



# **Quantification of sleep in dairy cows in three different stages of lactation**



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**Institutionen för husdjurens  
utfodring och vård**

**Examensarbete 356  
30 hp E-nivå**

**Swedish University of Agricultural Science  
Department of Animal Nutrition and Management**

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**Uppsala 2011**



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**Key words:** Sleep, EEG, cow management, transition period

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## Abstract

The aim of this project was to quantify the total sleep time in modern dairy cows during 24-hour periods and to investigate whether the total amount of sleep as well as time spent in different sleep stages varies between the dry period, early- and peak lactation. The distribution of sleep time between night and day was also examined. Furthermore, correlations between sleep and lying time, fluctuations in body temperature and heart beat were included in the aim. Eight dairy cows of the Swedish Red breed were used in this study, which was carried out between June and September 2010. In order to quantify sleep non-invasive electrophysiological recordings were performed. Simultaneously with collecting sleep data, body position and body temperature were also recorded. No heart rate data was obtained due to difficulties of maintaining electrodes for heart beat measurements attached to the skin. One 24-hour data collection was performed on each cow in the three different stages of lactation. The electrophysiological data was visually scored according to definitions of human sleep. Electrophysiological data from one of the eight cows mainly contained artefacts and all her data was excluded from the data set. Shorter sleep time was obtained in early- and peak lactation compared with the dry period. The rapid eye movement (REM) sleep time and REM sleep time in proportion of total sleep was higher in peak lactation compared to early lactation. It could also be concluded that cows sleep during larger proportion of the night compared with the day. Indications of a possible correlation between total sleep time and total lying time was found, but no relationship between sleep and fluctuations in body temperature. Possible correlations between sleep and heart beat could not be investigated since no heart rate measurements was not obtained.

## Sammanfattning

Syftet med studien var att kvantifiera den moderna mjölkkons totala sovtid under 24-timmars perioder och att utreda huruvida den totala sovtiden, samt sovtid i de olika sömnstadierna, varierar i sinperiod, tidig- och topplaktation. Även fördelningen av sovtid mellan natt och dag studerades. Syftet med studien var dessutom att undersöka eventuella korrelationer mellan sömn och liggtid, kroppstemperatur respektive hjärtfrekvens. Åtta mjölkkor av rasen svensk rödbråkig boskap användes för försöket som utfördes mellan juni och september 2010. Sömn registrerades med en icke-invasiv metod för elektrofysiologiska mätningar. Kroppsposition och kroppstemperatur uppmättes samtidigt som sömnregistreringar utfördes. På grund av svårigheter att fästa elektroderna för hjärtfrekvensregistrering erhöles ingen hjärtfrekvensdata. En 24-timmars datainsamling utfördes på varje ko i vardera laktationsstadium. Data från de elektrofysiologiska mätningarna klassificerades visuellt enligt sömndefinitioner för människor. Elektrofysiologisk data från en av de åtta korna bestod i huvudsak av artefakter och all hennes data togs bort från datamängden. Kortare sovtid uppmättes i tidig- och topplaktation jämfört med i sinperioden. REM sömn och andelen REM sömn av totala sovtiden var högre i topplaktation jämfört med i tidig laktation. Det kunde fastställas att kor har längre sovtid under natten än dagen. Indikationer av korrelationer mellan ligg- och sovtid påträffades, men inga samband mellan sömn och variationer i kroppstemperatur. Eventuella korrelationer mellan hjärtfrekvens och sömn kunde inte utredas på grund av utebliven hjärtfrekvensregistrering.

## Introduction

Dairy cows have for a long time been bred to produce high yields of milk. The large milk yields cause high pressure on the metabolism and it is often discussed whether the high milk yields interfere with animal welfare. Since there is a positive correlation between milk production and daily duration of rumination- and eating time (Dado & Allen, 1994) the cow has to spend a lot of time eating and ruminating in order to keep up with the high production levels. Energy requirement varies with the stage of lactation; there is a tremendous increase in energy requirement (Drackley *et al.*, 2005) and feed consumption (Jordan *et al.*, 1973; Ingvarlsen & Andersen, 2000; Bewley & Schutz, 2008) at onset of lactation. This change in energy requirement is also accompanied with increased time consumption for feeding- and ruminating after parturition (Ruckebusch, 1975). It is known that cattle are highly motivated to lie down (Metz, 1985; Jensen *et al.*, 2005) and having enough time to lie down seems to be an important factor for animal welfare (Fregonesi & Leaver, 2001; Jensen *et al.*, 2005; Cooper *et al.*, 2007) but little is known about how much time a dairy cow needs to sleep. Increasing milk yields and busy time budgets might lead to insufficient sleep time for the modern dairy cow. Sleep time in cows has only been quantified once, published by Ruckebusch (1972). Ruckebusch (1972) performed a study on four cows and the daily sleep time was found to be four hours, but stage of lactation was not specified in the publication. The limited access of sleep data could partly be due to the complexity of measuring sleep in dairy cows, historically the recording has been involving invasive methods while it is now possible to measure sleep in cattle with non-invasive methods (Hänninen *et al.*, 2008). It might not be possible to reach a conclusion regarding how much the modern dairy cow *needs* to sleep, but in order to achieve a deeper understanding about sleep behaviour in cows it seems necessary to update the knowledge about how much they sleep.

It is well known that sleep has a crucial role for vital body functions and sleep deprivation has been shown to have a negative impact on many physiological functions such as immune defence and metabolism (Rechtschaffen *et al.*, 1983; Bergmann *et al.*, 1989; Everson & Toth, 2000; Bryant *et al.*, 2004). During the transition period roughly one third (Duffield *et al.*, 2002) of the dairy cows are affected by similar health problems as caused by sleep deprivation, like metabolic disorders and infectious diseases (Mallard *et al.*, 1998; Drackley *et al.*, 2005). Due to increased time needed for feeding- and rumination at the onset of lactation the possible lack of sleep might be extraordinarily large in the transition period. In order to find a possible sleep depression in the periparturient period it is necessary to quantify sleep in different stages of lactation. If indications of insufficient sleep time during the transition period could be detected it might also be of interest to look for connections between health problems and sleep deprivation in dairy cows. Some studies claim that the prior waking experience, environmental- and physiological factors could influence the sleep time in the two different main sleep stages, non rapid eye movement (NREM)- and rapid eye movement (REM) sleep (Siegel, 2005; Zepelin *et al.*, 2005; Sanford *et al.*, 2003; Gonzalez *et al.*, 1995; Lesku *et al.*, 2006). There is a possibility that the physiological changes occurring in the periparturient period alters the sleep quality and the total sleep time requirement.

The aim of this project was to quantify the total sleep time in modern dairy cows during 24-hour periods and to investigate whether the total amount of sleep, as well as time spent in the different sleep stages, varies between the dry period, early- and peak lactation. The distribution of sleep time between night and day was also examined.

Furthermore, correlations between sleep and lying time, fluctuations in body temperature and heart beat were included in the aim.

## Literature review

### Sleep

#### *Sleep stages*

The measurement of changes in brain activity, through electroencephalogram (EEG), has been a crucial part of the sleep research during the last decades. Through electrodes attached on the scalp the EEG measures voltage changes in the brain (Allada & Siegel, 2008). NREM- and REM sleep are the two main stages of sleep, NREM sleep is sometimes called 'quiet sleep' while REM sleep could be referred to as 'paradoxical sleep' (PS) or 'active sleep'. Throughout this study the two main sleep stages will be referred to, as NREM- and REM sleep while the sum of NREM- and REM sleep will be called total sleep. NREM sleep is divided into four phases, which corresponds to increasing sleep depth (Staunton, 2005). When NREM sleep depth increases the metabolic- and muscle activity decreases (Åkerstedt & Nilsson, 2003). In humans, phases 3 and 4 correspond to the deepest NREM sleep phases, they are grouped together under the name 'slow wave sleep' or 'deep sleep' (Åkerstedt & Nilsson, 2003), while NREM sleep and 'slow wave sleep' are used as synonyms in nonhuman mammals (Zepelin *et al.*, 2005). Slow wave sleep is characterised by decreased respiration frequency, low heart rate and cerebral blood flow (Åkerstedt & Nilsson, 2003). The character of REM sleep, with the absence of muscle activity and a largely awake brain, is to a great extent diverging from the NREM sleep. EEG data from REM sleep is similar to an awakening individual (Allada & Siegel, 2008) and the metabolic activity in the brain is high (Siegel, 2005). During REM sleep the blood pressure, body temperature, heart- and breathing rate is increased to wakening levels (Åkerstedt & Nilsson, 2003). 10 to 50 % of the mammalian sleep is occupied with REM sleep (Zepelin *et al.*, 2005).

One important function of NREM sleep is considered to be the energy saving effects, the daily restitution for the entire physiological system is occurring during the NREM sleep (Berger & Phillips, 1995; Åkerstedt & Nilsson, 2003; Siegel, 2005). There is a clear connection between immune defence and sleep (Bryant *et al.*, 2004; Preston *et al.*, 2009), immune system activity is increasing during sleep (Åkerstedt & Nilsson, 2003). REM sleep seems to be essential for the brain and to have an impact on learning and memory (Capellini *et al.*, 2008). The function of NREM- and REM sleep is a complex topic and it is difficult to find a uniform explanation to why sleep is essential (Siegel, 2005; Preston *et al.*, 2009).

#### *Factors affecting sleep time in mammals*

There are multiple factors influencing the variety in total sleep time both between and within species such as; age, body size, reproduction strategies, energy requirement, environmental influences, recent sleep-wake history, pregnancy, previous waking experiences and temporary stress (Ruckebusch, 1974; Ruckebusch, 1975; Elgar *et al.*, 1988; Siegel, 2005; Zepelin *et al.*, 2005; Jha *et al.*, 2006; Capellini *et al.*, 2008; Langford & Cockram, 2010).

Most mammals, including cattle, display a polyphasic sleep pattern with sleep intervals disrupted by wakefulness, in a 24-hour cycle (Ruckebusch, 1972; Zepelin *et al.*, 2005).

