THE HAPPY HEALTHY COW

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The Happy Healthy Cow

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Chapter 1

General introduction

INTRODUCTION

In the dairy industry, there is an increased need to improve individual monitoring of the health and welfare of cows. The traditional method of direct observation by the farmer is being replaced by monitoring using wearable sensors (Lee and Seo, 2021). Several recent developments in the dairy industry show the need for this. The number of dairy farms has decreased, whereas herd size and milk production per cow have increased (Barkema et al., 2015). The natural behaviour of cows, climate control, emission of nitrogen components and reuse of waste have become more important elements in both governmental regulations and dairy farm management (Galama et al., 2020). The change of the housing system from tie-stall barns to free stall barns has impaired labour efficiency, but the larger herd sizes and the many aspects that need to be managed and controlled, have made close and frequent monitoring of individual cows essential.

Nowadays, a large variety of sensors are commonly used in dairy farms and play a crucial role in detecting changes in the behaviour or production of individuals (Stygar et al., 2021). Those sensors generate an enormous amount of data, requiring appropriate techniques and methods for data management, as well as to generate information relevant for the farmer. Besides the use by the farmer in day-to-day management, the data available from the sensors can also be used in the research field to monitor cows continuously over longer periods of time and to learn more about patterns of what one could call 'normal behaviour' for a cow with given characteristics, and about individual variations of physical parameters. Measuring important behaviours such as rumination, eating, drinking, defecating, urinating, sleeping, walking, standing, and lying of all cows in a herd continuously and automatically in a reliable, easy, and low-cost way would be of high value for the dairy industry and research. To be able to link sensor data to specific health or welfare problems the range of normal values and individual variation of the distinct behaviour patterns need to be better understood. This is where this thesis aims to contribute.

HEALTH OF DAIRY COWS

The average lifespan from birth to death of dairy cows is between 4.5 to 6 years in most developed dairy industries (De Vries and Marcondes, 2020). Cows calve on average for the first time at 2 years of age, which brings the productive lifespan of average dairy cows between 2.5 to 4 years (De Vries and Marcondes, 2020). In contrast, the natural life expectancy of dairy cattle is approximately 20 years (De Vries and Marcondes, 2020). Hadley et al. (2006) reported that up to 80% of all culling was due to health issues. Mastitis, clinical and subclinical, is one of the most frequent and most costly diseases in dairy cattle (De Vries and Marcondes, 2020). Cows with subclinical mastitis produce less milk and have elevated somatic cell counts. Also, the typical fresh cow diseases like retained placenta, metritis, displaced abomasum, and ketosis are frequent diseases in dairy cows. Metritis is associated with lower milk production and inefficient reproduction (De Vries and Marcondes, 2020). The transition period around calving is characterized by a negative energy balance, and an increased risk of ketosis. Low blood calcium levels are indicative for hypocalcaemia, a peripartum disease that is manifested clinically or sub clinically. Also, lameness negatively impacts reproductive efficiency and milk production of dairy cows (De Vries and Marcondes, 2020).

Most health issues have characteristics or signs that cannot be measured by sensors directly. For example, a well-functioning forestomach complex, composed of four structurally distinct compartments (rumen, reticulum, abomasum, and omasum) is vital for ruminants and is linked with cow health. The detection of aberrances in the functioning of the forestomach complex is not feasible with the current sensors available on the market.

Sensors can be useful to monitor individual animals in large herds by collecting data of each individual cow. On many farms, sensors are used to give a health alert to the farmer when, according to sensor data and their interpretation, a cow shows behaviour or physical characteristics that are not within an expected ‘normal’ range. Each sensor can measure other parameters of the animal and by monitoring those parameters 24/7, even small changes in an early phase of a disease or subclinical disease can be detected. To monitor, for example, fever and pain automatically measuring body temperature and heart rate could be helpful for relevant alerts. The analysis of behavioural elements such as the rumination time could be used as a marker to predict animals at risk of diseases, and as a first step to apply preventive veterinary practices in ruminant herds (Silva de Tarso, 2017).

There is large interest in not only generating general health alerts for the farmer but also to distinguish between health problems. Most studies using sensor data focus on giving an alert in case of only one specific disease or the reproduction status of a cow, but fewer studies focus on using sensor data to learn more about healthy cows.

WELFARE OF DAIRY COWS

Increasing concerns of consumers regarding the well-being of farm animals has led to the development of a variety of welfare programmes and certification systems (Lutz et al., 2021; Stygar et al., 2021). Several studies have evaluated distinct welfare standards and associated quality assurance programmes for dairy cows. Those programs are not always practical for detecting early-warning signals, which could result in implementation of preventive measures (Stygar et al., 2021). There is no universal definition of animal welfare. The ‘five freedoms’ concept defines that good welfare requires freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom to express normal and natural behaviours, and freedom from fear and stress (Lutz et al., 2021). The World Organization for Animal Health published another commonly used definition: ‘‘Animal welfare means the physical and psychological condition of an animal in relation to the conditions in which it lives and dies” (OIE, 2022). The most comprehensive Dynamic Animal Welfare Concept (DAWCon) combines and adds onto existing welfare concepts and states: “An individual is likely in a positive welfare state when it is mentally and physically capable and possesses the ability and opportunity to react adequately to sporadic or lasting appetitive and adverse internal and external stimuli, events, and conditions. Adequate reactions are elements of an animal's normal behaviour. They allow the animal to cope with and adapt to the demands of the (prevailing) environmental circumstances, enabling it to reach a state that it perceives as positive, i.e., that evokes positive emotions" (Arndt et al., 2022).

Cows are social animals that form dominant-subordinate relationships, to maintain a stable herd order and to reduce aggression. Under natural conditions, cows form groups interconnected by long-term, non-familial bonds that can span numerous years. Groups of mixed ages, regardless of sex, will have a social dominance structure that closely resembles those bonds in free-ranging herds (Hubbard et al., 2021). Dominance has been studied widely. Nowadays, more studies focus on the role of grooming and the relationships between affiliative and agonistic behaviours (Foris et al., 2019). Less is known about the roles of affiliative behaviour that has been associated with positive emotions, formation of social bonds and a calming effect on receiver cows (Foris et al., 2019). Reliably observing animal behaviour requires minimal human interference. Understanding social interactions between animals can be difficult and time-consuming using traditional methods of live observations and video recordings. The use of sensor technologies allows monitoring the cows continuously, non-invasive and in a way that is not labour-intensive. Animal welfare itself cannot be measured directly but must be reflected through a variety of measurements that represent multidimensionality (Lutz et al., 2021). Behaviour is highlighted as a crucial read-out parameter for animal welfare (Arndt et al., 2022). The results of a study by Adamie et al. (2022), support the use of already existing and routinely collected production economic and herd-management data from dairy cows to enable an analysis of farm animal welfare on a larger scale. Different measurements can be combined for greater accuracy. For example, sudden changes in activity, feeding and drinking could be detected by different sensors and combined to provide information that is more than the sum of its parts.

Sensors have the potential to provide insight to identify social structure, not previously possible in other ways. Also, when cows are not housed in a stable but are grazing in the field, they could be monitored with sensor technologies. A potential of such systems for monitoring social interactions between dairy cows is demonstrated in a study by Ren et al. (2021). Automated devices can be very helpful to quantify animal positioning patterns to understand social relationships, interactions between animals, group structure and to identify leaders that may influence group behaviour.

SENSORS MONITORING COWS ON DAIRY FARMS

The first commercially applied sensors in the dairy industry have been leg-mounted accelerometers, also called pedometers (Halachmi et al., 2019). Different versions of pedometers from several manufacturers have become available and generate summaries of lying time, number of steps and activity on the sensor in periods of 2 hours or daily. Activity drastically increases during oestrous of the cow and pedometers are widely used for oestrous detection (Halachmi et al., 2019). In daily management, those pedometers are also used for health alerts. When the sensor gives an alert that a specific cow has not stood up and has increased lying time, the farmer can examine the cow. In general, the sensor only detects a health alert and did not mention the exact health problem. Researchers started using those pedometers for lameness detection (Halachmi et al., 2019; O'Leary et al., 2020). More different pedometers became available of different trades and are still widely used on farms. In Chapter 2 and 3 we used the data of the Nedap Smarttag leg sensor (Fig. 1) to monitor the time standing, walking, and lying and number of times standing up from a lying position.

Nowadays, the neck collar sensor systems on dairy farms can monitor rumination time and eating time (Lee and Seo, 2021). Rumination is a very important behaviour in ruminants. Interestingly, before calving and when being in heat the rumination time decreases, but also when a cow has health problems. This indicates that rumination activity is an important factor to take into account for a sensor alert system. The Nedap Smarttag neck collar and SCR neck collar (Fig. 1 and 2) are examples of neck sensors used in Chapter 2 and Chapter 3, respectively, which both measure rumination activity.

Next to the wearable sensors introduced above, there are also sensors placed inside the cow, for example a bolus in the forestomach complex. The functioning of the forestomach complex is imperative for the entire health status of the cow. The contraction pattern depends on the activity of the animal, whether eating, ruminating, or resting, and it requires up to 50 seconds to complete a total cycle (Silva de Tarso, 2017). For nearly 100 years the function of the specific motility patterns of the forestomach of cows have been studied using direct observation, palpation, and pressure measurements (Sellers and Stevens, 1966). In the nineteen fifties and sixties, methods to measure pressure were developed, generally using rumen fistulated cows with balloons or fluid filled catheters (Okine et al., 1990; Sellers and Stevens, 1966). This research based on relatively few observations already showed potential associations between the contraction cycles and behaviour of the cow. The contraction pattern starts with a biphasic contraction cycle of the reticulum and only during rumination a third contraction of the reticulum occurs (Fig. 3).

Figure 1. Cows with SCR neck sensor and Nedap Smarttag leg sensor.

Figure 2. Screenshot of SCR neck sensor output

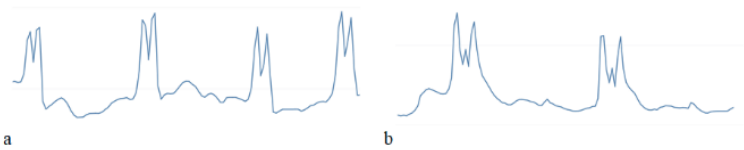

Figure 3.Pressure measured in the reticulum of a cow during eating (a) and rumination (b). During rumination, the typical extra peak is visible.

The used pressure measuring devices were not described in detail and no gold standard for measuring reticulorumen pressure is available. In Chapter 4, we study in detail such a pressure measuring device that can be used as a golden standard for measuring reticulorumen contractions. With the development of wireless sensors in the dairy field, a few orally applied sensors migrating to the reticulum became available measuring pH or temperature. A combined single rumen bolus could potentially include sensors detecting activity, temperature, pressure, and heartbeat (Knight, 2020). Chapter 5 describes the development of a wireless sensor bolus measuring reticular pressure and temperature (Fig. 4).

Figure 4. Wireless sensor bolus to measure reticulorumen contractions in a cow.

OBJECTIVES AND CONTENT OF THIS THESIS

The main objective of this thesis is to gain more insight into the opportunities of using sensor data to learn more about important health and welfare aspects of dairy cows. Neck and leg sensors are commonly attached to the cows on commercial dairy farms, providing the farmer alerts for health, for being in heat, and for the moment of calving. The sensors that are used monitor behavioural parameters of the cows 24/7 and generate a lot of data about each individual cow and the whole dairy herd. This data can be helpful for observing behaviour in detail without disturbing the animal. In most research studies that involve cow sensor data, the focus is on detecting specific diseases, but in this thesis, we focus on healthy cows (in any case not selected based on specific health problems). In the first two chapters we use data of commercially available validated sensors attached to the neck and leg that collect data about ruminating, eating, lying, walking, and standing time. In addition, we investigate the opportunities of the use of reticulorumen pressure data obtained from a new sensor still in development to measure important cow behaviours.

Chapter 2: “Heat stress in a temperate climate leads to adapted sensor-based behavioural patterns of dairy cows”. With global warming, heat stress increasingly affects the health and welfare of cows also in maritime and temperate climates. Most previous research on heat stress has focused on (sub)tropical climates. In this chapter, we use daily leg and neck sensor data to study the relation between the daily time budget of dairy cows and climatic variables in the Netherlands.

Chapter 3: “The effects of cow introductions on milk production and behaviour of the herd measured with sensors”. This chapter focuses on using data obtained from commercially available neck and leg sensors to study cow behaviour. Frequent introductions of fresh heifers and re-introductions of previously dried-off cows into the milking herd is a common management practice. Those regroupings potentially disturb the social hierarchy and behaviour of individuals with negative effects on welfare and productivity. Negative effects of regroupings on the introduced animals are reported in other studies. However, little is known about the effects on lactating cows in the herd. We address that gap in Chapter 3. The effect of cow introductions on sensor data of animals already in the herd is studied by analysing raw sensor data from neck and leg sensors and combining these data with milk data.

Chapter 4: ”Pressure measurement in the reticulum to detect different behaviours of healthy cows”. In this chapter, we study the relation between reticulorumen contractions and the monitoring of cow behaviours. We investigate if a purpose-built pressure measuring device can detect the known contraction patterns in the reticulorumen of rumen-fistulated cows and we monitor the contractions during rumination, eating, drinking, and sleeping.

Chapter 5: “Proof of principle and potential use of a bolus measuring reticular contractions and temperature”. In this chapter, we study the reticular contraction pattern during rumination, eating, resting, sleeping, urinating, and mooing with a wireless sensor bolus that is in development for this purpose. Also drinking behaviour is studied in detail with the bolus measuring temperature in the reticulum. We investigate a wireless single sensor bolus as a potential measuring device to monitor many parameters and combine data about the forestomach environment and behaviour of the animal.

Chapter 6: “General discussion”. In this chapter we give an overview of the most relevant results of this thesis and relate these results to opportunities for research and the dairy industry.

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Chapter 2

Heat stress in a temperate climate leads to adapted sensor-based behavioural 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 behaviour 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 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 3 data sets: (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°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 = (≥28°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 ≥72). To associate daily mean temperature and THI with sensor-based behavioural 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 analysed. 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 behaviour 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 behavioural adaptation to heat stress in dairy cows.

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 (Hansen, 2007; Kadzere et al., 2002). 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 because 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., 2012a).

Higher ambient temperatures during the dry period result 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 foetal 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 behaviour. By decreasing lying time and increasing standing time, cows expose a greater surface area to the air to cool as much as possible (Allen et al., 2015; Schütz et al., 2011). Increased standing time is associated with a higher risk for lameness (Cook et al., 2007; Cook and Nordlund, 2009). As the THI increases, DMI decreases, resulting in reduced milk production (Bohmanova et al., 2007; West, 2003). 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 (National Academies of Sciences and Medicine, 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 (Moretti et al., 2017; Soriani et al., 2013). 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 behaviour) 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 (Hut et al., 2019; Huzzey et al., 2005; Neave et al., 2017), 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 the 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 AMS and had no pasture access. On the other 5 farms, cows were milked with a 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 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, all 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.

Table 1. Details of the eight farms in this study, including the number of dairy cows, the type of milking system, AMS for automatic milking system and CMS for conventional milking system and the start date and end date of data collection.

Farm no.	No. of dairy cows	Milking system AMS/CMS	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

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., 2018a). 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.

Study Design

Sensor data were provided by Nedap Livestock Management (Groenlo, the Netherlands) per behavioural parameter in minutes per 15-min time block. These data were summed to create daily totals for each of the 5 behavioural 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: 200 d (DIM = 5): late lactation. Parity had 8 levels: 1, 2, 3, 4, 5, 6, 7, and ≥8. The individual cow data were divided into 3 data sets: (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 at various locations. For each farm, the recordings of the nearest weather station were used. The THI was calculated following the National Research Council (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 (Brügemann et al., 2012a; Kadzere et al., 2002). 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 ≥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 (≥28°C).

Grouping of temperature and THI values per increments of 3 and 5, and minimum and maximum temperature and THI values were analysed 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 analysed using generalized linear mixed models.

The temperature (mean/maximum) or THI (mean/ maximum) variable was included as the main effect, with a reference category 0 to 12°C for temperature and 30 to 56 for THI. All behaviours 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 were analysed 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 lsmeans (Lenth, 2016).

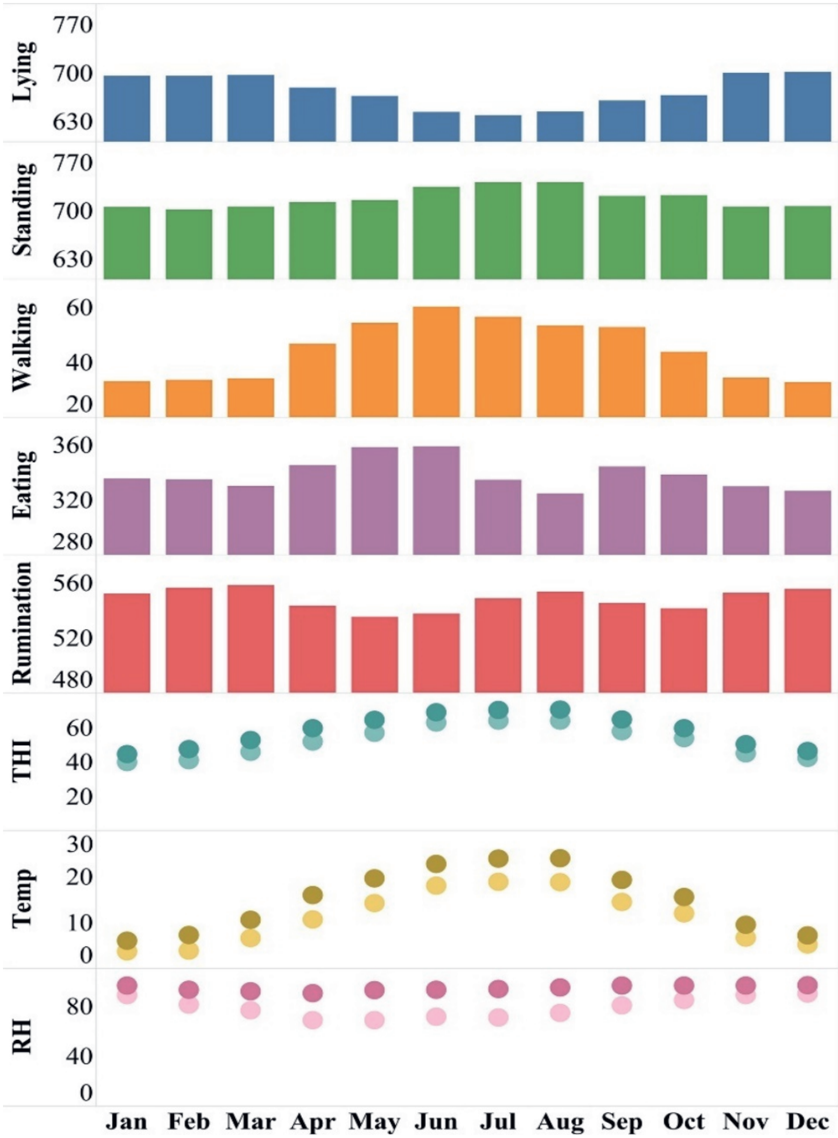

Figure 1. Overall sensor and climatic ta from 2017 to 2020 in means per month on 8 dairy farms in the Netherlands. Sensor data of 4,345 cow lactations consist of daily lying, standing, walking, eating, and rumination time in minutes per day. Climatic data consist of mean and maximum daily temperature-humidity 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.

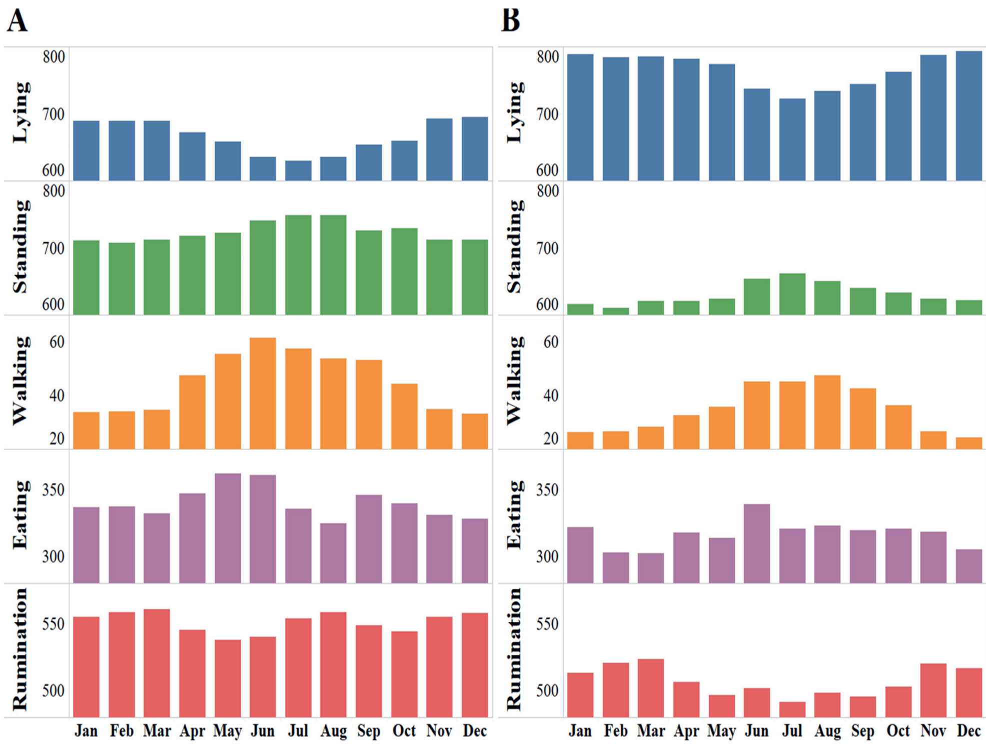

Figure 2. Daily sensor data from 2017 to 2020 of daily lying, standing, walking, eating, and rumination time in average minutes per day per month on 8 dairy farms in the Netherlands. Overview of monthly data of (A) lactating cows (n = 4,345 cow lactations); and (B) dry cows (n = 3,676 dry periods) is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

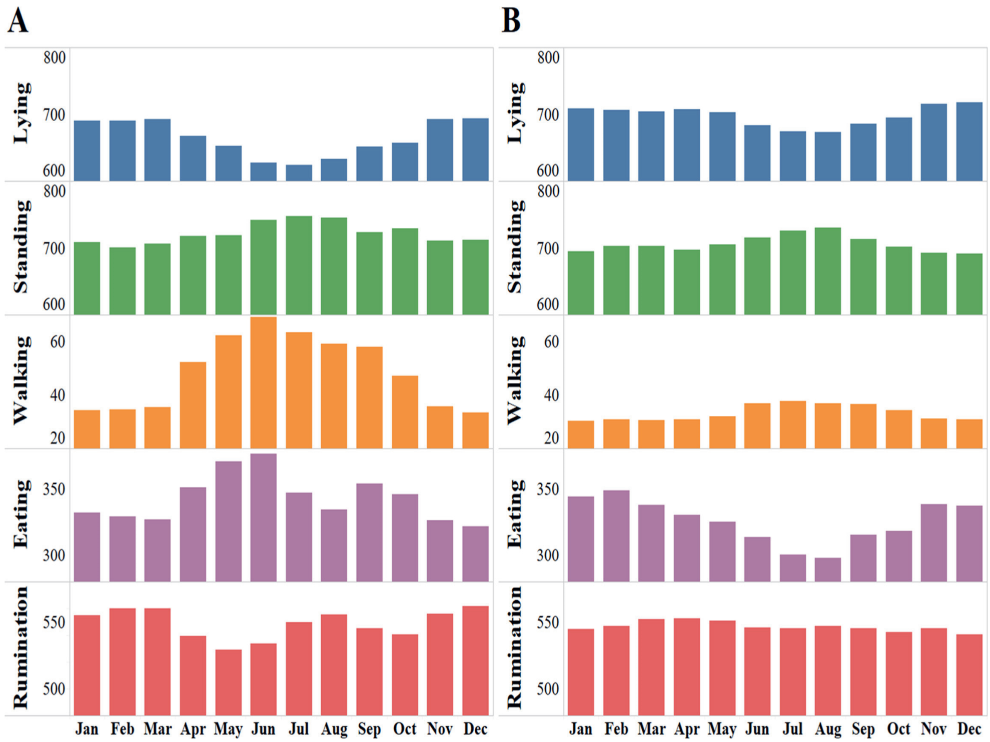

Figure 3. Daily sensor data from 2017 to 2020 of daily lying, standing, walking, eating, and rumination time in average minutes per day per month on 8 dairy farms in the Netherlands. Overview of monthly data of (A) cows with pasture access (n = 2,847), milked with a conventional milking system (CMS);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

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 Fig. 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 Fig. 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 had 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 (Fig. 3A) and lactating cows on AMS farms (Fig. 3B), as these 2 farm types differed in pasture access during the warm period of the year.

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 Fig. 4 and 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 ≥ 72 (Fig. 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 ≥72 (Fig. 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 ≥72 (Fig. 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 standing (Figure 4D, E, F). The standing time increased when the daily mean THI increase 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 (Fig. 4G, H, I). The AMS and dry cows only showed decreased walking time at THI ≥72, the highest THI class (Fig. 4H, I).

On average, lactating cows on CMS farms spent 323 min/d eating and those on AMS farms spent 348 min/d eating (Fig. 5A, B); dry cows spent 374 min/d eating (Fig. 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 totalled 75 min/d less time eating when THI was ≥72 (Fig. 5A).

Lactating cows housed on AMS farms spent 4 min/d less time eating when the average daily THI was ≥56, and 70 min/d less when the THI reached ≥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 ≥72 (Fig. 5C). Lactating cows on CMS farms spent around 573 min/d ruminating. Beginning at a THI of 68, their rumination time increased by 12 min/d beginning at a THI of 68 and increased by 14 min/d at a THI ≥72 (Fig. 5D). This is in contrast with lactating cows on AMS farms (542 min/d), where rumination decreased 9 min/d beginning at a THI ≥72 (Fig. 5E). In contrast, in dry cows (559 min/d), a decrease in rumination time of 5 min/d was observed, beginning at a THI of 56, and a decrease of 9 min/d occurred at a THI of ≥72 (Fig. 5F).

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 categorical classifications for temperature and THI showed similar effects as the presented results (results not shown).

