., 2013; Raboisson et al., 2014). This NEB is physiological because, after calving, the increase of the capacity of the rumen takes several weeks, while there is an immediate increase in the amount of energy required for milk production, resulting in a direct effect of insufficient dry matter intake (DMI) (Bauman and Bruce Currie, 1980; Esposito et al., 2014). The length and the severity of the NEB are related to the dry matter intake pre- and post partum (Butler, 2000). This NEB in the early post partum period is associated with other health problems as well. These include (sub)clinical milk fever, (sub)clinical ketosis, retained placenta, metritis, endometritis, inactive ovaries, cystic ovarian disease, displaced abomasum, mastitis and (subclinical) ruminal acidosis (Seifi et al., 2011; Suthar et al., 2013; Vina et al., 2017). As a consequence of these diseases reproductive performance can also be impaired (Ortega et al., 2015; Ribeiro et al., 2013; Dubuc and Denis-Robichaud, 2017). For example, Walsh et al. (2007) reported that, when subclinical ketosis occurred during the first two weeks after parturition, there was a decreased probability of pregnancy until approximately 165 days post partum (Walsh et al., 2007).

Reported health problems post partum are often associated with feeding- and social behavior during the transition period (Goldhawk et al., 2009). During this period, nutritional management is one of the key factors related to the NEB. The NEB has a direct effect on fertility and inadequate nutritional management can cause anestrus which is characterized by an absence of ovulation because preovulatory follicles are not capable to produce sufficient estradiol. These cows will not be inseminated (AI, artificial insemination) after the voluntary waiting period, have lower conception rates and a higher number of services per conception after resumption of the activity of the ovaries (Roche, 2006). Furthermore, other problems as lameness and retained placenta can be involved in a prolonged interval between calving and first AI (Barkema et al., 1994; van Werven et al., 1992). Combining all the above, a multi-level analysis is indicated, which combines risk factors, clinical parameters and reproductive indices (Jorritsma et al., 2003).

In the last decade the use of sensor technology has been widely introduced in dairy farm management, introducing the possibility to monitor cow behavior such as eating time. Measuring behavioral aspects with sensors could result in different outcomes because of sensor characteristics (Borchers et al., 2016). Furthermore, eating time could be related to dry matter intake (Halachmi et al., 2016) but differences in behavior per parity, for example, should be accounted for (Neave et al., 2017).

Several studies related behavioral aspects to metabolic diseases. Neck sensor data for instance, has resulted in an overall sensitivity of 91% for ketosis (Stangaferro et al., 2016a) and 78% for detection of cows with a metabolic disorder in combination with metritis (Stangaferro et al., 2016b). Decreased eating time is also related to metritis (Urton et al., 2005). Cows with subclinical ketosis and metritis already spent less time eating and ruminating a week before calving and continued to do so up to 3 weeks after calving (Schirmann et al., 2016).

In the present study, eating time from 4 weeks ante partum to 4 weeks post partum were chosen to represent the transition from ante to post partum. According to Kok et al. (2017) mean eating time in the weeks related to the transition period could be addressed as stable and non-stable (Kok et al., 2017). Eating time in week -4, -3, +3 and +4 were assessed as stable patterns which can be described by the mean eating time and the standard deviation of the eating time. Scheffer (2009) reported that it might be important to detect a change in volatility for processed data with a constant mean. A change in volatility might be a signal that in the (near) future a change in the mean can occur (Scheffer et al., 2009). Eating time in week -2, -1, +1 and + 2 can be described as non-stable for which the slope and residual variance were used. The goal of this study was to combine and associate these eating time variables of the transition period with the time to first service.

#### **MATERIALS AND METHODS**

#### Farms and animals

From 01 July 2014 to 30 April 2016, 2036 dairy cows on 17 dairy farms with free stall barns in the Netherlands were equipped with the "Nedap Smarttag Neck"-sensor from at least 28d pre partum until at least 28d post partum. Details of these farms, regarding number of cows per farm, type of bedding (mattress or deep litter bedding), type of milking system (automatic milking system (AMS) or conventional milking system (CMS)), production level and the interval between calving and first service on farm level are shown in table 1. The rations fed consisted of grass- and corn silage with additional concentrates. Due to the length of the study period (multi seasonal), no detailed composition of the ration can be given for each farm.

#### Sensors

To measure eating time, commercially available "Nedap Smarttag Neck" sensors (Nedap, Groenlo, The Netherlands) were attached to the neck collar of the cows. The "Nedap Smarttag Neck" uses a G-sensor, which uses the acceleration as a measure of movement and x-, y-, and z-axis (three-dimensional space) to determine the angle towards the floor. From this angle the head positioning was assessed and in combination

with the movement, the eating time was determined. A neural network is used to determine if the cow is eating per second. Eating time was recorded in a total of seconds per 15 minutes for each day (Van Erp-Van der Kooij et al. 2016). This is differentiated of rumination, because of the more prominent circular motion of the sensor caused by the intensive chewing while ruminating. Because of proprietary secrecy, exact algorithms cannot be disclosed but the sensor output was used as described below.

**Table 1.** details of the 17 farms used in this study. Per farm the number of dairy cows are shown, as well as the type of bedding of the cubicles M for mattress and D for deep litter. The type of milking system is shown as well, AMS for automatic milking system and CMS for conventional milking system. The average production in kilograms per cow per year is shown as well as the 10, 50 and 90 percentiles of interval between calving and first service on farm level (mean per year).

Farm no.	No. of dairy cows	Mattress (M) Deep litter (D)	Milking system AMS/CMS	Production (Kg milk/ cow/year)	Interval calving – first ins. in days percentile 10	Interval calving – first ins. in days percentile 50	Interval calving – first ins. in days percentile 90
1	143	M	CMS	8346	52	65	102
2	135	М	CMS	9009	57	74	122
3	108	D	CMS	8675	43	62	81
4	245	М	CMS	7300	51	64	106
5	156	D	AMS	9711	42	67	103
6	158	D	CMS	9195	47	75	123
7	144	D+M	AMS	9384	55	73	114
8	148	М	AMS	8971	56	96	162
9	300	D	CMS	10531	45	60	88
10	131	М	AMS	10069	44	64	101
11	204	D	CMS	8673	46	60	90
12	122	М	CMS	8924	62	77	120
13	136	М	AMS	10352	57	79	126
14	110	М	CMS	8774	63	79	134
15	112	М	CMS+AMS	8593	59	85	126
16	115	D+M	AMS	10800	50	76	121
17	120	D	CMS	8564	46	61	99

# Data

Data of 2027 cows, including 1578 multiparous cows (parity 2-13) and 449 primiparous cows, regarding individual eating time per day and the interval between calving and first service, were used. The interval between calving and first service was obtained from the data base of the Cattle Improvement Cooperative (CIC) (CRV, Arnhem, The Netherlands) and linked to the sensor data of each cow.

# **Statistical Analysis**

All analyses were performed using the software package R Studio version 3.3.3, (RStudio Team, 2016) The R Project. Cox proportional hazard models were developed for time to first service using farms, parity, mean daily eating time and their according standard deviations of week -4, -3, +3 and +4 and the slope and residual variance of week -2, -1, +1 and +2 as explanatory variables. All eating time variables used in this analysis are daily averages per week. According to Kok et al. (2017), weeks -4, -3, +3 and +4 can be regarded as a period with stable eating time patterns and therefore the mean eating time per day plus the mean daily standard deviation of these weeks, averaged over 7 days, were used. An increase in variance can serve as a signal that there is going to be a change of state for complex dynamical systems. In such systems these changes can be early warning signals that the system is approaching a state transition. Cows with a high variance as compared to cows with a lower variance might be in such an unstable state (Scheffer et al., 2009).

Weeks -2, -1, +1 and +2 can be regarded as weeks where the eating time is declining before calving and inclining after calving respectively and therefore the slope and residual variance were used (Kok et al., 2017). The slope was fitted over 7 days with a linear regression, indicating the mean decline (weeks -2 and -1) or incline (weeks +1 and +2) per day. The associated residual variance for that week indicates the stableness of the slope and was used to test the variance of eating time in these specified periods of declining or inclining eating time. To prevent bias in mean eating time sensor data from d0, the day of calving, was excluded. Week -1 included day -7 until day -1 and week + 1 included day +1 until day +7.

Univariable models were used to check all eating time variables independently of each other in relation with time to first service. Initially, all explanatory variables and interactions were offered to a multivariable Cox proportional hazard model. This full model contained all eating time variables and all pairwise interactions between primiparous or multiparous cows regarding the eating time variables per week were fitted in the full model to evaluate whether or not the relation between the eating time variables differed between primiparous and multiparous cows. If interactions remained in the model, independent variables were refitted in a nested version of the model to estimate the effect of eating time variables within each parity group. First service is the event of interest and the time to event is defined as the amount of days between calving and first service. Thereafter, Akaike's information criterion (AIC) was used for model fitted the data best. Non-explanatory variables were dropped based on a backwards stepwise procedure. Proportionality was checked using Schoenfeld residuals. Linearity was checked by plotting the martingale residuals versus the covariates.

As there was no proportionality of the hazard for some farms, farm was used as a strata in the model resulting in a farm-specific baseline hazard (Klein and Moeschberger,

2003). Results were considered significant when the 95% confidence interval (95% CI) of the hazard ratio did not include 1. Cows that were not inseminated were censored in the analysis at the day of culling relative to the day of calving (n=139). There was no fixed period of follow up defined because each cow was followed up until either first service or removal (day of culling). The interval (days) between calving and culling was used to define the day of censoring for each non-inseminated animal.

# RESULTS

#### Difference in eating time between primiparous and multiparous cows

The difference in mean daily eating time between primiparous (n=449) and multiparous cows (n=1578), starting from d-28 until d+28 and the difference in variation between primiparous and multiparous cows in sensor output is shown in figure 1a+b. A fairly stable daily mean eating time is shown in week -4 and -3 for primiparous and multiparous cows. Primiparous cows in this period spent 6.6 hours (h) per day on eating time on average. Multiparous cows spent a little over half an hour per day less on eating time in this period, 6.0h. In week -2 and -1 a drop in eating time of roughly 30 minutes for both groups occurs. On the day of calving, d0, an increase in eating time in both groups is shown to an average of 6h. The increase in eating time in the multiparous cow group is higher than the eating time of the primiparous cow group, resulting in more or less the same eating time on the day of calving in both groups. At d1 eating time is lowest for both groups with an average of 4.75h. Afterwards, both groups incline to a stable eating time per day in week 3 and 4 post partum. Eating time in the primiparous cow group increases to an average eating time per day of 5.75h and the eating time of the multiparous cow group increases to an average of 5h per day.



**Figure 1a.** Individual variation of the primiparous cows in the background (grey) with the difference in mean daily eating time, as measured by sensors, between primiparous (n=449, black line) and multiparous (n=1578, dashed line) cows from -28d until +28d on 17 commercial Dutch dairy farms. Daily means are based on a minimum of 287 and a maximum of 432 primiparous cows.



**Figure 1b.** Individual variation of the multiparous cows in the background (grey) with the difference in mean daily eating time, as measured by sensors, between primiparous (n=449, black line) and multiparous (n=1578, dashed line) cows from -28d until +28d on 17 commercial Dutch dairy farms. Daily means are based on a minimum of 1396 and a maximum of 1529 multiparous cows.

### Eating time variables related to the interval between calving and first service

The final model consisted of 1697 cows (296 primiparous and 1401 multiparous cows) in total, of which 1558 had the event first service and 139 cows did not have a first service and were thus censored in the analysis. For 330 cows there were no weekly mean eating time sensor data and were excluded from the model. Kaplan Meier curves per farm regarding the interval between calving and first service are shown in figure 2. The overall mean is at 83.44 days. Descriptive statistics of the analyzed eating time variables are presented in table 2. Based on the cumulative incidence curves in figures 3 and 4 the minimum, median and maximum days of time to first service are 22, 71 and 275 and for censoring time 23, 252 and 587 days respectively. Results of the univariable and final

model are presented in table 3. Results of the ante partum and post partum models are presented in the appendix.