According to Ruckebusch (1972), which published the only previous sleep quantification study in cows, 97 % of the total sleep time and 100 % of the REM sleep time is occurring during the night. Cows are capable of entering a state of NREM sleep, but not REM sleep, while standing, even though they are normally spending both NREM- and REM sleep in a recumbent position (Ruckebusch, 1974). Wakefulness can be divided into two different states; alert wakefulness and drowsing. Drowsing could be described as an intermediate state between wakefulness and sleep and is commonly occurring during rumination (Ruckebusch, 1972). According to Ruckebusch (1972) cows spend approximately one third of their time awake in the state of drowsing.

Species with high energy requirements spend a lot of time eating, which results in less time available for sleep. There is a negative correlation between energy requirements and sleep time when comparing mammalian species (Elgar *et al.*, 1988; Capellini *et al.*, 2008). Both NREM- and REM sleep time is shorter for herbivores compared with other mammals (Capellini *et al.*, 2008), which could be explained by the low energy density of their feed (Siegel, 2005) and the fact that they generally are more exposed to predator threats than non-herbivores (Allison & Cicchetti, 1976). Stress is affecting both the quality and quantity of sleep (Van Reeth *et al.*, 2000) and biological functions could be changed in order to cope with a stressful situation (Drackley *et al.*, 2005). Two different effects of stress have been found in rats; short intense stress has a sleep promoting effect with increased NREM- and REM sleep as a result (Marinesco *et al.*, 1999; Bouyer *et al.*, 1997) while chronic stress appeared to have a sleep disrupting effect (Cheeta *et al.*, 1997; Marinesco *et al.*, 1999). In cows, two different types of sleep alterations have been seen after potential stressful situations. Ruckebusch (1975), found a large decrease in sleep time as a response to separation between two cows and their calves, while Ruckebusch (1974) found an extensive rebound of both NREM- and REM sleep the first days after a long period of deprivation of lying down.

According to Siegel (2005) REM sleep time is positively correlated with sleep time and negatively correlated with body size. Anxiety, derived from anticipation of pain, has been shown to decrease the REM sleep proportion (Sanford *et al.*, 2003), while Gonzalez *et al.* (1995) found an increase in REM sleep time after acute stress. Furthermore Lesku *et al.* (2006) found a significant effect of predation risk, sleeping in more predator-exposed environment is associated with decreased REM sleep time. In contradiction, Capellini *et al.* (2008) found no indication that physiological- and environmental factors are affecting a specific sleep stage but rather the total sleep time.

### *Sleep deprivation*

Sleep deprivation has been shown to have negative effects on rats such as; increased metabolic activity, negative energy balance, reduced concentrations of thyroid hormone, increased catabolism, decrease in cerebral function, reduced resistance to infection and weight reduction (Bergmann *et al.*, 1989; Everson, 1995; Rechtschaffen *et al.*, 1983). Severe sleep deprivation in rats causes death within two to three weeks Rechtschaffen *et al.* (1983). It has also been shown that sleep disturbance is associated with increased mortality in humans (Kripke *et al.*, 1979; Nilsson *et al.*, 2001; Kripke *et al.*, 2002). Sleep deprivation studies have also been performed on cows. In a lying-, feeding- and selective REM sleep deprivation trial the cows were deprived from these certain behaviours 14- to 22 hours daily during eight weeks. When deprivation lasted 14 hours per day the cows were able to adapt, within five- to six days, to recover the daily feed consumption

and REM sleep time to levels occurring before the deprivation started. When daily deprivation time was enlarged the NREM sleep proportion increased, as well as the proportion of NREM sleep occurring while standing, in order to compensate for lost REM sleep time (Ruckebusch, 1974).

### **Lying behaviour**

Dairy cows and heifers lie down 12 to 14 hours per day (Wierenga & Hopster, 1990; Albright, 1993; Fregonesi & Leaver, 2001; Bencsik *et al.*, 2004; Munksgaard *et al.*, 2005; DeVries *et al.*, 2005; Jensen *et al.*, 2005). Some studies have suggested that when time for performing natural behaviours is limited, cows choose to spend a larger proportion of time lying down. There has been shown that they even prioritize to lie down before feeding (Metz, 1985; Munksgaard *et al.*, 2005). In contradiction, Cooper *et al.* (2008) and Ruckebusch (1974) concluded that cows are more motivated to rebound lost feed intake before lying time, after being deprived from both feed and the opportunity to lie down. Schutz *et al.* (2010) found that cows, during sunny conditions, prioritised to be standing in shade higher than to recover lost lying time.

There are many factors affecting lying time. High yield cows have shorter lying time, in advantage for feeding time, compared to low yield cows (Fregonesi & Leaver, 2001; Bewley *et al.* 2010), early lactation cows have shorter lying time compared to late lactation- and dry cows (Chaplin & Munksgaard, 2001; Bewley *et al.*, 2010). Housing system also influences time spent lying down, the general trend is that large and soft areas increase the lying time (Haley *et al.*, 2000; Fregonesi & Leaver, 2001).

### **Heat stress**

High ambient temperature induces heat stress in dairy cows and heat stress could affect physiological parameters as well as behaviour. When exposed to high temperature and humidity, dry matter intake declines (Coppock, 1985; West *et al.*, 2003). Since heat stress is higher when lying down (Berman, 2005), lying time decreases as temperature increases (Shultz, 1984; Overton *et al.*, 2002; Cook *et al.*, 2007; Schutz *et al.*, 2010). The external heat load is affecting the core body temperature of cows (Hillman & Willard, 2005; Schutz *et al.*, 2010), core body temperature increases when cows are lying down and as the core body temperature increases, cows are more likely to stand up in order to cool down (Hillman & Willard, 2005). Many factors are influencing the impact of heat stress on dairy cows. Environmental conditions and animal characteristics such as milk production, hair coat depth, air temperature, air humidity and heat exposed body surface, are important factors when estimating level of heat stress (Berman, 2005). The impact of heat stress is larger in high producing cows due to the positive correlation between milk production, dry matter intake and heat increment of feeding (Kadzere *et al.*, 2002).

### **Transition period**

In the transition from late pregnancy to early lactation the high producing dairy cows are exposed to a dramatic increase in nutrient requirement due to the high milk production, the net energy requirement is approximately doubled at the onset of lactation (Drackley *et al.*, 2005). Despite being offered high-energy diets, energy intake does not keep up with the high-energy requirement and most dairy cows enter a state of negative energy balance in early lactation (Coppock, 1985; Schröder & Staufenbiel, 2006; Bewley & Schutz, 2008; Walsh *et al.*, 2011). Milk yield (Schröder & Staufenbiel, 2006) and ability to increase dry matter intake after parturition (Maltz *et al.*, 1997) are

two of many factors that determines the severity of negative energy balance in early lactation.

During late gestation the dry matter intake is decreasing (Jordan *et al.*, 1973; Ingvarsen & Andersen, 2000; Drackley *et al.*, 2005), dry matter intake may be up to 30 % lower in that period compared with early dry period (Drackley *et al.*, 2005). Dry matter intake is at the lowest level on the day of the parturition (Marquardt *et al.*, 1976; Ingvarsen & Andersen, 2000) and postpartum the dry matter intake is increasing in support of lactation (Jordan *et al.*, 1973; Ingvarsen & Andersen, 2000; Bewley & Schutz, 2008). Peak milk yield is typically reached 5 to 8 weeks postpartum (Coppock, 1985; Ingvarsen & Andersen, 2000). Maximum dry matter intake is normally not reached until after peak lactation (Coppock, 1985; Maltz *et al.*, 1997; Ingvarsen & Andersen, 2000). The dry matter intake is increasing slower than milk production (Bewley & Schutz, 2008), the magnitude and rate of the increase in dry matter intake varies considerably and is influenced by many factors (Ingvarsen & Andersen, 2000). Regulation of dry matter intake in periparturient cows is widely discussed and the complex mechanism is not fully understood (Ingvarsen & Anderson, 2000; Drackley *et al.*, 2005). Despite the increase in feed consumption the transition cow is often losing weight (Jordan *et al.*, 1973) and there is also normally a drop in body condition score after calving (Bewley & Schutz, 2008), which might affect the animal health negatively (Bewley & Schutz, 2008).

Despite the nutritional challenges caused by the high energy requirement in early lactation, there are other stressors such as inadequate management, regrouping, heat stress, overcrowding, lameness, uncomfortable interior environment and rough handling that might affect the welfare of the periparturient dairy cow (Drackley *et al.*, 2005). Together with the immunosuppression occurring during the transition period (Mallard *et al.*, 1998), the high metabolic constraints and other stressors coupled to calving and commence of lactation are potentially factors contributing to the high rate of metabolic disorders and infectious diseases during the periparturient period (Drackley *et al.*, 2005).

## **Material and Method**

### **Animals and experimental design**

Data collection was conducted at Kungsängens Research Center in Uppsala between 23:th of June and 7:th of September 2010. Eight dairy cows of the Swedish Red breed were used in this trial. For detailed information about each cow see Table 1.

Three sessions of 24-hour electrophysiological recordings for identification of sleep pattern were performed on each cow. When possible, recording was performed on two cows at a time, using one set of equipment each. Body temperature-, heart rate- and body position (standing up or lying down) data was also collected during the recording sessions. The cows were fed pasture, silage *ad libitum* and individual amounts of concentrate; according to milk yield (Spörndly, 2003). During days when the cows were kept inside they had no access to pasture. The experimental procedure and all animal handling was approved by the Uppsala local ethics committee.

The first data collection session was carried out in the dry period, 11-15 days prior to expected day of parturition, which equalled 9-19 days prior to actual parturition. The second data collection session was performed 11-15 days postpartum, representing a period when milk yield is increasing, and the third data collection session was carried out 40-50 days postpartum, representing peak lactation. The time for the different data collection sessions was chosen based on previous lactation curves in the herd (Österman & Bertilsson, 2003; Österman *et al.* 2005).

### **Housing**

Data collection was carried out during the summer pasture period. Cows were taken in from pasture at least four days before each electrophysiological recording and kept in a tie stall barn for one to two days before being moved to the single pen, where the recording was carried out, two days prior to recording. For allowing the cow to get familiar with the equipment used for recording, the cow was prepared with a halter, an udder holder when moved to the parturition pen.