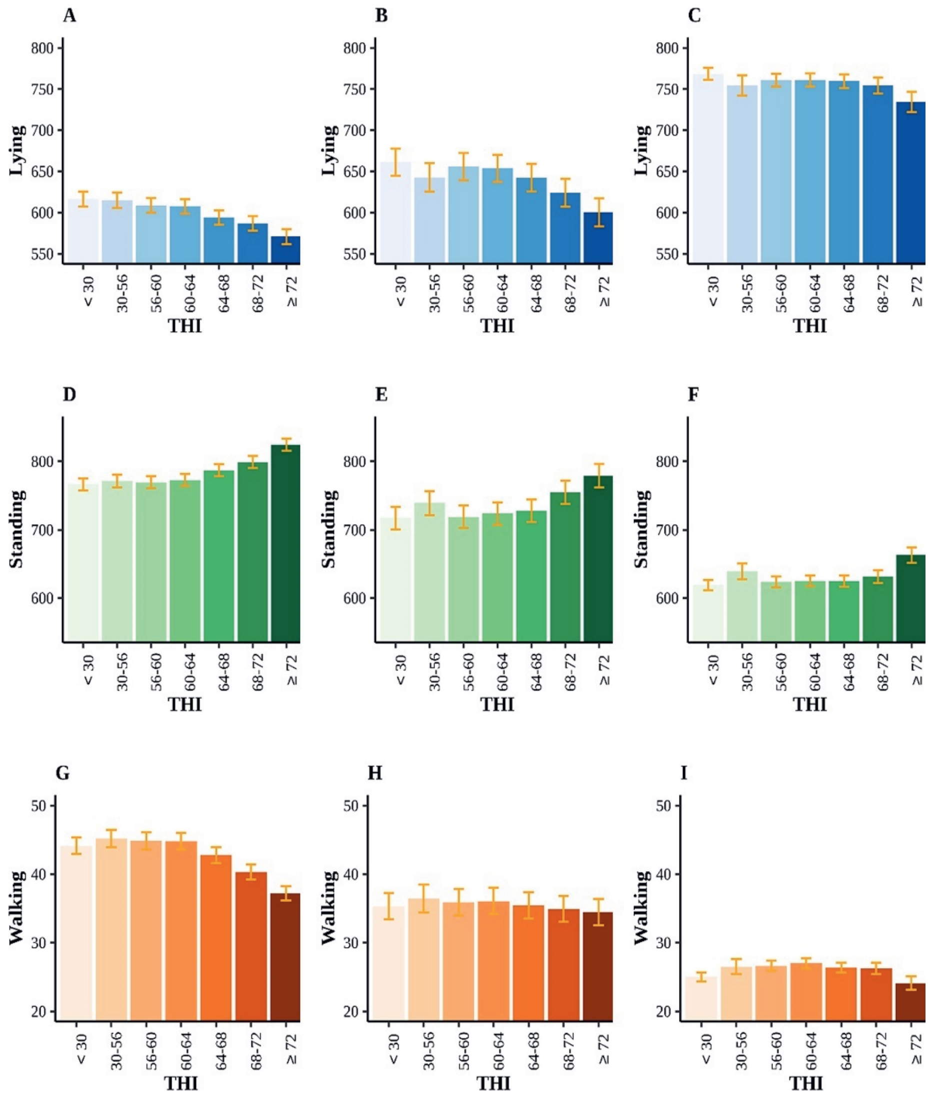

Figure 4. Predicted least squares mean with 95% confidence intervals of daily lying time (A–C), standing time (D–F), and median walking time (G–I) in minutes per day plotted against daily mean temperature-humidity index (THI). Left-hand panels present lactating cows on farms with conventional milking 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: ≥72. Colours darken as THI values increase.

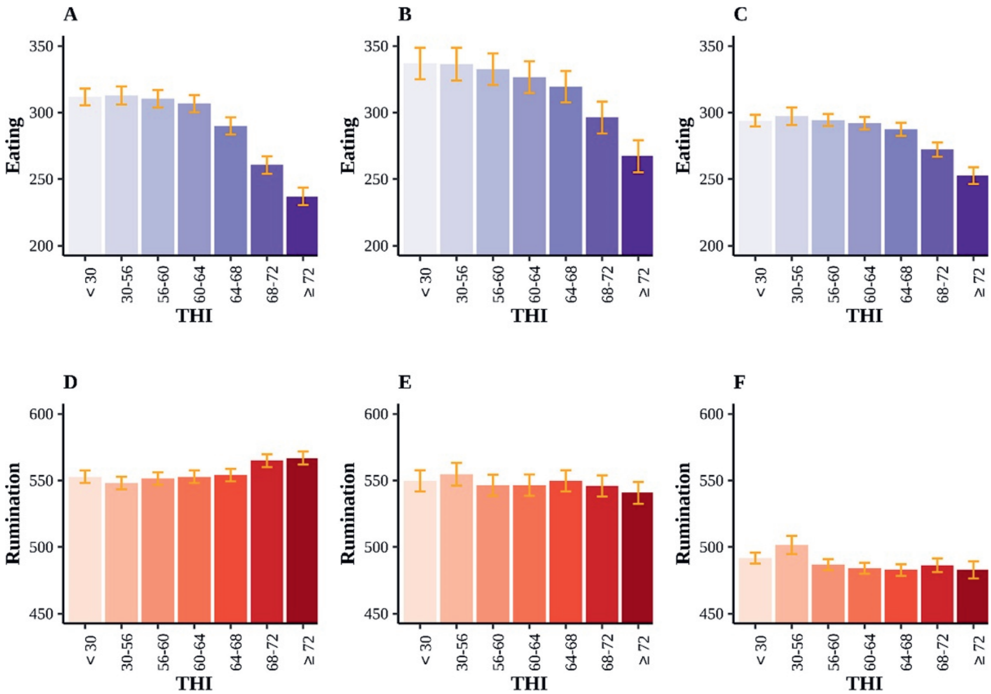

Figure 5. Predicted least squares mean with 95% confidence interval of daily eating time (A–C) and rumination time (D–F) in minutes per day plotted against daily mean temperature-humidity index (THI). Left-hand panels present lactating cows on farms with conventional milking systems (CMS, n = 2,847 cow 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: ≥72. Colors darken as THI values increase.

Table 2. Associations from three multivariable models between daily lying time (min/day) of cows from CMS farms with pasture access, cows from AMS farms without pasture access and dry cows from both farms without pasture access, with mean daily THI groups and mean daily ambient temperature (°C) groups. All results are corrected for covariates parity, herd, days in milk (lactating cows), year and day effects.

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

Table 3. Associations from three multivariable models between daily standing time (min/day) of cows from CMS farms with pasture access, cows from AMS farms without pasture access and dry cows from both farms without pasture access, with mean daily THI groups and mean daily ambient temperature (°C) groups. All results are corrected for covariates parity, herd, days in milk (lactating cows), year and day effects.

Standing time	Intercept	95% CI	Fixed effects		Estimate	95% CI
CMS	773	754; 792	THI	< 30	5	2; 7
				30-56	Ref.	
				56-60	3	2; 4
				60-64	6	5; 7
				64-68	20	19; 22
				68-72	33	31; 34
				≥ 72	58	55; 60
	773	754; 793	Temperature (°C)	< 0	4	3; 6
				0-12	Ref.	
				12-16	1	0; 2
				16-20	9	9; 10
				20-24	27	25; 28
				24-28	49	46; 51
				≥ 28	51	47; 56
AMS	727	702; 753	THI	< 30	22	16; 28
				30-56	Ref.	
				56-60	2	0; 4
				60-64	7	4; 9
				64-68	11	8; 13
				68-72	38	34; 41
				≥ 72	62	57; 67
	727	701; 753	Temperature (°C)	< 0	18	15; 20
				0-12	Ref.	
				12-16	1	-1; 3
				16-20	7	5; 10
				20-24	25	23; 28
				24-28	59	54; 64
				≥ 28	59	48; 69
Dry	680	663; 698	THI	< 30	20	11; 29
				30-56	Ref.	
				56-60	5	1; 8
				60-64	6	2; 10
				64-68	6	1; 11
				68-72	12	6; 19
				≥ 72	44	34; 54
	679	662; 696	Temperature (°C)	< 0	16	10; 22
				0-12	Ref.	
				12-16	3	-1; 7
				16-20	6	2; 11
				20-24	9	4; 15
				24-28	22	13; 30
				≥ 28	63	47; 80

Table 4. Associations from three multivariable models between daily walking time (ratio) of cows from CMS farms with pasture access, cows from AMS farms without pasture access and dry cows from both farms without pasture access, with mean daily THI groups and mean daily ambient temperature (°C) groups. All results are corrected for covariates parity, herd, days in milk (lactating cows), year and day effects.

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

Table 5. Associations from three multivariable models between daily eating time (min/day) of cows from CMS farms with pasture access, cows from AMS farms without pasture access and dry cows from both farms without pasture access, with mean daily THI groups and mean daily ambient temperature (°C) groups. All results are corrected for covariates parity, herd, days in milk (lactating cows), year and day effects.

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

Table 6. Associations from three multivariable models between daily rumination time (min/day) of cows from CMS farms with pasture access, cows from AMS farms without pasture access and dry cows from both farms without pasture access, with mean daily THI and mean daily ambient temperature (°C) groups. All results are corrected for covariates parity, herd, days in milk (lactating cows), year and day effects.

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

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 behaviour. With increasing daily temperature and THI, cows spent 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 behaviour of preference for dairy cows (Munksgaard et al., 2005). Reduced lying time (7 min/d less) was observed between 12°C 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 ≥28°C, and to 48 min/d less when the THI was ≥72. In a trial of 6 d, an increase in THI from 68.5 to 79 resulted in a decrease in lying time of 3 h/d (Nordlund et al., 2019). This is consistent with the 3 h/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 parlour. 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; Brügemann et al., 2012b; Rhoads et al., 2009). 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 of 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 behavioural parameters because one negative effect of heat stress is a 2-d delayed decrease in milk production (West, 2003).

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 (Herbut and Angrecka, 2018; Mader et al., 2006; Polsky and von Keyserlingk, 2017). 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 6 h/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., 2022). 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 ≥72 occurred less frequently during the 4-yr 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 ≥28°C occurred even less frequently due to the relatively constant high humidity. Furthermore, 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.

CONCLUSION

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 behavioural adaptation to heat stress of dairy cows.

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Chapter 3

The effects of cow introductions on milk production and behaviour of the herd measured with sensors

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ABSTRACT

This research paper addresses the hypothesis that cow introductions in dairy herds affect milk production and behaviour of animals already in the herd. In dairy farms, cows are commonly regrouped or moved. Negative effects of regroupings on the introduced animals are reported in other studies. However, little is known about the effects on lactating cows in the herd. In this research a herd of 53 lactating dairy cows was divided into two groups in a cross-over design study. 25 cows were selected as focal cows for which continuous sensor data were collected. The treatment period consisted of replacing non-focal cows three times a week. Many potentially influencing factors were taken into account in the analysis. Replacement of cows in the treatment period indeed affected the focal animals. During the treatment period these cows showed increased walking and reduced rumination activity and produced less milk compared to the control period. Milk production per milking decreased in the treatment period up to 0.4 kg per milking on certain weekdays. Lying and standing behaviour were similar between the control and the treatment period. The current study suggests that cow introductions affect welfare and milk production of the cows already in the herd.

INTRODUCTION

Cows live in complex hierarchical social structures. In many dairy farms, cows are kept in loose housing systems where they form relatively stable herds. A subset of the herd, consisting of youngstock and dry cows, is usually housed separately. This results in frequent introductions of fresh heifers and re-introductions of previously dried-off cows into the milking herd, while other cows leave for dry-off, health issues or culling. Repetitive regrouping of animals is, therefore, a common management practice (Bøe and Færevik, 2003). Some dairy farms introduce multiple animals at once, while others introduce one animal at a time. Previous studies showed that introducing heifers as a pair to the group diminished the anticipated negative effects of regrouping on the introduced animals (Gygax et al., 2009; Neisen et al., 2009; O'Connell et al., 2008). We hypothesize, however, that all regroupings may disturb the social hierarchy and behaviour of individuals already in the herd with negative effects on welfare and productivity.

Effects of regrouping on milk yield, feed intake, rumination time, lying time, lying bouts and standing bouts have been studied before (Brakel and Leis, 1976; Hasegawa et al., 1997; Schirmann et al., 2011; Smid et al., 2019; Von Keyserlingk et al., 2008). Most of these studies reported the effects of regrouping on the introduced animals. Schirmann et al. (2011) examined the short-term effects of regrouping on dry cows already in the herd, but only regarded a limited time span of 8 d. The effects of regrouping found in other studies lasted in duration from hours to weeks (Brakel and Leis, 1976; Hasegawa et al., 1997; Raussi et al., 2005; Von Keyserlingk et al., 2008). Interpretation is challenging, as changes could also be affected by other factors, like oestrus and weather conditions (Reith et al., 2014; Stone et al., 2017). Effects of regrouping on the behaviour of individual cows may be highly variable (Byskov et al., 2015; Ito et al., 2009; Schrader, 2002).

The more recent studies include sensor data to monitor health and behaviour (Halachmi et al., 2019; Leliveld and Provolo, 2020). The use of sensor data allows non-invasive, low work intensive analyses of a diverse range of behavioural aspects. Moreover, sensors allow effects to be studied continuously, facilitating detection of effects probably indiscernible in short observation periods.

The current study investigates the effects of regrouping on behaviour and milk production of animals in the receiving herd. Data were collected using sensors and were compared between a treatment period, when new cows were introduced, and a control period. To be able to detect even subtle changes many potentially influencing factors were taken into account.

MATERIALS AND METHODS

Experimental design

The study was performed from April 3, 2017, until May 29, 2017, in a herd used for teaching purposes at the Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands. Animal procedures were approved by and in accordance with the guidelines of the Dutch Committee of Animal Experiments.

A herd of 53 lactating dairy cows was divided into two groups by stratified randomization in order to create two groups (Group A and Group B) that were comparable with respect to average days in milk and parity. An additional eight cows that were not previously exposed to any of the other animals were housed separately.
Group A and B were managed as separate groups and enrolled in a cross-over study of two consecutive experimental periods of three weeks. Both periods were preceded by a one-week run-in period followed by three weeks of data collection. During the run-in period, cows were familiarized with the experimental design to optimize similarity between both test periods. In the first experimental period, Group A received the replacement treatment and Group B acted as control. In the second period this was reversed. The treatment consisted of replacing three cows twice a week and one cow, not previously exposed to any of the other animals, once a week. For details see online Supplementary Materials and Methods and Supplementary Fig. S1. Data were collected only from a subset of 25 cows, denoted as focal cows. The non-focal cows were used for replacements (Fig. 1). The focal animals were not pregnant and selected based on breed (Holstein Friesian) and health (no evident signs of lameness or other health impairments, somatic cell count <200 000 cells/ml). Five rumen-fistulated cows were excluded as focal animals.

Animals and husbandry

Group matching of the focal animals at the start of the experiment was based on days in milk (DIM) and parity. This resulted in two groups with comparable DIM with mean 97 (SD 30) and 98 (SD 34) and parity with mean 2.75 (SD 0.97) and 2.77 (SD 1.17) for the focal animals in group A and group B, respectively. The same matching criteria were used for grouping of the non-focal animals at the beginning of both test periods.

Group A and B were housed in separate but identical free stall housings. Both free-stall housings had slatted floors equipped with an automatic scraper and 27 cubicles with soft rubber mats. There was always more than one feeding and lying space available for each cow.

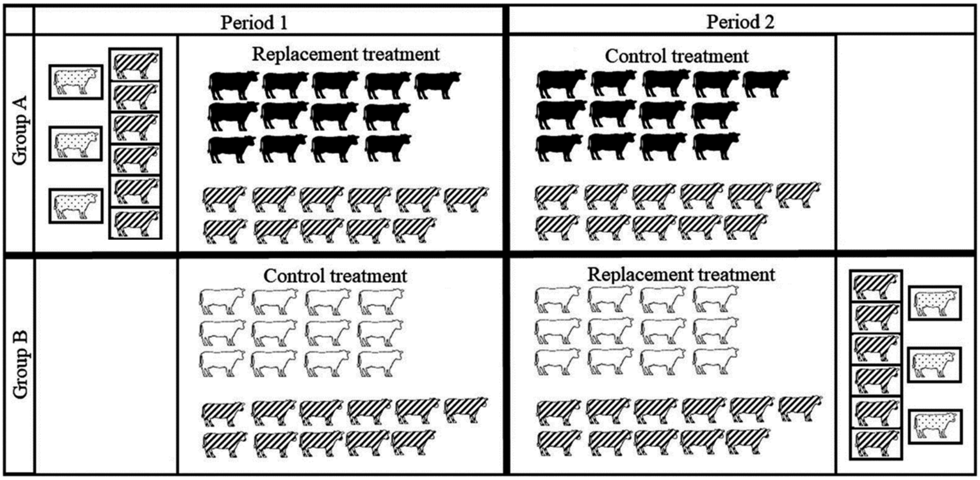

Figure. 1. Schematic overview of cows in the different stables during the experiment. An experiment of 2 × 3 weeks with a cross-over design where the effects of cow replacements on the remaining focal cows were studied. Spotted cows were non-focal animals not previously exposed to any of the other animals. Striped cows in the figure represent non-focal animals that had been used for replacements in the treatment period. Focal cows were used for data collection. Black cows represent focal animals in Group A and white cows represent focal animals in group B. Data was collected from focal animals not used for replacements.

The six non-focal cows from the animal replacement treatment group and the eight cows not previously exposed to any of the other animals were housed in an adjacent tie-stall barn. There was no visual, auditory or physical contact between those cows and the groups in the free-stall housing.

During the experiment, only the non-focal cows from group A and B were sometimes taken out of the group, individually, for a few hours a day for regular teaching activities such as physical examination, rectal examination and anatomy classes. The same small number of non-focal animals in both groups was taken out at the same time in both groups. All animals were accustomed to frequent handling by students. These teaching activities were comparable and equally balanced over both periods and between both groups.

The cows were milked twice a day in the same milking parlour (5 × 2 herringbone) between 06:00–08:00 h and 18:00–20:00 h. Group A was always milked first.
The diet consisted of 4.5 kg DM/day maize silage and ad libitum wilted grass silage at the feeding gate after morning and afternoon milking. A protein-rich supplement was supplied on top of the maize silage. One kg of concentrates was fed during each milking. Cows were provided with additional concentrates depending on milk production level by means of a computerized feed station. Fresh water was supplied ad libitum.

Measurements and data collection

Focal cows were equipped with a Smarttag leg sensor (Nedap livestock management, Groenlo, the Netherlands) The Smarttag leg sensors were strapped to each cow's left front leg just above the fetlock joint on March 31, 2017. The Smarttag leg sensor collected, within 15-minute time blocks, the number of minutes the cow spent lying down, standing and walking, and the number of transitions from lying down to standing. All data streams were synchronized to the daily clock time. Unrestricted movement started after unlocking the feed gate after milking and lasted until the gathering of the cows for the next milking. Data of 15-minute time blocks were averaged on an hourly basis to obtain one parameter for the day and one for the night. This resulted in 42 measurements in the first and second experimental period each for standing time, walking time, lying time, and number of lying-to-standing transitions for each focal cow.

Additionally, eight out of the 13 focal cows in group A and seven out of the 12 focal cows in group B were also equipped with an HR Tag neck sensor (SCR Dairy, Netanya, Israel). There were 15 neck sensors available for this research. The HR-Tag neck sensor was positioned on a collar behind the left jaw of the cow; the sensor monitored the number of minutes the cow spent on rumination in 2-hour time blocks. The 15 focal cows were equipped with sensors on March 31, 2017, and data were collected during the whole study period. Average rumination time per hour was calculated per day and per night as described for the Smarttag sensor, resulting in 42 measurements in both periods for each of the 15 focal cows. To assign rumination time within the free time, each 2-hour data time block of the neck sensor was reproportioned into 15-minutes blocks. All reported measurements are for unrestricted movement (free time).
A video camera system recorded the cows continuously during the whole experiment. Oestrous behaviour was determined by observations daily at 04:00–05:00 h, 10:00–11:00 h, 16:00– 17:00 h, and 22:00–23:00 h. Oestrous behaviour of a cow was defined as allowing another cow to mount, or when a cow attempted to mount another cow. Because a cow can already be restless during the hours before oestrus, the cow was defined to be in oestrus the day on which oestrous behaviour was observed, as well as the day before. Twelve cows in Group A vs. 13 cows in Group B were detected to be in oestrus during the total experiment.

Individual milk yield (kg) was recorded at each AM and PM milking in the milking parlour by the milking machine, with a precision of one decimal point.

Temperature and relative humidity were recorded hourly by the Dutch National Weather Service (KNMI) throughout the experiment at a location 2 km from the research location. The atmospheric temperatures ranged from −2.9°C to 31.1°C during the experiment. Heat and cold stress were defined based on the Temperature Humidity Index (see online Supplementary Materials and Methods).

At the start of the experiment, the categories for days in milk were classified as follows: 0–60 d (DIM = 0), 61–120 d (DIM = 1), 121–200 d (DIM = 2), 201–305 d (DIM = 3) and >305 d

(DIM = 4). The parity of each cow was classified into three groups: first, second, and third or higher.

Two focal cows did not provide complete data for the experiment, because of illness. One became lame in the second run-in period, and the other showed colic in the second week of the second period.

On 12 occasions milking data for an individual cow was missing due to technical issues; we used the average milk production per milking in the valid measurements of these cows as estimate for the missing data.

Statistical analysis

Cows were the sampling units in this research and individually randomized over both groups. In this study, data was collected from individual cows and differences in both groups were minimized; therefore, cow was taken as the experimental unit.

Average lying time, the median of log-transformed walking time, average standing time, and average rumination time, all in minutes per hour free time, were analysed using linear mixed effects models. Treatment was included as main effect and corrected for design related aspects such as week (1, 2, 3), group (A, B), period (1st, 2nd), day or night, weekday, and weekday-week interaction as fixed effects, when appropriate. As the composition of the groups changed on particular weekdays, we also included every day of the week as a weekday-treatment interaction.

Cow-related aspects such as heat or cold stress, parity, DIM, milk production (kg milk per milking), and daily presence of oestrus were included as fixed effects. Cow ID was included as a random effect to correct for multiple observations per cow. Similarly, the correlations between measurements are expected to depend on the day in the three-week period. Therefore, 'Day' (1–21) was included as a potential random effect but was checked to be included for each model beforehand. The treatment effect on milk production was analysed using a similar linear mixed effect model but without the correction for milk production as a cow related fixed effect. For all models, residuals were plotted to check for normality.

The average lying-to-standing transitions per hour were analysed using a generalized linear mixed model with a Poisson distribution, with random and fixed effects as before. A Poisson distribution is used because the outcomes are non-negative integer values that count the number of events. For the effects in the reduced models, 95% profile (log-) likelihood confidence intervals were estimated. When the treatment-weekday interaction was in the reduced model, a nested model was used to estimate the treatment effect for each day of the week. Data were analysed using R version 3.5.1 (2018-07-02) library lme4 (R Core Team, 2018). Akaike's information Criterion was used for model reductions where all variables could be dropped (Burnham and Anderson, 2002). When the treatment effect did not remain in the final reduced model, the treatment effect was forced in the model to achieve an estimate with a confidence interval.

RESULTS

Considering restricted and unrestricted time together, cows spent on average 8.5 h (SD 2.0) per day ruminating, 9.5 h (SD 3.2) lying down, 13.2 h (SD 3.4) standing and 0.7 h (SD 0.2) walking. The mean number of lying-to-standing transitions per day was 6.7 (SD 2.2). The average milk production in group A was 29 kg/day and in group B 31 kg/day. Online Supplementary Fig. S2 illustrates the variation of the sensor data and the milk production per milking over the experimental period for all focal cows. The following model results are based on time blocks with unrestricted animal movements only (see online Supplementary file for the full models).

Behavioural data

The mixed model analysis showed no difference in average lying time per hour between the treatment period and the control period (online Supplementary Table S1). The hour was higher during the night than during the day. Cows in oestrus spent on average less time lying per hour, as did cows during heat stress. The average standing time per hour was not different in the treatment and control period (online Supplementary Table S2). The average standing time was only affected by day or night, with a longer average standing time during the day.

For the lying-to-standing transitions, the full Poisson model could not be fitted, therefore, we started with a smaller model and excluded the following variables beforehand: treatment-weekday interaction, weekday-week interaction, milk production, DIM, and parity. The average number of lying-to-standing transitions per hour were not different between the treatment and control periods (online Supplementary Table S3). In general, transitions from a lying to a standing position occurred less frequently during the daytime than at night. Furthermore, no relevant effects on the average number of lying-to-standing transitions per hour were found for oestrus, heat or cold stress, period, group, weekday.

For the median walking time, the treatment-weekday interaction remained in the reduced model. The treatment effect for each day of the week was estimated with a nested version of the model. The median walking time of cows on Saturday and Sunday was increased in the treatment period compared to the control period (Table 1). During the weekend the median walking time per hour in the treatment period was 1.14 times higher than in the control period. The median walking time per hour was not different between the treatment and control group on the other days of the week. Overall, the median of the walking time was higher during the day than during the night. Cows in oestrus walked for longer periods of time. Cows walked more on the days that they experienced heat stress and milk production was also positively related with the median of the walking time per hour.

Table 1. Variables in the final model of walking time (*N* = 25) in minutes per hour during the unrestricted time period

Model	Intercept	(95% C.I.)	Fixed Effects	Estimate (ratio)	(95% C.I.)
Walking time	0.83	(0.65 to 1.19)	Treatment Monday	0.95	(0.88 to 1.04)
(min/h)			Treatment Tuesday	1.03	(0.95 to 1.12)
			Treatment Wednesday	1.08	(0.99 to 1.17)
			Treatment Thursday	1.08	(1.00 to 1.18)
			Treatment Friday	1.00	(0.92 to 1.09)
			Treatment Saturday	**1.14**	**(1.05 to 1.24)**
			Treatment Sunday	**1.14**	**(1.04 to 1.25)**
			Oestrus	**1.58**	**(1.47 to 1.70)**
			Day *vs.* Night	**1.15**	**(1.12 to 1.19)**
			Milk production	**1.02**	**(1.01 to 1.04)**
			Heat stress	**1.12**	**(1.07 to 1.18)**
			Monday *vs.* Sunday	**1.21**	**(1.07 to 1.36)**
			Tuesday *vs.* Sunday	**1.08**	**(1.04 to 1.22)**
			Wednesday *vs.* Sunday	1.04	(0.92 to 1.17)
			Thursday *vs.* Sunday	1.01	(0.90 to 1.14)
			Friday *vs.* Sunday	1.05	(0.93 to 1.18)
			Saturday *vs.* Sunday	0.92	(0.82 to 1.03)
			Week 2 *vs.* 1	1.09	(0.98 to 1.21)
			Week 3 *vs.* 1	**1.14**	**(1.01 to 1.29)**

Effects are shown for the focal cows remaining in the herd in a 2 × 3-week trial with a cross-over design where other cows were replaced. Walking time was corrected for weekday-week interaction and random effects included were 'cow' and 'day'. The treatment effect remained in the model during the model reduction steps. The model was nested for treatment. Variables are in bold excluding the H_0-value in the 95% confidence interval.