The final model included parity, mean eating time in week -4 and +3 and the interaction between parity and the mean eating time in week +4. One hour increase of mean eating time in week -4 resulted in a higher hazard for first service (HR 1.1 (95% CI (1.01-1.11)). One hour increase of mean eating time in week +3 resulted in a higher hazard for first service (HR 1.2 (95% CI (1.06-1.28)). Based on the nested version of the final model, the interactions between mean eating time in week +4 and parity showed that primiparous cows with one hour increase of mean eating time had a lower hazard for first service (HR 0.8 (95% CI (0.75-0.95)).



Figure 2. Kaplan-Meier plot of 17 commercial dairy farms in The Netherlands regarding the interval between calving and first service in days post partum.

a					
Period	Variables	Mean	Median	Minimum	Maximum
Week -4	Mean	6.63	6.74	0.25	10.22
	SD	0.77	0.72	0.10	4.16
Week -3	Mean	6.54	6.66	0.00	9.82
	SD	0.80	0.68	0.00	3.54
Week -2	Slope	-0.066	0.003	-5.301	2.096
	Residuals	0.744	0.453	0.006	12.128
Week -1	Slope	0.044	0.057	-5.192	2.259
	Residuals	0.766	0.463	0.004	8.890
Week +1	Slope	0.009	0.009	-0.941	1.456
	Residuals	0.578	0.385	0.004	8.232
Week +2	Slope	0.019	0.023	-0.556	0.562
	Residuals	0.353	0.271	0.001	2.312
Week +3	Mean	5.20	5.27	0.14	9.33
	SD	0.58	0.54	0.06	2.18
Week +4	Mean	5.29	5.30	0.25	9.76
	SD	0.59	0.54	0.00	2.04
Ь					
Period	Variables	Mean	Median	Minimum	Maximum
Week -4	Mean	6.02	6.06	0.06	11.58
	SD	0.75	0.68	0.03	3.70
Week -3	Mean	5.92	5.97	0.05	10.65
	SD	0.74	0.68	0.01	4.35
Week -2	Slope	0.019	0.016	-1.646	2.196
	Residuals	0.590	0.426	0.000	12.147
Week -1	Slope	0.038	0.039	-3.001	2.242
	Residuals	0.601	0.409	0.000	7.723
Week +1	Slope	-0.014	-0.004	-1.859	1.357
	Residuals	0.530	0.345	0.001	7.815
Week +2	Slope	0.009	0.006	-1.129	1.881

0.001

0.01

0.01

0.00

0.00

0.241

5.15

0.52

5.07

0.51

8.926

9.42

2.19

9.41

3.53

**Table 2 a and b.** Description of the eating time variables per week based on sensor data of 1697 cows (primiparous (n=296, table 2a) and multiparous (n=1401, table 2b)) on 17 commercial Dutch dairy farms followed between 28 days before calving to 28 days after calving. Mean and standard deviation (SD) are presented with 2 decimals and slope and residuals are presented with 3 decimals.

Week +3

Week +4

Residuals

Mean

Mean

SD

SD

0.352

4.99

0.56

4.97

0.56



Figure 3. Cumulative incidence for the time to first service in days with a 95% confidence interval based on 1 minus the Kaplan Meier estimated survival curve of 1697 cows on 17 commercial Dutch dairy farms.



**Figure 4.** Cumulative incidence for the time to culling for the censored cows (n=139) in days, based on 1 minus the Kaplan Meier estimated survival curve for censoring time on 17 commercial Dutch dairy farms.

Factor		Univariable analysis HR (95% CI)	Final reduced multivariable model HR (95% CI)
Parity			
Primiparous		1	1
Multiparous		0.8 (0.69-0.91)	0.5 (0.32-0.79)
Ante partum			
Week -4	Mean	1.1 (1.06-1.14)	1.1 (1.01-1.11)
	SD	1.0 (0.88-1.14)	_1
Week -3	Mean	1.1 (1.06-1.14)	-
	SD	0.9 (0.82-1.08)	-
Week -2	Slope	0.9 (0.68-1.17)	-
	Residuals	1.0 (0.90-1.08)	-
Week -1	Slope	1.0 (0.79-1.25)	-
	Residuals	1.0 (0.91-1.04)	-
Post partum			
Week +1	Slope	0.9 (0.73-1.13)	-
	Residuals	1.0 (0.91-1.08)	-
Week +2	Slope	0.8 (0.61-1.14)	-
	Residuals	1.1 (0.97-1.20)	-
Week +3	Mean	1.1 (1.07-1.14)	1.2 (1.06-1.28)
	SD	1.0 (0.83-1.25)	-
Week +4	Mean	1.1 (1.05-1.12)	-
	SD	1.0 (0.84-1.21)	-
Interactions	Primiparous x wee	ek + 4	0.8 (0.75-0.95)
	Multiparous x wee	ek + 4	0.9 (0.84-1.02)

**Table 3.** Results of the univariable and the final reduced multivariable Cox proportional hazard models for time to first service in days based on 1697 cows (296 primiparous cows and 1401 multiparous cows) on 17 commercial Dutch dairy farms. Hazard ratios (HR) with 95% confidence intervals (95%CI) are presented.

<sup>1</sup> Excluded from reduced model

# DISCUSSION

#### Eating time characteristics related to parity

Eating time differences between primiparous and multiparous cows are recorded in this study in the period of d-28 to d+28 around calving, showing more eating time for primiparous cows than multiparous cows in the entire transition period. According to another study, eating time is related to DMI (Halachmi et al. 2016) but a difference in eating time between primiparous and multiparous cows during the transition period is known from Neave et al. (2017) as well. In that study, a higher eating time was found in primiparous cows in comparison with multiparous cows, but the primiparous cows had a lower dry matter intake (DMI) than the multiparous cows (Neave et al., 2017).

Before parturition, a gradual decline of eating time in both primiparous and multiparous cow groups was recorded (Figure 1). This decline in eating time starts around 2 weeks before parturition as was observed in another study which shows similar patterns for DMI as measured by feed intake as our study with sensor output data (Kok et al., 2017). Most likely, the growing of the uterus reduces the volume of the rumen at the end of gestation up to one third (Habel, 1981). Besides the growing of the uterus, feed intake is reduced because of a rise of estrogen concentration in the blood (Grummer et al., 1990). Moreover, figure 1a+b shows a tremendous variation in sensor output for eating time of individual cows.

On the day of calving, d0, sensor data showed a steep incline in eating time for especially the multiparous cows. Visual observations suggested that this peak consisted for a large part of the licking of the calf. In this study, around 60% of the cows showed a peak in eating time at the day of calving. It is most likely that in 40% of the cases the calf was removed from the dam directly after calving. However, there was no data collected to corroborate this hypothesis. For this study the sensor data of the day of calving was excluded from the analysis to prevent information bias on mean eating time. After calving, eating time inclined in the first 2 weeks and stabilized around the end of the second week post partum as shown before (Kok et al., 2017).

#### Eating time related to the interval between calving and first service

In the final model multiparous cows had a lower hazard for first service (HR<1), indicating a longer interval between calving and first service compared with primiparous cows. This leads to differences in reproductive variables between primiparous and multiparous cows. Several reports have shown that a difference in follicular fluid was found between primiparous cows and multiparous cows. Compromised oocyte quality might be the result of these different microenvironments in which oocytes are developed (Bender et al. 2010, Van Vliet and Van Eerdenburg, 1996). Earlier reports showed differences in heat expression between primiparous cows and multiparous cows, however with contradicting outcomes (Adewuyi et al., 2006; López-Gatius et al., 2006; Van Vliet and Van Eerdenburg, 1996). However, the voluntary waiting period could be a confounder related to differences in production levels of individual cows.

The final reduced model showed that a higher mean eating time in week -4 was related to a higher hazard for first service (HR>1). The previous described gradual decline of feed intake accounts for every cow during the last part of gestation. Lower mean eating time could therefore indicate that these specific cows have health issues like lameness. Lameness is related with behavioral changes (Weigele et al., 2018). According to Thorup et al. (2016) lameness leads to a decrease in daily eating time and could therefore have an impact on subsequent reproductive successes (Thorup et al., 2016).

Chapter 4

In this study lameness data before calving was not collected but it could indicate that cows with a stable and higher mean eating time are not lame. These results indicate that less eating time before calving could result in a longer interval between calving and first service, which is consistent with another study where less eating time before calving is associated with subclinical ketosis (Schirmann et al., 2016).

Based on several studies a change in volatility could serve as an early warning system. Therefore, for weeks -4, -3, +3 and +4 the standard deviation per week were offered to the final model even though these outputs were non-significant in the univariable model. For the same reason, the slope and residual variance of weeks -2, -1, +1 and +2 were offered to the final model. None of these variables were significant in the final model (Scheffer et al., 2009; Van den Broek, 2015).

The mean eating time in week +3 had a higher hazard for first service (HR>1) and is thus related to a shorter interval between calving and first service. Cows with less eating time in this week could therefore still experience the consequences of post partum diseases with an effect on reproductive indicators. Diseases which could be related to this nadir of eating time are milk fever, downer cow syndrome and ketosis but also other factors as for example pain due to a difficult calving process (Mainau et al., 2014; Newby et al., 2013; Stangaferro et al., 2016a). For instance, earlier studies showed an apparent relationship between the post partum energy balance and the time of first ovulation and the nadir of NEB related to the start of the pulsatile LH secretion. The first ovulation can occur after the moment the LH secretion is no longer inhibited (Canfield and Butler 1990; Van Hoeck et al., 2011). A stabilized eating time with a higher mean in week +3 could therefore be the result of a successful transition period. Primiparous cows in this model had a minor effect with the mean eating time in week +4 with a HR<1 indicating that more eating time in this week results in a lower hazard for first service. A physiological explanation is unknown, however, an unclear difference in behavioral expression between primiparous and multiparous cows in this period could be responsible as underlying clarification (Neave et al., 2017). More likely, milk production of high producing primiparous cows is a confounder for time to first service resulting in higher eating times but a longer time to first service because of a longer voluntary waiting period. This management decision is supported by Stangaferro et al. (2018), which shows a higher profitability for primiparous cows with a longer voluntary waiting period (Stangaferro et al., 2018).

To understand the observed associations between eating time and reproductive health, more research is needed to study this effect systematically. In the present study, time to first service differed highly per farm (see figure 2). This can be a combination of feeding strategies, housing differences, voluntary waiting period combined with transition physiology and production levels. Future studies could investigate more clinical parameters per individual cow like the (difference between the) body condition score in the dry period and post partum, individual dry period length, the ketone body concentration, and especially the level and moment of expression of the first estrus post partum. Furthermore, sensors nowadays record multiple behavioral parameters as rumination time or number of eating bouts as well which can be taken into account to create a more multi-dimensional approach.

The present study showed that weekly eating time parameters are related to later events post partum but future studies should focus on prospective modeling to see if these relations are causal or confounding.

# CONCLUSIONS

In the transition period, ante partum mean eating time in week 4 before calving is related to the time to first service, as are mean eating time variables in week 3 and 4 post partum. Moreover, a difference between primiparous and multiparous cows was found. With these results, some new insights concerning sensor based eating time variables in the transition period could aid in the design of future studies to monitor and identify cows at risk for impaired reproductive parameters.

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# APPENDIX

**Table 4.** Results of the final reduced multivariable ante partum and post partum Cox proportional hazard models for time to first service in days based on 1697 cows (296 primiparous cows and 1401 multiparous cows) on 17 commercial Dutch dairy farms. Hazard ratios (HR) with 95% confidence intervals (95%CI) are presented.