### **Recording equipment**

Three types of electrophysiological recordings were performed simultaneously; electroencephalogram (EEG), electro-oculography (EOG) and electromyography (EMG). For the electrophysiological recordings an ambulatory recording device (Embla titanium, Embla Systems, Broomfield, USA) that weighed 0,3 kg and measured 4 x 8 x 12 cm (H x W x L) was used. The data was sampled at 256 Hz. For EEG recordings, four surface electrodes were placed two and two in lines drawn from the middle of the eye to the end of the horn base on each side, forming a square in the forehead (Figure 1 and 2), according to suggestions in Takeuchi *et al.* (1998). The reference electrode was adhered in the middle of the square and the ground electrode was attached just caudal the horn base (Figure 1 and 2) according to Hänninen *et al.* (2008). For EOG recordings, one surface electrode was placed below the right eye and one above the left eye, respectively (Figure 1). For EMG recordings, two surface electrodes were placed symmetrically on each side of the neck muscle (Figure 2) according to suggestions in Hänninen *et al.* (2008). While recording, a halter and an udder holder were used for enabling attachment of connecting wires used for attaching each electrode with the recording device. For heart rate measurements, electrocardiography (ECG) recordings were performed. Four surface electrodes were attached to the thorax, caudally the upper part of the left front leg. Three electrodes were placed in a line and one electrode was adhered caudally of the three other electrodes (Figure 3).

A girth was supposed to be used for protection and attachment of the ambulatory recording device but the girth turned out to be disturbing the electrophysiological recording. The disturbance was most likely due to that iron content of the girth interfered with the electronic signals in the device. Therefore the girth was not used in the experiment.

One day prior to electrophysiological recording, at the latest, a modified vaginal insert, (CIDR™; InterAg, Hamilton, New Zealand) with a temperature logger (Minilog-TX data logger, Vemco Ltd, Nova Scotia, Canada) attached to it, was inserted in the vaginal cavity, by an experimental technician. Before insertion, the vaginal region was cleaned with water and soap. At the same day an activity meter (IceTag3D, IceRobotics Ltd, Edinburgh, Scotland) was attached to one of the hind legs with a soft Velcro strap. The night before each data collection session the attachment of electrodes was prepared by

shaving the areas where electrodes would be adhered. An electric razor (Vega, Aesculap AG, Tuttinge, Germany), a safety-razor (Venus Divine, Gillette, Surrey, United Kingdom) and shaving foam (Satin Care, Gillette, Berkshire, United Kingdom) was used for the shaving.

Attachment of equipment started approximately 45 minutes prior to start time. Before adhering the surface electrodes the shaved areas were cleaned with 70% ethanol. The surface electrodes (Unilect, Unomedical Ltd, Stonehouse, Great Britain) were then adhered with cyanoacrylat glue (super glue) and connected to the ambulatory recording device with snap-on cables (Snap on-cables, Embla Systems, Broomfield, USA). Super glue was used for the first recording but was then replaced by tissue adhesive (3M Vetbond, 3M Animal Care Products, St. Paul, USA). To minimize discomfort for the cow, the snap-on cables were bundled and led to the recording device in fabric tubes, which were adhered to the udder holder. The recording device was attached to the udder holder on the back of the cow and a counterweight was attached to the udder holder on the opposite side, to balance the weight of the equipment. During days of data collection, the cows were fed silage *ad libitum* and individually amount of concentrate three times; approximately at 07.00 a.m., 12.00 p.m. and 05.00 p.m. Lactating cows were milked in the parturition pen, twice daily. The pen was cleaned while the cows were milked and while they were eating. Directly after each recording all equipment was removed, the shaved skin areas cleaned with 70% ethanol and the cows were taken back to pasture. Between the data collection sessions the shaved areas were checked for possible skin lesions, moisturiser was applied, as well as sun block if needed.

### **Data collection**

Each electrophysiological recording session started approximately at 6 am and lasted for 24-hours. Before each recording, the ambulatory recording device was connected to a computer, equipped with the software (RemLogic 2.0.1, Embla Systems, Broomfield, USA) needed for registering and handling the electrophysiological data. Downloading of electrophysiological data was enabled by connecting the recording device to the computer, once during the recording and also directly after recording was stopped.

Both the temperature logger and the activity meter were programmed to start logging when the electrophysiological recording started, at the latest. When activated the temperature logger registered body temperature (C°) every 10:th second. The activity meter registered body position i.e. if the cow was standing up or lying down. Registrations were made once every minute and obtained in percent; when lying down, registrations were made as 100% for the behaviour lying down and in the corresponding way for the behaviour standing. When changing from one behaviour to the other, intermediate percentage between 0-100% was registered.

Each data collection session was continuously recorded on two surveillance cameras and also manually observed by one person. The manual observation was mainly performed from a room nearby the parturition pen, by watching the ongoing camera recording, in order to minimise disturbances for the cow. On occasions the observer had to be seated in front of the parturition pen. The main task for the person observing the recordings was to prevent the cows from removing the equipment by scratching and to re-attach or replace electrodes if they came off. The person observing the recording also made sure that the ambulatory recording device was kept in place and not damaged. No

behaviour registrations were performed manually during the electrophysiological recordings.

### **Data analysis**

In order to distinguish between the different vigilance states (awake, NREM- and REM sleep) and to be able to quantify time spent in each state the electrophysiological data was visually scored. The scoring was made manually in the analysing software (RemLogic 2.0.1, Embla Systems, Broomfield, USA). Before scoring the EEG, EOG and EMG signals they were filtered using the following individual thresholds; EEG 0.3-30 Hz; EMG >10 Hz and EOG 0.15-15 Hz. The signals were analysed in 30-second intervals. One good quality signal of the EEG and EMG, and both EOG signals were selected for the scoring. The human sleep definition of different vigilance states from Rechtschaffen & Kales (1968) was used for the scoring. A power spectrum was calculated in order to make the visual scoring easier. REM sleep was scored when the EEG signal had a desynchronized pattern with large variations in amplitude, combined with a low muscle tone. NREM sleep was scored when the power spectrum showed values less than  $0.2 \mu V^2/Hz$  in the high frequencies (10-30 Hz), combined with a reduced muscle tone. In order to obtain durations occupied in NREM- and REM sleep the 30-second intervals scored with respectively state was summarised. For obtaining total sleep time the duration of NREM- and REM sleep time was summarised.

The body position and body temperature data was obtained as continuous registrations corresponding to time of day. To obtain the amount of time spent lying down during one electrophysiological recording the proportion of the behaviour was calculated and multiplied by total amount of recording time. Time intervals with electrophysiological data destroyed by artefacts in the data were deleted. All parameters were merged into one file and after transforming body position and body temperature data to data obtained from the electrophysiological recordings the intervals corresponding to deleted parts of electrophysiological data were excluded.

Due to the artefacts, 24-hour electrophysiological data was not obtained from all recording sessions. In order to obtain total sleep-, NREM sleep-, REM sleep- and lying time on a 24-hour basis, for recordings containing artefacts, some calculations were performed. The time each behaviour was performed was divided by total amount of obtained electrophysiological data during 24-hours (after exclusion of artefacts) and then multiplied by 24. All results for total sleep-, NREM sleep-, REM sleep- and lying time was given in hours of 24-hours. For calculating NREM sleep time as proportion of total sleep time, the amount of NREM sleep time was divided by total amount of sleep time. Same procedure was done for REM-sleep time. For calculating total sleep time as a proportion of time spent lying down the total sleep time was divided by total amount of time spent lying down.

To obtain the distribution of total sleep-, NREM sleep-, REM sleep- and lying time, during night- and daytime similar calculations as described above were performed. Amount of time performing each behaviour during night- and daytime respectively was divided by total amount of electrophysiological data obtained (after exclusion of artefacts) from night- and daytime. Night time was defined as time between 19.30 and 05.30, while daytime was defined as time between 05.30 and 19.30. Distribution of the different behaviours during night- and daytime was presented as the proportion of night (night

proportion)- and daytime (day proportion) that was used for the specific behaviour. Mean values and standard deviations for night- and day proportions for each sleep- and lying parameter were calculated for all three stages of lactation. In order to find possible differences between night- and day proportions for different behaviours within same stage of lactation, two tailed paired T-tests were performed. Differences were declared significant at  $P < 0.05$ . Same level of significance was used for all P-values throughout the entire data analysis. Proportion of total time occupied with one behaviour (total sleep, NREM sleep, REM sleep and lying) occurring during night time was also calculated. Amount of night time performing each behaviour was divided by total time performing the behaviour.

Mean values and standard deviations for total sleep-, NREM sleep-, REM sleep- and lying time was calculated for the three stages of lactation. For the total sleep time the overall mean value (for the entire data set) was also calculated. When analysing the body position data, lying time for six cows in each stage of lactation was used due to missing data. Missing data did not refer to the same individual cow in all stages of lactation; consequently there is a variation in which cows each stage of lactation contains in the aspect of body position data. In order to find possible differences in the aspect of total sleep-, NREM sleep-, REM sleep- and lying time between the three stages of lactation, two tailed paired T-tests were performed. Whether the proportion NREM- and REM sleep time of total sleep time differed between the three different stages of lactation was also tested, as well as if there were any variations in the proportion of total sleep time of total time spent lying down.

Average, longest and shortest duration of NREM- and REM sleep bouts were obtained from the sleep scoring data, for each electrophysiological recording. Mean values and standard deviations for respectively stage of lactation and parameter was calculated, as well as, the mean values and standard deviations for the entire data set. The different stages of lactation were compared by performing two tailed paired T-test on the average, longest and shortest NREM- respectively REM sleep bout durations.

Mean values and standard deviations for body temperature in the two different activity states (awake and asleep), were calculated from temperature registrations for each cow and data collection occasion. Differences between the mean values for body temperature in the two activity states, within the same data collection occasion, were calculated. Body temperature was plotted together with sleep sessions in order to find possible correlations between these two parameters.

## **Results**

### **Methodology**

#### *Excluded electrophysiological data*

24-hour data of EEG, EOG and EMG was successfully obtained from seven of the eight cows in all three stages of lactation. Electrophysiological data from one of the eight cows mainly contained artefacts and all her data was excluded from the data set. 2.2 % of the electrophysiological data from the seven remaining cows contained artefacts; these parts of data were excluded. For detailed information of amount remaining electrophysiological data in the different stages of lactation see Table 2. For distribution

of amount obtained electrophysiological data during night- respectively daytime see Table 3.