Rumination data

The average rumination time per hour of unrestricted movement was 28 min. Results of the mixed model analysis demonstrated that the average rumination time in the treatment period was one minute per hour more on Saturday and one minute per hour less on Thursday compared to the control period on these days (Table 2). During the day, the average rumination time was five minutes per hour lower compared to the night; when a cow was in oestrus the rumination time was four minutes lower. The increase of rumination time in the treatment period on Saturday was unexpected and because the data were based on only fifteen cows, we estimated the mean and standard deviation of the rumination time for individual cows. One cow had a large standard deviation compared to the others (data not shown). When the data were analysed without this specific cow, an overall treatment effect of one minute less rumination per hour remained (online Supplementary Table S4). '

Table 2. Variables in the final model for rumination time (N = 15) in minutes per hour during the unrestricted time period

Model	Intercept	(95% C.I.)	Fixed Effects	Estimate (ratio)	(95% C.I.)
Rumination time	27.54	(26.51 to 28.57)	Treatment Monday	−0.92	(−1.88 to 0.05)
(min/h)			Treatment Tuesday	0.38	(−0.58 to 1.35)
			Treatment Wednesday	−0.43	(−1.40 to 0.53)
			Treatment Thursday	**−1.09**	**(−2.06 to −0.13)**
			Treatment Friday	0.09	(−0.87 to 1.06)
			Treatment Saturday	**1.04**	**(0.08 to 2.00)**
			Treatment Sunday	−0.53	(−1.49 to 0.44)
			Day *vs.* Night	**−5.11**	**(−5.48 to −4.75)**
			Oestrus	**−3.86**	**(−4.72 to −2.99)**
			Monday *vs.* Sunday	0.38	(−0.60 to 1.35)
			Tuesday *vs.* Sunday	0.52	(−0.45 to 1.50)
			Wednesday *vs.* Sunday	0.63	(−0.34 to 1.61)
			Thursday *vs.* Sunday	**1.24**	**(0.26 to 2.21)**
			Friday *vs.* Sunday	0.67	(−0.31 to 1.65)
			Saturday *vs.* Sunday	−0.16	(−1.14 to 0.82)
			Week 2 *vs.* 1	−0.13	(−0.58 to 0.31)
			Week 3 *vs.* 1	**−0.81**	**(−1.25 to −0.36)**
			Period 2 *vs.* 1	**−0.91**	**(−1.28 to −0.54)**

Effects are shown for the focal cows remaining in the herd in a 2 × 3-week trial with a cross-over design where other cows were replaced. The random effect included in the model was 'cow'. The treatment effect remained in the model on specific weekdays during the model reduction steps and the model was nested for treatment. Variables are in bold excluding the H_0-value in the 95% confidence interval.

Milk production

The average milk production per milking was lower in the treatment period compared to the control period. On Tuesday, Wednesday and Thursday the average milk production decreased by 0.32–0.37 kg per milking in the treatment group (Table 3).

A number of variables showed associations with milk production, corrected for the day of the week. Milk production during the PM milking was higher than during the AM milking. Heat stress had a negative effect on milk production and cows in oestrus produced 0.49 kg less per milking. The milk production was higher for higher parity cows, decreased every week and was therefore on average lower in the second period for all cows.

Table 3. Variables in the final model for milk production (*N* = 25) in kg per milking, cows milked twice a day

Model	Intercept	(95% C.I.)	Fixed Effects	Estimate	(95% C.I.)
Milk production	10.04	(8.36 to 11.72)	**Treatment Monday**	**−0.37**	**(−0.62 to −0.13)**
(kg/milking)			**Treatment Tuesday**	**−0.33**	**(−0.56 to −0.08)**
			Treatment Wednesday	−0.15	(−0.39 to 0.09)
			Treatment Thursday	**−0.32**	**(−0.56 to −0.08)**
			Treatment Friday	−0.04	(−0.28 to 0.20)
			Treatment Saturday	0.00	(−0.24 to 0.24)
			Treatment Sunday	0.21	(−0.05 to 0.46)
			Monday *vs.* Sunday	**0.51**	**(0.22 to 0.90)**
			Tuesday *vs.* Sunday	**0.42**	**(0.21 to 0.89)**
			Wednesday *vs.* Sunday	**0.36**	**(0.05 to 0.73)**
			Thursday *vs.* Sunday	**0.09**	**(0.22 to 0.90)**
			Friday *vs.* Sunday	**0.07**	**(0.24 to 0.92)**
			Saturday *vs.* Sunday	0.17	(−0.07 to 0.61)
			Heat stress	**−0.49**	**(−0.65 to −0.32)**
			Milking PM *vs.* AM	**0.27**	**(0.17 to 0.36)**
			Parity 2 *vs.* 1	**3.82**	**(2.08 to 5.56)**
			Parity 3 *vs.* 1	**5.41**	**(3.69 to 7.13)**
			Period 2 *vs.* 1	**−0.60**	**(−0.71 to −0.49)**
			Group 2 *vs.* 1	**1.27**	**(0.38 to 2.16)**
			Week 2 *vs.* 1	**−0.90**	**(−1.19 to −0.61)**
			Week 3 *vs.* 1	**−0.83**	**(−1.13 to −0.53)**

Effects are shown for the focal cows remaining in the herd in a 2 × 3-week trial with a cross-over design where other cows were replaced. The random effect included in the model was 'cow' and the rumination time was corrected for weekday-week interaction. The treatment effect remained in the model during the model reduction steps and the model was nested for treatment. Variables are in bold excluding the H_0-value in the 95% confidence interval.

DISCUSSION

The results demonstrate the effects of regrouping cows in a dairy herd, when continuously monitored by sensors during a six-week experimental period. For this research we used two validated sensors widely used in the Netherlands (Nielsen et al., 2018b; Schirmann et al., 2009). Most previous studies focus on the effects of regrouping on the introduced animals only. One of the few studies that also examined the effects on animals already in the herd, reports that short-term effects on regrouped dry cows are more severe compared to the animals already in the group. The introduced animals can experience stress not only because of the new group, but also because of moving to another environment (Schirmann et al., 2011).

In our study walking time increased slightly after regrouping, corroborating the results of other studies (Hasegawa et al., 1997). Remarkably, the walking time was higher on Saturday and Sunday in the treatment group compared to the control group, while during the other days of the week no significant treatment effect was found. The absence of a treatment effect during weekdays may be related to the use of non-focal cows for educational purposes, which could have blurred a subtly increased activity of the focal animals. Even though walking, standing and lying time add up to 15 min/h of unrestricted movement, relatively large changes in walking time could amount to only small changes in the other behaviours.

Lying time was not different between the treatment and control periods, which is in line with earlier work (Schirmann et al., 2011). Several other studies focusing on the introduced animals showed variable effects on the first day after regrouping or no (Hasegawa et al., 1997; Schirmann et al., 2011; Smid et al., 2019; Talebi et al., 2014; Von Keyserlingk et al., 2008). A possible explanation is that dairy cows are highly motivated to lie down. Lying even appears to have a higher priority than eating and social contact in both early and late lactation cows (Munksgaard et al., 2005). In agreement with Smid et al. (2019), lying behaviour does not appear to be a sensitive indicator of regrouping disturbance.

Similar to our results for the remaining animals, Smid et al. (2019) found no effect on standing bouts after regrouping, while others report decreased frequencies in the exchanged animals (Hasegawa et al., 1997). This may be explained by study differences as the latter results are based on physical observations every five minutes on only one day a week after regrouping took place (Hasegawa et al., 1997). In agreement with others, we found no difference in standing time after introducing new cows in a herd (Hasegawa et al., 1997; Smid et al., 2019). In our study a small overall treatment effect on rumination time was found only after exclusion of one animal with highly variable rumination time. As the results were sensitive to a single individual in the group of 15 cows with rumination sensors, strong conclusions are difficult to draw. Other studies with similarly low numbers of remaining animals found either no effect (Hasegawa et al., 1997) or a reduction of rumination time by 9% (Schirmann et al., 2011), both with a 50% replacement rate.

We found an average drop in milk production on Tuesday, Wednesday and Thursday during the treatment period. Grant and Albright (2001) summarized that the effects of regrouping appear to be variable at reducing milk production. Temporarily reduced average milk yield of a few regrouped animals has a lower economic impact than a reduced average milk yield of the entire herd. A small drop of average milk yield of all the animals could be a serious loss in kg milk for a commercial dairy farm.

The experiment was performed in a research and education centre, comparable with most other studies. Dairy cows spend in general 7–10 h/day ruminating and approximately 10 h/day lying and (or) resting (Grant and Albright, 2001) both of which are in line with our observations.

In our study, no regroupings or teaching took place on Saturdays and Sundays while a treatment effect on walking time was found on these days. This suggests that effects on walking time may last for at least two days, which is in line with previous reviews that report that changes in social cow behaviour after regrouping normally return to basic levels within 3 to 7 d (Grant and Albright, 2001) or days to weeks (Bøe and Færevik, 2003).

We assumed that when we re-introduced animals that were housed separately from the herd for a week, this would be enough to disturb the social hierarchy of the herd. There is no agreement on the question whether habituation might influence the effect of regrouping (Bøe and Færevik, 2003; Raussi et al., 2005; Sowerby and Polan, 1978). As in most other studies, we used cows familiar with repeated regroupings.

In our opinion, a cross-over design is favourable over comparison of individual animals, which vary considerably in behaviour and presumably in coping to potentially stressful situations. Furthermore, we utilized a large quantity of repeated observations of two groups and the data were analysed by models to be able to utilize all the information of the whole study period and to correct for many influences, resulting in robust estimates of subtle effects.

In contrast to others (Brakel and Leis, 1976; Hasegawa et al., 1997), our study did not take hierarchical positioning of animals into account when assessing the effects of regrouping because we focused on sensor data, not physical observations. Any possible compounding effect of rank on the effect of regrouping was ignored. To this end, the results may have been influenced by the dominance level of animals used in replacements. However, we assume that these effects were mitigated by the three-week period, the use of repeated replacements of randomly chosen animals, and the inclusion of animals new to the herd.

In conclusion, introduction of new cows into a herd negatively influenced sensor-based behaviour and milk production of focal animals already established in the herd. The effects consisted of a slightly increased walking time and decreased milk production and standing behaviour did not seem to be very sensitive indicators of the disturbance from regrouping. Therefore, the common cow introductions in modern dairy production might negatively influence the milk production as well as the welfare of all the cows present in a herd. This study suggests that regrouping research should not focus solely on behavioural effects on the regrouped animals but should also take the entire herd into account.

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SUPPLEMENTARY MATERIALS AND METHODS

In total, 13 cows were selected as focal cows in Group A and 12 were selected as focal cows in Group B. At the beginning of both periods, from the 28 non-focal cows 11 cows were assigned to Group A and 11 to Group B and six cows were selected for the replacement treatment. These six cows were housed in a different housing away from the other animals for at least one week before using them to replace cows of the herd. The focal animals remained in their respective groups throughout the entire experiment. Before the start of the experiment, the cows in group A and B belonged to the same herd. On the first Monday of the run-in week of both periods, the cows were assigned to Group A and B immediately after the morning milking and feeding. After the run-in period of one week and the three weeks of data collection of the first period, the one-week run-in period of the second experimental period started immediately.

The replacement treatment consisted of the following weekly actions: 1) On Monday three cows were replaced by three of the six cows housed in the other housing, 2) On Thursday one cow was selected and replaced by one cow not previously exposed to any of the other animals, 3) On Friday three cows were replaced by three of the six cows housed in the other housing. The selection of cows to be replaced at each action was as follows: replaced cows were randomly selected from all non-focal animals that were in the group for at least one week. The cows were re-introduced in the order that they were originally removed from the group. The cows replaced by a cow not previously exposed to any of the other animals (i.e., action on Thursdays) were excluded for the rest of the experiment and kept separate from the herd.

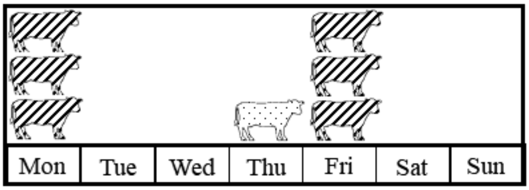
The Temperature Humidity Index (THI) was determined according to the formula:

$$\text{THI} = (1.8 * \text{temperature}(°\text{C}) + 32) - (0.55 - 0.0055 * \text{relative air humidity } (\%)) * (1.8 * \text{Temperature}(°\text{C}) - 26)$$

(Herbut and Angrecka, 2018)

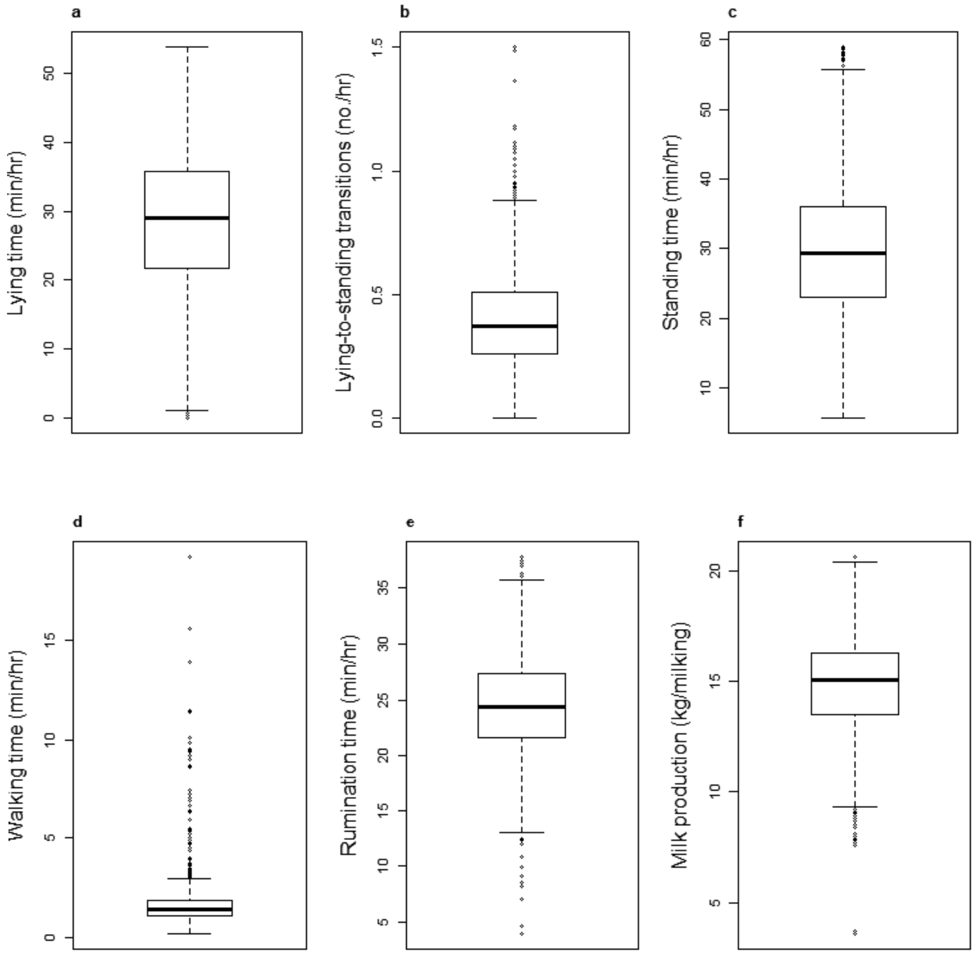
Cold stress was defined to occur at THI ≤ 40 and heat stress was defined to occur at THI ≥ 67 as follows (Brügemann et al., 2012). When THI exceeded 66 at least once during the day or night, all animals that day or night were defined as experiencing heat stress. Similarly, a day or night with a THI <40 at least once defined cold stress.

Supplementary Figure S1. Schematic overview of weekly cow replacement protocol in the treatment period in an experiment consisting of 2x3 weeks treatment weeks with a cross-over design. No cows were replaced in the control period. Spotted cows were not previously exposed to any of the other animals. Striped cows in the figure represent animals that had been housed separately from the other animals for at least one week. The cows in the figure represent the cows added to the group to replace the same number of animals at the specified days during the week.



SUPPLEMENTARY RESULTS

Supplementary Figure S2. Description of 24hr behaviour profile of 25 focal cows over the six weeks of observations. A: lying time, B: lying-to-standing transitions, C: standing time, D: walking time, E: rumination time (n=15) and F: milk production. Behavioural sensor data (A to E) are presented in minutes per hour during unrestricted movement. Boxplots are shown with median, interquartile range, which contains 50 % of the values, whiskers covering values up to 1.5 × interquartile range, and outliers (♦).



Supplementary Table S1. Variables in the final model for lying time (N=25) in minutes per hour during the unrestricted time period. Effects are shown for the focal cows remaining in the herd in a 2x3 week trial with a cross-over design where other cows were replaced. Random effects included were 'cow' and 'day'. The treatment effect was re-introduced into the final reduced model. Variables are in bold excluding the H_0-value in the 95% confidence interval.

Model	Intercept	(95% C.I.)	Fixed Effects	Estimate	(95% C.I.)
Lying time (min/hr)	30.20	(27.46 to 32.94)	Treatment	-0.04	(-0.73 to 0.65)
			Day vs Night	**-1.27**	**(-1.96 to -0.58)**
			Oestrus	**-2.02**	**(-3.59 to -0.44)**
			Heat stress	**-1.91**	**(-2.82 to -1.01)**

Supplementary Table S2. Variables in the final model for standing time (N=25) in minutes per hour during the unrestricted time period. Effects are shown for the focal cows remaining in the herd in a 2x3 week trial with a cross-over design where other cows were replaced. Random effects included were 'cow' and 'day'. Estimates are expressed as the median of the standing time. The treatment effect was re-introduced into the final reduced model. Variables are in bold excluding the H_0-value in the 95% confidence interval.

Model	Intercept	(95% C.I.)	Fixed Effects	Estimate	(95% C.I.)
Standing time (min/hr)	28.43	(25.76 to 31.11)	Treatment	-0.04	(-0.69 to 0.62)
			Day vs Night	**1.05**	**(0.39 to 1.70)**
			Heat stress	**1.79**	**(0.94 to 2.65)**

Supplementary Table S3. Variables in the final model for lying-to-standing transitions (N=25) in minutes per hour during unrestricted time periods. Effects are shown for the focal cows remaining in the herd in a 2x3 week trial with a cross-over design where other cows were replaced. The random effect included in the model was 'cow'. The treatment effect was re-introduced into the final reduced model. Variables are in bold excluding the H_0-value in the 95% confidence interval.

Model	Intercept	(95% C.I.)	Fixed Effects	Estimate (Ratio)	(95% C.I.)
Lying-to-standing (no./hr)	0.39	(0.33 to 0.45)	**Treatment**	**0.97**	**(0.93 to 1.01)**
			Day vs Night	**0.95**	**(0.91 to 0.99)**

Supplementary Table S4. Variables in the final model for average rumination time (N=14) in minutes per hour during unrestricted time periods. Effects are shown for the focal cows remaining in the herd in a 2x3-week trial with a cross-over design where other cows were replaced. The random effect included in the model was 'cow'. The treatment effect remained in the model during the model reduction steps. Variables are in bold excluding the H_0-value in the 95% confidence interval.

Model	Intercept	(95% C.I.)	Fixed effects	Estimate	(95% C.I.)
Rumination time (min/hr)	27.09	(26.16 to 28.03)	**Treatment**	**-0.38**	**(-0.76 to -0.00)**
			Day vs Night	**-4.69**	**(-5.05 to -4.32)**
			Oestrus	**-3.79**	**(-4.65 to -2.93)**
			Mon vs Sun	0.00	(-0.68 to 0.68)
			Tue vs Sun	**0.91**	**(0.26 to 2.22)**
			Wed vs Sun	0.57	(-0.11 to 1.24)
			Thu vs Sun	**0.90**	**(0.22 to 1.58)**
			Fri vs Sun	**0.92**	**(0.25 to 1.60)**
			Sat vs Sun	0.52	(-0.15 to 1.20)
			Week 2 vs 1	-0.13	(-0.57 to 0.32)
			Week 3 vs 1	**-0.64**	**(-1.08 to -0.20)**
			Period 2 vs 1	**-0.73**	**(-1.11 to -0.35)**

Supplementary Full model statements

The fixed part of the full model described in the section statistical analysis can be written as:

$\mu = \beta_0 + \beta_1 \times trt + \beta_2 \times w_2 + \beta_3 \times w_3 + \beta_4 \times gr + \beta_5 \times p + \beta_6 \times d + \beta_7 \times wd_2 + \ldots + \beta_{12} \times wd_7 + \beta_{13} \times w_2 \times wd_2 + \ldots + \beta_{24} \times w_3 \times wd_7 + \beta_{25} \times chs + \beta_{26} \times ccs + \beta_{27} \times p_2 + \beta_{28} \times p_3 + \beta_{29} \times dim + \beta_{30} \times mp + \beta_{31} \times eb + \beta_{32} \times trt \times wd_2 + \ldots + \beta_{37} \times trt \times wd_7$

μ is the mean of the variables average lying time, average standing time, logarithm of the average walking time, and average rumination time in minutes per hour free time. For average lying-to-standing transitions per hour free time, the linear part of the model is for *log (µ)*. For milk production per milking in kg milk, the fixed part of the model is similar, but without milk production as dependent variable.

Variable	Description
trt	1 for treatment, 0 otherwise
w_2, w_3	w_2 = 1 for week 2, 0 otherwise; w_3 similar
gr	1 for group B, 0 otherwise
P	1 for period 2, 0 otherwise
D	1 for day, 0 for night
wd_2, …, wd_7	wd_2 = 1 for weekday 2, 0 otherwise; wd_3…wd_7 similar
chs	1 for weather related heat stress, 0 otherwise
ccs	1 for weather related cold stress, 0 otherwise
p_2, p_3	p_2=1 for parity 2, 0 otherwise; p_3=1 for parity 3 or higher, 0 otherwise
dim	days in milk
mp	milk production in kg milk per milking
eb	1 for oestrus behaviour, 0 otherwise

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Chapter 4

Pressure measurement in the reticulum to detect different behaviours of healthy cows

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ABSTRACT

The aim of the current study was to investigate the relation between reticulorumen contractions and monitored cow behaviours. A purpose-built pressure measuring device was used and shown to be capable of detecting the known contraction patterns in the reticulorumen of four rumen-fistulated cows. Reticular pressure data was used to build a random forest algorithm, a learning algorithm based on a combination of decision trees, to detect rumination and other cow behaviours. In addition, we developed a peak-detection algorithm for rumination based on visual inspection of patterns in reticular pressure. Cow behaviours, differentiated in ruminating, eating, drinking, sleeping and 'other', as scored from video observation, were used to develop and test the algorithms. The results demonstrated that rumination of a cow can be detected by measuring pressure differences in the reticulum using either the random forest algorithm or the peak-detection algorithm. The random forest algorithm showed very robust performances for detecting rumination with an accuracy of 0.98, a sensitivity of 0.95 and a specificity of 0.99. The peak-detection algorithm could detect rumination robustly, with an accuracy of 0.92, a sensitivity of 0.97 and a specificity of 0.90. In addition, we provide proof of principle that a random forest algorithm can also detect eating, drinking and sleeping behaviour from the same data with performances above 0.90 for all measures. The measurement device used in this study needed rumen-fistulated cows, but the results indicate that behaviour detection using algorithms based on only measurements in the reticulum is feasible. This is promising as it may allow future wireless sensor techniques in the reticulum to continuously monitor a range of important behaviours of cows.

INTRODUCTION

A properly contracting reticulorumen is important for digestion of a ruminant, a prerequisite for health and welfare of the animal (Silva de Tarso, 2017). The reticulorumen is responsible for mixing and breaking down digesta and is the site of microbial digestion of plant fibre. Much of the function was discovered already nearly 100 years ago using direct observation, palpation and measuring of pressure (Sellers and Stevens, 1966). In the nineteen fifties and sixties, methods to measure pressure became more common, generally using rumen fistulated cows with balloons or fluid filled catheters (Balch et al., 1953; Amanda M Egert-McLean et al., 2019; Okine et al., 1989; Quigley and Brody, 1952; Sellers and Stevens, 1966). Measuring normal and abnormal contraction patterns is key to understanding the digestive performance and important for possible future continuous monitoring of ruminant health and welfare. Here, we improve on existing methods to show that these patterns can be quantified with algorithms based on measurement of changing pressure in the reticulorumen and that these patterns link to specific observed behaviour in cows (eating, rumination, drinking, sleeping). As described in (Braun and Rauch, 2008; Church, 1976; Sellers and Stevens, 1966), the contraction pattern of the reticulorumen consists of primary and secondary contraction cycles. The main function of the primary contraction cycle is to mix and transport ingesta back and forth between reticulum and rumen. The primary contraction cycle (A-wave) starts with a biphasic contraction of the reticulum followed by contractions of the different parts of the rumen in cranio-caudal direction. A third reticulum contraction occurs in the primary contraction cycle during rumination only (Fig. 1). There is a secondary contraction cycle (B-wave) of the forestomach complex that is important for the eructation of gases without involvement of the reticulum and ruminal atrium (Braun and Rauch, 2008; Church, 1976; Sellers and Stevens, 1966). We focus in this research on measurements of the A-wave in the reticulum.

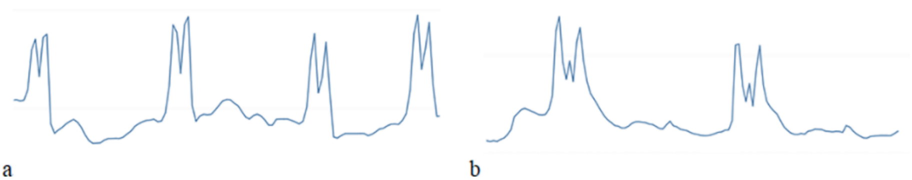

Figure 1. Reticular contractions during eating and rumination. Relative pressure differences illustrating two minutes of primary biphasic reticular contractions during eating (a), with a third contraction during rumination (b).

Previous research based on relatively few observations already showed potential associations between the contraction cycles and behaviour of the cow. For example, eating behaviour has been linked with a shorter time interval between two contraction cycles (Arai et al., 2019; Braun and Rauch, 2008; Church, 1976; Okine et al., 1998), whereby the higher frequency of contractions may play a major role in increased outflow rate (Okine et al., 1998). In contrast, stressed cows showed significantly longer intervals between contractions (Braun and Rauch, 2008), while during periods of apparent sleep an absence of rumen motility was reported (Church, 1976). Furthermore, previous research showed contradictory results regarding the interval between contraction cycles during resting compared to rumination (Braun and Rauch, 2008; Church, 1976; Okine et al., 1998). Comparing studies may be difficult because the state ‘resting’ is ambiguous. It is often defined as not-eating and not-ruminating but can still encompass a large variety of different behaviours, including actual resting.

Machine learning techniques can be valuable to link sensor data to animal behaviour (Neethirajan, 2020). Algorithms based on Random Forests and Neural Networks were applied earlier in behavioural classification of cows fitted with sensors (Smith et al., 2016; Vázquez-Diosdado et al., 2019). A random forest (RF) is a learning algorithm to solve classification or regression problems. It does so by considering a collection of decision trees and combining different ‘trees’ into ‘forests’. An RF is defined as a combination of tree predictors such that each tree depends on the values of a random vector sampled independently and with the same distribution for all trees in the forest (Breiman, 2001). An RF performs well compared to other algorithms and is easier to use and more forgiving with regard to overfitting and outliers (Horning, 2010; Silva de Tarso, 2017). Furthermore, RF are fast, flexible, and perform well even in the presence of a large number of features and a small number of observations (Ziegler and König, 2014). The application of RF creates opportunities for the use of sensor data in behaviour classification.