Factor		Final reduced multivariable ante partum model HR (95% CI)	Final reduced multivariable post partum model HR (95% CI)
Parity			
Primiparous		1	1
Multiparous		0.8 (0.63-0.96)	0.5 (0.30-0.75)
Ante partum			
Week -4	Mean	1.1 (1.07-1.16)	
	SD	_ 1	
Week -3	Mean	-	
	SD	0.9 (0.74-0.99)	
Week -2	Slope	-	
	Residuals	-	
Week -1	Slope	-	
	Residuals	-	
Post partum			
Week +1	Slope		0.8 (0.64-1.00)
	Residuals		-
Week +2	Slope		0.7 (0.54-1.01)
	Residuals		-
Week +3	Mean		1.2 (1.10-1.33)
	SD		-
Week +4	Mean		-
	SD		-
Interactions	Primiparous x week + 4		0.8 (0.75-0.95)
	Multiparous x week + 4		0.9 (0.85-1.03)

<sup>1</sup>Excluded from reduced model



# **Chapter 5**

Associations between body condition score, locomotion score, and sensorbased time budgets of dairy cattle during the dry period and early lactation

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# ABSTRACT

Lameness, one of the most important disorders in the dairy industry, is related to postpartum diseases and has impact on dairy cow welfare, leading to changes in cows' daily behavioral variables. This study quantified the effect of lameness on the daily time budget of dairy cows in the transition period.

In total 784 multiparous dairy cows from 8 commercial Dutch dairy farms were visually scored on their locomotion (score 1-5) and body condition (score 1-5). Each cow was scored in the early and late dry period as well as in week 4 and 8 postpartum. Cows with locomotion scores 1 and 2 were grouped together as a nonlame group, cows with score 3 were considered as moderately lame, and cows with score 4 and 5 were grouped together as severely lame. Cows were equipped with 2 types of sensors that measured behavioral parameters. The leg sensor provided number of steps, number of stand-ups (moving from lying to standing), lying time, number of lying bouts, and lying bout length. The neck sensor provided eating time, number of eating bouts, eating bout length, rumination time, number of rumination bout length. Sensor data for each behavioral parameter were averaged between 2 d before and 2 d after locomotion scoring.

The percentage of nonlame cows decreased from 63% in the early dry period to 46% at 8 wk in lactation; this decrease was more severe for cows with higher parity. Cows that calved in autumn had the highest odds for lameness. Body condition score loss of >0.75 point in early lactation was associated with lameness in wk 4 postpartum. Moderately lame cows had a reduction of daily eating time of around 20 min, whereas severely lame cows had a reduction of almost 40 min. Similarly, moderately and severely lame dry cows showed a reduction of 200 steps/d and severely lame cows in lactation showed a reduction of 600 steps/d. Daily lying time increased by 26 min and lying bout length increased by 8 min in severely lame cows compared with nonlame cows.

These results indicate a high prevalence of lameness on Dutch dairy farms, with an increase in higher locomotion scores from the dry period into early lactation. Time budgets for multiparous dairy cows differed between the dry period and the lactating period, with a higher locomotion score (increased lameness) having an effect on cows' complete behavioral profile. Body condition score loss in early lactation was associated with poor locomotion postpartum, whereas lameness resulted in less eating time in the dry period and early lactation, creating a harmful cycle.

Key words: dairy cow, lameness, locomotion score, sensor data, transition period

# **INTRODUCTION**

Lameness remains an underestimated problem in the dairy industry even as researchers have demonstrated that it affects a large percentage of dairy cows (Somers et al., 2003, Bicalho et al., 2009, Holzhauer et al., 2006). Lameness is usually caused by claw disorders (Barker et al., 2010, Solano et al., 2015, Randall et al., 2019) that are often painful (O'Callaghan et al., 2003, Bruijnis et al., 2012) and is associated with or followed by other diseases (Hernandez et al., 2002). However, claw disorders are not always clearly associated with lameness because cows are stoic prey animals (Blackie et al., 2013) and often mask the experience of pain until it is severe (O'Callaghan et al., 2003, Dyer et al., 2007). In addition to the impact on animal welfare, lameness is associated with economic losses (Enting et al., 1997), an increase in culling rates, a reduction in milk yield and has an effect on fertility (Green et al., 2002, Melendez et al., 2003, Amory et al., 2008).

A practical method to detect lameness is visual locomotion scoring. A locomotion score uses a scale of 1 to 5 to show differences between nonlame and lame cows, where 1 is a nonlame cow and 5 is a severely lame cow (Sprecher et al., 1997). Important factors that affect locomotion score are type, hardness and slipperiness of the walking surface (Van der Tol et al., 2005, Alsaaod et al., 2017, Telezhenko et al., 2017). These circumstances could result in a score related to mild lameness where a nonlame cow is actually just walking cautiously.

While lameness obviously affects a cow's movement (O'Callaghan et al., 2003) it affects a range of other types of behavior. Lameness was reported to be associated with variations in feeding behavior: less eating time but unaltered rumination time compared with nonlame cows (Thorup et al., 2016, Weigele et al., 2018). Other studies showed lower rumination time in new cases of lameness or variations in rumination time related to lameness (King et al., 2018, Steensels et al., 2017). Lame cows also showed longer lying times, fewer but longer lying bouts, and a higher variation in lying bout length (Chapinal et al., 2009, Ito et al., 2010, Solano et al., 2016). Thus, lameness most likely affects the daily time budget or behavioral patterns of dairy cows. It is not the sole factor, given that the time budget of transition cows differs pre- and postpartum (Kok et al., 2017, Hut et al., 2019), mainly due to the daily milking routine postpartum.

Others have studied the time budget of moderately lame cows on farms with sand or mattresses (Cook et al., 2004, Gomez and Cook, 2010); however, a complete sensor-based behavioral profile or time budget based on feeding, lying and walking behavior in relation to lameness seems lacking. A recent longitudinal study showed vulnerability to lameness to be highly related to previous cases of lameness (Randall et al., 2018), but these researchers did not analyze the transition period, when cows are generally more vulnerable to health problems (Drackley, 1999). The dry period has been identified as a time when cows are especially vulnerable to developing lameness. Cows

with a low body condition score (BCS) at dry off had higher odds of chronic lameness in the dry period and less cure from lameness (Daros et al., 2019). Loss of BCS in the dry period was shown to be a predisposing factor for transition disease and for reduced productive and reproductive parameters postpartum, but not for lameness (Chebel et al., 2018, Daros et al., 2020). Based on weekly body condition scoring in one herd and every 60 d in another herd, corrected for previous lameness, a BCS of <2.25 and <2, respectively, was associated with higher odds for lameness 1 to 3 wk or up to 4 months later (Randall et al., 2018).

Therefore, the goal of this study is twofold. The first is to use locomotion scores to get insight in the prevalence of high locomotion scores from the onset of the dry period until 8 weeks in lactation as well as the association with BCS and changes in BCS. The second is to quantify the effect of impaired locomotion on a daily time budget including parameters for feeding, lying, and walking behavior of dairy cows in the dry period and early lactation.

# **MATERIALS AND METHODS**

# Farms and animals

This study was conducted from November 1, 2016, to May 1, 2018, and included 1,326 dairy cows on 8 commercial dairy farms with freestall barns in the Netherlands. Details of these farms regarding herd size, type of bedding, type of milking system, production level, pasture access, and average dry period length are presented in table 1. All farms had a separate far-off and close-up group in the dry period and 1 lactational group for all cows in milk. Primiparous cows (n = 303) were excluded from this study because these animals do not have a transition period and because of behavioral differences compared to multiparous cows in the transition period (Hut et al., 2019). Some cows were excluded because their data were incomplete; analysis required 4 consecutive locomotion and body condition scores and a selection for complete sensor data for day d-2, -1, +1, and +2 relative to the day of scoring. Analysis included data of 784 multiparous cows. The numbers of cows per sensor-based behavior output are presented in Figure 1.

Farm	Herd size	DP cubicle bedding far off	DP cubicle/ yard bedding close up	Average DP length (25%-75% IQR)	Cubicle bedding lactation	Milking system	Pasture access	Production level (kg milk/ cow/ year)
1	170	Deep litter	Straw yard	41 (31-46)	Deep litter	AMS	No	10786
2	130	Deep litter	Straw yard	39 (30-41)	Deep litter	AMS	No	11177
3	110	Mattress	Mattress	45 (40-51)	Mattress	AMS+CMS	No	9341
4	110	Mattress	Straw yard	39 (33-43)	Mattress	CMS	Yes	9314
5	140	Deep litter	Deep litter	35 (30-40)	Deep litter	CMS	Yes	9256
6	170	Mattress	Mattress	37 (32-42)	Mattress	CMS	Yes	9243
7	175	Deep litter	Straw yard	42 (32-48)	Deep litter	CMS	Yes	9109
8	120	Mattress	Mattress	45 (37-49)	Mattress	CMS	Yes	9197

Table 1. Characteristics of 8 commercial dairy farms in the Netherlands used in this observational study.

AMS = automatic milking system. CMS = conventional milking system. DP = dry period. IQR= interquartile range. Deep litter is related to cubicle systems where a straw yard means a free-range area. Pasture access was for lactating animals only.

#### Sensors

To measure feeding behavior, commercially available Nedap Smarttag Neck sensors (Nedap, Groenlo, the Netherlands) were attached to the neck collar of the cows, and the commercially available Nedap Smarttag Leg sensors were attached to one of the front legs of the cows to measure walking and lying behavior. The Nedap Smarttag sensors use G-sensors, which utilize acceleration as a measure of movement and the x-, y-, and z-axis (3-dimensional space) to determine the angle. A proprietary neural network was used to determine whether the cow was displaying the specified behavior per minute. Behavioral parameters were recorded for each minute within every 15-min period in each day (Van Erp-Van der Kooij et al., 2016). The daily number of eating and rumination bout. Through the leg sensor, as was the average duration per eating and rumination bout. Through the leg sensor, the number of steps, number of stand ups (moving from lying to standing), lying time, number of lying bouts, and duration per lying bout, were measured (Nielsen et al., 2018).



**Figure 1.** Selection process of cows used for analysis in this study. Starting with 1,326 cows in total, 303 primiparous cows were excluded; 1,023 multiparous cows remained and were filtered for 4 consecutive locomotion scoring events, which resulted in 784 cows. Further selection was based on available sensor data for d -2 and d +2 relative to the day of scoring (complete sensor data of 4 d).
### Study design

At the beginning of the dry period, the end of the dry period, 4 weeks postpartum and 8 weeks postpartum, a trained veterinarian (PH) visually scored cows from all 8 herds for their locomotion score and BCS. Every scoring event was conducted on slatted concrete floors. Scoring took place in freestall systems where every cow was scored individually to assign a body condition and a locomotion score without any interference from milking routines. The distribution of all locomotion scores per farm and per scoring event of all multiparous cows with 4 consecutive scores is presented in table 2. Because of the final numbers of cows per locomotion score, cows with locomotion scores 1 and 2 were grouped together as a nonlame group, cows in group 3 were considered moderately lame, and cows with scores 4 and 5 were grouped together as severely. Sensor data from both sensors of every cow and every scoring event were collected from 7 d before until 7 d after every scoring event. Sensor data from the day of scoring were excluded from the analysis because of possible bias caused by the data collection during locomotion scoring. To exclude days where cows were still being milked to evaluate the early dry period and to exclude days where cows were already milked to evaluate the late dry period, only sensor data of 2 d before (d - 2) and 2 d after (d + 2) locomotion scores were used and averaged per day for analysis. These 4 d around the locomotion score were considered to represent precisely the daily time budget as affected by the potential lameness.

Body condition score was determined on a scale of 1 to 5 with 0.25-point increments (Ferguson et al., 1994) and was categorized into 3 groups based on 33% and 66% percentile values. These groups were <2.75, 2.75 to 3.25, and >3.25 for the early dry period; <3.0, 3.0 to 3.5, and >3.5 for the end of the dry period; <2.5, 2.5 to 3.0, and >3.0 for wk 4; and <2.25, 2.25 to 2.75, and >2.75 for wk 8. Changes in BCS were defined for 3 intervals: from the early dry period to the end of the dry period, from the end of the dry period to wk 4 postpartum and from wk 4 postpartum to wk 8 postpartum. The BCS change in the dry period (change dry) was categorized in 3 groups based on 33% and 66% percentile values as follows: BCS decrease (>0.00), a slight increase (0.00 - 0.25) and a moderate increase (>0.25). From the end of the dry period to 8 wk postpartum (change transition), BCS change was categorized as a severe decrease (>0.75), a moderate decrease (0.50 - 0.75), or a slight decrease (<0.50). From 4 to 8 wk postpartum (change post) BCS change was categorized as a moderate decrease (>0.25), a slight decrease (<0.25), or an increase (>0.00).