### *Health problems*

Production diseases affected some of the cows during the trial period. Due to health problems and high somatic cell counts, only one of the seven cows was let out on pasture between parturition and the data collection session in early lactation and three of the cows were even kept inside between the data collection session in early lactation and peak lactation. For detailed information about the health problems affecting the cows during the trial period and at how many days relative to calving each data collection session was performed see Table 4.

### *Equipment issues*

When using electrophysiological recording equipment on dairy cows in this trial, the main problem was to keep the electrodes in place. When a few *shorter test*- and one 24-hour electrophysiological recording were completed, the impression was that the super glue caused skin irritations. The super glue seemed to cause itching and the cows showed frustration when having the electrodes adhered with this type of glue. Two other types of glue were tested, collodion (Collodion adhesive, SLE Ltd, South Croydon, United Kingdom) and tissue adhesive (3M Vetbond, 3M Animal Care Products, St. Paul, USA). With collodion, the electrodes were not adhered properly and the cows obtained even worse skin irritations than when using super glue. Tissue adhesive appeared to have good attachment ability and seemed to have less impact on the cows. Tissue adhesive was used for all electrophysiological recordings except the first one. When using the tissue adhesive, no skin problems were observed, but the impression was still that the electrodes were itching and causing frustration among the cows. It was commonly occurring that the cows tried to scratch the electrodes off against something in their environment. For preventing the cows from removing the electrodes by scratching, they were occasionally manually sprayed with water in order to interrupt the behaviour. When electrodes fell off or were taken off by the cow they were replaced with new electrodes. The general impression was that the frustration, caused by itching electrodes, increased with time, number of recordings and with increasing air temperature. It also seemed like there were individual differences in the degree of frustration.

The experiment was conducted during a period with warm weather. Because of the high temperature, which induced increased sweating, persistent attachment of surface electrodes for ECG recordings was difficult to accomplish. The fact that the ECG electrodes were located in a position where the cows could reach them and were sweating was pronounced on hot days, also contributed to the difficulties with keeping the ECG electrodes in place. The complications with attachment of ECG electrodes resulted in stopped ECG recordings; no heart rate data was obtained and the analysis of this parameter was excluded from the experiment.

### *Body temperature*

Due to a high frequency of lost vaginal inserts in the dry period, body temperature data was only obtained from three of the seven cows from data collection in the dry period. The high frequency of lost vaginal inserts in the dry period was most likely due the enlargement of the vaginal cavity prior to calving. All body temperature data from data collection sessions in early lactation was obtained, except from 1377 (Table 2). Missing

data in early lactation was due to complications when downloading data. All body temperature data in peak lactation was obtained, with the exception for 7,5- and 6 hours missing data for cow 1165 and 1377 respectively, their vaginal insert did not stay in the vaginal cavity during the entire data collection.

### *Body position*

Body position data was successfully obtained from all data collection sessions, except three (Table 2). Missing data was in two cases due to complications when downloading data and in one data collection session, no recording equipment was attached to the cow.

## **Sleep-, body position- and body temperature data**

### *Total sleep time*

The total sleep time was longer in the dry period compared to early and peak lactation ( $P<0.005$  and  $0.05$  respectively). There was a trend indicating that total sleep time in early lactation was shorter than total sleep time in peak lactation ( $P=0.07$ ). For all individual cows, the longest time spent sleeping was obtained in the dry period. For six of the seven cows the longest total sleep time was obtained in early lactation, the exception was cow 1259 who obtained shortest total sleep time in peak lactation (Table 5a-b, Figure 4).

### *NREM- and REM sleep time*

Longer NREM sleep time was obtained in the dry period compared to early and peak lactation ( $P<0.01$ ). Electrophysiological data from each cow agree with this, since the longest total NREM sleep time was obtained in the dry period and the shortest in early lactation, for all individual cows, except 1259 who also had the longest NREM sleep time in the dry period but the shortest in peak lactation. There was no difference between early- and peak lactation ( $P>0.1$ ) in the aspect of NREM sleep time (Table 6a-b, Figure 5). NREM sleep time as a proportion of total sleep time during 24-hours (NREM sleep/total sleep) was larger in the dry period than peak lactation ( $P<0.01$ ). There was no difference between the proportion NREM sleep/total sleep in early lactation compared to the dry period and peak lactation ( $P>0.1$  and  $0.05$  respectively). Six out of seven cows obtained the smallest proportion NREM sleep/total sleep in peak lactation; exception was 1367 who had the smallest proportion in early lactation. Four of the seven cows had the largest proportion NREM sleep/total sleep in the dry period. The exceptions were cow 1259, cow 1377 and cow 1401, who had their largest proportion of NREM sleep/total sleep in early lactation (Table 7a-b).

The REM sleep time was longer in peak lactation compared to early lactation ( $P<0.01$ ). REM sleep time also tended to be longer in peak lactation compared with the dry period ( $P=0.08$ ). Moreover, six out of seven cows obtained the longest REM sleep time in peak lactation. The exception was cow 1259, who had the longest REM sleep time in the dry period. There was no difference between the amount of time spent in REM sleep between the dry period and early lactation ( $P>0.1$ ) (Table 8a-b, Figure 6). REM sleep time as a proportion of total sleep time (REM sleep/total sleep) follows the same pattern as the proportion NREM sleep/total sleep since the two variables depend on each other. Thus, the proportion of REM sleep/total sleep was larger in peak lactation compared to the dry period ( $P<0.005$ ) but there was no difference between early lactation and dry period or peak lactation ( $P>0.1$  and  $0.05$  respectively). Further, all cows except 1367,

obtained the largest proportion REM sleep/total sleep in peak lactation and four out of seven cows obtained smallest proportion REM sleep time in the dry period (Table 9a-b, Figure 7).

Average duration of REM sleep bouts was longer in peak lactation compared with the dry period and early lactation ( $P < 0.05$ ). Mean value for longest REM sleep bouts was also longer in peak lactation compared to the dry period and early lactation ( $P < 0.05$ ). No other differences between average, longest and shortest durations of NREM- and REM sleep bouts were observed (Table 14).

#### *Body position*

Body position data was obtained from six of the seven cows in each stage of lactation; missing data does not refer to the same cow in all stages of lactation (Table 2). Lying time (h/24-h) was shorter in early lactation compared to the dry period ( $P < 0.05$ ). No differences were found either between peak lactation and the dry period or early lactation ( $P > 0.1$ ). For four of the five cows, who had body position data from all three stages of lactation, the shortest time spent lying down was observed in early lactation. The exception was cow 1165, who had even shorter lying time in peak lactation (Table 10a-b, Figure 8). The proportion of total sleep time of time spent lying down (total sleep/lying) did not differ between the three stages of lactation even though early lactation tended to have smaller proportions than the dry period ( $P = 0.07$ ) (Table 11a-b, Figure 9).

#### *Sleep- and lying time distribution between night and day*

Total sleep-, NREM sleep-, REM sleep- and lying time occupied higher proportions of the night compared with the day, when comparing night- and day proportions within the same stage of lactation ( $P < 0.05$  to  $0.005$ ). For overall mean values ( $N = 21$ ) and standard deviations for night proportions of total sleep-, NREM sleep-, REM sleep- and lying time (Table 12).

#### *Body temperature*

Within individual 24-hour data collections, differences between mean values for body temperature while the cow was sleeping compared to mean values for body temperature while they were awake varied between  $0,005$  and  $0,297$  °C. During two data collection occasions lower mean value for body temperature were obtained while sleeping compared with mean value for time awake (Table 15). Some of the body temperature- and sleeping sessions, plotted together against time of day, indicated that temperature occasionally decreased before the cow fell asleep. On the other hand the pattern also suggested a possible increase in body temperature prior to, or while sleeping. After visual analysis of plotted data, the impression was that it did not seem to be any consistent correlation between body temperature and sleep (Figure 10). No statistical analysis was performed on the body temperature data.

## **Discussion**

### **Total sleep time**

The overall sleep duration of 3.5 hours in this study is similar to the four hour total sleep time published by Ruckebusch in 1972. When comparing the overall mean value of this study with Ruckebusch (1972) it looks like not much has changed in the aspect of sleep time in cows the last decades. Anyhow, this might not be completely true due to the fact

that, according to this study, there seems to be a difference in total sleep time in different stages of lactation. With the assumption that sleep time in dairy cows varies with the stage of lactation, it might not be applicable to compare overall mean values of sleep time without taking the stage of lactation into account. Another factor that may contribute to the difficulties with comparing the current study with Ruckebusch (1972) in the aspect of sleep time quantity is how the different vigilance states were scored. Even though the principles for how the sleep data were scored were similar the exact limits between different vigilance states and the scoring procedure might differ.

According to Rechtschaffen & Kales (1968) there are differences both between and within species in how the exact limits used for scoring sleep should be set and they suggested that separate scoring systems should be used for different species. Due to the fact that no previous studies have investigated how sleep in cattle should be defined, sleep scoring in the current study was performed according to the definitions of human sleep. The lack of a well-defined scoring system for cows might have resulted in some uncertainty of how well the sleep scoring is referring to the real vigilance state of the cows. Even though these possible differences are considered to be small it would be preferable to investigate the definitions of sleep in cattle more carefully in order to provide more reliable results in the future.

In agreement with Ruckebusch (1975), which saw tendencies of decreasing total sleep time after parturition there was a decrease in total sleep time between the dry period and early- and peak lactation respectively. In order to find a possible explanation to the reduced sleeping time after parturition, it is unavoidable to speculate in how transition related alterations might affect sleep. A major factor affecting the amount of time spent sleeping is likely to be limitations in time available for sleep. When the dry matter intake (Jordan *et al.*, 1973; Ingvarsen & Andersen, 2000; Bewley & Schutz, 2008), and as a consequence, time used for feeding and rumination (Ruckebusch, 1975) goes up after parturition, the remaining time for other behaviours, such as sleep, decreases. In addition to the increased feed consumption after parturition there is also a decline in dry matter intake during late gestation (Jordan *et al.*, 1973; Ingvarsen & Andersen, 2000; Drackley *et al.*, 2005), which is accelerating the difference in dry matter intake and also time consumption for feeding, before and after parturition. Thus, the large differences in dry matter intake between onset of lactation and late gestation might indirectly affect the sleep time by reducing time availability for the sleeping behaviour. If there is a negative correlation between energy requirement and sleep time also within species, in the same way as it is between species (Elgar *et al.*, 1988; Capellini *et al.*, 2008), such assumption could support the suggestion that increased energy requirement indirectly is causing sleep reduction.