The aim of the current study was to investigate the relation between reticulorumen contractions, as measured by pressure differences in the reticulum, and specific monitored cow behaviour (eating, rumination, drinking, sleeping). RF algorithms for detecting rumination and other behaviours was developed and evaluated. In addition, we developed a peak-detection algorithm for rumination based on visually inspection of differences in reticular pressure time series. We show that the RF algorithm based only on pressure differences in the reticulum can be used to detect several cow behaviours.

MATERIALS AND METHODS

Animals and animal observations

The study was performed from February 5, 2019, until April 15, 2019, in four rumen-fistulated Holstein Friesian cows at the Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands. Animal procedures were approved by and in accordance with the guidelines of the Dutch Committee of Animal Experiments. The cows belonged to the same herd and had free-stall housing. Cows used for measurements were housed in a tie-stall barn, together with an accompanying cow, the evening before the day of data collection, to allow adaptation to the experimental environment. All rumen-fistulated cows were used to tie-stall housing. Before the start of each measurement period the health of the cow was clinically examined by a veterinarian. The animals were 7 to 9 years old; three were lactating and one was a dry cow. For further cow characteristics see Table 1. All animals were used to being handled by the animal observer.

Table 1. Characteristics of the four rumen-fistulated Holstein Friesian cows.

Animal	1	2	3	4
Days in milk	309	189	dried off before calving	320
Lactation no.	5	4	3	3
Milk yield (kg/d)[a]	19	32	-	24
Milk fat (%)[a]	5.00	5.01	-	4.52
Milk protein (%)[a]	3.73	3.35	-	3.41
Concentrate (kg DM)	0.9	4.5	0	1.8
Corn silage (kg DM)	4.0	4.0	2.5	4.0
Soya bean expeller (kg DM)	0.77	0.77	0	0.77
Formaldehyde treated soya bean expeller (kg DM)	0.61	0.61	0	0.61
Wilted grass silage (kg DM)	12.2	12.2	13.5	12.2

[a] Milk yield, milk fat and milk protein based on the most recent test day before the start of the experiment.

During the experimental observation days, the cows were fed the same diet as they were used to in the free-stall housing and were milked twice daily. The diet consisted of 4 kg DM/day maize silage after morning and afternoon milking. Cows were fed according to Dutch standards (CVB, 2016). A protein-rich supplement was supplied on top of the maize silage. Cows were provided with concentrates depending on milk production level. Dried-off cows received 2.5 kg DM/day maize silage in the evening. Fresh water and wilted grass silage were supplied ad libitum to all animals. See Table 1 for more details. A video camera system recorded the cows during all measurements.

The video data were analysed by the same observer and cow behaviour was scored every second to be either rumination, eating, drinking, sleeping or other (defined as none of the other behaviours). Sleeping was defined as lying immobile in a sleeping position, in a ventral recumbency with the head retroflexed to the flank. The video data analysed by one human observer were used as the gold standard and were blindly scored independent of the pressure data, but there is always the chance of human error. In the process of data cleaning a second person checked a large part of the video data (JS) and did not observe any misinterpretations.

Measurement device

A purpose-built device to measure pressure differences in the reticulo-rumen was available. This device was originally developed for teaching (co-author AS) and is similar to approaches used in the literature that have previously been shown to measure pressure in the reticulorumen (Amanda M Egert-McLean et al., 2019; Okine et al., 1993; Quigley and Brody, 1952). In this device, four water-filled open-tipped catheters are used to measure pressure differences in four different compartments of the reticulorumen, namely the reticulum, the cranial, the dorsal and the ventral sac of the rumen. Each catheter is connected to a pressure transducer. The transducer converts the water pressure to an electronic signal and sends it to an amplifier. The amplifier is connected to a computer to store and edit the data. A waterfilled pressure bottle is connected to the transducer and a constant pressure from the bottle in the direction of the open tip of the catheter ensured that the catheter was continuously waterfilled. To minimize fluctuations in the baseline pressure of the compartments, the position of the transducer can be adjusted to approximate the height of the catheter inside the reticulum at all times. It was possible to flush the catheter if clogged. See Fig. 2 for a schematic overview of the device. The sensors measure pressure expressed in mV every 0.5 seconds. Using this device, data was collected during four hours at each measurement session between AM milking and PM milking. The proceedings did not visibly influence the rumen-fistulated cows in their normal behaviour.

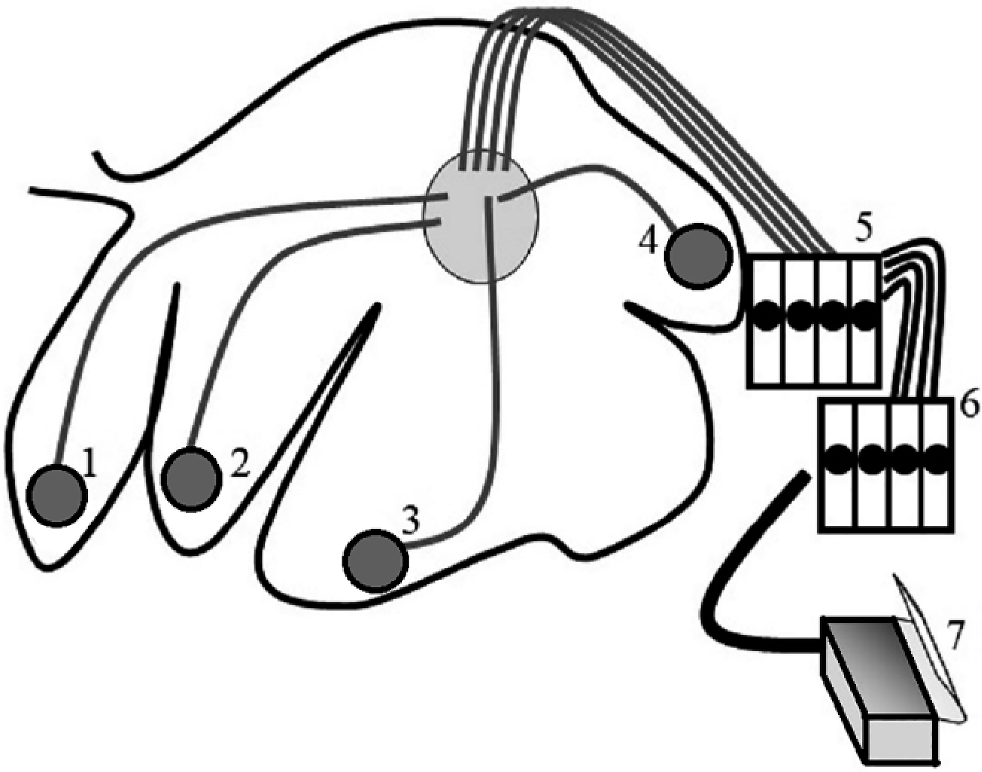

Figure 2. Schematic overview of the measurement device.
(1) Reticulum (2) cranial sac of the rumen (3) ventral sac of the rumen (4) dorsal sac of the rumen (5) pressure transducers (6) amplifier (7) computer

Data analysis

Cow behaviour, differentiated in ruminating, eating, drinking, sleeping and other, was scored from the video data and these observations were used to develop and test the algorithms based on reticulum pressure data. The pressure data were measured every 0.5 sec and matched to the behaviour data based on the time in seconds, resulting in a dataset per 0.5 sec.

Random forest algorithm. In short, we first normalized the pressure data, after which the data was partitioned in windows of 120 sec with an overlap of 119.5 sec. The video data was matched with each 0.5 sec window. For each sliding window containing 240 pressure timepoints, features based on frequency were computed using three different methods. Fourier transform, power spectral density and autocorrelation were used to generate 30 low-dimensional features (Bracewell, 1986; Kaur et al., 2011; Parák and Havlík, 2011). Based on these features, an RF classification model was developed to predict each behaviour separately. The full code can be found at (https://doi.org/10.5281/zenodo.4538933).

Each separate behaviour was predicted against a single class of all other behaviours, called the one-vs.-all technique (Smith et al., 2016). The behaviours sleeping and drinking were not observed frequently during the study period, resulting in relatively few datapoints where a cow was either sleeping or drinking. When training the RF on these very unbalanced datasets (i.e., 5% in the positive class against 95% in the negative class), the one-vs.-all-algorithm is mainly trained to predict the majority class well and not the minority class. To achieve a better balance in the data for sleeping and drinking behaviour, these minority classes were up-sampled three times in the data set with concomitant down-sampling of the majority class (Ziegler and König, 2014). Through visual inspection of the AUC-curves numbers of parameters were chosen for each RF to limit overfitting. The RF model was trained with 70% of the data and tested using the remaining 30% (70/30 RF). For rumination, two additional approaches were conducted to validate the RF (Rahman et al., 2018):

1. The N-fold Stratified Cross Validation (SCV) approach. Data from all experimental days and different animals were combined and randomly split into five subsets. After that, one subset became a test set, and the remaining subsets were combined to become the training set. The process was repeated for each subset.
2. Leave-Out-One-Animal (LOOA) validation approach. Data from one animal became the test set and data from the remaining animals were combined to become the training set. Each animal became a test animal in turn.

Peak-detection algorithm. A peak-detection algorithm was developed based on patterns detected by visual inspection of the reticular time series data during rumination (Fig.1). The full algorithm can be found at (https://doi.org/10.5281/zenodo.4538933). The algorithm is aimed at detecting sharp peaks in the pressure landscape and then characterizes patterns in terms of sets of consecutive peaks that determine a contraction cycle, specifically assessing the duration of such sets and the timing between them. The peak-detection algorithm identifies sharp peaks in the pressure signal by determining for each increase and decrease whether, the change surpasses a pre-set threshold value within a certain timespan. To detect the distinct three-peak pattern of pressure differences during rumination, specific intermediate signal features such as highest peak, peak value, baseline and contraction interval were calculated using a sliding window of 75 seconds. The algorithm based on these features was used to characterize whether a cow was ruminating per 0.5s. Occasionally, only two peaks were observed in a contraction cycle. However, in those cases the time period between the two peaks had the same length as that of a regular three-peak pattern.

Visual inspection of pressure data time series did not show typical peak patterns for the other behaviours when compared to the video data, precluding the similar development of algorithms for behaviour other than ruminating.

Analysis of algorithms

The performance of the RF algorithm and the peak-detection algorithm was evaluated using the video scores. Each datapoint of 0.5 seconds was scored by the algorithms to be positive or negative for a specific type of behaviour. The video scores were used as the true behaviour scores. A confusion matrix was constructed for each behaviour type, counting true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) for all predictions by the algorithms. Based on this, the following standard metrics were calculated for each behaviour:

Sensitivity (Se), is the fraction of video scores classified correctly by the algorithm as positive:

$$Se = \frac{TP}{TP+FN} \qquad (1)$$

Specificity (Sp), is the fraction of video scores classified correctly by the algorithm as negative:

$$Sp = \frac{TN}{TN+FP} \qquad (2)$$

Positive predictive value (PPV), is the fraction of algorithm scores classified as positive that are actually positive:

$$PPV = \frac{TP}{TP+FP} \qquad (3)$$

Negative predictive value (NPV), is the fraction of algorithm scores classified as negative that are actually negative:

$$NPV = \frac{TN}{TN+FN} \quad (4)$$

The F1-score combines sensitivity and PPV, and provides a single fraction reflecting the ‘goodness’ of a classifier in the presence of rare classes:

$$F1 = 2 * \frac{PPV*Se}{PPV+Se} \quad (5)$$

Accuracy (Acc) is the fraction of samples correctly classified by the algorithm:

$$Acc = \frac{TP+TN}{TP+TN+FP+FN} \quad (6)$$

We used the above formula to compute the classification performances of the classifiers for the different cow behaviours.

RESULTS

Detection of contraction pattern

The contractions in four different compartments of the reticulorumen were detected and we were able to follow the A-wave over the reticulorumen (Fig. 3). Each A-wave cycle could be detected in the reticulum first, followed by the cranial sac, the dorsal blind sac and finally the ventral sac of the rumen. A small pressure change was noted in the ventral ruminal sac at the moment of the contraction in the dorsal blind sac. This was likely due to noise caused by the measuring method.

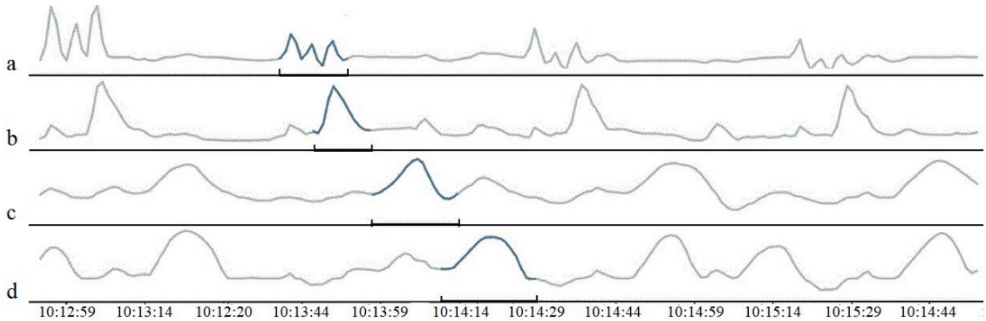

Figure 3. Pressure measured at four locations of the forestomach of a cow during rumination. (a) reticulum; (b) cranial ruminal sac; (c) dorsal blind sac; (d) ventral ruminal sac. The A-wave is highlighted in this figure and moves chronologically over the different compartments.

Descriptive aspects

In this experiment 57.5 hours of cow behaviour data were analysed resulting in 413,957 datapoints for the RF algorithm and 414,484 datapoints for the peak-detection algorithm. Four cows were used in this experiment. See Tables 2 and 3 for a summary of the number of observations per animal and per behaviour type, respectively.

The average time between two contraction cycles per behaviour type is given in Table 4; a difference in average time was found, with a wide overlap across behaviours. There was a significant difference ($P < 0.001$) of the average time between two contractions during rumination (48 sec) and eating (34 sec), as tested in a GLM model corrected for cow effect. However, even rumination and eating showed too much overlap to distinguish the two behaviours based on a cut-off value (Fig. 4).

Table 2. Number of observations per cow.

Cow ID	Observations [a]
2	109,288
13	72,564
21	97,337
84	135,295

[a] Number of datapoints measured per 0.5sec.

Table 3. Number of observations per behaviour.

Behavior	Observations [a]
Rumination	132,828
Eating	106,915
Drinking	1,570
Sleeping	13,294
Other	159,877

[a] Number of datapoints measured per 0.5sec.

Table 4. Time in seconds between two contraction cycles during different cow behaviours.

Behavior	Mean (SD)	Median (IQR)
Rumination	48 (12.1)	48 (12.5)
Eating	34 (12.7)	34 (13.0)
Drinking	35 (12.9)	34 (14.4)
Sleeping	41 (11.4)	39 (6.5)
Other	40 (23.1)	37 (14.5)

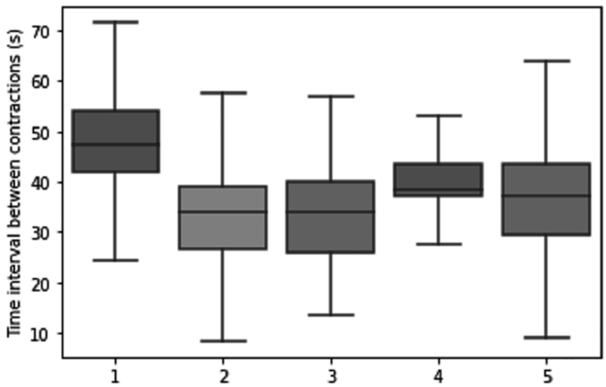

Figure 4. Boxplot of the time between two contraction cycles during the different behaviours. Time between two contraction cycles in seconds, during different behaviours: (1) Rumination; (2) Eating; (3) Drinking; (4) Sleeping; (5) Other. Boxplots depict the median and the upper and lower quartiles; whiskers depict quartiles ± 1.5 × the interquartile range (IQR); outliers not shown.

Detection of rumination

Rumination could also be detected with an RF algorithm trained on 70% of the dataset and tested on the other 30%. The RF algorithms showed very robust performances for detecting rumination, and also when repeated five times with a 5-fold stratified cross validation technique. All five RFs for rumination showed the same accuracy of 0.98, sensitivity of 0.95 and specificity of 0.99. In contrast, when the RF was trained on data of three cows and tested on the data of the other cow, in the LOOA validation the sensitivity and accuracy were lower and also the other metrics were more variable (see Table 5), possibly due to one aberrant animal with extreme low sensitivity of 0.56. The peak-detection algorithm could detect rumination with an accuracy of 0.92 a sensitivity of 0.97 and a specificity of 0.90. For other extracted metrics see Table 5.

Table 5. Performance of algorithms for rumination

Algorithm	Se	Sp	PPV	NPV	F1	Acc
Peak-detection	0.97	0.90	0.82	0.98	0.89	0.92
RF 70/30	0.94	0.99	0.98	0.97	0.96	0.98
RF SCV [a]	0.95 (0.002)	0.99 (0.000)	0.99 (0.001)	0.98 (0.001)	0.97 (0.002)	0.98 (0.001)
RF LOOA [b]	0.73 (0.105)	0.96 (0.019)	0.89 (0.067)	0.88 (0.076)	0.80 (0.057)	0.88 (0.049)

RF, Random Forest; SCV, Five-fold Stratified Cross Validation; LOOA, Leave-out-one-animal based on four cows.

[a] Average and standard variation over 5 folds.

[b] Average and standard deviation over four cows.

Proof of principle for detection of multiple behaviours

Four mutually exclusive behaviour classes were scored during the experiment: Rumination, Eating, Drinking and Sleeping. Table 6 illustrates the performance metrics of the one-vs.-all RF algorithms (RF 70/30) of these behaviours. Sleeping and Drinking had a small representation, resulting in unbalanced datasets that were resampled. Our data suggest that also these cow behaviours can be detected by measuring pressure differences in the reticulum.

Table 6. Random forest algorithms (RF 70/30) for different behaviours

Behavior	TP	FP	TN	FN	Se	Sp	PPV	NPV	F1	Acc
Rumination	37,410	570	83,940	2,196	0.94	0.99	0.98	0.97	0.96	0.98
Eating	29,231	903	91,006	2,976	0.91	0.99	0.97	0.97	0.94	0.97
Drinking [a]	467	17	454	4	0.99	0.96	0.96	0.99	0.98	0.98
Sleeping [a]	3,947	240	3,749	42	0.99	0.94	0.94	0.99	0.97	0.96

[a] Numbers reflect the resampled dataset to balance the data.

DISCUSSION

The measuring device used in this study indeed detected the expected contraction pattern in the reticulorumen of the rumen-fistulated cows (Braun and Rauch, 2008; Church, 1976; Stevens and Sellers, 1960). The results demonstrate that rumination activity of a cow can be detected by measuring pressure differences in the reticulum using either an RF algorithm or an algorithm based on visual inspection of reticulorumen pressure time series. A proof of principle is presented indicating that an RF algorithm can in addition detect eating, drinking and sleeping from the same data. These results are promising as they may allow future wireless pressure sensors in the reticulum to continuously monitor a range of important behaviours of cows.

Most previous studies that differentiated eating, rumination and resting based detection on the meantime interval between contraction cycles. We found that the time between two contraction cycles is shortest during eating, similar to other studies (Arai et al., 2019; Braun and Rauch, 2008; Church, 1976; Okine et al., 1998), but that the large variation around the means precluded setting a clear threshold to separate eating from other behaviour. In our study, the time interval between two contractions was defined as the time with no peaks. This is in contrast to the other studies where the time interval was the time between the last peak top of one contraction and the top of the first peak of the next contraction. Therefore, the exact time intervals in seconds were not comparable across studies. Furthermore, we did not find an absence of rumen motility during periods of apparent sleep as detected by others (Church, 1976). However, in our data there is no certainty of sleep because we scored sleeping based on direct observation and not on brain activity (Ternman et al., 2012).

The RF algorithm was able to differentiate between peaks and noise when trained to detect rumination, shown by a high specificity (99%). Likewise, the RF for eating had a high specificity (99%), indicating that the RF approach is also able to distinguish eating from other behaviours. Inspection of the features used by the RF for rumination and the RF for eating showed a clear difference in which features were important (https://doi.org/10.5281/zenodo.4538933). The different features used in the two RF's demonstrate that both behaviours have their own distinguishable pressure pattern. Studying the feature sets could provide additional insight into the differences in contraction pattern. The Stratified Cross Validation showed minimal variance in the performances, suggesting that the RF algorithm is robust. The weaker performance of the Leave-Out-One-Animal approach is possibly caused by the data of one of the four cows. This particular cow was the only dry cow in this experiment. Whether the dry period of the cow contributed to the difference needs more investigation. Potential causes that may account for the observed differences in reticulum contraction patterns compared to lactating cows, may be the diet and consequently the content of the reticulorumen, volume, or abdominal pressure by a pregnancy. Another reason could be that the reticular contraction patterns show important variability between individual cows and that the RF algorithm should be developed on more than three animals, or that more finetuning and filtering of the sensor data is necessary to allow comparison of different animals.

The peak detection algorithm showed that it was possible to distinguish between ruminating or not-ruminating with one algorithm independent of the specific animal. The algorithm functioned well to detect rumination for the complete dataset including data of all four tested animals. Other behaviours could not be detected by an algorithm based on visual inspection of patterns in the data. The specificity of the peak-detection algorithm for rumination was only 90% compared to a sensitivity of 97%. The reason for the relatively low specificity could be that the data was not normalized, resulting in noise being classified falsely as rumination peaks.

Drinking and sleeping could be detected with an RF algorithm with more than 90% sensitivity and specificity. Without the use of EEG techniques, the posture of the cow currently appears to be the most reliable way to score sleeping behaviour. Sufficient sleep time is important for the welfare of cows and essential for both an adequate metabolic system and the immune function. However, little is known about sleep in cattle (Ternman et al., 2012). Even though the datasets for sleeping and drinking were only small it still suggests that reticulum pressure could probably be used with an RF algorithm to detect other cow behaviours than rumination and eating.

In this study we presented a proof of principle for detecting different behaviours of cows by measuring pressure in the reticulum. The results suggest that with even standard default RF methods important behaviours could be detected. The behavioural types in this study were chosen because they have no overlap in the sense that no two of these behaviours are observed in the same individual simultaneously. For this proof of principle, the aim was to explore the relation between reticular pressure patterns and different cow behaviours, not to be able to predict what a cow was doing based on pressure patterns in the reticulum. If in further studies the multiple behaviours need to be classified at a particular moment, the corresponding set of binary classifiers can be combined by either ensemble methods or a multiclass algorithm. In addition, the behaviour types in this study were chosen because they can be scored as approximately binary (presence/absence) and are therefore well-suited for the approach. Because behaviour is generally not binary, future algorithms might improve by utilizing multiclass algorithms. In this study the peak-detection algorithm was developed based on visually observed patterns of pressure differences, while machine learning RFs are based on automatic extraction of features. With machine learning techniques like RF, patterns not apparent by visual inspection can be detected using higher order features. For example, the pressure data is transformed from a time domain to a frequency domain by Fourier Transformation. While this allows for the detection of more subtle and higher-order patterns, the abstract RF-approach complicates mechanistic interpretation of the patterns that are discovered.

It was not our aim to draw conclusions on the behaviour of healthy cows or on biological mechanisms underlying different types of behaviour. The aim was proof of principle for the monitoring of behaviour. There are limitations to the study and caution should therefore be taken before interpreting our results in a wider context beyond this proof of principle. First, the number of animals in our study was small. Second, it is unclear in what way pressure patterns of the rumen-fistulated cows, even if these animals are in all other aspects ‘healthy’, can be taken as representative of a normally functioning cow.

Furthermore, the fistula can also influence measurements in our set-up. The method itself may therefore limit biological interpretation of the patterns we were able to quantify. In addition, the algorithms, whether RF or informed by visual inspection do not immediately allow mechanistic interpretation of the patterns that are observed in terms of biological processes involved in different types of behaviour. This is less of a problem for monitoring when one has such high specificity and sensitivity as in our study, but clues to understanding the underlying biology are a more difficult challenge.

The future perspective is that behaviour detection based on measurement of only the A-wave in the reticulum is feasible and would therefore be relevant for wireless sensor techniques. Wireless ruminal sensors are available in commercial dairy farms and particularly in animal research (Denwood et al., 2018; Dijkstra et al., 2020; Falk et al., 2016). Most orally applied sensors migrate towards the reticulum and can measure temperature or pH in the reticulum continuously (Dijkstra et al., 2020). Measuring rumen motility by three axis acceleration data using a sensor bolus has also been explored (Arai et al., 2019). In another study, actual pressure differences are measured by a bolus every 10 sec (Kilic, 2011), which may not yet be frequent enough to monitor reticulorumen contraction waves sufficiently accurately for the identification of cow behaviour.
Measuring reticulorumen contractions is not only interesting for detection of different cow behaviours, but also for identifying cows with welfare problems or at risk for disease (Nogami et al., 2017; Silva de Tarso, 2017). For example, until now there is no valid method to score sleeping behaviour, which is important for cow welfare. The use of reticular pressure differences may be an important parameter as the basis for an index of sleeping behaviour in the near future. In addition, the frequency and amplitude of reticulorumen contractions are negatively influenced by many factors including metabolic diseases, anorexia and other diseases that cause pain or fever (Arai et al., 2019). The pH in the reticulorumen can also influence the reticulorumen contractions and acidosis has been shown to reduce the frequency of ruminal contractions, resulting in reticulorumen stasis (Amanda M. Egert-McLean et al., 2019). Reticulorumen stasis may lead to diseases such as displaced abomasum and ruminal tympany (Nogami et al., 2017). Therefore, reticulorumen motility is an important measurable characteristic to identify cows at risk for disease (Silva de Tarso, 2017).

CONCLUSION

Our results strongly suggest that measurements of pressure differences in the reticulum can be utilized to detect various behaviours of cows based on RF algorithms. Our data provided a proof of principle for future automatic monitoring of ruminating, eating, drinking, and sleeping behaviour of cows.

ACKNOWLEDGMENTS

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Chapter 5

Proof of principle and potential use of a bolus measuring reticular contractions and temperature

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INTRODUCTION

Precision livestock farming (PLF) technologies are increasingly important in the dairy industry. Numerous commercially available sensors monitor cow activity and detect oestrus, lameness, disease, and calving on farms worldwide (Knight, 2020). The use of sensors allows farmers to monitor large populations of animals for health (welfare) on a daily basis, both in stables and in the field, detect aberrancies in cow activity with individual animals, and even anticipate their occurrence. Additionally, those sensors are of great value for research to objectively study cow behaviour. The most commonly used sensors are attached to the cow's body at the neck or leg collecting information about activity, feeding behaviour, and physical condition and health (Stygar et al., 2021).