Calving season was modeled according to Sanders et al. (2009) with 3 mo per season (winter, spring, summer, and autumn). For example, January to March was considered to be winter.

The dry period length was based on the number of days between the first scoring event in the early dry period and the calving date. The dry period length was categorized into 3 equally distributed groups based on 33% and 66% percentiles as follows: <34 d, 34 to 43 d, and >43 d.

Farm	LS <sup>1</sup>	Begin dry	period	End dry p	period	Week 4 in	milk	Week 8 in	ı milk
1	1	2	2%	4	4%	1	1%	1	1%
	2	67	59%	61	54%	55	49%	58	51%
	3	17	15%	22	19%	24	21%	22	19%
	4	24	21%	26	23%	30	27%	30	27%
	5	4	4%	1	1%	3	3%	3	3%
2	1	5	6%	4	5%	1	1%	-	-
	2	39	50%	32	41%	40	51%	44	56%
	3	14	18%	17	22%	13	17%	12	15%
	4	21	27%	24	31%	23	29%	21	27%
	5	-	-	2	3%	2	3%	1	1%
3	1	-	-	-	-	-	-	-	-
	2	27	48%	16	29%	14	25%	14	25%
	3	3	5%	6	11%	6	11%	9	16%
	4	25	45%	31	55%	34	61%	31	55%
	5	1	2%	3	5%	2	4%	2	4%
4	1	9	11%	4	5%	3	4%	-	-
	2	45	57%	45	57%	33	42%	25	32%
	3	8	10%	5	6%	14	18%	17	22%
	4	17	22%	22	28%	26	33%	33	42%
	5	1	1%	3	4%	4	5%	4	5%
5	1	2	3%	-	-	-	-	-	-
	2	39	62%	42	67%	41	65%	38	60%
	3	12	19%	10	16%	9	14%	14	22%
	4	10	16%	12	19%	13	21%	11	17%
	5	2	3%	-	-	-	-	-	-
6	1	4	8%	2	4%	1	2%	-	-
	2	38	76%	39	78%	33	66%	30	60%
	3	5	10%	7	14%	11	22%	12	24%
	4	3	6%	2	4%	5	10%	8	16%
	5	-	-	-	-	-	-	-	-
7	1	11	8%	10	8%	4	3%	-	-
	2	75	57%	68	52%	70	53%	63	48%
	3	23	18%	15	11%	15	11%	24	18%
	4	25	19%	39	30%	44	34%	43	33%
	5	-	-	2	2%	2	2%	4	3%
8	1	-	-	-	-	-	-	-	-
	2	52	74%	53	76%	25	36%	23	33%
	3	6	9%	4	6%	16	23%	12	17%
	4	12	17%	13	19%	28	40%	31	44%
	5	-	-	-	-	1	1%	4	6%

**Table 2.** Distribution of locomotion scores per farm and scoring event of all multiparous cows with 4 consecutive scores.

1 Locomotion score. Each cow was scored in the early and late dry periods as well as in wk 4 and 8 postpartum. Cows with locomotion scores 1 and 2 were grouped together as nonlame, cows with score 3 were considered moderately lame, and cows with scores 4 and 5 were grouped together as severely lame.

## Statistical analysis

All analyses were performed using R (R Core Team, 2019) version 3.6.1, including packages: "lme4" (Bates et al., 2015), "magrittr" (Bache and Wickham, 2014), "dplyr" (Wickham et al., 2018), "tidyr" (Wickham and Henry, 2019), "multcompView" (Graves et al., 2019), "data.table" (Dowle and Srinivasan, 2019), "lsmeans" (Lenth, 2016), "effects" (Fox and Weisberg, 2018), "car" (Fox and Weisberg, 2011), "ggplot2" (Wickham, 2016) and "ggpubr" (Kassambara, 2018). All statistical analyses including code scripts can be downloaded at https://github.com/Bovi-analytics/ Hut-et-al-2020. Descriptive visuals can be downloaded at https://public.tableau.com/ profile/bovianalytics#!/vizhome/Hutetal\_2020/TransitionBodyConditionScore. The univariable analyses and final reduced models are presented in table 5 and 6 in the appendix.

Differences between lameness prevalences, defined as score 3, 4, and 5 combined, were tested by chi-squared test for the contrast dry versus lactating and Bonferroni corrected for the 4 scoring events against each other.

## **Association models**

For the association between BCS and lameness, 2 generalized linear mixed (binomial family with logit link) models were created for the locomotion scores at wk 4 and 8 comparing "healthy 1-2" versus "lame 3-5", "healthy 1-2" versus "lame 4-5" and "healthy 1-2-3" versus "lame 4-5". Only results from the first analysis ("healthy 1-2" versus "lame 3-5") are presented because results were comparable. Initially, all individual explanatory variables were tested in univariable models with herd as random effect. Only variables with P <0.1 were further analyzed in a multivariable model, including their mutual interactions. Thereafter, the likelihood ratio test on the Akaike information criterion was performed for model reduction to determine which final reduced model fitted the data best using the drop1 function. Final model effects were reported as odds ratios based on profile likelihoods. Multicollinearity was assessed with the variance inflation factor. There was no evidence of multicollinearity because every variance inflation factor value was <10 (Dohoo et al., 2003). Differences were reported with P-values, where P <0.05 was deemed significant and P <0.1 a trend.

### Sensor data models

All behavioral parameters were first checked for normal distribution and for linearity with quantile-quantile plotting. Except for the number of steps, all behavioral parameters displayed a normal distribution. To correct for skewness in the model concerning the number of steps, data were first log transformed and the final models were back transformed.

Generalized linear mixed models with a normal distribution were used for statistical analysis per behavioral parameter, corrected for animal within herd as random

effect. Initially, individual explanatory variables farm, calving season, and parity were tested in univariable models with animal within herd as random effect. Furthermore, the 3-way interaction between locomotion score, observation period (prepartum and postpartum), the observation event (begin dry (1<sup>st</sup> score prepartum), end dry (2<sup>nd</sup> score prepartum), 4 weeks in milk (1<sup>st</sup> score postpartum) and 8 weeks in milk (2<sup>nd</sup> score postpartum)) were offered. Thereafter, the likelihood ratio test on the Akaike information criterion was performed for full model reduction in order to determine which reduced model fitted the data best using the drop1 function. Farm and parity remained or were forced in all models. Final model effects were reported as means with 95% confidence intervals (95% CI) based on profile likelihoods. Differences between means were reported with P-values, where P<0.05 was deemed significant and P<0.1 a trend.

# RESULTS

# **Descriptives**

The distribution of locomotion scores per scoring event is presented in Figure 2A and shows the percentage of cows per locomotion score event. The percentage of locomotion scores per parity 2, 3 and >3 per scoring event is presented in Figure 2B. The lameness percentages (locomotion score 3, 4, and 5 combined) increased from 36% in the early dry period to 41% in the late dry period, 51% at 4 wk postpartum and to 54% at 8 wk postpartum. The percentages were different between the dry and lactation periods (P <0.001) as well as between the beginning of the dry period versus 4 wk postpartum (P <0.001), the beginning of the dry period versus 8 wk postpartum (P<0.001), the end of the dry period versus 4 wk postpartum (P = 0.006), and the end of the dry period versus 8 wk postpartum (P <0.001).

The categorized distribution of BCS per scoring event is presented in Figure 3A. The categorized change of the BCS between scoring events is presented in Figure 3B. In general, the BCS distributions indicate an increase in BCS during the dry period, a loss in BCS between the end of the dry period and 4 wk postpartum and an more or less equal distribution between cows losing and increasing in BCS between 4 and 8 wk postpartum.

Α

B



**Figure 2.** Distribution of multiparous cows (in %) with 4 consecutive scorings per locomotion score per scoring event (begin dry, end dry, 4 wk postpartum, 8 wk postpartum; A) and per parity group (2, 3, and  $\geq$ 4; B) on 8 commercial dairy farms in the Netherlands. Green = nonlame; orange = moderately lame; red = severely lame.



**Figure 3.** Distribution of the BCS of multiparous cows with 4 consecutive scorings per scoring event (begin dry, end dry, 4 wk postpartum, 8 wk postpartum; A) and the change in BCS between scoring events (B) on 8 commercial dairy farms in the Netherlands. Each BCS value on the x-axis corresponds with the bar to the left of that value.

The descriptive patterns of 2 sensor-based behavioral parameters are shown in Figure 4. These include the daily number of steps from the leg sensor and daily eating time from the neck sensor from day d -7 to d +7 around locomotion scoring (d 0). Other behavioral parameters from the leg sensor [number of stand ups (no./d), lying time (min/d), lying bouts (no./d) and lying bout length (min/bout)] are presented in the appendix in Figure 7A. The remaining neck sensor variables [eating bouts (no./d), eating bout length (min/bout), rumination time (min/d), rumination bouts (no./d) and rumination bout length (min/bout)] are presented in the appendix in Figure of eating time and number of steps around the 4 scoring events for nonlame cows per farm are illustrated in Appendix Figure 8 to present the baseline of nonlame cows and to show numerical differences in behavior in the dry and lactational periods.







## Association models

The final reduced models are presented in table 3. The final model for wk 4 included parity, calving season, and change in BCS between the end of the dry period and wk 4 postpartum (change transition). Cows with a large decrease in BCS of >0.75 point from the end of the dry period until 4 wk postpartum (change transition) had higher odds (OR=1.76) for lameness compared to cows with a decrease <0.50 (P=0.048). Cows calving in autumn had higher odds to be lame in wk 4 postpartum than cows calving in summer, winter, and spring. An increasing parity resulted in increasing odds for lameness in wk 4 postpartum.

The final model for week 8 only included calving season and parity (table 3). Cows calving in autumn and summer had higher odds of being lame in wk 8 postpartum than cows calving in winter and spring. An increasing parity resulted in increasing odds for lameness in wk 8 postpartum.

## Sensor data models

The statistical analysis showed that the overall time budget of dairy cows differed between the dry period and early lactation; these results are presented in table 4. All significant effects relative to nonlame cows are described per sensor-based behavioral parameter. First, as the model of the number of steps per day per locomotion score group (nonlame = 1 and 2; moderately lame = 3; severely lame = 4 and 5) shows, there was a significant difference between the dry and lactational periods. The daily number of steps declined by more than 200 steps for moderately and severely lame dry cows. In lactation, the number of steps declined by more than 600 steps for severely lame cows (Figure 5A). Severely lame cows had 26 min more lying time irrespective of the dry and lactating periods (Figure 5B). The number of lying bouts is shown in Figure 5C; there were significant differences between the dry and lactating periods, with 0.2 fewer lying bouts for severely lame cows in the lactating period. Lying bout length (Figure 5D) increased in severely lame cows; their lying bouts were 8 min longer, with no additional effect from dry or lactating period.

Eating time (Figure 6A) was lower in moderately lame and severely lame cows. Moderately lame cows spent 20 min less per day eating, and severely lame cows spent 38 min less in both the dry and lactational period. For number of eating bouts, moderately lame cows showed a trend of 0.4 fewer eating bouts, and severely lame cows had 0.8 eating bouts less per day in lactation (Figure 6B). The length of eating bouts (Figure 6C) was irrespective of the dry and lactational periods and was shorter in moderately lame cows (1.4 min less per bout) and severely lame cows (2.3 min less per bout). The rumination bout length (Figure 6D) of was 1.1 min shorter for moderately lame dry cows and 1.4 min shorter for severely lame dry cows.