To be able to strengthen the assumption that an increase in dry matter intake might be an important factor affecting time availability for sleep in different stages of lactation, it would have been preferable to record daily dry matter consumption, before, during and after the trial period. The dry matter consumption would enable detection of a possible relationship between feed intake and sleep time.

Another transition related factor that might contribute to the sleep time reduction after parturition is the state of negative energy balance, which is commonly occurring in early lactation (Coppock, 1985; Schröder & Staufenbiel, 2006; Bewley & Schutz, 2008; Walsh

*et al.*, 2011). The negative energy balance might last until after peak lactation due to the fact that maximum dry matter intake is normally not reached until after peak lactation (Coppock, 1985; Maltz *et al.*, 1997; Ingvarlsen & Andersen, 2000). Since peak lactation is commonly occurring 5 to 8 weeks postpartum (Coppock, 1985; Ingvarlsen & Andersen, 2000) it can be assumed that the cows still were in a state of negative energy balance when the data collection in peak lactation, 40 to 50 days after parturition, was performed. The negative energy balance is a potentially stressful situation for the transition cow (Drackley *et al.*, 2005). Since stress has been shown to have an impact on both quality and quantity of sleep in other species (Cheeta *et al.*, 1997; Marinesco *et al.*, 1999; Van Reeth *et al.*, 2000) and even in cows (Ruckebusch 1974; Ruckebusch, 1975), it is likely that the negative energy balance might have affected the sleep time in early- and peak lactation.

Except the large metabolic pressure and negative energy balance that the cows were possibly facing during the two data collections occurring post partum, there were some other potential stressors present in this trial. To prevent the cows from removing the equipment by scratching they were occasionally sprayed with water, in order to interrupt the behaviour. It would have been preferable to avoid this kind of interference with the cows during the electrophysiological recording, but this method was considered as the most efficient way to prevent the cows from removing the equipment. Both frustration caused by itching equipment and being sprayed with water may have been stressful for the cows. There is a large individual difference of how well dairy cows are coping with stress (Hopster *et al.*, 1998) and it is therefore difficult to predict to which extent stress did affect individual cows. One cow seemed to be affected by the potential stressful situation to a greater extent than the others. By standing mainly in one place of the parturition pen and avoiding being handled she showed clear signs of anxiety. Since it was electrophysiological data from this particular cow that mainly contained artefacts, and had to be excluded from the data, it may be suggested that anxiety caused the artefacts. Clearly, not all individuals cope well with the electrophysiological recordings

Since the frustration caused by itching electrodes seemed to increase with temperature, the impact of itching electrodes might be decreased if avoiding electrophysiological recordings on dairy cows during warm periods. Nevertheless it is still difficult to record sleep without affecting the animal (Langford & Cockram, 2010) and the result of electrophysiological recordings will probably always be affected by external factors such as equipment. The sleep time obtained in this trial might be slightly reduced due to the impact of recording procedure and equipment. However, since it was possible to find differences between sleep time in the different stages of lactation the impression was that the obtained sleep data was relatively uniform. The impression of a uniform data set could strengthen the suggestion that the data collection per se might have reduced the over all sleep time but that it did not affected the differences in sleep time between stages of lactation. Hence, sleep time might be slightly reduced due to impact of the data collection but the differences between stages of lactation are most likely not affected by the recording procedure and equipment.

Apart from the possible enhancement of itching electrodes the warm weather might have induced heat stress. Together with the fact that the warm weather is a potential stressor for dairy cows, heat stress also has behavioural impacts such as reduced lying

time (Shultz, 1984; Overton *et al.*, 2002; Cook *et al.*, 2007; Schutz *et al.*, 2010) and dry matter intake (Coppock, 1985; West *et al.*, 2003), which might have affected the result of this trial. Further, the effect of heat stress might have varied over the trial period. Due to the positive correlation between milk production, dry matter intake and heat increment of feeding (Kadzere *et al.*, 2002), the warm weather might have had a larger impact on the cows during the data collection sessions after parturition than during the dry period. The impact could be both in the aspect of increased perception of heat stress and itching electrodes.

Since the prior waking- and sleeping experience has an impact on sleep both in cows (Ruckebusch, 1974; Ruckebusch, 1975) and in other species (Gonzalez *et al.*, 1995; Sanford *et al.*, 2003; Jha *et al.*, 2006; Langford & Cockram, 2010), it would have been preferable to perform the electrophysiological recordings during more than 24-hours on each cow in each stage of lactation. There are two main limitations in extending electrophysiological recording time in dairy cows. First of all, it might be difficult to keep the recording equipment in place since the frustration, caused by the equipment, seemed to increase with time. Secondly, there is an extensive workload coupled to continuous manual observation of the electrophysiological recordings.

The fact that a larger proportion of night time, compared with daytime, is occupied with sleep is indicating that the sleep-wake cycle in dairy cows is affected by time of day, which is similar to findings made previously in both cows and sheep (Ruckebusch, 1972; Tobler *et al.*, 1991). In Ruckebusch (1972), 96 % of total sleep- and 100 % of REM sleep time occurred during night, corresponding values in this trial was 55- and 60 % respectively (Table 13). The difference in sleep time distribution during 24-hours could be a possible indication of an adaption to a more extensively rearing system, where management is commonly occurring during all 24-hours.

### **Sleep quality**

There is no consensus in literature regarding if there are any alterations in sleep quality as a response to physiological- and environmental factors, there are studies both supporting (Gonzalez *et al.*, 1995; Sanford *et al.*, 2003; Siegel, 2005; Zepelin *et al.*, 2005; Lesku *et al.*, 2006) and contradicting (Capellini *et al.*, 2008) that theory. In the current trial, the NREM sleep time in the different stages of lactation follows the same pattern as total sleep time, thus both NREM sleep- and total sleep time was higher in dry period compared with early- and peak lactation. REM sleep time, on the other hand, did not follow the same pattern as total sleep time. There are some indications throughout this study that are contradicting the suggestion that a positive correlation between total- and REM sleep time exists (Siegel *et al.*, 2005). First of all, the REM sleep as a proportion of total sleep time was higher ( $P < 0.01$ ), and also REM sleep time tended to be higher ( $P = 0.08$ ), in peak lactation compared with dry period, in contradiction to the higher ( $P < 0.05$ ) total sleep time in the dry period compared to peak lactation. Further, neither REM sleep time- nor proportion of total sleep time was higher in dry period compared with early lactation ( $P > 0.1$ ), even though the total sleep time was higher in the dry period compared to early lactation ( $P < 0.01$ ).

It has not been possible to explain these alterations of REM sleep time with support from the literature. Anyhow, it is tempting to speculate in whether the increased REM sleep time and proportion of total sleep time in peak lactation is an adaption to the

dramatic increase in energy requirement (Drackley *et al.*, 2005; Coppock, 1985; Schröder & Staufenbiel, 2006; Bewley & Schutz, 2008; Walsh *et al.*, 2011) and dry matter intake (Jordan *et al.*, 1973; Ingvarlsen & Andersen, 2000; Bewley & Schutz, 2008) occurring during the transition period. In that case, the REM sleep increase could be a compensation for the total sleep time reduction occurring after parturition. Another factor that could have influenced the quality and amount of sleep was the fact that some of the cows were affected by health problems, since the amount of NREM sleep has been shown to increase during infection (Toth & Krueger, 1989; Toth *et al.*, 1995; Lancel *et al.*, 1995; Bryant *et al.*, 2004; Preston *et al.*, 2009). Due to the small data set and the relatively low rate of health incidences, the effect of health problems has not been investigated further.

### **Lying behaviour**

The fact that lying time data is more easily obtained, compared to sleep data, makes it interesting to look for a correlation between these two parameters, with such a relationship it might be possible to get an estimation of sleep time by measuring lying time. It was not possible to find support for a correlation between lying- and total sleep time in dairy cows in literature. Anyhow, there are two factors in the result of this study that might support such a suggestion. Firstly, the proportion of total sleep time of time spent lying down (sleep/lying proportion) was not different in the three stages of lactation ( $P>0.05$ ), which indicates that the sleep/lying proportion is not changed even though the sleep time is altered. Secondly, without support from any statistical analysis, there is an impression that it exists a large variation between but not within individuals in the aspect of sleep/lying proportion, which could be considered as an indication of that each individual is having a quite constant relationship between these two parameters.

Assuming that factors affecting lying time have the same impact on sleep time there are some studies that might support the result of this study. It has for an example been shown that cows in early lactation spend less time lying down compared to late lactation- and dry cows (Chaplin & Munksgaard, 2001; Bewley *et al.*, 2010) and that high yield cows spend shorter time lying, in the advantage for feeding time, compared to low yield cows (Fregonesi & Leaver, 2001; Bewley *et al.* 2010). Thus, if this is true also for sleep time these two statements are supporting the result of reduced sleep time in early lactation found in this study, and also the theory of decreased sleep time in advantage for feeding. Further, it has been shown that the high motivation to lie down could be disregarded in the advantage of other needs such as feeding or avoiding heat stress (Ruckebusch, 1974; Cooper *et al.*, 2008; Schutz *et al.*, 2010), which support the idea that cows could reprioritise behaviours if needed.

### **Body temperature**

The general impression was that there seemed to be variations in how body temperature changed, prior and while sleeping, both between and within individuals. One possible suggestion to why no increase in body temperature during active periods was obtained in dairy cows, which has been found in other species (Refinetti & Menaker, 1992; Li & Satinoff 1995; Refinetti & Piccone, 2005), might be the fact that cows are sleeping during relatively short bouts. This has no support from the literature and needs to be investigated further.