Besides sensors attached to the cow, internal sensors collecting data from the rumen have been developed. A ruminal bolus usually resides in the reticulum after oral ingestion. For several years, a wireless ruminal bolus measuring pH and temperature in the reticulum has been commercially available (SmaXtec animal care GmbH, Graz, Austria). The pH data obtained from a ruminal bolus requires careful interpretation due to differences in pH between reticular locations that additionally appear to be diet dependent (Dijkstra et al., 2020). Over time, a substantial drift of non-retrievable rumen pH sensors is a major obstacle to their use in non-cannulated animals where ruminal sensors cannot be cleaned and recalibrated (Dijkstra et al., 2020). The temperature is recorded at 10-min intervals by this wireless ruminal bolus and enables an automatic, continuous, and labour-extensive monitoring of the reticular temperature. The data could be used to monitor individual body temperature and drinking of cows (Ammer et al., 2016). However, to monitor the exact amount of water intake to evaluate individual drinking patterns and behaviour it may be necessary to measure more frequently than 10-min intervals.

The rumen motility can be studied successfully with cameras (Song et al., 2019) and ultrasonography, but is very labour intensive (Braun and Schweizer, 2015). Although measuring normal and abnormal contraction patterns of the reticulum is especially important for studying the digestive performance of ruminants, an internal bolus to check reticular contraction patterns in ruminants is not commercially available.

Technologies to continuously monitor reticular contractions with wireless bolus sensors are in development. Wireless bolus sensors collecting data about reticular contractions are mostly based on a 3-axis accelerometer (Arai et al., 2019; Hamilton et al., 2019; Nogami et al., 2017). Arai et al. (2019) showed that a bolus-type wireless sensor, measuring acceleration of the y-axis with sampling rates of 1 Hz, could detect rumen motility. The average value of y-axis acceleration and peak acceleration were calculated every 10 sec. They found a higher frequency of ruminal contractions during feeding compared to resting, and the bolus detected ruminal atony after administration of xylazine.

A key indicator of cow health is the time spent for rumination. Hamilton et al. (2019) used a wireless sensor based on 3-axis acceleration data to provide an indication of rumination time with a sample frequency of 12.5 Hz in contrast to a sample frequency of 0.2 Hz for another acceleration-based prototype wireless bolus (Nogami et al., 2017). For the analysis of ruminal motility, the acceleration of the y-axis seems most appropriate (Arai et al., 2019). However, all bolus sensors thus far move freely in the lumen of the reticulum. This could complicate measurement of reticular motility based on 3-axis acceleration in a cow not standing still but, for example, walking in the field or mounting others during heat. Reticular contractions could be detected as well by measuring the actual pressure in the reticulum. In a proof of principle study, we previously showed that reticular pressure can be measured with a device based on water-filled open-tipped catheters in cannulated cows (Scheurwater et al., 2021) and found that to be able to detect the distinct three-peak pattern of pressure differences during rumination, the sampling frequency needs to be appropriate. A sample frequency of 2 Hz is sufficiently accurate for the identification of rumination (Scheurwater et al., 2021). Furthermore, potential associations between the reticulorumen contraction cycles and different types of cow behaviour have been investigated previously (Arai et al., 2019; Braun and Rauch, 2008; Church, 1976; Okine et al., 1998; Scheurwater et al., 2021). We previously found that the time between two contraction cycles is possibly shortest during eating, but the large variation precluded us from setting a clear threshold to separate eating from other behaviour (Scheurwater et al., 2021). An absence of rumen motility during periods of apparent sleep was detected by Church (1976) but not by Scheurwater et al. (2021). However, there is no absolute certainty of sleep when sleeping is scored based on direct observation and not on brain activity (Ternman et al., 2012).

A bolus measuring actual pressure and temperature with a sample frequency of 2 Hz would be of great value to study reticular contraction patterns. To analyse the large amount of pressure and temperature data generated in this way, machine learning techniques could be useful for detecting different behaviours in both healthy and unhealthy cows. Various machine learning techniques are studied for behavioural classification of cows using various types of individual-level animal data. Neural networks are used to identify and track cows and to classify behaviour based on video recordings (Ardö et al., 2018). The moment of calving could be predicted based on data of activity, lying and rumination collected by sensors attached to the neck and leg of dairy cows (Borchers et al., 2017; Liseune et al., 2021b). With a conventional neural network technique feeding behaviour of dairy cows has been recognized based on jaw movement data from a sensor attached to the neck (Chen et al., 2020). If a reticular bolus could accurately measure actual pressure and temperature in the reticulum, machine learning techniques might be used to link the contraction patterns to different cow behaviours.

In this study we provide proof of principle that a prototype bolus with a temperature and a pressure sensor can detect reticular contraction patterns. Furthermore, we study the possibility to use this bolus to measure temperature change in the reticulum during after drinking. We link the temperature and pressure measurements to other types of cow behaviours such as ruminating, eating, sleeping, urinating, and mooing.

MATERIALS AND METHODS

Measurement device

A prototype wireless bolus device was used to measure pressure and temperature in the reticulum with a frequency of 2 Hz (signal interval 0.5 sec) during ten observation days (day 1-3: signal interval fixed at 0.465 sec; day 5-8: signal interval fixed at 0.507 sec and file 9-10: signal interval fixed at 0.500 sec). The wireless bolus device (Spits Technology B.V.®) contains an absolute pressure sensor (TE connectivity®) with a resolution of 0.1 mBar and a temperature sensor (TE connectivity®) with a resolution of 0.01°C. This device works on a double-A battery, contains an SD card for data storage and uses LoRa peer-to-peer communication. The battery runtime was enough for one measuring day of 8 hours when measuring temperature and pressure twice per second. The battery was charged after each session. The device has a cylindric shape with a length of 11.5 cm and weighs 172 gram. See Fig. 1 for a picture of the wireless bolus device and the location in the reticulum. The head of the bolus was filled with water to transmit the pressure signal from the silicone membrane to the pressure sensor. In the interest of bolus development, another version of the bolus with a head filled with silicone has also been tested on one testing day. Only the data generated with the bolus with the head filled with water were included in the current study analysis. We do, however, report in Appendix S1 on the difference between the two versions because this helps to explain patterns found in the pressure data (see also first section of the Results).

Figure 1. Wireless bolus sensor and location in the cow

Experiment

The study was performed from January 18, 2021, until February 25, 2021, with four rumen-fistulated Holstein Friesian cows at the Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands. Animal procedures were approved by and in accordance with the guidelines of the Dutch Committee of Animal Experiments. The protocol for the animals and the animal observations was similar to that used in Scheurwater et al. (2021) and was in short as follows.

The four cows belonged to the same dairy herd with free-stall housing. Two hours before data collection, the experimental cow was moved to a tie-stall barn, together with an accompanying cow, to allow adaptation to the experimental environment. All cows were used to tie-stall housing and were used to being handled by the animal observer.

Before the start of each measurement, the health of the cow was clinically examined by a veterinarian. The rectal cow temperature, the drinking water temperature and environmental temperature were registered at the start, half-way through and at the end of each experimental day. One cow was 9 years old; the other three cows were 4 years old; two were lactating, one was a dry cow, and one was both lactating and dry. This cow was lactating on the first experimental day and in a dried-off state on the second experimental day. Due to a Salmonella-related abortion three days after the second experimental day, the cow was lactating again on the third and fourth experimental days. None of the cows showed any clinical signs of illness during the experimental days. For further cow characteristics see Table 1.

During the experimental days, the cows were milked twice daily and were fed the same diet as they were used to in the free-stall housing. Cows were fed according to Dutch standards (CVB, 2016), including a protein-rich supplement supplied on top of the corn silage. The base diet consisted of 4 kg DM/day corn silage after morning and afternoon milking. Cows were provided with concentrates depending on milk production level. Dried-off cows received 2.5 kg DM/day corn silage in the evening. Fresh water and wilted grass silage were supplied ad libitum to all animals. A water flow sensor (Gardena®) was used to measure the amount of water intake by the cow before and after a drinking moment. When a cow drank again within five minutes these moments were taking together as one drinking period. For each drinking period the start time and the end time of drinking were noted as well as the quantity of water intake.

Table 1. Characteristics of the four rumen-fistulated Holstein Friesian cows.

Animal-ID	7	8	21	25
Age	4 years	4 years	9 years	4 years
Data in experiment	134790 (18h 43min)	106815 (14h 50min)	94803 (13h 10min)	94755 (13h 10min)
Days in milk	125 d	472 d	Dried-off	190 d
KI date	-	14-6-2020 (218d[b])	18-8-2020 (153d[b])	10-1-2021(8d[b])
Lactation no[a]	2	1	4	2
Milk yield (kg/d)[b]	41	13/26[c]	-	26

[a] Milk yield based on the most recent test day before the start of the experiment
[b] Lactation number and pregnancy days at the start of the experiment
[c] Before dry-off, after abortion, respectively

A video camera system recorded the cows during the entire period of measurement. The video data were analysed by the same observer and labelled with behaviour classes with a resolution of one second based on the clock time of the video system, according to the definitions in Table 2. The video data analysed by a human observer were used as the gold standard and were blindly scored independent of the sensor data. In the process of data cleaning, a second person checked most of the video data and did not find any misinterpretations of the first observer.

Note that the timing of the bolus signal matched the video clock time exactly only on observation day 9 and 10 because on these days the signal rate was 0.5 seconds. On the other observation days, a discrepancy cannot be excluded because the signal rate was slightly higher (day 1-3) or slightly lower (day 5-8). Over a maximal observation period of eight hours this discrepancy can add up to a 15 second differences between bolus-time and video clock-time. Therefore, depending on the observation day, observations of behaviours can be shifted earlier or later compared to the bolus signal. This mostly affects the detection and interpretation of signals that would potentially indicate very-short duration behaviours, such as urinating, mooing and possibly sleeping.

Data analysis

In this experiment, data was collected on 20 days. From those 20 observational days only 10 days were included in the study based on an inclusion criterium of at least four hours of continuous measurement and the video recording being available during the entire period. In this experiment, 59 hours and 53 min of cow behaviour data were analysed resulting in 431,163 datapoints, based on cow 7 (on three days), cow 8 (on three days), cow 21 (on two days) and cow 25 (on two days).

Cow behaviour was scored from the video data and used to train and test the algorithms published by Scheurwater et al. (2021), Chapter 3 of this thesis, as an example of how the data obtained from this bolus can be linked to different cow behaviours. The Random Forest and Peak detection algorithm were developed for reticular pressure data measured with another device with water-filled open-tipped catheters. The pressure signal was in mBar instead of mVolt but showed a similar signal pattern during reticular contractions. The feature preparation for the RF algorithm was the same as used in Scheurwater et al. (2021) see Appendix 2. The correlation between drink amount and temperature drop in de reticulum is evaluated with a scatter plot and a regression line for those variables is calculated.

Table 2. Ethogram of cow behaviours.

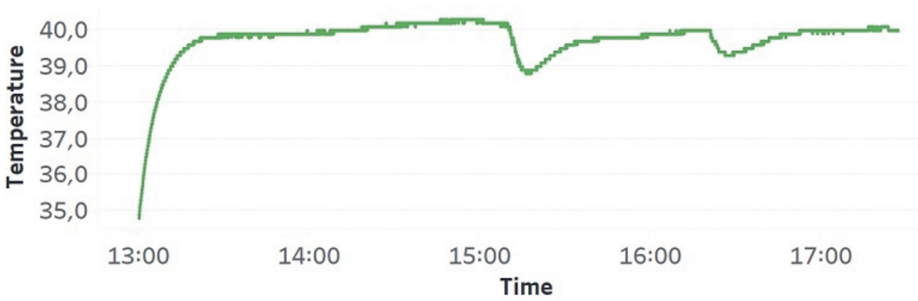
Behaviour Class	Description	Observed sample size (scored every sec)
Ruminating	The cow is masticating regurgitated feed, swallowing masticated feed or regurgitating feed	81,317
Eating	The cow is ingesting feed for at least five seconds	114,682
Sleeping	The cow is lying immobile, in a ventral recumbency with the head retroflexed to the flank and eyes closed.	3394
Drinking	The cow is ingesting water for at least five seconds	4,587
Mooing	The cow makes the characteristic deep resonant vocal sound of cattle.	3,166
Urinating	The cow discharges urine	1,107
Resting	The cow is standing or lying and is not showing any of the other behaviour types	211,940
Other	All other observed behaviour including playing with food or water, grooming, belching, sighing, defecating, standing up or lying down	10,970
Total		431,163

RESULTS

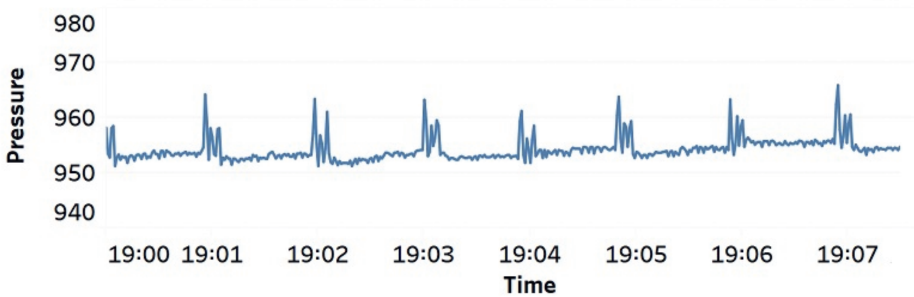
Detection of reticular contractions and reticular temperature

The reticular bolus was able to measure the temperature and the actual pressure in the reticulum. Fig. 2A shows a typical temperature pattern over a period of a few hours; Fig. 2B shows a typical contraction pattern over a period of eight minutes during rumination and Fig. 2C during eight minutes of eating. In Appendix 1 Fig. S1 we also show such patterns on the same time scale. In Fig. S1A data from one observational period using the bolus with a water-filled head is shown. The temperature and pressure follow a similar pattern. When the temperature increases the pressure increases as well and vice versa. The temperature sensor needed time to warm up after the bolus was inserted into the reticulum before it reflected the correct reticular temperature (this process took about 30 minutes; clearly visible in Fig. 2A). There is, on the long-time scale pictured in Fig. S1A, an overall increasing trend in the measured pressure signal, not visible on the short time scales depicted in Fig. 2. An explanation for this can be that in the bolus head filled with water, a small gas bell could form just behind the silicone membrane, for example due to osmosis from the bolus head to the rumen. Due to an increase in temperature this gas bell can expand and influence the measured pressure signal, which would show an overall increasing trend in the pressure measured by the bolus on top of the variation caused by cow behaviour. To investigate whether a different liquid behind the membrane can eliminate this background trend, we tried a silicon-based solution. The data in Supplementary file Fig. S1B shows that in a version of the bolus with a silicone-filled head the pressure is not influenced by the temperature in a way seen in the bolus with a water-filled head. Because most data available for this experiment was sampled with the bolus with the water-filled head we only focus on temperature data to measure drinking behaviour. The additional part of the experiment using a bolus with a silicone-filled head could explain the trend in the measured pressure signal on the long-time scale and gives implications for future bolus potencies.

A



B



C

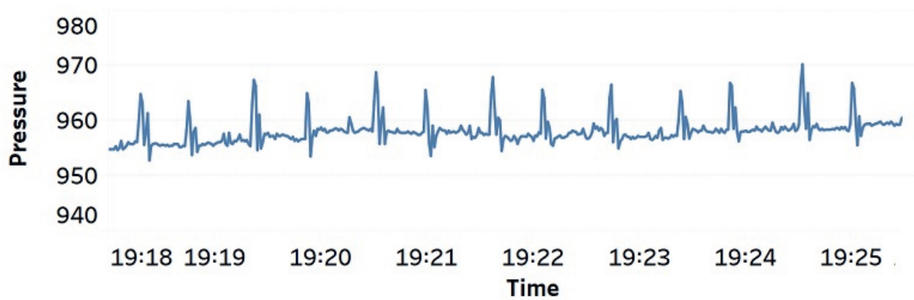
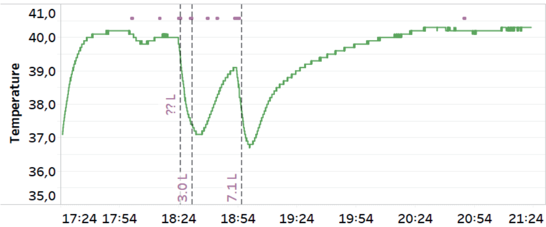


Figure 2. Reticular pressure and temperature measured with a bolus
In Fig. 2A the temperature measured in the reticulum with a sensor bolus is shown during one observation day. In Fig. 2B eight minutes of pressure data is shown during ruminating and in Fig. 2C during eating of the cow.

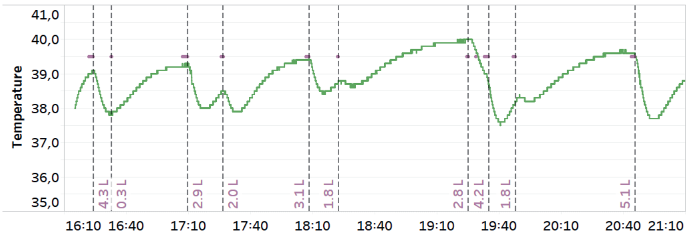
Drinking

Temperature in the reticulum of a cow could be detected with the reticular bolus. After drinking cold water, the drop in temperature was clearly detected in all drinking periods where the water intake was at least one litre. The temperature of the drinking water was on average 15.5 °C (SD 1.4). For each drinking moment the water intake in litres was measured (see Fig. 4). One can clearly observe differences in drinking pattern between cows and days, which was for example expressed in the number of drinking moments, the intervals between consecutive drinking moments and the quantity of water intake per drinking moment, (see Fig. 4). On observation day 9 the cow did not drink during the entire experiment. In Fig. 5AB the relation between the amount of water intake in litres and the drop in temperature in the reticulum is shown; in Fig. 5A the decrease in temperature (°C) and in Fig. 5B the decrease as a proportion of the temperature at the start of a drinking moment. In Fig. 5C the relation between the amount of water intake and the time it takes to reach the minimum temperature after drinking is shown. Fig. 5A and 5B show the same pattern, suggesting that the effect on the reticular temperature is not dependent on the temperature in the reticulum before drinking. When a cow drinks more litres of water the drop of reticular temperature measured by the bolus is larger and the decrease of temperature took longer. Cows drank on average 4.2 litres of water in this study with an SD of 2.3. Approximately 10 minutes after drinking, the temperature in the reticulum appears to reach its minimum value and then increases in one and a half hours to the temperature before drinking. In that period a cow often drinks again leading to a second drop (Fig. 4). The amount of water intake and the minimum temperature that was reached after drinking were stronger related than the amount of water intake and the duration of the temperature decrease after drinking.

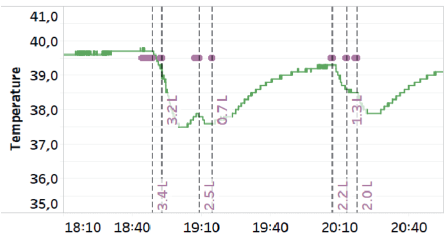
A Observation day 1 (cow 8)



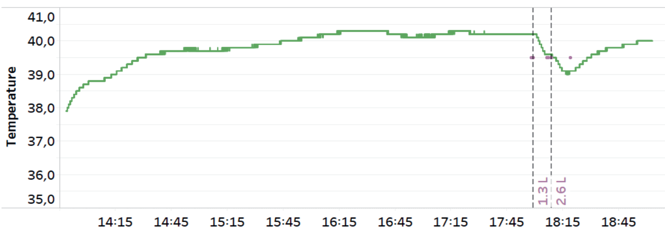
B Observation day 2 (cow 25)



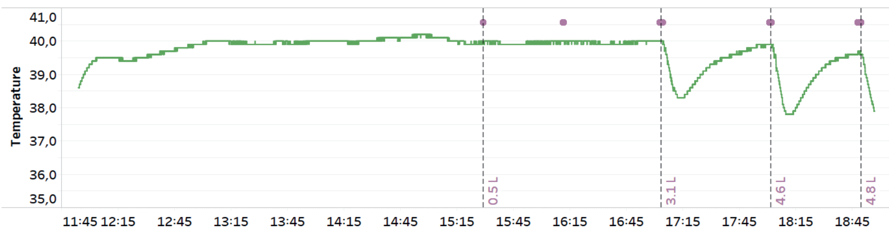
C Observation 3 (cow 21)



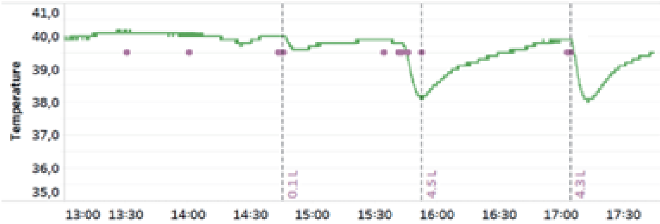
D Observation day 4 (cow7)



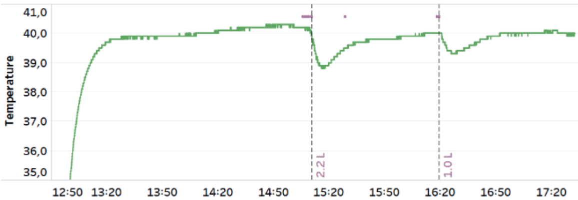
E Observation day 5 (cow 25)



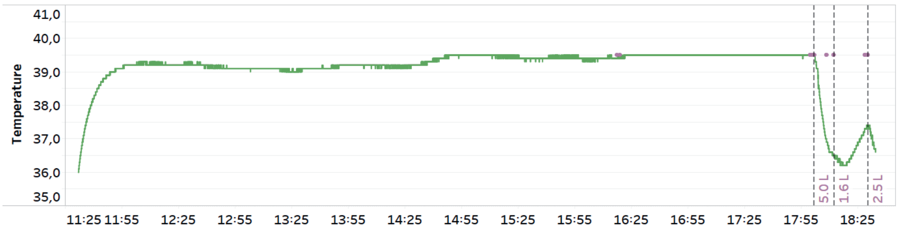
F Observation day 6 (cow 7)



G Observation day 7 (cow 8)



H Observation 8 (cow 21)



I Observation day 10 (cow 8)

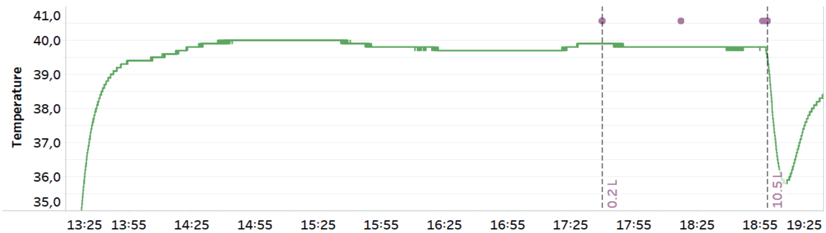


Figure 4. Reticular temperature and drinking
The green line represents the temperature measured with the reticular bolus; the independently observed drinking moments are highlighted in purple. For the drinking moments the measured amount of water intake is shown in litres. On observation day 9, the cow did not drink during the measurements.

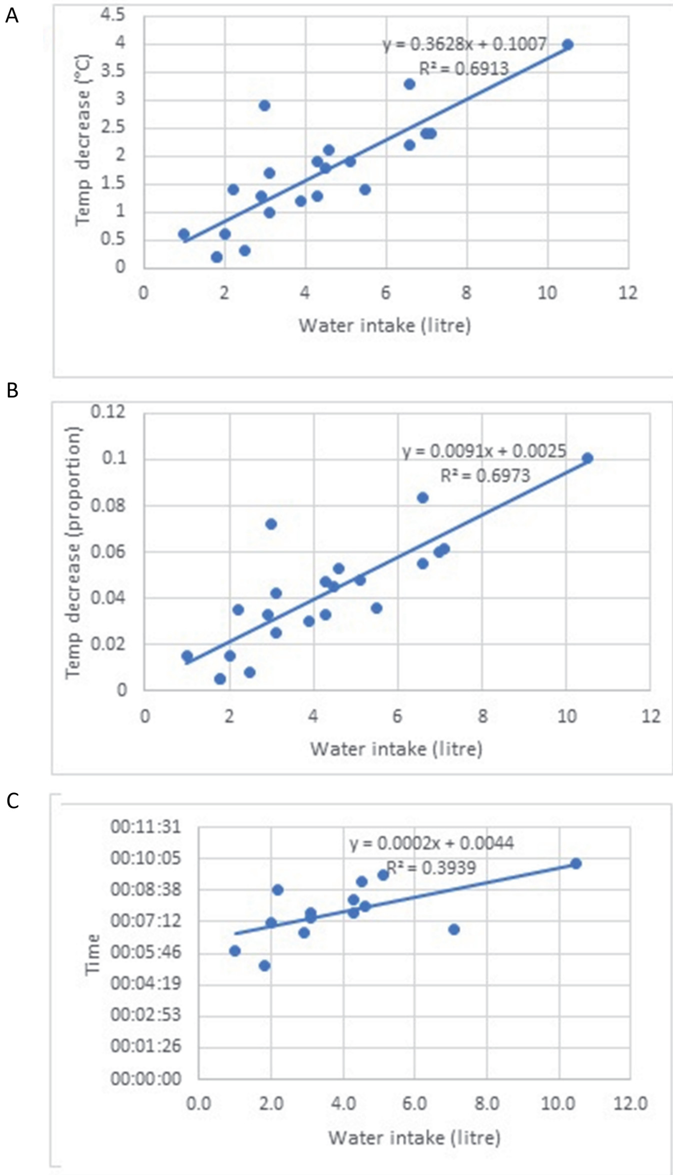


Figure 5. Drink amount and effect on temperature drop.

In Fig. 5A the relation between water intake in litres during a drink event and the absolute temperature decrease (°C) in the reticulum immediately after drinking measured with a bolus is shown. In Fig. 5B the relation between water intake and proportional decrease of temperature (°C) is shown. Fig. 5C shows the relation between the water intake in litres and the duration in seconds the temperature decreased after this drink.

Rumination

The reticular bolus detected the typical three-peak pattern of a reticulum contraction during rumination. The pattern of the pressure signal was comparable to that found in earlier studies (Arai et al., 2019; Dracy et al., 1972; Scheurwater et al., 2021; Sellers and Stevens, 1966). The height of the three peaks was very variable between the different experimental days and cows. Fig. 6 illustrates for each observation day one moment where the cow started ruminating and the three-peak pattern of a reticular contraction during rumination is detected.