	·			
Variable	Estimate	SE	Odds Ratio	P-value
Week 4				
BCS change transition				
Decrease < 0.50	Ref.			
Decrease 0.50 – 0.75	0.3018	0.1933	1.35	0.1185
Decrease > 0.75	0.5659	0.2343	1.76	0.0156
Calving season				
Autumn	Ref.			
Summer	-0.8321	0.2320	0.44	0.002
Winter	-0.9744	0.2353	0.38	< 0.001
Spring	-1.5533	0.2788	0.21	< 0.001
Parity				
2	Ref.			
3	0.6189	0.2111	1.86	0.0033
≥4	1.3732	0.2037	3.95	< 0.001
Week 8				
Calving season				
Autumn	Ref.			
Summer	-0.2903	0.2277	0.75	0.2024
Winter	-1.0419	0.2415	0.35	< 0.001
Spring	-1.3572	0.2676	0.26	< 0.001
Parity				
2	Ref.			
3	0.5204	0.2142	1.68	0.01
≥4	1.4659	0.2072	4.33	< 0.001

**Table 3.** Reduced final logistic regression models for the association between lameness at wk 4 and 8 postpartum (lame: scores 3, 4, and 5 vs. nonlame: scores 1 and 2) and recoded BCS with calving season, parity, and dry period length as fixed effects and herd as random effect.

**Table 4.** Predicted mean values and 95%CI for all sensor parameters based on reduced models with locomotion score group, pre- and postpartum, and first and second scores offered as explanatory variables to the full models and corrected for farm, calving season, and parity<sup>1</sup>.

Sensor parameter	Nonlame		Moderate	ely lame	Severely lame		
	Mean	95%CI	Mean	95%CI	Mean	95%CI	
Walking							
Number of stand ups (n/day)	10.3	9.95-10.6	10.3	9.98-10.8	10.4	9.98-10.8	
Number of steps (n/day)							
Pre partum*	3128	3013-3247	2894ª	2702-3100	2910ª	2753-3076	
Post partum	3722	3572-3879	3523	3311-3748	3116 <sup>a,b</sup>	2970-3270	
Lying							
Lying time (min/day)	679	667-691	682	665-699	705 <sup>a,b</sup>	691-720	
Lying bouts (n/day)							
Pre partum*	5.85	5.69-6.00	5.98	5.72-6.25	5.95	5.72-6.17	
Post partum	6.64	6.47-6.81	6.64 6.40-6.89		6.35 <sup>a,b</sup>	6.16-6.55	
Lying bout length (min/bout)	111	108-114	112	108-116	119 <sup>a,b</sup>	115-123	
Feeding							
Eating time (min/day)							
Pre partum*	362	355-370	342ª	331-352	325 <sup>a,b</sup>	316-334	
Post partum	346	339-354	326ª	316-336	309 <sup>a,b</sup>	300-318	
Eating bouts (n/day)							
Pre partum*	10.4	10.19-10.7	10.2	9.75-10.6	10.1	9.81-10.5	
Post partum	11.2	10.93-11.4	10.8ª	10.41-11.2	10.4 <sup>a,d</sup>	10.1-10.7	
Eating bout length (min/bout)	34.2	33.3-35.1	32.8ª	31.5-34.1	31.5-34.1 31.9ª		
Rumination time (min/day)	545	538-552	546	536-555	539	531-548	
Rumination bouts (n/day)	14.8	14.6-15.0	15.0	14.6-15.3	15.0	14.7-15.3	
Rumination bout length (min/bout)							
Pre partum*	37.7	36.9-38.4	36.6°	35.4-37.8	36.3ª	35.3-37.3	
Post partum	36.7	35.9-37.6	37.0	35.9-38.1	36.4	35.5-37.3	

<sup>a</sup>: Significantly different (P < 0.05) compared with nonlame

<sup>b</sup>: Significantly different (P < 0.05) compared with moderately lame

<sup>c</sup>: Trend (P < 0.1) compared with nonlame

<sup>d</sup>: Trend (P < 0.1) compared with moderately lame

<sup>1</sup>For the number of stand-ups, daily rumination time, and the number of rumination bouts, locomotion scores were forced in the reduced models.

\*: Significant effect (P < 0.05) of difference between pre- and post partum or  $1^{st}$  and  $2^{nd}$  score





Chapter 5

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# DISCUSSION

The locomotion scoring system (Sprecher et al., 1997) is a subjective scoring method with inter- and intraobserver variation (Channon et al., 2009). A limitation of our study is the unknown intraobserver reliability of the single scorer, which may have resulted in relatively low numbers of cows with score 1 and 5. Therefore, cows with score 1 and 2 were combined, as were cows with score 4 and 5; cows with score 3 were studied separately. The low number of cows with score 1 could be due to all cows having been scored on concrete slatted floors. Concrete is not their ideal walking surface and seems less suitable for claw health compared with straw yards (Van der Tol et al., 2005, Frankena et al., 2009). Although this recoding excluded the possibility of estimating the effect of each distinct score, our results indicate an effect on daily time budget with significant and biologically plausible differences. This is mainly the case for daily eating time, lying time, and number of steps. In contrast to the study of Grimm et al. (2019), which grouped locomotion score 1, 2, and 3 together as nonlame and score 4 and 5 as lame, our study also showed behavioral differences between score 1 and 2 and score 3.

Impaired locomotion increased for multiparous cows in lactation groups  $\geq 3$ from the early dry period until 8 wk postpartum, showing a large decrease of nonlame cows, especially after calving (Figure 2). The increasing number of lame and severely lame cows during the 4 scoring events could be related to the presence of chronic claw disorders (Bruijnis et al., 2012). The percentage of cows with impaired locomotion in our study was unfortunately still as high as reported 18 yr ago in a Dutch study (Somers et al., 2003). Comparable percentages of lame cows at the end of the dry period were seen by Daros et al. (2019). High locomotion scores have been associated with the weight of the calf in utero during the last part of gestation (Van Nuffel et al., 2016), udder size, and parity (Bölling and Pollott, 1998). A higher prevalence of sole ulcers has been reported in older cows (Holzhauer et al., 2008). Moreover, previous lameness could predispose cows for new cases of lameness (Randall et al., 2015). Lower feed intake is associated with an increase in lameness in high-producing cows (Gonzalez et al., 2008; Grimm et al., 2019). In early lactation, a loss in BCS related to the negative energy balance in older cows could include a decrease in digital cushion thickness (Bicalho et al., 2009; McArt et al., 2013, Macrae et al., 2019). Our results support an association between BCS loss in early lactation and lameness at 4 wk postpartum (Chebel et al., 2018; Daros et al., 2019; Randall et al., 2018).

We observed most lameness postpartum in autumn-calving cows and successively those that calved in summer, winter, and spring. Some of these autumn-calving cows had their dry periods during summer, whereas some were scored postpartum for locomotion in winter. Summer has been reported as a risk period for lameness (Sanders et al., 2009), but others found that lameness occurred more during winter (Cook, 2003; Espejo et al., 2006); therefore, the effect of season and climate is variable.

Sensor data from the leg sensor showed expected effects of lameness on walking and lying behavior, which is consistent with other studies (Ito et al., 2010; Westin et al., 2016). Others found a difference in leg activity between locomotion scores 1 and 2 (Thorup et al., 2015), indicating an underestimation in our study due to the combined analysis of locomotion scores 1 and 2. Sensor data from the neck sensor showed that lameness was associated with important changes in feeding behavior (i.e. less eating time in the dry period and in early lactation). Reduced eating time in the dry period has been related to a higher risk for metritis, ketosis, and other transition diseases in early lactation and a longer interval between calving and first service (Schirmann et al., 2016; Daros et al., 2020; Hut et al., 2019). Postpartum, a negative energy balance has a negative effect on reproduction and results in a decreased milk production (Esposito et al., 2014; Llonch et al., 2018). These studies indicate the importance of eating time in the dry and transition periods and the related feed intake. In our study, only severely lame dry cows showed shorter rumination bout length, whereas in lactation no association was found between locomotion score and rumination parameters, which is consistent with Thorup et al. (2016).

Behavioral differences as measured by sensor technology have been reported between cows in the dry period and those in early lactation and between primiparous and multiparous cows (Neave et al., 2017; Hut et al., 2019). In our study, lactating cows showed a higher number of steps, more lying bouts, less eating time with more eating bouts, and shorter rumination bout length compared with dry cows. We included calving season in our sensor data models because the 1.5-yr study period contained 6 mo of winter, 4 mo of spring, 3 mo of summer, and 5 mo of autumn. If we excluded calving season, these effects were picked up by a more evident contrast between dry and lactating animals (results not shown). We could not include effects of stocking densities in the dry period and early lactation, which may vary within farm by season, because these data were not collected. These farms do not have a policy to use overstocking, but the exact stocking densities per scoring moment were not recorded despite the known effect of stocking density on daily behavior of dairy cattle (Huzzey et al., 2006; Jensen and Proudfoot, 2017).

In this study, foot trimming data or lameness diagnosis was not taken into account due to practical constraints. To understand underlying causes of the incidence of high locomotion scores in transition cows, a weekly scoring interval followed by lameness diagnosis for scores  $\geq$ 3 should be implemented at least (Randall et al., 2015). Such scheme would allow a proper estimation of the incidence of diagnosed new cases of lameness that could be combined with the complete time budget of dairy cows as precisely measured with sensors. However, our study adds impaired locomotion as an explanation for reduced eating time in the dry period, with potential long-lasting effects on postpartum metabolic status and productive and reproductive successes.

# **CONCLUSIONS**

This study showed a high prevalence of locomotion scores 3 to 5 and an increase in locomotion scores 3 to 5 from the dry period up to 8 wk in lactation. Although the time budget of dairy cows differs between the dry and lactating periods using locomotion scores 1 and 2 (nonlame) as a baseline, more importantly, sensor data showed that daily eating time was reduced 38 min for locomotion scores 4 and 5 (severely lame) and 20 min for locomotion score 3 (moderately lame). This study shows that loss of BCS in early lactation is associated with an increased odds for lameness in wk 4 postpartum. Lameness is associated with less eating time in the dry period as well as in early lactation.

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# APPENDIX

**Table 5.** Variables used in the association models on lameness in week 4 and week 8 post partum in the univariable analysis and variables remaining in the final reduced models. Data were based on 784 multiparous cows in 8 commercial dairy farms in the Netherlands. Cows were scored 4 times: in the early dry period, at the end of the dry period, 4 weeks post partum and 8 weeks post partum.

Model	Variables	Univariable analysis	Final reduced model
Week 4			
	Parity	х	х
	BCS early dry	х	
	BCS end dry	х	
	BCS week 4	х	
	BCS change dry	х	
	BCS change transition	х	х
	Dry period length	х	
	Calving season	х	х
Week 8			
	Parity	х	х
	BCS early dry	х	
	BCS end dry	х	
	BCS week 4	х	
	BCS week 8	х	
	BCS change dry	х	
	BCS change transition	х	
	BCS change post partum	х	
	Dry period length	х	
	Calving season	х	х

BCS=body condition score. BCS change dry=BCS end – BCS early. BCS change transition=BCS week 4 – BCS end dry. Dry period length=number of days between first score and calving date. Calving season=summer, autumn, winter, spring.

Sensor data										
Variables		Leg sensor				1	Neck sensor			
	Number of steps	Lying time	Lying bouts	Lying bout length	Eating time	Eating bouts	Eating bout length	Rumination time	Rumination bouts	Rumination bout length
Locomotion score	x	x	x	x	x	x	x	x	x	x
Observation period	x	x	x	x	x	x	x	x	x	x
Observation moment	x	х	x	x	x	x	x	x	x	x
Calving season	x	x	x	x		x	x	x		x
OP:OM	x		x	x	x	x	x	x	x	
OP:LS	x		x	x		x				x
OM:LS				x	x					
OP:LS:OM				x						
Farm	x	x	x	x	x	x	x	x	x	x
Parity	x	x	x	x	x	x	x	x		x
Cow	x	x	x	x	x	x	x	x	x	x

**Table 6.** Variables that remained in the 10 final reduced sensor data models based on 784 multiparous cows in 8 commercial dairy farms in the Netherlands. Cows were scored 4 times: in the early dry period, at the end of the dry period, at 4 wk postpartum and at 8 wk postpartum.

LS=locomotion score, OP= observation period (dry/lactation), OM=observation moment (1<sup>st</sup>/2<sup>nd</sup> score), CS=calving season.