## Sleep deprivation

Due to the small number of individuals, relatively short recording durations and small number of repetitions on each individual, the result of this study should be considered as preliminary. It is not yet possible to conclude whether the dairy cow has the time she needs for sleep or if the high milk yields and modern dairy farm management is interfering with her sleep requirements. Sleep deprivation has in other species been shown to have multiple physiological effects such as increased metabolic activity, negative energy balance, increased catabolism, reduced immune defence, incidence of death and increased mortality (Kripke *et al.*, 1979; Rechtschaffen *et al.*, 1983; Bergmann *et al.*, 1989; Everson, 1995; Everson & Toth, 2000; Nilsson *et al.*, 2001; Kripke *et al.*, 2002). The fact that some of the sleep deprivation effects are similar to health problems that the transition cow is suffering from such as high metabolic pressure, negative energy balance and suppressed immune defence (Coppock, 1985; Mallard *et al.*, 1998; Drackely *et al.*, 2005; Mulligan *et al.*, 2006; Schröder & Staufenbiel, 2006; Bewley & Schutz, 2008; Walsh *et al.*, 2011;), might be considered as an indication of a possible connection between sleep deprivation and transition related health problems in dairy cows. In order to investigate this further, more extensive trials with sleep recording in dairy cows need to be performed and the sleep quantities need to be connected with measurements of transition related health problems.

## Conclusions

According to the results of this study total sleep time is shorter in both early- and peak lactation compared with the dry period. The largest factor causing the decreased sleeping time is suggested to be the dramatic increase in energy requirement, accompanied with a large dry matter intake and feeding- and rumination time in onset of lactation. Sleep quality was also altered after parturition, the REM sleep time and the proportion REM sleep time of total sleep time was higher in peak lactation compared with the dry period.

It could also be concluded that cows sleep during larger proportion of the night compared with the day. Indications of a possible correlation between total sleep time and total lying time was found, but no relationship between sleep and fluctuations in body temperature. Possible correlations between sleep and heart beat could not be investigated since no heart rate measurements was not obtained.

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## Appendix

**Table 1.** Age (number of years), number of lactations and total milk yield in previous lactation (kg energy corrected milk) for the eight cows, of the Swedish Red breed, used for electrophysiological recordings in three different stages of lactation.

Cow Id	Age	Lactation number	Milk yield previous lactation
1083	9	6	9745
1165	8	6	10 036
1228	7	6	7546
1259	7	5	8848
1367	5	3	8393
1377	4	3	7660
1401	3	2	5537
1417	3	2	9418

**Table 2.** Amount of data (h) obtained from electrophysiological-, body position- and body temperature recordings from seven cows, of the Swedish Red breed, in three different stages of lactation. Amount of data from electrophysiological recordings are time remaining after exclusion of artefacts.

Cow Id	Dry period	Early lactation	Peak lactation
Electrophysiological data			
1083	24,0	24,0	23,7
1165	23,7	24,0	24,0
1228	20,8	23,9	24,0
1259	24,0	24,0	24,0
1367	18,0	23,9	23,0
1377	24,0	24,0	24,0
1401	24,0	24,0	24,0
Body position data			
1083	24,0	24,0	24,0
1165	24,0	24,0	24,0
1228	-	-	24,0
1259	24,0	24,0	24,0
1367	24,0	24,0	24,0
1377	24,0	24,0	24,0
1401	24,0	24,0	-
Body temperature data			
1083	24,0	24,0	24,0
1165	-	24,0	16,5
1228	-	24,0	24,0
1259	24,0	24,0	24,0
1367	-	24,0	24,0
1377	24,0	-	18,0
1401	-	24,0	24,0

(-) No data obtained

**Table 3.** Distribution of amount of data (h) obtained during night- and daytime from electrophysiological recordings on seven cows of the Swedish Red breed in three different stages of lactation; dry period, early- and peak lactation. Amount of data are time remaining after exclusion of artefacts.

Distribution of electrophysiological data						
Cow Id	Dry period		Early lactation		Peak lactation	
	Night	Day	Night	Day	Night	Day
1083	10,0	14,0	10,0	14,0	10,0	13,7
1165	10,0	13,7	10,0	14,0	10,0	14,0
1228	8,1	12,7	10,0	13,9	10,0	14,0
1259	10,0	14,0	10,0	14,0	10,0	14,0
1367	6,3	11,7	10,0	13,9	9,5	13,5
1377	10,0	14,0	10,0	14,0	10,0	14,0
1401	10,0	14,0	10,0	14,0	10,0	14,0

**Table 4.** Time for electrophysiological recordings- and health problems given in days relative to calving (DRC) for seven cows, of the Swedish Red breed, in three different stages of lactation; dry period (-20 to 0 DRC), early (0 to +39 DRC)- and peak lactation (+40 to +50 DRC). Diagnosis, treatment type- and length (number of days) are specified for respectively health problem. High somatic cell count (SCC) was defined as when cow had California mastitis test (CMT) values higher than 3 on at least two teats.

Cow Id	Dry period			Early lactation			Peak lactation		
	Data collection (DRC)	Health problem (DRC)	Diagnosis and treatment	Data collection (DRC)	Health problem (DRC)	Diagnosis and treatment	Data collection (DRC)	Health problem (DRC)	Diagnosis and treatment
1083	(-20)	(-14)	Mastitis Antibiotics (5 days)	(+15)	(+7)	Mastitis Antibiotics (5 days)	(+43)	(+32)	Mastitis Antibiotics (5 days)
1165	(-17)			(+11)	(0)	Paresis Calcium injection (1 day)	(+45)		High SCC
1228	(-13)			(+15)		High SCC	(+50)		
1259	(-19)			(+14)		High SCC	(+42)		High SCC
1367	(-12)			(+13)			(+45)		
1377	(-10)			(+11)	(+6)	Fever Antinflammatory drug (1 day)	(+46)		
1401	(-16)			(+13)	(+1)	Mastitis Antibiotics (5 days)	(+41)		High SCC

**Table 5a.** Total sleep time given in hours per 24-hours for seven cows of the Swedish Red breed, in three different stages of lactation; dry period, early- and peak lactation. Mean values (N=7) and standard deviations (SD) for each stage of lactation and for the entire data set.

Cow Id	Total sleep time		
	Dry period	Early lactation	Peak lactation
1083	4,6	3,9	4,1
1165	2,5	1,5	2,0
1228	5,0	1,8	4,2
1259	6,0	3,7	3,0
1367	4,4	2,1	3,0
1377	3,1	1,5	2,7
1401	5,6	2,7	5,2
Mean	4,5	2,5	3,5
SD	1,3	1,0	1,1
Entire data set			
Mean	3,5		
SD	1,4		

**Table 5b.** P-values for comparison of total sleep time per 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values total sleep time		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,001	0,03	0,07

**Table 6a.** Non rapid eye movement (NREM) sleep time given in hours per 24-hours for seven cows, of the Swedish Red breed, in three different stages of lactation; dry period, early- and peak lactation. Mean values (N=7) and standard deviations (SD) for each stage of lactation.

Cow Id	NREM sleep time		
	Dry period	Early lactation	Peak lactation
1083	4,4	3,2	3,2
1165	2,1	1,1	1,5
1228	4,4	1,3	2,9
1259	5,1	3,5	2,4
1367	3,9	1,6	2,3
1377	2,7	1,3	1,7
1401	4,4	2,2	3,9
Mean	3,9	2,0	2,5
SD	1,1	1,0	0,8

**Table 6b.** P-values for comparison of non rapid eye movement (NREM) sleep time per 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values NREM sleep time		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,001	0,004	0,2

**Table 7a.** Non rapid eye movement (NREM) sleep time as proportion (%) of total sleep time (NREM sleep proportion) during 24-hours for seven cows of the Swedish Red breed, in three different stages of lactation ; dry period, early- and peak lactation. Mean values (N=7) and standard deviations (SD) for each stage of lactation.

Cow Id	NREM sleep proportion		
	Dry period	Early lactation	Peak lactation
1083	96	82	79
1165	84	77	76
1228	87	71	68
1259	85	95	80
1367	89	76	76
1377	88	89	62
1401	78	79	75
Mean	87	81	74
SD	5	8	6

**Table 7b.** P-values for comparison of non rapid eye movement (NREM) sleep time as proportion (%) of total sleep time (NREM sleep proportion) during 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values NREM sleep proportion		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,2	0,005	0,09

**Table 8a.** Rapid eye movement (REM) sleep time given in hours per 24-hours for seven cows, of the Swedish Red breed, in three different stages of lactation; dry period, early- and peak lactation. Mean values (N=7) and standard deviations (SD) for each stage of lactation.

Cow Id	REM sleep time		
	Dry period	Early lactation	Peak lactation
1083	0,2	0,7	0,9
1165	0,4	0,4	0,5
1228	0,7	0,5	1,4
1259	0,9	0,2	0,6
1367	0,5	0,5	0,7
1377	0,4	0,2	1,0
1401	1,2	0,6	1,3
Mean	0,6	0,4	0,9
SD	0,4	0,2	0,3

**Table 8b.** P-values for comparison of rapid eye movement (REM) sleep time as proportion (%) of total sleep time (REM sleep proportion) during 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values REM sleep time		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,3	0,08	0,008

**Table 9a.** Rapid eye movement (REM) sleep time as proportion (%) of total sleep time (REM sleep proportion) during 24-hours for seven cows of the Swedish Red breed, in three different stages of lactation; dry period, early- and peak lactation. Mean values (N=7) and standard deviations (SD) for each stage of lactation.

Cow Id	REM sleep proportion		
	Dry period	Early lactation	Peak lactation
1083	4	18	21
1165	16	23	24
1228	13	29	32
1259	15	5	20
1367	11	24	24
1377	12	11	38
1401	22	21	25
Mean	13	19	26
SD	5	8	6

**Table 9b.** P-values for comparison of rapid eye movement (REM) sleep time as proportion (%) of total sleep time (REM sleep proportion) during 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values REM sleep proportion		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,2	0,005	0,09

**Table 10a.** Lying time given in hours per 24-hours for seven cows of the Swedish Red breed in three different stages of lactation; dry period, early- and peak lactation. Mean values (N=6) and standard deviations (SD) for each stage of lactation.