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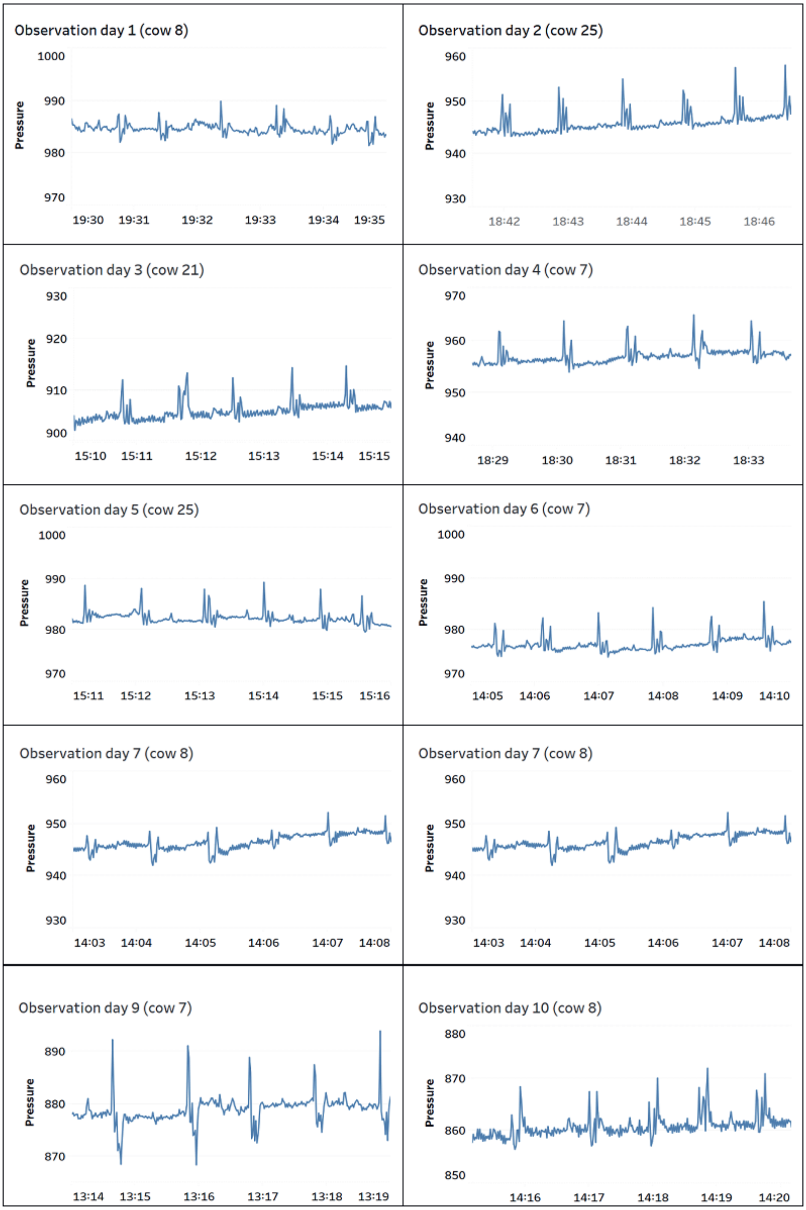


Figure 6. Reticular contractions during rumination
Pressure (mBar) signal is measured with the bolus in the reticulum during rumination on each observation day. Examples of the variation in three-peak patterns from all observation days and for all cows

Eating

By visual inspection of the pressure patterns, we did not observe a specific reticular pressure pattern when a cow was eating (Fig. 7). However, the time in seconds between two contractions appeared to be shorter during eating (Table 5) but was not significantly different most presumably due to the wide overlap across behaviours in time between contractions.

Figure 7. Reticular contractions during eating
Reticular pressure data is shown for periods of 12 minutes on two different observation days as measured with a sensor bolus in the reticulum. Different independently observed cow behaviours are marked including eating.

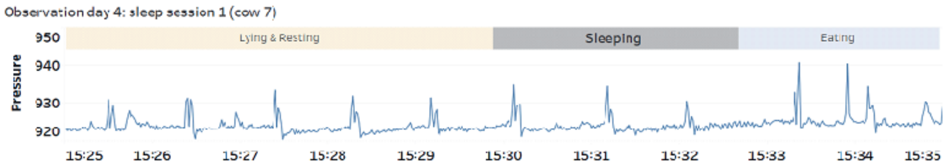
Table 5. Time in seconds between two contraction cycles during different types of cow behaviour

Behaviour	Mean (SD)	Median (IQR)
Rumination	50 (12.7)	51 (14.0)
Eating	38 (18.7)	35 (16.0)
Sleeping	50 (27.1)	44 (13.0)
Resting	42 (21.6)	39 (17.0)
Other	40 (23.1)	37 (14.5)

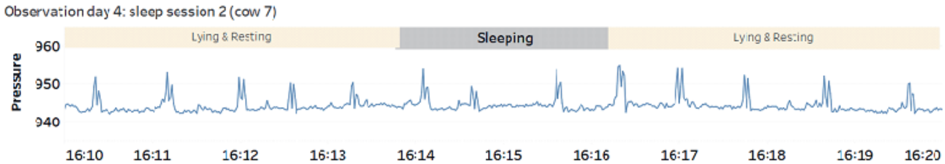
Sleeping

On four observation days sleeping behaviour was detected by video analysis, in total eight short sleep sessions were scored. As discussed in Scheurwater et al. (2021), there is no certainty of sleep because we scored sleeping based on direct observation and not on brain activity. Sleeping was defined as lying immobile in a sleeping position, in a ventral recumbency with the head retroflexed to the flank. In Fig. 8, all moments scored as sleeping on video recordings and the pressure data from the bolus are shown together. The moments scored as sleeping were only noticeably short periods of time including only a few reticular contractions. However, the data suggest that the amplitude of the pressure signal may be slightly lower and the time between two reticular contractions extended (Fig. 8).

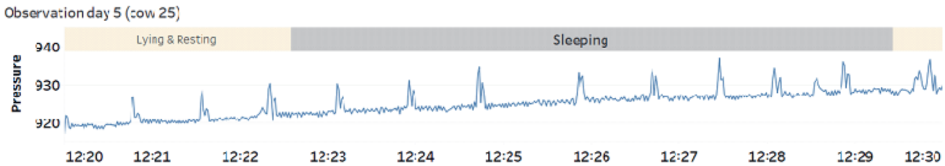
A


Observation day 4: sleep session 1 (cow 7)
Lying & Resting
Sleeping
Eating
Pressure
950
940
930
920
15:25
15:26
15:27
15:28
15:29
15:30
15:31
15:32
15:33
15:34
15:35

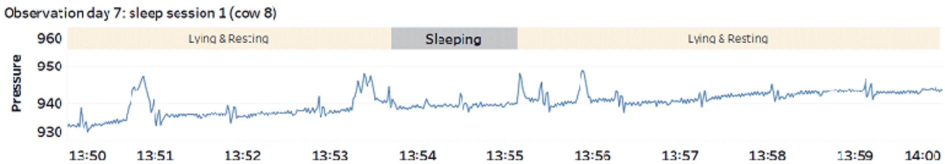
B


Observation day 4: sleep session 2 (cow 7)
Lying & Resting
Sleeping
Lying & Resting
Pressure
960
950
940
16:10
16:11
16:12
16:13
16:14
16:15
16:16
16:17
16:18
16:19
16:20

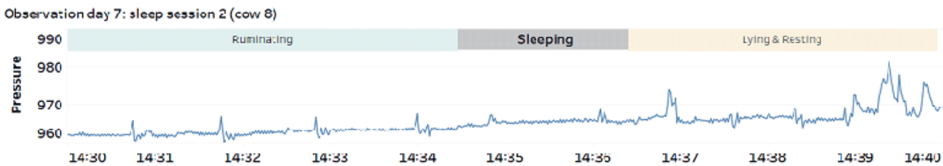
C


Observation day 5 (cow 25)
Lying & Resting
Sleeping
Pressure
940
930
920
12:20
12:21
12:22
12:23
12:24
12:25
12:26
12:27
12:28
12:29
12:30

D


Observation day 7: sleep session 1 (cow 8)
Lying & Resting
Sleeping
Lying & Resting
Pressure
960
950
940
930
13:50
13:51
13:52
13:53
13:54
13:55
13:56
13:57
13:58
13:59
14:00

E


Observation day 7: sleep session 2 (cow 8)
Ruminating
Sleeping
Lying & Resting
Pressure
990
980
970
960
14:30
14:31
14:32
14:33
14:34
14:35
14:36
14:37
14:38
14:39
14:40

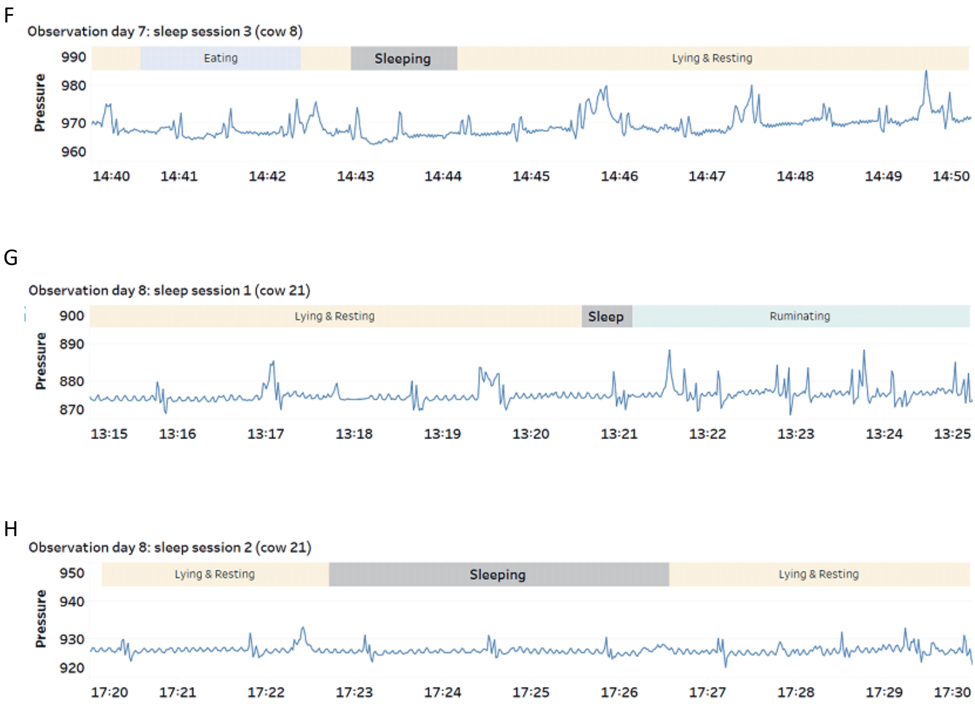
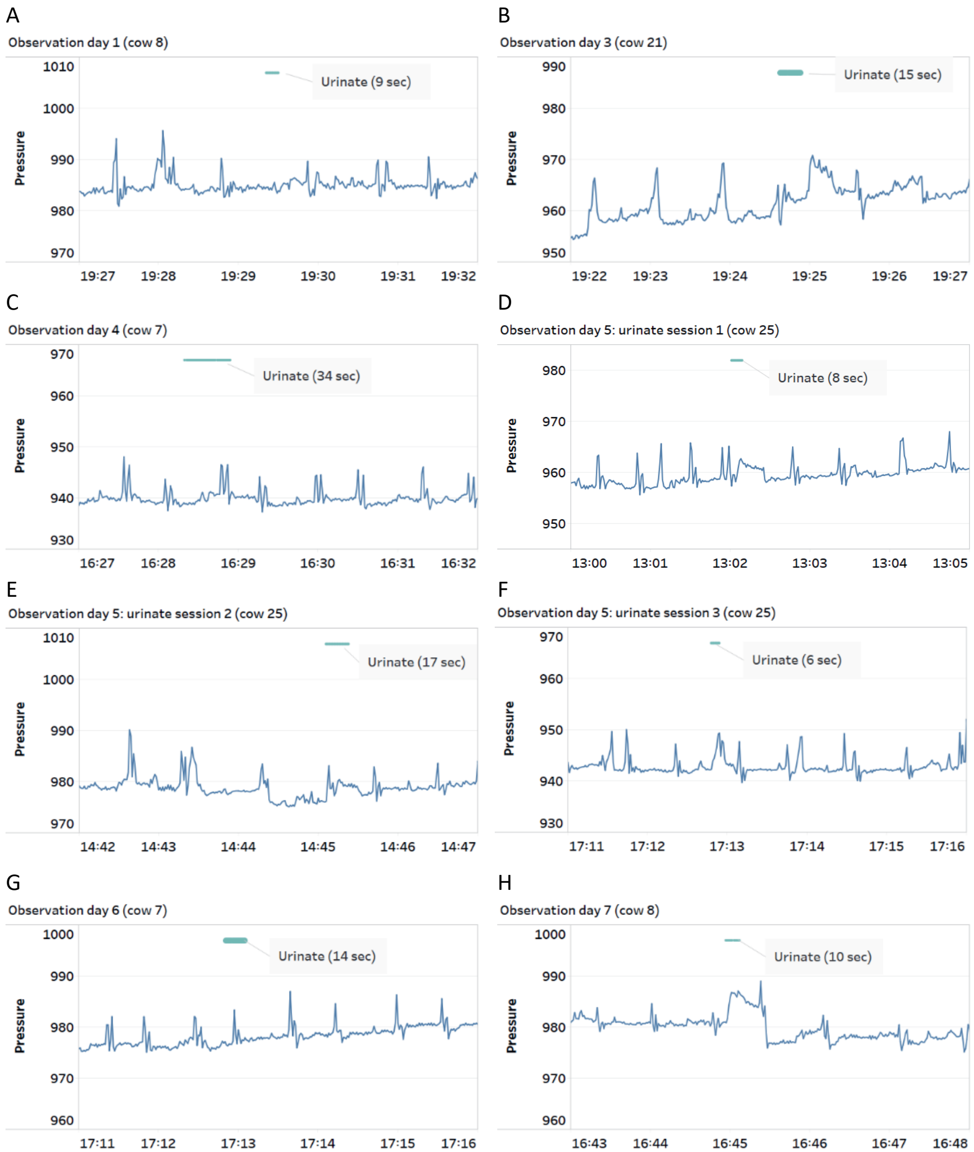


Figure 8. Reticular contractions during all sleep sessions.

Urinating

In total 25 urination events were scored during this study on eight observation days. The mean number of urination events was 2.4 (2.01 S.D., min 0 and max 6) per observation day. The mean duration of a urination event was 16 seconds (7.39 S.D., min 6 and max 30) in this study. These monitored events suggest that when a cow was urinating the reticular contraction frequency was not changed. The pressure data during a urination event seem to show at most of the events an increase in basal pressure level (Fig. 9). Urination is a short event and can take place during a reticular contraction or between two contractions. Mention that only on observation day 9 and 10 the monitored time matched exactly with the real time. On the other observation days there could be a discrepancy up to 15 seconds between the measured time and the real time, leading to the real urinate event to be maximal 15 seconds earlier or later.


A
Observation day 1 (cow 8)
Urinate (9 sec)
Pressure
1010
1000
990
980
970
19:27
19:28
19:29
19:30
19:31
19:32
B
Observation day 3 (cow 21)
Urinate (15 sec)
Pressure
990
980
970
960
950
19:22
19:23
19:24
19:25
19:26
19:27
C
Observation day 4 (cow 7)
Urinate (34 sec)
Pressure
970
960
950
940
930
16:27
16:28
16:29
16:30
16:31
16:32
D
Observation day 5: urinate session 1 (cow 25)
Urinate (8 sec)
Pressure
980
970
960
950
13:00
13:01
13:02
13:03
13:04
13:05
E
Observation day 5: urinate session 2 (cow 25)
Urinate (17 sec)
Pressure
1010
1000
990
980
970
14:42
14:43
14:44
14:45
14:46
14:47
F
Observation day 5: urinate session 3 (cow 25)
Urinate (6 sec)
Pressure
970
960
950
940
930
17:11
17:12
17:13
17:14
17:15
17:16
G
Observation day 6 (cow 7)
Urinate (14 sec)
Pressure
1000
990
980
970
960
17:11
17:12
17:13
17:14
17:15
17:16
H
Observation day 7 (cow 8)
Urinate (10 sec)
Pressure
1000
990
980
970
960
16:43
16:44
16:45
16:46
16:47
16:48

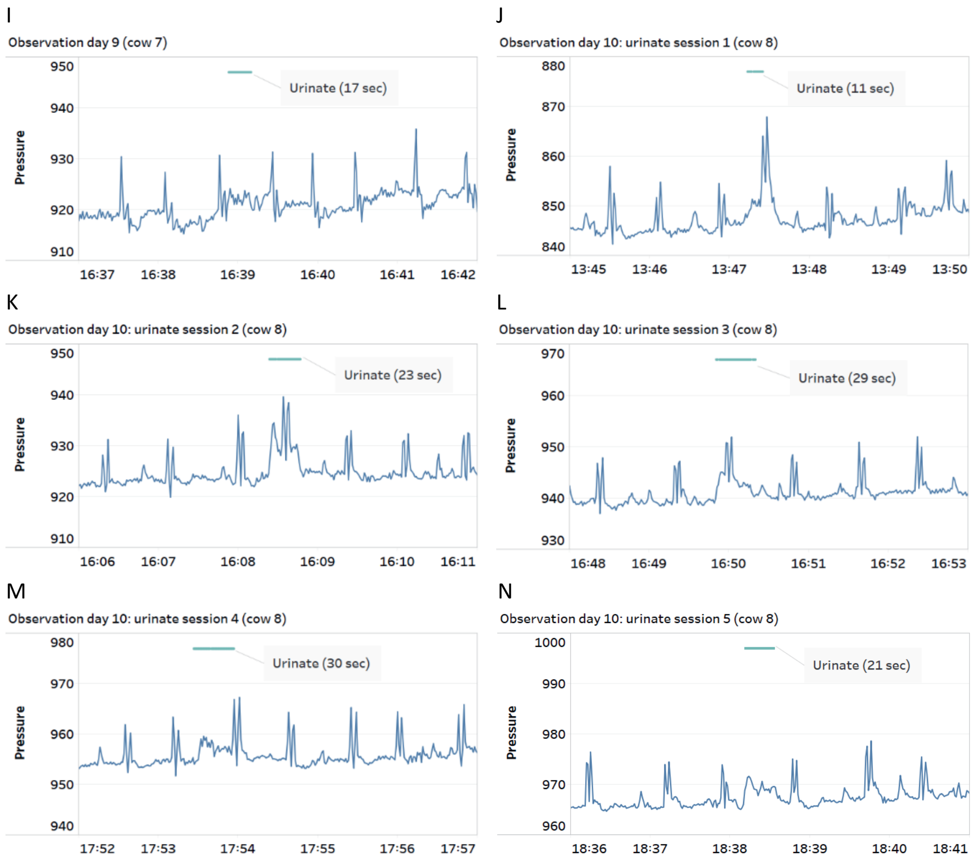
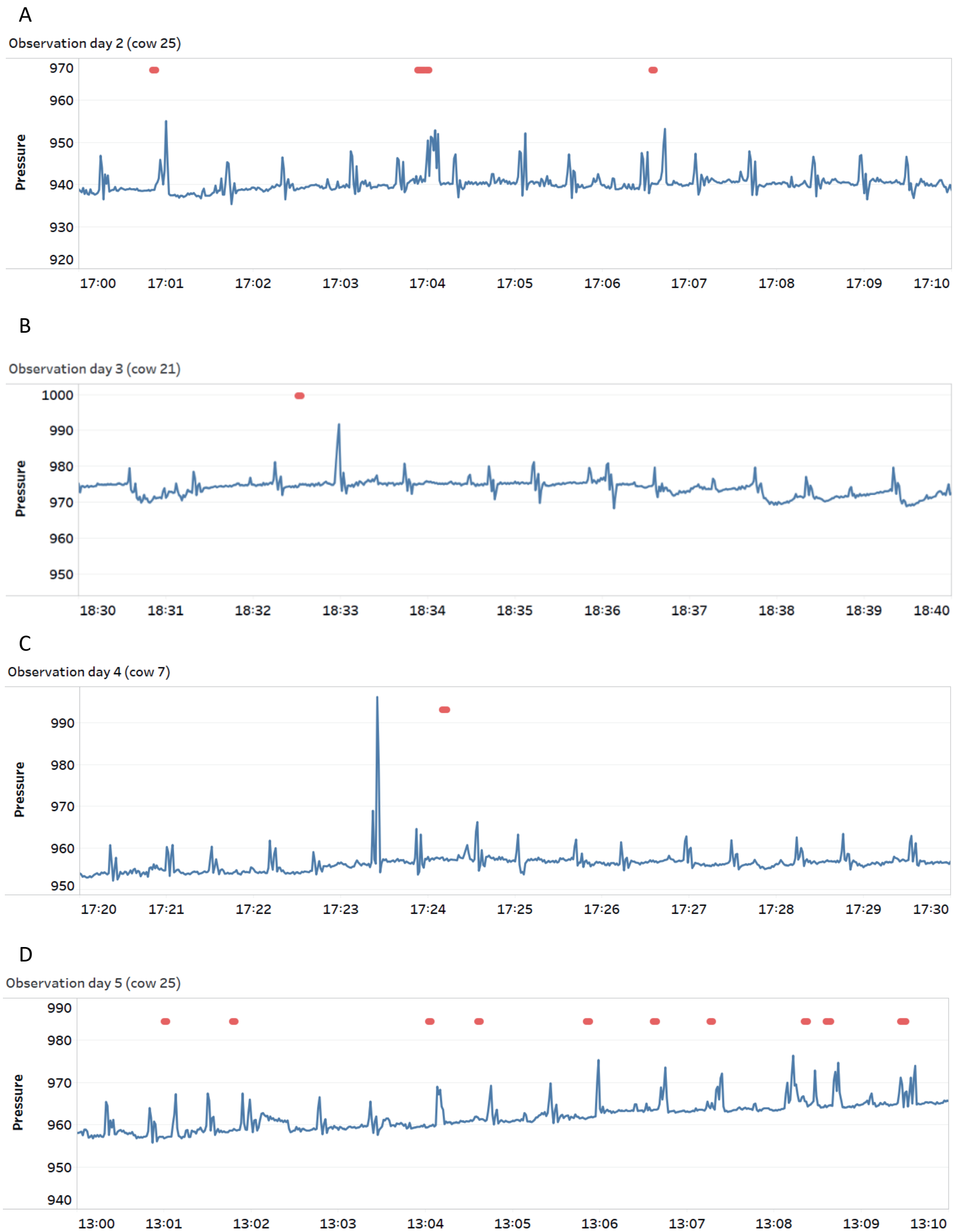


Figure 9. Reticular contractions during a selection of urination sessions
The reticular pressure measured with a bolus (blue line) is shown during urination. From each urination session (green bar) the duration in seconds is shown.

Mooing

The frequency of mooing during an observation day was variable. On two of the ten days no mooing was observed, on four of the days mooing was observed 1-2 times and on the other four days one of the cows was mooing very frequently during the whole day. The data from two of the four days with frequent mooing came from one specific cow and the other two days from another specific cow. The two other cows were only mooing maximum 1-2 times during the observation day. One mooing event takes only two or three seconds but can be repeated very often due to the restlessness of an animal caused by, for example, oestrus related behaviour. The short duration of a mooing event caused that mooing could occur in any moment of the reticular contraction cycle. The data suggest that mooing generates a pressure peak independent of the moment in the contraction cycle (Fig. 10). An extra peak is visible between two contractions and also when occurring at the moment of a contraction, an extra increase in pressure is visible. We see in Fig. 10 G and H, that the moment of mooing matches exactly in time with the peak pattern in pressure of the reticulum on observation day 9 and 10 (Fig. 10 G and H). As noted in the Materials and Methods section there is a discrepancy between the signal rate and the video time on the other observation days. This could explain deviations between mooing observation and the measured pressure signal on those days.


A
Observation day 2 (cow 25)
Pressure
970
960
950
940
930
920
17:00
17:01
17:02
17:03
17:04
17:05
17:06
17:07
17:08
17:09
17:10
B
Observation day 3 (cow 21)
Pressure
1000
990
980
970
960
950
18:30
18:31
18:32
18:33
18:34
18:35
18:36
18:37
18:38
18:39
18:40
C
Observation day 4 (cow 7)
Pressure
990
980
970
960
950
17:20
17:21
17:22
17:23
17:24
17:25
17:26
17:27
17:28
17:29
17:30
D
Observation day 5 (cow 25)
Pressure
990
980
970
960
950
940
13:00
13:01
13:02
13:03
13:04
13:05
13:06
13:07
13:08
13:09
13:10

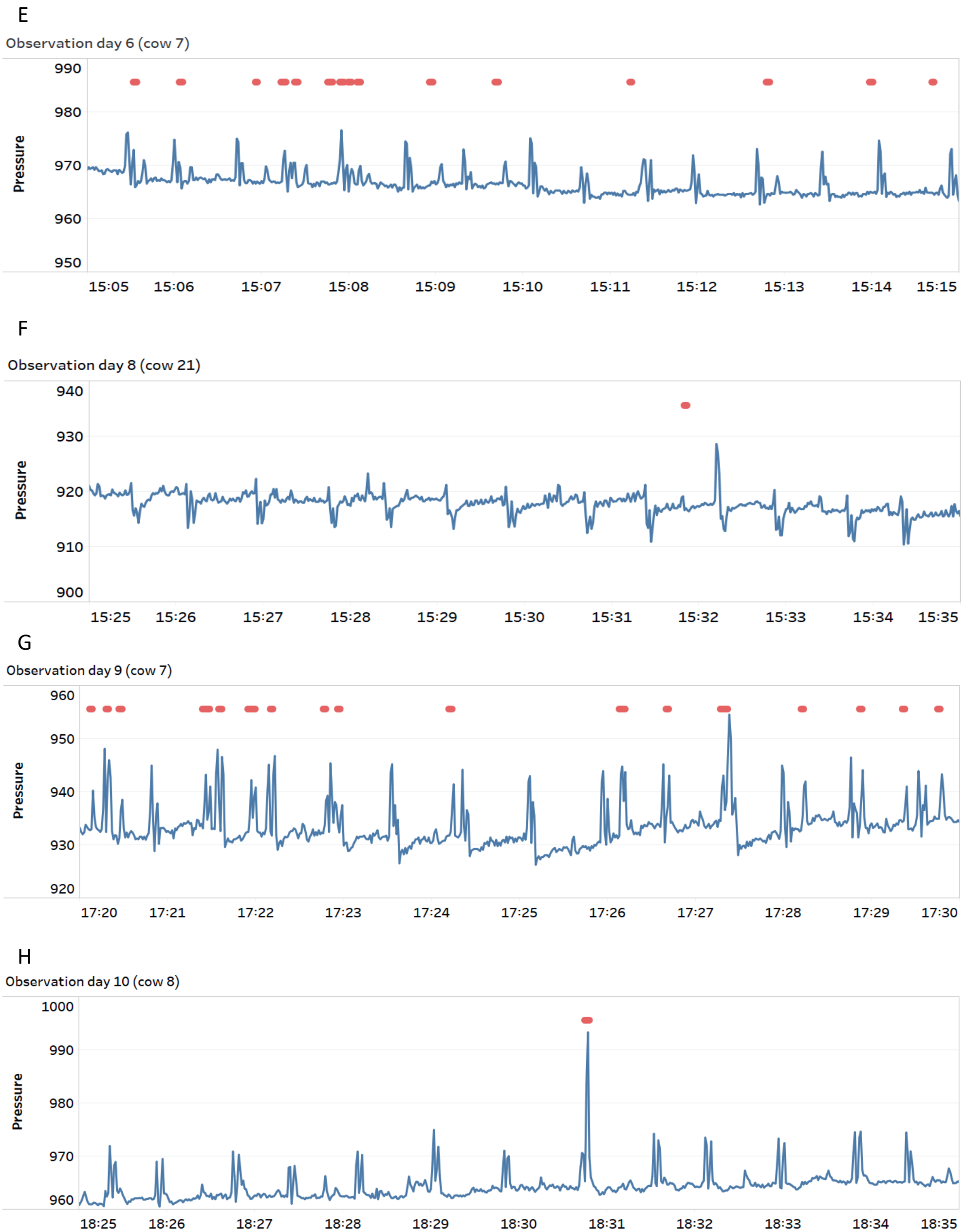


Figure 10. Reticular contractions during a selection of mooing sessions.
Red dots represent video-detected mooing events and the blue line the reticular pressure measured by a bolus.