**Figure 7.** Descriptive values per scoring event (begin dry, end dry, 4 wk postpartum, 8 wk postpartum) and per behavioral parameter (number of stand-ups, lying time, lying bouts, lying bout length) from the leg sensor (A) and data (eating bouts, eating bout length, rumination time, rumination bouts, rumination bout length) from the neck sensor (B) in 3 locomotion scoring groups (green: nonlame; orange: moderately lame; red: severely lame) from 7 d before until 7 d after the day of scoring (d 0). Number of multiparous cows with full sensor data per locomotion score group was as follows: begin dry period, n = 707 (scores 1 and 2: n = 378, score 3: n = 127, scores 4 and 5: n = 205); end dry period, total n = 717 (scores 1 and 2: n = 370, score 3: n = 112, scores 4 and 5: n = 235); 4 wk in milk, total n = 755 (scores 1 and 2: n = 398, score 3: n = 124, scores 4 and 5: n = 233); 8 wk in milk, total n = 752 (scores 1 and 2: n = 385, score 3: n = 213)].



**Figure 8.** Descriptive values averaged per scoring event (begin dry, end dry, 4 wk postpartum, 8 wk postpartum) for locomotion scores 1 and 2 for eating time (min/d) and steps (no./d) per farm (1–8, green lines) to present farm differences and behavior differences between the pre- and postpartum periods. Mean values per scoring event are presented in red.



# **Chapter 6**

**Summarizing Discussion** 

# MAIN FINDINGS OF THIS STUDY

This thesis describes the daily time budgets of dairy cows and how these time budgets are affected by rising environmental temperatures. Further, associations between sensor based behavioral variables during an extended transition period and two major health issues, reduced fertility and lameness, were presented.

In chapter 2, two types of complete time budgets are shown: the lactational time budget averaged per month over the course of lactation and the daily time budget with behavioral patterns over 24h. A main finding was the difference in time budgets between first parity cows and older cows before and after entering the milking herd. These findings showed that first parity cows have to adapt from the pre- to post-partum period, similar to a transition period. Introducing cows in the lactational herd led to major changes in daily behavioral patterns, potentially reflecting the effects of accomplishing the hierarchical order. This effect on daily behavioral patterns was largest in primiparous cows, indicating that these animals may be better housed in a separate group. The main finding from the daily time budget indicates that dairy cows are diurnal animals which eat, stand and walk during the day and lie down and ruminate during the night. For both types of time budgets, the behavioral patterns were quite comparable between farms. Thus, this is what high producing cows in free stalls with or without pasture access do in the Netherlands. These behavioral profiles can be used as a benchmark for comparable dairy farming systems. For other types of systems, e.g. seasonal breeding, full grazing, organic farming or tie stall housing, specific benchmarks should be studied.

In chapter 3, associations between daily time budgets and the daily Temperature Humidity Index (THI) and daily temperature were analyzed. Strikingly, behavioral adaptation increased with increasing THI and temperature, and was already present at the relatively low THI values and temperatures commonly found in the mild summers in the Netherlands. This adaptation was noticeable from a mean temperature of 12°C or a mean THI of 56 when dairy cows started spending less time lying, eating and walking, and spent more time standing.

Chapter 4 describes associations between eating time in the transition period and the interval between calving and first service. Besides differences between primiparous and multiparous cows, lower eating time in week 4 before calving and week 3 after calving was associated with a prolonged interval between calving and first insemination.

In chapter 5, associations between body condition score, locomotion score, and sensor-based time budgets during the dry period and early lactation were studied. The percentage of lame cows increased over the course of the scoring moments and was overall very high. There was a clear association between lameness and daily time budgets as well as with the expected loss of body condition in early lactation.





Where the further focus in this chapter will be on welfare, cow comfort, and resource efficiency, fertility remains an important management issue. Sensors should be used to monitor all estrus events after calving, starting from the first (silent) heat onwards to the end of the voluntary waiting period to monitor whether regular estrus cycles are present. If regular cycles are not present, or start later than expected, farmers could then be alerted to cows that might need fertility related interventions. In addition, farmers could make better informed decisions regarding the voluntary waiting period based on individual cow patterns of estrus cycles combined with production levels (Burgers et al., 2021a; Burgers et al., 2021b).

# SUMMARIZING DISCUSSION

## Welfare and sensors

The ultimate goal for the dairy industry is to accomplish true resource efficiency combined with societal acceptance and approval. The only way that society can accept and support the dairy industry is transparency throughout all the chains of this industry, with a focus on animal welfare. Therefore, the Welfare Quality protocol (WQ<sup>®</sup>) was established in Europe (Welfare Quality<sup>®</sup>, 2009a). This protocol defines evaluation criteria based on animal-, resource- and management-based measures. However, the welfare assessments mostly used in Europe are still predicated solely on resource-based parameters such as type of housing and stocking density, reducing their relevance (Stygar et al., 2022). For a thorough review of the present welfare assessment protocols worldwide, see Krueger et al. (2020).

Animal-based parameters are needed to assess animal welfare and to assess the effects of management interventions. The development of Precision Livestock Farming (PLF) technologies has increased the last decades and can assist in generating these animal-based parameters (Silva et al., 2021). To be able to study welfare and management interventions, a benchmark for these animal-based parameters needs to be established. The "Sense of Sensors" study generated sensor based behavioral variables which can be used as benchmarks for the dairy industry.

Dairy cow behavior varies over and during the productive cycle, and although the number of farms in this study seems limiting (N=8), the differences in cow behavior between these farms seemed less pronounced than, for instance, the behavioral differences between parities. Based on these 8 farms, access to pasture also seemed to influence daily time budgets, but could not be clearly distinguished due to confounding with milking system and the lack of precise data on actual grazing times. Furthermore, this thesis showed how the daily time budgets of dairy cows on commercial dairy farms were affected by lameness and heat stress, both negatively affecting dairy cow welfare.

## Lameness

Besides the increasing percentage of lame cows, from 37% in the early dry period until 54% at peak lactation, the overall percentage of lame cows is just too high. The welfare of lame cows is severely affected (Bruijnis et al., 2012). Further, in a period of 20 years, no improvement to lameness prevalence has been accomplished in the Netherlands, despite knowledge of the risk factors and insights regarding treatment and prevention (Somers et al., 2003; Holzhauer et al., 2006). With such a high percentages of lameness, especially in the dry period and in early lactation, production and reproduction efficiency are diminished via the cascade of direct and indirect effects on daily cow behavior, especially on eating time and thus feed intake. As we have shown, lameness also has a tremendous effect on daily time budgets of dairy cows. Lameness hurts and causes cows to lie down more and eat less. Infectious causes of lameness induce an inflammatory response, and non-infectious claw disorders can also induce this inflammatory state due to pain (Gleerup et al., 2015; Barragan et al., 2018). The circulating inflammatory mediators produced as a consequence of lameness have a negative effect on the appetite centers in the brain, resulting in a lower feed intake and putting lame cows in the dry period more at risk for developing transition diseases (Brown and Bradford, 2021; Horst et al., 2021). Therefore, lameness should be prevented at all times and, supported by our findings, starting in the dry period.

# Heat stress

Heat stress negatively affects dairy cow welfare and makes them more susceptible to illness (Bernabucci and Mele, 2014; Polsky and Von Keyserlingk, 2017). The adaptation of dairy cows to a rising environmental temperature also leads to lower productive and reproductive efficiency and therefore, less efficient use of natural resources. The combination of global warming and a genetically driven high milk production is resulting in increasing susceptibility to heat stress in high yielding cows. The societal demand for pasture access will lead to an increased risk of summertime heat stress for dairy cows in the Netherlands, where pasture generally lacks enough shade to allow them to escape from direct sunlight. Whether the common advice to avoid heat stress is to keep cows indoors during the day and let them graze at night fits with the preferred diurnal pattern of cows remains to be studied. This thesis showed the substantial effect of rising environmental Temperature Humidity Index (THI) and mean daily temperature on time budgets of dairy cows. It also showed that daily temperature is a solid parameter to study heat stress in dairy cows in the Netherlands, and probably in other countries with a temperate and maritime climate as well. It additionally showed that the behavioral adaptation of dairy cows was already present from a daily mean temperature of 12°C. With increasing temperature, the parameters of the daily time budgets were affected more severely, resulting in less lying and eating time as the most dominant effects. Like lameness, this heat stress could have direct and indirect effects, such as less feed intake in the dry period, making cows more susceptible to transition diseases. We showed that dairy cows spent less time eating with increasing ambient temperature. Heat stress induces an inflammatory response (Bagath et al., 2019; Marins et al., 2021) resulting in the previously described cascade of negative feedback on the appetite centers of the brain. We also showed that lying time decreased and standing time increased with increasing ambient temperatures. This adaptational response puts dairy cows more at risk for developing lameness due to longer weight bearing periods (Cook et al., 2007).

Based on the research in this thesis, it is advisable to support dairy cows in their adaptation to rising ambient temperatures from a mean daily temperature of 12°C in a temperate and maritime climate. The effects of heat abatement strategies, which should always be a combination of affecting convection, conduction, radiation and evaporation (Polsky and Von Keyserlingk, 2017), can be measured with cow based sensor data and becomes even more important when production efficiency increases.

#### **Priority for lameness detection**

PLF adds a new dimension to the detection of heat, health issues or other cow related problems (Stygar et al., 2021). Previously, the farmer or herd manager was the designated person to detect every issue. Farmers or farm personnel remain key, since the success of sensor based alerts still depends on the actions that take after such alert is generated. For example: if a system gives an alert for a cow that is lame but the person assessing the cow disagrees with the alert or ignores the alert, the cow will not be looked after. At the same time, cows are stoic prey animals (Blackie et al., 2013) and therefore they will try to show there is nothing wrong with them. Depending on the health issue, this can result in (overly) subtle deviations in sensor data. Sensor systems can thus miss diseases or other problems while the cow or group of cows is actually in trouble. Therefore, the keys for successful dairy cow management based on sensor based alerts are high accuracy and a low percentage of false positive and false negative alerts. To minimize the risk of ignoring valid alerts, providing veterinarians access to the sensor technology interface could assist farmers and farm personnel in recognizing subclinical or early stages of diseases and other disorders. This access also provides veterinarians the opportunity to analyze farm specific data to study when most alerts occur. Such analysis can corroborate farm-specific risk factors and evaluate interventions as prevention of diseases.

Lameness detection was one of the most time consuming issues in this thesis. Although the locomotion score (Sprecher et al., 1997) is an easy system to implement as only a trained observer is needed, this is a subjective method with high inter- and intra-observer variation (Channon et al., 2009). This method is mainly used in scientific research and not in practice. Moreover, farmers estimate the prevalence of lameness four times lower than experts do (Whay et al., 2002) and they are unaware of the effects of lameness on farm profitability and animal welfare (Leach et al., 2010). The implementation of sensor-based lameness detection can aid in rising the awareness of lameness prevalence on a farm level and take the lead in early detection of lameness on an individual cow level. This requires accurate algorithms to generate alerts for possibly lame cows. As these alerts are based on deviations in behavioral patterns, they are an indirect diagnostic tool. However, some promising results with high accuracy are still not used in practice (Alsaoud et al., 2015).

Another option which has potential to be used to detect lameness in practice is 3D imaging of the cow's gait (Hansen et al., 2018). Consecutive measurements of individual cow locomotion recorded by a camera system and analyzed with algorithms improved the classification rate based on the five point locomotion scoring system. This classification rate improved (over 80% accuracy) when a non-lame versus lame classification was adapted (Viazzi et al., 2014, van Hertem et al., 2014). For such a system to be adopted and implemented by farmers, a few things need to occur. We need to clarify the consequences of lameness and the effects on farm profitability. Farm level lameness prevalence is therefore needed, as well as purchase price and the return on investment (ROI) for a PLF sensor system. Finally, the performance of the system needs to be solid, as reflected in validity, reliability and sensitivity (van de Gucht et al., 2017a; van de Gucht et al., 2017b). Based on the results of this thesis, reduction of lameness should be a high priority issue to improve dairy cattle welfare. Development of PLF systems that are able to accurately monitor locomotion and detect lameness is sorely needed.