Cow Id	Lying time		
	Dry period	Early lactation	Peak lactation
1083	13,5	10,4	15,9
1165	15,8	14,8	13,9
1228	-	-	15,9
1259	15,4	10,3	13,3
1367	11,0	10,6	11,3
1377	15,0	11,4	11,9
1401	18,1	12,5	-
Mean	14,8	11,7	13,7
SD	2,4	1,7	1,9

(-) No data obtained

**Table 10b.** P-values for comparison of lying time during 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values lying time		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,01	0,4	0,2

**Table 11a.** Total sleep time as proportion (%) of the total lying time (total sleep proportion) for seven cows of the Swedish Red breed in three different stages of lactation; dry period, early- and peak lactation. Mean values (N=6) and standard deviations (SD) for each stage of lactation.

Cow Id	Total sleep proportion		
	Dry period	Early lactation	Peak lactation
1083	34	37	26
1165	16	10	15
1228	-	-	27
1259	39	36	22
1367	40	20	26
1377	20	13	23
1401	31	22	-
Mean	30,0	23,0	23,2
SD	9,9	11,3	4,4

(-) No data obtained

**Table 11b.** P-values for comparison of total sleep time as proportion (%) of total lying time (total sleep proportion) during 24-hours between three different stages of lactation; dry period, early- and peak lactation for seven cows of the Swedish Red breed.

P-values total sleep proportion		
Dry period-early lactation	Dry period-peak lactation	Early lactation-peak lactation
0,07	0,1	0,1

**Table 12.** Mean values (N=7) and standard deviations (SD) for total sleep-, non rapid eye movement (NREM) sleep-, rapid eye movement (REM) sleep- and lying time as proportion (%) of obtained amount electrophysiological recording data during night (between 7:30 p.m. and 5:30 a.m.)- and daytime (between 5:30 a.m. and 7:30 p.m.) for seven cows of the Swedish Red breed in three different stages of lactation; dry period, early- and peak lactation. P-values for comparison between night- and daytime proportions for each parameter within the three different stages of lactation.

	Total sleep		NREM sleep		REM sleep		Lying	
	Night	Day	Night	Day	Night	Day	Night	Day
Dry period								
Mean	23	15	20	13	3	2	75	53
SD	6	6	6	5	2	2	8	13
P-value	0,007		0,01		0,03		0,008	
Early Lactation								
Mean	14	8	11	6	3	1	55	44
SD	4	5	4	5	1	1	12	6
P-value	0,003		0,01		0,05		0,05	
Peak Lactation								
Mean	18	11	13	9	5	3	65	51
SD	6	4	5	4	2	1	9	9
P-value	0,002		0,03		0,01		0,009	

**Table 13.** Overall mean values (N=21) and standard deviations (SD) of proportion (%) of total sleep-, non rapid eye movement (NREM) sleep-, rapid eye movement (REM) sleep- and lying time occurring during night time for seven cows of the Swedish Red breed in three different stages of lactation; dry period, early- and peak lactation.

	Proportion occurring during night			
	Total sleep	NREM sleep	REM sleep	Lying time
Mean	55	54	60	48
SD	8	11	16	4

**Table 14.** Mean values (N=7) and standard deviations (SD) for average-, shortest- and longest duration (min) of non rapid eye movement (NREM)- and rapid eye movement (REM) sleep bouts for seven cows of the Swedish Red breed in three different stages of lactation; dry period, early- and peak lactation. Data collection was performed during 24-hours for each cow and stage of lactation. Shortest bout duration was 30s. P-values for comparison between the different stages of lactation.

NREM sleep bouts									
	Dry period		Early lactation		Peak lactation		Dry period- early lactation	Dry period- peak lactation	Early lactation- peak lactation
	Mean	SD	Mean	SD	Mean	SD	P-values		
Average	3,3	1,3	2,9	1,7	3,2	0,5	0,65	0,94	0,58
Shortest	0,5	0,0	0,5	0,0	0,6	0,2	-	0,17	0,17
Longest	11,5	3,6	10,8	3,6	11,3	2,9	0,97	0,74	0,84

REM sleep bouts									
	Dry period		Early lactation		Peak lactation		Dry period- early lactation	Dry period- peak lactation	Early lactation- peak lactation
	Mean	SD	Mean	SD	Mean	SD	P-values		
Average	2,8	0,8	3,3	0,4	4,2	0,9	0,19	0,01	0,05
Shortest	1,1	0,7	1,5	0,9	1,6	0,7	0,52	0,22	0,74
Longest	5,0	1,5	5,9	1,3	9,6	3,2	0,38	0,01	0,04

(-) No T-test could be performed, all shortest bout durations was the same (30s).

**Table 15.** Mean values and standard deviations (SD) for body temperature (C°) during time awake (awake) and time asleep (asleep) for seven cows of the Swedish Red breed, in three different stages of lactation; dry period, early- and peak lactation. Differences between mean values of body temperature (C°) for time asleep and time awake (asleep-awake) for each cow in each stage of lactation.

	Awake		Asleep		Difference asleep-awake
Cow Id	Mean	SD	Mean	SD	
Dry period					
1083	38,7	0,2	38,8	0,2	0,05
1165	-	-	-	-	-
1228	-	-	-	-	-
1259	39,0	0,2	39,1	0,1	0,03
1367	-	-	-	-	-
1377	38,6	0,2	38,7	0,1	0,08
1401	-	-	-	-	-
Early lactation					
1083	38,8	0,1	39,1	0,2	0,3
1165	38,8	0,2	38,8	0,2	0,04
1228	39,0	0,2	39,0	0,2	0,03
1259	38,9	0,1	38,9	0,1	-0,05
1367	39,2	0,3	39,2	0,3	0,03
1377	-	-	-	-	-
1401	39,0	0,2	39,1	0,1	0,07
Peak lactation					
1083	38,5	0,2	38,6	0,2	0,1
1165	38,4	0,2	38,4	0,3	-0,02
1228	38,7	0,2	38,8	0,2	0,05
1259	38,6	0,2	38,7	0,2	0,03
1367	39,0	0,2	39,1	0,2	0,06
1377	38,7	0,3	38,7	0,2	0,005
1401	38,7	0,1	38,7	0,1	0,03

(-) No data was obtained



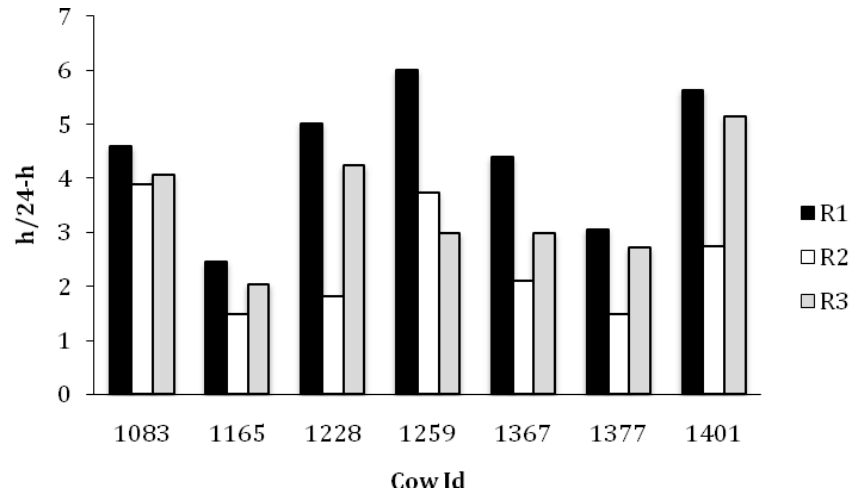
**Figure 1.** Attachment of surface electrodes for electrophysiological recordings on dairy cows; four electrodes forming a square in the forehead (EEG), reference electrode in the middle of the square (EEG) and right and left eye electrodes (EOG).



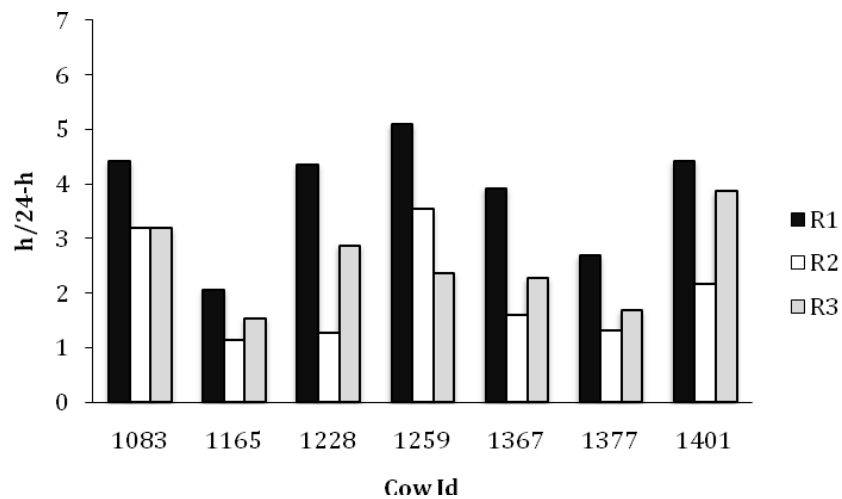
**Figure 2.** Attachment of surface electrodes for electrophysiological recordings on dairy cows; ground electrode (EEG), right eye electrode (EOG) and right neck muscle electrode (EMG).



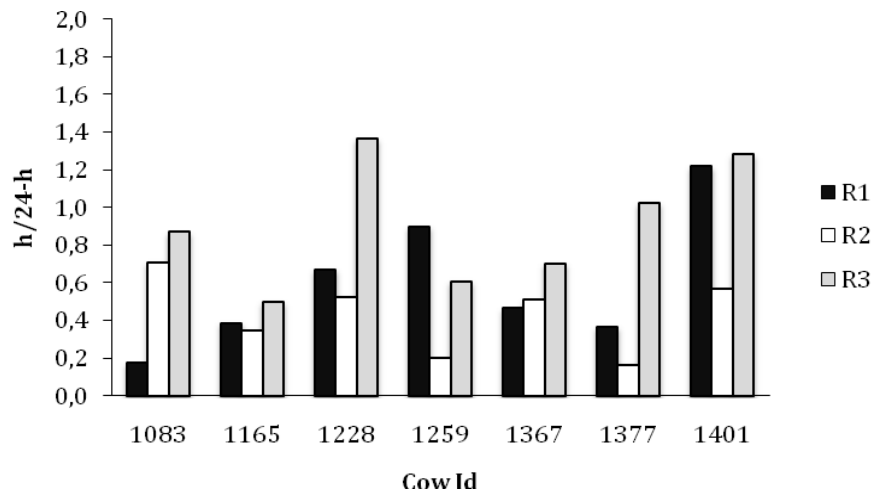
**Figure 3.** Attachment of surface electrodes for heart rate recordings (ECG) on dairy cows; four surface electrodes attached to the thorax on the left side.