DISCUSSION

The prototype bolus studied here was able to detect drinking patterns and reticular contraction patterns accurately via a bolus for measuring actual temperature and pressure with a frequency of 2 Hz. Temperature decreases were closely related to drinking behaviour and pressure measured seems to be related to cow behaviours rumination, eating, resting, sleeping, urinating, and mooing. The bolus could detect a specific pressure pattern for rumination. The prototype bolus has a potency for various applications, both in practice and in research settings to monitor, understand cow behaviour.

The bolus provides a reliable instrument to measure changes in drinking pattern in detail on a fine temporal scale and to monitor the water intake of individual cows. For example, it could be interesting to use this bolus to study the relation between heat stress and the change in drinking behaviour. The importance of access to sufficient drinking water for farm animal welfare is widely recognized and is reflected by the many studies on this topic (Golher et al., 2021). There is large individual difference in water intake between cows. For example, a dry pregnant cow drinks around 35 L/d water and a lactating cow with a milk yield of 28 kg/d drinks around 75 L/d water (Jensen and Vestergaard, 2021). Holstein dairy cows can drink at a rate of up to 24 L/min with higher intake rate when dairy cows drink from troughs (Jensen and Vestergaard, 2021). Dairy cows housed indoors drink between 5 and 20 times a day (Jensen and Vestergaard, 2021). In our study one cow did not drink during a period of six hours whereas another cow had 10 drinking bouts in five hours. It took up to 1.5 hour until the reticular temperature reached the level from before drinking depending on the amount of water ingested comparable to 2h in a study by Ammer et al. (2016). Furthermore, stocking density at the water bowls and social dominance have influence on water intake (Cardot et al., 2008; Jensen and Vestergaard, 2021). The cows in our study were housed individually and had ad libitum access to their own water bowl. In our study we also see a wide diversity in drinking behaviour ranging from drinking every hour a small amount of water, not drinking at all during the data collection, until drinking in one or two drinking bouts.

Rumination can be detected with the pressure sensor in the bolus. To detect the typical three-peak pattern during rumination the sampling frequency needs to be high enough.

When a cow appears to sleep the amplitude of the pressure signal seems to be a bit lower and the time between two reticular contractions extended, but we did not detect a total absence of rumen motility as detected by Church (1976). The different outcome of the studies could be caused by sleeping periods not being long enough or because the cows did not reach a sufficiently deep sleeping state during our study. More data is needed to study sleep behaviour in cows and the effect on reticular contractions and it can be helpful to monitor during the night when cows appear to sleep more frequently (Fukasawa and Takahashi, 2022). To define a true sleeping period, brain activity should be measured simultaneously to act as a gold standard instead of via observation by video as in the present study.

In our study, we detected extra peaks in reticular pressure during vocalization, but extra peaks could also be linked to other noise. Automatic detection of vocalization can be used as a non-invasive measure of wellness (Liseune et al., 2021a; Marshall et al., 2021). Results from other studies are also promising for the future use of vocalisation in automatic oestrus detection systems (Röttgen et al., 2020, 2018). The vocalisation rate could potentially be used as a suitable indicator of oestrus climax in automated detection devices (Röttgen et al., 2018). In all these studies the sound was recorded, and livestock noises had to be separated from external noises. The main challenge in using vocalisation to detect oestrus is correctly identifying the calling animal when animals are moving freely in large groups (Röttgen et al., 2018). A system for classifying cattle vocals and removing background noise with a convolutional neural network (CNN) is described by Jung et al. (2021). More research is needed to study the relation between the extra peaks and mooing. By measuring vocalisation with the reticular contractions no extra device recording sound with a microphone is necessary.

Since the growing concerns about nitrogen there has been an increase in research focusing on techniques to evaluate urination behaviour in grazing dairy cows (Marshall et al., 2021; Selbie et al., 2015; Shepherd et al., 2017; Shorten et al., 2022; Shorten and Welten, 2022). Different equipment attached to the cow to collect and measure urine at each urination event is studied (Marshall et al., 2021; Shepherd et al., 2017; Shorten et al., 2022; Shorten and Welten, 2022). In dairy cows there does not seem to be any relationship between frequency of urine eliminations and milk yield or feeding intensity. There are individual differences in urine eliminative behaviour, but in general the frequency of urine eliminations is lowest during resting periods (Aland et al., 2002). The differences in the frequency of urination between individuals underline the need for individual-based management (Aland et al., 2002). The results from our study suggest that it is possible to detect change in reticular pressure during a urination event. More data and research are needed to specify an algorithm to detect urination with a reticular bolus. The addition of a location sensor to the bolus, for example GPS, would be valuable for knowledge of where and when a cow is urinating and would allow for further fine-tuning of management systems to decrease nitrate leaching.

The bolus was still in the developing phase during this study. The pressure data available for this experiment were sampled with the bolus with a water-filled head, leading to an overall increasing trend in the pressure at the time scale of hours. A version of the bolus with a silicone-filled head would be better to use in future studies, because the pressure would not be influenced by a gas bell. Another aspect is the sampling frequency. The sampling frequency was around 2 Hz, resulting in a maximum difference of 15 seconds between sample time and real time over a period of eight hours. In the last two observational days the Real Time Clock (RTC) was used for the moments of sampling resulting in a minimum discrepancy between sample time and real time. In future research RTC should be used. Specifically for longitudinal measurements over hours, the influence of not having the exact time between samples increases. The increasing discrepancy between sample time and real time complicates especially pattern detection during short events like urination, mooing and sleeping. Short duration events can take place between two reticulorumen contractions or simultaneously with a reticulorumen contraction influencing the pressure change.

Pattern detection by, for example, machine learning techniques, of our data should take the (fixed and known) discrepancy into account.

In this study, we used rumen-fistulated cows. To be able to measure reticular contractions in non-cannulated cows with the same bolus, there are some technical challenges to resolve. The battery runtime was enough for one measuring day of eight hours when measuring temperature and pressure twice per second. Measuring twice per second will always be battery consuming and an issue for longer measurements. The frequency of sampling twice per second is needed to detect the distinct peaks in a contraction wave. It could be an option to measure at larger time intervals. For example, when a cow is ruminating, one could check every 15 minutes whether rumination continues. For use of the bolus in non-cannulated cows the data collection on an SD card will not be a satisfactory solution. The bolus now uses a LoRa peer-to-peer communication, but the signal sent out of the cow is not strong enough to detect further away from the animal. There is a maximum output power that is allowed for communication with LoRa (LoRaWAN, 2023). When cows walk freely through the stable, the distance to detect the signal needs to be more than a few meters. It could be a solution to equip the animals with a collar where the signal from the bolus is collected sent from inside the cow and send further to another location.

This is a proof-of-principle study to investigate whether the prototype bolus can detect patterns that can be linked to a range of cow behaviours. The bolus and its signal in pressure and temperature show to be promising in this regard. The interpretation of this promise should be treated with caution for several reasons and should be seen as indicators for specific further empirical research. Data from various observational days show large variation in amplitude and peak patterns during reticular contractions, and noise disturbance fluctuates. The bolus moves freely through the reticulum and the exact location or position of the bolus could influence the pressure signal. The exact position could also influence the noise by other pressure signals from outside the reticulum. For short-term behaviours like mooing, sleeping, and urinating only few events occurred. More events are needed to have sufficient data to create algorithms. Preliminary results (Appendix 2) demonstrated that rumination of a cow can be detected with a random forest algorithm. The random forest algorithm showed robust performances for detecting rumination but performed worse in a leave-one-out analysis, possibly because of the large variation in the data and the small number of cows in the study. With the random forest technique, we needed data from all cows in our train dataset to get reliable results for detecting rumination. Only four animals were used in this study and a dataset with more animals is needed to study the difference in reticular contraction pattern between animals. Probably, due to the large variation in peak height between observational days, the performance of the peak-detection algorithm published by Scheurwater et al. (2021), Chapter 4 of this thesis, was insufficient to set one value to define a peak. The performance of the peak detection algorithm on all data was poor, for this reason, but on the data of only one observational day, with clear peaks the performance of the algorithm was high.

In this study we limited ourselves to visual inspection of patterns. However, even with only visual detection differences in contraction patterns were observed during various important cow behaviours. This offers opportunities for further research, notably the development of more advanced algorithms such as machine learning techniques.

CONCLUSION

The prototype bolus studied here can accurately measure temperature and pressure in the reticulum of cows. The contraction pattern of the reticulum can be detected, and drinking can be monitored in detail. Rumination, eating, resting, sleeping, urinating, and mooing is detectable with measuring actual pressure with the bolus. For further development of the bolus and algorithms for monitoring the potential detectable behaviours more specific research is needed focusing on the specific behaviour.

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Appendix 1: Two versions of the bolus

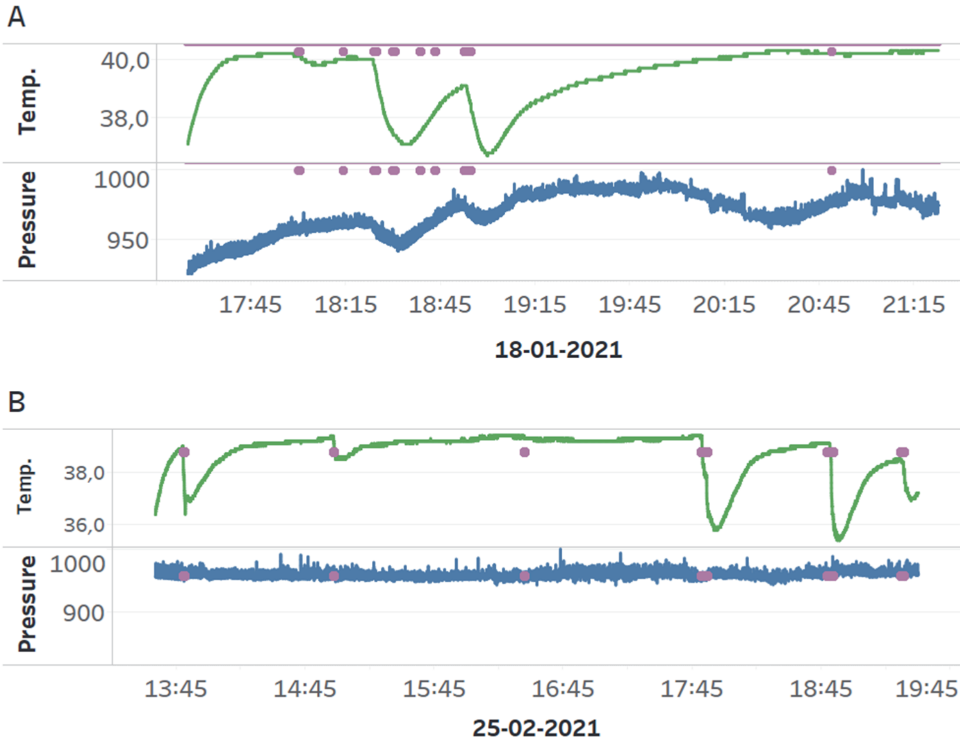

Figure S1. Reticular pressure and temperature measured with two versions of a reticular bolus.
A: data from a bolus with a bolus head filled with water. B: data from a bolus with a bolus head filled with silicone. The green line represents the temperature (°C) measured in the reticulum per 0.5sec. The blue line represents the pressure (mBar) per 0.5 sec and in purple the drinking moments scored with video recordings were shown.

Appendix 2: Algorithms for pattern detection

MATERIALS AND METHODS

The feature preparation for the RF algorithm was the same as used in Scheurwater et al. (2021) and was briefly as follows. We normalized the pressure data, after which the data was partitioned in windows of 120 sec with an overlap of 119.5 sec. For each sliding window containing 240 pressure timepoints, 30 low-dimensional features were generated based on frequency with an algorithm that comes from a notebook written by Marcos Duarte using Fourier transform, power spectral density and autocorrelation. Based on these features, an RF classification model was developed to predict each behaviour type separately. The full code can be found at (https://doi.org/10.5281/zenodo.10033989). Each separate behaviour type was predicted against a single class of all other behaviours, called the one-vs.-all technique. For rumination, we used a five-fold stratified cross and Leave-Out-One-Animal (LOOA) validation technique (Scheurwater et al., 2021).

RESULTS

Table S1 summarizes the results of analysis of the experimental data with a Random Forest algorithm and peak-detection algorithm similar as used in Scheurwater et al. (2021), Chapter 4 of this thesis. Rumination could be detected with an accuracy of 0.96, a sensitivity of 0.93, and a specificity of 0.98. The RF algorithm could detect rumination when trained on 70% of the dataset and tested on the other 30% with an accuracy of 0.99, a sensitivity of 0.99, and a specificity of 1.00. Similar as shown by Scheurwater et al. 2021, when the RF was trained on data of three cows and tested on the data of the fourth cow, the LOOA validation, the sensitivity and accuracy were much lower (see Table S2). The peak-detection algorithm detected rumination with an accuracy of 0.78, a sensitivity of 0.45, and a specificity of 0.93. However, an overall value for the gain to define a peak is difficult due to the diverse pattern of peak heights (Fig. 6). The peak-detection algorithm showed better results on the data of only 24-02-2021, the last experimental day within the study.

Table S1. Performance of algorithms for rumination.

Algorithm	Se	Sp	PPV	NPV	F1	Acc
Peak-detection (All files)	0.45	0.93	0.73	0.79	0.56	0.78
Peak-detection (only 24-02)	0.93	0.98	0.95	0.97	0.94	0.96
RF 70/30	1.00	0.99	0.97	1.00	0.98	0.99
RF SCV [a]	1.00 (0.001)	0.99 (0.000)	0.95 (0.002)	1.00 (0.000)	0.97 (0.001)	0.99 (0.000)
RF LOOA [b]	0.46 (0.260)	0.84 (0.065)	0.18 (0.087)	0.94 (0.046)	0.23 (0.081)	0.80 (0.037)

RF, Random Forest; SCV, Five-fold Stratified Cross Validation; LOOA, Leave-out-one-animal based on four cows.
[a] average and standard variation over 5 folds.
[b] average and standard deviation over four cows.

Table S2. Random forest algorithms (RF 70/30) for different types of behaviour.

Behaviour	TP	FP	TN	FN	Se	Sp	PPV	NPV	F1	Acc
Rumination	23452	927	104822	77	1.00	0.99	0.97	1.00	0.98	0.99
Eating	32399	1872	94810	197	0.99	0.98	0.95	1.00	0.97	0.98
Sleeping [a]	1010	9	1014	5	0.99	0.99	0.99	0.99	0.99	0.99
Mooing [a]	877	7	909	41	0.96	0.99	0.99	0.96	0.97	0.97
Drinking [a]	1355	22	1345	32	0.98	0.98	0.98	0.98	0.98	0.98
Urinating [a]	323	10	330	3	0.99	0.97	0.97	0.99	0.98	0.98
Resting	62553	1178	64154	1393	0.98	0.98	0.98	0.98	0.98	0.98

[a] numbers reflect the resampled dataset to balance the data

Chapter 6

General discussion

Introduction

The aim of this thesis was to investigate how sensors can contribute to making the health and welfare of a cow measurable. The potential of sensor data to enable more precise animal monitoring is widely acknowledged. However, on most farms, only the general alerts for health, being in heat and moment of calving are used to identify the animals that may need attention. Research using sensor data mostly focuses on early detecting diseases (Alipio and Villena, 2023). This thesis investigated the potential of a wider use of sensor data to monitor the health and welfare of cows on farms and in research.

The thesis can be seen as consisting of two parts:

In Part 1, we focused on commercially available sensors attached to the cow used in daily management and measuring standard characteristics. We showed the potential of these commercial sensors in characterising more complex health and welfare issues, such as heat stress and regrouping stress. We started with the former, more directly practice-driven question and used the data generated in the daily setting of eight commercial farms. The latter was a more science-driven question for which little is yet known. Hence, it makes more sense to explore this in an experimental setting where many aspects that might influence the results can be controlled. We again used a combination of different commercial sensors and moved from daily practice at a farm to a more experimental scientific exploration.

In Part 2, we then moved further into the experimental arena and dealt with developments for future sensors that can quantify individual cow characteristics within the animal. A proper functioning reticulorumen complex is essential for a healthy cow. With measuring by sensors attached to the animal we miss the information about the functioning of the interior of the reticulorumen. In Part 2 we focused on developing a sensor generating data about the cow from inside the reticulum. We provide proof-of-principle for two approaches. The first showing a method to measure reticulorumen pressure within the animal and linking these measurements to standard behaviours like rumination, eating, drinking and sleeping. The second is more ambitious and showed the development of a new bolus that can measure pressure and temperature within a cow. We showed proof-of-principle for such a device and showed that standard characteristics can be picked up by this sensor, just like a commercial 'on-cow sensor'. However, we also showed that the continuous detailed measurement of pressure and temperature can characterise relevant additional traits and patterns in standard traits that are much finer grained than from commercial sensors, allowing one to discover potentially new insights into health and welfare of the individual animal. This shows promise for future directions in monitoring individual cows housed in herds.

The thesis therefore moved from existing commercial sensors in their standard farm setting, used for a new question, to more experimental settings, first for new questions using the existing sensors, then leading to new developments that could be important for the future of sensor measurements and sensor innovation (from 'on-cow' to 'within-cow', from 'standard use in production settings' to 'wider use for more complex health and welfare issues', from production settings to experimental settings). In this final chapter, I will first summarize the main findings in the four research chapters. The studies have in common that they are about using sensor data of in principle happy and healthy cows (in any case cows were not selected because they were unhappy or unhealthy in specific ways). I will then use the experience with sensors in these chapters for a more general discussion of the need, possibilities, and challenges of characterizing a 'happy and healthy cow' using sensor data. I end with some remarks on possible future developments of sensors and the potential these have in a broader context of tracking cow health and welfare in herds.

Summary of the main findings

Chapter 2 used routinely collected sensor data available from 8 commercially dairy farms in the Netherlands to show that dairy cows in temperate climates begin to adapt their behaviour at a relatively low mean environmental temperature or THI. Adaptation to daily temperature and THI was already noticeable from a mean temperature of 12°C or a mean THI of 56. Data collected with neck and leg sensors on the cow during a period of 4 years was analysed in this study. The animals were equipped with a Smarttag neck sensor (measuring eating and rumination time) and a Smarttag leg sensor (measuring lying, standing, and walking time) both commercially available. The data streams were not synchronized to the daily clock time and daily data was used. With higher values for daily mean THI and temperature rumination time decreased, walking time decreased and cows spent less time lying and eating and more time standing.

Chapter 3 showed that cow introductions in dairy herds affect milk production and behaviour of animals already in the herd. To be able to detect even subtle changes, an experiment with a cross-over design was set up taking potentially influencing factors into account. Data was collected from individual cows with an on-cow neck and leg sensor and differences between both groups were minimized. The Smarttag leg sensor collected, within 15-minute time blocks, the number of minutes the cow spent lying down, standing and walking, and the number of transitions from lying down to standing. The HR-Tag neck sensor was positioned on a collar around the neck and monitored the number of minutes the cow spent on rumination in 2-hour time blocks. All data streams were synchronized to the daily clock time.

When cows were introduced to the herd, the cows already in the herd showed increased walking and reduced rumination activity and produced less milk.

Chapter 4 presented a proof of principle indicating that the known contraction patterns in the reticulorumen were detectable with a sensor in the reticulum of rumen-fistulated cows. Reticular pressure data was used to build algorithms to detect cow behaviours ruminating, eating, drinking, sleeping and 'other'. A sampling frequency of twice per second was frequent enough to detect the typical three peak contraction pattern during rumination and only measuring the A-wave in the reticulum would be enough to detect those different behaviours. There are two main limitations to this study. First, the number of animals is small and second, the wired pressure measurement system needs rumen-fistulated cows that are housed in a tie-stall barn. However, the results are promising for the development of future wireless pressure sensors in the reticulum to continuously monitor a range of important behaviours of cows measured by pressure differences in the reticulum, to detect specific cow behaviour (eating, rumination, drinking and sleeping).

In Chapter 5, we move further to a wireless bolus collecting data from inside the cow. We developed and tested a bolus measuring pressure differences and temperature in the reticulum. A proof of principle study is presented to investigate whether the prototype bolus can detect patterns that can be linked to eating, rumination, urinating, mooing, drinking, and sleeping. The prototype bolus can accurately measure temperature and pressure in the reticulum of cows and uses a LoRa peer-to-peer communication. The contraction pattern of the reticulum can be detected, and drinking can be monitored in detail. Rumination, eating, resting, sleeping, urinating, and mooing seem to be detectable with measuring actual pressure with the bolus. For further development of the bolus more specific research is needed.

Are there measurable parameters to define a happy, healthy cow?

In the introduction of this discussion, we suggest that we would like to measure the health and welfare of cows with sensors. However, health and welfare are not directly measurable parameters and there is a lot of overlap between possible read out parameters for both. When we conclude that a cow is healthy, in general we only mean that no signs of un-healthiness are detected. To define a happy cow is even more complicated because how to define the mental state of a cow? Most of the time, the focus is on avoiding negative welfare: the absence of disease, pain and suffering. However, the absence of signs of negative welfare does not mean that positive welfare is achieved (Council on Animal Affairs (RDA), 2021).

Farmers are trained to detect problems in the herd through years of experience and knowing the behaviour of their own individual cows. Each cow sends many signals about health and welfare, noticed by a farmer walking around in the stable, mostly during milking 2-3 times a day. Only a limited range of symptoms are directly visible, such as a swollen leg, skin problems, discharges, wounds, not being able to stand up, etc. Such visible signals can serve as proxies that an individual cow may need attention for more complex reasons that are then discovered upon closer examination, for example by a veterinarian. However, many reasons for attention may be missed because symptoms are vague, subtle, or not visible. Also, the inspection is a random assessment, only able to pick up signals that are visible at the precise moment that the inspection occurs. There are therefore likely to be many false negatives (cows with problems are missed) with a method based on visual signals alone, even for experienced farmers. In addition, this method is time consuming and is increasingly challenging in large herds with automatic milking systems, where the farmer is in general not there during milking.

Sensors can pick up signals that broaden the visual inspection, as well as allowing each individual animal to be monitored automatically, also in larger herds, and more frequently. The use of sensors adds signals to cow behaviour, a helpful read-out parameter for animal health and welfare. It therefore allows for a broader range of health and welfare alerts to be raised and will decrease false negatives, compared to the farmer's visual inspection. The health and welfare issues that can be detected directly increases, as do the indirect alerts in the sense that the broader range of signals highlight by proxy that an individual cow may need closer attention and examination. Sensors can not only be used as a diagnostic tool to detect specific health and welfare issues, but also more as a monitoring tool that raises an alert for the individual cows that may need attention by the farmer or a veterinarian to find a potential problem. It would be valuable to have sensors that cover a range of proxy variables that collectively have been shown to be indicative of the most common health and welfare issues faced by cows. Not only to be used for daily management on dairy farms, but also for monitoring cows for research purposes.

Nowadays, a variety of wearable sensors are commercially available for daily monitoring of cows in a herd. Lying, walking, standing, rumination and eating time are mostly monitored with sensors around the neck or leg and used to create an alert when the data deviates from the average, see Chapter 2 and 3. Most sensors work with alerts for health, heat and/or calving moment. The raw data and used algorithms are not available to the farmer. Some sensors correct for a herd effect, for example when all cows are running at the same time when moving from the stable to the pasture. Other sensors compare the data not only with a general mean value, but with the mean value for that specific animal. Milk production data of dairy cows is on most farms available as well; the availability of other milk parameters depends on the used milking system.

There are several issues though. For many health and welfare issues it is not known what should be measured for an individual cow. Even if it is known, it may be that the variables to be measured cannot be obtained in a non-invasive way (so, for example, without taking a blood sample to analyse, or performing exploration procedures). One can therefore often not measure what one would like to measure for a diagnosis, but also not for an alert. Instead of measuring what would be needed, we therefore measure what is possible and try to relate these variables to specific health/welfare issues or suspicions of those (alerts). Variables fall into two categories: directly/closely related to the specific health/welfare issue (such as fever, vaginal discharge, coughing, diarrhoea) and those variables that are indirect proxies (such as changes in eating behaviour, drinking behaviour, lying, standing, and walking behaviour). The sensors available currently are mostly for measuring those indirect proxies.

The challenge in monitoring by sensors and other inspections then is: which variables should we measure to collectively provide reliable evidence that the cow is healthy and happy, while at the same time identifying the few animals that might deviate? This challenge has many important and non-trivial aspects that need to be dealt with.

Structured consideration is needed to which health issues we want to be alerted to with priority. What are the indicators that one would like to have, that could be measured in principle? To define a healthy cow, a vet always checks respiration, pulse, temperature, ruminal contractions, mucous membranes, lymph nodes, and other obvious clinical abnormalities. The heart, lungs, rumen and udder are checked. Also, rectal exploration; vaginal discharges, or nasal discharges could contain valuable information; specific sounds like coughing could be indicative of respiratory problems and lameness suggests a locomotion problem. In milk, blood, faeces, urine and ruminal fluid, many parameters could be measured for information about the health of the cow. Most of those parameters are not measurable with sensors so far. When the number of those parameters that could be measured with sensors increases, more specific alerts could be generated, for example classified into groups, an alert for a locomotion problem or a digestion problem. More evidence can be collected to indicate a healthy cow.

Behaviour is a helpful read-out parameter for animal well-being. First, it must be defined which behaviour needs to be measured. In this thesis, cow behaviour is monitored in all chapters and the most prominent behaviours of a cow during the day were lying, ruminating, eating, standing, resting, drinking, walking, urinating, defecating, sleeping, mooing, and having social interactions.

The range of "normal" values and individual variation of the distinct behaviour patterns need to be better understood. There are many influencing factors on cow behaviour that need to be considered. In Chapter 2, we investigated the effect of climatic factors such as THI and temperature on cow behaviour. Cows live in hierarchical structured herds and have a lot of social interactions crucial for their well-being. In Chapter 3, we investigate the effect of introducing new cows on the cows already in the herd. We observed large variation in the behaviour of an introduced cow. Some cows introduced to the herd stayed quiet in the corner, while others confronted every cow they passed. I can imagine that the hierarchical position in the herd and the behaviour of the introduced cow, influenced the effect on the cows already in the herd. We did not find any differences between introducing one cow or three cows at the same time. However, it could be that the new cows were still recognised because they had been in the same herd before. The cows in our study were familiar with frequent regrouping. Despite that, we found effects of regrouping on the cows already in the herd. Dairy cows are highly motivated to lie down and lying behaviour does not appear to be a sensitive indicator of regrouping disturbances. Walking behaviour and rumination time are more sensitive indicators of the disturbance from regrouping. Also, milk production was negatively influenced by group disturbances. Chapter 3 demonstrates the added value of sensor data to monitor continuous cow behaviour 24/7 for research. To be able to measure social behaviour between cows, the distance and contact between individuals need to be measurable. Monitoring social behaviour like grooming or aggressive behaviour would be challenging to measure with sensors attached to the cow. Automatic video detection is probably more valuable.