## Sense of sensors: ruminating on the past

We have learned a great deal over the course of the "Sense of Sensors" project, and were in the fortunate circumstances to change the entire study during the data collection phase. At the beginning of this study, because a limited number of sensors was available per farm, we had to change sensors from cow to cow to focus on a period from six weeks before calving until 4 weeks after calving. We assumed that farmers would write down every health event and treatment of each individual cow. This appeared to be a heavy demotivator for farmers and disease registration did not occur.

For the second phase of this study, a specific timeline with cow-based measurements was created (fig. 1). At that moment, every cow from eight farms was equipped with both sensors (neck and leg) and our focus was to generate cow-based measurements. This generated a lot of data omitted from this thesis. It will allow for future analysis of a number of interesting topics, for instance the association of sensor based behavioral variables with the ketone body concentration. To study the association between sensor based behavioral variables and the calcium concentration, our data is probably less suitable. There is only one calcium measurement per cow. Since a new study has provided insights into the relationship between calcium metabolism postpartum and transient, delayed or persistent hypocalcemia (McArt and Neves, 2020) studying calcium concentrations in relation to sensor data has become more important. The number of calcium measurements needs to increase in the first and second week postpartum.

To be able to correct for demographic and geographic differences, farms in both phases were located throughout the Netherlands. However, daily cow behavior was reasonably comparable between farms and was merely based on differences between milking system (AMS, CMS) and pasture access. More importantly, as this thesis has shown, behavioral patterns and adaptational patterns also differ between parity groups. Every type of behavioral analysis should therefore factor in parity. Parity also affects production levels, and future research should focus on combining sensor data with production data. In that way, a more comprehensive understanding of associations between behavior, (re)production and welfare on a farm level can be gained.

One of the future perspectives of using sensor data is the prediction of specific events like diseases or production levels. The behavioral data from the "Sense of Sensors" study was used to create prediction models for the moment of calving (Liseune et al., 2021). This is a highly specific moment in the transition period, and an accurate prediction could possibly prevent severe effects of dystocia such as stillbirth as well as stress and injuries to the dam. This was addressed as one of the major issues in the study. Further, to cope here with missing sensor data, new imputation techniques were developed, allowing us to predict 65% of all calvings within 24h, with an accuracy of 77%. For the moment of calving, we used the time that the cow had calved, as reported by the farmer. This is not ideal, and to be able to detect dystocia, the moment of calving should have been the onset of calving based on imaging (for instance) combined with the actual time of birth (Rutten et al., 2017). This would allow specific and reliable algorithms to be created in the future. We hope that companies producing sensors will focus on thorough data collection with trustworthy and complete data sets.

# **Concluding remarks**

One way to meet the UN's "Sustainable Development Goals" is to improve the efficiency of milk production with a focus on resource efficiency. Resource efficiency can only occur when cows are healthy and able to express their genetic potential and normal behavior at all times. This can be aided by technology, which can also empower the dairy industry by generating insights and transparency for the public. A key goal in the efficient use of resources and the use of sensor technology, is to put animal welfare first, and so to contribute to a socially accepted dairy industry more able to influence policies in a positive manner and assist farmers to retain their social license to produce (Barkema et al., 2015). However, societal acceptance of the dairy industry will be become more at risk when so many cows experience transition diseases, are burdened by heat stress, and are in pain due to some degree of lameness. Moreover, cows that are sick, in pain or stressed will not produce the amount they could given their genetic potential and, therefore, optimal resource efficiency will not be accomplished. Only when farmers and their advisors are able to regard farm management from a cows' perspective is it possible to improve cow comfort and thus animal welfare, allowing cows to produce more with the same or even less input.

The world record for milk production in one year was established by dairy cow 'Selz-Pralle Aftershock 3918'. Her farmer said she has had not a single health issue since she calved for the first time. When achieving the world record, she was in her fifth lactation. On 'her' farm, management is focused on cow comfort, and after investing in sensor technology, this farmer was able to see the difference in behavior between the world champion and his other cows. When animal welfare is on the highest level and disease is absent, dairy cows are able to express their full genetic potential, and it is our turn to enable that. Monitoring dairy cow behavior: there is more than meets the sensor.
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**Nederlandse Samenvatting** 

### Belangrijkste bevindingen van deze studie

Dit proefschrift beschrijft dagelijkse gedragspatronen van melkkoeien op basis van sensormetingen. Gedragspatronen worden mede bepaald door pariteit. Daarnaast hebben vruchtbaarheid en locomotie invloed op deze gedragspatronen, naast (een stijgende) omgevingstemperatuur.

Het "Sense of Sensors"-project is gestart om sensordata van transitiekoeien beter te begrijpen. De transitie heeft betrekking op de overgang tussen niet melkgevend (droogstand) en melkgevend. De transitieperiode is een periode van drie weken voor tot en met drie weken na afkalven. Het uiteindelijke doel is een succesvolle transitie voor elke koe. Gezien het feit dat 75% van de zieke koeien in de eerste weken na afkalven ziek wordt, hebben veel koeien geen succesvolle transitie. Van een aantal transitieziekten is bekend dat koeien met dergelijke problemen al vóór afkalven andere gedragspatronen vertonen. Als we die gedragspatronen beter kunnen begrijpen, dan komt dat het welzijn van koeien ten goede. Voordat we gedragsafwijkingen konden gaan monitoren, was het allereerst van belang om normale gedragspatronen in kaart te brengen én vervolgens hoe deze patronen worden beïnvloed door externe (bijv. type bedrijf, omgevingstemperatuur) en interne factoren (bijv. pariteit, ziekte en/of kreupelheid).

Het "Sense of Sensors"-project was een samenwerking tussen Nedap Livestock Management, Vetvice, Wageningen Universiteit en Universiteit Utrecht. Het project bestond uit twee fases. Fase 1 betrof een studie op 17 melkveebedrijven in Nederland waarbij gebruik werd gemaakt van twee sensoren per koe: een hals-sensor en een pootsensor. De hals-sensor registreerde data met betrekking tot vreettijd en herkauwtijd. De pootsensor registreerde data met betrekking tot ligtijd, sta-tijd en looptijd. Per koe werd van zes weken voor afkalven tot en met vier weken na afkalven het gedrag continu gemonitord door beide sensoren. De resulterende sensordata werd vervolgens gecombineerd met vruchtbaarheidsgegevens en andere koe-gebonden informatie, die de veehouder bijhield, zoals bijvoorbeeld het afkalfmoment en behandelingen. Vanwege missende gegevens en een gebrek aan koe-gebonden informatie, werd voor Fase 2 besloten om acht van deze 17 bedrijven intensiever te volgen. Hiervoor werd elke koe met beide sensoren uitgerust en werd elk bedrijf gedurende een periode van anderhalf jaar wekelijks bezocht om koe-specifieke gegevens te verzamelen, zoals locomotiescores, conditiescores en bloedmonsters ten behoeve van de analyse van hypocalcemie en ketose. Van deze acht bedrijven hadden vijf bedrijven een conventioneel melksysteem (CMS) met weidegang en drie bedrijven hadden een automatisch melksysteem (AMS) zonder weidegang. Al deze gegevens samen hebben geleid tot een dataset bestaande uit meer dan een miljard observaties. De dataverzameling vond plaats tussen 1 november 2016 en 11 november 2020.

Hoofdstuk 2 beschrijft gedragspatronen van melkkoeien op twee verschillende tijdschalen 1) daggemiddelden per maand van droogstand tot eind lactatie om de dagelijkse gemiddelde gedragspatronen gedurende een lactatiecvclus te bestuderen en 2) twee-uurs gemiddelden per 24 uur voor lacterende dieren om dagelijkse gedragspatronen en het dag-nacht ritme te bestuderen. De gedragspatronen van de eerste tijdschaal laten het verloop van de vijf gedragingen (vreettijd, herkauwtijd, ligtijd, sta-tijd, looptijd) zien, van één maand voor afkalven tot en met tien maanden na afkalven. Om afwijkingen te bestuderen, is het van belang om eerst inzicht in normaalwaardes te krijgen. Opvallend was dat de gedragspatronen over de lactatiecyclus een gelijksoortig verloop vertoonden over alle bedrijven met verschillende systemen. De introductie van koeien na afkalven in de melkgevende koppel, vooral de introductie van eerstekalfs-koeien, laat zien dat deze dieren extra aandacht behoeven. Eerstekalfs-koeien laten namelijk de grootste verandering zien wanneer sensordata in de maand voor afkalven wordt vergeleken met de sensordata uit de eerste maand na afkalven. De ligtijd van eerstekalfs-koeien daalt met 215 minuten, terwijl de ligtijd van ouderkalfskoeien met ongeveer 120 minuten daalt. De daling van de ligtijd is terug te zien in een evenredige stijging van de sta-tijd, hetgeen betekent dat deze jonge dieren onvoldoende ligtijd hebben en dat er grotere belasting op de klauwen plaatsvindt in een al zeer kritische periode: de transitieperiode. De sensordata laten het vinden van een plek in de hiërarchie van de koppel zien. Het tweede type gedragspatroon - op basis van dezelfde gedragingen -, is het dagelijkse gedragspatroon over 24 uur. Deze resultaten laten zien dat, melkkoeien circadiaanse gedragspatronen hebben waarin ze overwegend overdag eten, staan en lopen en 's nachts liggen en herkauwen. Beide typen gedragspatronen waren opnieuw zeer vergelijkbaar tussen de bedrijven, met relatief weinig verschil tussen bedrijven met een CMS en weidegang enerzijds en bedrijven met een AMS zonder weidegang anderzijds. Deze gedragspatronen geven dus een goed beeld van wat koeien in dergelijke systemen in Nederland doen en kunnen derhalve worden gebruikt als referentie voor vergelijkbare melkveehouderijsystemen. Andersoortige manieren waarop melkvee wordt gehouden, met bijvoorbeeld seizoensgebonden afkalfpatronen, of volledige weidegang zoals in Nieuw-Zeeland of Ierland, of in aangebonden stallen, hebben eigen specifieke referenties nodig om de normale gedragspatronen te kunnen vergelijken.

In hoofdstuk 3 zijn associaties tussen de dagelijkse gedragspatronen en de dagelijkse gemiddelde temperatuur-luchtvochtigheidsindex (THI) én de dagelijkse gemiddelde temperatuur geanalyseerd. De THI is een variabele zonder eenheid met waardes tussen 0 en 100 en bestaat uit een verhouding tussen temperatuur en luchtvochtigheid. Op de warmere dagen daalde de vreettijd (gemiddelde vreettijd ligt rond zes uur per dag) met 92 minuten per dag en nam de ligtijd (gemiddelde ligtijd ligt rond 11 uur per dag) met 56 minuten per dag af. De gedragsadaptatie was reeds zichtbaar vanaf een omgevingstemperatuur van 12°C of een gemiddelde THI van 56. Melkkoeien gingen bij hogere waardes minder liggen en eten terwijl de sta-tijd

toenam. Ook nam de herkauwtijd af, dit was echter alleen te zien in de data van de droogstaande koeien en de melkgevende koeien van de AMS-bedrijven. Met stijgende waardes in THI en temperatuur nam tevens de looptijd af. Deze resultaten laten zien dat melkkoeien hun gedragspatronen in een gematigd klimaat al aanpassen bij een relatief lage temperatuur of THI en ze dienen dan ook ondersteund te worden vanaf deze temperatuur via straling, stroming, geleiding én verdamping. In de vergelijking tussen de aanpassing in gedragspatronen naar zowel stijgende temperatuur als stijgende THI, bleken de resultaten sterk overeen te komen. In het gematigde en maritieme klimaat van Nederland is de dagelijkse gemiddelde temperatuur dus geschikt om de adaptatie in het gedrag van koeien ten gevolge van hittestress te bestuderen.