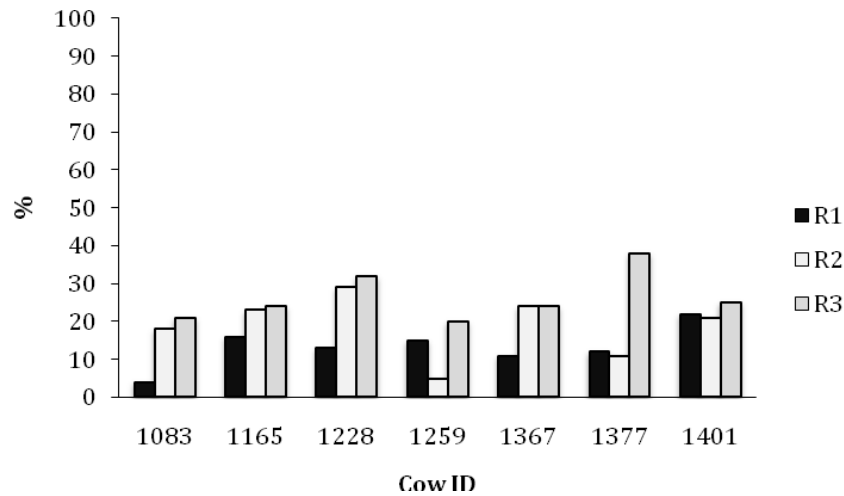
**Figure 4.** Total sleep time given in hours per 24-hours (h/24-h) for seven cows of the Swedish Red breed, in three different stages of lactation; dry period (R1), early (R2)- and peak lactation (R3).



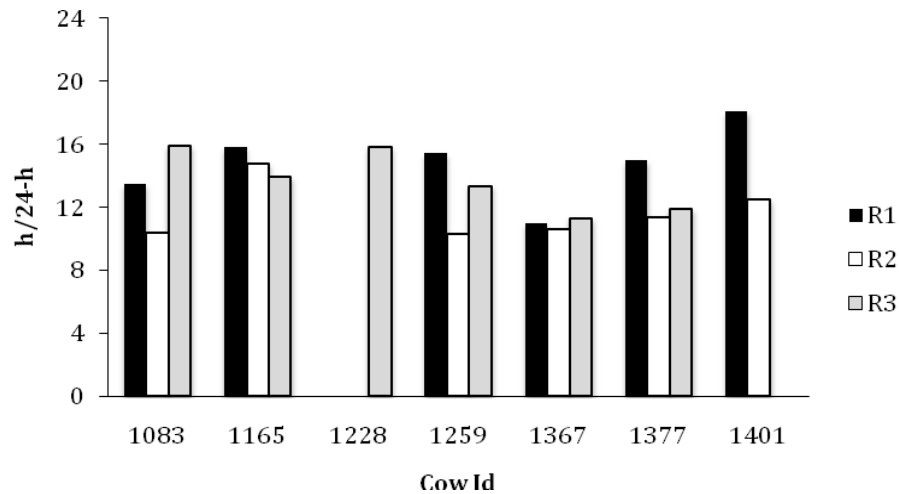
**Figure 5.** Non rapid eye movement (NREM) sleep time given in hours per 24-hours (h/24-h) for seven cows of the Swedish Red breed, in three different stages of lactation; dry period (R1), early (R2)- and peak lactation (R3).



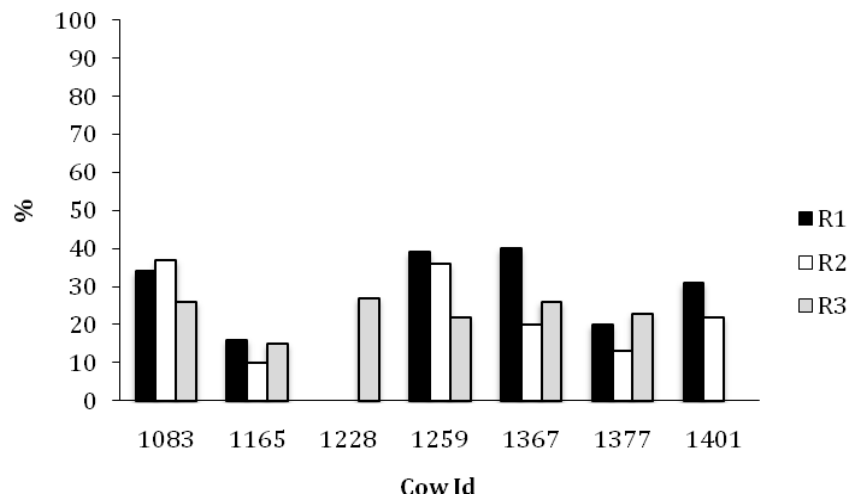
**Figure 6.** Rapid eye movement (REM) sleep time given in hours per 24-hours (h/24-h) for seven cows of the Swedish Red breed, in three different stages of lactation; dry period (R1), early (R2)- and peak lactation (R3).



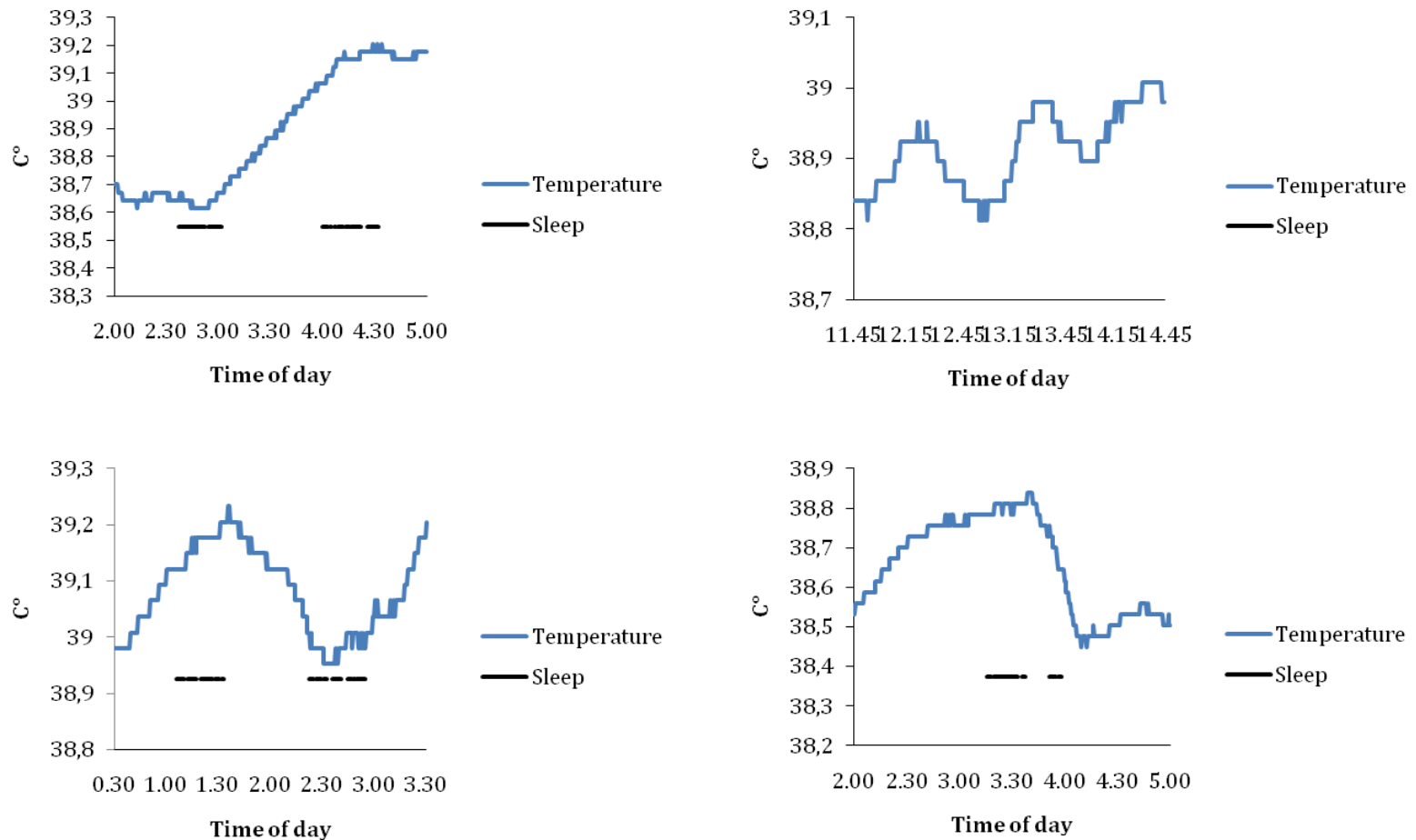
**Figure 7.** Rapid eye movement (REM) sleep time as proportion (%) of total sleep time during 24-hours for seven cows of the Swedish Red breed in three different stages of lactation; dry period (R1), early (R2)- and peak lactation (R3).



**Figure 8.** Lying time given in hours per 24-hours (h/24-h) for seven cows of the Swedish Red breed, in three different stages of lactation; dry period (R1), early (R2)- and peak lactation (R3). Data for cow 1228 in R1 and R2 is missing, as well as data for cow 1401 in R3.



**Figure 9.** Total sleep time as proportion (%) of total lying time per 24-hours for seven cows of the Swedish Red breed, in three different stages of lactation; dry period (R1), early (R2)- and peak lactation (R3). Data for cow 1228 in R1 and R2 is missing, as well as data for cow 1401 in R3.



**Figure 10.** Body temperature (C°) and sleep sessions plotted against time of day, for four different cows during 3 hours of one day. The cow was in peak- or early lactation and of the Swedish Red breed.

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