For many (or even all) direct and indirect indicators, their 'normal' values, ranges and patterns may vary naturally in time throughout different lactation phases for an individual cow and may differ between cows. Alerts may also require combinations of variables as most issues will be multifactorial in the way they affect the proxies (in ways that are unknown). Several indicators that we can measure will be (strongly) correlated. It is a challenge to determine whether important dimensions are missed by focussing on the current set of variables that sensors can measure. Work is needed to determine how the current set is correlated and what type of measurable information would augment that in profound ways. Since what we can measure not necessarily (and often not at all) relates directly to specific health/welfare issues, one needs to get a sense of how much a measured variable should deviate from what is "normal" for a given individual, to indicate a potential (set of) underlying issue(s). Some variables we can measure in principle give an indication that this is an indirect assessment. For example, temperature is in principle a measurable indicator. In cattle, temperature can be measurements in the rectum, ear canal, rumen and vagina (Godyń et al., 2019). Which type of measurement reflects the temperature that we would like to know? In Chapter 5, we measured temperature in the reticulum. In my opinion, to be able to measure fever in the reticulum, smart algorithms are essential due to the large variation and fluctuations due to drinking.

There are technical challenges even for measuring variables that one could measure in principle. Can we measure them reliably, accurately, and at a frequency that is sufficiently high for our purpose? In Chapter 4 and 5, we investigated measuring reticular pressure patterns. To be able to get reliable data from inside the reticulum there are different technical issues to be solved. The sensor needs to stay in place, without the cow excreting the bolus again via rumination or via the further intestinal tract. There are sensors using 3-axis accelerometers to measure contractions, being complicated by the fact that the sensor freely moves through the reticulum. Another complexity is that the reticulorumen fluid breaks down not only feed particles but can damage the bolus and its functioning if used for a longer time. Because the sensor is in the reticulum, the bolus needs to stay in the reticulum and cannot be cleaned. The battery life span is another major issue. To be able to detect a signal sent from inside the reticulum outside the cow, the signal needs to be strong enough. Furthermore, frequent sampling is needed to differentiate different behaviours, like e.g., rumination and feeding. Both together will be battery consuming and an issue for longer measurements. However, long functioning is crucial since a sensor bolus remains in the non-fistulated cow's reticulum for a lifetime.

Sensors can be helpful tools to monitor cows continuously day and night. However, sensors may not disturb the cow in performing their normal behaviour (no negative impact on the welfare of the cow). The sensor needs to be robust and safe for the cow and comfortable to wear. Many of the commercially available sensors have not yet been independently tested under rigorous scientific conditions (Knight, 2020) and there is a lack of clear evidence of the economic benefits of using sensor systems (Rutten et al., 2013).

Another challenge is the enormous amount of data that needs to be managed. When sensors gather data 24/7, this data needs to be collected, processed and analysed to provide the right information. Artificial intelligence and machine learning algorithms can make use of this extensive data to generate an alert for the farmer that an animal needs attention (Neethirajan, 2020). For example, several models based on Conventional Neural Networks have been developed to evaluate changes in animal behaviour or are used for animal identification (Achour et al., 2020; McDonagh et al., 2021; Shen et al., 2020). In addition to this, big data and machine learning can help detect animal diseases earlier than conventionally possible (Neethirajan, 2020).

Possible future developments

In this final section, I reflect on potential future developments and innovations in the use of sensors for monitoring health and welfare of dairy cows in herds and experiments. When we are interested in monitoring the health and welfare of a cow with sensors, we need to measure many aspects. Sensors cannot take over a check by a veterinarian but can generate helpful information.

Especially when cows are grazing, they could be monitored easily outside by sensors. I think that sensors will become increasingly important in daily dairy cow management. Ideally, a sensor bolus in the reticulum would measure not only temperature and pressure but also analyse the fluid contents. However, there are some major technical challenges to overcome, like to measure reliably the whole life of a cow without running out of battery and without being able to clean the bolus. The frequency needs to be high to detect the distinct peaks in a contraction wave, but is could be an option to check only every 15 minutes whether the behaviour continues to save the battery. It could be a solution to equip the animals with a collar where the signal from the bolus is collected, sent from inside the cow, and sent further to another location. There, data about the environment could be collected and relevant sounds, for example coughing or mooing. The location of the cow can be registered and the distance from other cows. I think, especially with infectious diseases, this could be relevant and it could also make social behaviour more measurable.

There is increasing interest in understanding how we can provide quality of life to farm animals. Cows form complex social relationships in a herd and dominance is widely studied. Grooming and other affiliative behaviours could improve their well-being and need more attention. Most studies observe video or live observations, limited to short periods of time, because these methods are time-consuming. Sensor development focuses more on health and reproduction, and less on welfare. The use of sensors offers a promising complement to behaviour studies, as they can capture long-term animal monitoring data.

The data from the reticular bolus could be combined with a leg sensor with a 3-axis accelerometer. Maybe together, information about lameness can be collected. The important thing is that the used equipment does not interfere with the well-being of the cow. I think that, for example, sleeping would be behaviour to measure with sensors in future, relevant for determining health and well-being. Even though cows sleep only short periods, from other animals the importance of sufficient sleep is known, and a recent study showed comparable results in cows (Kull et al., 2019). A combination of data from more sensors at the same time (bolus and leg/or neck) could possibly make it easier to monitor sleep behaviour of cows.

With machine learning algorithms, the different data sets can be combined and translated into valuable data about the cow. I expect that the strength in monitoring with sensors will be in an inventive combination of various non-invasive sensors and that monitoring welfare and social behaviour with sensors needs more attention instead of only focusing on production. Especially, the potential for the further development of parameters that enable the measurement and monitoring of positive animal welfare needs to be investigated. Group dynamics play an important role in social interactions and a stable social group can serve as an important basis for positive welfare experiences (Council on Animal Affairs (RDA), 2021).

I envisage that in the future cows will be monitored 24/7 with sensors and the farmer will receive a specific alert when a cow needs attention, ideally before a cow will be suffering severe health or welfare problems. In this way, sensors will play a major and essential role in defining happy and healthy cows and in maintaining that status through timely management changes, preventive measures, and interventions.

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Summary

SUMMARY

This thesis is about the potential of a wider use of sensor data to get more insight into a variety of important welfare and health aspects of dairy cows. Sensors could provide information about each individual animal 24/7, not only when housed in a stable but also outside in the field. Many parameters could be monitored simultaneously by using different sensors, and large data sets could be generated. In all studies included in this thesis, the focus is on sensor data used to study healthy dairy cows (in any case cows are not selected based on specific health problems). In Chapter 2 and 3, different combinations of commercially available sensors attached to the neck and leg of the cow are used to monitor cow behaviour 24/7. Chapter 4 and 5 focus on the development of a sensor bolus measuring from inside the reticulum of the cow.

There are many potentially disturbing factors that even healthy dairy cows must deal with in daily life. One of those factors is the climatic condition of the environment, where high temperatures commonly lead to heat stress in cows. In Chapter 2, the use of sensors made it possible to study the effect of the weather on the time spent eating, ruminating, lying, standing, and walking by a large group of cows on eight commercial dairy farms in the Netherlands during a period of four years. Specifically, we looked at temperature and humidity, where the latter is measured as the so-called temperature-humidity index (THI). Chapter 2 demonstrates that dairy cows in temperate climates begin to adapt their behaviour already at a relatively low mean environmental temperature (>12°C) or THI (>56). From a mean temperature of 12°C, dairy cows started spending less time lying and eating and spending more time standing. Rumination time decreases, although only in dry cows and cows on farms with automatic milking systems. With higher values for the daily mean THI and temperature, walking time decreases as well. In the temperate maritime climate of the Netherlands, the results indicate that daily mean temperature suffices to study the effects of behavioural adaptation to heat stress in dairy cows.

Another disturbing factor for cows is regrouping since cows live in relatively stable herds on dairy farms and form relatively complex hierarchical social structures. Repetitive regrouping is common on most dairy farms and could lead to stress. Negative effects of regrouping have been reported in the literature but these focused on effects on the animals being introduced into the herd. We have studied the effects on the animals already in the herd when a new animal is introduced. In Chapter 3 we show that cow introductions in dairy herds negatively affect milk production and behaviour of the animals already in the herd. Many potentially influencing factors were taken into account in the analysis for a precise comparison between the two groups. During the period of replacement of cows (the treatment period) indeed the walking time increased, the rumination time decreased and milk production of the other animals in the herd were affected. During the treatment period these cows showed increased walking time and reduced rumination activity and produced less milk in the treatment period, up to 0.4 kg per milking on certain weekdays compared to the control period. Within 15-minute time blocks, the number of minutes the cow spent lying down, standing and walking, and the number of transitions from lying down to standing was collected by a leg sensor and the rumination time was collected by a neck

sensor in 2-hour time blocks. Sensor data of 25 dairy cows were included in this 2×3-week experiment. Using sensors, we could use a large quantity of repeated observations of two groups resulting in robust estimates of subtle effects. An average drop in milk production on Tuesday, Wednesday and Thursday was found during the treatment period. Even if the drop in milk production is small per animal, the economic impact could be a serious loss in kg milk for a commercial dairy farm. This study suggests that regrouping research should not focus solely on the regrouped animals but should take effects on all animals in the entire herd into account.

The use of sensor data largely increases the possibilities to study behaviour of cows in detail without disturbing the animals. In Chapter 2 and 3 the data we used were generated by commercially available sensors. However, there is not a sensor commercially available that could generate reliable detailed data from inside the animal. Sensors around the neck and leg are mostly used to generate data to study the types of behaviour regarded in Chapter 2 and 3. In the second part of this thesis we focus on the development of such an internal sensor that could generate data from inside the reticulum of a cow. A properly contracting reticulorumen is important for the health of a dairy cow. The function was discovered already nearly 100 years ago and methods to measure reticuloruminal pressure became more common in the nineteen fifties and sixties, generally using rumen fistulated cows with balloons or fluid filled catheters. The pressure measuring devices used were not described in detail and no gold standard for measuring reticulorumen pressure is available. In Chapter 4 a purpose-built pressure measuring device is presented that can detect the contraction patterns in the reticulorumen of rumen-fistulated cows. The contractions are measured during rumination, eating, drinking, and sleeping. The measuring device is described in detail and can be used as a golden standard for measuring reticulorumen contractions. A random forest algorithm, an artificial intelligence learning algorithm, was built to detect rumination and other cow behaviours based on reticular pressure. In addition, we present an algorithm for rumination based on visual inspection of patterns and peaks in reticular pressure data is presented (peak-detection algorithm). Chapter 4 demonstrates that rumination of a cow can be detected by measuring pressure differences in the reticulum using either the random forest algorithm or the peak-detection algorithm. The random forest algorithm showed very robust performances for detecting rumination with an accuracy of 0.98, a sensitivity of 0.95 and a specificity of 0.99. The peak-detection algorithm could detect rumination robustly, with an accuracy of 0.92, a sensitivity of 0.97 and a specificity of 0.90. In addition, we provide proof of principle that a random forest algorithm can also detect eating, drinking, and sleeping behaviour from the same data with performances above 0.90 for all measures. The data provide a proof of principle for future automatic monitoring of ruminating, eating, drinking, and sleeping behaviour of cows. A few orally applied wireless sensors migrating to the reticulum became available to measure pH or temperature. However, reticular pressure needs to be measured more frequently to distinguish between rumination and other behaviours. Chapter 4 indicates that behaviour detection using algorithms based on only frequent measurements in the reticulum is feasible. This is promising as it may allow future wireless sensor techniques in the reticulum to continuously monitor a range of important behaviours of cows, since wireless sensors will always migrate to the reticulum.

A sensor measuring actual pressure and temperature in the reticulum with a sample frequency of 2 Hz would be of great value to study reticular contraction patterns. Chapter 5 describes the development of such a wireless sensor bolus accurately measuring actual reticular pressure and temperature.

In total, 59 hours and 53 min of cow behaviour data were analysed resulting in 431,163 datapoints, based on four cows. Cow behaviour was scored from video data and used to train and test the same algorithms used in Chapter 4 of this thesis. The experimental data is analysed with the random forest algorithm and peak-detection algorithm. The random forest and peak-detection algorithms were developed for reticular pressure data measured with the device with water-filled open-tipped catheters described in Chapter 4. The pressure signal was in mBar instead of mVolt but showed a similar signal pattern during reticular contractions. Chapter 5 presents proof of principle that a prototype bolus with a pressure sensor can detect the reticular contraction patterns during rumination. Furthermore, the possibility to use this bolus to measure temperature change in the reticulum after drinking is investigated in this chapter.

The bolus provides a reliable instrument to measure drinking and changes in drinking patterns on a fine temporal scale and to monitor the water intake of individual cows. The temperature and pressure measurements can be linked to other types of cow behaviours such as ruminating, eating, sleeping, urinating, and mooing as well.

In all studies described in this thesis, sensor data is used to monitor the behaviour of dairy cows. The use of sensor technology allows monitoring the cows continuously, non-invasively and in a way that is not labour-intensive. Animal welfare itself cannot be measured directly but types of welfare-relevant behaviour can be derived from the data. Large numbers of animals can be monitored for years like in Chapter 2 or in small detail like in Chapter 3 to be able to find even subtle changes. This thesis shows that dairy cows in the temperate maritime climate of the Netherlands begin to adapt their behaviour at a relatively low mean environmental temperature and indicate that daily mean temperature suffices to study the effects of behavioural adaptation to heat stress in dairy cows. Furthermore, this thesis demonstrates that cow introductions affect welfare and milk production of the cows already in the herd and that regrouping research should not focus solely on behavioural effects on the regrouped animals but should also take the entire herd into account. And finally, the thesis shows that a single wireless sensor inside the reticulum can have potential value as a measuring device to monitor many variables and allow insight from combining data about the forestomach environment and behaviour of the animal.

Summary

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Dit proefschrift gaat over het gebruik van sensordata bij koeien om meer inzicht te krijgen in belangrijke gezondheids- en welzijnsaspecten. Sensoren kunnen 24/7 informatie verzamelen over ieder dier. Niet alleen over koeien die in de stal lopen, maar ook over dieren buiten in de wei. Door het combineren van meerdere sensoren kunnen verschillende parameters tegelijkertijd worden gemonitord en kunnen grote datasets worden gegenereerd. De focus ligt bij alle studies in dit proefschrift op het gebruik van sensordata bij gezonde melkkoeien (in ieder geval koeien die niet geselecteerd zijn op basis van specifieke gezondheidsproblemen). In hoofdstuk 2 en 3 worden combinaties van commercieel verkrijgbare poot- en neksensoren gebruik, om het gedrag van de koe continu te monitoren. In Hoofdstuk 4 en 5 gaat het over de potentie en de ontwikkeling van een nieuwe sensor: een sensorbolus die data genereert vanuit de netmaag.

Er zijn diverse verstorende factoren vanuit de omgeving waar koeien in het dagelijks leven mee te maken krijgen. Eén van die factoren is het weer. Het is bekend dat koeien niet goed tegen warmte kunnen. Hoge temperaturen leiden snel tot “hittestress”. Met de opwarming van de aarde en de verandering van het klimaat wordt dit een steeds relevanter thema, ook in gematigde maritieme klimaatzones zoals in Nederland. Het gebruik van sensoren maakt het mogelijk om veel koeien tegelijkertijd over langere tijd te monitoren. In Hoofdstuk 2 is hier gebruik van gemaakt en is gekeken naar het effect van het weer op het gedrag van koeien. Hiervoor is de sensordata van een grote groep melkkoeien op 8 commerciële melkveebedrijven geanalyseerd. Het effect van het weer op de tijd die wordt besteed aan de gedragingen eten, herkauwen, liggen, staan en lopen is bestudeerd gedurende een periode van vier jaar. Concreet hebben we gekeken naar temperatuur en luchtvochtigheid, waarbij deze laatste wordt gemeten als de zogenoemde temperatuur-vochtigheidsindex (THI). Hoofdstuk 2 laat zien dat bij melkkoeien in een gematigd klimaat, zoals in Nederland, al adaptatie van het gedrag optreedt bij een relatief lage gemiddelde omgevingstemperatuur (>12°C) of THI (>56). De koeien gaan minder lang liggen en eten, en de koeien staan meer. De herkauwtijd neemt af, maar alleen bij droge koeien en koeien op bedrijven met automatische melksystemen. Bij hogere gemiddelde waarden voor THI en temperatuur per dag neemt ook de looptijd af. Uit deze studie blijkt dat in het gematigde zeeklimaat van Nederland de gemiddelde temperatuur per dag voldoende is om de effecten van hittestress op het gedrag van melkkoeien te monitoren.

Een andere veelvoorkomende situatie waar koeien last van kunnen hebben is als er dieren uit de koppel gaan of als er nieuwe dieren worden toegevoegd. Koeien leven in een relatief stabiele groep met een complexe hiërarchische sociale structuur. Herhaaldelijk hergroeperen is op de meeste melkveebedrijven gebruikelijk en kan verstorend werken. Er zijn verscheidene onderzoeken naar de negatieve effecten van hergroepering bij koeien, maar deze gaan over de effecten op de nieuwe dieren die in de koppel worden geïntroduceerd. In Hoofdstuk 3 van dit proefschrift onderzoeken we juist het effect van het introduceren van nieuwe dieren op de dieren die zich al in de koppel bevinden. De introductie van nieuwe koeien heeft naast een effect op het gedrag, ook een negatief effect

op de melkproductie van de dieren al aanwezig in de koppel. Het gaat maar om marginale veranderingen, maar doordat het om de hele koppel gaat kan het bij elkaar opgeteld toch wel degelijk een economisch impact hebben.

Hoofdstuk 4 en 5 gaan over het meten vanuit de netmaag van een koe. Er is geen gouden standaard beschikbaar om drukverschillen te meten bij koeien in de pens. Dit terwijl een goed werkend voormagencomplex cruciaal is voor herkauwers. Honderd jaar geleden werd hier al aandacht aan besteed in diverse studies, echter de gebruikte systemen zijn niet in detail beschreven. Er wordt al lang gesuggereerd in de literatuur dat op basis van de contractiepatronen van het voormagencomplex van de koe onderscheid tussen verschillende gedragingen van de koe te maken is. Echter ook hiervoor geldt dat goede wetenschappelijke studies er over ontbreken. Tegenwoordig zijn er een paar oraal in te brengen draadloze sensorbolussen beschikbaar die naar het reticulum migreren en daar elke tien minuten de pH en/of temperatuur meten. De reticulaire druk moet echter frequenter worden gemeten om onderscheid te kunnen maken tussen bijvoorbeeld herkauwen en ander gedrag. In hoofdstuk 4 is een system beschreven en getest om reticulaire contractiepatronen gedetailleerd te kunnen meten, middels een zelf ontwikkeld system met water gevulde open-punt katheters.

Hoofdstuk 4 laat zien dat het herkauwen van een koe kan worden gedetecteerd met dit system door het meten van drukverschillen in het reticulum. Door visuele inspectie van patronen en pieken in de reticulaire druk is een algoritme (piekdetectie algoritme) opgesteld dat wordt gepresenteerd in dit hoofdstuk voor het detecteren van herkauwen. Ook is er met de Machine Learning techniek Random Forest een tweede algoritme opgesteld om koegedrag te koppelen aan druksignalen uit de netmaag. Het Random Forest algoritme laat zeer goede prestaties zien voor het detecteren van herkauwen met een nauwkeurigheid van 0,98, een gevoeligheid van 0,95 en een specificiteit van 0,99. Het piekdetectie algoritme kon herkauwen ook goed detecteren, met een nauwkeurigheid van 0,92, een gevoeligheid van 0,97 en een specificiteit van 0,90. Daarnaast staat in Hoofdstuk 4 een proof of principle dat met een Random Forest algoritme ook eet-, drink- en slaapgedrag kan worden gedetecteerd uit dezelfde drukdata met prestaties boven de 0,90 voor alle metingen. De gegevens bieden een proof-of-principle voor toekomstige automatische monitoring van herkauw-, eet-, drink- en slaapgedrag van koeien. Hoofdstuk 4 laat zien dat gedragsdetectie, met behulp van algoritmen die alleen gebaseerd zijn op metingen in de netmaag, mogelijk is en dat het niet nodig is om in de pens zelf te meten. Dit is belangrijk, omdat dit de mogelijkheden vergroot om in de toekomst met draadloze sensortechnieken continu koegedrag te kunnen monitoren. Immers zullen draadloze sensoren altijd naar de netmaag migreren. Een sensorbolus die de druk en temperatuur in de netmaag meet met een meetfrequentie van 2 Hz zou van grote waarde zijn.

Hoofdstuk 5 gaat over een prototype draadloze sensorbolus die nauwkeurig de druk en temperatuur meet in de netmaag. In totaal werd koegedrag geanalyseerd van vier koeien.

Het gedrag van koeien werd gescoord op basis van videogegevens en deze videodata werd gebruikt om dezelfde algoritmen te trainen en te testen als gebruikt in Hoofdstuk 4 van dit proefschrift. De experimentele gegevens worden geanalyseerd met het Random Forest algoritme en het piekdetectie algoritme. Het Random Forest- en piekdetectie algoritme zijn ontwikkeld voor drukdata uit de netmaag, gemeten met een apparaat met water gevulde katheters met open punt, beschreven in Hoofdstuk 4. Het druksignaal vertoonde een soortgelijk signaalpatroon tijdens netmaag contracties. Hoofdstuk 5 presenteert dat een prototype bolus met een druksensor contractiepatronen in de netmaag tijdens het herkauwen kan detecteren. Verder wordt in dit hoofdstuk getoond dat deze bolus gebruikt kan worden om de temperatuursverandering in de netmaag na het drinken nauwkeurig te meten.

De bolus biedt een betrouwbaar instrument om het drinken en veranderingen in het drinkpatroon op een nauwkeurige tijdsschaal te meten en de wateropname van individuele koeien te monitoren. De temperatuur- en drukmetingen kunnen ook worden gekoppeld aan ander koegedrag, zoals herkauwen, eten, slapen, urineren en loeien. In alle onderzoeken uit dit proefschrift, worden sensorgegevens gebruikt om het gedrag van melkkoeien te monitoren. Door het gebruik van sensortechnologie kunnen de koeien continu en non-invasief worden gemonitord op een manier die niet arbeidsintensief is. Welzijn kan niet direct worden gemeten, maar uit de sensordata kan welzijnsrelevant gedrag worden afgeleid. Grote aantallen dieren kunnen jarenlang worden gevolgd, zoals in Hoofdstuk 2, of in nauwkeurig detail, zoals in Hoofdstuk 3, om zelfs subtiele veranderingen te kunnen detecteren. Dit proefschrift laat zien dat melkkoeien die leven in een gematigd maritiem klimaat zoals in Nederland hun gedrag al beginnen aan te passen bij een relatief lage gemiddelde omgevingstemperatuur. Ook blijkt uit deze studie dat de dagelijkse gemiddelde temperatuur voldoende is om de effecten van gedragsaanpassing aan hittestress bij melkkoeien te onderzoeken. Bovendien laat dit proefschrift zien dat de introductie van koeien het welzijn en de melkproductie van de koeien die zich al in de koppel bevinden beïnvloedt en dat onderzoek zich niet alleen zou moeten richten op gedragseffecten op de geïntroduceerde dieren, maar ook rekening moet worden gehouden met de effecten op de rest van de dieren. En ten slotte laat het proefschrift zien dat een enkele draadloze sensor in de netmaag potentiële waarde heeft als meetinstrument bij koeien door het combineren van gegevens over het voormagencomplex en het gedrag van het dier.

Dankwoord

DANKWOORD

Nu is het tijd voor de laatste woorden van mijn proefschrift. Een raar idee, dat er een eind aan is gekomen na maar liefst zeven jaar. Eindelijk, zouden de meeste mensen denken, maar net als bij mijn studie diergeneeskunde, vind ik het vooral jammer. Ik heb nog genoeg ideeën, maar het is af en dat was nooit gelukt zonder de bijdragen van de mensen om me heen. Daarom is het nu de hoogste tijd om deze mensen, in willekeurige volgorde, te bedanken.

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Aan het enthousiasme van Arend kan niemand tippen. Alles wat ik weet over het voormagencomplex van de koe en de functie daarvan heb ik geleerd van Arend. Het is het door jou ontwikkelde “bolletjes systeem” dat we hebben beschreven in hoofdstuk 4. Zonder jou was het vierde hoofdstuk er dus zeker niet geweest. Je hebt je maximaal ingezet voor de metingen en heel wat uren doorgebracht naast de koe. Zelfs de minder geïnteresseerde studenten wist je te motiveren. Ook de bolus heb je helpen ontwikkelen en je hebt veel mee gedacht over hoe we dingen op konden lossen. Ik ben dan ook trots dat we met het artikel uit hoofdstuk 4 eindelijk jouw systeem vast hebben kunnen leggen op papier.

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weer boven water kreeg wat ik nodig had. Ook Maurits verdient een extra vermelding. De nauwkeurigheid waarmee hij de beelden heeft gescoord en de metingen heeft uitgevoerd waren van groot belang. Hierdoor hadden we data die we daadwerkelijk konden gebruiken. Bedankt voor al je inzet en werk. Verder wil ik Tim bedanken voor zijn hulp tijdens mijn hele experiment in de loopstal. Samen hebben we het hele praktische stuk uitgevoerd en ik heb fijn met je samengewerkt. Het was altijd gezellig en ik wil je ook bedanken voor het bekijken van alle beelden.

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About the author

ABOUT THE AUTHOR

Josje Scheurwater was born on the 18th of January 1985 in Dordrecht, the Netherlands. She obtained her gymnasium diploma from Johan de Witt gymnasium in Dordrecht in 2003. After that, she started with a Technical Mathematics study at the Twente University. She always had the intention to combine veterinary medicine with mathematics. Josje realized that a veterinary study was better to start with, so she changed directions and went to Spain to work in the meantime. In 2004, she started studying Veterinary Science at Utrecht University. During her study she developed a passion for dairy cattle. For her master thesis she went to Buenos Aires, in Argentina. After she graduated as a equine vet in 2012, she worked in a mobile equine clinic for two years. In 2014, Josje started with a propaedeutic year in Applied Mathematics at the Technical University in Delft and started her own equine clinic. In 2016, she started as a PhD-student at the department of Farm Animal Health of the Faculty of Veterinary Medicine of Utrecht University. During her PhD, Josje continued with her own equine clinic. Her PhD project called Happy Healthy Cow, was about sensor data use in cows. She worked on developing a wireless sensor bolus during the whole promotion project. For the first studies she used other sensors, but the last study was about a prototype of this wireless sensor bolus.