In hoofdstuk 4 worden de associaties tussen verschillende vreettijdparameters in de transitieperiode en het interval tussen afkalven en eerste inseminatie, besproken. Voor deze studie hebben wij de transitieperiode iets ruimer genomen, namelijk van vier weken voor afkalven tot en met vier weken na afkalven. De gedragsdata van deze weken hebben we vervolgens gerelateerd aan het interval tussen afkalven en eerste inseminatie. Naast de verschillen in vreettijd tussen eerstekalfs- en ouderekalfs-koeien (ouderekalfskoeien besteden minder tijd aan vreten), hing een lagere vreettijd in week vier voor afkalven en in week drie na afkalven samen met groter interval tussen afkalven en eerste inseminatie. Deze resultaten geven het belang van vreettijd in de transitieperiode aan, ook al is het interval tussen afkalven. Tijdens dit interval maakt een koe zich op voor de pieklactatie, dient zij weer vruchtbaar te worden én vinden de meeste problemen plaats: transitieziekten.

In hoofdstuk 5 worden de associaties tussen de conditiescore, de locomotiescore (LS) en de gedragspatronen op basis van sensordata gepresenteerd. Elke koe werd gescoord op kreupelheid (score 1-5) en lichaamsconditie (score 1-5) aan het begin en einde van de droogstand en ook in week vier en in week acht na afkalven. Koeien met LS 1 en 2 werden gegroepeerd in de categorie "niet kreupel", koeien met LS 3 waren "matig kreupel" en koeien met LS 4 en 5 waren gegroepeerd in de categorie "ernstig kreupel". Het percentage kreupele koeien nam toe over het verloop van de vier scoremomenten: 36% was kreupel aan het begin van de droogstand, 41% was kreupel aan het einde van de droogstand, 50% was kreupel in week vier na afkalven en 54% van de koeien was kreupel in week acht na afkalven. Op basis van het dagelijkse gedragspatroon vraten matig kreupele koeien gemiddeld 20 minuten minder, waar ernstig kreupele koeien 40 minuten minder vraten. Matig en ernstig kreupele koeien zetten in de droogstand 200 stappen per dag minder terwijl de ernstig kreupele koeien in lactatie dagelijks 600 stappen minder zetten. Vergeleken met niet kreupele koeien nam de ligtijd met 26 minuten toe bij kreupele koeien en de duur per ligmoment nam toe met acht minuten. Het verlies van lichaamsconditie aan het begin van de lactatie was geassocieerd met kreupelheid ( $LS \ge 3$ ) na afkalven, terwijl kreupelheid resulteerde in verminderde vreettijd in de droogstand en aan het begin van de lactatie, wat resulteert in een vicieuze cirkel.

In hoofdstuk 6 worden de belangrijkste resultaten uit dit proefschrift gecombineerd met recente inzichten uit de wetenschappelijke literatuur. De resultaten laten het grote belang zien van betere, betrouwbare, automatische kreupelheidsdetectie. Voor de dataverzameling van dit proefschrift, heeft de kreupelheidsdetectie verreweg het meeste tijd gekost. Dit maakt het praktisch gezien onhaalbaar om op bedrijfsniveau de kreupelheidsprevalentie en -incidentie goed in kaart te brengen. Door middel van het gebruik van 3D-camerabeelden worden door andere onderzoeksgroepen hoopvolle resultaten geboekt voor de toekomst. Feitelijk is er in Nederland, ondanks alle kennis over klauwproblemen en aandoeningen, inclusief behandeling en preventie, weinig verbetering in het grote aantal kreupele koeien, met name in en rondom de transitieperiode. Dit is op alle vlakken onwenselijk. Ten eerste wordt het welzijn van koeien door kreupelheid aangetast, ten tweede is een kreupele koe minder efficiënt, zal zij minder produceren en vatbaarder zijn voor andere ziekten en aandoeningen, waaronder een verminderde vruchtbaarheid. Bovendien passen deze aantallen kreupele koeien niet in een maatschappelijk verantwoorde en geaccepteerde veehouderij.

Hittestress is een tweede onderdeel dat te allen tijde voorkomen dient te worden. Naarmate de temperatuur (en luchtvochtigheid) stijgt, zal een koe zich in haar gedrag dusdanig proberen aan te passen opdat zij haar warmte kwijt zal blijven kunnen. Hittestress is het moment dat de koe haar warmte niet meer kwijt kan en dit leidt tot fysieke klachten. Hittestress komt in toenemende mate voor door een combinatie van een stijging van omgevingstemperatuur en een hogere melkproductie van de koe. We hebben in dit proefschrift laten zien dat koeien hun gedragspatronen al eerder dan verwacht aanpassen aan een stijgende gemiddelde dagelijkse omgevingstemperatuur, namelijk vanaf 12 graden Celsius. Het is bovendien bekend dat koeien die in de droogstand hittestress ervaren, minder productief zijn gedurende de hele lactatie én dat hun kalveren gevoeliger zijn voor ziekten én minder productief zijn tijdens hun lactatie.

De resultaten en overwegingen in dit proefschrift tonen het grote belang van sensordata aan. Sensordata kunnen een grote bijdrage leveren aan het optimaliseren van de huidige melkveehouderij, waar de koe in gezondheid en welzijn en de veehouder in een efficiëntere bedrijfsvoering van zullen profiteren.



**Review Committee** 

Prof. dr. P. R. van Weeren Professor of Equine Musculoskeletal Biology, Faculty of Veterinary Medicine, Utrecht University.

Prof. dr. ir. G. van Schaik Professor in Monitoring and Surveillance of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University

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Chair: Prof. dr. P. J. M. Rottier Emeritus professor and advisor in the Division of Virology, Faculty of Veterinary Medicine, Utrecht University.



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Het "Nedap-Vetvice project", de naam van dit project voordat het werd omgedoopt tot "Sense of Sensors" is destijds geïnitieerd door Jan Hulsen (Vetvice) en Rutger Woolderink (Nedap). Op basis van jullie onderlinge vertrouwen, is er uiteindelijk een prachtig project ontstaan. Dit boekje is er een resultaat van en er zit nog zoveel meer in de data die we hebben verzameld. Jan, als geen ander integreer je de diergeneeskunde, praktijk, bedrijfskunde én wetenschap. Voor dit project, en voor mij, heb je een rol van onschatbare waarde gespeeld. Moeiteloos leg je de vinger op de zere plek en met dezelfde moeite heb je de oplossing paraat. In de afgelopen jaren zijn we tegen veel knulligheden aangelopen. Op de momenten dat ik het even niet meer zag zitten, wist je me te motiveren. Dankjewel. Linda, Rudy en Rudy, ontzettend bedankt voor alles wat jullie voor dit project hebben gedaan. Arnold, dankjewel voor alles én voor alle keren dat je ons en onze studenten wilde ontvangen in Groenlo, ze hebben hun ogen uitgekeken.

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verder (en niet teveel lullen tijdens de Top2000), is dit deel van "Sense of Sensors" eindelijk af. Voor mij net zo'n bevalling als veel van jullie onderzoeksstages 😟. Jullie hebben me geïnspireerd met onbevangen en bevlogen ideeën en nog honderden onderzoeksvragen. De dataverzameling is alweer even ten einde, maar ik denk met weemoed terug aan elke dinsdag en donderdag waar we voor dag en dauw, vaak met bus 40, een rondje oost of zuidwest Nederland gingen rijden. De nostalgie van bus 40, de oude bus van Dick, vooral voor de veterinair student, een icoon. Lampies die te pas en te onpas aan én uit gingen, een oliepeil waar geen pijl op te trekken was en bovenal de cassettespeler. Nog een generatie die weet waarom je altijd een potlood in de buurt van een cassette moet hebben. Wat waren we ontsteld dat deze bus op een dag bleek afgevoerd. Tim, Nils, Erik, Jilles, Aron, Willem, Anton, Rein, Samantha, Amerins, Marcel, Adriaan, Niels, Tom, Marco, Hanna, Arjen, Samantha, Leslie, Gerben, Feline, Wouter, Dianne, Caroline, Sascha, Ruben, Esther, Femke, Anne Sophie, Gerben, Tjitske, Anne-Miek, Susanne, Romy, Lotte, Lianne, Coen, Mariëlle, Jessica, Gerwin, Pim, Sjoerd, Robin, Jessy, Marleen, Rebecca, Tanja, Marieke, Marleen, Lisa, Melanie, Lena, Annemieke, Frederike, Daniëlle, Dyon, Jolien, Anne, Sarah en Meike: heel veel dank!

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List of Publications

### Peer reviewed publications

Hut, P. R., J. Scheurwater, M. Nielen, J. van den Broek, and M. M. Hostens. 2022. Heat stress in a temperate climate leads to adapted sensor-based behavioral patterns of dairy cows. J. Dairy Sci. 105:6909-6922. https://doi.org/10.3168/jds.2021-21756.

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Hulsen, J., N. Vreeburg, F. van Eerdenburg, G. Hooijer, P. Hut, A. Harbers, and E. Stassen. 2018. Making sense of sensors in transition cow care. Hoard's Dairyman. April 25.

Hut, P.R., R. Jorritsma, en W. Gruenberg. 2019. Klinische problemen rond het kalven door een fosfortekort. Tijdschrift voor Diergeneeskunde. 144(2).

Santifort, K., D. Verduijn, en P. Hut. 2022. Een Holstein-Friesian kalf met neurologische verschijnselen. Tijdschrift voor Diergeneeskunde. 147(5).

### **Conference presentations**

Hut, P. R., J. Scheurwater, M. Nielen, J. van den Broek, and M. M. Hostens. 2022. *Heat stress in a temperate climate leads to adapted sensor-based behavioral patterns of dairy cows.* World Buiatrics Conference, Madrid, Spain.

Hut, P. R., J. van den Broek, J. Hulsen, A. Harbers, G. A. Hooijer, E. N. Stassen, and F. J. C. M. van Eerdenburg. 2018. *Fluctuation of eating time in the dry period affects fertility parameters in subsequent lactation*. World Buiatrics Conference Sapporo Japan.

Hut, P. R., L. Paagman, J. van den Broek, J. Hulsen, A. Harbers, G. A. Hooijer, E. N. Stassen, and F. J. C. M. van Eerdenburg. 2018. *Hypocalcemia and acetonemia are highly correlated in dairy cows until the fourth lactation, then the table turns*. World Buiatrics Conference, Sapporo, Japan.

Hut, P. R., J. van den Broek, J. Hulsen, A. Harbers, G. A. Hooijer, E. N. Stassen, and F. J. C. M. van Eerdenburg. 2018. *Sense of sensors: behavioral aspects of dairy cattle in the transition period related to their locomotion scores*. World Buiatrics Conference, Sapporo, Japan.

Hut, P. R., L. Paagman, J. van den Broek, J. Hulsen, A. Harbers, G. A. Hooijer, E. N. Stassen, and F. J. C. M. van Eerdenburg. 2018. *Sense of sensors: several behavioral parameters in the dry period are associated with calcium concentrations within 48h postpartum.* World Buiatrics Conference Sapporo, Japan.

Heiltjes, A. S., F. A. J. Manders, P. R. Hut, A. Harbers, J. Hulsen, G. A. Hooijer, and E. Stassen. 2017. *Disease registration: 'From torment to triumph, for farmer and scientist!'* International Society for Economics and Social Sciences of Animal Health (ISESSAH), Aviemore, Scotland.



Biography



Peter Hut was born in Groningen, the Netherlands, on the 1<sup>st</sup> of September 1986. He was raised in Zuidlaren, a village in the northern part of the Netherlands. He graduated at the Maartens College in Haren in 2005 and started studying Veterinary Medicine at Utrecht University the same year. After graduation in 2013, he worked in a veterinary practice with a focus on dairy cattle and afterwards in the dairy cattle feed industry. In 2015, he started as a resident for the European College of Bovine Health Management at the Faculty of Veterinary Medicine, Utrecht University, and passed the exam in 2020. During his residency, he also joined the "Sense of Sensors" project, a collaborative research project between Nedap Livestock Management, Vetvice, Wageningen University & Research and Utrecht University, which resulted in this thesis. Peter started working at CowManager B.V., Harmelen, the Netherlands in May 2022 as product manager. Peter is married to Margot and they have two sons: Maurits and Philip.

